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Host plant cultivar, leaf positions and nutrition affect the expression of Marssonina blotch resistance in apple

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ABSTRACT

Three experiments were conducted under polyhouse conditions during 2010-2011, using inoculated one year old grafted plants of apple, to determine the influence of host plant cultivars, leaf position and nutrition on the development of Marssonina blotch disease. None of the tested apple cultivars was resistant to Marssonina blotch, however, Granny Smith and Gibbson's Golden showed moderately resistant reaction. The commercial cultivars Royal Delicious, Golden Delicious, Vance Delicious and Scarlet Spur were found highly susceptible. The upper leaves were more resistant than the lower leaves when one year old plants of Starking Delicious cultivar were tested. The progression of Marssonina blotch from the base of the plant upwards on apple plants could be due to difference in leaf resistance. In addition, macronutrient combinations with full dose of nitrogen, registered good growth of potted apple plants, consequently, reduced the severity of disease, which was increased by its half and double dosages.

Key words: Apple, disease severity, host factors, Marssonina blotch, *Marssonina coronaria*

Marssonina blotch caused by *Marssonina coronaria* (Ell. et J.J. Davis) J.J. Davis, imperfect stage of *Diplocarpon mali* (Harada *et al.*, 1974) is a major foliar disease of apple (Lee *et al*., 2011), which may affect seedling in the nurseries and adult plants in the field. This disease was first observed in Japanese orchards in 1904 and identified to be caused by *Marssonina mali* (P. Henn) S. Ito (Miyake, 1907), however, Parmelee (1971) identified the causal agent as *Marssonina coronaria* (EII. et J.J. Davis) J.J. Davis (Syn. *Marssonina mali*). The disease is widely-distributed, being reported in North America, Oceana, and Asia (Harada *et al*., 1974; Lee and Shin, 2000). In India, Marssonina blotch in apple has been relatively a new threat to its successful cultivation in Himachal Pradesh and was first recorded in the year 1992 in some orchards of Kotkhai area in the state (Sharma *et al*., 2004). Marssonina blotch appeared in epiphytotic form in Himachal Pradesh in 1996 and threatened the apple cultivation by causing premature leaf fall (Sharma, 2001). All the commercial Delicious cultivars viz., Royal Delicious, Golden Delicious, Red Delicious, Rich-a-Red and Red Gold etc. are highly susceptible to this disease (Li *et al*., 2012). It affects the fruit size, colour, quality and fruit set adversely by affecting the tree vigour and fruit bearing capacity of the trees at the same time. Appearance of fruit spots on the produce makes it unsaleable in the market leading to direct economic loss to the growers (Sharma *et al*., 2011). The research reported in this paper was performed to elucidate the effect of host cultivars, leaf position and nutrition on disease development.

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MATERIALS AND METHODS

The present work consisted of three experiments to evaluate host response to Marssonina blotch disease and was conducted during 2010-2011 at experimental farm of Department of Mycology and Plant Pathology, College of Horticulture, Dr. Y. S. Parmar University of Horticulture and Forestry, Solan. All the experiments were conducted under well equipped polyhouse conditions as the testing disease resistance in the field only produces reliable results if the environmental conditions are favorable for the disease development (Coelho *et al*., 2009).

Under each experiment one year old grafted plants of apple were inoculated with the standardized conidial suspension ($5x10^4$ conidia/ml) subjected to 24 h of leaf wetness and incubated further for 21 days in ambient environmental conditions in polyhouse. Each treatment was replicated thrice. Observations on disease appearance (+ or –), incubation period (days), latent period (days), conidial production (number of spores per lesion) and disease severity (%) were recorded separately for each treatment (Sharma *et al*., 2005). Requirement of infection period for disease development (Sharma, 2004) was kept in mind for maintaining congenial set of environmental conditions for disease development under polyhouse. The least significant difference was used to compare the values under different conducted experiments (Gomez and Gomez, 1984).

Inoculum of *M. coronaria* was collected from an infected apple plant cultivar Starking Delicious and maintained on plants of same cultivar in polyhouse with average night/day temperatures of 20 and 25^0C, respectively. Planting material and pathogen inoculum were maintained in Fruit Pathology Laboratory, Dr. Y. S. Parmar University of Horticulture and Forestry, Solan. The plants were irrigated, fertilized and their insect/pests were managed using standard practices.

Effect of host plant cultivar on disease development

Eleven different apple cultivars (both commercial and pollinizers), viz Scarlett Spur, Super Chief, Vance Delicious, Red Chief, Red Fuji, Gale Gala, Golden Delicious, Granny Smith, Gibbson's Golden and Starking Delicious were grafted on seedling root stock and maintained under polyhouse conditions. The cultivars were inoculated and evaluated for their reaction to Marssonina blotch disease under semi-controlled conditions during the year 2010. The disease reaction for different cultivars was recorded by using the following scales:

Category	Disease index (%)	Reaction
1	0-6	Resistant (R)
2	6-20.9	Moderately resistant (MR)
3	21-40.9	Moderately susceptible (MS)
4	41-60.9	Susceptible (S)
5	More than 60.9	Highly susceptible (HS)

Effect of leaf position on disease development

Potted apple plants of Starking Delicious were inoculated with the standardized conidial suspension ($5x10^4$ conidia/ml). Youngest unfolding leaf were tagged with cotton thread and subjected to high humidity for 24 h of leaf wetness and incubated further for 21 days in ambient environmental conditions in polyhouse.

Effect of host nutrients on disease development

Nutrient status of the potting mixture for primary nutrients was determined by analysing soil sample in the laboratory of Department of Soil Science and Water Management, Dr. Y.S. Parmar University of Horticulture and Foretry, Nauni, Solan. Additional quantity of N, P and K were supplied in different combinations to the potted apple Starking Delicious plants. The fertilizer doses were calculated on the basis of soil weight. Each nutrient was calculated for its full, half and double dose. Nine different combinations were prepared and replicated three times. Plants without additional fertilizer doses were maintained as control. They were then inoculated with standardized conidial suspension to evaluate effect of host nutrition on diseases development.

RESULTS AND DISCUSSION

Effect of host plant cultivar on disease development

All cultivars developed disease symptoms (Table 1). First visible disease symptoms on Early Red 1, Scarlet Spur, Super Chief, Vance Delicious, Red Chief, Red Fuji, Gale Gala, Golden Delicious and Starking Delicious appeared on 11^{th} day of inoculation. However, on Granny Smith and Gibbson's Golden cultivars symptoms appeared on the 12^{th} day. Sporulation of pathogen on Early Red 1, Scarlet Spur, Super Chief, Vance Delicious, Red Chief, Red Fuji, Gale Gala, Golden Delicious and Starking Delicious was observed on 12^{th} day of inoculation, whereas on Granny Smith and Gibbson's Golden cultivars, it occurred on the 14^{th} day. These genotypes were categorized into different reaction classes on the basis of per cent disease severity after inoculation. None of the cultivars of apple was rated resistant to Marssonina leaf blotch, however, Granny Smith and Gibbson's

Table 1. Effect of host cultivars on Marssonina blotch disease development in apple under semi-controlled conditions

Apple cultivar	Conidial production ($x10^4$/ml)	Disease severity (%)	Disease reaction*
Early Red 1	25.33	47.48 (43.56)	S
Scarlet Spur	19.52	37.71 (37.89)	MS
Super Chief	28.19	75.74 (60.50)	HS
Vance Delicious	29.21	76.13 (60.76)	HS
Red Chief	17.31	30.44 (33.49)	MS
Red Fuji	15.74	28.64 (32.36)	MS
Gale Gala	14.59	28.31 (32.15)	MS
Golden Delicious	30.71	78.11 (62.11)	HS
Granny Smith	23.34	20.06 (26.61)	MR
Gibbson's Golden	21.11	20.41 (26.86)	MR
Starking Delicious	34.68	80.00 (63.44)	HS
CD (p=0.05)	2.17	0.79 (0.51)	

Figures in parentheses are arc sine transformed values
*R-Resistant, MR-Moderately resistant, MS- Moderately susceptible, S-Susceptible and HS-Highly susceptible

Golden showed moderately resistant (MR) reaction with per cent disease severity of 20.06 and 20.41, respectively. Cultivars Red Chief, Red Fuji and Gale Gala were moderately susceptible (MS); disease severity ranging from 28.31 to 37.71 per cent, while the remaining cultivars showed susceptible (S) to highly susceptible (HS) disease reaction. Sharma *et al*. (2011) also reported the commercial cultivars Starking Delicious, Golden Delicious, Vance Delicious and Scarlet Spur to be highly susceptible (HS) to Marssonina blotch.

Effect of leaf position on disease development

The results revealed that younger leaves (at 0 – 4 position) did not develop disease under the given circumstances, however, disease appeared on the older leaves (Table 2). First visible disease symptoms and sporulation of the pathogen were observed on the oldest leaves of apple plants. These studies also revealed that maximum (63.63%) disease severity and conidial production (34.12 x 10^4 conidia/ml) were recorded on oldest leaf of position 10^{th}, whereas, no disease was recorded on 0 to 4^{th} leaf even after 21 days of incubation period. Similar results have also been reported by Thakur *et al.* (2005) .

Table 2. Effect of leaf position on Marssonina blotch disease development in apple under semi-controlled conditions

Leaf position*	Conidial production ($x10^4$/ml)	Disease severity (%)
0	0.00	0.00 (0.00)
1	0.00	0.00 (0.00)
2	0.00	0.00 (0.00)
3	0.00	0.00 (0.00)
4	0.00	0.00 (0.00)
5	6.61	12.75 (20.86)
6	7.44	19.99 (26.55)
7	10.38	32.67 (34.85)
8	13.78	40.72 (39.64)
9	22.55	51.27 (45.73)
10	34.12	63.63 (52.94)
CD (p=0.05)	2.56	4.32 (2.69)

*- Position from the tip downward, with 0 being the unfolded leaf ; Figures in parentheses are arc sine transformed values

Effect of host nutrients on disease development

The data presented in Table 3 revealed that disease appeared in all the nutrient combinations. First visible disease symptoms appeared on 11^{th} day after inoculation when the plants were treated with $N_2P_1K_1$, $N_2P_2K_2$, $N_2P_3K_3$, $N_3P_1K_1$, $N_3P_2K_2$, $N_3P_3K_3$ nutrient combinations and control. However, disease symptoms appeared on 12^{th} day when plants were treated with $N_1P_1K_1$, $N_1P_2K_2$ and $N_1P_3K_3$ combinations. Sporulation of pathogen started on 12^{th} day when plants were supplied with $N_2P_1K_1$, $N_2P_2K_2$, $N_2P_3K_3$, $N_3P_1K_1$, $N_3P_2K_2$, $N_3P_3K_3$ nutrient

Table 3. Effect of host nutrition on Marssonina blotch disease development in apple under semi-controlled conditions

Nutrient combinations*	Conidial production (x10^4/ml)	Disease severity (%)
$N_1P_1K_1$	16.69	38.12 (38.13)
$N_1P_2K_2$	17.92	40.52 (39.53)
$N_1P_3K_3$	19.60	44.40 (41.79)
$N_2P_1K_1$	23.72	65.11 (53.82)
$N_2P_2K_2$	26.06	56.03 (48.47)
$N_2P_3K_3$	27.19	60.05 (50.81)
$N_3P_1K_1$	31.28	70.26 (56.96)
$N_3P_2K_2$	32.39	75.11 (60.08)
$N_3P_3K_3$	32.98	80.16 (63.55)
Control	36.21	80.66 (63.92)
CD (p=0.05)	4.15	3.78 (2.22)

Figures in parentheses are arc sine transformed values
*- as per general recommendations
where;
N_1 : Full dose of nitrogen; N_2: Half dose of nitrogen; N_3: Double dose of nitrogen
P_1 : Full dose of phosphorus; P_2: Half dose of phosphorus; P_3: Double dose of phosphorus
K_1 : Full dose of potassium; K_2: Half dose of potassium; K_3: Double dose of potassium

combinations as well as in control. However, it occurred on 13th day when plants were treated with $N_1P_1K_1$, $N_1P_2K_2$ and $N_1P_3K_3$ combinations. Maximum conidial production (36.21x10^4 conidia/ml) was recorded on control plants i.e. nutritionally deficient plants, whereas, minimum was recorded on plants with $N_1P_1K_1$ combination (16.69x10^4 conidia/ml). It is evident from the present investigations that nutrient combinations with full dose of nitrogen, registered good growth of apple plant, consequently, reduced the severity of Marssonina blotch ranging from 38.12 to 44.40 per cent. The combinations with half dose of nitrogen showed weak growth of plant and resulted in appearance of deficiency symptoms i.e. yellowing of older leaves. When the leaves turned yellow, photosynthetic activities automatically went down. As a result, the plants became weak and prone to test pathogen that could be due to reduction in disease resistance and consequently, this might have led to high disease pressure (60.05-65.11%) under these combinations. Finally, combination with double dose of nitrogen registered more vegetative growth of plants. Plants also showed burning symptoms due to high dose of nutrients. Such type of nutrient combinations registered high disease severity as compared to others (70.26-80.16%). Half dose combinations of phosphorus and potassium along with the control showed deficiency symptoms on the foliage. Potassium showed marginal scorching and phosphorus showed pinkish spots on the leaves, consequently, plants became more susceptible to the test pathogen. However, no information is available in the literature to support these results particularly in case of Marssonina blotch of apple. However, similar observations have been recorded by Meszka and Bielenin (2009) on apple scab.

It is concluded from the present investigations that the progression of Marssonina blotch from the base of the plant upwards on apple plants could be due to difference in leaf resistance due to host age, type and nutritional status. The data show that in *M. coronaria,* epidemiological efficiency is influenced extensively by host factors such as susceptibility, leaf position and poor or excess nutrition. Combined effect of these factors and virulent nature of pathogen generate high disease pressure on apple plants. Increased fertilizer application especially nitrogen, in apple production, may in fact increase epidemics if not well managed. Deployment of resistant cultivars in combination with appropriate nutrition and other cultural practices are suggested suitable that could reduce inoculum pressure.

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Identification of potent strains of *Trichoderma* showing antagonism to *Rhizoctonia solani* in rice and isolation of partial antifungal chitinase gene

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ABSTRACT

Thirty soil samples were collected from sheath blight infected rice fields of Punjab Agricultural University, Ludhiana for identification of potent strains of *Trichoderma* showing antagonism to *Rhizoctonia solani* Kuhn. *Trichoderma* strains were grown on PDA for 3-5 days and visually observed for characters like colony colour, colony type, growth rate, discoloration of media, number of sclerotia, parasitism of sclerotia, extent of parasitism of sclerotia, coverage of Petriplate and effectiveness. The comparisons of attributes were made with taxonomic key for the genus *Trichoderma* leading to tentative identification of 38 *Trichoderma* strains. The dual culture analysis of *Trichoderma* strains with *R. solani* led to detection of 24 antagonistic *Trichoderma* strains. The strain designated as S-15 was observed to be most potent and the microscopic analysis revealed *R. solani* to be growth inhibitor. The genomic DNA was isolated from the 24 antagonistic *Trichoderma* strains for isolation of chitinase gene(s). The authentication of genomic DNA was done through PCR using housekeeping actin gene specific primers, resulting in amplification of expected 137bp product in sample S-15. The internal PCR primers were designed to isolate antifungal chitinase gene from S-15. The results revealed two independent amplicons of approximately 1020bp and 771bp size on 1.5 per cent agarose gel with primer SB-14F/R and SB-35F/R, respectively corresponding to two partial chitinase gene(s). The partial chitinase genes were sequenced and authenticated through BLAST for homology with other antifungal genes.

Key words: Sheath blight, rice, *Trichoderma*, antagonism, *Rhizoctonia*, chitinase

Rice sheath blight is caused by a fungus belonging to *Rhizoctonia* sp. that survives in soil from year to year as a hard, weather-resistant structure called a sclerotium (Wrather and Sweets, 2009). The damage can range from partial infection of the lower leaves with little effect on grain development to premature plant death. The disease causes a yield loss of 6 per cent across lowland rice fields in tropical Asia, and more importantly no natural resistance is available against the disease (http://irri.org/our-science/better-varieties/disease-and-pest-resistant-rice, unpublished). Simple agronomic and pathological practices *e.g.* plant spacing, use of foliar fungicides, burning stubbles, rotation and alternate cropping are being used to control the disease (Wrather and Sweets, 2009). Phytotoxic organic arsine compounds are effective for inhibiting infection, lesion enlargement and mycelial growth. However, extensive use of fungicides and chemicals has increased the soil and water pollution level.

The mycoparasitic fungus *Trichoderma* has been shown to antagonize plant pathogens and is effective biological control agent against foliar diseases (Benitez *et al.,* 2004). The first clear cut induced resistance by *Trichoderma* against *Botrytis cinerea* in tobacco was demonstrated by Bigirimana *et al.* (1998). The different species of *Trichoderma* are capable of parasitizing a large number of plant pathogenic fungi that make them useful as biofungicides (Mukhopadhayay *et al.,* 1992; Harman and Bjorkmann, 1998). Most biocontrol agents are from *T. harzianum, T. viride* and *T. hamatum* sp. The strong antifungal effects

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of *Trichoderma* sp. are because they produce extracellular chitinase and glucanase enzymes that hydrolyse chitin, β-1,3 glucans, linear homopolymer of β-1,4-N-acetyl-D-glucosamine, β-1,6 glucans which are the main constituents of fungal cell wall. *Trichoderma* sp. has evolved numerous mechanisms that are involved in attacking other fungi. These mechanisms include competition for space and nutrients (Elad *et al.,* 1999), mycoparasitism (Haran *et al.,* 1996; Lorito *et al.,* 1996), production of inhibitory compounds (Sivasithamparam and Ghisalberti, 1998), inactivation of the pathogen's enzymes (Roco and Perez, 2001) and induced resistance (Kapulnik and Chet, 2000). More than 50 different *Trichoderma* based agriculture products can be found as registered in many countries in five continents and are sold and applied to protect and improve yield of vegetables, ornamentals and fruit trees (Lorito, 2005). *Trichoderma* is completely safe and in 55 years of research there has never been a recorded adverse reaction on humans and livestock (Anonymous, 2005).

The efficacy of biocontrol agents is a limiting factor due to low field performance, suggesting thereby a need for permanent expression of such enzymes in the plant. Therefore, an alternate strategy to harness the antifungal effects of *Trichoderma* sp. can be through isolation of gene(s) encoding chitinase and glucanase that hydrolyse the main constituents of fungal cell wall. These genes can then be used in generation of gene cassettes and introduction into the host plant genome to engineer transgenic plants with a view to allow them to produce antifungal proteins as their intrinsic proteins. An endochitinase encoding gene, *ech*42, was the first gene cloned from *Trichoderma* to be introduced into tobacco and potato (Lorito *et al.,* 1998). It provided near total protection against foliar pathogens *Alternaria alternata, A. solani, B. cinerea* and the soil borne pathogen *R. solani.* The present study was undertaken with objectives to identify local potent strains of *Trichoderma* having antagonism to *R. solani* of rice and to isolate corresponding antifungal chitinase gene(s).

MATERIALS AND METHODS

Collection and isolation of *Trichoderma* sp.

Identification of potent strains of *Trichoderma* showing antagonism to *R. solani* of rice was carried out at the Rice Pathology Laboratory, Department of Plant Breeding and Genetics; and the isolation of antifungal chitinase genes was carried out at the Molecular and Tissue Culture Laboratory, School of Agricultural Biotechnology, Punjab Agricultural University (PAU), Ludhiana. Thirty soil samples were collected from rice fields of PAU, Ludhiana infected with sheath blight disease. The soil samples were numbered 1 to 30. The serial dilution technique was used for isolation of *Trichoderma*. The appropriate dilutions (1ml aliquot) were pour plated onto *Trichoderma* selective media (TSM) and Petriplates were incubated at 28 ±2°C for 5-7 days (Elad *et al.,* 1981). The *Trichoderma* colonies were transferred to PDA plates till pure cultures were obtained. The pure cultures of *Trichoderma* sp. were grown at 25°C for about 4-5 days on PDA and examined for typical morphological culture characteristics under microscope.

Screening based on antagonism between *Trichoderma* sp. and *R. solani*

The fungal isolates were cultured in Petriplates with PDA for seven days. Discs of 5mm diameter were cut and removed from the growing borders of the colonies and transferred to another Petriplate with PDA. Dual cultures were carried out by using one week old cultures of *R. solani* and *Trichoderma* sp. on PDA. The PDA medium was inoculated with a 5mm diameter disc of antagonist *i.e. Trichoderma* positioned diametrically opposite to a 5mm diameter disc of the pathogen. The distance between discs was approximately 5cm. The cultures were grown at 28 ±2°C and measurements were taken after four days. In the control treatment, a sterile agar disc was placed in Petridish instead of *Trichoderma* isolates. There were three replications for each treatment. At the end of the incubation period, radial growth was measured. The efficiency of *Trichoderma* sp. in suppressing radial growth was studied. The antagonistic *Trichoderma* strains were selected and grown on PDA slants and kept for further use at 4°C.

Genomic DNA isolation and PCR amplification

The selective fungal mat of *Trichoderma* sp. was obtained on Potato Dextrose Broth (PDB), after incubation at 28°C for 5 days. Genomic DNA was isolated from fungal mat using a modified CTAB

method (Kim *et al.,* 1989) and quantified using spectrophotometer analysis. The genomic DNA samples were standardized to 20 ng/µl in TE buffer. The isolated genomic DNA samples were verified through PCR using housekeeping actin gene specific forward primer designated as Act_F1 5› ACT CTG GTG ATG GTG T 3›, and reverse primer designated as Act_R1 5› CGA GTA ACC ACG CTC C 3› with anticipated 137bp actin amplicon size. Several internal PCR primers were designed (data not shown) after homology analysis using Basic Local Alignment Search Tool (BLAST) and Clustalw2 on already cloned chitinase genes (Supplementary Fig. 1). Two sets of PCR primers *i.e.* first set designated as SB-14F 5' GTC TAC TTC ACC AAC TGG TGA GC 3' and SB-14R 5' CCA GCT TCC AGA TCC GAT TC 3' with expected amplicon size of 1020bp were designed from AF188924.1. The second set of primers was designed from EF635427.1 designated as SB-35F 5' AGC ACC GAT GCC AAC CGC AAG AAC TTT 3' and SB-35R 5' TGT GGC TTG TTC CGA TCA AGG AGT CGG A 3' with expected amplicon size of 771bp. The primers were designed using Bio-tool Kit software and Primer 3 and custom synthesized from Integrated DNA Technologies, Inc, Coralville, IA, USA. The 25 µl PCR reaction mixture comprised of 2.5 µl of 10X PCR buffer, 2.5 µl of 25 mM MgCl2, 0.5 µl dNTPs mix, 0.5 µl Taq DNA polymerase, 2.0 µl of primer, 2.0 µl of genomic DNA and 15 µl water. The reagents were obtained from Promega. The PCR amplifications were accomplished in a programmable DNA thermal cycler (Mastercycler Gradient-Eppendorf™) programmed as follows: Step 1-Denaturation at 95°C for 1 min; Step 2-Primer annealing at 50°C for 1 min; Step 3-Extension at 72°C for 2 min; Step 4-Repeated steps 1 to 3 for 25 times; Step 5-Final extension at 72°C for 7 min; Step 6-Store at 4°C. The PCR products were electrophoresed on 1.5 per cent (w/v) agarose gel at constant voltage (75V) for about 1hr. The gel was visualized under UV transilluminator and recorded with gel documentation system (Avegene). The amplicon size was ascertained by comparing with that of the standard marker (Fermentas 1Kb DNA ladder). The PCR fragments were eluted from agarose gel, purified using Wizard PCR clean up system (Promega, USA) essentially according to manufacturer's instructions and ligated into pGEM-T vector (Promega) for sequencing.

RESULTS AND DISCUSSION

Identification and screening of *Trichoderma* sp.

Identification of *Trichoderma* was carried out from 30 soil samples collected from sheath blight infected rice fields of PAU. Soil samples were cultured *in vitro* on TSM using serial dilution technique to isolate *Trichoderma* strains. The preliminary identification of *Trichoderma* strains was carried out by growing on PDA for 3-5 days and characters like colony colour, colony type, growth rate, discoloration of media, number of sclerotia, parasitism of sclerotia, extent of parasitism of sclerotia, coverage of Petriplate and effectiveness were observed visually. Comparison of attributes with taxonomic key for the genus *Trichoderma* such as shape, size, arrangement and development of conidiophores or phialides led to tentative identification of *Trichoderma* sp. Thirty eight *Trichoderma* strains were identified from 22 soil samples. *Trichoderma* strain identified from soil sample number 1 was named S-1b. In similar manner, the other identified strains were numbered. Few soil samples carried more than one *Trichoderma* strains and belonged to *T. harzianum* (Fig. 1) and *T. viride* (Fig. 2). The present study is consistent with Rahman *et al.* (2009) where a total of 135 *Trichoderma* isolates from different habitats were isolated using serial dilution technique. Similarly, twenty isolates of *Trichoderma* sp. were isolated by Asran-Amal *et al.* (2005) using dual culture from cotton (*Gossypium barbardense* L.) roots and evaluated *in vitro* as antagonists against isolates of *R. solani.*

Thirty-eight pure *Trichoderma* strains were identified and co-cultured on PDA with pure culture of *R. solani* for antagonism studies. Co-culturing was done by placing *Trichoderma* and *R. solani* stubs at poles on the same petridish. Thirty-eight separate experiments were conducted to study antagonistic activities of all the isolated *Trichoderma* strains. After the co-culturing, 24 *Trichoderma* strains were selected on the basis of antagonistic activity against *R. solani*. The selected strains showed different colony colour *i.e.* green, dark green or light green (Table 1), and two strains *i.e.* from sample 1, 4, 5, 6, five *Trichoderma* strains from sample 9, five

Table 1. Characteristics evaluated for *Trichoderma* sp. identification

Isolate no.	Colony colour	Colony type	Growth rate	Discoloration of media	No. of sclerotia	Parasitism of sclerotia	Extent of parasitism	Coverage of Petriplate	Effectiveness	*Trichoderma* sp.
S-1b	Dark green	Fluffy	+++	Yellowish	++++	Yes	Incomplete	50-70%	++	*T. harzianum*
S-1c	Light green	Fluffy	++	Light orange	++++	Yes	Incomplete	50-75%	+	*T. harzianum*
S-4a	Dark green	Very fluffy	+++	Yellowish	++	Yes	Full	Full	+++	*T. harzianum*
S-4b	Light green	Fluffy	++	Light yellow	+++	Yes	Full	Full	+++	*T. harzianum*
S-5a	Dark green	Very fluffy	++++	Yellowish orange	+++	Yes	Incomplete	50%	+++	*T. harzianum*
S-5b	Dark green	Fluffy	++++	Yellowish orange	++	Yes	Full	Full	++++	*T. harzianum*
S-6a	Light green	Fluffy & patchy	++++	White with red spots	++	Yes	Full	Full	++++	*T. viride*
S-6b	Light green	Suppressed	+++	Yellowish	++	Yes	Full	Full	++	*T. viride*
S-9b	Light green	Fluffy	++	Yellowish	++	Yes	Partial	70-80%	++	*T. viride*
S-9c	Dark green	Fluffy	+++	Yellowish	+++	Yes	Full	Full	+++	*T. harzianum*
S-9e	Dark green	Very fluffy	+++	Yellowish	+++	Yes	Full	Full	+++	*T. harzianum*
S-9f	Light green	Suppressed	++	Yellowish	+++	Yes	Full	Full	+++	*T. viride*
S-9g	Light green	Suppressed	++	Yellowish	+	Yes	Full	Full	++++	*T. viride*
S-10a	Dark green	Fluffy	+++++	Yellowish	+	Yes	Full	Full	++++	*T. harzianum*
S-10b	Dark green	Suppressed	+++++	Yellowish	+	Yes	Full	Full	++++	*T. viride*
S-10c	Dark green	Fluffy	++++	Yellowish	+	Yes	Full	Full	++++	*T. harzianum*
S-10d	Dark green	Fluffy	+++++	Yellowish	+	Yes	Full	Full	++++	*T. harzianum*
S-10e	Dark green	Fluffy	+++++	Yellowish	+	Yes	Full	Full	++++	*T. harzianum*
S-11a	Dark green	Suppressed & patchy	++++	White with red spots	+	Yes	Full	Full	++++	*T. harzianum*
S-11e	Dark green	Suppressed & patchy	++++	White with red spots	+	Yes	Full	Full	++++	*T. harzianum*
S-11c	Very dark green	Suppressed & patchy	++++	Light orange	+	Yes	Full	Full	+++++	*T. harzianum*
S-12a	Dark green	Fluffy & patchy	+++++	Yellowish orange	++++	Yes	Full	Full	++	*T. harzianum*
S-14	Yellowish green	Suppressed	+++++	Yellowish orange	+	No	Nil	Full	+++	*T. viride*
S-15	Dark green	Fluffy	+++++	Light yellow	+	Yes	Full	Full	+++++	*T. harzianum*

Note: +++++ Highly effective; +++ Moderately effective; + Least effective in suppressing the growth of *R. solani*

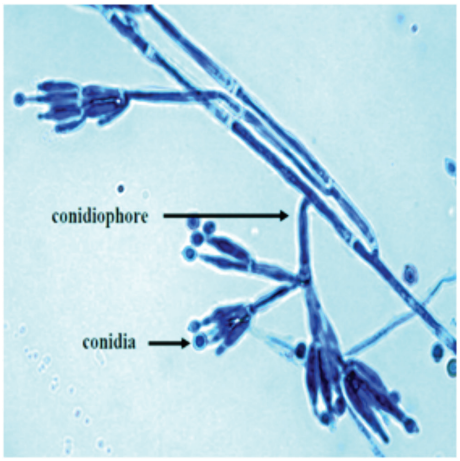

Fig. 1. Identification of *T. harzianum* under microscope (1000X), stained with lactophenol cotton blue

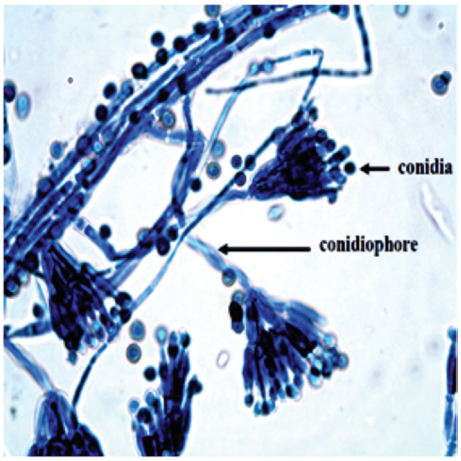

Fig. 2. Identification of *T. viride* under microscope (1000X), stained with lactophenol cotton blue

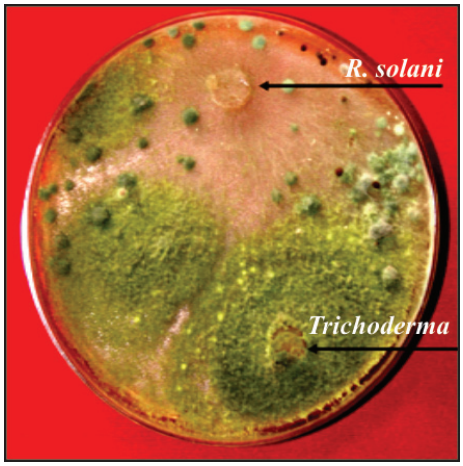

Fig. 3. *In vitro* evaluation of *Trichoderma* (S-15) against *R. solani*

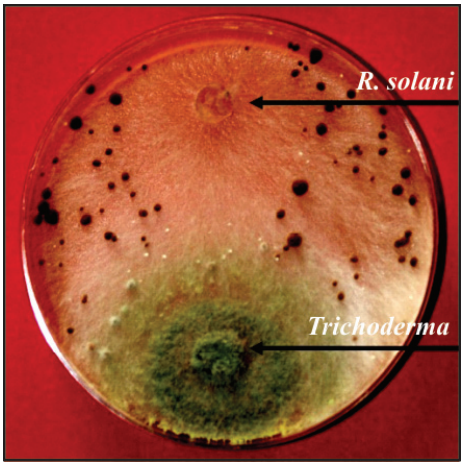

Fig. 4. *In vitro* evaluation of *Trichoderma* (S-1b) against *R. solani*

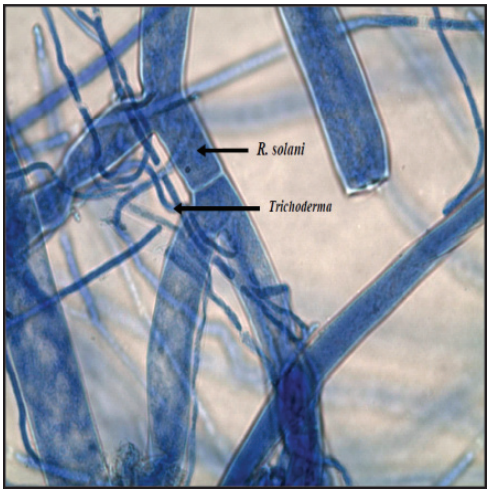

Fig. 5. *In vivo* hyphal evaluation of *Trichoderma* antagonism against *R. solani*, under microscope

Fig. 6. Pure *Trichoderma* strains maintained on PDA slants

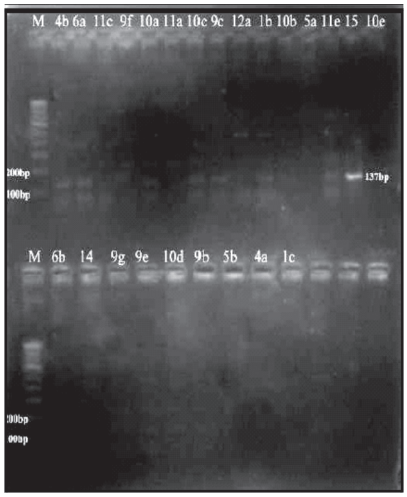

Fig. 7. PCR amplification of 137bp product from sample S-15 with actin primers

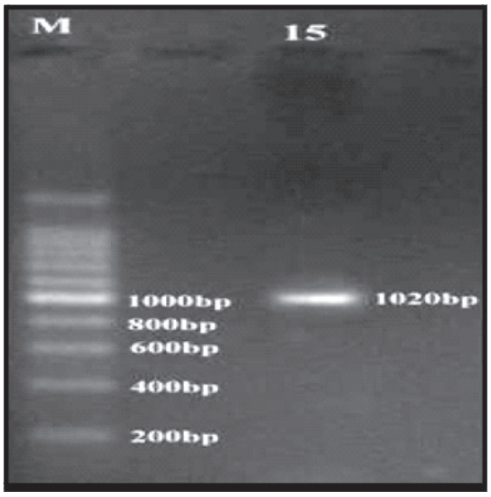

Fig. 8. PCR amplification of 1020bp product from sample S-15 with primer SB-14F/R

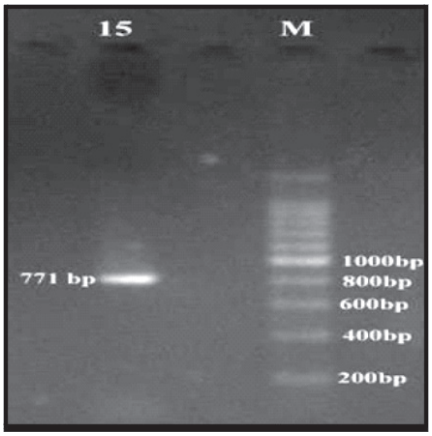

Fig. 9. PCR amplification of 771bp product from sample S-15 with primer SB-35F/R

Trichoderma strains from sample 10, three from sample 11, one each from sample 12, 14, 15 were selected. The characteristics of all 24 selected antagonistic *Trichoderma* strains are given in Table 1. Colony types were also studied that divided strains into categories like very fluffy, fluffy, fluffy and patchy, suppressed and patchy (Table 1). The growth rate was also observed as coverage of media on the Petriplate after 5 days at 28°C. All the strains discoloured the media on which they were grown like sample S-1b discoloured the PDA media as yellowish. Number of sclerotia varied from 1-4 and all the strains showed parasitism of sclerotia except sample S-14. Further, extent of parasitism of sclerotia was studied as full, incomplete and nil. Different levels of effectiveness were shown by all the strains as high, moderate and least. Some strains *e.g.* sample S-15, were able to cover the whole plate while the other designated as S-1b covered only partially (50-70%). Strains covering full plate (Fig. 3), showed very quick response in antagonizing *R. solani* when both were allowed to grow simultaneously, while the other ones were slow in their action to suppress the growth of *R. solani* (Fig. 4). Most potent strain was sample S-15 while the least potent strain was sample S-1b. The microscopic analysis of *Trichoderma* inhibiting the growth of *R. solani* by growing over it is shown in Fig. 5. The screened strains were then maintained on PDA slants and kept at 4°C for further experiments (Fig. 6). The dual culture method was used in this study to identify 24 antagonistic *Trichoderma* strains, this method has also been followed by Goes *et al.* (2002) for identifying 14 antagonistic

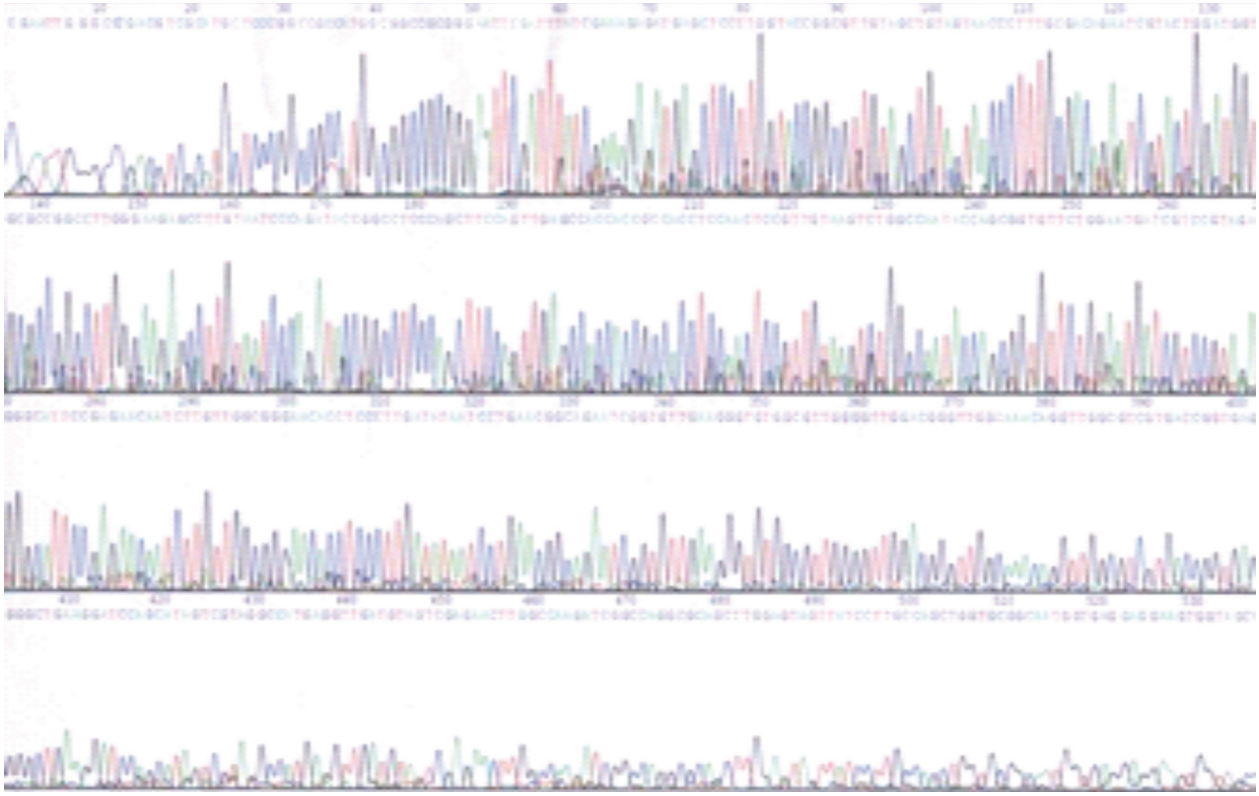

Fig. 10. Electropherogram of 1020 bp amplicon

Description	Max score	Total score	Query cover	E value	Ident	Accession
Hypocrea lixii isolate YZ2203 endochitinase 42 gene, complete cds	586	586	81%	6e-164	99%	HQ287004.1
Hypocrea lixii isolate SH3204 endochitinase 42 gene, complete cds	586	586	81%	6e-164	99%	HQ287001.1
Hypocrea lixii isolate HA2202 endochitinase 42 gene, complete cds	586	586	81%	6e-164	99%	HQ286999.1
Hypocrea lixii isolate ZQ2302 endochitinase 42 gene, complete cds	586	586	81%	6e-164	99%	HQ286997.1
Hypocrea lixii isolate ZQ3101 endochitinase 42 gene, complete cds	586	586	81%	6e-164	99%	HQ286996.1
Hypocrea lixii isolate SH4102 endochitinase 42 gene, complete cds	586	586	81%	6e-164	99%	HQ286994.1
Hypocrea lixii isolate SH4103 endochitinase 42 gene, complete cds	586	586	81%	6e-164	99%	HQ286993.1
Hypocrea lixii isolate FZ1302 endochitinase 42 gene, complete cds	586	586	81%	6e-164	99%	HQ286989.1
Hypocrea lixii isolate DLY1202 endochitinase 42 gene, complete cds	586	586	81%	6e-164	99%	HQ286987.1
Trichoderma harzianum chit-HAR2 gene for endochitinase-HAR2, complete cds	586	586	81%	6e-164	99%	AB041752.1
Hypocrea lixii isolate SZ3105 endochitinase 42 gene, complete cds	580	580	80%	3e-162	98%	HQ287003.1
Hypocrea lixii isolate SZ1303 endochitinase 42 gene, complete cds	580	580	80%	3e-162	98%	HQ286995.1
Hypocrea lixii isolate SG3303 endochitinase 42 gene, complete cds	580	580	81%	3e-162	98%	HQ286992.1
Hypocrea lixii isolate NC3206 endochitinase 42 gene, complete cds	575	575	81%	1e-160	98%	HQ287000.1
Trichoderma saturnisporum 42kDa endochitinase mRNA, complete cds	575	575	81%	1e-160	98%	GU290064.1
Hypocrea lixii chitinase precursor, gene, complete cds	575	575	80%	1e-160	98%	FJ596157.1
Trichoderma saturnisporum 42kDa endochitinase gene, complete cds	569	569	81%	6e-159	98%	GU290065.1

Fig. 11. Blast results of extracted DNA sequence

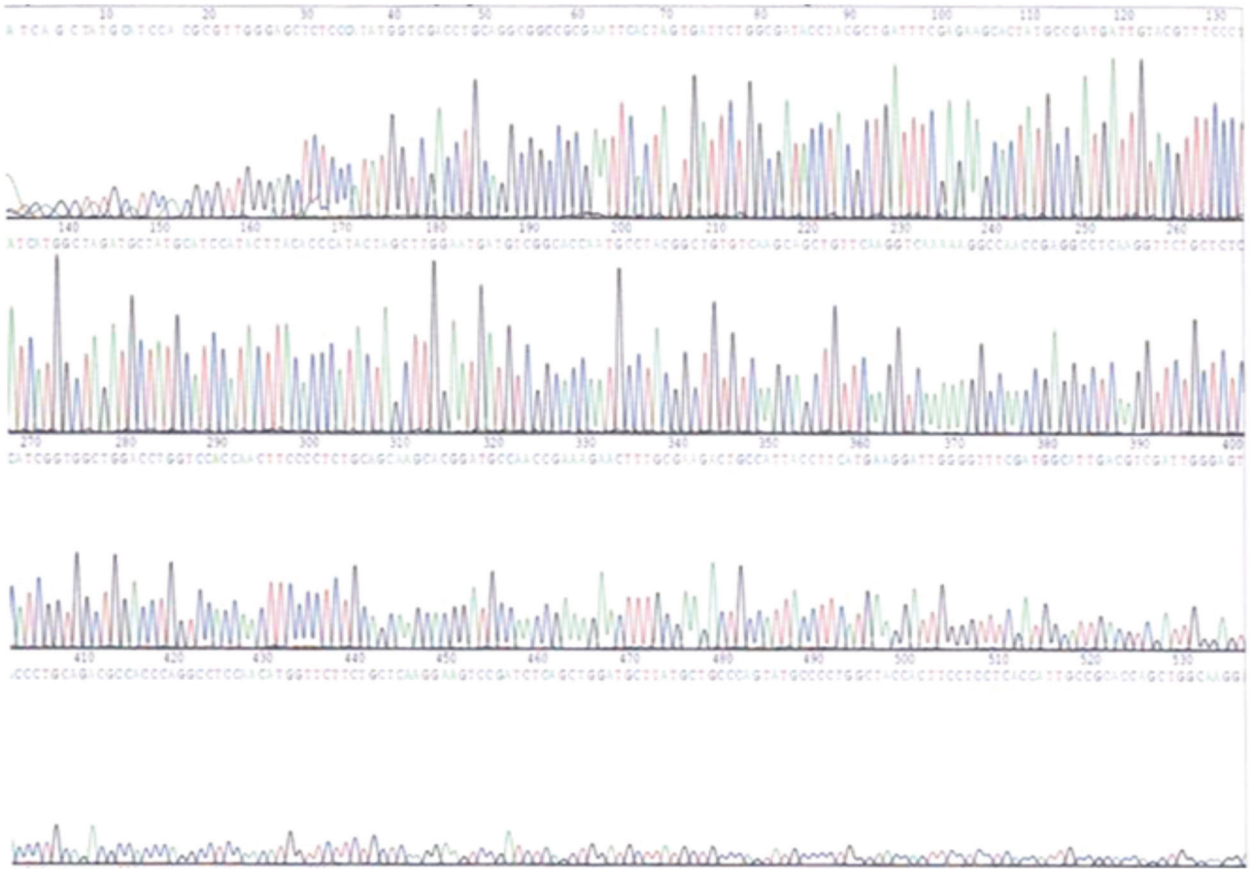

Fig. 12. Electropherogram of 771 bp amplicon

Description	Max score	Total score	Query cover	E value	Ident	Accession
Hypocrea lixii isolate YZ2203 endochitinase 42 gene, complete cds	1284	1284	90%	0.0	99%	HQ287004.1
Hypocrea lixii isolate SH3204 endochitinase 42 gene, complete cds	1284	1284	90%	0.0	99%	HQ287001.1
Hypocrea lixii isolate SZ1303 endochitinase 42 gene, complete cds	1284	1284	90%	0.0	99%	HQ286995.1
Hypocrea lixii isolate SH4102 endochitinase 42 gene, complete cds	1284	1284	90%	0.0	99%	HQ286994.1
Trichoderma harzianum chit-HAR2 gene for endochitinase-HAR2, complete cds	1284	1284	90%	0.0	99%	AB041752.1
Hypocrea lixii isolate SZ3105 endochitinase 42 gene, complete cds	1273	1273	90%	0.0	99%	HQ287003.1
Hypocrea lixii isolate NC3206 endochitinase 42 gene, complete cds	1267	1267	90%	0.0	99%	HQ287000.1
Hypocrea lixii isolate SG3303 endochitinase 42 gene, complete cds	1267	1267	90%	0.0	99%	HQ286992.1
Hypocrea lixii isolate FZ1302 endochitinase 42 gene, complete cds	1267	1267	90%	0.0	99%	HQ286989.1
Hypocrea lixii isolate DLY1202 endochitinase 42 gene, complete cds	1267	1267	90%	0.0	99%	HQ286987.1
Hypocrea lixii isolate HA2202 endochitinase 42 gene, complete cds	1262	1262	90%	0.0	99%	HQ286999.1
Hypocrea lixii isolate ZQ2302 endochitinase 42 gene, complete cds	1262	1262	90%	0.0	99%	HQ286997.1
Hypocrea lixii isolate ZQ3101 endochitinase 42 gene, complete cds	1262	1262	90%	0.0	99%	HQ286996.1
Hypocrea lixii isolate SH4103 endochitinase 42 gene, complete cds	1262	1262	90%	0.0	99%	HQ286993.1
Hypocrea lixii chitinase precursor, gene, complete cds	1262	1262	90%	0.0	99%	FJ596157.1
Hypocrea lixii isolate HA1102 endochitinase 42 gene, complete cds	1168	1168	90%	0.0	96%	HQ286998.1
Hypocrea lixii isolate HA1302 endochitinase 42 gene, complete cds	1168	1168	90%	0.0	96%	HQ286991.1

Fig. 13. Blast results of extracted DNA sequence

Trichoderma strains against pathogenic fungus *R. solani*. In similar manner, Rini and Sulochana (2007) evaluated 11 antagonistic *Trichoderma* isolates against *R. solani* using this method.

Isolation of partial antifungal chitinase genes from antagonistic *Trichoderma* sp.

The 24 selected antagonistic *Trichoderma* strains were grown on PDB medium. Incubation of *Trichoderma* strains on PDB medium led to induction of fungal mat and was used for genomic DNA isolation following CTAB method. The concentration of 24 genomic DNA samples was brought to 20ng/µl by diluting in TE buffer. The dilution step was done to maintain uniformity of template DNA in the PCR mix. The CTAB method is routinely used for fungal DNA isolation (Wattanalai *et al.,* 2004; Alias *et al.,* 2009). The authenticity and purity of genomic DNA samples was verified by PCR using housekeeping actin gene specific primers. An expected 137bp product was amplified in sample S-15 (Fig. 7). Based on the preliminary result, attempt was made to isolate antifungal chitinase gene(s) from the sample S-15 using PCR approach. The chitinase gene specific internal PCR primers designed based on homologus regions identified from BLAST results of already cloned chitinase genes were used for PCR amplifications. The PCR of S-15 revealed two independent amplicons approximately 1020bp (Fig. 8) and 771bp (Fig. 9) on 1.5 per cent agarose gel with primer SB-14F/R and SB-35F/R, respectively corresponding to two partial chitinase gene(s). The PCR fragments were eluted, purified from agarose gel, ligated into pGEM-T vector and got commercially sequenced. The electropherogram of 1020 bp fragment is shown in fig 10 and the BLAST result demonstrates approximately 99.0 per cent homology with cloned endochitinase genes (Fig. 11). Likewise, the electropherogram of 771 bp fragment (Fig. 12) and the BLAST results reveal approximately 99.0 per cent homology with cloned endochitinase genes (Fig. 13). These results confirm cloning of partial chitinase genes from S-15. While attempting to isolate chitinase genes from *Trichoderma* sp., several partial chitinase genes were isolated by various workers *e.g*. 180bp long *ech42* gene was cloned from *T. asperellum* with GenBank accession no. AJ563359.1. Similarly, another 791bp long *ech42* gene was cloned from *T. viride* vide accession no. AY665695. Another 653bp long *ech42* gene was cloned from *T. hamatum*, accession no. DQ087237. The 42 kDa chitinase gene from *T. viride* (GenBank accession number AF050098) was isolated using PCR approach, after designing primers specifically based on GenBank homology to a gene encoding 42 kDa endochitinase in *T. harzianum* (Baek *et al.,* 1999), pointing that homology based PCR primers can be used to isolate chitinase genes. The *in vitro* amplification of flanking regions of a known partial chitinase genomic sequences using inverse PCR can obtain full length gene sequence. Joshi *et al.* (1998) used inverse PCR technique to obtain a 900 bp long product from a small 153 bp partial intronic region of human glutamic acid decarboxylase (GAD) gene. The inverse PCR approach was followed to clone a chitosanase gene (*csn*) from *Penicillium* sp. based on the highly conserved regions of fungal chitosanase (Zhu *et al.,* 2012). The partial chitinase genes obtained in this study could be used in inverse PCR or as probes for screening genomic and cDNA libraries to isolate antifungal chitinase gene(s) from local potent *Trichoderma* strains.

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Bio-efficacy of different fungicides in managing blast of rice caused by *Pyricularia grisea*

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ABSTRACT

***In vitro* evaluation of different fungicides indicated that tebuconazole proved most effective as it completely inhibited the colony growth of *P.grisea* at 10 µg/ml whereas azoxystrobin + difenconazole, propiconazole and difenconazole completely inhibited the colony growth of the pathogen at 25 µg/ml. Rest of fungicides *viz.* zineb, tricyclazole, kasugamycin and azoxystrobin proved least effective even at a concentration of 200 µg/. Under pot culture conditions, tebuconazole @ 0.1 per cent was found to be highly effective in reducing leaf blast severity followed by propiconazole @ 0.1 per cent. Under field conditions, the efficacy of propiconazole, tricyclazole, tebuconazole and azoxystrobin + difenconazole was at par and proved to be effective fungicides in reducing the leaf and neck blast severity and increasing the yield.**

Key words: Rice, blast, bio-efficacy, fungicides, management

Rice blast caused by *Pyricularia grisea* Sacc. (teleomorph *Magnaporthe grisea* (Hebert) Barr.) continues to be a serious constraint in all the rice ecosystems of the country (Muralidharan, 2006) resulting in serious losses in yield (Ou, 1985). Losses of grains to the tune of 75 per cent has been reported in India (Padmanabhan, 1965). In Punjab, the disease is posing a serious threat on presently grown susceptible basmati rice cultivars. Although, host plant resistance is one of the most important components of disease management programme, however , in the absence of any durable resistance in presently grown high yielding cultivars, an effort was made to determine the efficacy of some triazole and strobilurin fungicides against the pathogen both under laboratory and artificial epiphytotic conditions in the field.

MATERIALS AND METHODS

Eight fungicides, *viz* Indofil Z-78 (zineb 75%), Kasu-B (kasugamycin 3%), Folicur 25 EC (tebuconazole 25%), Tilt 25 EC (propiconazole 25%), Score 25 EC (difenconazole 25%), Baan 75 WP (tricyclazole 75%), Amistar 250 SC (azoxystrobin 25%), Amistar Top 325 SC (azoxystrobin 18.2% + difenconazole 11.4%) each @ 0.1, 1, 10, 25, 50, 100 and 200 µg ml^{-1} (on a.i. basis) were evaluated in the laboratory against *Pyricularia grisea* following growth inhibition technique. Oat meal agar (OMA) medium amended with respective concentration of each fungicide was poured in sterilized Petri dishes aseptically. The stock solutions of standard fungicides were prepared on active ingredient basis and the required concentrations were subsequently made from stock solutions by adding required amount of sterilized distilled water.The Petri dishes containing unamended medium served as control. Three Petri plates were used for each treatment. The Petri dishes inoculated with 5mm mycelial bit taken from 8 days old culture of *P. grisea* were wrapped with cellophane film to minimize the chances of contamination and incubated at 25±1^{0}C temperature. The colony diameter was measured after 10 days of inoculations. The efficacy of the fungicides was expressed in terms of ED_{50} and ED_{90} values.

Efficacy of fungicides each @ 0.1 per cent was also evaluated against blast of rice under artificial epiphytotic conditions in the field. Nursery of Pusa basmati 1121 cultivar was raised during June, 2011 and 2012 in the field area as per standard agronomic practices. After 30 days, seedlings of basmati rice

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were transplanted in small plots of 3×3 m size as per standard agronomic practices (Anonymous, 2011). Each treatment was replicated thrice in a randomized block design. Untreated check was also maintained. After tillering stage, the plants in the field were sprayed with different fungicide treatments 24 hours before inoculations with the pathogen. The inoculations were done with conidial suspension of the *P.grisea* (3×10^6 conidia/ ml) raised on *makra* grass (*Eleusine indica*) in 250 ml Erlenmeyer flasks during evening to ensure uniform development of the disease. During day time, the inoculated plots were regularly sprayed with ordinary water after every two hours continuously for two days to facilitate infection and development of the disease. Two fungicide sprays were given at 15 days interval. The observations on disease were taken 10 days after the last spray on severity of leaf blast and incidence of neck blast in each treatment as per standard scale (IRRI, 1996). The data on paddy yield were recorded at harvest and expressed as paddy yield in quintals per acre.

RESULTS AND DISCUSSION

In vitro evaluation of fungicides

The data (Table 1) revealed that tebuconazole proved to be the most effective as it completely inhibited the colony growth of *P. grisea* at 10µg/ ml. whereas azoxystrobin + difenconazole, propiconazole and difenconazole completely inhibited colony growth of the pathogen at 25µg/ ml. Rest of the fungicides *viz.* zineb, tricyclazole, kasugamycin and azoxystrobin proved least effective even at a concentration of 200 µg/ml as per cent inhibition in colony growth was 78.29, 81.84, 90.18 and 75.16 respectively. The data revealed that ED_{50} values for azoxystrobin + difenconazole, azoxystrobin, propiconazole, tebuconazole and difenconazole were 0.3, 0.3, 0.5, 0.7 and 0.8 µg/ml, respectively. Whereas, in case of zineb, kasugamycin and tricyclazole ED_{50} values were quite high with 85, 74 and 101 µg/ml, respectively. Least ED_{90} value of 4 µg/ml was recorded in case of tebuconazole followed by ED_{90} values of 15, 18 and 20 µg/ml of propiconazole, difenconazole and azoxystrobin + difenconazole respectively. ED_{90} value in the range of 190 to 249 µg/ml was recorded in case of kasugamycin, tricyclazole and zineb. Azoxystrobin recorded highest ED_{90} value of 1140 µg/ml. Mohan *et al.* (2010) reported that Tilt 25 EC (propiconazole), Amistar top 325 SC (azoxystrobin + difenconazole), Score 25 EC (difenconazole) and Folicur 25 EC (tebuconazole) were found significantly effective over other treatments.

Evaluation of fungicides under pot culture conditions

Initially fungicides were evaluated against rice blast on Pusa Basmati 1121 potted plants under pot culture conditions during 2011 crop season. After tillering stage, the plants in the pots were sprayed with different fungicide treatments 24 hours before inoculations with the test pathogen. Three replications (3 potted plants/replicate) were kept for each treatment. The data on per cent leaf severity recorded 15 days after spray are presented in Table 2.

The perusal of the data indicated that all the fungicide treatments were significantly superior than untreated control in reducing rice blast. Tebuconazole (0.1%) followed by propiconazole (0.1%), azoxystrobin + difenconazole (0.1 %) and difenconazole (0.1%) proved significantly superior in controlling the disease with disease severity values of 6.5, 7.0, 7.3 and 8.0 per cent, respectively compared with 40.3 per cent in untreated control. Kasugamycin (0.1 %), azoxystrobin (0.1%) and zineb (0.25%) proved least effective in controlling the disease.

Field evaluation of fungicides

The perusal of the data (Tables 3) indicated that all the fungicide treatments were significantly superior than untreated control in reducing the leaf and neck blast severity and increasing the paddy yield during 2011 and 2012 crop seasons. During both the years i.e. 2011 and 2012, propiconazole @ 0.1 per cent was found to be effective in reducing the disease with respective average leaf blast severity of 7.5 and 6.1 per cent and neck blast incidence of 6.2 and 8.0 per cent. The other fungicide treatments which were at par were tricyclazole @ 0.1 per cent with respective leaf blast severity of 8.9 and 7.3 per cent and neck blast incidence of 7.0 and 8.2 per cent, azoxystrobin + difenconazole @ 0.1 per cent with leaf blast severity of 9.6 and 8.0 per cent and neck blast incidence of 7.3 and 7.6 per cent and tebuconazole

Table 1. Inhibition in colony growth of *Pyricularia grisea* at different concentrations of fungicides

Fungicide	Per cent inhibition in colony growth at different concentrations (µg/ml)							ED values (µg/ml)	
	0.1	1	10	25	50	100	200	ED_{50}	ED_{90}
Indofil Z-78 (Zineb 75%)	5.12 (13.05)	11.7 (19.97)	17.05 (24.33)	25.08 (29.94)	40.72 (39.63)	53.65 (47.07)	78.29 (62.20)	85	249
Baan 75 WP (Tricyclazole 75%)	2.98 (9.43)	11.19 (19.51)	27.61 (31.67)	33.83 (35.54)	43.03 (40.98)	49.50 (44.69)	81.84 (64.75)	101	227
Kasu-B (Kasugamycin 3%)	3.54 (8.99)	4.89 (10.34)	5.44 (13.06)	9.24 (16.72)	14.69 (22.07)	86.64 (68.56)	90.18 (71.74)	74	190
Amistar 250 SC (Azoxystrobin 25%)	47.62 (43.61)	57.56 (49.32)	59.14 (50.24)	67.50 (55.22)	69.99 (56.77)	73.59 (59.05)	75.16 (60.08)	0.3	1140
Score 25 EC (Difenconazole 25%)	14.68 (22.47)	59.24 (50.31)	75.94 (60.60)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)	0.8	18
Amistar Top 325SC (Azoxystrobin 18.2% + difenconazole 11.4%)	46.37 (42.90)	62.61 (52.28)	71.62 (57.78)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)	0.3	20
Tilt 25 EC (Propiconazole 25%)	40.9 (39.74)	63.92 (53.06)	85.13 (67.31)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)	0.5	15
Folicur 25 EC (Tebuconazole 25%)	8.89 (16.95)	75.73 (60.48)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)	0.7	4.0

Figures in parentheses represent per cent growth inhibition in arc sine transformed value
CD (p= 0.05)
Fungicide = 0.87, Concentration = 0.81, Fungicide x Concentration = 2.30

@ 0.1 per cent with leaf blast severity of 10.0 and 7.8 per cent and neck blast incidence of 7.3 and 8.2 per cent compared with leaf blast severity of 30.5 and 24.6 per cent and neck blast incidence of 18.3 and 20.4 per cent in untreated control during 2011 and 2012 crop season, respectively. Difenconazole @ 0.1 per cent showed leaf severity of 12.3 and 10.3 per cent and neck blast of 9.6 and 9.0 per cent. Highest leaf severity of 16.6 and 15.3 per cent and neck blast incidence of 11.3 and 13.0 per cent were recorded in case of kasugamycin @ 0.1 per cent. It was closely followed by leaf severity of 17.0 and 15.3 per cent and neck blast incidence of 10.8 and 11.3 per cent in case of zineb @ 0.25 per cent. All the treatments significantly improved the paddy yield in comparison to control. Maximum yield of 15.9 and 14.6 q/acre was recorded in propiconazole @ 0.1 per cent and it was closely followed by 15.6 and 14.8 q/

Table 2. Efficacy of different fungicides against blast of rice under artificial inoculation conditions under pot culture conditions

Fungicide	Conc. (%)	Average disease severity on leaves (%)	Disease control (%)
Indofil Z-78 (Zineb 75%)	0.25	12.4	69.2
Baan 75 WP (Tricyclazole 75%)	0.1	8.0	80.1
Kasu-B (Kasugamycin 3%)	0.1	18.3	54.6
Amistar 250 SC (Azoxystrobin 25%)	0.1	14.6	63.7
Score 25 EC (Difenconazole 25%)	0.1	8.3	79.4
Amistar Top 325SC (Azoxystrobin 18.2% + difenconazole 11.4%)	0.1	7.3	81.9
Tilt 25 EC (Propiconazole 25%)	0.1	7.0	82.6
Folicur 25 EC (Tebuconazole 25%)	0.1	6.5	83.9
Control	-	40.3	-
CD (p=0.05)		2.62	

Table 3. Efficacy of different fungicides against blast of rice under artificial epiphytotic conditions in field during 2011 and 2012 crop seasons

Fungicide	Conc. (%)	Average leaf blast severity (%)			Average neck blast (%)			Grain yield (q/acre)		
		2011	2012	mean	2011	2012	Mean	2011	2012	mean
Indofil Z-78 (Zineb 75%)	0.25	17.0	15.3	16.15	10.8	11.3	11.05	13.9	12.8	13.35
Baan 75 WP (Tricyclazole 75%)	0.1	8.9	7.3	8.10	7.0	8.2	7.60	15.6	14.2	14.90
Kasu-B (Kasugamycin 3%)	0.1	16.6	15.3	15.95	11.3	13.0	12.15	14.2	13.1	13.65
Amistar 250 SC (Azoxystrobin 25%)	0.1	14.3	12.0	13.15	9.3	10.6	9.95	14.0	13.8	13.90
Score 25 EC (Difenconazole 25%)	0.1	12.3	10.3	11.30	9.6	9.0	9.30	14.8	14.0	14.40
Amistar Top 325SC (Azoxystrobin 18.2% + difenconazole 11.4%)	0.1	9.6	8.0	8.80	7.3	7.6	7.45	15.6	14.8	15.20
Tilt 25 EC (Propiconazole 25%)	0.1	7.5	6.1	6.80	6.2	8.0	7.10	15.9	14.6	15.25
Folicur 25 EC (Tebuconazole 25%)	0.1	10.0	7.8	8.90	7.3	8.2	7.75	15.3	14.2	14.75
Control		30.5	24.6	27.55	18.3	20.4	19.35	11.6	11.0	11.30
CD(p=0.05)		2.57	2.34		2.42	2.42		2.1	1.63	

acre in azoxystrobin + difenconazole @ 0.1 per cent during 2011 and 2012 crop seasons, respectively . Tricyclazole, tebuconazole and difenconazole each @ 0.1 per cent also significantly improved the paddy yield in comparison to control where paddy yield of 11.6 and 11.0 q/acre was recorded during 2011 and 2012 crop seasons, respectively.

The pooled data (Table3) of both the crop seasons indicated that propiconazole @ 0.1 per cent was found to be superior over all other tested fungicides with leaf blast severity 6.80 per cent and neck blast incidence of 7.10 per cent followed by tricyclazole @ 0.1 per cent with leaf blast severity of 8.10 per cent and neck blast incidence of 7.60 per cent and azoxystrobin + difenconazole @ 0.1 per cent with leaf blast severity of 8.80 per cent and neck blast incidence of 7.45 per cent compared to leaf blast severity of 27.55 per cent and neck blast incidence of 19.38 per cent in untreated control. Tebuconazole @ 0.1 per cent, difenconazole @ 0.1 per cent and azoxystrobin @ 0.1 per cent were the next best fungicides in controlling both leaf and neck blast. Zineb @ 0.25 per cent and kasugamycin @ 0.1 per cent proved inferior where respective leaf blast severity of 16.15 and 15.95 per cent and neck blast incidence of 11.05 and 12.15 per cent were recorded. Maximum grain yield of 15.25 q/acre was recorded in propiconazole @ 0.1 per cent followed by 15.20 q/acre in azoxystrobin + difenconazole @ 0.1 per cent, 14.90 q/acre in tricyclazole @ 0.1 per cent, 14.75 q/acre in tebuconazole @ 0.1 per cent, 14.40 q/acre in difenconazole @ 0.1 per cent and 13.90 q/acre in azoxystrobin @ 0.1 per cent compared to untreated control where only 11.30 q/acre paddy yield was recorded (Table 3). The minimum grain yield of 13.35 q/acre was recorded in zineb @ 0.25 per cent and 13.65 q/acre in kasugamycin @ 0.1 per cent.

The present studies indicated that propiconazole @ 0.1 per cent followed by azoxystrobin + difenconazole @ 0.1 per cent and tricyclazole @ 0.1 per cent were the most effective fungicides in reducing the leaf and neck blast severity and increasing the grain yield. Paul and Coulombe (1993) evaluated the efficacy of iprobenfos, carbendazim, metam, edifenphos, tricyclazole, carbendazim + metiram and pyroquilon for the control of rice blast disease. Among these fungicides, tricyclazole proved to be

highly effective in controlling the blast disease. Singh *et al.* (2010) reported that carpropamid and tricyclazole were found to be the most promising fungicides in reducing the neck blast incidence. The promising efficacy of tricyclazole in controlling blast of rice has also been reported by Prajapati *et al.* (2004).

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Variability in single spore isolates of *Fusarium oxysporum* f. sp. *ciceri*

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ABSTRACT

Nine isolates of *Fusarium oxysporum* f. sp. *ciceri,* incitant of wilt of chickpea (*Cicer arietinum* L.) were studied for their discernible characters. The isolates tested for their pathogenic nature using susceptible JG-62 variety of chickpea were found virulent in sterilized as well as in unsterilized soil. The isolate Sriganganagar was highly virulent. There was practically no direct correlation between the cultural characters and virulence of any of the isolates. The mycelial growth of eight isolates of *Fusarium oxysporum* f. sp. c*iceri* on Czapek's dox agar basal medium with different carbon and nitrogen sources varied. Forty varieties/germplasm of chickpea screened against the highly virulent isolate Sriganganagar in sick soil in pots revealed resistance and susceptibility.

Key words: *Cicer arietinum*, variability, resistance, susceptibility, *Fusarium oxysporum*

Fusarium oxysporum f. sp. *ciceri* incitant of wilt disease in chickpea (*Cicer arietinum* L.) is a destructive disease in major chickpea growing districts of Rajasthan resulting into considerable economic losses. Annual yield losses due to wilt have been estimated 10%–90% (Jimenez-Diaz *et al*., 1989; Singh and Reddy, 1991). Persistence of the pathogen in soil and its capacity to survive there for years even in the absence of host (Haware *et al*., 1996) renders its management difficult. The isolated pathogenic strains may vary morphologically, physiologically and in pathogenicity, thus studies were undertaken to find out the causes for variability in disease development and to screen varieties/ germplasm for resistance.

MATERIALS AND METHODS

The samples collected at seedling stage of the crop and pathogen was isolated and purified. A large number of single spore cultures were raised from isolates of different collections on 2 per cent potato dextrose agar (PDA) slants. These were grouped initially on the basis of their cultural characters. Eight isolates of *Fusarium oxysporum* f. sp. c*iceri* obtained from chickpea areas of Rajasthan and one from CCSHAU, were given the code on the basis of the place of their collection as BKR (Bikaner), HNG (Hanumangarh), CHR (Churu), SGN (Sriganganagar), SKR (Sikar), JPR (Jaipur), JOB (Jobner), JJN (Jhunjhunu) and HAU (Hisar).

Morphological variability

Nine isolates of *Fusarium oxysporum* f. sp. c*iceri* were transferred on 2 per cent PDA in Petri dishes to study their cultural characters such as the colour of aerial mycelium, colony growth and pigmentation of substratum and sporulation.

For studying the intensity of sporulation two discs, each having diameter of 2 mm, were cut at random with a sterilized mechanical cutter and suspended in 20 ml of sterilized distilled water in 50 ml Erlenmeyer flasks. The flasks were shaken thoroughly on an automatic shaker for 20 minutes and the suspension passed through folded muslin cloth to eliminate mycelium and the substratum. The number of spores per milli-liter of water was calculated by means of heamocytometer and grouped as moderate (1.5-25×10^6 spores / ml), good (25-50×10^6 spores / ml) and very good (51 to 89.2×10^6 spores / ml). After microscopic studies of mycelium and spores, the colony diameter of each isolate was measured in cm.

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Pathogenic variability and screening for disease resistance

For pathogenecity tests, isolates were multiplied on sand maize flour medium (10 gm maize flour, 90 g sand and 20 ml distilled water in each flask) incubated at 28±1ºC for 15 days and then added to steam sterilized soil and to unsterilized soil in a ratio of 1 : 200 in disinfected earthen pots. Controls were prepared without inoculum. Seed samples were surface sterilized with 0.1 % mercuric chloride for 2 minutes. Ten seeds of susceptible variety JG-62 were sown in each pot. After 40 days of sowing, the suspected pathogen *Fusarium oxysporum* f. sp. c*iceri* was isolated and purified from the wilted plants. In a second experiment, 40 varieties of chickpea were tested against the highly virulent isolate Sriganganagar for screening their resistance against wilt.

Effect of carbon sources

To find out the effect of various carbon sources on growth of nine isolates of *Fusarium oxysporum* f. sp. c*iceri*; the sucrose content of basal medium Czapek's dox agar was substituted by adding different sources of carbon on equivalent basis (12.63g in 30g of sucrose). Inoculated Petri dishes containing basal medium supplemented with different carbon sources were incubated at 28±1°C for 10 days and the mycelial growth of each isolate was recorded. Carbon sources used were dextrose ($C_6H_{12}O_6$), glycerol ($C_3H_8O_3$), maltose ($C_{12}H_{22}O_{11}$), sucrose ($C_{12}H_{22}O_{11}$) and control being without carbon source.

Effect of nitrogen sources

To observe the effect of various nitrogen sources on growth of nine isolates of *Fusarium oxysporum* f. sp. c*iceri*, the sodium nitrate of basal medium Czapek's dox agar medium was substituted by adding different sources of nitrogen on equivalent basis (329 mg in 2 g of sodium nitrate) to study the effect of different nitrogen sources on the growth of different ten isolates. Nitrogen sources studied were sodium nitrate ($NaNO_3$), ammonium nitrate (NH_4NO_3), asparagine ($C_4H_8N_2O_3.H_2O$), leucine ($C_6H_{13}NO_2$) and control being without nitrogen source.

RESULTS AND DISCUSSION

Morphological characteristics

The data (Table 1) revealed that the color of the mycelium in different isolates was as: Isolate Sriganganagar: mycelium white with violet substrate, Isolate Bikaner: mycelium white with crushed violet substrate, Isolate Churu: white to pinkish mycelium with mulberry purple substrate, Isolate Hanumangarh: white mycelium with violet substrate, Isolate Hisar: cream mycelium with café cream substrate, Isolate Jhunjhunu: cream mycelium with gold brown substrate, Isolate Jobner: white mycelium with white to cream substrate, Isolate Jaipur: white mycelium with cream substrate and Isolate Sikar: cream mycelium with gold brown substrate. The sporulation was very good in isolates Bikaner, Hanumangarh and Sriganganagar; good in Churu, Hisar, Jhunjhunu, Sikar and moderate in remaining isolates Jaipur and Jobner. The colony diameter of isolates *viz.* Sikar, Jhunjhunu, Jaipur, Churu, Jobner, Bikaner, Hanumangarh, Sriganganagar and Hisar was 8.90, 8.60, 8.13, 7.60, 7.23, 7.13, 7.06, 6.96 and 4.06 cm, respectively after ten days growth.

Table 1. also reveals that there was practically no direct correlation between the cultural characters and virulence. The isolate Sriganganagar having Very good aerial mycelium with suppressed growth was highly pathogenic. The isolates Bikaner with scanty and fibrous, mycelium; Hanumangarh with scanty, fibrous and suppressed mycelium; Jobner with suppressed, fast appraised to substratum mycelium; Jaipur with suppressed, fibrous growth but fast, Sikar with fluffy fast growth but dark coloured dot in the centre, were moderately pathogenic. The rest two isolates *viz*. Churu and Hisar with different mycelial character were less pathogenic.

A study of the number of septa and frequency of macro and micro conidia and other spore characters of isolates showed that macro conidia were upto 5 septate, micro conidia more in number, which were non septate, oval to cylindrical, straight to curved shaped. Terminal or intercalary chlamydospores were produced in all the cultures either in single or in pair or in chain.

Table 1. Characteristics of eight isolates of *Fusarium oxysporum* f. sp. *ciceri* on PDA after 10 days growth

Isolate	Cultural characters			Sporulation	Colony diameter (cm)*
	Mycelium colour	Substrate pigmentation	Mycelial growth		
BKR	White	Crushed Violet	Scanty and fibrous	+++	7.13
CHR	White to Pinkish	Mulberry purple	Suppressed, Profusely dense	++	7.60
HAU	Cream	Café cream	Growth suppressed good	++	4.06
HNG	White	Violet	Scanty, fibrous, suppressed	+++	7.06
JJN	Cream	Gold brown	Fluffy, very good, mycelium	++	8.60
JOB	White	White to Creamy white	Suppressed, fast, appraised to substratum	+	7.23
JPR	White	Creamy white	Suppressed, fibrous growth but fast	+	8.13
SGN	White	Violet	Very good arial mycelium, fibrous, Suppressed	+++	6.96
SKR	Cream	Golden brown	Fluffy, fast but dark coloured dot in the centre	++	8.90

*Mean of three replication
+ Moderate sporulation production
++ Good sporulation production
+++ Very good sporulation production

CD (p=0.05) 0.801
General mean 7.30

Pathogenicity and screening

All nine isolates tested for their pathogenicity towards susceptible JG-62 variety of chickpea were found virulent in sterilized and in unsterilized soil. Earthen pots (25 cm diameter) were filled with sterilized soil collected from chickpea cultivated areas of Agronomy Farm, Bikaner. Sand maize flour medium (10 gm maize flour, 90 g sand and 20 ml distilled water in each flask) was autoclaved in 250 ml Erlenmeyer's flasks. Each flask was incubated with pure culture isolate of *Fusarium oxysporum* f. sp. *ciceri* separately and incubated at 28±1ºC for 15 days. Fungus infested sand maize flour medium was mixed in soil of each pot with a ratio of 1 : 200. The pots were watered regularly and kept moist for two days. Similarly, the pots filled with unsterlized soil were also made sick for testing the pathogenicity. Koch's postulates were proven for each isolate. Symptoms of wilt appeared after 40 days of germination in sterilized soil while it was delayed upto 50 days in unsterilized soil. The wilt incidence (%) in sterilized and unsterilized soil varied from 33.33 to 100 & 28.57 to 93.33. Isolate Sriganganagar was highly virulent, followed by Hanumangarh, Bikaner, Jaipur, Sikar, Jobner, Jhunjhunu, Hisar and Churu. In unsterilized soil the reaction of isolates was less and delayed as compared to sterilized one.

Forty varieties/germplasm lines of chickpea were screened against the highly virulent isolate Sriganganagar in sick soil in pots and the different reactions were recorded. The nine varieties *viz.* C-235, RSG-573, RSG-581, Avrodi, BG-1107,BGD-1004, BG-1088, H-82-2 and IPC-98-53 remained free from wilt or having incidence between 1 to 10 per cent were termed as highly resistant to wilt. Three varieties/germplasm lines *viz*. BG-1003, PG-97121 and RSG-963 having incidence between 11 to 20 per cent were termed as resistant. Seven varieties/germplasm lines *viz*. BG-377, CSJ-140, GJG-9807, IPC-98-1, PG-96409, PG-97128 and RSG-974 having incidence 21 to 30 per cent were categorized as moderately resistant. Twelve varieties/germplasm lines *viz*. BG-1098, BG-1103, BG-256, BJ-1105, BG-1105, CSJ-172, CSJ-195, CSJ-239, CSJ-253, IPC-99-1, PBG-1, and WCG-97-37 had 31 to 51 per cent disease incidence termed as susceptible. Nine varieties/germplasm lines viz. GL-94022, GNG-1382, JG-62, L-550, PG-92307, PG-95421, PG-95424, PBG-204, and RSG-897 were highly susceptible as incidence of disease ranged between 51 to 100 per cent.

Physiological characteristics

Effect of carbon sources: The data (Table 2) revealed that mycelial growth of nine isolates of *Fusarium oxysporum* f. sp. c*iceri,* grown on Czapek's dox agar basal medium with different

Table 2. Mycelial growth (cm) of nine isolates of *Fusarium oxysporum* f. sp. c*iceri* grown on Czapek (Dox) agar basal medium with different carbon and nitrogen sources

Isolate	Carbon sources					Nitrogen sources				
	Dextrose	Glycerol	Maltose	Sucrose	Control	Sodium nitrate	Ammonium nitrate	Asparagines	Leucine	Control
BKR	7.16	6.03	5.00	7.06	0.76	7.76	6.43	5.16	6.40	4.03
CHR	4.06	3.00	2.10	4.03	0.33	3.16	2.83	3.06	4.20	2.70
HAU	3.10	1.36	1.66	2.96	0.43	2.26	1.60	2.10	2.23	1.50
HNG	8.00	6.00	5.26	7.93	0.83	7.13	6.16	5.83	7.26	5.10
JJN	4.83	2.30	2.23	3.76	2.20	5.53	3.16	4.43	4.83	2.26
JOB	8.06	7.26	4.90	7.93	1.96	7.53	7.03	6.70	5.23	4.00
JPR	7.60	5.83	4.96	7.33	2.00	6.66	5.26	4.90	4.90	3.70
SGN	6.83	5.03	5.46	6.63	1.00	6.50	6.00	5.26	6.73	4.76
SKR	5.20	1.86	4.10	5.60	0.70	5.30	3.46	4.66	4.33	2.20

	CD (p=0.05)		CD (p=0.05)
Isolates	0.16	Isolates	0.14
Carbon sources	0.12	Nitrogen sources	0.11
Isolates x Carbon sources	0.36	Isolates x Nitrogen sources	0.32

carbon sources was different. Isolate Bikaner had 7.16, 6.03, 5.00 and 7.06 cm growth on dextrose, glycerol, maltose, sucrose carbon sources, respectively. These carbon sources enhanced the growth of isolate Bikaner as compared to control (0.76 cm). The isolates Hanumangarh, Jobner, Jaipur, Sriganganagar and Sikar have better growth on different carbon sources. The mycelial growth of isolate Churu, Hisar and Jhunjhunu was comparatively less on the four carbon sources. All the carbon sources supported better growth of all the nine isolates. But the growth of isolates was less on maltose as compared to other carbon sources on different carbon sources.

Effect of nitrogen sources: Mycelial growth of nine isolates of *Fusarium oxysporum* f. sp. c*iceri* grown on Czapek's dox agar basal medium with different nitrogen sources also varied. Isolates Bikaner, Hanumangarh and Jobner were having significantly better growth on all the four nitrogen sources *viz.* sodium nitrate, ammonium nitrate, asparagine and leucine as compared to control. Jobner isolate was having maximum mycelial growth i.e. 7.53, 7.03, 6.70 and 5.23 cm on sodium nitrate, ammonium nitrate, asparagine and leucine, respectively. Isolates Jhunjhunu, Jaipur, Sriganganagar and Sikar also have good growth on different nitrogen sources. The mycelial growth of Churu and Hisar was retarded. There was a significant difference among the isolates, nitrogen sources and their interactions (Table 3).

Fusarium oxysporum f. sp. c*iceri* isolates varied morphologically and in their pathogenic nature in the present investigations as earlier observed by Honnareddy and Dubey (2006) while studying pathogenic and genetic variability of *Fusarium oxysporum* f. sp. c*iceri* isolates collected from different parts of India. Similarly, Mandharea *et al* (2011) and Arvayo-Ortiz *et al.* (2011) also observed pathogenic and morphological variation in isolates of *Fusarium oxysporum* f. sp. c*iceri,* thereby, confirming the findings of present investigations. The variation on the growth of isolates of *Fusarium oxysporum* f. sp. c*iceri* on different nutritional sources was also observed. Sajid Farooq *et al.* (2005) reported glucose as best source of carbon and peptone of nitrogen while studying physiological studies of *Fusarium oxysporum* f. sp. *ciceri.* Khilare and Ahmed (2011) found different nutritional sources *viz.* glucose, potassium nitrate, di-potassium hydrogen orthophosphate, many amino acids (especially hydroxyproline), ferric oxide most favorable for the growth of the *Fusarium oxysporum* f. sp. *ciceri.* Khan *et al.* (2012) tested variation in growth and physiological characters of *Fusarium oxysporum* f. sp. *ciceri* in five different

solid media. They found glucose and alanine as carbon and nitrogen sources best for the growth of pathogen, confirming the present findings.

Chaudhry *et al.* (2007) screened one hundred and ninety six chickpea germplasm lines/cultivars for resistance to wilt but could not find any resistant or immune. Ahmad *et al.* (2010) screened 321 genotypes from different sources and found considerable variation among the genotypes. At seedling stage, disease incidence varied from 0 to 29.3% whereas at reproductive stage ranged from 0 to 57%. Eighty two genotypes showed steady resistance at both stages. Thus, the present study is significant for breeding programme to develop varieties resistant to pathogenic isolates of *Fusarium oxysporum* f. sp. *ciceri.*

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Evaluation of antagonists and natural plant extracts in controlling collar rot of groundnut caused by *Aspergillus niger*

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ABSTRACT

Forty five isolates of *Trichoderma* spp. were isolated from soil samples collected from groundnut growing fields of north-coastal zone of Andhra Pradesh and of them 10 morphologically different isolates were identified. The native isolates of *Trichoderma* spp. significantly inhibited the growth of *Aspergillus niger* over control. The isolates T_1, T_2, T_{18}, T_{21} and T_{40} recorded more inhibition than others in dual culture and seeded agar methods. The extent of inhibition was more pronounced in seeded agar method than in dual culture method. The results revealed that all the ten bioagents tested *in vitro* applying dual culture technique against *A. niger* significantly inhibited the mycelial growth of the test pathogen over untreated control. However, *Trichoderma* isolate T_{21} recorded minimum colony diameter (2.07 cm) and highest inhibition (75.44%) of mycelial growth of *A. niger* over untreated control followed by the T_1 and T_{40} which recorded mean colony diameter of 2.50 cm, 2.53 cm and mean mycelial inhibition of 70.34 %, 69.99%, respectively. Where as in seeded agar methods maximum inhibition was recorded in T_1 (85.00%) followed by T_2 (81.93%) and T_{21} (81.11%). The native isolates of *Trichoderma* spp. showed a clear cut superiority over commercial formulations. But the commercial formulation of bacterial antagonist *Bacillus subtilis* showed significantly more antagonistic effect on the growth of *A. niger* than native isolates. Evaluation of different botanicals by poisoned food technique showed that all plant extracts tested *in vitro* were found significantly effective in reducing the percentage mycelial growth of *Aspergillus niger* over untreated control. However, plant extract (@ 5, 10 and 15% of garlic, recorded lowest mean colony diameter (1.57 cm) and highest mean mycelial growth inhibition (81.51%) followed by *Polyalthia longifolia, Annona squamosa* and *Pongamia glabra* which recorded the mycelial growth of 3.38 cm, 3.75 cm, 3.87 cm and the mean mycelial growth inhibition of 60.18%, 55.87%, 54.44% respectively. Among the concentrations and plant extracts, *A. sativum* (@10 and 15%) was most effective in reducing the growth of *A. niger* (100%) followed by *Polyalthia longifolia @15%* (77.73%), *Annona squamosa* @ 15% (74.61%) and *Pongamia glabra* @ *15%* (70.33%). When plant extracts compared with the isolates of *Trichoderma* spp., *P. fluorescens* and *B. subtilis* showed inhibition of growth of the *A. niger* which ranged from 62.95 to 100.00 per cent. *A. sativum* showed the highest inhibition of growth of *A. niger* (100.0%) followed by *B. subtilis* and *Trichoderma* T_1 isolate, which caused 84.52 and 84.49 per cent growth reduction of *A. niger*, respectively.

Key words: Groundnut, collar rot, *Trichoderma,* garlic, *Aspergillus*

Groundnut (*Arachis hypogaea* L.) is an important annual oil seed crop. India at global level and Andhra Pradesh at national level holds key position in groundnut area and production. A large number of diseases attack groundnut in India. Among them, collar rot of groundnut caused by *Aspergillus niger* Van Teighem is one of important seed and soil borne diseases causing seedling losses up to 50 per cent (Chohan, 1969). Collar rot largely account for the pre and post-emergence death of the seed and seedlings. Infected seeds become black and do not germinate. After germination, infection on collar region of plants results in seedling wilt.

Several workers have tried to manage this damage by seed dressing with different fungicides (Raju *et al*., 2002). However, it was observed that seed dressing provided protection only for a limited period. Though chemical fungicides have played an important role in increasing groundnut production and management of collar rot, their indiscriminate

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use for the control of disease has threat to the environment, crop ecology, soil biology and human health. Thus, other alternative disease management options were considered and among these biological control appears promising. But limited work has been done on successful exploitation of bio-control agents and plant extracts for the management of collar rot disease. *Trichoderma* have been used as biological control agents against soil-borne plant pathogenic fungi (Kucuk and Kivank, 2003). Plant extracts may be used as an alternative source for controlling soil-borne diseases since they comprises a rich source of bioactive substance (Hadian, 2012; Gurjar *et al.,* 2012). The main objective of the present study was to find a *Trichoderma* strain and botanicals that will act as the best bio-control agent for effectively inhibiting the growth of *A. niger*.

MATERIALS AND METHODS

Isolation and maintenance of microbes

The causal organism of collar rot disease, *Aspergillus niger* was isolated from infected groundnut plants showing typical symptoms of collar rot on potato dextrose agar medium in Petri dishes aseptically and incubated at room temperature (28 ± 2^0C) in the laboratory and the pure pathogen culture was made by the hyphal tip method (Dhingra and Sinclair, 1995).

Soil samples collected during the survey were used for isolation of native isolates of *Trichoderma* spp. by serial dilution pour plate technique (Johnson and Curl, 1972) using *Trichoderma* selective medium. The isolates of *Trichoderma* spp. were identified based on their colony and morphological characters. Different isolates were maintained throughout the study by periodical transfers on PDA media under aseptic conditions, to keep the culture fresh and viable.

In vitro antagonism between bio-agent *Trichoderma* and pathogen *A. niger*

The antagonistic effect of the different *Trichoderma* spp. isolated from soil samples was tested against the growth of *A. niger* by dual culture technique (Dennis and Webster, 1971) and seeded agar method (Dhingra and Sinclair, 1995). For each isolate, three replications were maintained. Then the plates were incubated at room temperature (28 ± 2^0C). Petri dishes inoculated with only *A. niger* served as control. Five days after incubation, the colony diameter of the pathogen and *Trichoderma* spp. were measured and per cent inhibition of the pathogen over control was calculated (Odebode and Sobowale, 2001**;** Skidmore and Dickinson, 1976).

Comparison of native isolates of *Trichoderma* spp. with commercial formulations

The native isolates of *Trichoderma* spp. were compared with commercially formulated products of *T. viride* (Biocure F), *T. harzianum* (Biocure B)*, Pseudomonas fluorescens* (Ecoderma) and *Bacillus subtilis* (Biotok) to find out their relative antagonistic potential against mycelial growth of *A. niger* by dual culture technique. Three replications were maintained and PDA inoculated with *A. niger* alone served as control.

Evaluation of plant extracts against *A. niger*

Plant extracts of the different plant species *viz. Annona squamosa* (kernel), *Allium sativum* (bulb), *Polyalthia longifolia* (seed), *Ocimum sanctum* (leaf), *Curcuma longa* (rhizome), *Piper betle* (leaf), *Azadirachta indica* (leaf and seed), *Pongamia glabra* (seed) and *Acacia arabica* (bark) were tested. Fresh plant materials of different plant species were thoroughly cleaned, surface sterilized with 2% sodium hypochlorite and washed well with sterile water. The predetermined plant parts were grounded along with sterile water at the rate of 1:1 w/v using pestle and mortar and the macerate was filtered through a Whatman No.1 filter paper under sterilized condition to get the clear plant extract (100%). The extract of each plant species at three concentrations *viz*., 5, 10 and 15 per cent were tested against the *A. niger* by poisoned food technique (Nene and Thapliyal, 1971). Three replications were maintained for each plant extract. The colony diameter was recorded and per cent inhibition of growth of *A. niger* over control estimated.

The plant extracts of *Annona squamosa* (15%), *Allium sativum* (10%), *Polyalthia longifolia* (15%) and *Pongamia glabra* (15%) found effective at higher concentrations were compared with T_1, T_2, T_{18}, T_{21}, T_{40} isolates of *Trichoderma* spp*., Pseudomonas fluorescens* and *B. subtilis* through poisoned food technique as used. Each treatment was replicated three times.

The data obtained on *in vitro* per cent growth inhibition of test fungus were analysed following completely randomized design (CRD) and factorial completely randomized block design was adopted for the experiments conducted to study the effect of plant extracts against *A. niger* (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Effect of native *Trichoderma* isolates on *Aspergillus niger in vitro*

Forty five isolates of *Trichoderma* spp. were isolated from 29 soil samples collected from groundnut growing fields of north-coastal zone of Andhra Pradesh and of them effective 10 morphologically different isolates (T_1, T_2, T_{18}, T_{20}, T_{21}, T_{22}, T_{27}, T_{29} T_{38} and T_{40}) were identified. The results (Table 1) revealed that *Trichoderma* isolate T_{21} had maximum growth inhibition of *A. niger* (75.44 %) which was significantly superior over all other isolates. The next best isolates were T_1 (70.34%), T_{40} (69.99%) and T_{18} (67.62%) which were significantly superior to the remaining isolates and were at par with each other. T_{38} isolate (53.74%) was least effective in dual culture method, T_1 isolate in seed agar method inhibited 85.00 per cent growth of *A. niger* which was significantly superior to all other isolates followed by T_2 (81.93%) and T_{21} (81.11%). T_{27} isolate (72.84%) was least effective. Even though all the 10 isolates significantly inhibited growth of *A. niger* over control, differences were found among the isolates in respect of their relative antagonistic potential. The isolates T_{21}, T_1, T_{40}, T_{18} and T_2 showed more per cent inhibition of growth of 75.44, 70.34, 69.99, 67.62 and 66.79 per cent, respectively than others in dual culture method. Whereas, in seeded agar method the isolates T_1, T_2, T_{21}, T_{18} and T_{40} showed more per cent inhibition of growth of 85.00, 81.93, 81.11, 78.44 and 76.44 per cent, respectively (Table 1 and Plate 1).

There are many well documented studies on the antagonistic effect of *Trichoderma* spp. against the growth of *A. niger* (Raju and Krishnamurthy, 2002; Subbiah and Indra, 2003; Gajera *et al.,* 2011).

Comparison of native *Trichoderma* isolates with commercial formulations of *T. viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis*

Native isolates of *Trichoderma* spp. were significantly superior to the commercial formulations of *T. viride, T. harzianum* and *P. fluorescens* in inhibiting the growth of *A. niger* ((Table 2) except *B. subtilis* which recorded maximum growth inhibition (77.21%) and was at par with T_{21} isolate (75.21%). *P. fluorescens* was least effective which recorded 31.88 per cent inhibition over control. Significant differences were found between the native isolates

Table 1. Screening of *Trichoderma* native isolates against *Aspergillus niger in vitro*

Trichoderma isolate	Dual culture method		Seeded agar method	
	Mycelial growth of *A. niger* (cm)	Inhibition over control (%)	Mycelial growth of *A. niger* (cm)	Inhibition over control (%)
T_1	2.50	70.34	1.27	85.00
T_2	2.80	66.79	1.53	81.93
T_{18}	2.73	67.62	1.80	78.44
T_{20}	3.27	61.21	2.10	75.20
T_{21}	2.07	75.44	1.60	81.11
T_{22}	2.90	66.60	1.93	77.33
T_{27}	3.17	62.40	2.30	72.84
T_{29}	3.50	58.48	2.20	74.02
T_{38}	3.90	53.74	2.00	76.38
T_{40}	2.53	69.99	1.80	76.44
Control	8.43	-	8.47	-
C.D (p=0.05)	0.254		0.184	

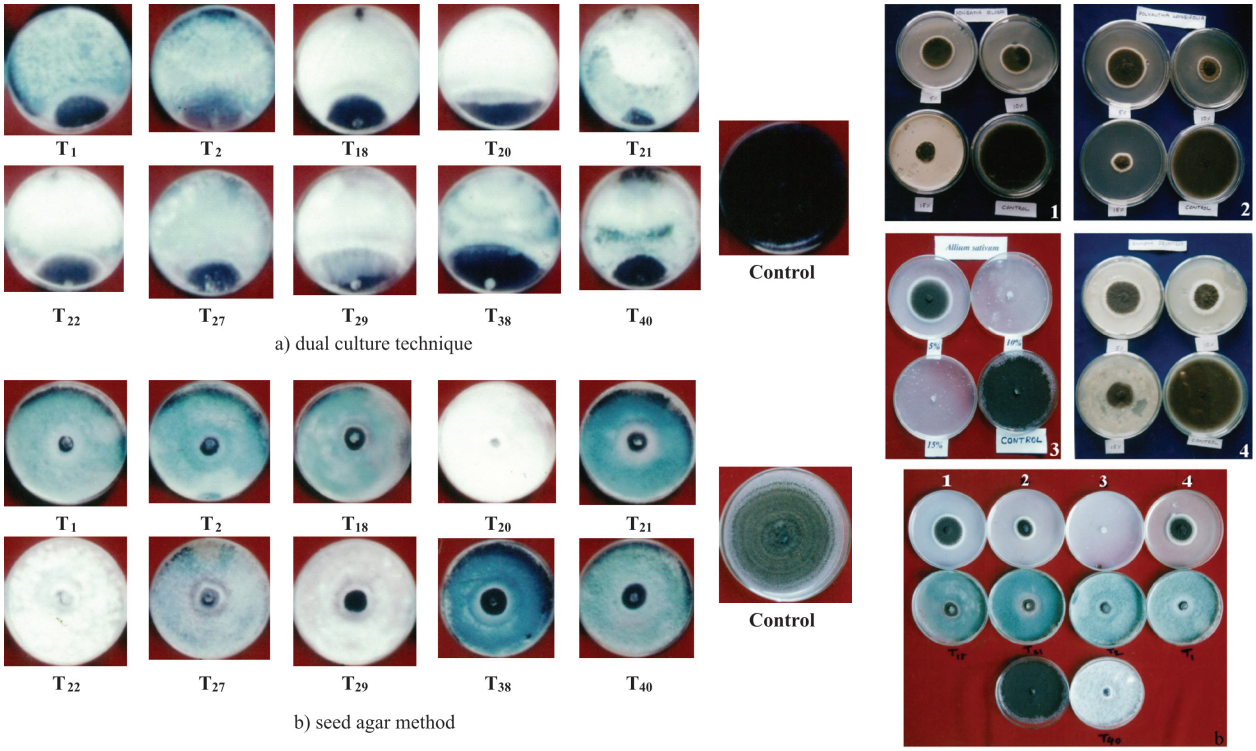

Plate 1. (a) Inhibition of growth of *A. niger* by plant extracts (b) Comparison of inhibition of growth of *A. niger* by plant extracts and native isolates of *Trichoderma*

Table 2. Comparison of native *Trichoderma isolates* with commercial formulations

Biocontrol agent	Mycelial growth of *Aspergillus niger* (cm)	Inhibition over control (%)
Native isolates		
Trichoderma isolate (T_1)	2.52	70.25
Trichoderma isolate (T_2)	2.79	67.06
Trichoderma isolate (T_{18})	2.71	68.00
Trichoderma isolate (T_{21})	2.10	75.21
Trichoderma isolate (T_{40})	2.56	69.78
Commercial formulations		
Trichoderma viride	3.50	58.68
Trichoderma harzianum	3.77	55.49
Pseudomonas f luorescens	5.77	31.88
Bacillus subtilis	1.93	77.21
Control	8.47	-
CD (p=0.05)	0.150	

of *Trichoderma* and commercial formulations of *T. viride, T. harzianum, P. fluorescens* and *B. subtilis* in respect of antagonistic effect against *A. niger*. However, the results showed a clear cut superiority of native isolates of *Trichoderma* over the commercial formulations of *T. viride, T. harzianum* and *P. fluorescens*. The formulated product of *B. subtilis* and T_{21} isolate showed significantly more antagonistic effect against the growth of *A. niger* than the remaining biocontrol agents. Commercial formulations of *P. fluorescens* were observed to be least effective than all other biocontrol agents.

Ramarathinam *et al*. (2000) reported antagonistic effect of commercial formulation of *Trichoderma*

Table 3. Effect of plant extracts on growth of *Aspergillus niger in vitro*

Plant extract	Concentration of plant extract						Mean	
	5%		10%		15%			
	1	2	1	2	1	2	1	2
Annona squamosa	5.10	40.23 (39.36)	4.00	52.76 (46.58)	2.17	74.61 (59.75)	3.75	55.87 (48.56)
Allium sativum	4.73	44.54 (41.86)	0.00	100.00 (90.00)	0.0	100.00 (90.00)	1.57	81.51 (73.95)
Polyalthia longifolia	4.90	42.58 (40.73)	3.37	60.24 (50.91)	1.90	77.73 (61.84)	3.38	60.18 (51.16)
Ocimum sanctum	8.53	00.00 (00.00)	7.87	7.09 (15.40)	6.80	20.32 (26.77)	7.73	9.13 (14.06)
Curcuma longa	8.53	00.00 (00.00)	8.41	0.00 (0.00)	5.63	33.99 (35.65)	7.54	11.33 (11.88)
Piper betle	7.67	10.15 (18.53)	7.13	15.74 (23.36)	6.70	21.15 (27.37)	7.16	15.68 (23.09)
Azadirachta indica (Leaf extract)	8.20	3.90 (11.20)	7.67	9.44 (17.82)	6.30	24.84 (29.88)	7.38	12.73 (19.64)
A. indica (seed extract)	8.53	0.00 (0.0)	8.03	5.11 (12.98)	5.90	30.85 (33.74)	7.48	11.99 (15.57)
Pongamia glabra	5.03	41.01 (39.82)	4.07	51.97 (46.13)	2.53	70.33 (57.00)	3.87	54.44 (47.65)
Acacia arabica	7.43	12.89 (21.02)	6.50	23.22 (28.80)	5.63	33.98 (35.65)	6.52	23.36 (28.49)
Control	8.53	0.00 (0.0)	8.47	0.00 (0.00)	8.53	0.00 (0.00)	8.51	0.00 (0.00)
Mean	7.01	17.75 (19.32)	5.96	29.59 (30.18)	4.73	44.34 (41.61)		
			Colony diameter		Per cent inhibition			
			CD (p=0.05)		CD (p=0.05)			
Plant extract			0.0086		0.9902			
Concentration			0.0619		0.5271			
Plant extract X concentration			0.2053		1.7151			

Figures in parentheses are angular transformed values; 1 Colony diameter (cm); 2 Inhibition of mycelial growth over control (%)

and directly correlated with agroclimatic conditions of particular site and season. In the past, *Trichoderma* spp. have proved to be potential bio-control agents in controlling several soil borne diseases under both green house and field conditions (Elad *et al*., 1981; Cook and Baker, 1983). *Trichoderma*, an antagonistic fungi inhibits a range of test pathogens by a different mechanisms of antagonism.

Effect of plant extracts on growth of *Aspergillus niger in vitro*

All the test plant extracts at three concentrations were significantly effective in reducing the growth of *A. niger* when compared to control (Table 3 & Plate 2a). The inhibition of growth of *A. niger* ranged from 1.57 to 7.73 cm as against 8.51 cm in control. Irrespective of the concentrations, *Allium sativum* was proved to be the most effective botanical and recorded the highest reduction of growth (81.51%) which was significantly superior to all other plant extracts. The next best treatment was *Polyalthia longifolia* (60.18%) followed by *Annona squamosa* (55.87%) and *Pongamia glabra* (54.44%). *Ocimum sanctum* was least effective in reducing the fungal growth (9.13%). The plant extracts irrespective of the species were found to be most effective at 15 % concentration. Maximum reduction of mycelial growth (41.61%) was observed at 15 % concentration which was significantly superior over rest of the concentrations. The interaction between plant extracts and concentrations was significant. All the plant extracts were increasingly effective in reducing the mycelial growth with increase in concentrations. Maximum reduction of mycelial growth (100%) was observed with *Allium sativum* at 10 % concentration which was significantly superior over all other combinations followed by *Polyalthia longifolia* (77.73%), *Annona squamosa* (74.61%) and *Pongamia glabra* (70.33%) at 15 per cent concentration whereas no mycelial growth reduction (0.0%) was observed with *Ocimum sanctum* (5%), *Curcuma longa* (10%) and *Azadirachta indica* seed extract (5%).

Table 4. Comparison of the plant extracts with *Trichoderma* spp., native isolates *Psueodomonas fluorescens* and *Bacillus subtilis*

Plant extract/ biocontrol agent	Mycelial growth (cm)	Inhibition over control (%)
Annona squamosa (15%)	2.23	73.87
Allium sativum (10%)	0.00	100.00
Polyalthia longifolia (15%)	1.93	77.37
Pongamia glabra (15%)	2.57	69.87
Pseudomonas fluorescens	3.16	62.95
Bacillus subtilis	1.29	84.52
Trichoderma isolate (T_1)	1.32	84.49
Trichoderma isolate (T_2)	1.54	81.19
Trichoderma isolate (T_{18})	1.85	78.31
Trichoderma isolate (T_{21})	1.69	80.76
Trichoderma isolate (T_{40})	1.88	77.96
Control	8.53	-
CD (p=0.05)	0.230	

The findings of the present study conformed the observations of Arun *et al*. (1995) who reported the fungitoxic activity of *Allium sativum* against the *A. niger*, Sobti *et al*., 1995 reported the antifungal activity of seed extract of *Polyalthia longifolia* on *A. niger*.

Comparison of plant extracts with biological control agents

Allium sativum (10%) had maximum inhibiting effect (100.00%) on *A. niger* which was significantly superior to all other plant extracts and bio-control agents and it was followed by *Bacillus subtilis* and T_1 isolate which recorded 84.52 and 84.49 per cent growth inhibition over control, respectively and were at par with each other. *Psuedomonas fluorescens* was least effective and recorded 62.95 per cent inhibition over control (Table 4 & Plate 2b).

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Histopathological studies for powdery and downy mildew pathogens of grapevine

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ABSTRACT

Grapevine (*Vitis vinifera* L.) is an essential commercial fruit crop and one of the most widely cultivated crops in the world. Powdery and mildews seriously affect the grapes cultivation and cause higher economic loss. Present study was conducted to find out the histological changes due to the infection by these pathogen. Microtomy sections of healthy grapevine leaves showed regular arrangement of epidermal cells, cortical parenchyma cells, conducting vessels and pith cells. In the infected leaf by *Plasmopara viticola,* thickening of cell wall was pronounced and cytoplasm was dense and granular. The infection by *Uncinula necator* led to collapse of epidermal cells, disruption of mesophyll and guard cells. In addition, large cavities were also found in the cytoplasm.

Key words: Powdery mildew, downy mildew, histopathology, grapevine

India is the 12th largest grape producing country in the world with the production of 1235.00 thousand tonnes. Production of grapevines is threatened by biotic and abiotic stresses. Fungal infections reduce mostly the yield and damage fruit and wine quality. Fungal diseases *viz.,* downy mildew, powdery mildew and anthracnose have been major constraints in grapevine cultivation (Naqvi *et al.*, 2013). Powdery mildew caused by *Uncinula necator* is an important endemic fungal diseases on commercial grapevine varieties and it is not possible to harvest good quality fruits without prophylactic measures (Rao, 1991). Downy mildew caused by *Plasmopara viticola* is the most destructive and explosive disease of grapevine. It has been recorded in 91 countries from temperate to tropical zones (CMI, 1988). It was first reported in India by Syndow and Butler (1912) from Pune.The objective of the present study was to find out the effect of infection of powdery and downy mildew pathogens on the plant morphology, which in turn helps in elucidating how the fungus colonizes leaf surface, palisade and vascular tissues.

MATERIALS AND METHODS

Histopathological studies

Microtome sectioning of healthy as well as infected leaves was carried out as described by Johnson (1940). For this purpose, grapevine leaves from healthy and infected plants were collected from the field.The leaf bits were fixed in 5 parts of 30 per cent formalin, 5parts of glacial acetic acid and 90 parts of 70 per cent ethanol for 29 hours at 4^0C.The leaf bits were then transferred subsequently to 50, 60 and 70 per cent ethanol for one hour.

Dehydration

The leaf bits were gradually dehydrated in tertiary butyl alcohol (TBA) series. Later the samples were washed in running tap water for 10 -15 min followed by washing in ethanol 10, 50, 70 and 100 per cent for 5 min, respectively. Then the samples were transferred to xylene I, xylene II for 5 min each and stained with toluidine blue (1g/100ml).The slides were mounted in DPX and left undisturbed for 24 hrs and photographed in an image analyser.

RESULTS AND DISCUSSION

Studies with *P.viticola*

The histopathological and molecular mechanisms by which the *P. viticola* infects grapevine along with infection cycle are unknown. A number of studies deal with the description and visualization of the oomycete's morphology and

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development. So, the present study on histopathology were undertaken with following results. Microtomy sections of healthy grapevine leaves showed regular arrangement of epidermal cells, cortical parenchyma cells, conducting vessels and pith cells. The infection by *P.viticola* led to several histological changes *viz*., pronounced thickening of cell wall and dense and granular cytoplasm. This finding is supported by other reports. The epidermal cells and cortical parenchyma were collapsed and larger cavities were also seen (Plate 1). The histochemical studies of grapevine downy mildew infected leaves showed a small amount of yellow autoflouroscence in the stomatal cells. (Dai *et al*., 1995). Allegre *et al.* (2006) found that abnormal opening of grapevine stomata following infection by *P. viticola*, a deregulation and they suspected because young infected cuttings subjected to water starvation wilted. Chaerle *et al*. (2001) revealed that the wilting has not been described in the vineyard, perhaps because natural infections are generally more limited than the artificial ones. The *P. viticola* emerges through stomatal pores, this abnormal opening of stomata probably facilitates the emergence of sporangiophores and pathogen dissemination. In some plant–pathogen interactions, transpiration is reduced, owing to defoliation, obstruction of stomata and xylem elements (Bassanezi *et al*., 2002) or stomatal closure (Langcake and Lovell, 1980; Kortekamp, 2005)

Studies with *U.necator*

However, only a few studies have focused on histopathology in this plant–pathogen interaction by producing symptoms despite the key role of these internal plant structures modification in the *U.necator* infection cycle on grapes. So, the present study on histopathology were undertaken with following results. The microtome sections of healthy leaves of grapevine revealed the presence of thick and intact epidermal layer with cylindrical palisade and round spongy parenchyma cells, whereas the sections of diseased leaves showed more damage to leaf structures. The infection by *U.necator* led to

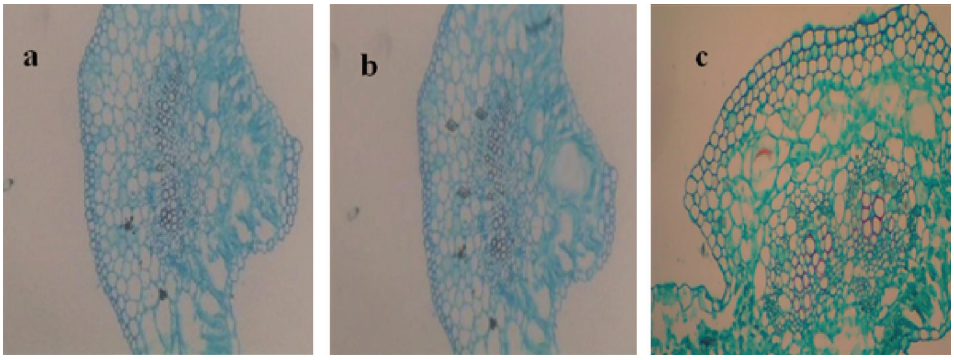

Plate 1. Histopathological studies of downy mildew infected grapevine leaves
(a) The epidermal cells and cortical parenchyma were collapsed with larger cavities (b) Thickening of cell wall was pronounced and cytoplasm was dense and granular (c) Irregular arrangement of epidermal cells

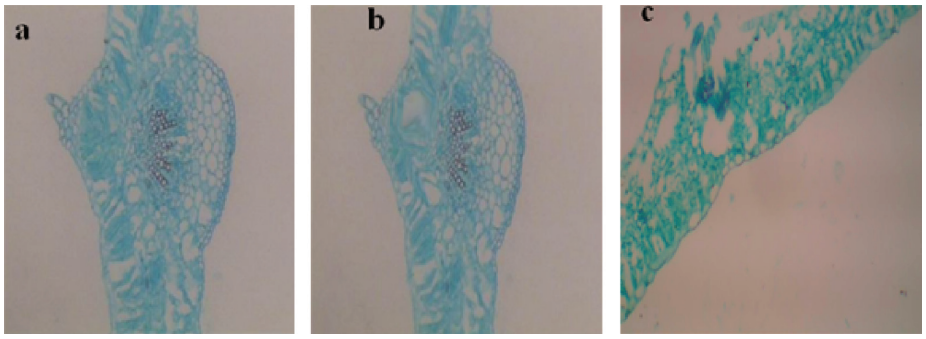

Plate 2. Histopathological studies of powdery mildew infected grapevine leaves
(a) Presence of thick and intact epidermal layer with cylindrical palisade and round spongy parenchyma cells, (b) Disruption of mesophyll layer (c) Disruption of guard cells with large cavities

collapse of epidermal cells, disruption of mesophyll and guard cells. In addition large cavities were also found in the cytoplasm(Plate 2).

The results of the present histopathological study will be usefull to find out the effect of infection caused by powdery mildew and downy mildew pathogens on the grapevine, which in turn helps in finding out the colonization of fungus in plant tissues which causes infection and extent of damage. It enhances the efficacy of suitable means of managing the pathogen causing diseases.

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Evaluation of botanicals in the management of buckeye rot of tomato

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ABSTRACT

Seven botanicals in the form of powder and crude extract were evaluated *in vitro* against *Phytophthora nicotianae* var *parasitica* causing buckeye rot of tomato . Aqueous extract of marigold leaves completely inhibited the growth of the pathogen followed by turmeric, black pepper, garlic powder which inhibited the growth of the pathogen to the tune of 50.34, 31.93 and 11.09%, respectively. Cent per cent inhibition was recorded at all concentrations when crude extract of *Eupatorium adenophorum* (kali basuti/ snake root) was tested against the pathogen without sterilization while the crude extract from *Melia azedarach* resulted in 100 per cent inhibition at 1.0 per cent and higher concentrations only.

Key words : *Phytophthora*, tomato, botanicals, extract, buckeye rot

Tomato *(Solanum lycopersicum*), a member of solanaceae family, is one of the most important " protective foods" both because of its special nutritive value and also because of its widespread production. It is the world's largest vegetable crop after potato and sweet potato, but it tops the list of canned vegetables. In India, the tomato covers an area of 8, 80,000 ha with an annual production of 18.2 million metric tonnes whereas in Himachal Pradesh, the area under tomato cultivation is 9,930 ha with production of 0.41million metric tonnes (Anonymous, 2013).The crop is generally grown during the winter months (Oct.-April) in the plains of India and summers (April-Oct.) in hilly regions of the country**.** Buckeye rot caused by *Phytophthora nicotianae* (Breda de Haan) var. *parasitica* (Dastur) Waterhouse is one of the most important fungal diseases which affects mostly the fruits during both spring and winter crops. In the areas where the fruiting period coincides with the onset of monsoon rains, the crop is severely infected. Under high humidity and good rainfall conditions, the incidence of buckeye rot alone may go up to 90 per cent (Gupta and Thind, 2006). In India, losses from this disease range between 35 and 40 per cent, which may rise with the prevalence and severity of disease depending upon the favourable weather conditions (Jain *et al.* 1961).

Keeping in view the importance of organic cultivation in modern day agriculture and adverse impact of chemicals on soil, plant and human health, the present work has been planned as botanicals being biodegradable and selective in their toxicity are considered valuable in controlling plant diseases and with minimal adverse impact on environment.

MATERIALS AND METHODS

Collection and preservation of botanicals

Five botanicals namely garlic (*Allium sativum*) (leaves, cloves-dried and lyophilized powder), lantana(*Lantana camara*) (leaves), local marigold (*Tagetes* sp.) (leaves), turmeric (*Curcuma longa*) (leaves and rhizome powder) and black pepper (*Piper nigrum*) (seed powder) were used in the present study. Leaves of all the botanicals were collected from the surroundings of Palampur and were oven dried by spreading them on the shelves of hot air oven over two to three layered blotting sheets at 50°C for 5 to 6 hours for two to three days. After drying, the respective plant material was grounded in a blender to obtain fine dry powder. Sufficient powdery biomass was stored in paper bags (tassel bags) at room temperature for further use.

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Cloves of garlic were oven dried at 60°C for 1 hour for three consecutive days and lyophilized. Black pepper and turmeric powder were procured from the market (Patanjali Ayurved Limited,Uttarakhand,India).

Preparation of aqueous extracts

Fifty gram fine powder of each botanical was soaked overnight in 100ml of sterilized distilled water (1:2 w/v) in 500 ml conical flask. The extract obtained was filtered twice through a double layer of muslin cloth through Whatman No.1 filter paper to get clear filtrate (Ashlesha, 2012). This was considered as standard aqueous extract.

Preparation of crude extracts

Crude extracts of botanicals prepared in the pilot plant system located at Biopesticide Lab, Department of Entomology, CSKHPKV Palampur were used. Pilot plant system is mainly used for extraction of botanical formulation. This system is based on the green technology in which CO_2 gas is used for extraction. Without using any solvent, the crude extract is extracted from leaf powder of *Eupatorium adenophorum* (kali basuti) and *Melia azedarach* (darek) plants with the help of CO_2 gas. The CO_2 gas is converted into super critical state which helps in extraction of crude extract from fine powdered plants.

***In vitro* evaluation of botanicals**

Plant extracts were evaluated against the pathogen using poisoned food technique with and without autoclaving (Yadav and Yadav, 2009). Desired concentrations of botanicals were mixed with equal quantity of double strength sterilized potato dextrose agar medium and poured aseptically in sterilized Petri plates. Medium mixed with equal quantities of sterilized distilled water without any treatment served as control. Seven days old mycelial bits (5mm) of *Phytophthora nicotianae* var. *parasitica* were placed in the centre of plates. Each treatment was replicated thrice and incubated at 25±1°C. Regular observations were made and finally colony diameter was measured after the control plates were completely covered by the pathogen. Per cent inhibition of mycelial growth was calculated by using formula given by McKinney (1923).

RESULTS AND DISCUSSION

Aqueous extracts of five botanicals as detailed above were evaluated *in vitro* against the pathogen under sterilized conditions by poisoned food technique. Data as presented in the Table 1 indicate that aqueous extract of garlic, lantana and turmeric leaves supported the growth of the pathogen to the tune of 19.73,19.73 and 18.84 %, respectively and aqueous extract of marigold leaves completely inhibited the growth of the pathogen where as turmeric extract resulted in maximum inhibition up to 50.34% followed by black pepper (31.93%), garlic lyophilized powder (11.09%) and garlic oven dried powder (8.87%). Results thus indicate that marigold leaf extract is most effective against

Table1. Effect of botanicals on mycelial growth of *Phytophthora nicotianae* var. *parasitica*

Botanical (Leaves & powder) @ 50 per cent concentration	Mycelial growth (mm)*	Growth inhibition (%)
Garlic (L)	90.00(9.54)	19.73
Marigold (L)	0.00(1.00)	100
Lantana (L)	90.00(9.54)	19.73
Turmeric(L)	70.67(8.47)	18.84
Garlic oven dried powder (GODP)	68.50(8.34)	8.87
Garlic lyophilized powder (GLP)	66.83(8.24)	11.09
Black pepper powder (BP)	51.17(7.22)	31.93
Turmeric powder (TP)	37.33(6.19)	50.34
Control	75.17(8.73)	
CD(p=0.05)	0.16	

* Mean of three replications
Figures in parentheses are square root transformed values

the pathogen followed by turmeric powder extract. Mycelial inhibition in case of all other extracts evaluated was found statistically insignificant as compared to control. In case when these botanicals were tested without autoclaving, provided complete inhibition of the mycelial growth of the pathogen. Earlier workers have reported efficacy of various botanicals against *Phytophthora* species but very little work has been done against the buckeye rot pathogen. Jagtap *et al.* (2012) found out the efficacy of leaf extracts of botanicals *viz.* neem (*Azadirachta indica*), mehendi (*Lawsonia inermis*), eucalyptus (*Eucalyptus cinarium*), acacia (*Acacia catechu*), glyricidia (*Glyricidia sepium*), dhatura *(Datura stramonium*), lantana (*Lantana camera)* against *Phytophthora* spp. under *in vitro* conditions using 'poisoned food technique' and 'dual culture technique', respectively. *L. camera* was significantly more effective in inhibiting the mycelial growth (44.54%) of *Phytophthora citrophthora* and *Datura stramonium* against *Phytophthora nicotianae*, inhibiting mycelial growth (57.78%), both at 5% concentration. Shashidhara *et al.* (2008) reported that *duranta* and garlic (10%) resulted in 35.5 and 26.6 per cent mycelial inhibition of *P. capsici*, respectively. Huang *et al.* (2006) reported aqueous extracts of *Eugenia caryophyllata*, *Coptis chinensis* and *Glycyrrhiza uralensis* to be most effective against *Phytophthora capsici*, *Botrytis cinerea*, *Verticillium dahliae*, *Fusarium oxysporum* and *Sclerotinia sclerotiorum*. Zang *et al.* (2009) found *Ocimum basilicum* var. *pilosum* to be most inhibitory against *Botrytis cinerea*, *F. oxysporum*, *S. sclerotiorum*, *F. solani* and *Phytophthora capsici*.

Only crude extracts, without any addition of antibiotics, derived from *Eupatorium* (kali basuti) and *Melia azedarach* (darek) were evaluated against *Phytophthora nicotianae* var. *parasitica* @ 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 per cent concentrations with and without autoclaving and the results pertaining to inhibition percentage have been presented in Table 2. A perusal of data indicates that cent per cent inhibition was recorded at all concentrations when *Eupatorium* was tested against the pathogen without sterilization and *Melia azedarach* resulted

Table 2.Effect of botanicals (crude extract) on the mycelial growth of *Phytophthora nicotianae* var. *parasitica*

Conc.(%)	*Eupatorium* **sp.**				*Melia azedarach*				Mean
	Autoclaved		Without autoclaving		Autoclaved		Without autoclaving		
	Growth (mm)*	Growth inhibition (%)	Growth (mm)	Growth inhibition (%)	Growth (mm)	Growth inhibition (%)	Growth (mm)	Growth inhibition (%)	
0.5	65.33 (8.14)	27.41	0.00 (1.00)	100.00	62.17 (7.94)	30.92	1.58 (1.60)	98.24	32.27 (4.67)
1.0	45.67 (6.83)	49.26	0.00 (1.00)	100.00	55.33 (7.51)	38.52	0.00 (1.00)	100.00	25.25 (4.08)
1.5	24.00 (4.99)	73.33	0.00 (1.00)	100.00	38.00 (6.24)	57.78	0.00 (1.00)	100.00	15.50 (3.31)
2.0	0.00 (1.00)	100.00	0.00 (1.00)	100.00	19.17 (4.48)	78.70	0.00 (1.00)	100.00	4.79 (1.87)
2.5	0.00 (1.00)	100.00	0.00 (1.00)	100.00	0.00 (1.00)	100.00	0.00 (1.00)	100.00	0.00 (1.00)
3.0	0.00 (1.00)	100.00	0.00 (1.00)	100.00	0.00 (1.00)	100.00	0.00 (1.00)	100.00	0.00 (1.00)
Control	90.00 (9.54)								90.00 (9.54)
Mean	32.14 (4.64)		12.86 (2.22)		37.81 (5.39)		13.08 (2.31)		
		Product	Conc.	Interaction					
CD(p=0.05)		(0.07)	(0.09)	(0.18)					

* Mean of three replications

Figures in parentheses are square root transformed values

in 100 per cent inhibition at 1.0% and higher concentrations, 98.24% inhibition was observed at 0.5% concentration.

The per cent inhibition increased with increasing concentration from 0.5 to 3.0% when crude extracts of these botanicals were tested against the pathogen. *Eupatorium* crude extract resulted in 27.41% inhibition of mycelial growth of the pathogen upto 0.5 % concentration which enhanced to 49.26% at 1% concentration and 73.33% at 1.5% concentration and ultimately to 100 per cent inhibition at 2.0% and higher concetration. Whereas *Melia* was found to be inhibitive to the extent of 30.92 % at 0.5% concentration, which further increased to 38.52, 57.78, 78.70 and 100% at 1, 1.5, 2 and 2.5% concentrations, respectively. Statistically the crude extracts were significantly different when tested under both sterilized and unsterilized conditions but when these were tested by autoclaving *Eupatorium* was statistically significant at 1.0 per cent and higher concentrations and *Melia* was found effective at 2.0% and higher concentration.

Ashlesha *et al.* (2013) evaluated cow urine distillates of various botanicals *in vitro* against bell pepper pathogens including *Phytophthora nicotianae*. *Murraya koenigii* (curry tree) and *Vitex negundo* (chaste tree) provided the complete inhibition in mycelial growth of *Phytophthora nicotianae* at 10% concentration. Cow urine distillates of botanicals were found more inhibitory to pathogens than cow urine distillate alone. Zhang *et al*. (2006) reported that petroleum ether extract of *E. adenophorum* recorded the highest (44.42%) inhibitory activity against *Phytophthora infestans*. Sterilized and non-sterilized leaf juice of *E. adenophorum* exhibited more than 50% and reaching up to 95% inhibitory activity to the pathogenicity of *P. infestans* when mixed with 25% metalaxyl. Yadav and Yadav (2009) observed that extracts of 6 plants showed the highest fungitoxic activity against *Phytophthora nicotianae* var. *parasitica* at 30% concentration. Maximum growth inhibition of the pathogen was recorded with *Aegle marmelos* (88.3%) followed by *Murraya koenigii* (82.6%), *Lawsonia inermis* (81.3%), *Argemone mexicana* (80.1%), *Catharanthus roseus* (72.5%), and *Melia azedarach* (68.7%). The fungitoxic activity of *Eupatorium* sp. and *Melia azedarach* might be due to the presence of carotene, flavones glycoside and ranuncoside (Hussain *et al*., 2011) and triterperoid,benzoic acid , β- sitosterol (Jabeen *et al*., 2011) and when sterilized these alkaloids / constituents might have been less toxic due to their easily biodegradable nature (Fawcett and Spencer, 1970).

The results of the present investigation are encouraging in that the local botanicals *in vitro* inhibited the growth of the *Phytophthora nicotianae* var. *parasitica* isolated from buckeye rot infected tomato fruits. Leaf extract of marigold and crude extract of *Eupatorium* were found to inhibit the growth of the pathogen completely and provide suitable organic substitute to chemicals in the management of this deadly disease. Contrary to the problems associated with the use of synthetic chemicals, botanicals are environmentally non pollute, locally available, easily accessible, non phytotoxic, systemic ephemeral, readily biodegradable, relatively cost effective and hence constitute a suitable plant protection strategy in the plant disease management.

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In vitro antagonistic potentials of native *Trichoderma* spp. against stem rot of French bean caused by *Sclerotium rolfsii* in Manipur

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ABSTRACT

Ten isolates of three different *Trichoderma* spp. were isolated from the rhizosphere of French bean from different locations of Manipur. Among these, five were *T.viride* (Salam, Samurou, Lilong, Khurai and Thangmeiband), four were *T.harzianum* (Kongba, Basikhong, Khoijuman and Nambol) and one was *T.hamatum* (Thoubal). Mycelial growth inhibition of *Sclerotium rolfsii* in dual culture by *Trichoderma* isolates ranged from 88.00 to 74.67 per cent. Effect of volatile compound produced by *Trichoderma* spp. against *S. rolfsii* ranged from 10.22 per cent to 41.98 per cent where the highest inhibition was recorded with isolate ThmFB3 (41.98 per cent) and for non-volatile compound it was 31.77 per cent at 7.5 % v/v concentrations and 70.22 per cent at 15 % v/v by the isolate TvFB1. Ecological adaptability of four potent *Trichoderma* isolates showed variation in the colonization of sclerotia under three different soil conditions *viz*., unsterilized, sundried and sterilized soil, where the highest colonization of TvFB1 (44.00 per cent) was observed in sterilized soil. The rhizosphere competence of four potent isolates of *Trichoderma* spp. *viz*., TvFB1, ThrFB2, ThmFB3 and ThrFB4 showed variation in the levels of colonization under three different soil conditions. It was observed that the rate of colonization by *Trichoderma* isolates increased upto 45 days after sowing and reduced at 60 days after sowing.

Key words*: Sclerotium rolfsii*, volatile compounds, non-volatile compounds, French bean, antagonistic potential

French bean also known as green bean or string bean or snap bean or squeaky bean is the unripe fruit of specific cultivated varieties of the common bean (*Phaseolus vulgaris* L.) (David, 2005). It is an important and highly profitable vegetable crop of North Eastern Hill Region of India. Generally, it is cultivated for vegetable purpose round the year except winter months (October-January) in foothills as well as in the mid altitudinal areas of all the North Eastern States. However, at present its average yield is low in farmer's field as compared to its potential yield due to several biotic and abiotic factors. Biotic factors that affect Frenchbean include diseases caused by fungi, bacteria, virus and insect pests. Among the fungal diseases, stem rot caused by *Sclerotium rolfsii* Sacc. is one of the important constrains as the pathogen is a soil inhabitant, polyphagous, facultative parasite (Aycock, 1966; Sarangthem *et al*., 2012). Biological controls in recent times have been accepted as more natural and environmentally acceptable alternative to the existing chemical treatments. In this context *Trichoderma* spp has been investigated as an important antagonistic soil fungus having the ability to reduce disease incidence caused by the phytopathogenic fungi, particularly the soil borne fungi. *Trichoderma*, a saprophytic fungus is known to be one of the best candidates of biocontrol agents (Elad, 2000). Hence an attempt was made to evaluate the antagonistic potentials of promising native *Trichoderma* isolates from different places such as Salam, Kongba, Thoubal and Basikhong of Manipur against *Sclerotium rolfsii*.

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MATERIALS AND METHODS

French bean plants showing characteristics symptoms of stem rot were collected from the farmers' fields in Imphal East and West, Thoubal and Bishnupur districts of Manipur during July-August, 2012. Isolations were made from diseased samples and fungus associated with the samples were identified by following standard procedure (Kokub

et al., 2007). *Trichoderma* spp. were isolated from soil samples collected from the rhizosphere of French bean at different locations of Manipur by using soil dilution plate technique (Dhingra and Sinclair, 1995).

The mycelial growth inhibition assay of the test plant pathogen was done by dual culture technique using ten numbers of *Trichoderma* isolates with five replications each and per cent inhibition of radial growth of pathogen was calculated as per the followng formulae adopted by Garcia (1991):

Per cent inhibition of radial growth, %IRG = 100 $[(R_1-R_2)/R_1]$

where R_1 = the farthest radial distance grown by the pathogen in the direction of the antagonist (control)

R_2= the distance grown on a line between inoculation positions of the pathogen and the antagonist.

The effect of volatile and non-volatile (7.5% and 15% v/v) compounds produced by *Trichoderma* isolates was studied by following methods of Dennis and Webster (1971a, 1971b).

Ecological fitness of four potential isolates of *Trichoderma* was evaluated by taking soils from four places of Manipur area by following the method of Papavizas and Lumsden(1982) and per cent sclerotia colonisation was calculated. Competitive parasitic ability of *Trichoderma* isolates against *Sclerotium rolfsii* was studied by following standard procedure (Rodriguez-Kabana *et al*., 1974).

The rhizosphere competence of test *Trichoderma* isolates was studied under net house condition on French bean (*Phaseolus vulgaris)* using unsterilized, sun dried and sterilized soils. Two kg potting mixture of soil (unsterilized, sun dried and sterilized soil) and farm yard manure (2:1 v/v) was mixed thoroughly with 20 g of rice bran + mustard cake (20%) formulation of *Trichoderma* isolates (1 x 10^8 cfu/g of product) and filled into the earthen pots. Twenty five seeds of French bean were sown per pot. The experiment was replicated four times and seeds of test crop were allowed to germinate. The rhizosphere soil was collected by gently uprooting the test crops and brushing the soil adhered to roots after 15 days (Pan and Bhagat, 2008). The observation on rhizosphere competence of *Trichoderma* isolates was recorded at 15 days interval upto 60 days and population of *Trichoderma* spp. was estimated by soil dilution plate technique (Dhingra and Sinclair,1995).

RESULTS AND DISCUSSION

Ten numbers of *Trichoderma* spp. were isolated and identified based on Rifai (1969) morphology and taxonomic keys. The species was identified as *Trichoderma viridi* (5nos- TvFB1, TvFB5, TvFB6, TvFB8, TvFB10), *Trichoderma harzianum* (4 nos- ThrFB2, ThrFB4, ThrFB7, ThrFB9) and *Trichoderma hamatum* (1no- ThmFB3). All the *Trichoderma* spp. showed different degree of growth inhibition of *S. rolfsii* Sacc. in dual culture technique(Table1). Among the ten isolates of *Trichoderma* spp. tested, maximum percentage inhibition was recorded with isolate TvFB1 (88.00 per cent) and minimum was observed with the isolate TvFB10 (76.67 per cent). The results are in confirmation with Rekha *et al*. (2012) who found that growth of *S. rolfsii* in dual culture was suppressed by all the three *Trichoderma* spp. (*T. viride*, *T. harzianum* and *T. hamatum*). The inhibitory effect of volatile compounds produced by *Trichoderma* isolates against *Sclerotium rolfsii* ranged from 10.22 to 41.98 per cent. The highest inhibition of growth was observed by the isolate ThmFB3 and lowest by TFvB8. The effect of non-volatile compounds produced by *Trichoderma* spp. at two different concentrations *viz*.,7.5 % (v/v) and 15 % (v/v) were studied against *S. rolfsii* (Table 1). Results showed that the per cent inhibition of radial growth of *S. rolfsii* Sacc. by ten different isolates of *Trichoderma* spp. ranged from 0.00 to 31.77% at 7.5% v/v concentrations and from 0.00 to 70.22% at 15% v/v of non-volatile compounds produced by *Trichoderma* isolates. The highest per cent inhibition at 7.5 and 15% v/v concentration was recorded with the isolate TvFB1 where the inhibition percentage was 31.77 per cent and 70.22 per cent, respectively. However, isolate TvFB10 showed no inhibition against the pathogen in both the concentrations. It is important to mention that *Trichoderma* spp. are known to produce a number of antibiotics suchvas trichodermin, trichodermol, harzianum A, hrazianolide (Dennis and Webster, 1971a,b; Kucuk and Kivanc, 2004) as well as some cell wall degrading enzymes such as chitinases, glucanases that break down polysaccharides, chitins and beta glucanase destroying cell wall (Elad, 2000).

Table 1. *In vitro* screening and comparative effect of volatile and non-volatile compounds of *Trichoderma* isolates on growth of *S. rolfsii*

Isolates	Per cent radial growth inhibition			
	Dual culture technique	Volatile	Non volatile	
			(7.5%)	(15%)*
TvFB1	88.00	36.29	31.77 (5.67)	70.22 (8.40)
ThrFB2	87.33	36.09	18.89 (4.34)	25.33 (5.07)
ThmFB3	86.89	41.98	15.33 (3.97)	20.44 (4.53)
ThrFB4	86.44	36.02	12.67 (3.56)	18.22 (4.24)
TvFB5	85.78	35.18	9.56 (3.11)	16.22 (4.07)
TvFB6	85.44	21.33	3.56 (1.85)	9.56 (3.00)
ThrFB7	85.11	25.11	5.33 (2.32)	7.33 (2.73)
TvFB8	83.78	10.22	6.00 (2.41)	16.22 (4.04)
ThrFB9	79.33	26.67	2.89 (1.63)	14.22 (3.78)
TvFB10	74.67	20.44	0.00 (0.71)	0.00 (0.71)
CD (p=0.05)	4.28	1.66	1.07	1.03

*Mean of five replications
Values in parentheses are $\sqrt{x+0.5}$ transformed values

The effect of volatile compounds produced by *Trichoderma* spp. against *S. rolfsii* is aided by large variety of volatile secondary metabolites produced by *Trichoderma* such as Ethylene, Hydrogen cyanide, Aldehydes and Ketones which play an important role in controlling the plant pathogens (Vey *et al.,* 2001). The present findings also showed varying degree of inhibition on growth of the pathogen (*Sclerotium rolfsii*) in culture media.

Competitive parasitic ability of the four potential isolates of *Trichoderma* against *S. rolfsii*, in three different conditions of soils, *viz.,* unsterilized, sun dried and sterilized soils revealed that the colonization percentage of sclerotia of *S. rolfsii* by TvFB1, ThrFB2, ThmFB3 and ThrFB4 in unsterilized soil was 12, 16, 12 and 8%, respectively, while in sundried soil the colonization percentages were 24, 20, 16 and 12%, respectively (Table 2). The degrees of colonization in sterilized soil by TvFB1, ThrFB2, ThmFB3 and ThrFB4 were 44, 24, 20 and 16%, respectively. Among the three different conditions of soil, per cent colonization by all *Trichoderma* isolates of the sclerotia of *S. rolfsii* was found to be more in sterilized soil followed by sun dried and unsterilized soil. Increase in colonizing ability in sterilized soil may be due to non existence of other competitive microbes as during soil sterilization all microbes has been killed. The parasitic or saprophytic ability of antagonists, *Trichoderma* spp. also depends upon the ability to parasitize or colonize the resting structures especially the soil borne pathogens. Existence of

Table 2. Competitive parasitic ability of *Trichoderma* isolates against sclerotia of *Sclerotium rolfsii*

Per cent colonization of sclerotia of *S. rolfsii* by *Trichoderma* isolates*			
Trichoderma isolate	Unsterilized soil	Sundried soil	Sterilized soil
TvFB1	12.00 (3.00)	24.00 (4.01)	44.00 (5.42)
ThrFB2	16.00 (3.36)	20.00 (3.25)	24.00 (4.49)
ThmFB3	12.00 (3.00)	16.00 (3.36)	20.00 (4.13)
ThrFB4	8.00 (2.23)	12.00 (2.60)	16.00 (3.36)
Control	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
CD (p=0.05)	N.S	N.S	N.S

*Mean of five replications, N.S= Non significant
Values in parentheses are $\sqrt{x+0.5}$ transffcormed value

Trichoderma spp. in various soils, depends on their ability to degrade various organic substrates in soil, metabolic versatility, and resistance to metabolic inhibitors. This finding of varied colonizing ability of *Trichoderma* isolates are in accordance with Henis *et al.* (1983) who have shown that strains of *Trichoderma* spp. varied in their ability to colonize the sclerotia of *S. rolfsii*.

The rhizosphere competence of four potent isolates of *Trichoderma* spp. viz., TvFB1, ThrFB2, ThmFB3and ThrFB4 was found to be increased upto 45 days of sowing and then decreased (Table 3). The rhizosphere colonization by isolate TvFB1at 15, 30, 45 and 60 days after sowing was 0.21x10^8 cfu/g to 0.52x10^8 cfu/g in unsterilized soil, 0.28x10^8 cfu/g to 0.61x10^8 cfu/g in sundried soil and 0.54x10^8 cfu/g to 0.87x10^8 cfu/g in sterilized soil, respectively. The colonization of isolateThrFB2 at 15, 30, 45 and 60 days after sowing were 0.22 x10^8 cfu/g to 0.45 x10^8 cfu/gin unsterilized soil, 0.32 x10^8 cfu/g to 0.80 x10^8 cfu/g in sundried soil and 0.37 x10^8 cfu/g to 0.97 x10^8 cfu/g in sterilized soil, respectively. The colonization of isolate ThmFB3at 15, 30, 45 and 60 days after sowing were 0.19x10^8 cfu/g to 0.41x10^8 cfu/g in unsterilized soil, 0.30x10^8 cfu/g to 0.80x10^8 cfu/g in sundried soil and 0.42x10^8 cfu/g to 0.77 x10^8 cfu/g in sterilized soil, respectively. The colonization of isolate ThrFB4 at 15, 30, 45 and 60 days after sowing were 0.19 x10^8 cfu/g to 0.38 x10^8 cfu/g in unsterilized soil, 0.27 x10^8 cfu/g to 0.83 x10^8 cfu/g in sundried soil and 0.41 x10^8 cfu/g to 0.76 x10^8 cfu/g in sterilized soil, respectively. No colonization was observed in untreated control. The level of rhizosphere colonization among the four potent isolates of *Trichoderma* spp. was found significantly different.

Increase in the rate of colonization upto 45 days of sowing and reduction later may be result of the decrease in the amount of nutrients available to the antagonist. During active growth phase, the different root exudates serve as source of nutrients to introduced antagonist (Benitez *et al.,* 2004). Benitez *et al.* (2004) reported that *Trichoderma* has a strong capacity to mobilize and take up soil nutrients, thus making it more efficient and competitive than many other soil microbes. *Trichoderma* or *Gliocladium* introduced through seed must be multiplied in the rhizosphere if they are to inhibit pathogens that infect roots some distance away from the cotyledon attachment (Papavizas, 1985; Ahmad and Baker, 1987), it may suppress pathogens causing seed rot and seedling diseases but not those that cause root diseases.

Rhizosphere competence of antagonists is a prerequisite for the biological control of soil borne plant pathogens. Several research papers reveal the fact that success of bio agent introduced in soil does not guarantee the control of target pathogen(s)

Table 3. Rhizosphere colonization of four potent *Trichoderma* isolates in Frenchbean

Trichoderma isolates	Population of *Trichoderma* isolates* (x10^8cfu/g soil)											
	Unsterilized soil				Sundried soil				Sterilized soil			
	15 DAS	30 DAS	45 DAS	60 DAS	15 DAS	30 DAS	45 DAS	60 DAS	15 DAS	30 DAS	45 DAS	60 DAS
TvFB1	0.21 (0.84)	0.55 (1.00)	0.96 (1.20)	0.52 (1.04)	0.28 (0.87)	0.71 (1.09)	1.20 (1.29)	0.61 (1.04)	0.54 (1.01)	1.07 (1.25)	1.70 (1.47)	0.87 (0.97)
ThrFB2	0.22 (0.84)	0.53 (1.00)	0.95 (1.20)	0.45 (0.96)	0.32 (0.90)	0.90 (1.18)	1.12 (1.27)	0.80 (1.13)	0.37 (0.92)	1.03 (1.23)	1.47 (1.40)	0.97 (1.20)
ThmFB3	0.19 (0.84)	0.51 (1.00)	0.91 (1.18)	0.41 (0.94)	0.30 (0.89)	0.90 (1.26)	1.12 (1.29)	0.80 (1.16)	0.42 (0.95)	1.16 (1.20)	1.54 (1.42)	0.77 (1.12)
ThrFB4	0.19 (0.82)	0.50 (1.00)	0.83 (1.14)	0.38 (0.93)	0.27 (0.87)	0.87 (1.16)	1.09 (1.25)	0.83 (1.14)	0.41 (0.94)	0.94 (1.19)	1.52 (1.41)	0.76 (1.11)
Control	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
CD (p=0.05)	0.008	0.012	0.012	0.034	0.004	0.112	0.006	0.004	0.008	0.006	0.004	0.007

*Mean of five replications
Values in parentheses are $\sqrt{x+0.5}$ transformed values

because plants, physicochemical and biological factors affect establishment and antagonism of introduced bio agents .In this context, it is presumed that to ensure success of introduced bioagents, they should be isolated from the local area where they exist. The present study is focused on this theme, that after isolation and identification of local rhizospheric fungi and testing their antagonistic potential under *in vitro* conditions they can be prescribed for application in fields which is already a known and conducive environment. Among the ten isolates of *Trichoderma* spp. from rhizosphere of French bean crop, some isolates showed excellent capability to inhibit the growth of pathogen in *in vitro* conditions. The results reported, suggest that the isolates of *T.harzianum* and *T. viride* were more capable of influencing the growth of tested pathogen in dual culture. This result is a pioneer information that particular isolate from a particular location can be employed in bulk for treatment of disease incidence. Furthermore, it can be tested against other pathogens. The present findings showed that native *Trichoderma* spp. can be used effectively for the management of the soil borne pathogen such as *Sclerotium rolfsii*. and also further efforts are onto to evaluate the performance of promising isolates under fields conditions.

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Evaluation of botanicals, cow urine and biocontrol agents against *Rhizoctonia solani* inciting leaf blight of Kalmegh *(Andrographis paniculata)*

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ABSTRACT

The efficacy of different plant extracts, cow urine and bio-agents was tested against *Rhizoctonia solani,* the incitent of leaf blight of Kalmegh *(Andrographis paniculata)*. Garlic extract at 15% was found to be most effective with 78.89 % inhibition followed by thuja extract at 15.0 % concentration with 75.56 % inhibition. Cow urine at the high concentration was found to be more effective in inducing cent per cent inhibition. *Trichoderma harzianum* (*Th-4*) caused maximum 88.71 % *in vitro* inhibition followed by *Th-12* with 78.63% where as *Pseudomonas fluorescens* (*Psf*) was least effective.

Keywords: Kalmegh, *Rhizoctonia solani,* botanicals, cow urine and bio-agent

Kalmegh (*Andrographis paniculata* Nees.) is one of the most important medicinal crops of tropical and subtropical region of the country and is more grown in Tarai and Bhabhar region of Uttarakhand. Kalmegh is also known as a hepatoprotective drug and immunomodulator. Kalmegh is adversely affected by many biotic and abiotic factors, major one is leaf blight caused by *Rhizoctonia solani* (Anonymous, 2007-08). The symptoms of leaf blight were noticed in all the aerial parts of plants including leaves, petioles and sometimes on stem resulting in burning appearance on the edges of the leaves. Later on Leaves turned dark brown to black colour and dried out.

MATERIALS AND METHODS

Preparation of plant extract and cow urine

The extracts of tulsi *(Ocimum tenuiflorum),* eucalyptus (*Eclipta globules*), thuja (*Thuja occidentalis)*, neem (*Azadirachta indica*), garlic (*Allium sativum*), and tobacco (*Nicotiana tobaccum*) were tested on mycelial growth of *Rhizoctonia solani*. Plant parts were thoroughly washed with sterile distilled water and air dried. One hundred gm of plant parts was homogenized in 100 ml each of sterile distilled water, acetone and methanol for 10-15 min. The macerates were individually filtered through double layered cheese cloth and centrifuged at 10,000 g for 15 minutes. The supernatant was decanted and passed through bacterial filters under vacuum. The desired concentration of the extracts was prepared by mixing separately in sterilized distilled water and stored in refrigerator for further use (Gangopadhyay *et al.,* 2010).

The efficacy of selected plant extracts for their antifungal properties was assayed by poisoned food technique (Grover and Moore, 1962). Each botanical was tested at 5, 10 and 15 per cent concentrations Twenty ml of poisoned medium were poured in each Petri plate and three replications were maintained. After solidification, 5 mm mycelial discs cut with the help of sterilized cork borer from 3 days old culture of the test fungus were centrally inoculated in each Petri plate. Unamended PDA plates, inoculated with test pathogen, served as control. All the inoculated plates were incubated at 28 $\pm 1^0$ C .The observation on colony diameter in mm was recorded at every 24 hrs interval till the check Petri plates were fully covered with the growth of the test fungus. The data were statistically analyzed.

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The efficacy of five strain of *Trichoderma viz*., Th-1, Th-3, Th-4, Th-12 and *Th* -PBT 23 and *Pseudomonas* was tested against *R. solani* following dual culture technique (Morton and Strobe, 1955). The per cent inhibition was determined and data analysed statistically.

RESULTS AND DISCUSSION

Effect of plant extracts and cow urine on mycelial growth of *R. solani*

All the plant extracts were more or less inhibitory to radial growth of the pathogen (Table 1 and Fig. 1, 2 &3**).** Presence of plant extracts affected the normal growth of the test fungus. The effectiveness of the extracts increased with an increase in concentration and maximum inhibition was recorded at 15%. Among the botanicals significantly, the maximum inhibition (78.89 %) in mycelial growth was recorded with garlic extract at 15 % followed by thuja extract (75.56 %) and tulsi extract (54.44 %) after 48 hr of incubation. Whereas at this concentration, cow urine completely inhibited the mycelial growth. Cow urine and garlic were observed to be highly significant as compared to other. Shinde and Patel (2004) reported that garlic extract at 10% concentration showed complete growth inhibition of *R. solani*.

Effect of bioagents on mycelial growth of *R. solani*

The growth of antagonist overlapped the mycelia of the test pathogen after 72 hr of inoculation (Table 2). Periodic observations on the colonization of the test pathogen by the selected bioagents indicated their varied antagonistic potentiality. The data

Table 1. *In vitro* effect of plant extracts and cow urine on the mycelial growth inhibition of *R. solani*

Treatment	Per cent mycelial growth inhibition					
Conc (%)	5		10		15	
	G	I	G	I	G	I
Tulsi	64.00	28.89	50.33	44.08	41.00	54.44
Eucalyptus	72.40	19.56	57.83	35.74	48.50	46.11
Thuja	42.50	52.78	32.50	63.89	22.00	75.56
Neem	70.20	22.00	62.50	30.56	52.00	42.22
Garlic	37.23	58.63	27.50	69.44	19.00	78.89
Tobacco	81.23	9.74	64.16	28.71	47.40	47.33
Cow urine	11.50	87.22	8.00	91.11	0.00	100.00
Check	90	0.00	90.00	0.00	90.00	0.00
CD (p=0.05)		1.36	-	1.30	-	1.33

G= Average mycelial growth in (mm)
I= Average mycelial growth Inhibition (%)

Table 2. *In-vitro* efficacy of *T. harzianum* and *P. fluorescens* on mycelial growth of *R. solani*

Treatment	Average of mycelial growth (mm) at different incubation hrs				Av. per cent inhibition
	24hrs	48hrs	72hrs	96hrs	
Th-1	10.23	26.63	42.30	67.50	75.55
Th-3	10.90	26.50	43.50	68.53	76.66
Th-4	13.23	34.83	54.00	79.83	88.71
Th-12	12.86	30.30	47.26	70.63	78.63
Th -PBT 23	8.50	28.16	39.66	64.16	71.66
Psf	6.70	13.63	26.50	35.16	39.44
Check	15.03	36.56	73.86	90.00	0.00
CD (p=0.05)	0.63	1.02	2.73	3.02	

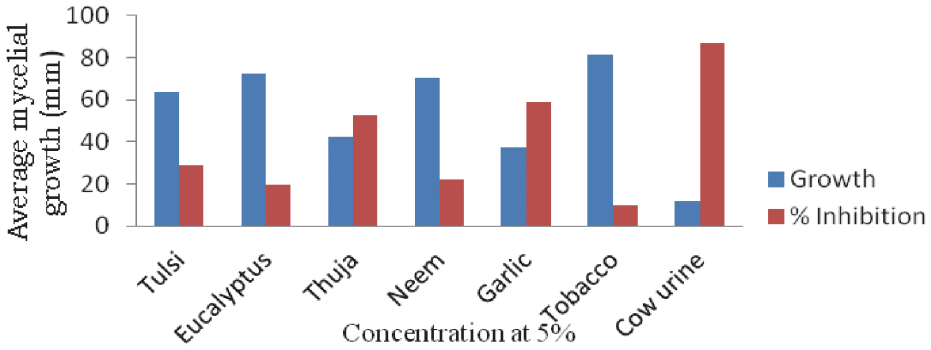

Fig. 1. Effect of botanicals and cow urine at 5% concentration on growth of *R. solani*

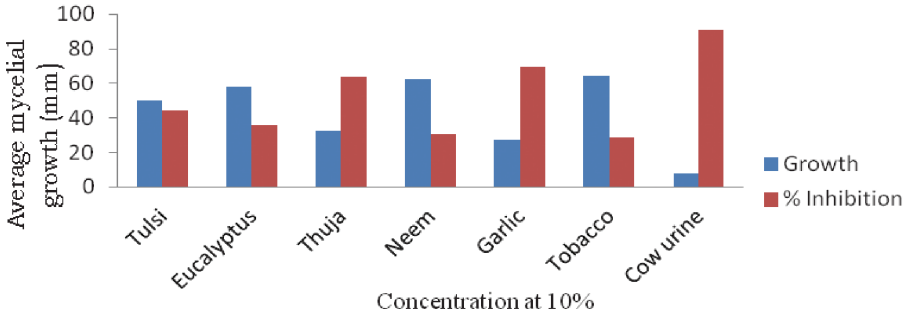

Fig. 2. Effect of botanicals and cow urine at 10% concentration on growth of *R.solani*

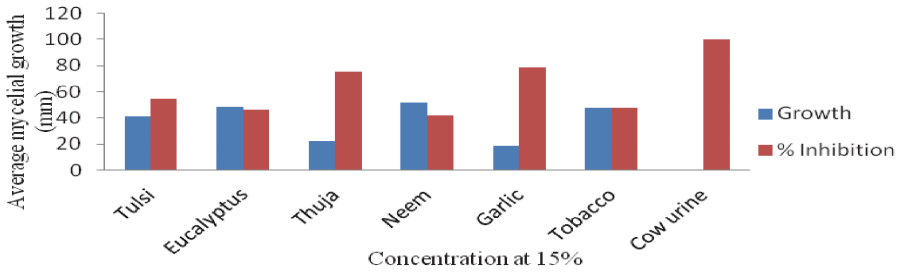

Fig. 3. Effect of botanicals and cow urine at 15% concentration on growth of *R. solani*

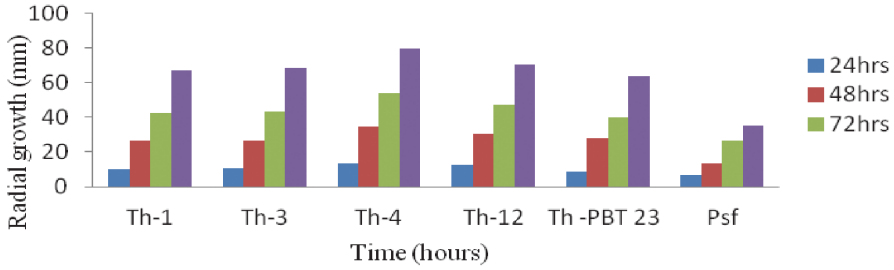

Fig. 4. Comparison between the average radial growth pattern of *R. solani* due to *T. harzianum* and *P. fluorescens* in medium at 28±1^{0}C

revealed that all five antagonists inhibited the growth of *R. solani* significantly and inhibition zones were visible after 96 hr. A demarcation line was produced between the test fungus and bioagent after 72 hr. After 96 hr, *Th*-4 inhibited maximum (88.71 %) followed by *Th*-12 (78.63 %), while minimum inhibition was recorded with PBT-23, (71.66 %). Shrama *et al.* (2001) reported that the biocontrol fungi started inhibiting the growth of *R. solani* after 7 days and covered the entire Petri dish within 10 days of inoculation.

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Identification of resistant genotypes and weather effects on disease development in mungbean

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ABSTRACT

Sixty five mungbean genotypes were screened for resistance to anthracnose disease under epiphytotic conditions. Considerable variation among the genotypes against the disease was observed. Among the sixty five genotypes, two (LGG-460 and TMV-37), one (GM-9926) and twenty five genotypes were found resistant, moderately resistant and moderately susceptible respectively to disease. Rests of the genotypes were found susceptible to highly susceptible. Simple correlation analysis between the relative progress of anthracnose depicted positive correlation with rainfall, wind speed, minimum temperature, vapour pressure and relative humidity of mungbean varieties. Bright sun shine hours and maximum temperature were found negatively correlated with the relative progress of the disease.

Key words: Mungbean, genotypes, anthracnose, correlation, *Colletotrichum truncatum*

Mungbean [*Vigna radiata* (L.) Wilczek] is one of the important pulse crops of India. The crop suffers from many economically important diseases caused by fungi, bacteria, viruses, nematodes and among these, anthracnose, (*Colletotrichum truncatum* (Schw.) Andrus and Moore), is one of the most important fungal diseases of mungbean. The pathogen causes losses both in terms of yield and quality (Sharma *et al*., 2008). Losses in yield range between 24 to 67 per cent (Deeksha and Tripathi, 2002) depending on the severity, stage of infection, genotypes and environmental conditions. The losses are much higher when the pathogen infects the crop before flowering. Infection of pods directly damages the seeds and reduces its germinability. Pod infection may result in complete seed yield loss. Though, there are fungicides that can reduce disease development, but they are not economical. Therefore, it is necessary to identify disease resistant varieties.

MATERIALS AND METHODS

Sixty five mungbean genotypes (Table 1) obtained from Indian Institute of Pulse Research, Kanpur and Department of Plant Pathology, B. A. College of Agriculture, Anand were screened against anthracnose disease during *Kharif* 2011 and 2012 under field conditions. Each test entry was sown in a row of 5 m with 30 cm apart and 10 cm distance from plant to plant with variety IR 16 as susceptible check after every 5 test entries. Initial plant count was taken 10-15 days after sowing. The data on severities of anthracnose on 10 randomly selected plants in each row were recorded. The disease was scored on 0-9 scale (Mayee and Datar, 1986) before flowering, pod formation and at physiological maturity and per cent disease index (PDI) was calculated.

Effect of weather on disease development

The experiment on effect of weather on disease development was conducted during *Kharif* 2011-12 and 2012-13 (sown during third week of July) at experimental site of Department of Plant Pathology, B. A. College of Agriculture, AAU, Anand, Gujarat under disease epiphytotic conditions. The varieties IR 16, K-851 and Meha were sown in rows 30 cm apart and 10 cm distance from plant to plant in 5 x 3 m/plot. The cultivars were replicated thrice in a randomized block design (RBD). Standard

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Table 1. Reaction of mungbean genotypes against anthracnose under field conditions*

Disease score	Disease reaction	Name of the genotypes
0	Immune	Nil
1	Resistant	LGG-460 and TMV-37
3	Moderately resistant	GM-9926
5	Moderately susceptible	PDM-262, PDM-11, PDM 84-143, IPM 02-3 RED, PDM-87, PDM-139, IPM 02-14, BRS-2435, SAPTARI LOCAL, K-851, GM - 02-06, GM - 02-08, GM - 02-09, GM - 02-10, GM - 02-14, GM - 02-16, GM - 0 2-17, GM - 02-19, GM - 02-21, GM - 03-04, GM - 03-08, GM - 03-11, GM - 03-12, GM - 03-15 and GM - 03-16
7	Susceptible	PDM-288, IPM 02-1, IPM 99-125, MEHA, IR-16, GM-3, GM-4, GM-9703, GM – 9917, GM - 9918, GM – 9925, GM – 9926, GM - 02-01, GM - 02-02, GM - 02-03, GM - 02-04, GM - 02-05, GM - 02-07, GM - 02-11, GM - 02-12, GM - 02-13, GM- 02-15, GM - 02-18, GM - 02-20, GM - 03-01, GM - 03-02, GM - 03-03, GM - 03-05, GM - 03-06, GM - 03-07, GM - 03-09, GM - 03-10, GM - 03-13 and GM - 03-14
9	Highly susceptible	GM- 9705, GM - 2k-5 and GM - 2k-14

*Mean 2011 and 2012

agronomical practices were followed to raise the crop. Observations on disease were recorded on 10 randomly selected plants using 0-9 scale at weekly interval up to maturity of the crop and PDI calculated. Relative progress of disease (RPD) was determined by subtracting previous week disease intensity from the present week disease intensity.

Observation on meteorological factors (temperature, rainfall, relative humidity, number of rainy days and vapour pressure and sunshine) were recorded at observatory of Department of Meteorology, B.A. College of Agriculture, AAU, Anand and correlated with the disease intensity.

RESULTS AND DISCUSSION

The results (Table 1) showed that there were considerable differences among the genotypes for the level of resistance against the disease. Among the sixty five genotypes screened, none of the genotypes were found to be immune. However, two genotypes viz., LGG-460 and TMV-37 showed resistant reaction, one genotype i.e. GM-9926 showed moderately resistant, while rest of the genotypes were found moderately susceptible to highly susceptible. Kulkarni *et al*. (2009) reported that in green gram, the genotypes *viz.*, TM-96-2 and TARM-18 were resistant to anthracnose

Table 2. Development of anthracnose on selected mungbean genotypes over a period of time*

Std Week	K-851		IR-16		Meha		BSS	RF	WS	Max. Temp.	Min. Temp.	VP	RH
	PDI	RPD	PDI	RPD	PDI	RPD							
32	0	0	0	0	0	0	1.1	116.0	6.4	30.9	25.5	24.7	89.1
33	2.4	2.4	1.95	1.95	2.5	2.5	2.3	156.8	6.7	29.9	24.9	25.2	89.6
34	6.77	4.37	6.85	4.9	8.65	6.15	2.6	70.5	4.5	31.5	25.2	24.9	85.5
35	12.1	5.33	11.1	4.25	11	2.35	2.8	90.9	4.5	31.9	25.5	26.0	90.5
36	17.85	5.75	16	4.9	12.95	1.95	2.8	132.5	5.9	31.3	25.3	25.3	90.2
37	21.35	3.5	17.85	1.85	15.55	2.6	2.4	29.5	4.9	30.6	25.0	24.4	88.6
38	22.2	0.85	19.3	1.45	15.65	0.1	6.1	24.9	4.3	31.9	24.5	23.7	82.2
39	23.95	1.75	20.7	1.4	16	0.35	8.0	3.0	3.5	32.9	23.9	21.5	74.0
40	24.05	0.1	22	1.3	17	1	8.6	0.0	3.4	35.3	24.4	21.3	69.2
41	24.1	0.05	22.6	0.6	17.15	0.15	8.4	0.0	2.8	36.7	22.4	19.3	63.7

*Mean data of *Kharif,* 2011 and 2012, PDI= Per cent disease intensity, RPD= Relative progress of the disease

Table 3. Simple correlation between relative progress of anthracnose and weather factors in mungbean varieties*

Varieties	BSS	RF	WS	Max. Temp.	Min. Temp.	VP	RH
IR-16	-0.393	0.394	0.166	-0.331	0.493	0.586	0.486
K-851	-0.546	0.472	0.308	-0.505	0.565	0.691**	0.648**
Meha	-0.485	0.307	0.194	-0.392	0.446	0.509	0.426

*Pooled data of *Kharif*, 2011 and 2012, ** Correlation is significant at the 0.05 level

disease. Rathaiah and Sharma (2004) reported that in greengram, the cultivars *viz.* MLTG-2 and TARM-18 were highly resistant to *C. truncatum*. In the present studies, the two genotypes identified as resistant may be exploited in breeding programme aimed at the development of anthracnose resistant varieties of mungbean.

Simple correlation between the relative progress of anthracnose, weather factor and three mungbean varieties revealed positive correlating with rainfall, wind speed, minimum temperature, vapour pressure and relative humidity with anthracnose disease. Whereas, bright sun shine hours and maximum temperature did not have significant correlation with the relative progress of the anthracnose. Present results corroborate the earlier findings of Thakur (1988) who reported that the intensity of the mungbean anthracnose had been found negatively correlated with the maximum temperature. In the present study, K-851 showed maximum positive correlation with vapour pressure and relative humidity. It is clearly indicated that anthracnose development in mungbean plants depends on the prevailing weather condition at a particular time.

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Evaluation of botanicals against *Ralstonia solanacearum* causing bacterial wilt of solanaceous crops

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ABSTARACT

***In vitro* studies were conducted on aqueous and organic extracts of 18 botanicals at varying concentrations against *R. solanacearum* isolates obtained from tomato, brinjal and capsicum through paper disc, plate disc and spectrophotometer methods. White weed (*Ageratum houstonianum*), siam weed (*Chromolaena odorata*) and mint *(Mentha viridis*) at 100% concentration of extract caused maximum inhibition against all the tested isolates of *R. solanacearum* by paper disc method. The spectrophotometer method revealed that margosa (*Azadirachta indica)* extract at all the concentrations was most inhibitory against tomato isolate followed by buttercup (*R. muricatus*) and white weed (*Ageratum houstonianum*). The aqueous extract of margosa with 0.20, 0.24, 0.37 and 0.64 OD value at 100, 50, 25 and 10% concentrations with corresponding cfu/ml values $1.64x10^2$, $1.96x10^2$, $3.03x10^2$ and $5.24x10^2$, was most inhibitory followed by white weed and buttercup against capsicum and brinjal isolates. In case of test chemicals, streptocycline was most inhibitory followed by copper oxychloride. The aqueous extract of buttercup at all the concentrations was most effective to tomato isolate of *R. solanacearum* whereas aqueous extract of margosa and buttercup was found most inhibitory against brinjal and capsicum isolates, respectively. *In vitro* efficacy of margosa at 100% concentration showed maximum inhibition against tomato and capsicum isolates by plate disc method. Maximum inhibition was shown to brinjal isolate (6.67 mm) with the organic extract of mint (*Mentha viridis)* at 100% concentration. The organic extracts of margosa followed by buttercup and white weed were at par and found most effective against tomato isolate by spectrophotometer method. In case of brinjal and capsicum isolates, however buttercup at all the four concentrations was found to be most inhibitory followed by white weed and margosa.**

Key words: Solanaceous crops, *Ralstonia solanacearum*, botanicals, aqueous and organic plant extracts

Bacterial wilt caused by *Ralstonia solanacearum* Yabuuchi (Smith) (syn. *Pseudomonas solanacearum* (Smith) is one of the major constraints in the production of solanaceous vegetable crops in the tropical, subtropical and warm temperate regions of the world. The bacterium has a wide host range that includes over 200 plant species belonging to 50 botanical families (Hayward *et al*., 1991). It limits production of diverse crops such as tomato, brinjal (egg plant), bell pepper (capsicum), chilli (hot pepper) and potato and is responsible for substantial yield losses worldwide (Hayward *et al*., 1991). The disease is well established in the mid hill sub-humid areas of Himachal Pradesh and appears endemically every year (Sood and Singh, 1993; Gupta *et al.,* 1998). The race 1 biovar III of *R. solanacearum* is prevalent in Himachal Pradesh. (Kalha and Sood, 1994) which affects solanaceous crops and the infected field is uncultivable due to its long survival in soil.

The bacterial wilt disease is difficult to manage due to broad host range of *R. solanacearum* with exceptional ability to survive in the soil and roots of non host plants including several weeds. Furthermore all the commercial cultivars including hybrid varieties are highly susceptible; and chemical control of the disease is not feasible. Since the northwestern Himalayan region of India is rich in wild medicinal and aromatic flora, many botanicals can be tried as alternative biodegradable components for the control of bacterial wilt. More than 2, 00,000 species of higher plants are said to possess medicinal properties (Tiwari *et al.,* 1998).

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However, not much information is available on the antibacterial activity of these and other botanicals alongwith commercially available organic formulations against *R. solanacearum.* Once found effective, they can be used as additional components for inclusion in integrated management system of bacterial wilt.

MATERIALS AND METHODS

Collection and preservation of botanicals

The 18 plants species used in the present study *viz.*, goat weed *(Adhatoda vasica*), garlic (*Allium sativum*), holy basil (*Ocimum sanctum)*, siam weed (*Chromolaena odorata)*, mint (*Mentha viridis)*, curry patta (*Murraya koengii)*, marigold (*Tagetes erecta*), wild sage (*Lantana camara)*, hemp (*Cannabis sativus)*, garden spurge (*Euphorbia hirta*), buttercup (*Ranunculus arvensis)*, buttercup (*Ranunculus scleratus)*, buttercup (*Ranunculus muricatus*), sweet flag (*Acorus calamus)*, stink weed (*Datura stramonium* Linn.), white weed (*Ageratum houstonianum)*, aloe (*Aloe vera)* and margosa (*Azadirachta indica)* were collected locally.

The whole plants (roots, stem, leaves and flowers) of buttercup and white weed were collected from the bunds of fields around Palampur during March to July, 2005 and 2006. Leaves of other botanicals *viz.* wild sage, garden spurge, hemp, stink weed, curry patta, goat weed and siam weed were collected from the wastelands near Palampur during February to September, 2005 and 2006. The leaves of the botanicals namely marigold, aloe, mint and garlic were collected from kitchen gardens. The leaves of sweet flag were collected from the sides of water channels during March-May and margosa leaves were collected from village Saloh (Una).

The leaves and whole plant material of all the tested plants were oven dried at 50°C for 5 to 6 hours for two to three days. The leaves of malabar nut, holy basil, siam weed, mint, garden spurge, white weed and hemp were shade dried at room temperature for five to six days. After drying, the respective plant material was ground in a wearing blender to obtain fine dry powder. The powdery biomass of all the botanicals was stored in paper bags at room temperature for further use.

Preparation of plant extract

Fifty gram fine powder of each botanical was soaked overnight in 100 ml of sterilized distilled water (1:2 w/v) and 85% ethanol to get the aqueous and organic plant extract in a conical flask covered with aluminum foil, filtered through a double layered muslin cloth. The filtrate was sterilized with the help of a glass filter (300 ml). The sterilized extract was stored in another sterilized flask (250 or 500 ml) and marked as mother extract. The mother extract with 100 % concentration was further diluted to the requisite concentrations of 50, 25 and 10% by adding sterilized distilled water proportionately and stored in the refrigerator for further use.

In vitro evaluation

The aqueous and organic plant extract were evaluated *in vitro* by paper disc, spectrophotometer and plate count methods. Streptocycline and Copper oxychloride at 100 µg/ml and 0.25% concentration, respectively and their combination were included as check treatments.

Paper disc method

The sterilized circular discs (6 mm dia.) of Whatman No. 1 were dipped in each of the concentration of a plant extract and test chemical for a few seconds. Likewise, the discs dipped in sterilized distilled water served as control. Three discs from each treatment were picked up and placed equidistantly in a Petriplate containing seeded 2,3,5-triphenyl tetrazolium chloride (TZC) medium. The three discs in a single Petriplate comprised three replications of each treatment. The Petriplates were then placed in the lower most shelf of a refrigerator (5°C) for half an hour, thus allowing plant extracts and test chemicals to diffuse into the medium. The plates were then shifted to the incubator at 28 $\pm$ 1°C. The inhibition zone (mm) was measured with the help of a scale after 48 hours of incubation. The mean inhibition zone was worked out for each treatment and compared with the control.

Spectrophotometer method

Plant extracts and organic formulations along with test chemicals streptocycline and copper oxychloride were evaluated *in vitro* for antimicrobial activity against *R. solanacearum* as per method

given by Manav and Thind (2001). The turbidity of bacterial growth in each treatment was measured by recording optical density (OD) with the help of a spectrophotometer (Bousch and Lomb) at 600 nm wavelength. Each time, the spectrophotometer scale was set at 'zero' optical density with blank which consisted of a double strength TZC broth mixed with equal quantity of sterilized water. A standard curve was prepared by recording OD of a range of known bacterial concentration and their corresponding cfu/ml.

Plate count method

Plant extracts, organics and test chemicals alongwith inoculated and uninoculated control were also evaluated by pour plate technique. In each flask containing 100 ml of seeded TZC medium, 5 ml of each concentration of a plant extract was added, separately before pouring. The ingredients were mixed thoroughly by shaking the contents gently. Twenty to twenty five ml each of the mixture was aseptically poured in three sterilized Petriplates. The plates were incubated at 28 $\pm$ 1°C for 48 hours. The inoculated and uninoculated plates without plant extracts served as control. After incubation, the number of *R. solanacearum* colonies formed in each plate was counted with the help of a 'STUART' colony counter. Mean colony count per treatment was then worked out.

RESULTS AND DISCUSSION

The results of *in vitro* efficacy of aqueous plant extracts against tomato, brinjal and capsicum isolates of *R. solanacearum* by paper disc method (Table 1) showed that white weed (*Ageratum houstonianum)* at 100% concentration produced maximum inhibition zone of 7.67 mm against tomato isolate of *R. solanacearum* followed by buttercup (*Ranunculus muricatus)*, margosa (*Azadirachta indica)* and mint (*Mentha viridis)* with inhibition zones of 7.17, 7.00 and 7.00 mm, respectively which were significantly lower than that of white weed. At 50% concentration, margosa showed maximum inhibition zone of 7.00 mm followed by mint and buttercup (*Ranunculus muricatus)*. At 25% concentration, only white weed (5.83 mm), margosa (5.50 mm), buttercup (5.33 mm), mint (5.16 mm) and wild sage (4.67 mm) produced inhibition zones against the tomato isolate; and margosa, buttercup and mint were statistically at par. At 10% concentration however, only mint showed inhibition zone and other botanicals did not produce any inhibition zone. Almost same tend was observed in other isolates with varying degree of behaviour towards the aqueous plant extracts. A fairly large number of plants are known to possess antibacterial properties against *R. solanacearum* (Hanudin, 1987; Hutagalung, 1988). Out of three species of buttercup, *R. muricatus* was found better irrespective of the method of evaluation as compared to other two species *R. scleratus and R. arvensis*.

In vitro evaluation of aqueous extracts by spectrophotometer method (Table 2) revealed that margosa extract at all the four concentrations was most inhibitory against tomato isolate of *R. solanacearum* followed by buttercup (*R. muricatus*) and white weed. The OD values of margosa at 100, 50, 25 and 10% concentration were 0.22, 0.31, 0.45 and 0.75 with corresponding cfu/ml of $1.80x10^2$, $2.54x10^2$, $3.69x10^2$ and $6.15x10^2$, respectively. The results of capsicum isolate were similar to brinjal isolate and aqueous extract of margosa with 0.20, 0.24, 0.37 and 0.64 OD value at 100, 50, 25 and 10% concentration with corresponding cfu/ml values $1.64x10^2$, $1.96x10^2$, $3.03x10^2$ and $5.24x10^2$, respectively was most inhibitory followed by white weed and buttercup (*R. muricatus)*. This finding is in agreement with Satish *et al.* (1999) who also did not find the aqueous extracts of *Lantana camara* and margosa (*A. indica*) effective against any of the pathovars of *Xanthomona campestris*.

The aqueous extract of buttercup (*R. muricatus*) at all concentrations was most inhibitory to tomato and capsicum isolates followed by margosa and white weed by plate count method (Table 2). In case of brinjal isolate, however the aqueous extract of margosa at all the four concentrations was found most inhibitory with 181.33, 347.33, 528.66 and 726.66 cfu/ml colony count, respectively followed by buttercup species *R. muricatus* and *R. arvensis.* The antimicrobial activity of plant extracts has been reported to vary with the test organism and the part of the plant used for preparing the plant extracts (Gourinath and Manoharachary, 1990). The relative inhibitory effect of a plant extract against a particular pathogen depends upon the content and type(s) of phenols, alkaloids, flavonoids, tannins,

Table 1. In vitro efficacy of aqueous and organic plant extracts against tomato, brinjal and capsicum isolates of R. solanacearum by paper disc method

Plant	Concentration (%)/µg ml-1	Inhibition zone (mm)*					
		Tomato isolate		Brinjal isolate		Capsicum isolate	
		Aqueous extracts	Organic extract	Aqueous extracts	Organic extract	Aqueous extracts	Organic extract
Goat weed (*Adhatoda vasica*)	100	6.63	6.50	5.50	5.67	6.37	6.67
	50	5.67	5.33	5.00	4.50	5.50	5.17
	25	0.00	4.67	3.17	0.00	3.83	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
Garlic (*Allium sativum*)	100	5.00	5.17	0.00	2.37	0.00	0.00
	50	3.17	4.50	0.00	0.00	0.00	0.00
	25	0.00	0.00	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.33	0.00	0.00	0.00
Holy basil (*Ocimum sanctum*)	100	0.00	0.00	0.53	5.50	0.00	0.00
	50	0.00	0.00	0.00	4.83	0.00	0.00
	25	0.00	0.00	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
Siam weed (*Chromolaena odorata*)	100	0.00	0.00	8.00	5.50	6.33	5.50
	50	0.00	0.00	5.50	4.83	5.17	4.30
	25	0.00	0.00	3.40	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
Mint (*Mentha viridis*)	100	7.00	5.67	7.50	6.67	7.17	5.00
	50	6.17	5.50	4.50	5.33	5.50	4.00
	25	5.16	0.00	0.00	0.00	0.00	0.00
	10	5.00	0.00	0.00	0.00	0.00	0.00
Curry patta (*Murraya koengii*)	100	4.50	0.00	5.67	5.67	5.50	4.50
	50	0.00	0.00	4.50	3.67	4.50	4.17
	25	0.00	0.00	3.33	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
Marigold (*Tagetes erecta*)	100	4.50	0.00	0.00	0.00	0.00	0.00
	50	0.00	0.00	0.00	0.00	0.00	0.00
	25	0.00	0.00	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
Wild sage (*Lantana camara*)	100	6.33	6.00	6.83	6.27	6.17	0.00
	50	6.00	5.00	4.33	4.33	5.33	0.00
	25	4.67	0.00	1.90	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
Hemp (*Cannabis sativus*)	100	5.17	0.00	0.83	0.00	0.00	0.00
	50	5.00	0.00	0.00	0.00	0.00	0.00
	25	0.00	0.00	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
Garden spurge (*Euphorbia hirta*)	100	0.00	0.00	0.00	1.73	0.00	0.00
	50	0.00	0.00	0.00	0.00	0.00	0.00
	25	0.00	0.00	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00

Buttercup (*Ranunculus arvensis*)	100	6.50	5.33	4.50	5.00	5.50	6.33
	50	6.00	4.67	3.50	4.00	4.50	6.00
	25	5.83	0.00	1.06	0.00	0.00	3.50
	10	0.00	0.00	0.40	0.00	0.00	0.00
Buttercup (*Ranunculus scleratus*)	100	5.50	5.00	5.00	4.50	5.00	5.83
	50	5.00	0.00	3.33	4.17	4.33	4.67
	25	0.00	0.00	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
Buttercup (*Ranunculus muricatus*)	100	7.17	6.50	5.67	5.83	6.00	6.17
	50	6.03	5.50	4.00	3.33	4.17	5.17
	25	5.33	4.33	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
Sweet flag (*Acorus calamus*)	100	5.17	5.17	5.83	4.00	4.50	4.17
	50	0.00	0.00	3.50	3.17	4.17	0.00
	25	0.00	0.00	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
Stink weed (*Datura stramonium*)	100	4.33	0.00	3.50	3.17	4.17	0.00
	50	0.00	0.00	2.50	2.17	0.00	0.00
	25	0.00	0.00	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
White weed (*Ageratum houstonianum*)	100	7.67	7.17	6.83	5.83	6.33	5.67
	50	6.50	5.33	5.33	4.17	5.17	3.50
	25	5.83	0.00	3.50	2.63	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
Aloe (*Aloe vera*)	100	0.00	0.00	0.00	0.00	0.00	0.00
	50	0.00	0.00	0.00	0.00	0.00	0.00
	25	0.00	0.00	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
Margosa (*Azadirachta indica*)	100	7.00	7.50	7.06	6.00	6.50	6.83
	50	7.00	6.83	5.00	4.83	4.33	4.50
	25	5.50	5.17	3.33	3.50	0.00	0.00
	10	0.00	0.00	0.00	0.00	0.00	0.00
Streptocycline**	0.25	7.97	7.97	7.97	7.97	7.97	7.97
	0.125	6.00	6.00	6.00	6.00	6.00	6.00
	0.062	4.67	4.67	4.67	4.67	4.67	4.67
	0.025	2.87	2.87	2.87	2.87	2.87	2.87
Copper oxychloride	100+0.25	4.50	4.50	4.50	4.50	4.50	4.50
	50+ 0.125	3.17	3.17	3.17	3.17	3.17	3.17
	25+ 0.062	2.88	2.88	2.87	2.87	2.87	2.87
	10+ 0.025	1.67	1.67	1.67	1.67	2.40	2.40
Streptocycline** + Copper oxychloride	100+0.25	6.37	6.37	6.37	6.37	6.37	6.37
	50+ 0.125	3.83	3.83	3.83	3.83	3.83	3.83
	25+ 0.062	3.87	3.87	3.87	3.87	3.87	3.87
	10+ 0.025	0.00	0.00	0.00	0.00	0.00	0.00
Control	-	0.00	0.00	0.00	0.00	0.00	0.00
CD (p=0.05)	-	**0.37**	**0.30**	**0.40**	**0.35**	**0.30**	**0.29**

*Average of three replications

Table 2. ***In vitro*** **efficacy of aqueous and organic plant extracts against tomato, brinjal and capsicum isolates of** ***R. solanacearum*** **by spectrophotometer and plate count methods**

Plant	Concentration (%)/µg ml^{-1}	Tomato isolate				Brinjal isolate				Capsicum isolate			
		Aqueous extracts		Organic extracts		Aqueous extracts		Organic extracts		Aqueous extracts		Organic extracts	
		Spectrophotometer method cfu/ml (OD*)	Plate count method cfu/ml*	Spectrophotometer method cfu/ml (OD*)	Plate count method cfu/ml*	Spectrophotometer method cfu/ml (OD*)	Plate count method cfu/ml	Spectrophotometer method cfu/ml (OD*)	Plate count method cfu/ml*	Spectrophotometer method cfu/ml (OD*)	Plate count method cfu/ml*	Spectrophotometer method cfu/ml (OD*)	Plate count method cfu/ml*
Goat weed (*Adhatoda vasica*)	100	5.08x10^2 (0.62)	517.00	4.92 x10^2 (0.60)	433.33	4.92 x10^2 (0.60)	437.33	4.75 x10^2 (0.58)	416.66	5.24 x10^2 (0.64)	515.66	4.67 x10^2 (0.57)	473.33
	50	5.33x10^2 (0.65)	641.66	5.41 x10^2 (0.66)	501.66	5.57 x10^2 (0.68)	453.33	5.33 x10^2 (0.65)	443.33	5.57 x10^2 (0.68)	608.33	5.33 x10^2 (0.65)	535.00
	25	5.41 x10^2 (0.66)	860.33	6.06 x10^2 (0.74)	740.00	6.47 x10^2 (0.79)	716.66	5.82 x10^2 (0.71)	566.66	6.47 x10^2 (0.79)	743.33	5.82 x10^2 (0.71)	630.33
	10	6.31 x10^2 (0.77)	840.00	6.88 x10^2 (0.84)	867.00	7.29 x10^2 (0.89)	866.66	6.72 x10^2 (0.82)	660.33	6.97 x10^2 (0.85)	883.33	6.80 x10^2 (0.83)	790.66
Garlic (*Allium sativum*)	100	5.98 x10^2 (0.73)	410.00	5.98 x10^2 (0.73)	263.33	6.15 x10^2 (0.75)	413.33	4.67 x10^2 (0.57)	350.00	6.47 x10^2 (0.79)	275.00	6.06 x10^2 (0.74)	250.66
	50	6.06 x10^2 (0.74)	719.00	6.31 x10^2 (0.77)	528.33	6.72 x10^2 (0.82)	649.33	5.49 x10^2 (0.67)	435.66	6.56 x10^2 (0.78)	476.66	6.15 x10^2 (0.75)	319.00
	25	6.97 x10^2 (0.85)	829.00	7.05 x10^2 (0.86)	751.66	6.88 x10^2 (0.84)	748.33	6.39 x10^2 (0.78)	631.66	6.88 x10^2 (0.84)	581.66	6.56 x10^2 (0.80)	487.00
	10	7.05 x10^2 (0.86)	1031.00	7.62 x10^2 (0.93)	824.33	7.54 x10^2 (0.92)	818.33	7.21 x10^2 (0.88)	786.66	7.54 x10^2 (0.92)	670.00	7.38 x10^2 (0.90)	529.33
Holy basil (*Ocimum sanctum*)	100	8.28 x10^2 (1.01)	421.66	6.97 x10^2 (0.85)	300.66	7.13 x10^2 (0.87)	408.33	6.88 x10^2 (0.84)	280.00	7.05 x10^2 (0.86)	375.00	6.72 x10^2 (0.82)	290.00
	50	9.18 x10^2 (1.12)	650.00	7.05 x10^2 (0.86)	451.33	7.46 x10^2 (0.91)	488.33	7.46 x10^2 (0.91)	388.33	7.62 x10^2 (0.93)	478.33	7.29 x10^2 (0.89)	397.00
	25	1.16 x10^3 (1.42)	887.00	8.93 x10^2 (1.09)	566.66	8.36 x10^2 (1.02)	710.00	7.95 x10^2 (0.97)	533.33	9.18 x10^2 (1.12)	518.66	7.95 x10^2 (0.97)	435.66
	10	1.12 x10^3 (1.37)	916.66	1.13 x10^3 (1.38)	728.33	1.04 x10^3 (1.27)	785.00	9.10 x10^2 (1.11)	706.00	1.18 x10^3 (1.44)	566.66	9.59 x10^2 (1.17)	520.00
Siam weed (*Chromolaena odorata*)	100	7.05 x10^2 (0.86)	344.33	6.64 x10^2 (0.81)	375.00	6.97 x10^2 (0.85)	436.66	6.39 x10^2 (0.78)	344.00	7.38 x10^2 (0.90)	367.00	6.72 x10^2 (0.82)	293.66
	50	7.54 x10^2 (0.92)	596.66	6.72 x10^2 (0.82)	463.33	7.70 x10^2 (0.94)	500.00	7.05 x10^2 (0.86)	421.33	8.11 x10^2 (0.99)	428.66	6.88 x10^2 (0.84)	379.33
	25	8.85 x10^2 (1.08)	716.00	7.79 x10^2 (0.95)	582.00	8.93 x10^2 (1.09)	640.00	7.54 x10^2 (0.92)	493.66	9.10 x10^2 (1.11)	522.00	7.54 x10^2 (0.92)	440.33
	10	10.4 x10^3 (1.28)	867.66	8.85 x10^2 (1.08)	859.00	9.67 x10^2 (1.18)	848.66	9.02 x10^2 (1.10)	805.00	1.04 x10^3 (1.28)	655.33	9.18 x10^2 (1.12)	455.66
Mint (*Mentha viridis*)	100	5.57 x10^2 (0.68)	478.33	5.57 x10^2 (0.68)	403.66	5.49 x10^2 (0.67)	460.00	5.41 x10^2 (0.66)	440.33	5.49 x10^2 (0.67)	457.00	5.16 x10^2 (0.63)	395.00
	50	6.23 x10^2 (0.76)	578.00	6.31 x10^2 (0.77)	519.33	5.74 x10^2 (0.70)	646.66	6.06 x10^2 (0.74)	508.00	5.98 x10^2 (0.73)	520.66	6.06 x10^2 (0.74)	481.33
	25	6.23 x10^2 (0.76)	852.00	7.46 x10^2 (0.91)	595.33	6.47 x10^2 (0.79)	908.33	6.64 x10^2 (0.81)	659.00	6.72 x10^2 (0.82)	641.33	6.80 x10^2 (0.83)	561.00
	10	7.23 x10^2 (0.88)	897.66	8.44 x10^2 (1.03)	840.00	7.38 x10^2 (0.90)	921.00	7.62 x10^2 (0.93)	773.33	7.29 x10^2 (0.89)	758.33	7.62 x10^2 (0.93)	758.66
Curry patta (*Murraya koengii*)	100	5.57 x10^2 (0.68)	570.00	5.90 x10^2 (0.72)	493.33	5.00 x10^2 (0.61)	560.00	5.16 x10^2 (0.63)	490.00	5.57 x10^2 (0.68)	543.00	6.56 x10^2 (0.68)	508.33
	50	5.74 x10^2 (0.77)	703.00	6.23 x10^2 (0.76)	593.33	6.31 x10^2 (0.77)	730.00	5.82 x10^2 (0.71)	647.00	6.56 x10^2 (0.80)	566.66	5.82 x10^2 (0.71)	550.00
	25	6.88 x10^2 (0.84)	796.66	6.39 x10^2 (0.78)	583.33	7.46 x10^2 (0.91)	856.66	6.97 x10^2 (0.85)	692.33	6.97 x10^2 (0.85)	647.33	6.72 x10^2 (0.82)	573.33
	10	7.54 x10^2 (0.92)	856.00	7.79 x10^2 (0.95)	733.33	8.11 x10^2 (0.99)	867.33	7.46 x10^2 (0.91)	773.66	7.70 x10^2 (0.94)	735.00	7.29 x10^2 (0.89)	683.33
Marigold (*Tagetes erecta*)	100	6.15 x10^2 (0.75)	425.33	6.47 x10^2 (0.79)	310.00	6.31 x10^2 (0.77)	395.66	6.72 x10^2 (0.82)	406.66	6.80 x10^2 (0.83)	405.66	6.80 x10^2 (0.83)	370.00
	50	6.97 x10^2 (0.85)	515.00	7.05 x10^2 (0.86)	391.66	7.13 x10^2 (0.87)	608.33	7.05 x10^2 (0.86)	517.00	7.13 x10^2 (0.87)	486.66	7.05 x10^2 (0.86)	438.33
	25	7.54 x10^2 (0.92)	742.33	7.21 x10^2 (0.88)	660.00	7.95 x10^2 (0.97)	758.66	7.70 x10^2 (0.94)	584.00	7.70 x10^2 (0.94)	517.33	7.54 x10^2 (0.92)	500.66
	10	8.20 x10^2 (1.00)	831.66	8.85 x10^2 (1.08)	718.66	8.93 x10^2 (1.09)	849.66	9.18 x10^2 (1.12)	788.00	9.02 x10^2 (1.10)	685.00	8.36 x10^2 (1.02)	639.00

wild sage (*Lantana camara*)	100	4.42 x10² (0.54)	349.33	4.10 x10² (0.50)	223.33	4.51 x10² (0.55)	333.33	4.26 x10² (0.52)	203.33	4.42 x10² (0.54)	266.66	4.10 x10² (0.50)	210.00
	50	5.33 x10² (0.65)	426.66	4.75 x10² (0.58)	420.00	5.82 x10² (0.71)	415.00	5.00 x10² (0.61)	342.66	6.06 x10² (0.74)	373.33	5.41 x10² (0.66)	366.66
	25	6.15 x10² (0.75)	530.33	6.31 x10² (0.77)	514.00	6.72 x10² (0.82)	578.33	6.72 x10² (0.82)	436.66	6.56 x10² (0.80)	431.00	6.39 x10² (0.78)	426.66
	10	6.88 x10² (0.84)	782.33	7.70 x10² (0.94)	785.66	7.62 x10² (0.93)	808.33	7.79 x10² (0.95)	494.66	7.54 x10² (0.92)	570.00	7.62 x10² (0.93)	501.33
Hemp (*Cannabis sativus*)	100	4.92 x10² (0.60)	590.00	4.59 x10² (0.56)	266.66	4.51 x10² (0.55)	481.66	4.34 x10² (0.53)	491.33	4.92 x10² (0.60)	273.33	4.59 x10² (0.56)	262.00
	50	5.82 x10² (0.71)	745.00	6.23 x10² (0.76)	405.00	5.57 x10² (0.68)	766.66	5.57 x10² (0.68)	700.00	5.90 x10² (0.72)	370.00	5.74 x10² (0.70)	312.00
	25	6.97 x10² (0.85)	910.00	6.88 x10² (0.84)	525.33	7.05 x10² (0.86)	859.33	6.72 x10² (0.82)	839.00	6.56 x10² (0.80)	450.66	6.47 x10² (0.79)	405.00
	10	7.54 x10² (0.92)	914.00	7.70 x10² (0.94)	810.00	7.62 x10² (0.93)	928.33	7.54 x10² (0.92)	885.66	7.79 x10² (0.95)	648.00	7.46 x10² (0.91)	580.66
Garden spurge (*Euphorbia hirta*)	100	6.97 x10² (0.85)	744.33	6.64 x10² (0.81)	526.66	7.21 x10² (0.88)	718.33	6.39 x10² (0.78)	680.00	7.13 x10² (0.87)	531.66	6.56 x10² (0.80)	473.66
	50	8.93 x10² (1.09)	822.33	7.70 x10² (0.94)	693.33	7.70 x10² (0.94)	814.00	7.21 x10² (0.88)	755.00	7.05 x10² (0.86)	584.00	6.80 x10² (0.83)	539.66
	25	9.75 x10² (1.19)	1021.00	8.85 x10² (1.08)	766.66	8.85 x10² (1.08)	940.00	8.77 x10² (1.07)	787.00	8.03 x10² (0.98)	668.00	8.44 x10² (1.03)	628.66
	10	1.27 x10³ (1.55)	1021.00	1.01 x10³ (1.24)	913.33	1.00 x10³ (1.22)	980.33	9.67 x10² (1.18)	978.33	9.67 x10² (1.18)	8781.00	9.43 x10² (1.15)	768.33
Buttercup (*Ranunculus arvensis*)	100	4.34 x10² (0.53)	280.33	4.75 x10² (0.58)	336.66	3.77 x10² (0.46)	245.00	3.93 x10² (0.48)	250.00	3.85 x10² (0.47)	293.33	3.77 x10² (0.46)	238.33
	50	4.83 x10² (0.59)	328.33	6.23 x10² (0.76)	421.66	4.92 x10² (0.60)	381.66	5.33 x10² (0.65)	311.66	5.00 x10² (0.61)	341.66	5.65 x10² (0.69)	308.00
	25	5.74 x10² (0.77)	412.00	6.97 x10² (0.85)	476.66	6.31 x10² (0.77)	398.33	6.88 x10² (0.84)	440.33	6.23 x10² (0.76)	463.00	6.72 x10² (0.82)	390.00
	10	7.13 x10² (0.87)	700.00	8.20 x10² (1.00)	560.00	7.38 x10² (0.90)	556.66	7.79 x10² (0.95)	550.00	6.72 x10² (0.82-)	695.33	7.54 x10² (0.92)	450.66
Buttercup (*Ranunculus scleratus*)	100	4.51 x10² (0.55)	298.00	4.83 x10² (0.59)	238.33	3.44 x10² (0.42)	265.33	4.42 x10² (0.54)	246.66	3.52 x10² (0.43)	277.33	4.51 x10² (0.55)	208.33
	50	4.67 x10² (0.57)	485.00	5.49 x10² (0.67)	456.66	4.26 x10² (0.52)	482.00	5.49 x10² (0.67)	380.00	4.59 x10² (0.56)	367.33	5.41 x10² (0.66)	367.33
	25	5.98 x10² (0.73)	588.33	6.72 x10² (0.82)	528.33	5.00 x10² (0.61)	513.33	6.39 x10² (0.78)	418.33	5.00 x10² (0.61)	443.33	6.06 x10² (0.74)	464.66
	10	7.05 x10² (0.86)	835.66	7.21 x10² (0.89)	743.33	6.88 x10² (0.84)	765.33	7.62 x10² (0.93)	575.00	6.64 x10² (0.81)	645.33	7.95 x10² (0.97)	538.33
Buttercup (*Ranunculus muricatus*)	100	1.88 x10² (0.23)	170.66	2.78 x10² (0.34)	135.33	3.11 x10² (0.38)	189.66	2.05 x10² (0.25)	181.33	3.44 x10² (0.42)	158.66	1.64 x10² (0.20)	136.66
	50	2.87 x10² (0.35)	248.33	3.11 x10² (0.38)	250.00	3.52 x10² (0.43)	240.33	2.46 x10² (0.30)	244.00	3.93 x10² (0.48)	211.33	2.70 x10² (0.33)	253.66
	25	4.26 x10² (0.52)	512.33	4.10 x10² (0.50)	341.66	5.16 x10² (0.63)	466.66	4.10 x10² (0.50)	396.66	5.74 x10² (0.70)	428.33	4.10 x10² (0.50)	427.00
	10	5.41 x10² (0.66)	641.00	6.15 x10² (0.75)	531.66	6.23 x10² (0.76)	640.66	4.67 x10² (0.57)	553.33	6.56 x10² (0.80)	523.00	5.90 x10² (0.72)	516.66
Sweet flag (*Acorus calamus*)	100	6.15 x10² (0.75)	516.33	5.33 x10² (0.65)	476.66	4.83 x10² (0.59)	412.33	5.74 x10² (0.70)	390.00	6.56 x10² (0.80)	433.33	5.33 x10² (0.65)	351.66
	50	7.38 x10² (0.90)	556.66	6.06 x10² (0.74)	480.00	6.06 x10² (0.74)	511.66	6.23 x10² (0.76)	428.66	6.88 x10² (0.84)	528.33	5.82 x10² (0.71)	427.00
	25	7.70 x10² (0.94)	605.00	7.05 x10² (0.86)	766.66	7.21 x10² (0.88)	648.33	6.80 x10² (0.83)	471.66	7.62 x10² (0.93)	673.00	7.21 x10² (0.88)	538.66
	10	9.43 x10² (1.15)	845.66	8.44 x10² (1.03)	955.00	7.87 x10² (0.96)	861.66	7.70 x10² (0.94)	590.66	7.87 x10² (0.96)	759.66	8.77 x10² (1.07)	674.66
Stink weed (*Datura stramonium*)	100	5.74 x10² (0.70)	534.06	5.16 x10² (0.63)	382.00	5.24 x10² (0.64)	503.33	4.34 x10² (0.53)	478.33	5.24 x10² (0.64)	405.66	4.51 x10² (0.55)	464.00
	50	5.98 x10² (0.73)	630.66	5.82 x10² (0.71)	435.00	6.15 x10² (0.75)	652.33	5.33 x10² (0.65)	531.66	6.31 x10² (0.77)	461.66	5.33 x10² (0.71)	462.33
	25	6.64 x10² (0.81)	727.33	6.97 x10² (0.85)	498.33	7.05 x10² (0.86)	680.00	6.39 x10² (0.78)	591.66	7.13 x10² (0.87)	522.33	6.80 x10² (0.83)	490.66
	10	7.70 x10² (0.94)	855.33	7.54 x10² (0.92)	676.00	8.61 x10² (1.05)	875.00	8.44 x10² (1.03)	763.66	9.34 x10² (1.14)	665.00	7.38 x10² (0.90)	684.33
White weed (*Ageratum houstonianum*)	100	2.87 x10² (0.35)	327.33	2.95 x10² (0.36)	324.00	2.37 x10² (0.29)	318.33	2.05 x10² (0.25)	305.66	2.62 x10² (0.32)	338.33	2.13 x10² (0.26)	289.00
	50	2.95 x10² (0.36)	465.00	3.52 x10² (0.43)	308.66	3.11 x10² (0.38)	441.66	3.11 x10² (0.38)	365.00	3.36 x10² (0.41)	391.66	3.60 x10² (0.44)	641.66
	25	4.26 x10² (0.52)	512.33	5.24 x10² (0.64)	417.33	4.26 x10² (0.52)	498.33	4.67 x10² (0.57)	441.66	5.00 x10² (0.61)	427.33	5.16 x10² (0.63)	465.00
	10	5.33 x10² (0.65)	709.33	6.47 x10² (0.79)	706.66	5.74 x10² (0.70)	695.00	6.80 x10² (0.83)	548.33	5.33 x10² (0.65)	500.00	6.06 x10² (0.74)	539.00

Aloe (*Aloe vera*)	100	6.80 x10^2 (0.83)	565.00	5.90 x10^2 (0.72)	511.66	5.65 x10^2 (0.69)	603.33	5.24 x10^2 (0.64)	514.00	6.47 x10^2 (0.79)	564.00	5.49 x10^2 (0.67)	494.00
	50	7.70 x10^2 (0.94)	626.66	7.05 x10^2 (0.86)	578.33	6.64 x10^2 (0.81)	653.00	6.56 x10^2 (0.80)	566.33	6.97 x10^2 (0.85)	595.00	5.98 x10^2 (0.73)	563.33
	25	8.11 x10^2 (0.99)	836.66	7.29 x10^2 (0.89)	703.33	7.87 x10^2 (0.96)	738.66	7.21 x10^2 (0.88)	634.66	7.46 x10^2 (0.91)	629.33	7.05 x10^2 (0.86)	626.66
	10	9.18 x10^2 (1.12)	866.66	9.02 x10^2 (1.10)	893.33	8.44 x10^2 (1.03)	921.66	8.20 x10^2 (1.00)	720.00	8.61 x10^2 (1.05)	678.66	8.28 x10^2 (1.01)	683.33
Margosa (*Azadirachta indica*)	100	1.80 x10^2 (0.22)	240.66	2.37 x10^2 (0.29)	245.00	2.05 x10^2 (0.25)	181.33	3.03 x10^2 (0.37)	175.00	1.64 x10^2 (0.20)	341.66	2.29 x10^2 (0.28)	340.00
	50	2.54 x10^2 (0.31)	402.33	2.95 x10^2 (0.36)	296.66	2.46 x10^2 (0.30)	347.33	3.52 x10^2 (0.43)	271.66	1.96 x10^2 (0.24)	354.00	2.78 x10^2 (0.34)	349.33
	25	3.69 x10^2 (0.45)	506.66	4.34 x10^2 (0.53)	433.33	3.03 x10^2 (0.37)	528.66	4.59 x10^2 (0.56)	403.33	3.03 x10^2 (0.37)	433.33	4.18 x10^2 (0.51)	460.00
	10	6.15 x10^2 (0.75)	685.00	5.08 x10^2 (0.62)	566.66	4.26 x10^2 (0.52)	726.66	5.24 x10^2 (0.64)	524.00	5.24 x10^2 (0.64)	484.00	5.00 x10^2 (0.61)	581.66
Streptocycline**	0.25	2.78 x10^2 (0.34)	218.33	2.78 x10^2 (0.34)	218.00	2.78 x10^2 (0.34)	218.33	2.78 x10^2 (0.34)	218.33	2.78 x10^2 (0.34)	218.33	2.78 x10^2 (0.34)	218.33
	0.125	3.85 x10^2 (0.47)	330.00	3.85 x10^2 (0.47)	330.00	3.85 x10^2 (0.47)	330.00	3.85 x10^2 (0.47)	330.00	3.85 x10^2 (0.47)	330.00	3.85 x10^2 (0.47)	330.00
	0.062	5.90 x10^2 (0.72)	723.33	5.90 x10^2 (0.72)	723.33	5.90 x10^2 (0.72)	723.33	5.90 x10^2 (0.72)	723.33	5.90 x10^2 (0.72)	723.33	4.10 x10^2 (0.72)	723.33
	0.025	6.97 x10^2 (0.85)	783.33	6.97 x10^2 (0.85)	783.33	6.97 x10^2 (0.85)	783.33	6.97 x10^2 (0.85)	783.33	6.97 x10^2 (0.85)	783.33	6.97 x10^2 (0.85)	783.33
Copper oxychloride	100+ 0.25	7.13 x10^2 (0.87)	361.66	7.13 x10^2 (0.87)	361.66	7.13 x10^2 (0.87)	361.66	7.13 x10^2 (0.85)	361.66	7.13 x10^2 (.87)	361.66	7.13 x10^2 (0.87)	361.66
	50+ 0.125	7.29 x10^2 (0.89)	421.66	7.29 x10^2 (0.89)	421.66	7.29 x10^2 (0.89)	421.66	7.29 x10^2 (0.89)	421.66	7.29 x10^2 (0.89)	421.66	7.29 x10^2 (0.89)	421.66
	25+ 0.062	8.36 x10^2 (1.02)	616.66	8.36 x10^2 (1.02)	616.66	8.36 x10^2 (1.02)	616.66	8.36 x10^2 (1.02)	616.66	8.36 x10^2 (1.02)	616.66	8.36 x10^2 (1.02)	616.66
	10+ 0.025	8.52 x10^2 (1.04)	415.00	8.52 x10^2 (1.04)	915.00	8.52 x10^2 (1.04)	915.00	8.52 x10^2 (1.04)	915.00	8.52 x10^2 (1.04)	915.00	8.52 x10^2 (1.04)	915.00
Streptocycline** + Copper oxychloride	100+ 0.25	2.54 x10^2 (0.31)	363.33	2.54 x10^2 (0.31)	363.33	2.54 x10^2 (0.31)	363.33	2.54 x10^2 (1.31)	363.33	2.54 x10^2 (0.31)	363.33	2.54 x10^2 (0.31)	363.33
	50+ 0.125	3.11 x10^2 (0.38)	418.33	3.11 x10^2 (0.38)	418.33	3.11 x10^2 (0.38)	418.33	3.11 x10^2 (0.38)	418.33	3.11 x10^2 (0.38)	418.33	3.11 x10^2 (0.38)	418.00
	25+ 0.062	4.34 x10^2 (0.53)	517.00	4.34 x10^2 (0.53)	517.00	4.34 x10^2 (0.53)	517.00	4.34 x10^2 (0.53)	517.00	4.34 x10^2 (0.53)	517.00	4.34 x10^2 (0.53)	517.00
	10+ 0.025	6.64 x10^2 (0.81)	678.33	6.64 x10^2 (0.81)	678.33	6.64 x10^2 (0.81)	678.33	6.64 x10^2 (0.81)	678.33	6.64 x10^2 (0.81)	678.33	6.64 x10^2 (0.81)	678.33
Control 1 (Inoculated)	-	1.51 x10^3 (1.85)	983.33	1.51 x10^3 (1.85)	983.33	1.51 x10^3 (1.85)	983.33	1.51 x10^3 (1.85)	983.33	1.51 x10^3 (1.85)	983.33	1.51 x10^3 1.85)	983.33
Control 2 (Uninoculated)	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD (p=0.05)		0.38	34.20	0.54	32.99	0.43	19.56	0.40	26.95	0.52	25.54	0.46	22.61

*Average of three replications

** Concentration in µg ml^{-1}

Figures in parentheses are OD values to the corresponding cfu/ml

castic acid present in that particular plant material.

In case of test chemicals, streptocycline was most inhibitory followed by copper oxychloride and their combination. The results of *in vitro* efficacy of organic plant extracts against tomato, brinjal and capsicum isolates of *R. solanacearum* are presented in Table 1 and 2. Margosa (*Azadirachta indica)* at 100% concentration showed maximum inhibition against tomato and capsicum isolates followed by white weed, buttercup (*Ranunculus muricatus)* and goat weed. However, the margosa was statistically superior to rest of the three. At 50% concentration also, the organic extract of margosa was found maximum inhibitory followed by buttercup (*R. muricatus)* and white weed.

The organic extract of margosa was significantly more inhibitory than that of goat weed and buttercup against capsicum isolate. At 50% concentration, buttercup (*R. arvensis*) formed the maximum inhibition zone of 6.00 mm followed by goat weed (5.17 mm) and buttercup (*R. muricatus*) with 5.17 mm inhibition zone. At 25% concentration, only the organic extract of buttercup species *R. arvensis* formed the inhibition zone (3.50 mm) against the test isolate. The extracts of marigold, hemp and aloe did not form any inhibition zone against all the isolates of *R. solanacearum* at the three concentrations. The antibacterial activity of ethanol extract (1:1) of the leaves and seeds of *Datuira metel* and *Datura stramonium* against *Xanthomonas campestris* pv. *malvacearum* was reported earlier (Bambawale *et al*., 1995). Bacterial leaf blight of rice (*X. oryzae* pv. *oryzae*) has also been reported to be controlled by spraying of aqueous extract of goat weed (*Adhatoda vasica*) leaves (Madhiazhagen *et al*. 2002).

Similar to aqueous extracts, the Organic extract of margosa followed by buttercup (*R. muricatus)* and white weed were at par and found most inhibitory against tomato isolate by spectrophotometer method. The OD values at 100, 50, 25 and 10% concentration of organic extract of margosa were 0.29, 0.36, 0.53 and 0.62 with corresponding cfu/ml values of $2.37x10^2$, $2.95x10^2$, $4.34x10^2$ and $5.08x10^2$, respectively (Table 2). In case of brinjal and capsicum isolates, however buttercup (*R. muricatus)* at all the four concentrations was found to be most inhibitory followed by white weed and margosa. In case of test chemicals again, streptocycline (100µg/ml) + copper oxychloride (0.25%) with lower OD value in all the three isolates was most inhibitory followed by streptocycline (100µg/ml) + copper oxychloride (0.25%) alone.

The organic extract of buttercup (*R. muricatus*) at all the four concentrations was found to be most inhibitory against tomato isolate by plate count method. In case of brinjal isolate, the organic extract of margosa however, was found most inhibitory followed by buttercup (*R. muricatus*) and wild sage with correspondingly increased number of colony count. In case of capsicum isolate, the organic extract of buttercup (*R. muricatus*) with lesser number of cfu/ml at the four concentrations was most inhibitory followed by buttercup (*R. scleratus*) and wild sage with higher cfu/ml.

The *in vitro* efficacy of mainly margosa, buttercup and white weed and to some extent of wild sage, malabar nut, sweet flag against *R. solanacearum* found in the present study is suggestive of their usefulness as cover crops in rotation cropping system or as partner crops in mixed cropping system for the management of bacterial wilt since it is well established that crop rotation is the most effective method for the management of soil-borne diseases like bacterial wilt. The antimicrobial effects of the compounds present in the plant extracts, margosa based formulations may also be attributed to the fact that the substances released from the plants suppress directly the growth of pathogens and development of diseases and/or indirectly affect the soil microorganisms such as antagonistic bacteria and plant growth promoting rhizobacteria (PGPR), thereby affecting the severity of the diseases (Masaoka *et al*. 1993).

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Evaluation of elite breeding lines and some land races for resistance to emerging pathotypes of *Xanthomonas oryzae* pv. *oryzae*

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ABSTRACT

One hundred sixty three entries from National Screening Nursery-1 (NSN-1), 540 entries from National Screening Nursery-2 (NSN-2) and 912 entries from rice germplasm (land races) were evaluated against the prevalent pathotypes *viz.* PbXo-1 and PbXo-7 and emerging pathotypes PbXo-9 and PbXo-10 of the bacterial blight pathogen in Punjab, India under artificial inoculation conditions. Two years data revealed that thirteen entries from NSN-1, 40 entries from NSN-2 and three land races *viz.* Acc. no. 441, 784 and 867 were found resistant to all the four pathotypes. The evaluation data of all the tested entries (1615) suggested that 163 (10.1%) and 151 (9.3%) of the entries were resistant to established pathotypes PbXo-1 and PbXo-7, respectively as compared to 60 (3.9%) entries were resistant to emerging pathotype PbXo-9. Most of the entries which were resistant to PbXo-9 also showed resistance to all the other three pathotypes. Similarly, only 4.5% (73) entries showed resistant/ moderately resistant reaction to PbXo-10 which was found to be virulent on newly identified gene *Xa38*. These resistant entries can either be released as new varieties or further utilized as donors in multiple disease resistance breeding programmes.

Key word: Bacterial blight, pathotype, rice, resistance, *Xanthomonas oryzae* pv. *oryzae*

Bacterial blight (BB) of rice (*Oryzae sativa* L.) caused by *Xanthomonas oryzae* pv. *oryzae* [(Ishiyama), Swings *et al.*] (*Xoo*) is widely distributed and endemic to most parts of Asia (Nino-Liu *et al.,* 2006). Losses due to its outbreak can reach up to 50% depending upon stage of the crop, weather conditions, location and the cultivar used (Mew *et al.,* 1993). The disease appears every year in varying degrees throughout the Punjab state in India causing yield losses from 60 to 70% during epiphytotic years (Raina *et al.,* 1981). Chemical control of BB in the tropical monsoon climate of Asia is impractical and no truly effective bactericide is commercially available for its control. Development and deployment of host plant resistance is the only effective means of BB management. Currently, more than 38 *Xa/xa* gene (s) in rice conferring resistance against *Xoo* have been designated globally (Nino-Liu *et al.,* 2006; Wang *et al.*, 2009; Bhasin *et al.,* 2012). Since the effectiveness of these genes varies in time and space and relatively few are currently being used in rice improvement. The genetic base of resistance to BB in the working germplasm at the national level is quite narrow and transfer of BB resistance gene (s) from many of wild *Oryza* species especially, those without AA genome, poses many practical problems. A previous report on genetic diversity showed that more alleles were detected in land races than in cultivars. Although, rice resistance breeding has gained large achievements in managing bacterial blight disease, but due to high variability with respect to virulence of the pathogen, breeding of resistant cultivars always confronts difficulties in terms of durability of resistance. The pathogen is highly variable and more than 30 races/ pathotypes of the bacterium have been reported worldwide (Adhikari *et al.,* 1999; Noda *et al.*, 2001). The *Xoo* population from the state has been already classified into seven distinct pathotypes by inducing differential reactions on a set of near-isogenic lines, international, national differentials and regional cultivars (Lore *et al.,* 2011). Three new pathotypes of *Xoo* have been identified during

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2009-10 which resulted in to breakdown of the resistance of varieties cultivated in Punjab state. So keeping in view the above mentioned facts, elite breeding lines and land races were evaluated against dominant and emerging pathotypes of *Xoo* under artificial inoculation conditions, so that resistance sources can be used in disease resistance breeding programme.

MATERIALS AND METHODS

Plant materials

A total of 163 entries from National Screening Nursery-1 (NSN-1) comprising advance varietal trials (AVT), 540 entries from National Screening Nursery-2 (NSN-2) comprising of initial varietal trials (IVT) and 912 entries from rice germplasm (land races) along with resistant check IET 8585 (Ajay) and susceptible check TN1 were received from Directorate of Rice Research, Hyderabad and transplanted in paired rows with 20 x 20 cm spacing. Agronomic practices and fertilizer application was as per PAU recommendations (Anonymous, 2010). These entries were evaluated against the prevalent pathotypes *viz.* PbXo-1 and PbXo-7 and emerging pathotypes PbXo-9 (Tar 949) and PbXo-10 (Tar-950) of the BB pathogen, under artificial inoculation conditions during year 2011-12 and only selected entries were grown during year 2012-13 for further evaluations.

Artificial inoculation

For bacterial blight inoculation, four virulent pathotypes *viz*. PbXo-1, PbXo-7, PbXo-9 (Tar949) and PbXo-10 (Tar-950) of *Xoo* were isolated on Waki Moto medium. Seventy two hours old single colony culture of the pathotypes was used for artificial inoculations. Before the inoculation, each pathotype was tested on a set of near isogenic lines/ a set of differentials for their virulence/avirulence to *Xa/xa* genes. All the test genotypes were inoculated at maximum tillering stage by clip-inoculation technique (Kauffman *et al*., 1973) with bacterial suspension of approximately 10^9 cells/ ml. Ten plants of each test entry were inoculated with four pathotypes separately.

Disease assessment

Reaction of plants was recorded 14 days after inoculation according to Standard Evaluation System (0-9 scale) for rice (IRRI, 1996). Plants were characterized as resistant or susceptible based on scale 0-3 (resistant); 5 (moderately resistant); 7-9 (susceptible).

RESULTS AND DISCUSSION

Testing of *Xoo* pathotypes

Virulence of the pathotypes was tested on a set of near isogenic lines/ differentials before inoculations to the elite breeding lines/germplasm. Virulence/avirulence of the four pathotypes *viz*. PbXo-1, PbXo-7, PbXo-9 and PbXo-10 to *Xa/xa* genes indicated that all the pathotypes were virulent on bacterial blight resistant genes *Xa1, Xa3, Xa4, xa5, Xa7, Xa10* and *Xa11*. PbXo-1 was avirulent to *xa8* and rice cultivars PR114, PR116 and PR118, however, PbXo-7, the most dominant pathotype virulent to *xa8* and had already broken down the resistance of these cultivars grown in the state. PbXo-9 is virulent to most of the single *Xa/xa* gene (s) including *Xa21* and also broke down the resistance in cultivars PR120, PR113 and PR111 in the state. Another emerging pathotype, PbXo-10 has been found to be virulent on *Xa38*, a new BB resistant gene identified from *Oryza nivara* (Cheema *et al.,* 2008).

National Screening Nursery-1 (AVT)

Among 163 advanced lines (NSN-1) tested, 13 lines viz. IET 22108 (MGD-107), IET 22108 (PAU3879-87-1-1), IET22123 (R1528-1058-1-110-1), IET22144 (ORS-327), IET 22841 (RP 5130-12-3-5-21-3), IET 22842 (RP5130-136-5-5-33-5), IET21513 (PAU 3105-45-3-2), IET 22226 (PAU3761-26-3-1), IET 22231 (PAU3371-26-1-3), IET 22289 (Pusa 1592-06-5-2), IET 22069 (TM 05091), IET 22075 (JGL 17183) and IET 22086 (JGL 17196) showed resistant (R) reaction to all the four *Xoo* pathotypes (Table1), whereas 46 and 42 entries showed resistant reaction to pathotype PbXo-1 and PbXo-7, respectively (Fig. 1). Seventeen entries showed resistant and 15 entries showed moderately resistant (MR) reaction to pathotype PbXo-10, however, minimum number of entries (13) showed resistant reaction to highly virulent pathotype PbXo-9 as well as resistant to all the pathotypes (Table1). Some entries IET22222, IET22225 and 22074 were

Table 1. Breeding lines and rice germplasm showing resistance to four pathotypes of *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight in rice during the year 2011-12 and 2012-13

Trial	Total entries tested	Number of entries resistant to four pathotypes	IET/Accession no.
National Screening Nursery-1 (Advanced Varietal Trial)	163	13	IET 22108, IET 22108, IET 22123, IET 22144, IET 22841, IET 22842, IET 21513, IET 22226, IET 22231, IET 22289, IET 22069, IET 22075 and IET 22086
National Screening Nursery-2 (Initial Varietal Trial)	540	40	IET 22490, IET 22506, IET 22507, IET 22508, IET 22533, IET 22541, IET 22542, IET 22550, IET 22551, IET 22584, IET 22592, IET 22600, IET 22601, IET 21940, IET 21726, IET 21936, IET 22627, IET 22628, IET 22629, IET 22630, IET 22631, IET 22632, IET 22633, IET 22634, IET 22635, IET 22636, IET 22657, IET 22702, IET 22715, IET 22716, IET 22791, IET 22793, IET 22790, IET 22752, IET 22753, IET 22754, IET 22755, IET 22764, IET 22766 and IET 22767
Rice germplam/land races	912	3	Acc. 441, Acc. 784 and Acc. 867

found resistant to the emerging pathotype PbXo-10, but susceptible (S) to PbXo-9 (Table2).

National Screening Nursery-2 (IVT)

Out of 540 breeding lines tested, 108 and 105 lines showed resistance to PbXo-1 and PbXo-7 respectively. It is pertinent to mention here that although PbXo-7 has been considred as most dominant pathotype in Punjab (Lore *et al.,* 2011), the newly emerged pathotype PbXo-9 was found to be more virulent as only 43 entries were found to be resistant to it as compared to 105 entries resistant to PbXo-7 (Fig. 1). Fifty two entries were found resistant and 23 were moderately resistant to another emerging pathotype PbXo-10. Among the emerging pathotypes, PbXo-10 was avirulent on IET 22307, IET 22545, IET 22547, IET 22576 IET 22583, IET22691, IET 22792, IET 22765 and IET 22770, however, PbXo-9 was virulent. In contrast to PbXo-10 was virulent on entries IET 21895, IET 21723, IET 22560 and IET 22566 and PbXo-9 was avirulent (Table 2).

Rice germplam (land races)

Out of 912 rice germplasm lines/land races tested, only three accessions *viz*. Acc nos. 441, 784 and 867 were found resistant to all the pathotypes. Diverse reaction was observed to the emerging pathotypes as Acc no. 312 showed susceptible reactions to both the emerging pathotypes PbXo-9 and PbXo-10, but R/MR reactions to PbXo-1 and PbXo-7. Acc nos. 812, 122 and 214 exhibited R/MR to three pathotypes (PbXo-1, PbXo-7 & PbXo-9), but susceptible to PbXo-10. On the other hand, Acc no. 819 was found resistant to PbXo-10, but susceptible to PbXo-9 (Table 2).

It is concluded that from a total of 1615 entries evaluated against four pathotypes (PbXo-1, PbXo-7, PbXo-9 & PbXo-10) of *Xoo* in this study, 163 (10.1%) and 151 entries (9.3%) showed R/MR reaction to the PbXo-1 and PbXo-7, respectively. However, lowest number of resistant entries (60 and 73) was observed in the case of newly emerging pathotypes, PbXo-9 and PbXo-10, respectively (Fig. 1).

Bacterial blight is a major disease of rice in Punjab and number of bacterial blight resistant cultivars has been released in the state (Anonymous, 2010). The pathogen is highly variable and breakdowns the resistance of the released cultivars in state. Seven pathotypes of the *Xoo* have been reported in Punjab (Lore *et al.,* 2011). More than 30 races/pathotypes of the bacterium have been reported worldwide (Adhikari *et al.,* 1999; Noda *et al.*, 2001). Pathotype PbXo-7 was the most dominant in Punjab state and virulent to known *Xa* genes namely *Xa* 1, *Xa* 3, *Xa* 4, *xa* 5, *Xa* 7, *xa* 8, *Xa* 10 and *Xa* 11 and mega varieties PR 114, PR 116 and PR 118 having genetic resistance to four pathotypes namely PbXo-1, PbXo-2, PbXo-3 and PbXo-4 (Lore *et al.,* 2011). The entries were selected based on the resistance to pathotype PbXo-7 and further evaluated for emerging pathotypes PbXo-9 and PbX0-10. The

Table 2. Some breeding lines and rice germplasm showing diverse reaction to the emerging pathotypes of *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight in rice during the year 2011-12 and 2012-13

Entry IET No.	Designation	Bacterial blight (Pathotypes) (Score 0-9)			
		PbXo-1	PbXo-7	PbXo-9	PbXo-10
22107	IR78091-6-2-3-1-1	1	3	7	3
22222	CB 05-031	3	3	7	3
22225	NPG-209	3	3	7	3
21579	R2085-RF-69	3	3	7	3
22074	OM 5240	3	3	7	3
21895	TTB 303-1-26	3	5	3	7
22307	CR-2285-3-2-1-2-1	3	3	7	3
22545	R1530-1546-1-418-1	3	3	7	3
22547	RP 5129-17-8-3-2	3	3	7	3
22560	CN 1740-5-3-3-2-MLD 9	3	3	3	7
22566	UPR 3528-12-1-1	3	3	3	7
22576	AAIR 203	3	3	7	3
22583	SKL-3-22-19-31-55-11	3	3	7	3
21723	GNV 05-01-1	3	3	3	7
22691	OR 2131-1	3	3	7	3
22792	JGL18038	3	3	7	3
22765	NLR 40058	3	3	7	3
22770	CR2995-1-2-3-1-1	3	3	7	3
Acc. 122	-	3	5	5	7
Acc. 214	-	3	5	5	7
Acc. 812	-	3	3	3	7
Acc. 819	-	5	5	7	3
IET 8585	IET4141/CR 98-7216 (*xa5*+*xa13*) (Resistant check)	3	3	3	3
TN-1	*Xa14* (Susceptible check)	9	9	9	9

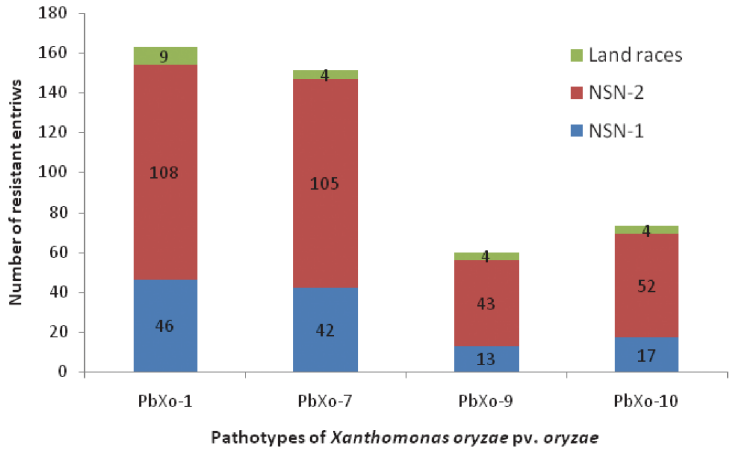

Fig. 1. Number of resistant entries from different trials against four pathotypes of *Xanthomonas oryzae* pv. *oryzae*

pathotype PbXo-10 was virulent and PbXo-9 was avirulent on newly identified gene *Xa* 38 (Cheema *et al.,* 2008), however, both the pathotypes were virulent on cultivar PR 120 which was resistant to all the earlier identified seven pathotypes (Lore *et al.,* 2011). Pathotype PbXo-9 was virulent on BB resistant cultivars PR111, PR113, PR115 and PR120 along with the entire known *Xa/xa* gene (s) except *xa13* and *Xa38*. This pathotype was also found to be virulent on pyramided genes *Xa4+xa5*. In the present study, only 3.7% entries showed R/MR to PbXo-9 as compared to 9.3% gave R/MR to the PbXo-7. The entries showing resistance to the PbXo-9 also showed resistance to the other three pathotypes, however, some exception entries i.e. IET 21895, IET 22560, IET22566 and land race Acc no. 812 showed resistance to PbXo-9 but susceptibility to PbXo-10. The rice breeding lines in NSN-1, NSN-2, alongwith land races possess diverse sources of resistance against different pathotypes of *Xoo* in Punjab. Sixty seven accessions of wild *Oryza* species were identified to be resistant or moderately resistant against the seven pathotypes of *X. oryzae* pv. *oryzae* (Vikal *et al.*, 2007). Similarly, Hunjan *et al.,* (2010) evaluated advanced stage breeding lines of rice comprising of 127 entries (non basmati) and 43 entries (basmati) for multiple disease resistance. Of these, 41 entries (non basmati) and 5 (Basmati) were found to possess genetic resistance to all the seven pathotypes of bacterial blight pathogen, but these resistance entries might be susceptible to the emerging pathotypes PbXo-9 and PbXo-10.

The sources of resistance identified in this study from advanced breeding lines and land races of rice can be used as such or exploited in disease resistance breeding programmes for the development of durable bacterial blight resistant cultivars in Punjab state of India.

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Present status of bacterial top rot disease of sugarcane in Indian Punjab

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ABSTRACT

Top rot/ red stripe disease is caused by *Acidovorax avenae* subsp. *avenae* poses a constant threat to the successful cultivation of sugarcane in Punjab. It is quite severe on variety CoJ 85 during the past 4-5 years in the state. During the survey based on the average of three months, the maximum prevalence of top rot was 56.0% and 54.3% in Amritsar district on variety CoJ 85 in 2011 and 2012, respectively. Maximum disease incidence of 4.2% and severity 65.8% was observed on variety CoJ 85 in the month of August 2011 and 4.4% and 72.0% in 2012, respectively. However, least disease incidence and severity was recorded on variety CoJ 88. It was concluded that an early variety CoJ 85 was more susceptible to the top rot disease than the other varieties.

Key words: Sugarcane, top rot, bacteria, Punjab, *Acidovorax avenae* subsp. *avenae*

Sugarcane (*Saccharum officinarum* L.) is one of the main sugar producing crops that contributes nearly 75% of the total sugar pool at the global level. It is the prime source of sugar in India and holds the prominent position as the commercial cash crop. In Punjab, it occupies an area of about 81 thousand hectare with an average cane productivity of 750 quintals/ ha (Anonymous, 2012).

Bacterial top rot incited by *Acidovorax avenae* subsp. *avenae* once considered to be the minor disease is gaining importance these days. The disease occurs in two forms i.e., leaf stripes and top rot, which may occur singly or together under field condition during relatively high atmospheric humidity. Highest losses from the top-rot phase up to 15 per cent have been reported by Egan and Hughes (1958) and Martin and Wismer (1961).

Chaudhary *et al.* (1999) reported up to 38 per cent and 40 per cent reduction in cane yield and recoverable sugar, respectively by top rot. They further observed that the disease incidence was higher in the month of June and July when temperature and humidity were high. In north India, Rana and Shukla (1968) reported the appearance of disease only from July to August.

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Since then disease has not received any attention in our country and no work on disease incidence and severity on different varieties is available in India. Keeping this in view, a survey was conducted for two years in major sugarcane growing districts of Punjab.

MATERIALS AND METHODS

Surveys were conducted for recording the prevalence and disease severity of top rot in Sugarcane crop in the district of Amritsar, Jalandhar, Ludhiana and Gurdaspur of Punjab state in the month of June, July and August during 2011 and 2012. From each selected field, incidence of top rot was recorded amongst 300 randomly selected plants. In total, 221 fields during 2011 and 240 fields during 2012 were surveyed from four districts representing different agro- climatic regions of the state. The plants exhibiting sunken, water-soaked areas in the internodes, having retarded growth and with brown-to-red in colour were considered as top rot affected plants. The disease intensity was calculated as:

Disease Intensity (Per cent) = Sum of all numerical grades* X 100/ Total No. of leaves observed X Maximum grade

*Grades:1 = 1-5%, 2 = 6-20%, 3 = 21-50%, 4 = 51-70%, 5 = >71%

Table 1a. Prevalence of top rot disease in different districts of Punjab

Location/ District	Variety	No. of fields surveyed		Prevalence of disease (%)							
		2011	2012	2011				2012			
				June	July	August	Average	June	July	August	Average
Gurdaspur	CoJ 85	17	20	6	65	83	51.3	5	61	90	52.0
	CoJ 88	13	12	0	0	8	2.7	0	0	10	3.3
	Co 89003	13	11	0	8	15	7.7	3	12	18	11.0
	CoS 8436	08	11	0	13	25	12.7	0	35	15	16.7
	CoH 119	14	09	0	14	21	11.7	2	16	20	12.7
Amritsar	CoJ 85	19	22	5	68	95	56.0	7	62	94	54.3
	CoJ 88	07	08	0	14	0	4.7	0	10	3	4.3
	Co 89003	10	12	0	30	30	20.0	4	28	34	22.0
	CoS 8436	10	15	0	20	30	16.7	0	24	32	18.7
	CoH 119	06	07	0	33	17	16.7	0	35	20	18.3
Ludhiana	CoJ 85	15	18	0	13	20	11.0	4	15	22	13.7
	CoJ 88	05	07	0	0	0	0.0	0	0	0	0.0
	Co 89003	13	12	0	15	8	7.7	3	12	10	8.3
	CoS 8436	12	15	0	17	17	11.3	0	20	18	12.7
	CoH 119	04	02	0	0	25	8.3	0	5	20	8.3
Jalandhar	CoJ 85	20	25	5	60	65	43.3	6	55	72	44.3
	CoJ 88	10	12	0	20	0	6.7	0	18	0	6.0
	Co 89003	14	10	0	21	14	11.7	0	20	18	12.7
	CoS 8436	06	10	0	33	0	11.0	0	35	5	13.3
	CoH 119	05	02	20	20	20	20.0	10	22	15	15.7
Overall average				1.8	23.2	24.65	16.56	2.2	24.25	25.8	17.42

Table 1b. Prevalence of top rot disease in different varieties cultivated in Punjab

Variety	Prevalence of disease (%)									
	2011					2012				
	Gurdaspur	Amritsar	Ludhiana	Jalandhar	Average	Gurdaspur	Amritsar	Ludhiana	Jalandhar	Average
CoJ 85	51.3	56.0	11.0	43.3	40.4	52.0	54.3	13.7	44.3	41.1
CoJ 88	2.7	4.7	0.0	6.7	3.5	3.3	4.3	0	6.0	3.4
Co 89003	7.7	20.0	7.7	11.7	11.8	11.0	22.0	8.3	12.7	13.5
CoS 8436	12.7	16.7	11.3	11.0	12.9	16.7	18.7	12.7	13.3	15.3
CoH 119	11.7	16.7	8.3	20.0	14.2	12.7	18.3	8.3	15.7	13.7

RESULTS AND DISCUSSION

The data presented in Table 1a revealed the maximum prevalence of top rot to the extent of 95% and 94% in Amritsar district on variety CoJ 85 in August 2011 and 2012, respectively followed by 83% and 90% in Gurdaspur district on the same variety. Overall disease prevalence was maximum on variety CoJ 85 with 40.4% and 41.1% in 2011 and 2012, respectively and least on variety CoJ 88 with 3.5% and 3.4% (Table 1b).

The highest disease incidence of 4.2% and 4.4% was observed on variety CoJ 85 during the month of August in both the years followed by Co 89003 (2.5% and 3.2%, respectively) and least (1.0%) on variety CoJ 88 (Table 2). Similarly the disease severity was also maximum 65.8% and 72.0% on variety CoJ 85 in the month of August in both the years and least (2.0%) on CoJ 88 . The overall top rot

Table 2. Incidence and severity of top rot disease on different sugarcane varieties in Punjab

Variety	No. of fields surveyed		Disease incidence (%)						Disease severity (%)					
	2011	2012	2011			2012			2011			2012		
			June	July	August	June	July	August	June	July	August	June	July	August
CoJ 85	71	85	1.0	3.3	4.2	1.5	3.5	4.4	4.0	51.5	65.8	4.8	53.5	72.0
CoJ 88	35	39	0.0	1.0	0.0	0.0	2.0	1.0	0.0	8.5	2.0	0.0	8.0	2.0
Co 89003	50	45	0.0	2.2	2.5	1.0	2.5	3.2	0.0	18.5	16.8	0.0	20.5	18.0
CoS 8436	36	51	0.0	2.1	2.2	0.0	2.2	2.8	0.0	20.8	18.0	0.0	18.5	20.5
CoH 119	29	20	0.0	1.1	2.4	0.0	2.3	2.0	0.0	16.8	20.8	0.0	19.8	22.5
Overall aaverage			0.0	1.9	2.3	0.5	2.5	2.7	0.8	23.2	24.7	1.0	24.1	27.0

disease incidence (0-0.5%) and severity (0.8-1.0%) was minimum in the month of June and maximum incidence (2.3-2.7%) and severity (24.7-27.0%) in the month of August.

During both the years in the state the relative humidity (RH) remained below 70% in the month of June and maximum more than 90% in the month of August. This factor is critical for the development of the disease. Similar results were reported by Grillo and Azevido (1939) reported that the top rot as a characteristic symptom developed under very humid condition of more than 85% and might cause appreciable injury. In contrast, higher disease incidence of top rot during the month of June and July was reported by Chaudhary *et al.* (1999) when temperature and humidity were relatively high. They further observed that there was reduction in cane yield and recoverable sugar upto 38 per cent and 40 per cent, respectively.

Based on the studies carried out, it was concluded that the disease is increasing with changing climatic conditions. Proper management of this disease should be adopted so that the economy of the farmers could not be affected.

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Integrated management of seed-borne viruses of tomato through chemicals and dry heat seed treatment

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ABSTRACT

The management studies conducted during the investigations showed that tomato viruses can be effectively managed by seed treated with chemicals like sodium hypochlorite, trisodium phosphate and hydrochloric acid (HCL). These chemicals reduced the disease incidence of viral infection on the tomato seedling with increase in concentration. No disease incidence was observed when seeds were treated with sodium hypochlorite and HCL at concentration of 1.0 per cent for 5 min. and 10 min., respectively. The highest germination percentage (86 per cent) was recorded when the seeds were given pre-sowing trisodium phosphate treatment (0.5%) for 5 minutes. It was observed that the germination percentage and reduction in disease incidence were directly proportional to the concentration of chemicals and duration of treatments. The infected seeds were also treated at different temperature (64°C, 72°C and 80°C) and durations for 48 and 72 hours for inactivation of these viruses. The per cent disease incidence and seed germination were significantly reduced with increase in temperature and its duration. The dry heat treatment significantly reduced the disease incidence but seed germination was reduced significantly. Thus the chemical seed treatment proved better than dry heat treatment.

Key words: Tomato, seed-brone, viruses, chemicals, dry heat, seed treatment

Tomato (*Solanum lycopersicum* L.), a member of family Solanaceae is one of the important vegetable crops. It is the second horticultural species in terms of cultivated surface area and production volume after potato. It is grown throughout the year because of favorable agro-climatic conditions in one or other part of the country. In India, tomato occupies an area of about 0.9 million hectare with an average yield of 20.6 t/ha and total production of 18.653 MT (Anonymous, 2012) and in Punjab, tomato is cultivated in an area of about 6510 ha with an average yield of 24.66 t/ha and total production of 1.60 lac t (Anonymous 2013).

Tomato is vulnerable to the attack of many biotic and abiotic stresses among which viruses occupy important place as more than 159 viruses are known to infect tomato (Anonymous, 2010). Out of these 9 viruses *viz*., *Tomato mosaic virus* (TMV), *Cucumber mosaic virus* (CMV), *Tomato leaf curl virus* (TLCV), *Tomato yellow leaf curl virus* (TYLCV), *Potato leaf curl virus* (PLRV), *Potato virus X* (PVX), *Potato virus Y* (PVY), *Tomato spotted wilt virus* (TSWV) and *Tomato bushy stunt virus* (TBSV) attack tomato crop in India (Verma, 1988). Infection of more than one virus is common on this crop and results in serious crop losses. The high incidence of TMV and ToMV is common on tomato during rainy season tomato crop (July to October due to favorable environment conditions (Xu Zhihao *et al.,* 2000).

Viral diseases are an important limiting factor in many crop production systems, because of non-availability of antiviral products, therefore control strategies rely only on genetic resistance or sanitary measures or vector control eradication of diseased crops to prevent virus spread. Seed borne viruses infecting tomato mainly includes TMV, ToMV and CMV. Among these TMV and ToMV are mainly surface contaminants rather than internally seed borne (Sevik and Tohumcu (2011). Efforts have been made by different workers to eliminate these viruses by using various surface disinfectants. Seed coat virus was eliminated by acid extraction or trisodium phosphate treatment, but not only by thorough washing in detergent solutions. TMV in endosperms was not affected by acid or Na_3PO_4

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treatments, but was inactivated slowly during storage (Taylor *et al.,* 1961). The objective of the present study was to manage the seed borne viruses of tomato using chemical and thermotherapy based measures.

MATERIALS AND METHODS

Seed source

The seeds of tomato var. Punjab Chhuhara were obtained from Vegetable Farms, Punjab Agricultural University, Ludhiana and were sown in earthen pots (25 cm diameter) filled with a mixture of sterilized soil and well decomposed farmyard manure in the ratio of 2:1 in cage under insect - proof conditions. At 3-4 leaf stage, these healthy tomato plants were mechanically inoculated with the sap of tomato leaf samples showing symptoms like mosaic, mottling, blistering, puckering, leaf deformations, vein clearing *etc.* collected from the field. Then the inoculated tomato plants showing the symptoms of stunting, yellowing with dark green mottled areas on leaves, smalling, curling and puckering of leaves were selected and tomato fruits from these selected plants were harvested for seed extraction. Extracted seeds then tested for the presence of TMV and ToMV using DAS-ELISA. The seed sample showing positive results with DAS-ELISA was selected for further treatments.

Chemotherapy and thermotherapy

One hundred infected tomato seeds were treated with sodium hypochlorite, trisodium phosphate and hydrochloric acid using two concentrations *viz.*, 0.5% and 1.0%. The seeds were immersed in disinfectant solution for10 min. and 15 min. durations and then dried for some time in shade. The virus infected tomato seeds were also given dry heat treatment at 64^{o}C, 72^{o}C and 80^{o}Cfor 48 and 72 hours durations. Treated seeds were sown in the earthen pots filled with mixture of sterilized soil and well decomposed farmyard manure in the ratio of 2:1.The pots were kept in cage under insect-proof conditions. The untreated diseased tomato seed served as control.

Observations were recorded on seed germination, type of symptoms, incubation period, plant age at the time of initiation of symptoms and disease incidence.

The per cent disease incidence was calculated by the following formula:

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plants}}{\text{Number of plants observed}} \times 100$$

RESULTS AND DISCUSSION

The Table 1 revealed that seed treatment with sodium hypochlorite, trisodium phosphate and hydrochloric acid (HCL) influenced the seed germination adversely over control but reduced disease incidence with increase in concentration of these chemicals. The rate of germination significantly increased with increase in the duration of treatment. The highest germination percentage (86%) was observed with trisodium phosphate treated seeds at 0.5 per cent concentration for 5min. Seed germination was higher in case of untreated seeds along with disease incidence (Table 1). No disease symptoms were observed in sodium hypochlorite and HCL treated seeds at a concentration of 1.0 per cent and duration of 5 min. and 10 min., respectively. Broadbent (1976) observed that seed treatment with HCL and trisodium phosphate was successful to eradicate these viruses from seed coat of tomato seeds. Cordoba-Selles *et al.,* (2007) evaluated 10% trisodium phosphate solution (TP), 3 g/liter pectinase solution (from Rhizopus sp., SIGMA-ALDRICH, Germany), 3 g/liter pectinase solution supplemented with 2% HCl and 30% commercial bleach (PHB) as tomato seed treatments for their ability to prevent seed transmission of PepMV. They found that PepMV virus was largely eradicated by immersing the seeds in trisodium phosphate (10%) for 3 hours.Ling (2010) observed efficacy of seed-disinfecting treatments through bioassay on *Nicotiana benthamiana* plants. He observed that longer soaking time at 0.5% and 1% conc. of sodium hypochlorite, trisodium phosphate at conc. of 10 and 20 per cent and HCL at conc. of 1.25per cent completely deactivated the plant viruses from tomato seeds without any effect on seed germination.

In case of thermotherapy,the data presented in Table 2 revealed that the infected seed when treated at different temperature of 64^{o}C, 72^{o}C and 80^{o}C for 48 and 72 hours, the rate of per cent disease incidence was significantly reduced with increase in temperature and duration of treatment. However, seed germination was significantly reduced with

Table 1. Seed treatment of virus infected seeds of tomato with different chemicals

Chemical	Concentration (%)	Duration (min.)	Germination (%)	Disease symptoms		Plant age at the time of symptoms appearance (days)	Per cent disease incidence
				Types of symptoms	Incubation period (days)		
Sodium hypochlorite	0.5	5	78 (8.88)	Mosaic, mottling	15-16 (4.06)	15-16 (4.06)	12.82 (3.64)
		10	82 (9.11)	Mosaic	17-19 (4.35)	17-19 (4.35)	9.70 (3.27)
Sodium hypochlorite	1.0	5	74 (8.66)	No visible symptoms	− (1.00)	− (1.00)	− (1.00)
		10	80 (9.00)	Mosaic	19-21 (4.58)	19-21 (4.58)	15.00 (4.00)
Trisodium phosphate	0.5	5	72 (8.54)	Mosaic, puckering	16-17 (4.18)	16-17 (4.18)	11.11 (3.47)
		10	86 (9.32)	Mosaic	20-21 (4.63)	20-21 (4.63)	16.27 (4.15)
Trisodium phosphate	1.0	5	76 (8.77)	Mosaic, mottling	18-19 (4.41)	18-19 (4.41)	15.78 (4.09)
		10	82 (9.11)	Mosaic, puckering	14-16 (4.00)	14-16 (4.00)	7.31 (2.88)
Hydrochloric acid	0.5	5	72 (8.54)	Mosaic	20-21 (4.63)	20-21 (4.63)	11.11 (3.47)
		10	78 (8.88)	Mottling	17-19 (4.35)	17-19 (4.35)	5.11 (2.47)
Hydrochloric acid	1.0	5	70 (8.42)	Mosaic	21-23 (4.79)	21-23 (4.79)	17.14 (4.25)
		10	72 (8.54)	No visible symptoms	− (1.00)	− (1.00)	− (1.00)
Control	−	−	88 (9.43)	Mosaic, mottling and puckering	13-17 (4.00)	13-17 (4.00)	29.54 (5.52)
CD (p=0.05)	−	−	0.99	No visible symptoms	0.98	0.98	0.84

Figures in parentheses are the values for square root transformation and CD is applicable to those only.

Table 2. Seed treatment of virus infected seeds of tomato at different temperatures for different durations

Temperature (°C)	Duration (hours)	Germination (%)	Disease symptoms		Plant age at the time of symptoms appearance (days)	Per cent disease incidence
			Types of symptoms	Incubation period (days)		
64	48	58 (7.68)	Mosaic	17-19 (4.35)	17-19 (4.35)	10.34 (3.36)
	72	54 (7.41)	Mosaic	20-21 (4.63)	20-21 (4.63)	11.11 (3.47)
72	48	52 (7.28)	Mosaic	19-21 (4.58)	19-21 (4.58)	7.69 (2.94)
	72	46 (6.85)	No visible symptoms	− (1.00)	− (1.00)	− (1.00)
80	48	44 (6.70)	Mosaic	21-23 (4.79)	21-23 (4.79)	9.09 (3.17)
	72	42 (6.55)	No visible symptoms	− (1.00)	− (1.00)	− (1.00)
Control	-	84 (9.21)	No visible symptoms	− (1.00)	− (1.00)	26.19 (5.21)
CD (p=0.05)	-	0.14	No visible symptoms	0.14	0.14	0.12

Figures in parentheses are the values for square root transformation and CD is applicable to those only.

increase in temperature and duration of treatment. Broadbent (1976) explained that heat treatment for three days at 70°C or one day at 80°C of dried tomato seeds had also been successful to control TMV. Ling (2010) observed that virus infectivity on tomato seeds was totally destroyed at 72°C and 80°C when exposed for 48 or 72 hours. Germination of tomato seeds was delayed by 1 day on dry heat treatment, however, germination rate was not significantly affected.

In the present study, it was observed that the seed borne tomato viruses can be effectively managed by seed treatment with sodium hypochlorite and hydrochloric acid at the concentration of 1% with time duration of 5min. and 10 min., respectively. Also the germination percentage and reduction in disease incidence were directly proportional to the concentration and duration of treatment. The results of thermotherapy treatment showed that seeds treated at different temperatures (64°C, 72°C

and 80°C) and different durations for 48 and 72 hours, the rate of per cent disease incidence was significantly reduced with increase in temperature and duration of treatment. However, per cent seed germination was significantly reduced with increase in temperature and duration of treatment. Thus, the chemical seed treatment was considered better than dry heat seed treatment.

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Prevalence of chilli leaf curl disease in Punjab

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ABSTRACT

Roving surveys of major chilli growing areas of Punjab conducted during 2012-2013 revealed wide range of leaf curl symptoms in addition to vein clearing, puckering, crinkling and stunting of the plants. Incidence of leaf curl disease varied from 11 to 64 per cent maximum being in central zone of Sangrur and Patiala district of central zone, whereas, it was minimum in Ferozpur and Taranatran district of southwest zone . Leaf curl severity was maximum during July-August than April-May due to white fly (*Bemisia tabaci*) population build up in June- July. Almost all the varieties/ hybrids grown in this tract were susceptible to the chilli leaf curl complex. Though the disease incidence varied with the cultivar. The maximum disease incidence was observed in hybrid CH-1 and CH-3 (63-64%) whereas, it was minimum in hybrid Bio, Pepsi, MH1 and Soldier (13-17%).

Key words: chilli, leaf curl, whitefly, survey, Punjab

Chilli (*Capsicum annuum* L.) is an important spice crop grown for its green fruits used for table purpose and ripe as dried condiment. Chilli belongs to the genus *Capsicum,* family Solanaceae. It has originated in Mexico, Southern Peru and Bolivia (Villalon, 1981). In India Chilli is mainly cultivated in Andhra Pradesh, Karnataka, Maharashtra, Orissa, Rajasthan, Tamil Nadu, West Bengal and Punjab states over an area of 804790 hectares with total production of 1276300 metric tonnes of dry chilli with productivity of 1.5 metric tonnes per hectare (Anonymous, 2012-13).

Chilli crop suffers from a large number of viral, fungal, bacterial, nematode and phytoplasmal diseases, of these viruses are known to cause heavy losses (Villalon, 1981). Viral diseases are an important factor contributing to low yields and reduced fruit quality and 100 per cent losses of marketable fruit have been reported (Marte and Wetter, 1986). Natural occurrence of more than 45 viruses, including *Pepper leaf curl virus, Pepper veinal mottle virus* and *Pepper vein bending virus* have been reported from chilli worldwide (Prakash and Singh, 2006). Among all whitefly (*Bemisia tabaci*) transmitted chilli leaf curl disease (syn. Pepper leaf curl disease, PepLCD) is the most destructive in terms of incidence and yield loss (Muniyappa and Veeresh, 1984).

Leaf curl disease of chilli has also been reported from India and the extent of yield losses due to this disease ranges between 25 to 80 per cent (Ravi, 1991). The PepLCD infected plants shows symptoms similar to tomato leaf curl, such as yellowing, leaf curling, a reduction in leaf size. The major viruses reported with the disease are *Tomato leaf curl New Delhi* (ToLCNDV), *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) (Hussain *et al*., 2004, Tsai *et al.,*2006) in Pakistan and Indonesia, respectively. In India, *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl Joydebpur virus* (ToLCJV), *Chilli leaf curl India virus* (ChiLCIV) has been recently shown to be associated with chilli leaf curl disease (Khan *et al.,* 2006; Senanayke *et al.,* 2006; Shih *et al.,* 2007).

MATERIALS AND METHODS

Survey for chilli leaf curl disease was undertaken in four districts of the Punjab *viz.* Sangrur (Malerkotla and Dhuri), Patiala (Nabha and Sanaur) of central zone, Ferozepur (Makhu and Malwala kadeem,) and Tarntaran (Patti and Jandiyalaguru) of southwest zone. The surveys were conducted during July- August 2012 in central zone and April-May 2013 in southwest zone. Roving survey method was

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adopted to know the chilli leaf curl disease incidence at above mentioned places. The disease diagnosis in the field was based on typical symptoms. Under laboratory conditions the PCR based assay was used for detection and confirmation of virus using begomovirus specific universal primers (Wyatt and Brown, 1996). The per cent disease incidence was recorded at random involving different locations (four corners S1,S2,S3, S4 and one central patch (SC), 1m² each) in each field by counting total number of plants and number of plants showing leaf curl symptoms using the formula given below:

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

Besides the disease incidence, location of field using Global Positioning System (GPS) apparatus (Garmin,USA), variety grown, date of sowing, crop rotation like parameters were also recorded.

RESULTS AND DISCUSSION

Chilli is grown in diverse agroclimatic conditions of Punjab. Roving survey (Fig. 1) revealed that the leaf curl disease is prevalent in all chilli growing areas. The incidence varies with locations ranging from 11 to 64 per cent. The virus incidence was more in central zone (64 %) as compared to south-west zone (11 %). It can be inferred from Table 1 that chilli leaf curl disease appears in severe form in central zone i.e Sangrur and Patiala in the months of July-August as compared to Ferozpur and Tarantaran districts where the incidence was less when surveyed in April-May. The disease incidence was more in Sanaur block (64%) followed by Nabha (63%) and Malerkotla (61%) blocks. The popular crop rotation in south west zone is chilli-paddy. The chilli crop is planted early in October-November followed by paddy in June; therefore the cropping season does not coincide with white fly peak season

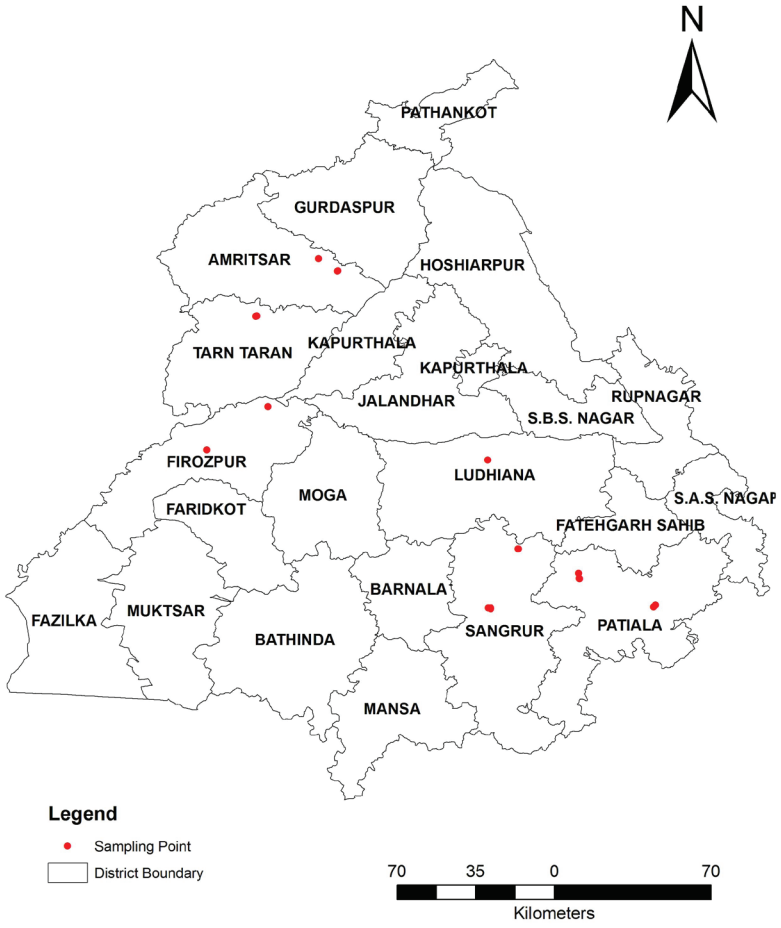

Fig. 1. Global position reading based map showing surveyed chilli field of different agro-climatic zones

Table 1. Prevalence and incidence of leaf curl disease of chilli in major chilli growing areas of Punjab

No. of fields	District: Sangrur				Date of observation:- 13-07-2012 and 16-07-2012							
	Block	GPS Location	Area (acres)	Name of variety/hybrid	Date of sowing	Crop rotation	S1	S2	S3	S4	SC	Average incidence%
1	Malerkotla	N-30°32'58.3" E-75°56'8.3"	1.5	CH-1	December	Chilli-Cauliflower	70	65	50	55	45	57.00
2		N-30°32'59.5" E-75°56' 8.8"	1.0	CH-1	December	Chilli-Cauliflower	75	55	60	65	50	61.00
3		N-30°32'58.5" E-75°56'9.4"	0.5	CH-1	December	Chilli- Coriander	60	75	55	55	45	58.00
1	Dhuri	N-30°18'35.7" E-75°49'31.8"	1.0	CH-1	December	Chilli-Potato	70	65	50	55	40	56.00
2		N-30°18'50.5" E-75°49'31.1"	1.0	CH-1	December	Chilli-Maize	75	60	50	55	45	57.00
3		N-30°18'50.1" E-75°48'56.7"	1.0	CH-1	December	Barley-Chilli	60	70	65	50	50	59.00
	District: Patiala					Date of observation:- 3-08-2012 and 11-08-2012						
1	Nabha	N-30°25'52.6" E-76°10'47.5"	1.0	CH-1 and CH-3	December	Chilli-Potato	60	75	60	55	45	59.00
2		N-30°27'06.5" E-76°10'40.9"	6.0	CH-1 and CH-3	December	Chilli-Potato-Onion	65	60	70	60	55	62.00
3		N-30°25'49.0" E-76°10'55.7"	6.0	CH-1 and CH-3	December	Chilli-Potato-Mellon	60	65	75	65	55	63.00
1	Sanaur	N-30°19'0.7" E-76°28'42.9"	4.0	CH-1	December	Chilli-Potato-Maize	70	65	75	60	55	64.00
2		N-30°19'29.7" E-76°29'8.8"	1.5	CH-1	December	Chilli-Potato-Wheat	65	70	60	55	50	60.00

	District: Ferozepur					Date of observation:- 19-4-2013 and 28-4-2013						
1	Malwa Kadeem	N-30°54'18.9" E-75°48'48.2"	4.0	Lalima and Soldier	October	Chilli-Basmati	20	20	15	20	10	17.00
2		N-30°56'47.1" E-74°41'15.8"	24	Soldier	October	Chilli-Basmati	20	15	15	10	05	13.00
3		N-30°56'47.1" E-74°41'15.8"	4.0	Soldier and MH-1	October	Chilli-Basmati	20	10	10	15	05	14.00
1	Makhu	N-31°7'11.7" E-74°55'57.4"	1.0	Soldier and Bio	October	Chilli-Basmati	25	20	20	15	10	18.00
	District: Tarntaran					Date of observation:-9-05-2013 and 15-05-2013						
1	Patti	N-31°28'52.7" E-74°53"4.6"	3.0	Soldier	October	Chilli-Paddy	20	10	20	25	10	15.00
2		N-31°28'57.6" E-74°53'15.2"	2.0	Soldier	October	Chilli- Berseem	15	20	20	10	5	14.00
3		N-31°28'57.2" E-74°53'15.1"	1.5	Pepsi	October	Chilli- Paddy	10	20	20	20	10	16.00
1	Jandiyala guru	N-31°39'46.2" E-75°12'41.1"	0.5	Soldier and MH-1	October	Chilli- Berseem	20	10	10	15	05	14.00
2		N-31°39'52.2" E-75°12'47.4"	1.0	Soldier	October	Chilli-Potato	20	15	10	15	05	13.00
3		N-31°42'46.1" E-75°8'38.4"	0.5	Soldier	October	Chilli-Potato	20	15	20	15	10	16.00

i.e June-July. Therefore, the chilli crop of south west zone bears early fruiting and escape from leaf curl disease. In central zone the crops rotation is vegetable rich and chilli growing period (Dec-Sept) coincides with peaks of whitefly population, the vector of leaf curl disease results in more incidence. Due to high relative humidity and minimum temperature the whitefly population increases in the month of July- August as compared to other months in Punjab region (Singh *et al.,* 2014). The maximum disease incidence was observed in hybrid CH-1 and CH-3 (63-64%) whereas, minimum in hybrid Bio, MH-1, Pepsi and Soldier (13-17%). The hybrid CH-1 and CH-3 released by PAU, Ludhiana is popular among local farmers and also possess tolerance to leaf curl disease. Since the incidence was very high on these hybrids, the authenticity of hybrids (CH-1 and CH3) available with farmers is in question as these are being developed and sold by local growers. The important symptoms recorded were upward as well as downward curling, leathry and brittle leaves, reduction in leaf size, thickening of veins along with vein clearing, puckering, crinkling and stunting of the plants. This symptom variability could be attributed to the cultivation of different hybrids/variety, virus, stage of the plant and climatic factors. Similar observations were also made by Shih *et al.* (2007) where wide range of symptom variability was observed in chilli. The total DNA from symptomatic plants showing typical symptoms of begomovirus amplified a desired size product (~575 bp) with set of universal primers by PCR, which confirms the association of begomovirus. This primer has been extensively and efficiently used for the detection of begomoviruses from differet hosts (Wyatt and Brown, 1996). Almost all the varieties/hybrids grown in this tract are susceptible to the chilli leaf curl complex. Further studies are required to demonstrate the effect of crop rotation on survival and carryover of begomoviruses.

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Sources of resistance to *Heterodera avenae* in wheat

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ABSTRACT

Wheat varieties recommended for cultivation in Punjab and wild wheat genotypes (15 entries of *Aegilops tauschii*) were evaluated for their reaction to three populations of the cereal cyst nematode (CCN), *Heterodera avenae* collected from different wheat based cropping systems *i.e.* wheat-maize, wheat-cotton and wheat-rice being followed in Punjab, India. Out of the eighteen wheat cultivars including bread wheat, *durum* wheat and triticale, none has been found resistant to *H. avenae.* However, moderately resistant reaction was recorded in two cultivars *i.e.* PBW 509 (wheat-cotton and wheat-rice populations) and a triticale cultivar, TL 2908 (wheat-maize and wheat-rice populations). While in wild wheat genotypes, resistance to *H. avenae* has been demonstrated to all the three populations of the nematode species in three entries of *Ae. tauschii viz*., AT 41, AT 202 and AT 242.

Key words: Cereal cyst nematode, *Heterodera avenae*, wheat, resistance, *Aegilops tauschii*

Wheat (*Triticum aestivum* L.) is the most important human food grain and ranks second in total production as a cereal crop behind maize. It is the staple food for nearly 40 per cent of world's population and provides about 20 per cent of world's food calories. Globally, a loss of 10% of world crop production has been estimated as a result of plant nematode damage (Whitehead, 1998). About 90 species of plant parasitic nematodes have been reported to be associated with wheat crop. Out of the economically important nematodes, cereal cyst nematode (CCN), *Heterodera avenae* Woll. is the most important and the most studied plant-parasitic nematode on wheat (Toktay, *et al.,* 2013). In the wheat crop, losses up to 100% have been reported in India (Van Berkum and Seshadri, 1970). The nematode can be managed by cultural practices, chemicals, using CCN resistant cultivars *etc*. However, resistance is considered to be the economically effective method of managing CCN. Resistance in wheat to CCN has been reported from CIMMYT, Australia and France, but only a few varieties with CCN resistance are grown commercially (Rivoal and Cook, 1993; Bekal *et al.,* 1998; Nicol *et al.,* 2001). From India, resistance against CCN has been reported in a few varieties only and one *i.e.* RAJ MR 1 has been recommended for cultivation (Bhatti and Dahiya, 1992; Sharma and Sharma, 2000). As *H. avenae* has different biotypes and the Punjab population of the nematode is different to those from Haryana, Delhi and Rajasthan, the bread wheat varieties that are resistant to populations of CCN from other states may be susceptible to the Punjab population (Bishnoi *et al.,* 2004). Hence, it was planned to identify resistant varieties amongst the recommended wheat varieties in Punjab and also in wild wheat genotypes to the local nematode populations of *H. avenae*.

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MATERIALS AND METHODS

Plant material : Seed of one set of test plants consisted of eighteen wheat varieties including bread wheat, *durum* wheat and triticale recommended for cultivation in Punjab (Anon., 2011) and another set of fifteen entries of wild wheat (*Aegilops tauschii*) were procured from Wheat Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Two varieties, PBW 343 and PBW550 were used as susceptible checks. All the varieties were evaluated for their reaction to the CCN populations collected from different wheat based cropping systems *i.e.* wheat-maize, wheat-cotton and wheat-rice being followed for more than ten years (in the same field) in Punjab.

Screening : Four seeds of each of the variety/ line were sown in 15 cm pots, containing 1 kg

Table 1. Grading scale for categorization of cultivar reaction

Rating index	Cysts per plant	Host response
1	0 cysts	Highly Resistant (HR)
2	Up to 4 cysts	Resistant (R)
3	4.1 to 9.0	Moderately Resistant (MR)
4	9.1 to 20	Susceptible (S)
5	More than 20	Highly Susceptible (HS)

of CCN infested soil with initial population of 2 cysts/100g of soil in November, 2012 separately for each population. The experiment was replicated four times and pots were kept on the bench in glass house for ten days and after germination of seed these were kept outside the glass house. Irrigation was given as and when required. Observations were recorded in the month of March. Number of white cysts were determined by estimating the number of cysts per 250cc soil using the Cobb's Decanting and Sieving technique (Cobb, 1918) after washing roots and soil using 25 and 60 mesh sieves. (pore size of 250 µm). Reaction was categorized from highly resistant to highly susceptible depending upon the number of white cysts obtained and grading was done according to the scale adopted by All India Coordinated Wheat and Barley Improvement Project (Table 1).

RESULTS AND DISCUSSION

The data recorded on average number of white cysts of *H. avenae* populations of wheat – maize, wheat - cotton rotation fields on the recommended wheat varieties (Table 2) revealed that out of the eighteen wheat cultivars including bread wheat, *durum* wheat and triticale none has been found free from nematode infection of all the three populations of *H. avenae.* All the varieties have shown

Table 2. Reaction of wheat varieties recommended for cultivation in Punjab to three populations of *H. avenae*

Variety	Wheat - maize population		Wheat - cotton population		Wheat - rice population	
	Cysts / 250 cc soil	Reaction	Cysts / 250 cc soil	Reaction	Cysts / 250 cc soil	Reaction
HD 2967	13.00	S	9.75	S	15.25	S
PBW 621	10.50	S	9.25	S	9.25	S
DBW 17	10.00	S	6.25	MR	10.25	S
PBW 550	13.25	S	10.50	S	10.00	S
PBW 502	22.25	HS	21.25	HS	22.50	HS
PBW 343	11.25	S	11.75	S	12.00	S
WH 542	10.75	S	18.25	S	9.75	S
PBW 590	20.25	HS	17.50	S	16.75	S
PBW 509	9.75	S	7.25	MR	4.75	MR
PBW 373	9.75	S	15.75	S	9.25	S
PBW 527	9.50	S	7.25	MR	12.00	S
PBW 175	12.50	S	9.25	S	20.25	HS
PDW 314	16.50	S	15.00	S	7.25	MR
WHD 943	14.00	S	10.00	S	12.00	S
PDW 291	13.00	S	10.50	S	10.75	S
PDW 233	21.50	HS	16.75	S	14.75	S
TL 2908	7.75	MR	9.25	S	7.50	MR
TL 1210	11.75	S	9.75	S	9.50	S
CD (p=0.05)	2.38	-	2.01	-	2.13	-

nematode multiplication. None of the variety was found resistant, however only moderately resistant reaction was recorded. Only two cultivars were found moderately resistant to two populations of *H. avenae*, one was the late sown bread wheat PBW 509 to wheat-cotton and wheat-rice populations and other was triticale TL 2908 to wheat-maize and wheat-rice populations. Bread wheat DBW 17 and PBW 527 were moderately resistant to wheat-cotton population and *durum* wheat PDW 314 to wheat-rice population. Rest of the cultivars were found to be either susceptible or highly susceptible to all the three populations. The susceptible cultivars; PBW 509, PBW 373 and PBW 527 were statistically at par with the moderately resistant cultivar TL 2908, though they were numerically different from each other against wheat - maize population and in wheat - cotton population, PBW 621, PBW 175 and TL 2908 (susceptible cultivars) were statistically at par with PBW 509 and PBW 527 (moderately resistant cultivars). Similarly, the cultivars PBW 621 and PBW 373 susceptible to wheat - rice population, were statistically at par with the moderately resistant cultivar PDW 314 and TL 2908, though numerically different from each other.

Multiple resistance in wheat to different diseases and nematode pests has been depicted in several lines of CIMMYT (Singh and Rajaram, 2002). Resistance in some of the wheat, oat and barley cultivars to *H. avenae* pathotypes, Ha 1, Ha 2 and Ha 3 has also been earlier reported (Nicol, 2002). Moderately resistant reaction has also been reported in varieties; PDW306, PDW304, WH1024, HW1095, SWL36, DWR28, AKDW2997-16, HPW286, PDW312(d), HPW296, HPW308, PBW621, MP4106, MPO1220(d), NIAW1415, DDK1037, DDK1039, PDW317, NIDW 295, DDK1009, DBW31, PDW312, HW1095, T2961(T), HUW609, DDW19, UAS320(D), HI8699(D), MPO1226(D), TL2966(T), DDK 1038(DIC.), MACS2998, MACS5012, HI8692(d), HPW347 and HI 1568 under Advanced Varietal Trial (AVT) of All India Coordinated wheat and barley Improvement Project against the mixed population of *H. avenae* from Punjab (Kaur *et al.,* 2008; Kaur and Sharma, 2012).

Resistance to three populations of *H. avenae* has been demonstrated in three entries of *Ae. tauschii viz.,* AT 41, AT 202 and AT 242 in the present study, while AT 36, AT 55, AT 56, AT 195, AT 201 and AT 243 were moderately resistant as against susceptible

Table 3. Reaction of wild wheat genotypes (*Ae. tauschii*) to three populations of *H. avenae*

Entry	Wheat- maize population		Wheat- cotton population		Wheat- rice population	
	Cysts / 250 cc soil	Reaction	Cysts / 250 cc soil	Reaction	Cysts / 250 cc soil	Reaction
AT 36	7.00	MR	6.75	MR	5.75	MR
AT 41	3.75	R	3.25	R	3.00	R
AT 55	6.25	MR	5.75	MR	4.75	MR
AT 56	9.50	S	7.00	MR	5.75	MR
AT 169	13.25	S	12.50	S	9.00	S
AT 178	13.75	S	10.25	S	9.75	S
AT 186	5.25	MR	4.00	R	3.75	R
AT 187	11.50	S	10.75	S	9.25	S
AT 195	7.25	MR	7.25	MR	5.75	MR
AT 201	7.00	MR	8.00	MR	7.25	MR
AT 202	3.50	R	3.25	R	3.00	R
AT 203	14.50	S	11.75	S	6.50	MR
AT 242	4.00	R	3.50	R	4.00	R
AT 243	6.75	MR	5.75	MR	4.75	MR
AT 244	11.50	S	9.50	S	8.00	MR
PBW 343	16.75	S	13.75	S	21.50	HS
PBW 550	14.25	S	16.75	S	17.00	S
CD (p=0.05)	1.96	-	1.74	-	1.72	-

to highly susceptible check wheat varieties. AT 244 was the only entry which was moderately resistant to wheat-maize and wheat-cotton populations of *H. avenae* but susceptible to wheat-rice population. Rest of the entries were susceptible to all the populations tested (Table 3). The moderately resistant to wheat - maize population, AT 186 was statistically at par with the resistant genotypes AT 41, AT 202 and AT 242, and all the resistant genotypes were different statistically from all the other cultivars in wheat - cotton population. In case of wheat - rice population, out of the moderately resistant genotypes; AT 55 and AT 243 were statistically at par with the resistant genotypes; AT 186 and AT 242, though they were numerically different from each other.

Wild wheat cultivars, *Ae. tauschii, Ae. ventricosa, Ae. variabilis, Ae. triunclatis, Ae. longissima* and *Ae. geniculata* are well known resistance sources against cereal cyst nematode. Source of cereal cyst nematode resistance genes has been designated in *Ae. tauschii, Ae. triuncialis, Ae. variabilis, Ae. ventricosum, Secale cereale* and *T. aestivum* (Nicol, 2002; Williams *et al.,* 2003; Barloy *et al.,* 2007). Kaur *et al.,* (2008) have also recorded resistance in seven accessions of *Ae. tauschii viz.* AT104, AT138, AT186, AT264, AT270, AT272 and AT282) to a mixed population of *H. avenae* of Punjab.

From the present studies, it can be inferred that none of the cultivated wheat varieties under investigation exhibited highly resistant/ resistant reaction to *H. avenae*. However, in wild wheat resistance is found in a number of genotypes (*Ae. tauschii*), which can be further used for incorporating resistance to *H. avenae* in wheat varieties having the desirable attributes.

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Impact of planting time on grain yield and neck blast incidence in tall and semi dwarf genotypes of *basmati* rice (*Oryza sativa*)

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Haryana state is well known for cultivation and export of *basmati* rice which gets a premium price in the market for its excellent aroma, cooking quality and palatability. *basmati* rice varieties are occupying about 65% of the total area under rice in the state. Time of planting is one of the most important management factors which have a significant bearing on the crop yield. Blast continues to be an economically important disease in all the rice ecosystems of the country (Muralidharan, 2006), which is known to affect different parts of the rice plant viz. leaf, node, neck and the collar region of leaf and stem. Of these, neck blast caused by *Pyricularia grisea* Sacc. (*Magnaporthe grisea* (Hebert) Barr) has become a major constraint in realizing the potential yield of *basmati* rice varieties grown for export purpose in the state (Ram Singh *et al.,* 2004). Although the effect of planting time on grain yield and neck blast incidence has been studied in traditional tall and photo-sensitive *basmati* rice variety *Taraori Basmati* (Dodan and Ram Singh, 1995) but no such information is available on semi-dwarf (photo-insensitive) varieties as well as on other tall and promising (photo-sensitive) genotypes of *basmati* rice. Keeping above facts in view, present study was carried out to determine the optimum time of transplanting for different *basmati* rice genotypes in order to minimize neck blast incidence and to obtain maximum grain yield.

Field investigations were carried out at CCS Haryana Agricultural University Rice Research Station, Kaul during rainy (*Kharif*) season of 2008 and 2009. The treatments consisted of three dates of transplanting (25 June, 10 July and 25 July) and five *basmati* rice genotypes viz. CSR 30, HKR 03-408, HKR 04-487 (tall and photo-sensitive), *Pusa Basmati* 1 and *Pusa Basmati* 1121 (semi dwarf and photo-insensitive). Among these, *Pusa Basmati* 1121, CSR 30, *Pusa Basmati* 1 are recommended for general cultivation in the state while HKR 03-408 and HKR 04-487 have been found to be the promising cultivars. These genotypes were selected based on variation with respect to photo-sensitivity, plant stature and nutritional requirement.

The treatments were laid out in split plot design with four replications keeping the transplanting dates in main plots and genotypes in sub-plots. Staggered transplanting of 30 days old seedlings of all the genotypes was done in puddled field at a spacing of 20 x 15 cm at 15 days interval starting from 25 June and the crop was raised following recommended package of practices. The data on grain yield (t/ha) and its attributes were recorded at the crop maturity. Neck blast incidence was recorded in percentage under natural conditions by counting the number of infected and healthy tillers in a randomly selected 25 hills/replicate (plot) of each treatment. The data were subjected to statistical analysis following Panse and Sukhatme (1978). When averaged over the genotypes, neck blast incidence was significantly higher under late planting (July 25) as compared to earlier planting dates, thus corroborating the findings of Dodan and Ram Singh (1995) who reported the lowest incidence of neck blast and the highest grain yield of a photo-sensitive variety *Taraori Basmati* in early planted crop. The lower blast incidence in the early planted crop has been attributed to higher temperature and a low relative humidity (Ou, 1985) and accumulation

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of more silicon in host epidermis (Osuna-Canizaler *et al*., 1991). Comparison of grain yield (averaged over transplanting dates) of different genotypes revealed that *Pusa Basmati* 1121 gave the highest grain yield and proved significantly superior to all other genotypes during both the years. *Pusa Basmati* 1, the other photo-insensitive genotype, also significantly out yielded the photo-sensitive genotypes during 2009 but failed to do so during 2008 obviously because of higher incidence of neck blast during this year. Among photo-sensitive genotypes, HKR 03-408 and HKR 04-487, being at par, yielded significantly higher than CSR 30.

Interaction between transplanting dates and genotypes was found significant for neck blast incidence as well as for grain yield (Table 1) which revealed that maximum neck blast, irrespective of the time of transplanting, was recorded in *Pusa Basmati*-1 followed by *Pusa Basmati* 1121 and CSR 30, while minimum incidence was observed in HKR 03-408 and HKR 04-487. In general, increase in neck blast incidence under late planting was more in tall *Basmati* genotypes as compared to semi-dwarf ones. These observations indicated that response of rice cultivars under different planting dates varied with respect to their photo-sensitivity. Grain yield of *Pusa Basmati*-1 and *Pusa Basmati* 1121 (photo-insensitive genotypes) and CSR 30 (photo-sensitive genotype) decreased significantly under late planting (July 25). But the yield of the other two photo-sensitive genotypes *viz*. HKR 03-408 and HKR 04-487 did not decrease under late planting which might be due to lower incidence of the disease in these genotypes as compared to CSR 30 (Table 1), thus indicating that these promising genotypes can be transplanted over a longer period.

From the findings of the present study, it can be concluded that both tall and semi-dwarf varieties of *Basmati* rice should be transplanted up to 10 July in order to minimize the incidence of neck blast and

Table 1. Effect of interaction between transplanting time and genotypes on neck blast incidence (%) and grain yield (t/ha)

Genotypes	Transplanting dates							
	2008				2009			
	June 25	July 10	July 25	Mean	June 25	July 10	July 25	Mean
CSR 30	15.88 (23.40) *2.91*	15.80 (23.34) *2.82*	27.80 (32.00) *2.53*	19.83 (26.25) *2.75*	30.38 (33.44) *3.71*	28.57 (32.21) *3.66*	42.44 (40.95) *3.27*	33.90 (35.53) *3.55*
HKR 03-408	4.21 (11.73) *3.10*	2.75 (9.52) *3.08*	15.99 (23.70) *3.13*	7.65 (14.98) *3.10*	8.98 (17.19) *4.19*	9.61 (17.60) *4.16*	14.35 (22.61) *4.13*	10.98 (19.25) *4.16*
HKR 04-487	6.61 (14.65) *3.08*	3.20 (10.40) *3.01*	12.04 (20.44) *3.02*	7.28 (15.16) *3.04*	7.28 (15.6) *4.13*	15.34 (22.90) *4.26*	19.17 (26.17) *4.12*	13.93 (21.56) *4.17*
Pusa Basmati 1	53.19 (46.73) *3.20*	57.74 (49.25) *2.97*	65.35 (54.21) *2.51*	58.76 (50.06) *2.89*	35.15 (36.26) *4.77*	30.00 (33.24) *4.82*	43.45 (41.31) *3.85*	36.20 (36.94) *4.48*
Pusa Basmati 1121	38.16 (38.21) *4.57*	45.50 (42.42) *4.58*	41.43 (40.03) *3.72*	41.70 (40.22) *4.29*	19.30 (26.05) *5.11*	15.81 (23.46) *5.27*	20.98 (27.23) *4.55*	18.70 (25.58) *4.98*
Mean	23.61 (26.94) *3.37*	25.00 (26.99) *3.29*	32.52 (34.08) *2.98*		20.28 (25.72) *4.38*	19.87 (25.90) *4.43*	28.08 (31.69) *3.98*	

The figures in parentheses represent angular transformed values and figures in italics represent grain yield

	Disease incidence		Grain yield	
	2008	2009	2008	2009
CD (p=0.05) for transplanting dates	2.67	2.91	0.23	0.25
CD (p=0.05) for cultivars	2.60	2.64	0.10	0.16
CD (p=0.05) (interaction) for cultivars at same date	4.51	4.58	0.16	0.28
CD (p=0.05) (interaction) for dates in same cultivar	4.83	5.00	0.27	0.35

to get higher grain yield. However, the promising tall genotypes like HKR 03-408 and HKR 04-487 performed better even under delayed planting.

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A novel technique for isolation of *Phytophthora nicotianae* var. *parasitica* from infested soils

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Phytophthora spp. causes damping off of seedlings, root rot, collar rot, foot rot and gummosis as major disease accounting for widespread decline and death of citrus plants in nursery as well as in orchards (Naqvi and Singh, 1999). The disease causes heavy damage to Kinnow plants by reducing their life expectancy, quality and yield (Thind and Sharma, 1996). Epidemiological studies have been hampered by the lack of an efficient technique to isolate this fungus from soil. Direct isolation from soil, even on selective media, has not been successful, presumably because of low number of propagules . A major problem is that the presence of fast growing other organisms such as *Pythium* which tends to inhibit growth of the target pathogen (Nechwatal *et al.,* 2001). Isolation of *Phytophthora* from necrotic plant tissue is more difficult, because most species of *Phytophthora* have poor saprophytic capabilities. Use of baiting technique markedly increases the frequency of isolation, when samples are relatively clean and secondary invaders are absent, thus making direct isolation feasible onto media without the use of antibiotics (Drenth and Sendail, 2004). The purpose of this study was to generate easier, cheap and authentic information regarding *Phytophthora* isolation.

Detection and/or isolation of *Phytophthora* from plant tissue are relatively simple and successful if the tissue is fresh and recently infected. Positive soil sample collection is prime requirement for isolation of *Phytophthora*. Rhizosphere soils with roots were taken for isolation of *Phytophthora nicotianae* var. *parasitica*. Soil samples were collected from affected citrus nurseries and orchards. Collected freshly infected roots from the edge of an actively growing lesion. Roots were washed with sterilized water. To one part of infected soil, three parts of water was added to make the soil slurry in flat plastic boxes of 7.5cm depth (Plate 1a). Debris over the water layer were removed with the help of sieve. Water level was kept 2 inches above the soil in container. Leaves of different maturity levels of rough lemon (*Citrus jambhiri* Lush) were used as bait and floated over the water layer (lower side of leaves touching the water). The leaves were washed with teepol before floating on soil water mixture. These containers after covering with the perforated polyethylene sheet were incubated at 25$\pm$1°C for 3-4 days in incubator. Leaves were regularly examined for the appearance of water soaked lesions. The leaves developing water soaked lesions were removed from the slurry, washed with water and surface sterilized and isolates were taken on selective V8 juice agar medium. Pure culture was obtained after 2-3 sub-culturings of the axenic culture of *Phytophthora*. For maintaining and multiplication, both V8 juice agar (4.43 % (v/v)) and pea extract (250g peas + 15g sucrose + 10g agar for one litre) medium were used. Cultures were subcultured every 1-2 weeks to maintain vigour. Sporangia were induced after every 2 months at 18 to 20^{o}C in incubator to check the purity and vigour of cultures (Plate. 1e).

The baiting bioassay detected *Phytophthora* in naturally infested soils of citrus nurseries and orchards. Duration of baiting (*i.e.* the period when leaves were floated over flooded container containing mixture of soil and water) affected both detection of *Phytophthora* and incidence of contaminants. The best time to take out the bait was found to be when water soaked lesions were 2-6 mm in diameter. Mature leaves though took more time

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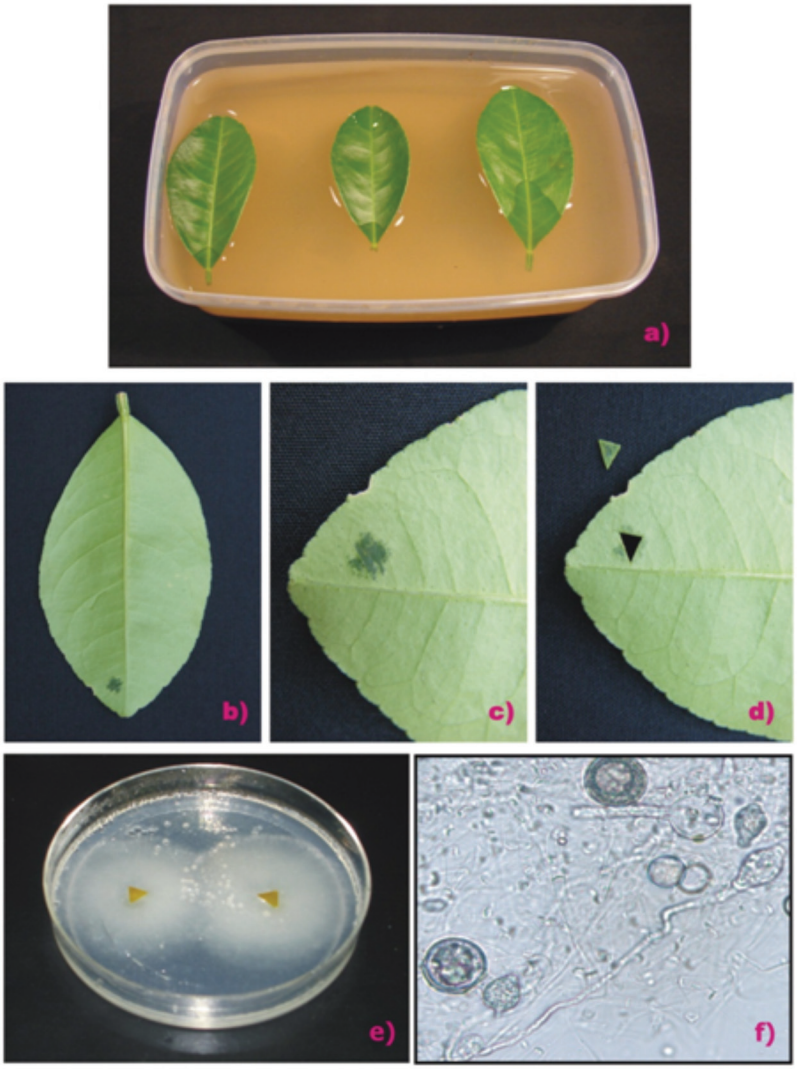

Plate. 1. Various steps in *Phytophthora* isolation from infected soils
a) rough lemon leaves floating on soil water slurry; b) & c) water soaked lesion formed on leaf ; d) cutting of leaf bit from lesion; e) fungal growth from bits cultured on V8 juice agar medium; f) identification of fungus species from sporangia and oospores

to develop lesions as compared to younger leaves, but higher percentage of mature leaves resulted in isolation of *Phytophthora*. The temperature during baiting did not have a dramatic effect on the detection of *Phytophthora* or incidence of contamination.

Phytophthora is a slow growing fungus on artificial medium and on routine isolating media used for fungus isolation, other fast growing fungi from the soil or infected plant part usually cover its growth. A number of techniques are used for the detection of multiple species of *Phytophthora* within a sample (Gallegly and Hong, 2008). With the development of a number of media for selective isolation of this pathogen, it becomes easy to work with *Phytophthora* species. *Phytophthora* can be detected by plating diseased tissue onto selective agar containing antibacterial and antifungal antibiotics allowing outgrowth of *Phytophthora* from the tissue and identification by morphological characters (Tsao, 1983; Kox *et al.,* 2007). More than 35 selective media have been developed and tried by various workers (Tsao, 1983).

The result of present study will make isolation of *Phytophthora* from citrus nurseries and orchards simple and cost effective, without the use of any antibacterial and antifungal chemicals in media to supress the growth of competing organisms. Besides, this technique can be applied to other crop species

affected by soil borne *Phytophthora* diseases.

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Comparative growth, cultural characteristics of *Sclerotium rolfsii* isolates from selected locations of Tamil Nadu

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Peppermint (*Mentha piperita* L.) is an important aromatic perennial herb grown throughout the world, and about 70% of the International annual requirement is met from crops raised in the central region of the Indo-Gangetic plains. Peppermint, serves as a source of menthol, menthone, isomenthone *etc.* and these constituents are used in medicinal preparation, toothpaste, mouthwash, perfumery, cosmetics and as flavoring agents.

In India, peppermint is grown throughout the year and affected by fungal diseases caused by *Rhizoctonia bataticola, Verticillium dahliae*, *Colletotrichum cocodes, Rhizoctonia solani* and *Sclerotium rolfsii*. Of these, collar rot caused by *Sclerotium rolfsii* Sacc. is a major constraint in the pepper mint cultivation in Tamil Nadu as it causes considerable damage to the crop. The present study was conducted to know the effect of different culture media on the growth and sclerotial production of *S. rolfsii* isolates.

The collar rot samples were collected from major mint growing tracts of Tamil Nadu *viz.* Coimbatore, Dindigul, Erode, Hosur, Krishnagiri, Namakkal, Salem and Theni districts. The infected plant material was washed, cut into 5 mm segments including the advancing margins of infection. The segments were surface sterilized in 0.5% sodium hypochlorite solution for 5 min. and rinsed thrice in sterilized distilled water, dried in between sterilized filter paper placed (3 pieces per plate), inoculated on fresh potato dextrose agar (PDA) medium (Ainsworth, 1961) impregnated with streptomycin, and incubated for seven days at 28±2°C. The fungal growth on 5th day, was cut by inoculation loop and transferred aseptically to the PDA slants and allowed to grow at room (28±2°C) temperature to obtain the pure culture of the fungus. The culture thus obtained was stored in refrigerator at 5°C for further studies and was sub cultured periodically. The purified isolates were identified based on morphological and colony characteristics (Watanabe, 2002) and designated as I_1 to I_8.

The effect of nine solid media *viz.,* Carrot agar, Corn meal agar, Czapek's dox agar, Malt extract agar, Oat meal agar, Potato dextrose agar, Richards agar, Rose bengal agar and Yeast extract agar was studied on the growth of different isolates of the pathogen. Fifteen ml of molten media were dispensed into each of 90 mm sterile Petri plates. Mycelial discs (5 mm) taken from the advancing margins of seven days old culture of *S. rolfsii* by the aid of cork borer were separately placed each at the centre of the plate containing the above mentioned medium. The inoculated plates were incubated at room temperature (28±2°C) for nine days and the diameter of the mycelial growth of pathogen was measured after five days of incubation. Further, the plates were examined for the cultural characteristics like growth of mycelium, sclerotial initiation, colour and shape of the sclerotia. Each plate was replicated thrice.

The inoculated plates were incubated at room temperature (28±2°C) and the mycelial growth of the pathogen was recorded at various intervals (24, 48, 72, 96, 120 and 144 h) and the colour and number of sclerotia per plate were recorded after 144, 192 and 216 h

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Table 1. Growth and sclerotial formation by *S. rolfsii* isolates on PDA plates after different hours of incubation (28 ± 2°C)

Isolate	Mycelial growth (mm)						Colour of sclerotia			No. of sclerotia / plate		
	24 h	48 h	72 h	96 h	120 h	144 h	144 h	192 h	216 h	144 h	192 h	216 h
I_1	-	-	25	61	80	90	W	LB	DB	240	261	278
I_2	-	-	21	59	73	90	W	LB	DB	216	210	182
I_3	-	-	17	50	67	90	OW	LB	DB	140	172	111
I_4	-	-	20	57	71	90	W	LB	DB	130	116	102
I_5	-	-	12	40	59	90	W	LB	DB	108	177	152
I_6	-	-	18	51	68	90	OW	LB	DB	132	181	143
I_7	-	-	14	45	60	90	OW	LB	DB	103	129	115
I_8	-	-	13	41	70	90	W	LB	DB	220	246	223
CD (p=0.05)	-	-	2.12	2.58	2.64	-	-	-	-	4.23	4.20	4.21

W-White; OW-Off white; LB-Light brown; DB-Dark brown; - = No growth

In all, eight isolates were isolated and designated as I_1 to I_8 and based on the virulence test the isolate-I_1 was the highly virulent one.

Growth and sclerotial formation of different isolates grown on PDA plates revealed that mycelial growth of different isolates varied considerably. Initially in the first two days of incubation, there was no mycelial growth in all the isolates tested in the present study. Whereas, the whole plate was covered with mycelium after six days of incubation in all the isolates tested.

The formation of sclerotia was initiated at six days of incubation. Initially they were white in colour and at the end of 9th day the colour became light brown in all the isolates. The number of sclerotia /plate varied between the isolates. The highest number of sclerotia per plate was observed in the isolate-I_1 whereas, the least number of sclerotia was observed in the isolate- I_4 (Table 1).

Initially white colour sclerotia were formed. Then the colour changed from white to light brown and dark brown as they attained maturity after utilization of nutrients, the plates became dry. However, dark brown coloured sclerotia survived for longer period. The change in colour of sclerotia might also be due to utilization (or) exhaustion of nutrients (Yaqub and Shahazad, 2005; Azhar Hussain *et al*., 2003).

It was further observed in the present studies that isolates with heavy mycelial growth produced more number of sclerotia. These findings were consistent with the early investigations made by Tripathi and Khare (2006) and Santha *et al*. (2012).

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Management of collar rot of groundnut caused by *Aspergillus niger*

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Groundnut (*Arachis hypogae* Link) is an important oilseed crop and world's largest source of edible oil. The major constraints in its production are unreliable rainfall pattern, poor germination or early mortality of seedlings due to seed and soil borne diseases. Collar rot caused by *Aspergillus niger* is a major disease of groundnut crop in India and conventionally controlled through seed dressing chemicals, the efficacy of which lasts for 3-4 weeks only (Gangopadhyay *et al*., 1996; Parakhia and Akbari, 2004). An attempt was, therefore, made to control this disease through alternative means by amendments, bio-control agents and neem formulation.

A field experiment with groundnut susceptible cv. MH 4 was conducted in a sick plot to find out the effect of different treatments viz., pre-sowing application of half and fully decomposed poultry manure (2 tons/acre), seed treatment (ST) with *Pseudomonas maltophila* strain 4, *Pseudomonas fluorescens* and *Rhizobium* @ 200 g/32 kg seed, ST with Nimbecidine @ 20 ml/kg seed and Captan 3g/kg seed. The crop was grown with 30 x 15 cm spacing in plot size of 5 m x 4 m. Each treatment was replicated thrice. All the recommended agronomic practices were adopted during the crop period. The disease incidence was recorded periodically and pod yield was recorded at harvest. The data were analyzed statistically using ANOVA.

The results from the study revealed that all the treatments were effective in controlling the collar rot disease. The least disease incidence of 8.7 per cent and highest seed yield (12.6 q/ha) were recorded in soil application of fully decomposed poultry manure compared to 26.5 per cent disease incidence and 6.9 q/ha pod yield in control. The present findings fall in line with Raj Kumar and Raja (2007) who reported decreased charcoal rot incidence in sunflower with

Table 1. Effect of different treatments on the control of collar rot in groundnut

Treatment	Dose	Method of application	Disease incidence (%)***	Disease control (%)	Pod yield (q/ha)***
Poultry manure	2 t/acre (half decomposed)	PSA*	10.0	62,3	11.6
"	2 t/acre(fully decomposed)	PSA	8.7	67.2	12.6
Nimbecidine	20 g/kg seed	ST**	13.4	49.4	9,9
P. maltophila strain-4	200 g/32 kg seed	ST	11.2	57.7	11.2
Rhizobium GN-1	200 g/32 kg seed	ST	18.0	32.1	9.3
P. fluorescens	200 g/32 kg seed	ST	18.4	30.6	9.0
Captan	3 g/kg seed	ST	17.0	35.8	9.2
Control	-	-	26.5	-	6.9
CD (p = 0.05)			1.33		

*Pre sowing application, **Seed treatment, ***Average of two years data

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increased poultry litters. Seed treatment with Captan could result in 35.8 per cent disease control. Seed treatment with *Pseudomonas maltophila* also gave promising results

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Assessment of mungbean genotypes for resistance to yellow mosaic and crinkle diseases

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India has largest pulse production area of about 24 m ha with production of grains only upto 14.87 m tons per annum. The productivity of pulses staggers around 600 Kg/ha, well below the world average productivity 846 Kg/ha during last 15-20 years due to the effect of several biotic constraints (Biswas *et al.,* 2009). Of the biotic constraints, diseases caused by viral pathogens namely mungbean yellow mosaic and crinkle diseases are major bottlenecks in its cultivation. As a result of infection, the plants bear very less flowers and pods. The pods are deformed and smaller in size. The seeds are also shriveled and lighter in weight there by affecting the yield drastically (Nene, 1969). Though, this disease can be kept under check by controlling the vector (white fly) population through chemicals but a better way to manage this disease is through host resistance. Screening of cultivars is important to select the resistance against yellow mosaic and crinkle viral diseases to be further exploited in breeding programme

With a view to search for host resistance, advance entries of mungbean were evaluated against yellow mosaic and crinkle at CCS Haryana Agricultural University, Regional Research Station, Bawal during the year 2008 following recommended package and practices for the crop. Observations were recorded at the time of maturity of the crop by counting the total number of plants and diseased plants for calculating the per cent disease incidence (Mote *et al.,* 1994). Based on per cent disease incidence, the plants were categorized for yellow mosaic and crinkle as 0-10% = Resistant (R); 11-20% = Moderately Resistant (MR); 21-30% = Tolerant (T); 31-50% = Susceptible (S) and 51-100% = Highly Susceptible (HS).

It is evident from the Table 1 that seven genotypes viz., KM8 203, KM8 205, KM8 208, KM8 217, KM8 218, KM8 222, KM8 232 were completely free from yellow mosaic. Seven elite lines (MH 707, MH 708, MH 709, MH 717, MH 719, Satya and Basanti) in MLT I, five (MH 534, MH 564, MH 748, Satya and Basanti) in MLT II, 15 (KM8 201, KM8 202, KM8 206, KM8 211, KM8 214, KM8 215, KM8 216, KM8 219, KM8 220, KM8 221, KM8 224, KM8 225, Muskan, Satya and Basanti in IVT and only one (KM8 124) in AVT fell under resistant category to yellow mosaic as the disease incidence in these genotypes was less than 10 per cent. The genotypes MH 714, MH 717, MH 719 and Satya in MLT I, MH 614 and Muskan in MLT II, KM8 216, KM8 217, KM8 221, KM8 222, KM8 223 and Muskan in IVT and KM8 121, KM8 123 and KM8 124, in AVT were observed resistant to crinkle disease. The remaining genotypes had variable degree of incidence to both the diseases hence were rated moderately resistant/ tolerant or susceptible/highly susceptible. Pathak and Jhamaria (2004) observed two varieties ML 5 (2.2%) and MUM 2 (3.1% disease incidence) resistant to yellow mosaic. The varieties/advance entries found resistant in the present study can be recommended for cultivation in yellow mosaic prone areas for minimizing the losses and further exploitation in the breeding programs.

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Table 1. Reaction of mungbean genotypes against yellow mosaic and crinkle diseases in different trials

MLT I

Yellow mosaic

MH 702 (20.6)*, MH 705 (12.9), MH 707 (9.1), MH 708 (8.8), MH 709 (6.5), MH 714 (11.1), MH 715 (10.3), MH 717 (5.3), MH 719 (4.4), MH 721 (20.4), MH 724 (54.5), MH 729 (27.7), MH 732 (11.3), MH 735 (11.5), MH 736 (30.5), MH 740 (21.4), MH 742 (16.6), Asha (11.1), Muskan (13.2), Satya (4.5) and Basanti (Nil).

Crinkle

MH 702 (17.6), MH 705 (16.1), MH 707 (18.2), MH 708 (20.5), MH 709 (12.9), MH 714 (6.7), MH 715 (11.8), MH 717 (5.3), MH 719 (8.7), MH 721 (16.7), MH 724 (18.2), MH 729 (33.3), MH 732 (14.5), MH 735 (15.4), MH 736 (25.0), MH 740 (21.4), MH 742 (13.3), Asha (20.6), Muskan (19.1), Satya (5.6) and Basanti (11.6).

MLT II

Yellow mosaic

MH 318 (11.9), MH 421 A (16.4), MH 510 (14.3), MH 512(11.5), MH 534 (8.7), MH 539 (18.4), MH 560 (13.6), MH 562 (27.5), MH 564 (3.6), MH 565 (14.3), MH 612 (24.2), MH 613 (26.8), MH 614 (14.5), MH 745 (12.5), MH 748 (8.9), Asha (21.1), Muskan (10.2), Satya (15.7) and Basanti (14.3).

Crinkle

MH 318 (19.0), MH 510 (14.3), MH 421 A (19.4), MH 512(11.5), MH 534 (19.6), MH 539 (20.4), MH 560 (12.3), MH 562 (19.6), MH 564 (8.3), MH 565 (19.0), MH 612 (19.7), MH 613 (24.4), MH 614 (8.1), MH 745 (10.0), MH 748 (23.5), Asha (13.3), Muskan (9.1), Satya (14.3) and Basanti (13.2).

IVT

Yellow mosaic

KM 8 201 (1.9), KM 8 202 (6.0), KM 8 203 (NIL), KM 8 205 (NIL), KM 8 206 (1.9), KM 8 208 (NIL), KM 8 211 (3.8), KM 8 213 (19.2), KM 8 214 (3.8), KM 8 215(5.3), KM 8 216 (1.6), KM 8 217 (NIL), KM 8 218 (NIL), KM 8 219 (1.8), KM 8 220 (8.6), KM 8 221 (6.7), KM 8 222 (NIL), KM 8 223 (10.6), KM 8 224 (4.8), KM 8 225 (5.1), KM 8 226 (22.5), KM 8 227 (22.7), KM 8 232 (NIL), KM 8 235 (9.8), Asha (13.3), Muskan (9.1), Satya (14.3) and Basanti (13.2).

IVT

Crinkle

KM 8 201 (24.1), KM 8 202 (12.0), KM 8 203 (32.3), KM 8 205 (18.2), KM 8 206 (20.8), KM 8 208 (10.5), KM 8 211 (25.0), KM 8 213 (30.8), KM 8 214 (9.4), KM 8 215(21.1), KM 8 216 (7.9), KM 8 217 (9.8), KM 8 218 (18.0), KM 8 219 (16.4), KM 8 220 (17.1), KM 8 221 (6.7), KM 8 222 (7.5), KM 8 223 (7.0), KM 8 224 (23.8), KM 8 225 (17.9), KM 8 226 (10.0), KM 8 227 (29.5), KM 8 232 (16.3), KM 8 235 (19.5), Asha (13.3), Muskan (9.1), Satya (14.3) and Basanti (13.2).

AVT

Yellow mosaic

KM 8 121 (12.9), KM 8 123 (11.7), KM 8 124(7.4), KM 8 125 (13.9) and KM 8 126 (10.6)

Crinkle

KM 8 121 (6.5), KM 8 123 (8.3), KM 8 124(9.9), KM 8 125 (11.6) and KM 8 126 (18.2)

* Figures in parentheses show per cent disease incidence

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Alternate method for management of groundnut collar rot (*Aspergillus niger*)

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Groundnut (*Arachis hypogaea* L.), is one of the important oil seed crops of the world. India is a major groundnut producing country and ranks second in the world. Andhra Pradesh at national level holds a key position in area and production. Groundnut is attacked by several pathogens. Among them, collar rot of groundnut caused by *Aspergillus niger* van Tieghem is a very common and destructive seed and soil borne disease in India causing seedling losses up to 50 per cent, largely account for the pre and post-emergence death of the seed and seedlings.

Though several fungicides have been recommended for managing this pathogen, but with a limited success. Induction of natural disease resistance using physical, biological and/or chemical elicitors has received increasing attention over recent years, it being considered a preferred strategy for disease management. A lot of information is also available on use of different groups of non-conventional chemicals with little or no direct toxicity that could reduce disease incidence significantly in many crops. In this context, an experiment was conducted to reduce the collar rot incidence through developing systemic defense mechanism in host by using non-conventional chemicals.

The experiment was conducted at department of Plant Pathology, Agricultural College, Bapatla on efficacy of non-conventional chemicals on pre and post emergence damping off and collar rot incidence caused by *A. niger*. The pathogen was isolated form infected groundnut plants and maintained on potato dextrose agar. The pathogen was mass multiplied on sand maize meal (3:1) medium (Waller *et al*., 2002). The whole medium was used as inoculum. The plots were inoculated by adding 100g of 50% inoculum mixture. Ten non-conventional chemicals *viz*., lithium sulphate, cupric sulphate, cupric chloride, boric acid, zinc sulphate, ferrous sulphate, manganese sulphate, salicylic acid, barium nitrate and barium chloride were tested at 10^{-2}, 10^{-3} and 10^{-4} M concentrations in pot culture experiment. Surface sterilized (0.1% sodium hypochlorite) seeds were soaked for 24 hours in the chemical solutions. Each pot was sown with ten groundnut seeds of JL 24 and maintained in poly house. Seeds soaked in sterile distilled water served as control. Factorial completely randomized block design was followed with three replications. The observations on pre and post-emergence damping off up to 15 days and collar rot incidence up to 50 days were recorded and expressed in the disease percentage and disease control over control.

Pre and post-emergence damping off: Perusal of data (Table 1) revealed that the disease incidence was significantly influenced by chemicals and interaction of chemicals and concentrations but concentrations were found to be non significant. Irrespective of the concentrations tested, lithium sulphate followed by barium nitrate were most effective and significantly superior to all other treatments by recording 32.91 and 35.51 per cent disease incidence, respectively followed by cupric sulphate (39.41%), cupric chloride (40.58%) and barium chloride (41.73%) and were at par with each other whereas boric acid was found to be least effective (51.01%).

The interaction between chemicals and their concentrations was found significant. Differences among the chemicals in respect of their ability to

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Table 1. Effect of seed soaking in heavy metal salts and weak acids on groundnut pre and post-emergence damping off caused by *Aspergillus niger*

Chemical	Concentration of chemicals						Mean
	10^{-2} M		10^{-3} M		10^{-4} M		
	Pre and post-emergence damping off (%)	Disease control (%)	Pre and post-emergence damping off (%)	Disease control (%)	Pre and post-emergence damping off (%)	Disease control (%)	
Lithium sulphate	32.06 (34.36) *	43.43	21.67 (27.52)	63.88	45.00 (42.12)	20.59	32.91 (34.67)
Cupric sulphate	43.22 (41.07)	23.73	46.67 (43.09)	22.22	28.33 (32.14)	50.00	39.41 (38.77)
Cupric chloride	44.40 (41.76)	21.65	42.34 (40.58)	29.43	35.00 (36.18)	38.24	40.58 (39.51)
Boric acid	48.05 (43.88)	15.21	51.67 (45.96)	13.88	53.33 (46.92)	5.89	51.01 (45.59)
Zinc sulphate	49.99 (44.99)	11.79	43.33 (41.16)	27.78	51.67 (45.96)	8.82	48.33 (44.04)
Ferrous sulphate	47.21 (43.38)	16.69	38.33 (38.16)	36.11	48.33 (44.04)	14.72	44.63 (41.86)
Manganese sulphate	31.03 (33.66)	45.24	46.67 (43.08)	22.21	55.00 (47.88)	2.94	44.23 (41.54)
Salicylic acid	49.45 (44.86)	12.21	43.64 (41.32)	27.27	41.67 (40.17)	26.47	45.02 (42.12)
Barium nitrate	46.53 (42.99)	17.89	35.00 (36.23)	41.67	25.00 (29.92)	55.88	35.51 (36.39)
Barium chloride	35.18 (36.37)	37.92	41.67 (40.10)	30.55	48.33 (44.04)	14.72	41.73 (40.17)
Control	56.67 (48.84)	-	60.00 (50.79)	-	56.67 (48.85)	-	57.78 (49.49)
Mean	44.01 (41.47)		42.82 (40.73)		44.39 (41.66)		
				CD (p=0.05)			
Chemicals				3.95			
Concentration				NS			
Chemicals X concentration				6.83			

* Figures in parentheses are angular transformed values

reduce disease incidence were influenced by the concentrations. Similarly, the differences observed among the concentrations varied with the type of chemical used. But the test compounds showed a fairly strong effect at low concentrations than at higher concentrations. Maximum disease control of 63.88% was observed with lithium sulphate (10^{-3} M) followed by 55.88% and 50.00% with barium nitrate (10^{-4}M) and cupric sulphate (10^{-4}M), respectively; which were most effective and significantly superior over all other treatments.

Collar rot: The collar rot incidence was significantly reduced by all the chemicals tested compared to control (Table 2). Barium nitrate followed by lithium sulphate, cupric chloride and ferrous sulphate were most effective and significantly superior to all other treatments by recording 17.46, 18.02, 18.26 and 18.99% collar rot incidence, respectively, which were at par with each other. Boric acid was least effective by recording 27.59 per cent disease incidence. The effect of concentrations on collar rot incidence irrespective of chemicals was found to be non significant.

The interaction between chemicals and concentrations was found to be significant. Differences among the chemicals were influenced by the different concentrations. Similarly, the effect of different concentrations varied with the different chemicals. Maximum disease control of 71.57% was observed with lithium sulphate (10^{-3}M) over control followed by 68.16%, 66.69%, 62.22%, 56.58% and 54.05% with barium nitrate (10^{-4}M), cupric sulphate (10^{-4}M), manganese sulphate (10^{-2}M), cupric chloride (10^{-4}M) and zinc sulphate (10^{-4}M), respectively which were most effective and significantly superior to all the other treatments.

The results are in agreement with the findings of Dasgupta *et al*. (2000) who observed that incidence of pre and post-emergence damping off and collar rot of groundnut effectively reduced when seeds were pre soaked in heavy metal salts which could be due to the rapid development of antifungal substances in

Table 2. Effect of seed soaking in heavy metal salts and weak acids on groundnut collar rot incidence caused by Aspergillus *niger*

Chemical	Concentration of chemicals used						Mean
	10^{-2} M		10^{-3} M		10^{-4}M		
	Collar rot (%)	Disease control (%)	Collar rot (%)	Disease control (%)	Collar rot (%)	Disease control (%)	
Lithium sulphate	16.98 (24.29)*	55.70	12.63 (20.79)	71.57	24.44 (29.46)	40.55	18.02 (24.84)
Cupric sulphate	23.33 (28.86)	39.13	17.77 (24.91)	60.01	13.69 (21.70)	66.69	18.26 (25.16)
Cupric chloride	31.67 (33.86)	17.37	23.33 (28.64)	47.50	17.85 (24.80)	56.58	24.28 (29.09)
Boric acid	33.33 (35.00)	13.04	26.11 (30.61)	41.25	23.33 (28.86)	43.25	27.59 (31.49)
Zinc sulphate	30.00 (30.08)	21.73	28.87 (32.36)	35.03	18.89 (25.74)	54.05	25.92 (30.39)
Ferrous sulphate	20.55 (26.88)	46.39	16.98 (24.29)	61.79	19.44 (26.06)	52.71	18.99 (25.74)
Manganese sulphate	14.48 (22.33)	62.22	24.44 (29.46)	45.00	30.00 (33.08)	27.02	22.97 (28.29)
Salicylic acid	30.55 (33.51)	20.29	27.78 (31.74)	37.49	24.44 (29.14)	40.55	27.59 (31.46)
Barium nitrate	19.44 (26.06)	49.28	19.84 (26.20)	55.35	13.09 (21.20)	68.16	17.46 (24.49)
Barium chloride	23.33 (28.64)	39.13	21.74 (27.66)	51.10	26.11 (30.61)	36.49	23.73 (28.98)
Control	38.33 (38.08)		44.44 (41.75)		41.11 (39.83)		41.29 (39.89)
Mean	25.64 (30.05)		23.99 (28.95)		22.94 (28.22)		
				CD (p=0.05)			
Chemicals				4.07			
Concentration				NS			
Chemicals X Concentration				6.69			

* Figures in parentheses are angular transformed values

treated plants. It appeared that the seed soaking with these 10 compounds uncommonly used for plant protection and also having no or little fungitoxicity at the concentrations employed could substantially limit the disease development in groundnut (Terrya and Joyceb, 2004; Rajasekharam *et al.,* 2007).

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Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *melonis* in muskmelon wilt

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Muskmelon (*Cucumis melo* L.) is an important vegetable crop and is known to be attacked by number of pests, fungi, virus and nematodes during different stages of its growth, thus, reducing its production. Of the various soil borne pathogens limiting yields of crop, root-knot nematode (RKN), is an important pathogen (Ploeg and Phillips, 2001) causing yield losses upto 37.5 per cent in melon crop (Jain *et al.*, 2007). Nematode-fungal disease complexes involving root-knot nematode are common. This association may be synergistic, additive or antagonistic with respect to disease development and yield loss. Both *Meloidogyne* sp. and *Fusarium* sp. have been reported to be associated in muskmelon and hence the present studies were undertaken to study the interaction of these pathogens in wilt disease complex.

Cultures of *Meloidogyne* sp. and *Fusarium* sp. were obtained from diseased muskmelon plants and identified as *Meloidogyne incognita* (Eisenback *et al.*, 1981; Jepson, 1987) and *Fusarium oxysporum* f. sp. *melonis* (Leslie and Summerell, 2006). A monospecific population of *M. incognita* was maintained on tomato plants. Fungus was isolated from diseased plants and maintained on Potato Dextrose Agar (PDA) medium. Pathogenicity of the culture was confirmed on susceptible variety (Punjab Sunehri) of muskmelon. The inoculated plants showed typical symptoms of the wilt disease. For the pot experiments, *F. oxysporum* f. sp. *melonis* was multiplied on sterilized sand-maize medium. Muskmelon seeds were sown in sterilized soil to raise seedlings. Twenty five day old seedlings of muskmelon variety (Punjab Sunehri) were transplanted in pots filled with 2kg sterilized soil. The treatments were: T1 - *M. incognita* alone; T2 - *F. oxysporum* f. sp. *melonis* alone; T3 - *M. incognita* seven days prior to *F. oxysporum* f. sp. *melonis*; T4 - *F. oxysporum* f. sp. *melonis* seven days prior to *M. incognita*; T5 - Simultaneous inoculations of *M. incognita* and *F. oxysporum* f. sp. *melonis*; T6 - Control (without pathogen inoculation). Nematode inoculum @ two juveniles/g of soil and fungal inoculum @ 8g/kg of soil were added after three days of transplanting by removing the soil around the root zone and after inoculation, the roots were covered gently with sterilized soil. Each treatment was replicated thrice along with control. Sixty days old crop was uprooted and observations were recorded on plant growth parameters viz., shoot length, root length, number of branches, fresh shoot weight and fresh root weight. Nematode population in soil as well as in roots (root galling index) was also recorded. Root galling index (RGI) was graded on a scale of 0-5 as given by Hussey and Janssen (2002). Disease severity of plants in pots was recorded on 0-4 scale (Kaur, 2005).

The experiment was laid under completely randomized block design. The data were analysed statistically. Significant differences were determined using least significant difference (LSD) test at probability level of 0.05.

Observations on root gall index and percent wilt disease intensity revealed that single or combined inoculations of *M. incognita* and *F. oxysporum* f. sp. *melonis* had significantly affected plant growth parameters, nematode population and wilt disease as compared to uninoculated control.

Of the two pathogens when inoculated singly, *M. incognita* (T1) caused significant reduction in plant growth parameters viz., shoot length and weight, root length and weight and number of branches

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compared to T2 treatment i.e. *F. oxysporum* f. sp. *melonis* and control (Table 1). Shoot length in *M. incognita* inoculated treatment was observed to be 58.7 cm as compared to 62.8 cm in *F. oxysporum* f. sp. *melonis* treatment. Root length was also reduced by 31 per cent in *M. incognita* inoculated pots. Nematode population and root galling index was observed to 566.5/250cc soil and 5.0, respectively in T1 (*M. incognita* alone). Wilt disease intensity was observed to be 42.7 per cent in *F. oxysporum* f. sp. *melonis* alone treatment (T2).

In combined inoculations of both pathogens, simultaneous as well as sequential; greater reduction of plant growth parameters was observed as compared to individual inoculations. Maximum decrease in shoot length and weight (49.7 per cent and 40.1 per cent) was observed in simultaneous inoculations of both pathogens (T5). Decrease in root length and root weight was also higher in this treatment (53.7 and 57.9 per cent, respectively). In case of sequential inoculations of both pathogens, significantly higher reduction in shoot length (51.5 cm) and root length (16.3 cm) was observed in inoculation of *M. incognita* seven days prior to *F. oxysporum* f. sp. *melonis* (T3) as compared to 55.7 cm and 18.8 cm shoot and root length, respectively in T4 treatment (i.e., inoculation of *F. oxysporum* f. sp. *melonis* seven days prior to *M. incognita*).

Significant higher reductions in root-knot nematode populations were observed in combined inoculations of both pathogens as compared to single inoculations (Table 2). Soil nematode population (212.5/250cc soil) and root galling index (2.5) were observed to be minimum in concomitant inoculation of *M. incognita* and *F. oxysporum* f. sp. *melonis* (T5). In sequential inoculations, reduction in root galling index (RGI=3.3) and nematode population (312.3/250cc soil) were higher in inoculation of *F. oxysporum* f. sp. *melonis* seven days prior to *M. incognita* (T4) in comparison to inoculation of *M. incognita* seven days prior to *F. oxysporum* f. sp. *melonis* (T3). Wilt disease intensity was higher in simultaneous inoculations of *M. incognita* and *F. oxysporum* f. sp. *melonis* followed by sequential inoculation of *M. incognita* seven days prior to *F. oxysporum* f. sp. *melonis* (T3).

The present study revealed greater reduction in growth parameters in combined inoculations. The

Table 1. Influence of single or sequential inoculation of *M. incognita* and *F. oxysporum* f. sp. *melonis* in pots on plant growth parameters and nematode population and wilt disease intensity of muskmelon

Treatment	Shoot				Root				No. of branches	Nematode population/ 250cc soil	Root gall index (RGI)	Disease intensity (%)
	Length (cm)	Per cent decrease	Fresh weight (g)	Per cent decrease	Length (cm)	Per cent decrease	Fresh weight (g)	Per cent decrease				
T1- *M.incognita* alone	58.7	39.2	38.9	27.4	15.6	31.2	8.4	48.7	12.5	566.5 (6.3)	5.0	-
T2- *F. oxysporum* alone	62.8	34.9	42.7	20.3	19.3	14.9	10.9	29.2	15.7	-	-	42.7 (41.0)
T3- *M. incognita* (7 days prior to *F. oxysporum*)	51.5	46.6	35.9	33.0	16.3	33.5	6.2	61.0	10.7	370.5 (5.9)	4.0	67.8 (56.2)
T4- *F. oxysporum* (7 days prior to *M. incognita*)	55.7	42.3	39.4	26.4	18.8	23.7	7.1	55.3	17.7	312.3 (5.7)	3.3	45.3 (44.9)
T5- *M. incognita* and *F. oxysporum* simultaneously	48.5	49.7	32.1	40.1	10.5	53.7	6.7	57.9	14.0	212.5 (5.4)	2.5	84.3 (67.5)
T6- Uninoculated Control	96.6	-	53.6	-	22.7	-	15.9	-	19.3	-	-	-
C.D. (p=0.05)	3.76		3.17		2.41		0.73		2.52	(0.13)	0.6	(14.1)

***** Figures in parentheses are arc sine transformed values

results are in agreement with earlier observations of Safiuddin and Shahab (2012) and Sharma and Khan (2013) who observed that nematode inoculation prior to fungus and simultaneous inoculation of nematode and fungus significantly reduced the plant growth. Present study also showed that presence of root-knot nematode along with fungus adversely affected the root-knot disease and are in conformity with Akhtar *et al.* (2007) who studied that the nematode development and multiplication were adversely affected by *F. solani* irrespective of time of inoculation. Reduction of nematode population build up and number of galls in presence of fungus might be due to reduced root system and fungal disruption of nematode feeding sites. Abd-El-Fattah *et al.* (2012) had also observed increase in wilt disease intensity due to combined presence of both pathogens. In the present study also wilt disease intensity was observed to be higher in combined inoculations..

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Utilization of native strains of bioagents for ecofriendly management of black scurf of potato

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Potatoes are widely cultivated, and could contribute to reduce worldwide food shortages (El-Kot, 2008). Potato crop is grown in more than 75,000 ha in Punjab. With private buyers like Potato Growers Associations, PepsiCo, etc., there is wider scope of diversification for this crop, especially in rain-fed areas of Hoshiarpur district, where rice is replacing the Kharif maize crop. Unfortunately, the crop is prone to various diseases which severely reduces its production. The disease which most commonly affects the potato tubers, sprouts, stems and stolons is black scurf. The management of *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris*), the cause of potato black scurf, is complex due to its soil-borne nature and high level of survival. This pathogen is present in most of the soils and if once established in a field, remains there for an indefinite period (Rauf *et. al.* 2007). The disease commences by seed or soil-borne inoculum and both inocula are equally damaging. Presently, it is not possible to entirely control this disease, but severity may be limited by following a combination of crop protection strategies for successful disease management. The present study was undertaken to evaluate and identify effective bio-agents for control of black scurf of potato.

Pot experiment was conducted during *Kharif* 2012 in completly randomized block design with three replications in 25 x 12.5 cm pots using infected seed tubers of a susceptible variety 'Khufri Pukhraj'. *Trichoderma harzianum* (Th), *Trichoderma viride* (Tv*), Pseudomonas fluorescens (Pf)* and three new isolates of *Trichoderma harzianum* (T2, T3 and T6) and their combinations were evaluated through dip and spray methods. Dip and dry seed treatment, spraying with bio-agents was done before sowing. Mollasses @ 20g /lit of water were added in water for increasing adherence capacity of bio agents. Control pots without any treatment and with recommended treatment of Moncern @ 2.5 ml/ lit. of water for ten minutes as dip treatment were maintained. The disease intensity (based upon 0 - 4 scale) and yield of tubers in each replication were recorded.

All the strains exhibited antagonistic activity against *R. solani,* however a wide variation was found in the biocontrol potential. Results (Table 1) indicated that minimum disease intensity(1.67 %) was found in the combination of *T. harzianum* and *P. fluorescens* @ 5gm each/kg seed and 5 gm each/kg of soil before sowing along with the recommended treatment (diping in Moncern @2.5 ml /lit of water). Dipping of tubers in 20 gm/lit. of *T. harzianum* alone and *T. harzianum* (seed+soil) @ 10gm Th/kg of seed and 10gm Th/kg of soil before sowing, each showed 3.33% of disease intensity in comparison to 58.33% in the untreated control. Combination of *T. harzianum* and *P. fluorescens* @ 5 gm each/kg of seed and 5 gm each/kg of soil before sowing also recorded 181.6 gm tuber yield followed by 176.66 gm in case of *T. harzianum-Th* (seed+soil) @ 10gm of Th/kg of seed and 10 gm/kg of soil before sowing whereas it was 102.3gm in untreated control.

Efficacy of bio-agents against black scurf of potato has been reported earlier also by various workers (Tsror *et al*., 2001; Larkin, 2008). Wilson *et al.,* (2008) advocated that mixture of beneficial microorganisms reduced stem canker, black scurf, and common scab on tubers by 18–33% and increased yield by 20–23%. Tariq *et al.,* (2010) also observed that two *Pseudomonas* spp. *viz.* StT2 and StS3 were found most effective against black scurf disease of potato with 65.1 and 73.9 %

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Table 1. Black Scurf incidence and yield of potato tubers as affected by the treatment with bioagents

Treatment	Dose	Per cent disease intensity	Weight of potato tubers (gm/pot)
Th Dip*	20 gm/lit	3.33	173.33
Th Spray	20 gm/lit	18.33	170.0
Tv Dip	20 gm/lit	18.33	160.0
Tv Spray	20 gm/lit	25.00	125.0
Pf Dip	20 gm/lit	11.67	163.33
Pf Spray	20 gm/lit	33.33	146.6
T2 Dip	20 gm/lit	25.00	139.0
T2 Spray	20 gm/lit	33.33	131.0
T3 Dip	20 gm/lit	25.00	121.33
T3 Spray	20 gm/lit	41.67	113.33
T6 Dip	20 gm/lit	11.67	133.33
T6 Spray	20 gm/lit	25.00	120.0
Pf + T2 Dip	10 gm each/lit	25.00	127.0
Pf + T2 Spray	10 gm each/lit	18.33	115.33
Pf + T3 Dip	10 gm each/lit	18.33	163.33
Pf + T3 Spray	10 gm each/lit	11.67	142.66
Pf + T6 Dip	10 gm each/lit	25.00	151.0
Pf + T6 Spray	10 gm each/lit	25.00	141.0
Pf + Tv Dip	10 gm each/lit	5.00	121.66
Pf + Tv Spray	10 gm each/lit	11.67	103.33
Pf + Th Dip	10 gm each/lit	3.33	162.66
Pf + Th Spray	10 gm each/lit	5.00	145.0
Tv + Th Dip	10 gm each/lit	11.67	169.33
Tv + Th Spray	10 gm each/lit	18.33	133.66
Th + Pf (seed + soil)	5 gm each/kg of seed and 5 gm each/kg of soil before sowing	1.67	181.66
Th (seed + soil)	10 gm Th/kg of seed and 10 gm Th /kg of soil before sowing	3.33	176.66
Moncern 250 SL (Recommended treatment)	Moncern @ 2.5 ml/lit. of water for ten minutes as dip treatment	1.67	172.0
Control	No treatment	58.33	102.33
CD (p=0.05)		3.14	4.03

Th =*Trichoderma harzianum,* Pf= *Pseudomonas fluorescens,* TV= *Trichoderma viride,* T2,T3 and T6 = *Trichoderma harzianum* strains; * For 10 minutes

biocontrol efficacy along with 87.3 and 98.3% yield increase, respectively. These results suggest that *Pseudomonas* isolates have excellent potential to be used as effective biocontrol agents promoting plant growth with reduced disease incidence.

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Phytophthora fruit rot of pomegranate – a new report from Himachal Pradesh

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Pomegranate (*Punica granatum* L.) belonging to the family Punicaceae, is one of the favourite table fruits of tropical, subtropical and sub-temperate regions of the world. In India, pomegranate is grown to a limited extent in selected locations in almost all states, but is commercially cultivated in Maharashtra and Karnataka. During the last decade, pomegranate crop has witnessed a tremendous growth potential in Himachal Pradesh particularly in mid-hill zone (915-1523 m amsl) of Kullu and Mandi districts where it is cultivated on commercial scale. Its cultivation has now spread to other parts of the state where it has emerged as the main crop replacing stone fruits, apple and pear due to high yield potential and regular bearing habit besides high cost: benefit ratio. Presently around 2000 h area is occupied by this crop in the State. The crop is adversely affected by various foliar, fruit and soil borne diseases resulting in huge losses to the growers (Khosla and Bhardwaj, 2011). Amongst the fruit rots caused by *Coniella granati* (Sharma, 1998), *Phomopsis aucubicola* and *Phytophthora* sp, the rot caused by *Phytophthora* sp is most devastating because of its fast spread under high humidity conditions particularly in orchards located in mid-hill zone experiencing high rainfall (>1200mm/ year). Therefore, the present study was undertaken to find out the exact etiology of the disease.

Periodic survey of pomegranate growing areas was conducted during the years 2011 – 2013 to record the prevalence of the disease. Samples of infected leaves and fruits were collected and the associated pathogen was isolated on potato dextrose agar (PDA) medium. The cultures were purified by hyphal tip method and sent to National Center of Fungal Taxonomy, Inderpuri, New Delhi for identification. For proving pathogenicity, apparently healthy almost four months old fruits of cv. Kandhari Kabuli surface sterilized with rectified spirit were used. In one set of experiments, five such fruits were inoculated without injury while in another set of five fruits, injuries with sterilised needle were inflicted. Five mm dia mycelial discs of 7d old culture were placed on uninjured and injured fruits, covered with wet cotton plug and tied with parafilm. The inoculated fruits were kept in Petri dishes on a wet towel of sterile blotting paper and covered with a sterile glass jars in which high humidity was maintained by spraying distilled water. After 48 h, the cotton plugs were removed and the fruits were kept under jars at room temperature for symptom development. Sterile PDA plugs were placed on another five injured and uninjured fruits to serve as control.

In the fields during the month of July, water soaked light brown lesions initiating from the periphery of the leaf lamina which later turning dark brown and watery were frequently observed (Plate 1a). Such infected leaves later fell off in abundance. On fruits, the initial symptoms were observed on middle or lower portion as water soaked round lesions with light brown colouration (Plate1b). Later, such fruits were found covered with white fluffy cottony growth resulting into total rotting of the fruits (Plate1c). High incidence was observed in dense orchards and in the fruits near to the soil. Majority of such rotted fruits dropped and few remained hanging as dark brown mummified fruits on the trees (Plate1e). A similar white cottony fluffy growth of the mycelium mostly at distal end was also observed on the late emerging flowers

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Plate 1. a= Water soaked brown rotting of leaf ; b=initial rot symptom on fruit; c=arachnoid mycelial growth on fruit: d=fungal growth on flower; e=mummified fruits hanging on tree; f= fluffy mycelium in PDA slants; g=rose pattern growth of fungus in Petri dish; h=abundant sporangiophores with pyriform to ellipsoid sporangia (x100); i= sporangia with papilla (x100); j=arachnoid mycelial growth on fruit in pathogenicity test

(Plate1d). Such flowers later dried and dropped.

The survey conducted for three years revealed that the disease was more in orchards located in high rainfall areas and having dense canopy and also in the orchards where farmers had utilised the orchard floor between the rows for raising the tomato or capsicum crop. The disease was recorded to the tune of 13.6 % in Paniyala area of Bilaspur district, 4.1% in Bajaura, 2.4% in Jarar area of Kullu district and 21.6% in Balh area of Mandi district. A species of *Phytophthora* was consistently obtained from affected tissues of the leaf and fruit samples collected from these areas. In the culture medium a fluffy white colonies with arachnoid aerial mycelia were obtained (Plate1f) which grew in rose petal pattern (Plate1g), rather slow, covering whole Petri plate in 18 days when incubated at 25±1^{0}C. Mycelium was irregular, sparingly branched having aerial and submerged hyaline hyphae, slender, 2.77 to 7.4 µm in dia, often undulate, the walls stout and hyaline. Chlamydospores present, which were thick walled globose to ellipsoid 25.9 µm to 31.45 µm in dia and 3.7 x 25.9 µm in size, terminal on short lateral branches, solitary. Sporangiophores simple and were differentiated. Sporangia with short pedicel produced abundantly, 27.5 to 55.5 x 20.35 to 33.3 µm in size, terminal, pyriform to ellipsoid, hyaline, thin walled with prominent papilla on distal apex (Plate 1h-i). Each sporangium contained 8-12 kidney shaped zoospores with two unequal flagella attached to concave side. Size of zoospores ranged from 9.23 to 12.95 x 1.85 to 2.77 µm. Oogonia and antheridia were not observed. On the basis of morphological features, the pathogen was tentatively identified as *Phytophthora nicotianae*. The identity was confirmed by National Center of Fungal Taxonomy Inderpuri, New Delhi under NCFT Acc. No. 4470.11. Under pathogenicity tests, water soaked lesions with light brown colouration of fruit tissues started appearing on 3rd day of inoculation on injured fruits and on 5th day in uninjured fruits. A white fluffy mycelial growth started appearing on 5th and on 8th day in injured and uninjured fruits, respectively and the entire fruit was covered with cottony arachnoid mycelium within 10-12 days (Plate 1j). No such symptoms were however, observed on control fruits. The same pathogen was reisolated from fruits having typical fluffy mycelial growth.

Phytophthora nicotianae is a destructive pathogen widely distributed in tropical and warm-temperate regions (Hall, 1993) and is pathogenic to wide range of plant species (Erwin and Ribeiro, 1996). Sporulation of *Phytophthora* on lesion is favoured by high humidity and high temperature (Alvarez *et al.,* 2007). It may be due to this reason that a high incidence of this disease was observed under high humidity (in orchards having dense canopy) and high rainfall and temperature conditions prevailing in areas of Bilaspur district and Balh area of Mandi district of Himachal Pradesh. Jamadar *et al*. (2009) have however, reported the occurrence of *Phytophthora nicotianae* fruit rot of pomegranate as minor disease in Karnataka but Naema and Sharma (2006) have earlier reported a severe fruit rot of pomegranate caused by *P. nicotianae* var. *nicotianae* from Jabalpur, Madhya Pradesh. The present report however, constitutes the first record of pomegranate fruit rot caused by *P. nicotianae* from this region of the country.

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PCR based detection of latent infection of *Puccinia striiformis* f. sp. *tritici* in wheat

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Stripe rust (yellow rust), caused by *Puccinia striiformis* f. sp. *tritici,* is one of the most important diseases of wheat throughout the world (Saari and Prescott, 1985; Stubbs, 1985). This disease occurs regularly in varying proportions in north-western plain and hill zone of India. The onset of disease starts in the months of December-January with initial disease foci forming in sub-mountainous wheat growing areas of Punjab. In these situations, monitoring of this disease plays a key role in timely intervention for its effective management. Its infection usually takes place during the months of December-January and due to low prevailing temperature, its symptoms appear in many fields towards the end of January. This necessitates the use of a rapid and reliable method for detection of latent infection of pathogen as early as possible for its timely management. In the recent years, DNA based detection techniques have been employed successfully to detect plant pathogens in a quick and reliable manner (Edel, 1998; Ward, 1994; Mutasa *et al.,* 1995). In the current study, the validation of PCR based protocol (previously given by Wang *et al.*, 2007) to detect the latent infection of *P. striiformis* f. sp. *tritici* from wheat plants before the appearance of symptoms was undertaken.

Ten days old seedlings of susceptible cultivar Agra local were grown in small earthen pots and were artificially inoculated with uredospores of *P. striiformis* f. sp. *tritici*. The pots were provided with artificial humidity by covering with polythene bags. The inoculated pots were kept at 10±2° C for 48 h and then shifted to muslin cloth chamber in pot house. The leaf samples were collected for PCR assay from 4th to 15th days after inoculation. The symptoms of rust appeared after 16 days of inoculation. To further test the protocol, eighteen wheat leaf samples from apparently healthy plants in the month of January were collected from Wheat Research Farm of Punjab Agricultural University Campus. Additionally, wheat growing areas in Sub-mountainous agro-ecological zone of Punjab were surveyed and a total of 36 leaf samples were collected randomly from apparently healthy plants from thirteen locations. At least two leaf samples (100- 150 m apart) were collected from one location. All the leaf samples collected were washed with sterile distilled water to remove the surface contamination.

Individual leaf samples were cut in to small pieces, transferred to silica gel in 2 ml micro tubes. After 48 hours, the samples were taken out, two metallic beads per samples added and crushed over cover slip glasses. The samples were further crushed with Tissulizer for 10 minutes at 10000 rpm/ min. The total genomic DNA from the processed samples was isolated using CTAB method. Eight hundred (800) µl of CTAB buffer was added to crushed samples, mixed thoroughly and kept at 65 ºC on water bath for 1 hour. After incubation, 700 µl chloroform + isomylalcohol (24:1) was added to each sample and kept on shaker for 30 minutes. The samples were centrifuged at 10000 rpm for 10 minutes and the supernatant was transferred to fresh 1.5 ml micro tube. Approximately equal volume of chilled isopropanol was added and mixed properly and kept at -20ºC for 20 minutes. The samples were then centrifuged at 14000 rpm for 15 minutes to precipitate the DNA. The DNA pellets were washed twice with 500 µl of ethyl alcohol (70%) to remove any residual salts. The DNA pellets were

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air dried for two to three hours. After that the 100 µl of 1X TE (Tris EDTA buffer-10 mM Tris HCl, 1mM EDTA, pH 8.0) buffer was added and the tubes were left overnight at 4ºC before storing at -20ºC for further analysis. To remove the contaminant RNA from the DNA thus obtained, RNase (Promega Inc.) was added to a final concentration of 10 µl/ml, mixed and then incubated at 37ºC for 45 minutes. The quantity and quality of DNA was assessed by using Tecan 2000 Nanoquant plate. All the samples showed good quality DNA with A260 and 280 ratio between 1.79-1.83.

The oligonucleotide primer pair, Pst2 (F-5'GTCTGTAAGATGTTAGATGC3' and R-5'ATGCTGGCAGTGTGGTTG3') which is specific to *P. striiformis* f. sp. *tritici* as given by Wang *et al* (2007) was used to amplify an expected product size of 470bp. Amplifications were carried out in 30 µl reactions containing 25 ng/µl of template DNA, 1 µM of each primer, 6 µl of GoTaq green reaction buffer, 2.5 mM $MgCl_2$, 0.1mM of PCR nucleotide mix and 1U of Taq DNA polymerase, and the volume was topped up with nuclease free water. All biochemicals otherwise mentioned were sourced from Promega Inc., USA.

For *in vitro* amplification PCR was performed in a 96 well PCR plate (Axygen Inc.) in Eppendorf Master cycler ProS. The PCR plate was covered with thermo seal (Genexy Labs.) in order to avoid evaporation. The PCR amplification cycle consisted of initial denaturation for 3 min at 94º C followed by 34 cycles of denaturation at 94º C for 50 seconds, annealing at 54º C for 90 seconds, extension at 72º C for 2 minutes followed by a final extension at 72º C for 10 minutes. The PCR products were resolved on Ethidium bromide stained agarose gel prepared in 0.5X TBE buffer and photographed on gel documentation system (Syngene G:Box,UK) with "Gene Snap" software programme.

To detect the latent infection of *P. striiformis* f. sp. *tritici*, periodic samples were collected from inoculation up to appearance of the disease in artificially inoculated wheat seedlings of cultivar Agra local. The latent infection was detected with PCR, using specific primer Pst2. The PCR amplification of the DNA samples from infected leaves produced a single band of 470bp, which is specific to *P. striiformis* f. sp. *tritici*. During the current experimentation, latent infection of the pathogen was detected from the artificially inoculated leaves right from three days after inoculation. The results have been presented in Fig. 1.

To validate the results under field conditions, PCR was also performed on the leaf samples collected from Wheat Research Farm, PAU and farmers' field. The samples represented initial stage of rust infection, where in suspected flecks may or may not be actually due to infection of *P. striiformis* f. sp. *tritici*. Out of 17 samples collected from Wheat Research Farm, the latent infection was detected in 14 samples (Figure 2) using PCR assay. All the samples which showed symptoms having suspected flecks on the PAU farm were also found to be positive in PCR assay. It is further reported here that the suspected plants in which the pathogen was detected by PCR assay, developed rust symptoms later in two weeks time.

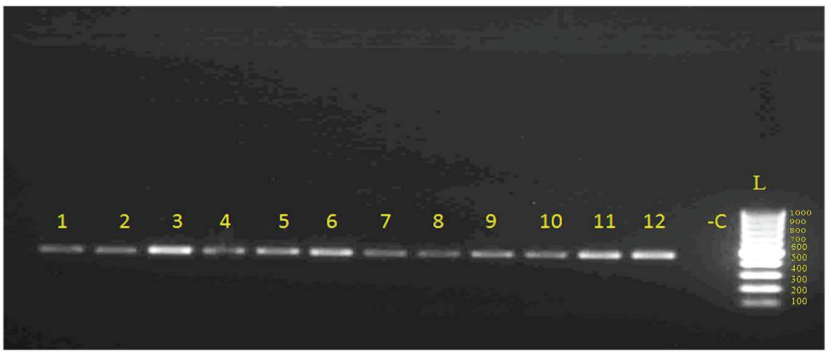

Fig. 1. PCR amplification of total genomic DNA isolated from artificially inoculated wheat Seedlings of cv. Agra local with *P. striiformis* f. sp. *tritici* using primer pair Pst2, Lanes 1-12 Contain amplified PCR products of from inoculated samples. Lanes 13 Contains (Negative control) Lanes 14 Contains100 bpDNA ladder (Promega Inc., USA)

Fig. 2. PCR amplification of total genomic DNA isolated using primer PST2 from samples infected with *P. striiformis* f. sp. *tritici,* Lanes 1-17 contain amplified PCR products from samples collected from Wheat Research Farm, PAU, Lanes 18 contains (positive control) PCR amplified product of DNA isolated from fresh uredospores of *P. striiformis* f. sp. *tritici,* Lane 19 is negative control and lane 20 contains 100 bpDNA ladder (Promega Inc., USA)

Fig. 3. PCR amplification of total genomic DNA isolated from 17 samples out of collected, From farmers' fields suspected to be infected with *P. striiformis* f. sp. *tritici* using specific primer PST2 from samples, Lanes 1-17 contain amplified PCR products of *P. striiformis* f. sp. *tritici*, from samples collected from farmers' fields. Only two samples viz. 4 and 5 showed amplification. Lane 18 and 19 contain positive and negative controls, respectively, Lane 21contains 100 bpDNA ladder (Promega Inc., USA), No amplification was observed in samples F18-36 (gel picture not shown here)

Out of 36 samples which were collected from farmers' fields, the pathogen was detected in only two samples with PCR assay (Figure 3) even though suspected flecks' symptoms were observed in 15 samples. During the follow-up survey of the same villages after three weeks, the visible symptoms of stripe rust appeared in those fields.

PCR based detection of latent infection of stripe rust can be very helpful in predicting the onset of this diseases in sub-mountainous wheat growing area of Punjab. The results of the present investigation showed that this technique can be employed for application of timely intervention strategies to manage this disease. Wang *et al.* (2007) developed this PCR based method for sensitive detection of the *Puccinia striiformis* f. sp. *tritici* where in stripe rust was also detected in the dormant stage by PCR assay in samples of wheat leaves taken during the winter season. They tested seven different isolates of *Puccinia striiformis* f. sp *tritici* which yielded a specific band of 470bp. The latent infection of *P. striiformis* was also detected with a pair of specific primer PSF by Zhao *et al.* (2007). Freeman *et al.* (2002) reported that PCR assay is the more sensitive method for early detection of the pathogen *Sclerotinia sclerotiorum* with specific primer.

This PCR based method will be useful for early detection of yellow rust prior to appearance of symptoms in the sub-mountainous areas of Punjab and cooler areas of Himachal Pradesh and Jammu and Kashmir, where initial foci of the disease appear. Once the pathogen is detected, the measures for its management can be timely adopted

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Management of grapewine downy mildew by new fungicide molecule upf 509 (azoxystrobin 8.3 % w/w + mancozeb 64.7 5 w/w) along with *Pseudomonas fluorescens*

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Grapevine (*Vitis vinifera* L.) is an important commercial fruit crop and one of the most widely cultivated crops in temperate, sub-tropical and tropical regions of the world. Grapevine cultivation offers a great economic potential due to its higher yield and monetary returns owing to the export to Gulf, European countries and to some extent West Asian countries. Fungal diseases *viz.,* downy mildew [*Plasmopara viticola* (Berk and Curtis) Berlese and De toni], powdery mildew [*Uncinula necator* (Schw.) Burn] and anthracnose [*Gloeosporium ampelophagum* (Pass) Sacc. (Perfect stage: *Elsinoe ampelina* (DeB) Shear)] are found to be the major constraints in grapevine cultivation. Recently, several fungicides *viz.,* propicanozole, fenarimol, bupirimate, penconozole, dimethomorph, triademeton, pyrazophos, hexaconazole, chlorothalonil and flusilazole were introduced in India for control of powdery mildew pathogens.

The new fungicides tested against downy mildew are metalxyl and Fosetyl- Al group of fungicides. Because of variability in the pathogen, repeated use of fungicides resistance to metalaxyl is reported.

In the quest to find newer and more efficacious molecules, the present investigation was carried out using a new formulation UPF 509 (a combination of azoxystrobin 8.3 % w/w + mancozeb 64.7 5 w/w) of United Phosphorus Limited, Mumbai.

Two field trials were conducted in the grapevine variety Muscat at Theenampalayam village of Coimbatore district to test the efficacy of different fungicide and *P. fluorescens* against downy mildew diseases of grapevine during 2011 and 2012. The trials were laid out in a randomized block design (RBD) with three replications with a plot size of 3x2 m (8 vines per plot). The package of practices were followed as per the farmers' practices and the treatments were given as UPF 509 99.6+800 g ai ha^{-1} (0.24%),UPF 509 124.5+1000 g ai ha^{-1} (0.30%),UPF 509 149.4+1200 g ai ha^{-1} (0.36%), mancozeb 75% WP 1500 g ai/ ha (0.40%), azoxystrobin 23% SC 125 g ai ha^{-1} (0.10%), hexaconazole 2% SC 60 g ai ha^{-1} (0.60%), metalaxyl 8% + mancozeb 64% WP 2000 g ai ha^{-1} (0.50%)*P. fluorescens* (Pf 1) (0.2%) and healthy control.

First spray was given with the initiation of the disease and subsequent sprays were given at 15 days interval for 2 times. The severity of downy mildew infection was recorded a week after each spray. The observations on the disease incidence were recorded separately for leaves, inflorescence and fruits. The observations were recorded by selecting 10 samples from each replication at random for all the three categories. Per cent disease index (PDI) was calculated (Vincent, 1927) using the score chart and the results were expressed in terms of per cent disease reduction over control

Two field trials at Theenampalayam village of Coimbatore district were laid out to study the phytotoxicity effect of UPF 509. The fungicide UPF 509 was sprayed at the concentration of 249+2000 g a.i/ha and compared with other treatments. The phytotoxicity symptoms were recorded a week after each spray. The leaves were regularly examined for

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injury to leaf tip, leaf surface, wilting, vein clearing, necrosis, epinasty and hyponasty. Leaf injury was graded based on visual rating on a 1-10 scale (CIB, 1989).

The results of two seasons showed that all the treatments were found to be more effective in comparison to untreated control. However, the foliar spray of UPF 509 at 0.36 per cent provided the maximum disease control of downy mildew in leaves, inflorescence and fruits during both the seasons. This was followed by UPF 509 at 0.30 per cent and 0.24%. The combination of metalaxyl+ mancozeb was found to be better than mancozeb alone in reducing the downy mildew disease. Similarly, the spray treatment of UPF 509 at 0.36% also recorded the maximum yield of 25.83 t/ha which was at par with the spray treatment of UPF 509 at 0.30 per cent with yield of 25.80 t/ha. UPF 509 at 0.24 per cent dose proved the next best treatment (24.56t/ha) and more effective than rest of the treatments. Untreated control yielded only 15.52t/ ha during the 1st season. In the second season also UPF 509 at 0.36% per cent concentration recorded the maximum yield of 27.96 t/ ha which was on par with the spray treatment of UPF 509 @ 0.30 per cent (27.92 t) and followed by the treatment of UPF 509 at 0.24 per cent. Besides fungicides, *P. fluorescens* (0.20%) also controlled the disease and increased the yield but was less effective than the fungicidal treatments. Untreated control yielded only 17.78 t/ ha during the second season.

Jamadar *et al.* (2008) reported the effectiveness of the Crystal 50 WDG at 1125 g ai ha^{-1} in the management of downy mildew. Where as Wicks and Hitch (2002) evaluated the strobilurin fungicide, Amistar (azoxystrobin) for control of downy mildew of grapes and recorded that Amistar (0.5g/l) was more effective than Flint (0.5g/l) and Thiovit (2g/l) in controlling this disease. Wong and Wilcox (2002) reported that azoxystrobin applied as protectant provided cent per cent control of *U. necator* at the recommended rate of 250 µg ai ml^{-1}.

Table 1. Effectiveness of representative spray of different fungicides and *P.fluorescens* on the incidence of downy mildew disease in grapevine fruits and yield (Season-I)

Treatment	Disease index (%)*									Yield (t/ha)	Yield increase over control
	Leaves			Inflorescence			Fruits				
	1	2	3	1	2	3	1	2	3		
UPF 509 (0.24%)	48.91^{a} (44.37)	18.52^{b} (25.48)	71.70	6.49^{a} (14.75)	4.92^{b} (12.81)	80.66	5.29^{b} (13.29)	4.06^{b} (11.62)	83.40	24.56^{b}	58.24
UPF 509 (0.30%)	47.65^{a} (43.62)	13.21^{a} (21.30)	79.81	6.82^{b} (15.13)	3.24^{a} (10.36)	87.26	4.16^{a} (11.76)	2.49^{a} (9.07)	89.82	25.80^{a}	66.23
UPF 509 (0.36%)	49.29^{b} (44.54)	13.01^{a} (21.130	80.12	8.59^{e} 17.03	3.12^{a} (10.17)	87.74	5.89^{c} (14.04)	2.42^{a} (8.94)	90.11	25.83^{a}	66.43
Mancozeb 75% WP (0.40%)	46.94^{a} (43.22)	24.12^{d} (29.40)	63.15	7.21^{c} (15.57)	6.45^{d} (14.70)	74.65	8.94^{g} (17.36)	7.25^{e} (12.81)	70.37	22.13^{c}	42.59
Azoxystrobin 23% SC (0.10%)	51.14^{b} (45.63)	29.32^{e} (32.77)	55.20	8.86^{f} (17.31)	8.21^{f} (16.64)	67.50	7.96^{f} (16.38)	7.83^{f} (16.24)	68.00	21.62^{d}	39.30
Hexaconazole 2% SC (0.60%)	52.31^{b} (46.32)	32.63^{f} (34.83)	50.15	7.56^{d} (15.95)	7.41^{e} (15.79)	70.92	7.02^{e} (15.36)	6.92^{d} (15.24)	71.72	19.02^{f}	22.55
Metalaxyl 8% + Mancozeb 64% WP (0.50%)	55.65^{c} (48.22)	21.52^{c} (27.63)	67.12	6.38^{a} (14.62)	6.10^{c} (14.30)	76.03	6.32^{d} (14.55)	5.88^{c} (14.03)	75.97	20.35^{e}	31.12
P. fluorescens (0.20%)	57.82^{d} (49.49)	37.20^{g} (37.57)	43.17	9.49^{g} (17.85)	9.20^{g} (17.650	63.85	9.25^{h} (17.70)	8.95^{f} (17.40)	63.42	18.00^{g}	15.97
Control	58.22^{d} (49.73)	65.46^{h} (54.02)	-	11.32^{h} (19.64)	25.45^{h} (30.29)	-	9.89^{h} (18.24)	24.47^{g} (29.64)	-	15.52^{h}	-

1- Before spray 2 – After spray 3- Disease control *Mean of three replications; Values in parentheses are arc sine-transformed values; In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Table 2. Effectiveness of representative spray of different fungicides and *P.fluorescens* on the incidence of downy mildew disease in grapevine fruits and yield (Season-II)

Treatment	Leaves			Inflorescence			Fruits			Yield t/ha	Yield increase over control
	1	2	3	1	2	3	1	2	3		
UPF 509 (0.24%)	3.16^{c} (10.23)	2.01^{b} (8.14)	83.23	2.78^{c} (9.59)	1.29^{b} (6.52)	79.29	2.89^{d} (9.78)	1.41^{b} (6.81)	83.40	26.31^{b}	47.97
UPF 509 (0.30%)	2.96^{b} (9.90)	1.05^{a} (5.88)	91.24	2.91^{e} (9.81)	0.54^{a} (4.21)	91.33	2.66^{c} (9.38)	0.42^{a} (3.71)	89.82	27.92^{a}	57.03
UPF 509 (0.36%)	4.53^{e} (12.28)	1.03^{a} (5.82)	91.40	3.65^{h} (11.01)	0.52^{a} (4.13)	91.65	3.53^{g} (10.82)	0.40^{a} (3.62)	90.11	27.96^{a}	57.25
Mancozeb 75% WP (0.40%)	3.94^{d} (11.44)	3.56^{d} (10.87)	70.30	2.16^{a} (8.44)	1.99^{d} (8.10)	68.05	2.96^{e} (9.90)	2.53^{e} (9.15)	70.37	24.35^{c}	36.95
Azoxystrobin 23% SC (0.10%)	2.81^{a} (9.64)	2.75^{c} (9.54)	77.06	2.54^{b} (9.16)	1.72^{c} (7.53)	72.39	2.18^{a} (8.48)	1.74^{c} (7.57)	68.00	23.18^{d}	30.37
Hexaconazole 2% SC (0.60%)	5.18^{g} (13.15)	4.93^{f} (12.82)	58.88	2.86^{d} (9.73)	2.31^{e} (8.74)	62.92	3.11^{f} (10.15)	2.95^{f} (9.88)	71.72	21.05^{f}	18.39
Metalaxyl 8% + Mancozeb 64% WP (0.50%)	4.66^{f} (12.46)	4.05^{e} (11.60)	66.22	3.01^{f} (9.98)	2.68^{f} (9.42)	56.98	2.51^{b} (9.11)	2.25^{d} (8.62)	75.97	22.69^{e}	27.61
P. fluorescens (0.20%)	5.77^{h} (13.89)	5.29^{g} (13.29)	55.87	3.41^{g} (10.63)	3.02^{g} (10.00)	51.52	3.91^{h} (11.40)	3.53^{g} (10.82)	63.42	20.01^{g}	12.54
Control	6.58^{i} (14.77)	11.99^{h} (20.25)	-	5.79^{i} (13.92)	6.23^{h} (14.45)	-	4.86^{i} (12.66)	8.36^{h} (16.80)	-	17.78^{h}	-

1- Before spray 2 – After spray 3- Disease control;*Mean of three replications;Values in parentheses are arc sine-transformed values; In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

In the present study, the new formulation, UPF 509 at 0.30 per cent was compared with its 2x dose *i.e* 0.60 % concentration to study its reaction to the plants. The observations on the leaf tip, surface injury, wilting, vein clearing, necrosis, epinasty, hyponasty and fruit injury were recorded from both the seasons and it was found that UPF 509 even at 0.60% (2 x dose) did not show any phytotoxicity symptoms. Similarly, all the other fungicides tested also did not exhibit any phytotoxicity symptoms. However, contrasting reports were presented by some workers (Lange, 2004; Miles *et al.,* 2006; Yi *et al.,* 2003).

The foliar spray of UPF 509 @0.36% at 15 days interval could be recommended for the management of downy mildew disease and for maximizing the yield of grapewine

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Evaluation of French bean germplasm against bean rust (*Uromyces appendiculatus*)

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French bean (*Phaseolus vulgaris* L.) is one of the most important leguminous Kharif pulse and vegetable crops, grown throughout the hills of India. In Himachal Pradesh, it is grown for green pods during May to October in the mid and high hills which are transported to the Northern plains where they fetch premium prices being an off-season crop. With the introduction of pencil type of beans which are fibreless, meaty type, tender and therefore brings lucrative returns to the farmers. The area under this crop has increased tremendously but rust (*Uromyces appendiculatus* (Pers.) Unger) which was not prevalent earlier in the state was first time noticed in 2006 on pencil type of beans (Gupta *et al.,* 2008). In India, the losses in green pod yield due to this disease ranged in between 4.7 to 69.0 per cent whereas in Himachal Pradesh, the severity of this disease ranged from 15 to 80 per cent (Gupta *et al.,* 2008). Though the disease can be kept in check with elaborate chemical sprays (Shukla and Sharma, 2009) but the cost involved is very high besides environmental pollution. The use of host resistance is the only feasible means of controlling the disease.

During 2010 and 2011 crop seasons, 21 cultivars or germplasm lines of French bean were screened against rust at the experimental farm of Department of Plant Pathology, Nauni, under natural epiphytotic conditions and to confirm the resistance if any, these cultivars/ germplasm lines were further screened under laboratory conditions.

Fourteen seeds of each French bean cultivar or line available in the Department of Vegetable Crops of the University and local collections obtained from Sikkam were sown in 2 m long rows in replicated field trial in both the years, maintaining row to row distance of 45 cm and plant to plant distance of 15 cm. Highly susceptible cultivar 'Falguni' was planted in two rows around the experimental plots as well as in a single row alternating with 10 rows of cultivars/lines. The severity of the disease was recorded on a 0-5 scale where 0= apparently healthy foliage; 1 = small brown uredosori covering 1% or less leaf area; 2 = few spots on leaves, typical uredosori covering 1 to 10% leaf area; 3 = uredosori covering up to 25% leaf area with no stem infection; 4 = uredosori covering up to 40% leaf area on both surfaces with 10% stem infection; 5 = >40% of leaf area covered under lesions with lesions on stems, branches, petioles and defoliation starts to occur and disease severity was calculated.

The different cultivars/ germplasm lines were graded as highly resistant, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible depending on 0, 0.01–5.0, 5.1-10.0, 10.1–25.0, 25.1– 40.0 and > 40% disease severity, respectively. The apparent infection rate for each cultivar or germplasm or line was calculated as per Van der Plank **(**1963).

Under artificial inoculation conditions, the plants of all 21 cultivars/ germplasm lines were raised in plastic pots (10 cm diameter) filled with sterilized soil where 3 seeds of each cultivar/ germplasm lines were sown in each pot. Plants (21 days old) were then subjected to inoculation with urediniospores by leaf coating method. The inoculated plants were transferred to polythene chamber maintaining high humidity (>90 % by misting) to provide leaf wetness for 24 h after which they were shifted to net house bench (20±2°C) for symptom development. Plants were observed regularly for the appearance of the

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Table 1. Reaction of Frenchbean germplasm lines to rust under natural epiphytotic and artificial inoculation conditions and level of resistance/ susceptibility in relation to apparent infection rate and incubation period

Germplasm line	Disease severity (%)		Disease reaction		Apparent infection rate (r) /unit/day	Incubation period (h)
	1	2	1	2		
Jureli Bodi	0.00	33.30	HR	S	0.00	240
Alapa Trey	0.00	0.00	HR	HR	0.00	NSA
Sing Tamey	0.00	0.00	HR	HR	0.00	NSA
Powell	0.00	20.00	HR	MS	0.00	264
Everest	0.00	4.00	HR	R	0.00	288
Surya	0.00	20.00	HR	MS	0.00	264
Rajguni	0.00	20.00	HR	MS	0.00	264
Local Sel.	0.00	0.00	HR	HR	0.00	NSA
Contender	0.00	20.00	HR	MS	0.00	264
VRJ-125	21.67	93.30	MS	HS	0.0231	168
Baspa	22.86	73.30	MS	HS	0.0259	204
Arka Suvidha	23.31	26.66	S	HS	0.0221	252
SR-6	23.06	66.60	MS	HS	0.0339	204
Jawala	25.65	86.60	HS	HS	0.0664	192
Laxmi	26.99	53.30	S	HS	0.0421	216
HVR-137	29.99	86.60	S	HS	0.0693	192
SR-1	27.46	86.60	S	HS	0.0671	192
HVR-701	41.67	100.00	HS	HS	0.0873	168
Falguni	61.86	86.60	HS	HS	0.0944	192
Kailash	42.84	53.30	HS	HS	0.0698	216
Crhew Sebhi	71.41	100.00	HS	HS	0.0793	168

1=Natural epiphytotic, 2=Artificial inculation

symptoms. Incubation period (h) and severity of the disease for all the cultivars/ germplasm lines were recorded.

Cultivars/ germplasm lines *viz.*, Jureli Bodi, Alapa Trey, Sing Tamey, Contender, Powell, Everest, Surya, Rajguni and Local Selection were found to be highly resistant to the disease under natural epiphytotic conditions (Table 1) while VRJ-125, Baspa, Arka Suvidha and SR-6 exhibited moderately susceptible reaction whereas rest were susceptible to highly susceptible. Levels of resistance and susceptibility of different cultivars/ lines revealed that irrespective of the spread of the disease on leaves, apparent infection rate increased with the progressive decrease of resistance falling into a category of highly susceptible disease reaction (Table 1). The moderately susceptible cultivars/lines VRJ-125, Baspa, Arka Suvidha and SR-6 exhibited minimum infection rate 0.0231 to 0.0339/ unit / day, respectively. Highest 'r' (0.0698 to 0.0944/unit/day) values were observed in case of highly susceptible cultivars/lines Kailash, Crhew Sebhi, HVR-701 and Falguni, respectively. It is also evident from the Table 1 that the lines exhibiting moderately susceptible reaction exhibited lower values of apparent infection rates compared to the cultivars/lines ranging in their reactions from susceptible to highly susceptible.

Germplasm lines-pathogen interaction in relation to incubation period indicated that the incubation period in different cultivars/ lines varied from 168 to 288 h. Longest incubation period of 288 h was observed in Everest followed by Contender, Powell, Surya, Rajguni, Arka Suvidha, HVR-137, Jureli Bodi, Laxmi, Kailash and SR-6 with incubation periods of 264, 252, 240, 216 and 204 h, respectively. Interestingly, Alpa Trey, Sing Tamey and Local Selection (hypersensitive reaction),

which stayed disease free under natural epiphytotic conditions, also remained disease free under *in vitro* studies while Everest, Rajguni, Contender, Powell, Surya and Jureli Bodi which were highly resistant under natural epiphytotic conditions, ranged from resistant to susceptible under *in vitro* conditions as indicated by the lower incubation period and disease severity.

Among the 21 cultivars/ lines screened for resistance against the pathogen, Alpa Trey, Sing Tamey and Local Selection were found highly resistant both under natural epiphytotic as well as laboratory conditions which remained disease free while rest were moderately susceptible to highly susceptible. The results of the studies on relationship between levels of resistance/ susceptibility of the cultivars/ lines, apparent infection rate (r) for the development of the disease further indicated that the apparent infection rate was inversely proportional to the degree of resistance. Results of the *in vitro* studies of cultivars/ germplasm lines-pathogen interaction in relation to incubation period generally reflected that the cultivars/ lines which were rated as resistant or moderately susceptible under *in vitro* conditions were found to have longer incubation periods. These findings are in consonance with Verma *et al.* (2001) and Aghora *et al.* (2007) who also reported the longer incubation period in the moderately resistant cultivars/ lines of bell pepper.

The foregoing discussion clearly indicates that Alapa Trey, Sing Tamey and Local Selection were found highly resistant under field as well as laboratory conditions.

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