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Efficacy of bio--agents, fungicides and plant extracts on leaf spot of *Piper longum* caused by *Botryodiplodia theobromae*

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Abstract
The leaf spot of *Piper longum* L. caused by *Botryodiplodia theobromae* Pat. was found to appear as few tiny spots on lower leaves, which increased in number and size gradually and also, affected upper leaves, finally various spot merged covering the entire leaf and ultimately leaf turned yellow and dried. There was fast development of disease from May to September and maximum disease severity was recorded in the month of September (31.1per cent) Among various fungicides tested against *Botryodiplodia theobromae* the most effective was Carbendazim at various concentration 0.1%, 0.2% and 0.3%; among other fungicides Propiconazole, Blitox -50 and SAAF were also effective. Among various botanicals extract, Brahmi extract was most effective in suppression of growth of target pathogen, the next most effective extract was mundookprani, which also markedly supressed the growth of pathogen. All the native and commercial isolates of *Trichoderma viride* and *Trichoderma harzianum* were found highly effective in supression of growth of pathogen. However native isolates of *Trichoderma viride* and *Trichoderma harzianum* showed significantly stronger antagonistic effect against the pathogen as compared to commercial isolates of the both antagonists (*Trichoderma viride* and *Trichoderma harzianum*). Among all the isolates, *Trichoderma viride* native showed the strongest antagonistic effect. The most effective chemical and botanical and the strongest native antagonists -*Trichoderma viride* may be utilised for evolving integrated disease management strategy against leaf spots of *Piper longum* caused by *Botryodiplodia theobromae.*

Keywords: Bio-agents, fungicides and plant extracts, *Piper longum and Botryodiplodia theobromae*

Introduction
Piper longum L. is the most extensively used medicinal plant in the Ayurvedic system of Medicine. It is used in over 320 classical compound medicinal formulations and in many modern herbal formulations. Chemical analysis of dried long pepper spike showed moisture-9.5%, protein -12.2, starch -39.5%, fibre -5.9%, acid insoluble ash -4.25%, fixed oil -6.6%, volatile oil -1.55%, and piperine -0.5-4.5%. Long pepper also contains essential oil and piperine. Chemical investigation on spike showed presence of alkaloid piperine, piplartine, piper longumine and piper longuminine. Roots contain alkaloids piperine, piplartine, piperlongumine, piper longuminine, of which piper longumine is major ones. Other constituents in root include triacontane, dihydrostigmasterol, a steroid reducing sugars and glycosides. The dried fruits on steam distillation yield 0.7% essential oil with spicy odor. It is also a component of various modern herbal formulations means for digestion, dyspepsia, asthma, bronchitis, chest congestion etc. In traditional Indian medicine, pungent root is useful as heating, stomachic, laxative, anthelmintic, carminative, improves the appetite, useful in bronchitis, abdominal pain, diseases of spleen, tumours. Ripe fruits are sweetish pungent, stomachic, laxative, antidiarrhoeic, antidysenteric. It is also useful in asthma, bronchitis, fevers, leucoderma, urinary discharges, tumours, piles diseases of spleen, inflammation, leprosy, jaundice and tuberculous glands. Dried immature spike and matured root are extensively used in acute and chronic bronchitis and cough for getting gradual relief. Kumari *and* Jha (2014) reported that the leaf spot of *Piper longum* L. was caused by *Botryodiplodia theobromae* Pat. in Bihar. So far no systematic work has been done on management of leaf spot of *Piper longum* caused by *Botryodiplodia theobromae*. Hence the present study was eco-friendly management strategy against leaf spot of *Piper longum* L.

Material and Methods
Experimental site
The study was conducted during *kharif* season, 2013 at herbal garden Pusa, Samastipur, Bihar

and laboratory works was done in Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India.

Isolation and identification of Pathogen

Fungal pathogen was isolated from infected *Piper longum* leaves. The infected portion of leaf were surface sterilized with 0.1% mercuric chloride solution for 30 seconds, thrice rinsed with sterilized distilled water, and then transferred aseptically into PDA tube. These PDA tubes were incubated at 25 ± 2^0C.

Isolation and purification of native and commercial *Trichoderma viride* and *Trichoderma harzianum*

The native isolates of *Trichoderma viride* and *Trichoderma harzianum* available in laboratory of the Plant Pathology Department were multiplied and maintained on PDA for further study. Likewise isolates from the commercial formulation of *Trichoderma viride* and *Trichoderma harzianum* were multiplied and maintained on PDA for further study.

Evaluation of botanical extracts against *Botryodiplodia theobromae*

The effect of botanical leaf extracts against *Botryodiplodia theobromae* was assessed by poison food technique. The botanical leaf extracts were evaluated at 6, 8 and 10 per cent prepared from standard solution, 100 g of leaves was weighted separately and ground in 100ml of sterilized distilled water and squeezed with two layers of muslin cloth. Extract obtained in such a manner served as standard extract. 6, 8 and 10 ml of the standard extract was separately poured in 250ml flask containing 94, 92 and 90 ml of sterilized molten PDA to get desired concentrations.

Evaluation of fungicides

The effect of fungicides on *Botryodiplodia theobromae* was assessed by poison food technique. Fungicides was evaluated at 0.1, 0.2, and 0.3 per cent concentration. For preparation of different concentrations of fungicides 100, 200 and 300 mg were separately weighed and thoroughly mixed in 100 ml of PDA to get 0.1, 0.2 and 0.3 per cent concentrations, respectively. Fifteen ml of desired concentration of fungicide was poured in each sterilized Petri-plates and cooled and then after inoculated with 5mm disc of fungal pathogen.

Percentage inhibition of mycelia growth of test pathogen was calculated using the formula.

$$I = \left(\frac{C - T}{C} \right) \times 100$$

Where, I = Percent growth inhibition
C =Colony diameter in check Petri-plate.
T =Colony diameter in the treated Petri-plate.

Results and discussion

Efficacy of fungicides on *Botrydiplodia theobromae*

In the present study, various fungicides were evaluated for their effect on *Botrydiplodia theobromae* isolated from *Piper longum*. The radial growth of the pathogen was significantly inhibited by various fungicides i.e. mancozeb, saaf (mancozeb + carbendazim) and Blitox-50. Initially similar growth of the pathogen was observed after 24 hours in all the treatments; but after 24 hours there were significant inhibition of radial growth of the pathogen due to fungicides. Among these fungicides, Blitox-50 was found to be most inhibitory to the target fungus. Initially the slow growth of the pathogen was found in case of Blitox-50 (0.3%) after 24 hours, which further developed slowly and reached to 8.80 mm after 72 hours. Other fungicides i.e. Saaf and Mancozeb at the concentrations of 0.1 %, 0.2 % and 0.3 % were also found to be highly effective and suppressed the growth of the pathogen.

In the present study, the two other fungicides- Propiconazole and Carbendazim were also evaluated against *Botrydiplodia theobromae.* Initially the minimum growth of the pathogen was found in case of Carbendazim (0.3%) after 24 hours, which further developed slowly and reached to 8.26 mm after 72 hours, other fungicide i.e. Propiconazole was also found to be highly effective and suppressed the growth of the pathogen when used at the concentrations of 0.1%, 0.2 %, 0.3 %. The findings of present study showed similarity with the finding of Brown (1984) who found that application of Carbendazim suppressed the growth of *Botryodiplodia theobromae* and effectively controlled stem rot of citrus. Likewise Maunder and Pataki (1997**)** also evaluated various fungicides against *Botryodiplodia theobromae* causing fruit rot of guava and found that imazalil and benomyl were highly effective in suppressing the disease.

Efficacy of plant extract on *Botrydiplodia theobromae*

In the present investigation, the extracts of *Neem, Tulsi, Brahmi* and *Mundookparni* were evaluated at 6, 8 and 10% concentrations against *Botryodiplodia theobromae***.** Initially the minimum growth of the test-fungus was found in case of *tulsi* (10%extract) after 24 hours which further developed slowly and reached to 9.25 mm after 72 hours. However *Brahmi* (10% extract) was most effective with least growth (5.71mm) of the pathogen after 72 hrs. The extracts of neem and mandookprani used at 6%, 8% and 10 % were also found to be highly effective resulting in poor growth of the pathogen. Earlier also various workers have reported the inhibitory effect of extracts of different botanicals against *Botryodiplodia theobromae* and other plant pathogens. Mota *et. al.* (2002**)** evaluated the extracts of *Lippia sidoides* and *Azadirachta indica*, essential oils of *Piper aduncum, Pilocarpus microphyllus* and *Vanilosmopsis arborea*, and 2-tridecanone, a secondary compound of *P. microphyllus* against the fungus- *Lasiodiplodia theobromae* and found that these extracts were effective in inhibiting mycelial growth of *Lasiodiplodia theobromae*. Pramod *et. al.* (2007) also studied the effect of different botanicals on *Botryodiplodia theobromae* and reported that extract of *Catharanthus roseus* and *Lawsonia alba* markedly inhibited the germination of spore. Thus the present findings conform to the findings of earlier workers. The inhibitory effect of botanical extracts against *Botryodiplodia theobromae* in the present study and studies of earlier workers might have been due to presence of antimicrobial metabolite in the extracts.

Efficacy of bio-agents against *Botrydiplodia theobromae*

Native and commercial isolates of *Trichoderma viride* and *Trichoderma harzianum* were evaluated for their antagonistic potential against *Botryodiplodia theobromae.* Both *Trichoderma viride* and *Trichoderma harzianum* isolates, whether native or commercial, exhibited strong antagonistic effect on the target pathogen in dual culture. The strongest antagonistic effect was expressed by *Trichoderma viride* (native) where growth of pathogen was confined to only 15 mm diameter after 7 days, thus inhibited by 70.77 % as

compared to control (pathogen alone). The next most potential antagonistic was *Trichoderma harzianum* (native) which was found to inhibit the pathogen growth by 53.24 %. It is also clear that the commercial strain of *Trichoderma viride* and *Trichoderma harzianum* showed lesser antagonistic effect as compared to native ones. The growth of target pathogen in dual culture with *Trichoderma viride* (commercial) was 37.33 mm and that with *Trichoderma harzianum* (commercial) was 36.84 mm, there by pathogen was inhibited by 37.50 % and 38.33 % by the respective antagonists. The antagonistic effect of *Trichoderma viride* and *Trichoderma harzianum* on *Botryodiplodia theobromae* and other plant pathogens has been reported by earlier workers also. Verma and Sharma (2007**)** evaluated various bio-control agents viz., *T. viride, T. harzianum, T. hamatum,* and *T. virens* against the pathogen *Fusarium solani* causing mango seedling wilt and reported that *T. harzianum* caused the maximum (72%) inhibition, followed by *T. virens* (71%), *T. viride* (67%) and *T. hamatum* (58%). Naik *et al*. (2012) evaluated the antagonistic potential of various bioagents against *Botryodiplodia theobromae* and *Fusarium solani* together causing root disease complex in mulberry and found that the the bio-agents *Verticillium chlamydosporium*, *Paecilomyces lilacinus* and *Trichoderma harzianum* were effective in suppressing the growth of the pathogens.

The strong inhibition of growth of *Botryodiplodia theobromae* by *Trichoderma viride* and *Trichoderma harzianum* in the present study might be attributed to production of antimicrobial metaboles by these antagonists. Besides faster growth rate and myco-parasitic activity of these antagonists may also be responsible for their inhibitory effect. The greater antagonistic potential of native *Trichoderma* isolates may be due to their better adaptation in their native environment.

Conclusion

Among fungicides tested against *Botryodiplodia theobromae* the most effective was Carbendazim at various concentration 0.1%, 0.2% and 0.3%; among other fungicides Propiconazole, Blitox -50 and SAAF were also effective. Among botanicals extract, Brahmi extract was most effective in suppression of growth of target pathogen, the next most effective extract was mundookprani, which also markedly supressed the growth of pathogen. All the native and commercial isolates of *Trichoderma viride* and *Trichoderma harzianum* were found highly effective in supression of growth of pathogen. However native isolates of *Trichoderma viride* and *Trichoderma harzianum* showed significantly stronger antagonistic effect against the pathogen as compared to commercial isolates of the both antagonists (*Trichoderma viride* and *Trichoderma harzianum*.

Table 1: Effect of Mancozeb, SAAF and Blitox-50 on radial growth (mm) of *Botryodiplodia theobromae* at 24 hours interval

Treatment No.	Treatments	Radial growth (mm) of *Botryodiplodia theobromae*		
		24 hrs	48 hrs	72 hrs
T_0	Control (untreated)	7.40	16.90	22.56
T_1	Mancozeb (0.1%)	6.60	7.90	9.43
T_2	Mancozeb (0.2%)	6.20	7.30	9.23
T_3	Mancozeb (0.3%)	6.05	7.21	9.00
T_4	Saaf (0.1%)	7.20	8.40	9.05
T_5	Saaf (0.2%)	7.10	8.10	8.98
T_6	Saaf (0.3%)	6.86	7.43	8.76
T_7	Blitox-50 (0.1%)	7.90	8.20	9.26
T_8	Blitox-50 (0.2%)	7.60	8.13	9.08
T_9	Blitox-50 (0.3%)	6.90	7.98	8.80
CD at 5%		0.16	0.21	0.27
CV %		1.34	1.40	1.54

Table 2: Effect of Propiconazole and Carbendazim on radial growth (mm) of *Botryodiplodia theobromae* at 24 hours interval.

Treatment No.	Treatments	Radial growth (mm) of *Botryodiplodia theobromae*		
		24 hrs	48 hrs	72 hrs
T_0	Control (untreated)	5.60	14.03	20.50
T_1	Propiconazole (0.1%)	5.21	6.16	11.25
T_2	Propiconazole (0.2%)	5.26	5.33	9.56
T_3	Propiconazole (0.3%)	5.14	5.36	8.33
T_4	Carbendazim (0.1%)	5.36	5.72	9.30
T_5	Carbendazim (0.2%)	5.13	6.16	8.83
T_6	Carbendazim (0.3%)	4.40	6.10	8.26
CD (0.05)		0.44	0.99	0.97
CV%		4.81	7.88	4.97

Table 3: Effect of *Neem, Tulsi, Brahmi* and *Mundookprani* extract on radial growth (mm) of *Botryodiplodia theobromae* at 24 hours interval by poison food technique

Treatment No.	Treatments	Radial growth (mm) of *Botryodiplodia theobromae*		
		24 hrs	48 hrs	72 hrs
T_0	Control (untreated)	9.38	15.15	21.41
T_1	Neem (6%)	8.00	14.15	18.20
T_2	Neem (8%)	7.20	13.19	18.32
T_3	Neem (10%)	6.60	12.30	17.31
T_4	Tulsi (6%)	6.00	7.33	10.30
T_5	Tulsi (8%)	5.10	7.40	9.78
T_6	Tulsi (10%)	5.08	5.26	9.25
T_7	Brahmi (6%)	6.10	6.65	6.81
T_8	Brahmi (8%)	5.90	5.90	6.10
T_9	Brahmi (10%)	5.10	5.27	5.71
T_{10}	Mundookprani (6%)	6.65	7.15	7.31
T_{11}	Mundookprani (8%)	6.20	6.56	7.10
T_{12}	Mundookprani (10%)	5.53	6.40	6.80
CD		0.24	0.27	0.14
CV		2.28	1.64	0.78

Table 4: Per cent Inhibition of *Botryodiplodia theobromae* as influenced by dual culture with *Trichoderma viride* (native and commercial) at 24 hours interval

Time	Radial growth (mm) of *Botryodiplodia theobromae*	
	%Inhibition (Native)	%Inhibition (Commercial)
24 Hrs.	13.65	11.27
48 Hrs.	41.03	38.94
72 Hrs.	44.27	34.34
96 Hrs.	53.22	26.56
120 Hrs.	60.28	28.30
144 Hrs.	65.52	26.84
168 Hrs.	69.79	27.27
192 Hrs.	73.53	27.00
216 Hrs.	76.44	25.79
240 Hrs.	78.51	28.33
264 Hrs.	80.39	27.62
288 Hrs.	81.66	27.60
312 Hrs.	83.05	28.73
F- test	S	S
S. Ed. (±)	1.02	1.39
C. D. (P = 0.05)	2.10	2.86

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