Wilt of Castor caused by *Fusarium oxysporum* f. sp. ricini: Detection and Pathogenecity in Castor (*Ricinus Communis* L) Seed

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Abstract- The present investigation was undertaken with the main objective to determine detection on seed borne *Fusarium oxysporum* f.sp. *ricini* and its Pathogenicity were conducted at Department of Applied Botany, PlantPathology laboratory, Kuvempu University, Jnanasahyadri, Shankaraghatta, Shivamogga, Karnataka. Castor is one of the important non edible oilseed crops in India. Wilt of castor is caused by *F. oxysporum* f. sp. *ricini.* The objective of this work were to estimate the incidence of *F. oxysporum* f. sp. *ricini* in castor seed using different seed health test methods. A total 69 samples were collected from retail shops, APMC markets, fields and farmers of different agro-climatic regions of Karnataka during-2011. The collected samples, ten samples show a higher incidence of *F. oxysporum* f. sp. *ricini* and other fungi, were selected for PDA, Water agar and 2,4-D methods. The incidence of seed infection was 19.7 percent on a selective medium for standard blotter method (SBM), Potato dextrose agar medium 13.1 percent (PDA), Water agar medium 8.9 percent and 2,4-D 11.0 percent (2, Dichloro phenoxy acetic acid) methods respectively. Determine the rate of pathogenicity under green house conditions. *F. oxysporum* f. sp. *ricini* showed the symptoms of wilts were observed in 1-10 percent wilts in one, 10-30 percent in two month seedlings and 30-60 percent wilts in three month old plants, no wilts observed in water treatment plants. Among the sample collected field samples show a higher incidence of *F. oxysporum* f. sp. *ricini*. The seed health test methods, SBM is most superior for isolating the *F. oxysporum* f. sp. *ricini*, pathogenic and saprophytic fungi. The importance of infected seed and its pathogenicity were discussed. *F. oxysporum* f. sp. *ricini* is a causal agent of wilt disease in castor crop.

Index Terms- Castor, Seed health tests, Pathogenicity, *F. oxysporum* f. sp. *ricini.*

I. INTRODUCTION

Castor (*Ricinus communis* L.) is one of the important non edible oilseed crops and considered as the ancient non edible oilseed crop. It is indigenous to eastern Africa and most probably originated in Ethiopia (Weiss 1971) [16]. This crop is widely distributed throughout the tropics and sub-tropics and is well adapted to the temperate regions of the world. Castor is cultivated over on area of 20161 hectares with a production 17493 tones and productivity 193 kg/ha in Karnataka (Anon 2011) [2]. Castor plant is affected by number of fungal diseases. The important diseases are wilt-*Fusarium oxysporum* f.sp.*ricini*, leaf spot & blight-*Alternaria ricini*, cercospora leaf spot-*Cercospora ricinella*, root rot, stem rot & charcoal rot-*Macrophomina phaseolina*, seedling blight-*Phytophthora parasitica*, capsule rot-*Cladosporium oxysporum*, fruit rot & Gray rot-*Botrytis ricini*, rust-*Melamspora ricini*, powdery mildew-*Leveillula taurica*, phyllosticta leaf spot-*Phyllosticta bosensis*, angular leaf spot-*Botrytis* sp., damping off-*Phythium aphanidermatum* (Svirdvi, 1989) [15] (Andreva, 1979) [1]. These diseases are reduces the yield, production and germination up to 30-50%. Seed-borne fungi are carried over by infected seeds. Therefore, the present study was conducted to detection of *F. oxysporum* f. sp. *ricini* and other mycoflora of castor seeds and their Pathogenicity was studied.

II. MATERIALS AND METHODS

Collection of safflower seed samples

The seeds of safflower were collected from different locations of Karnataka state during kharif-2011. A total of sixty nine samples were collected from fields, farmers, retail shops and APMC markets of Bellary, Bidar, Chitradurga, Chikmagalore, Davanagere, Dharwad, Gulabarga, Haveri and Raichur districts of Karnataka. The samples were collected and brought to the plant pathology laboratory of Applied Botany, Kuvempu University and stored in cloth bags room temperature for subsequent studies.

Detection of seed-borne *F. oxysporum* f. sp. *ricini* other fungi by seed health tests

a. SBM Method: Seed samples were analyzed for the detection of seed-borne fungi by blotter method following ISTA, 1993 with some modifications. In this method, three layers of blotter paper were soaked in sterilized water and placed at the bottom of the Petri plates. One hundred seeds were sterilized in 0.2% sodium hypochlorite solution for 2 to 3 minutes and seeds taken randomly from each sample and were placed in five Petri plates (10 seeds per plate). The Petri plates with seeds were then incubated at for seven days in the laboratory. The plates were kept under alternating cycles of 12 hrs light and 12 hrs darkness for seven days. After incubation, the distilled water was added

every fourth day to the blotter so as to keep it sufficiently moist (Ataga and Aquishi, 1996) [3]. The germination and fungi associated with the seeds were recorded during the incubation period. The incubated seeds were examined under stereo binocular microscope to ascertain the presence of fungi. Some times were not apparent even after seven days of the incubation. In such condition, the Petri plates were allowed for further incubation. A temporary slide was prepared from each colony, which could not be identified stereo binocular microscope and examined under Labomed vision 2000 microscope. In fewer cases, the fungi from the incubated seeds were transferred to PDA medium in Petri plates aseptically and incubated under controlled temperature (28±1^{o}C) for 3 to 10 days and than examined under Labomed vision 2000 microscope.

b. PDA Method: For potato dextrose agar method, 100 seeds were sterilized with 0.2% sodium hypo chlorite solution for 2 to 3 minutes. Then, the seeds were plated on sterile glass Petri plates containing PDA medium. Ten seeds per Petri plates and than the plates were incubated at 40^{o}C in alternating cycles of 12 hrs light and 12 hrs darkness for seven days. After incubation eighth days the seeds were examined by stereo binocular microscope.

c. Water agar Method: For agar plate method, 100 seeds were sterilized with 0.2% sodium hypo chlorite solution for 2 to 3 minuets. Seeds were plated on sterile glass Petri plates containing (2.5%., i.e., 12.5 gms in 1000 ml of distilled water) water agar medium. These Petri plates were incubated at 25±2^{o}C for seven days. After seven days these seeds were examined under stereo binocular microscope (Neergaard, 1977) [13]

d. 2, 4-D Method: In this method, 100 seeds were sterilized with 0.2% sodium hypo chlorite solution for 2 to 3 minuets. The three layers of blotter paper discs were dipped in 0.2% of 2,4-Dichloro Phenoxy acetic acid solution. Ten seeds were placed equidistantly on moist blotter discs using sterilized forceps in laminar air flow wood under aseptic conditions. The plates were incubated room temperature for seven days. The observations were taken on the seventh day and then seeds were examined under stereo binocular microscope (Limonrad, 1985) [9].

Screening of seeds for associated mycoflora

The incubated seeds were screened on eighth day using stereo binocular and labomed vision 2000 compound microscope. The germination, associated fungi were recorded and identified with the help of standard guides and manuals like (Booth, (1977) [17], (Sigourd and Funder, 1961) [18], (Subramanium, 1983)[19] and Barnett, 1960)[19].

Pathogenicity test

The pathogenicity test was carried out at the department experimental plot during kharif-2011. The discoloration local variety of castor seed samples were disinfected by 2% sodium hypochloride solution for 2-3 minutes and in the distilled water before sowing the seeds. The experimental plot were prepared by 25 x 25 meter (row and column). One hundred seeds were selected in ten replicates. Seeds were sown directly in the month of August-2011. Proper agronomical practices were followed for raising the plants.

Artificial inoculation to plants

Healthy seedlings of castor were raised in the departmental experimental field. Eight days old pure culture of *F. oxysporum* f. sp. *ricini* inoculums was prepared from PDA slants. Before spraying, the leaves were washed with sterile distilled water and 10^{4} conidial suspension was sprayed to one month seedlings (30 days), before flowering (60 days) and after flowering (90 days). The plants were maintained in five replicates of five per row. The conidial suspension was applied with the help of sprayer on abaxial and adaxial surface of leaves (Bhale *et al*, 2001) [4], (Bhale *et al* 1999) [5].The distilled water sprayed plants served as a control. The plants were maintained in green house. The severity of the disease was assessed by using 0-9 scale (Mayee and Datar, 1986) [9] and percent disease index calculated using the formula

Percent disease index = [Sum of individual ratings/ (No. of leaves examined Maximum disease index)] x 100

III. RESULTS AND DISCUSSION

Seed health testing

Results of four types of methods used to detect *F. oxysporum* f. sp. *ricini* and other mycoflora shown in (Table 1). The standard blotter method were more sensitive in detection of *F. oxysporum* f. sp. *ricini* than the PDA, Water agar and 2, 4, Dichloro phenoxy acetic acid mediums. Similarly, visual sporulation of the fungus on the seed was generally heavier in the SBM methods than in Water agar method. However, the standard blotter method was the most effective and revealed a higher incidence of seed infection than the other methods. This method was also easy quick for recording the presence of *F. oxysporum* f. sp. *ricini* on the seed.

Table 1: Incidence of *F. oxysporum* f. sp. *ricini* in seed health test methods of castor

Seed health testing methods	% seed infection								
	F.oxy	**A. ric**	**A.alt**	**M.pha**	**C. ric**	**C.glo**	**A.nig**	**A.fla**	**A.och**
SBM	19.7	11.9	16.1	11.1	13.0	21.6	12.0	21.0	19.0
PDA	13.1	9.8	13.2	8.7	9.1	19.8	11.1	13.5	16.6
Water agar	8.9	9.7	11.8	6.5	11.1	6.6	9.7	8,9	2.6
2,4-D	11.0	5.9	6.8	8.8	9.2	11.1	10.0	9.3	3.3
SD	4.6757 ±	2.4984 ±	3.8870 ±	1.8786 ±	1.8457 ±	7.1219 ±	1.0551 ±	5.9270 ±	8.6341 ±

SE	2.3378	1.2453	1.4231	1.5594	1.8911	3.1850	0.5275	3.4219	4.1256

Average values of 100 seeds per method (Ten replicates of 100 seeds).
F.oxy- F.oxysporum f.sp. *ricini*, **A.ric-***Alternaria ricini*, **A. alt-***Alternaria alternata*
M.pha-*Macrophomina phaseolina*, **C. ric-***Cercospora ricini*, **C.glo-***Chaetomium globosum*
A.nig-*Aspergillus* niger, **A.flav-***Aspergillus flavus*, **A.och-** *Aspergillus ochraceus*

Pathogenicity test

Inoculums sprayed plants showed the symptoms *F. oxysporum* f. sp. *ricini* in 2-6 days. *F. oxysporum* f. sp. *ricini* sprayed plants showed 10-30 percent wilts in one, two month seedlings and 30-60 % observed in three month old plants and no wilts observed in water treatment plants (Table 2).

Table 2: Artificial inoculation of *F. oxysporum* f. sp. *ricini* on castor seedlings during kharif-2011

Name of the pathogen	Infected plants			
	Germ %	Seedlings (1 month)	Before flowering (2 months)	After flowering (3 months)
F. oxysporum f. sp. *ricini*	72	00	03	06
Water treatment	90	00	00	00

Data based on 100 seed samples .

The importance of *F. oxysporum* f. sp. *ricini* infected seed and its pathogenicity role was confirmed in this study. Detection of oilseeds pathogens on seed is commonly carried out by the using routine standard blotter method. However, this study showed that the standard blotter method developed for *F. oxysporum* f. sp. *ricini* was the most sensitive (Chattopadhyay, 2000) [7]. The antibiotics in to the medium not only did not inhibit the growth of *F. oxysporum* f. sp. *ricini* but also suppressed the growth of other fungi *A. ricini*, *C. ricini*, *M. phaseolina*, *C.globosum*, *A. alternata*, *A. niger*, *A.flavus* and *A. ochraceus*, were frequently observed in the SBM method, that could mask the sporulation of *F. oxysporum* f. sp. *ricini* on seed. This fact facilitated the detection of the target fungus and gave a higher record of incidence. Many researchers (Naik 1994) [10], (Nanda and Prasad 1974) [11] and (Svirdvi 1989) [15] studied in diseases of castor and causal agent of wilt disease in castor crop.

Many researchers have worked on effect of temperature, relative humidity, fruit age, inoculums load on repeated sub cultured inoculums on the development of phomopsis fruit rot of brinjal at temperature of 25^{o}C, RH=90%, fruit early age (5-10 days old), higher inoculums load (> 120 spores/ml) (Sugha, 2002) [13]. *Sclerotium rolfsii* on chilli, which gross actively only in moist soil at moderate to high temperature (30-35^{o}C). Maximum disease intensity (30.72 and 30.81%) was recorded from the second fortnight of October to the second fortnight of November , when temperature varied between a maximum of 28.7-32.2 ^{o}C and minimum of 15.5 – 20.3 ^{o}C; Relative humidity ranged between 62-74 maximum and 32-46 % minimum (Bhale *et al*, 2001) [4]. The minimum disease intensity (9.37 and 10.37%) was observed in July reported in alternaria leaf spot and fruit rot of brinjal (Suryavamshi and Deokar) [14]. Disease severity, weather factors are favorable in development of leaf blight and spots of castor, temperature and relative humidity between 24-26 ^{o}C and 47.3-51.2 percent respectively (Chander Mohan and Thind. 2001) [6].

IV. CONCLUSION

F. oxysporum f. sp. *ricini* is a causal agent of wilt disease of castor. Detection of *F. oxysporum* f. sp. *ricini* and other fungi plays an important role in determining the quality and longevity of seeds. Microbial invasion can lead to the rotting, loss of seed viability, germination and oil quality. This is due to the environmental factors like rainfall, temperature, humidity and in growth stages of the crop. Seed-borne fungi are important from economic point of view as they render losses in a number of ways. Some of the fungi infect the seed and cause discoloration of the seed. Several seed-borne pathogens are known to be associated with wheat seed which are responsible for deteriorating seed quality and weight during storage. Seed borne pathogens of castor are responsible to cause variation in plant morphology and also reducing yield up to 15-90 % if untreated seeds are grown in the field.

REFERENCES

[1] Andreeva LT. 1979. *Fusarium* wilt of *Ricinus communis*. Zashchita Rastenil, **7**: 22.

[2] Anonymous. 2011 Directorate of agricultural statistics in principles crops of Karnataka, Seshadri road, Bangalore, 2008.

[3] Ataga AE, and Akueshi CO.1996. Fungi associated with sunflower in Nigeria. Seed Research, 24(1): 64-65.

[4] Bhale MS. Khare D. Raut ND. and Singh D. 2001. Seed borne diseases objectionable in seed production and their management. Scientific publishers, India, Jodhpur: 2-182.

[5] Bhale MS, Usha Bhale and Khare MN. 1999. A method of testing virulence in Chilli anthracnose pathogen. Journal Mycology and Plant Pathology, 29: 253-254.

[6] Chander Mohan and Thind. 2001. Relative performance of spray schedules of systemic and non systemic fungicides against late blight of tomato. Plant Disease Research, 16: 247-250,

[7] Chattopadhyay C. 2000. Seed borne nature of *Fusarium oxysporum* f. sp. *ricini* and relationship of castor wilt incidence with seed yield. Journal Mycology and Plant Pathology. 30: 265,

[8] Limonard. 1985. Ecological aspects of seed health testing, Reprint from Proc. ISTA. 33: 17-7.

[9] Mayee CD. and Datar VV. 1986. Technical bulletin-1. Marathwad Agricultural University, Parbhani. Phytopathometry: 46.

[10] Naik, M.K. 1994. Seed borne nature of *Fusarium oxysporum* in castor. Indian J. Mycol. Pl. Pathol., 24**(**1): 62-63.

[11] Nanda, S and Prasad, N. 1974. Wilt of castor, a new record. J. Mycol. Pl. Pathol. 24: 62-63.

[12] Neergaard P. 1977. Seed pathology, Vol. I & II. Mac Millan, London: 850&250.

[13] Sugha SK Narendar Kaushal and Suman Kumar. 2002. Factors affecting development of phomopsis fruit rot of brinjal, Indian Phytopathology. 56: 26-29.

[14] Suryavamshi AV and Deokar CD. 2001. Effect of fungicides on growth and sporulation of fungal pathogens fruit rot of Chilli, Research Notes: 181-182,

[15] Svirdvi, A.A. 1989. Fusarium wilt of castor bean and its pathogenecity. Fitopatologiya. 23(1): 91-94.

[16] Weiss. (1971. Castor, sesame and safflower. Leonard Hill, London: 1-901.

[17] Booth C. 1977. Fusarium laboratory guide to the identification of the major species. Commonwealth Mycological Institute. Kew. Surrey. England: 1-237.

[18] Sigourd and Funder. 1961. Practical mycology, manual for identification of fungi. A.W. Broggers, Bltrykkeri, AISOSIO-Norway: 1-145.

[19] Subramanian CV. 1983. Hypomycetes Taxonomy and Biology. Academic Press. London, I & II: 1-930.

[20] Barnett HL. 1960. Illustrated genera of imperfect fungi. Burgees publishing company IInd edition, West Virginia: 1-225.

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