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Gray mold of castor caused by *Botrytis ricini*: Detection and pathogenicity in castor

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Abstract

Castor is one of the important non edible oilseed crops in India. The objective of this work was to estimate the incidence of *Botrytis ricini* on castor seed using different seed health test methods. A total sixty nine samples were collected from retail shops, APMC markets, fields and farmers of different agro-climatic regions of Karnataka kharif during - 2011. Among all the collected samples, five samples show a higher incidence of *B. ricini* and other fungi, were selected for PDA, Water agar and 2,4-D methods. The incidence of seed infection was 14.8 percent on a selective medium for standard blotter method (SBM), Potato dextrose agar medium 8.8 percent (PDA), Water agar medium 7.8 percent and 2,4-D 11.4 percent (2,4, Dichloro phenoxy acetic acid) methods respectively. Determine the rate of pathogenicity under green house conditions. *B. ricini* showed the symptoms of gray mold were observed in zero percent in one month seedlings, 0-10 percent in two month seedlings and 10-40 percent gray mold in three month old plants, no gray mold observed in water treatment plants. Among the sample collected field and farmers samples show a higher incidence of *B. ricini* and other fungi. The seed health test methods, SBM is most superior for isolating the *B. ricini*, pathogenic and saprophytic fungi. The present study is concentrated on detection of *B. ricini* and its pathogenicity were discussed. *B. ricini* is a causal agent of gray mold disease of castor crop.

Keywords: Castor, Seed health tests, Pathogenicity, *Botrytis ricini*, SBM

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, 1976). This crop is widely distributed throughout the tropics and sub-tropics and is well adapted to the temperate regions of the world (Dange, *et al*., 2005). Castor is cultivated over on area of 20161 hectares with a production 17493 tones and productivity 193 kg/ha in Karnataka (Anonymous, 2011). 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* (Rangaswamy and Mahadevan, 2005).These diseases are reduces the yield, production and germination up to 30-50% (Kumar *et al*., 2007). Therefore, the present study was conducted to detection of *B. ricini* and other mycoflora of castor seeds and their Pathogenicity was studied.

Materials and methods

Scope of the Study

The present experiment was carried out at Department of Applied Botany, Plant Pathology laboratory, Kuvempu University, Shankaraghatta, Shivamogga Karnataka during *kharif* season- 2011. Castor seed samples (local variety) collected from different castor growing districts of Karnataka *viz.*, Bellary, Bidar, Chitradurga, Chikmagalore, Davanagere, Dharwad, Gulabarga, Haveri, Mysore, Chamarajanagar, Tumkur, Bangalore-rural, Bangalore-urban, Kolar, Dhrarwad and Raichur districts.

Collection of castor seed samples

The seeds of castor 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, Mysore, Chamarajanagar, Tumkur, Bangalore-rural, Bangalore-urban, Kolar, Dhrarwad 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 *B. 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 Akueshi, 1996). 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 ($28\pm1^{o}C$) 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°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°C for seven days. After seven days these seeds were examined under stereo binocular microscope (Neergaard, 1977).

d. 2, 4-D Method:

In this method, 100 seeds were sterilized with 0.2% sodium hypo chlorite solution for 2 to 3 minutes. 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 (Limonard, 1968).

Screening of *B. ricini* and 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; Barnett, 1960; Sigourd and Funder, 1961and Subramanian, 1983).

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) leveled and ploughed. 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 *B 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 ten replicates of ten per row. The conidial suspension was applied with the help of sprayer on abaxial and adaxial surface of leaves, stem and inflorescence (Bhale *et al*., 1999 and Bhale *et al*., 2001). 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) and percent disease index calculated using the formula

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

Results and discussion

Seed health testing

Results of four types of methods used to detect *B. ricini* and other mycoflora shown in (Table 1). The standard blotter methods were more sensitive in detection of *B. ricini* than the PDA, Water agar and 2, 4, Dichloro phenoxy acetic acid mediums. Significant differences in occurrence of seed mycoflora were observed and the results indicated that irrespective of the locations and sources, a

total of nine fungal species *viz., Alternaria ricini. F.oxysporum* f.sp. *ricini, Alternaria alternata, Botrytis ricini, Cercospora ricini, Macrophomina phaseolina, Aspergillus flavus, Aspergillus niger*, and *Aspergillus ochraceus* belonging to two genera were detected from local variety of castor beans. Out of nine fungal species recorded, the occurrence of *B. ricini, F.oxysporum* f.sp. *ricini* and *Alternaria ricini* was found predominant in the seed samples analyzed from sixteen districts (14.8%). The present study revealed that occurrence of seed borne *B. ricini* and other fungi may vary depending up on the location and sources of collection from different farmers and fields. The present findings are in conformity with earlier reports of (Dange *et al*., 2005 and Bhattiprolu & Bhattiprolu, 2006) who reported that variation in the occurrence of seed borne *B. ricini* and other fungi according to geographic location in castor crop.

Similarly, visual sporulation of the fungus on the seed was generally heavier in the SBM methods than in Water agar method, PDA and 2,4-D methods. However, the standard blotter method was the most effective and revealed a higher incidence of seed infection than the other methods. Among the collected seed samples, farmers and fields samples shows a higher incidence of *B. ricini*. The Chitradurga, Tumkur, and Chickmangalore locations are higher incidence of *B. ricini* and other fungi than Raichur and Bellary districts etc. This method was also easy quick for recording the presence of *B. ricini* on the seed.

Pathogenicity test

Inoculums sprayed plants showed the symptoms *B. ricini* in two to three months. The first symptoms are visible as bluish spots on the inflorescences, on both female and male (before anthesis) flowers, and on developing fruits. On fruits, the symptoms can evolve to circular or elliptic, sunken, dark coloured spots that can result in rupture of the capsule. The symptoms on the male flowers, before anthesis, are small, pale brown, necrotic spots, which can evolve to larger brown spots with a darker edge. The infected flowers and young capsules became softened due the fungal colonization and mycelial growth is, at first, pale gray and later dark olivaceous. A profuse sporulation is usually observed in such stage. When the infection starts on immature capsules, they become rotten; if the infection starts later, with fully developed capsules, the seeds usually became hollow, with coat discoloration and weight loss. Determine the rate of pathogenicity under green house conditions, *B. ricini* showed the symptoms of gray mold were observed in zero percent in one month seedlings, 0-10 percent in two month seedlings and 10-40 percent gray mold in three month old plants, no gray mold observed in water treatment plants (Table 2).

The importance of *B. 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 *B. ricini* was the most sensitive. The antibiotics in to the medium not only did not inhibit the growth of *B. ricini* but also suppressed the growth of other fungi *A. ricini, F. oxysporum* f.sp. *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 *B. ricini* on seed. This fact facilitated the detection of the target fungus and gave a higher record of incidence.

Table 1: Incidence of *B. ricini* in seed health test methods of castor.

Seed health testing methods	% seed infection								
	B.ric	**F.oxy**	**A.alt**	**M.pha**	**C. ric**	**A. ric**	**A.nig**	**A.fla**	**A.och**
SBM	*14.8	19.4	11.0	12.4	13.6	16.8	11.6	4.8	5.0
PDA	8.8	17.8	11.2	9.6	10.8	10.8	7.6	6.2	3.4
Water agar	7.8	8.2	13.4	8.4	10.6	15.8	9.8	6.2	6.4
2,4-D	11.4	8.6	7.6	6.4	10.2	7.0	5.2	5.6	5.0
SD	1.0748	5.9273	2.3944	2.5086	1.5534	4.4136	2.7682	0.6633	1.2261
	±	±	±	±	±	±	±	±	±
SE	0.2371	2.9636	1.1972	1.2543	0.7767	2.5482	1.3841	0.2966	0.6130

Average values of five samples and 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*

Table 2: Artificial inoculation of *B. 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)**
Alternaria ricini	72	00	01	04
Water treatment	90	00	00	00

Data based on 100 seed samples.

Many researchers (Brigham, 1961; Karan, 1966; Raoof, *et al.*, 2003 and Tirupathi *et al*., 2006) studied on seed borne nature of *B. ricini*, diseases of castor and causal agent of gray mold disease of castor crop.

These symptoms are usually more frequent when a period of low relative humidity unfavorable to fungal sporulation occurs soon after the fungus penetrates the host tissues. 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 *et al*., 2002). *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*., 1999 and Bhale *et al*., 2001). 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, 2001). 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).

Conclusion

Results from the present investigation indicated that there was variation in mycoflora from one locality to another. Mycoflora of seed varied from place to place due to change in conditions prevailing during seed development, harvesting and storage. Out of four methods adopted for

detection of seed borne *B. ricini* and other fungi, standard blotter method (SBM) was proved to be superior to other methods as the total fungal colonies was more in standard blotter method. Out of nine fungal species recorded, *B. ricini* was found predominant in the samples analysed from sixteen districts of Karnataka. Detection of *B. 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 castor 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 15-60 % if untreated seeds are grown in the field. *B. ricini* is a seed-borne and causal agent of gray mold disease of castor crop.

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