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DETECTION OF SEED-BORNE *MACROPHOMINA PHASEOLINA* THE ROOT-ROT FUNGUS LOCATION AND TRANSMISSION IN CASTOR (*RICINUS COMMUNIS* L) KARNATAKA

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ABSTRACT: An investigate to detect the seed-borne *Macrophomina phaseolina* and other fungi, location and its transmission were conducted in the Department of Applied Botany, Plant Pathology laboratory, Kuvempu University Shivamogga during 2006. A total 130 seed samples of Castor were collected from farmers, fields and APMC markets of different agro climatic regions of Karnataka kharif during-2006. *M. phaseolina* and other fungi were detected by both SBM, PDA, WA and 2, 4-D methods. *M. phaseolina* was detected in all seed health tests and the pathogen incidence ranged from (11-38%) in SBM different seed samples. Detection of *M. phaseolina* other fungi shows forty five fungal species belonging to four genera were recorded in local variety of Castor beans. The seed component tests, seed coat and cotyledons showed highest incidence of *M. phaseolina*. The present study reveals that the disease transmission is more during kharif-2006 (32.4%) season than 2007 (19.0%). The pathogen was found both seed-borne and seed transmitted in Castor. *M. phaseolina* the causal agent of root-rot and charcoal rot disease of castor.
Key words: Castor, SBM test, Location, Transmission, *M. phaseolina.*

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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 [22]. 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 [1]. 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* [16]. It appears at all crop growth stages but more conspicuous during early stage. Root-rot and charcoal rot of castor caused by *M. phaseolina* is considered to be a major devastating disease in India.

In the present work, the occurrence and detection of *M. phaseolina* and other mycoflora on castor seeds in various samples collected from different regions of Karnataka. Location, seed to seedling transmission and their frequency of mortality, recovery of pathogens and its significance were studied.

METHODOLOGY

Collection of Castor seed samples

A total of 130 samples were collected from castor during kharif, 2006 (Table, 1). Seeds were harvested from mature castor plants, farmers, fields, retail shops and APMC markets in different agro climatic regions of Karnataka state. Seed collections were made during May to July 2006. The seeds were stored in cloth bags at room temperature 23±2^{o}C for further investigation studied.

Table-1. Details of Castor seed collection in Karnataka during kharif-2006

Name of the districts	Variety	Source of seed sample collection				Number of sample collected
		Farmers	Fields	Retail shops	APMC markets	
Bangalore-urban	Local	1	6	1	-	8
Bangalore-rural	Local	1	6	1	-	8
Bellary	Local	2	-	-	1	3
Bidar	Local	2	-	1	1	4
Bijapur	Local	2	-	2	1	5
Chitradurga	Local	11	2	-	-	13
Chikamagalur	Local	1	2	-	4	7
Chamarajanagar	Local	-	-	-	5	5
Davanagere	Local	7	-	1	1	9
Dharwad	Local	-	2	1	2	5
Kolar	Local	5	-	-	-	5
Haveri	Local	3	4	-	5	10
Hassan	Local	1	-	2	5	8
Gulbarga	Local	-	-	1	-	1
Mandya	Local	8	-	1	2	11
Mysore	Local	3	2	-	3	8
Shivamogga	Local	-	4	-	-	4
Tumkur	Local	1	-	-	9	10
Raichur	Local	1	-	2	3	6
Total		49	28	13	42	130

Detection of seed-borne *M. phaseolina* and other fungi:

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 and placed at the bottom of the Petri plates. 100 seeds were sterilized with 0.2% sodium hypo chloride solution for 2 to 3 minuets and seeds taken randomly from each sample and were placed in ten Petri plates (Ten seeds per plate). The Petri plates with seeds were than incubated at room temperature for seven days in the laboratory. The plates were alternating cycles of 12 hrs light and 12 hrs darkness for seven days. Sterile distilled water was aseptically added to each Petri plates under incubation every third day in order to keep the blotter is sufficiently moist [15]. Germination and fungi associated with the seeds were recorded during the incubation period. Each of the incubated seeds was 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. Fungi were identified by preparing temporary slides 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 then examined under labomed vision 2000 compound microscope.

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 [7, 8, 9, 10 11, 14, 19, 20].

Location of the *M. phaseolina* in Castor

For investigation presence of the pathogen in castor seeds, component plating method [21, 23, 3] was used. The individual seed components were excised after soaking the surface sterilized seeds 0.2% sodium hypochloride (NaOCl) for three min, in sterile distilled water for five hours. The seed coat, cotyledons and embryonic axis (Plumule and radicle) were dissected aseptically using forceps and needles on blotter. Each component was dipped separately in 0.2% sodium hypochloride solution (NaOCl) for 50 to 90 seconds and was plated on SBM method, and incubated as described the above. One hundred seeds were dissected in each sample and ten replication were maintained. The plates incubated at 25 ± 2^0C for room temperature. After eight day observation of these plates under stereobinacular microscope. Fungal infection in different seed components was determined based on the appearance of the fungus on the SBM and the percentage of infection was calculated.

Disease transmission studies in the field

Among the five samples shows a higher incidence of *M. phaseolina* were selected for disease transmission. One hundred seeds of each sample were used for determining the seed to seedlings in the experimental plots. Before sowing the seeds the experimental plot were prepared by 10 x 10 meter (row and columns). Each sample selected 100 seeds in ten replicates. Sterilized seeds were directly sowing in the fields in the month of July -2006. The proper agronomical practices were followed for raising the plants. All the seeds have germinated after 7-10 days. In experimental plots, 15 plants were randomly selected by selecting five leaves randomly in each plant. The severity of the disease was assessed by using 0-9 scale [24] and percentage of diseases index was calculated by using the formula.

$$\text{\% of disease Index (PDI)} = \frac{\text{Sum of individual ratings}}{\text{No. of leaves examined X Maximum disease grade (9)}}$$

Seed to seedling transmission of *M. phaseolina* of pathogens were studied.

Recovery of pathogens from diseased plants

Seeds were collected from experimental plots in rabi seasons. Seeds were subjected for seed health test by SBM. In experimental plot for recovery of pathogens were studied. These seeds yielded the *M. phaseolina*. The study shows that *M. phaseolina* are transmitted from seed to seedlings and to the seed [21].

RESULTS AND DISCUSSIONS

Detection of seed-borne *M. phaseolina* and other fungi:

Sporulation of *M. phaseolina* occurred after 6 to 9 days on surface of the seed coat of castor seeds. The incidence of *M. phaseolina* ranged between 11-38% in tested seed samples. Incidence of other fungi was recorded in. The analysis of seed-borne fungi of castor showed forty five fungal species belonging to four genera namely, *Alternaria ricini, A. alternata,A. tenuis, Fusarium moniliforme, Macrophomina phaseolina, Curvularia lunata, Sclerotinia sclerotiorum, Chaetomium globosum, Chaetomium* sp.,*Cladosporium herbarum,Cladosporium cladosporioides, C. chlorocephalum, C. fulvum, Cunnighamella elegans, Botryodiplodia acerina, Stachybotrys chartarum, S. atra, Phoma glomerata, Pestaliopsis macrotricha, Myrothecium roridum, Gliocladium roseum, Helminthosporium bipolaris, Phytophthora* sp., *Popullospora sphaerosperma, Oedocephalum caprophyllum, Verticillium dahliae, Cercospora ricini., Aspergillus ochraceus, A. niger, A. flavus, A. versicolor, A. candidus, A. fumigatus, A. terreus, Penicillium digitatum, Penicillium* sp., *Haplosporangium* sp., *Trichoderma harzianum, T. viride, Neurospora glabra, Nigrospora* sp., *Rhizopus stolonifer. R.nigricans* and Mycella sterillae respectively.

Root-rot and Charcoal rot disease caused by *M. phaseolina*

M. phaseolina produces characteristic root-rot and charcoal rot symptoms appears in young plants during the period from sprouting to formation of two or three real. The disease appears at all phases as color rot, stem blight and root rot. Initially the infected plant shows the sign of water shortage. Within a week the leaves and petiole drop down and finally within a fortnight, the entire plants dries up and get easily pulled up. The patches of dried plants are seen in severely infected field. The tap root shows sign of drying and root bark shreds-off easily, rotting sometimes spreads partly above the ground, at an advanced stage, sclerotial body is seen as minute black dots on the surface woody tissues and in pith region.

The pathogen is known to survive soil borne to through the diseased debris. The pathogen is also known to survive on the infected seeds. The disease is well characterised by the presence of numerous black microsclerotia varying from 100 µm to 1 mm in stems, leaves and roots and 50 µm -300 µm in culture. Under the microscope they appear as round to ovate black dots which, when lighted from above, have relatively large, smooth, rounded bumps on their surface, a bit like an artichoke. Clear images of microsclerotia were obtained by using optical and electron microscopes. Pycnidia may also sometimes be seen. These are black and globose varying from 100 µm to 250 µm in length with a truncate ostiole, the spores of which are pointed at one end. The hyphae are septate, usually containing numerous vesicles. The fungus is very common in the tropics and usually infects roots of variety of crop plants and causes seedling, stem rot, charcoal rot and root rot diseases. The fungus also capable of surviving in soils for considerable periods by means of the sclerotia or pycnidia.

Location of *M. phaseolina* in Castor seeds

Component plating revealed that the infection of *M.phaseolina* in the seeds of castor seed is mainly located externally as well as internally in the cotyledons, seed coat and rarely in embryonic axis. In castor seeds *M. phaseolina* (11-38%) in the SBM method. *M. phaseolina* ranged (2-12%) in seed coat, (0-8%) in cotyledons and (0-1%) in plumule and radical during kharif-2006. In majority of the infected seeds, sporulation of *M. phaseolina* was first observed in the and seed coat and cotyledons region of Castor seeds (Table 2).

Table-3. Location of *M.phaseolina* in different seed component plating method kharif 2006.

Place of collection	% of infection in SBM	In percentage		
		Seed coat	Cotyledons	Embryonic axis
Jalahalli	11.0	4.0	5.0	0.0
Hebbala	22.0	6.0	4.0	0.0
Nelamangala	38.0	12.0	8.0	1.0
Mylnalli	33.0	7.0	3.0	0.0
Anekal	21.0	8.0	6.0	0.0
Yeshvanthpura	16.0	3.0	0.0	0.0
Dudda	14.0	2.0	0.0	0.0
Chikkaballapur	26.0	12.0	3.0	0.0
Thubina kere	22.0	11.0	3.0	0.0
Bannur	15.0	2.0	1.0	0.0
Mean	21.8	6.7	3.3	0.1
SD	8.146165	3.769615	2.45153	0.3
SE	2.715388	1.86543	0.817177	0

Data based on 100 seeds for each sample.

Disease transmission studies in the field

Transmission studies revealed that the reduction of yield in naturally infected seeds and reconfirmation of transmission in experimental plot of oilseed crop. The root rot symptoms shows, tap root shows sign of drying and root bark shreds-off easily, rotting sometimes spreads partly above the ground, at an advanced stage, sclerotial body is seen as minute black dots on the surface woody tissues and in pith region. The pathogen is known to survive soil borne to through the diseased debris. The pathogen is also known to survive on the infected seeds. In kharif 2006, the castor seeds having (11-38%) infection in SBM of *M.phaseolina* and when sown in experimental plot resulted (32.4%) average transmission (Table 3). The seeds collected from disease transmitted plants sown again in kharif, 2007 season for reconfirmation of transmission. *M. phaseolina* results at the (19.9%) average transmission in all five seed samples (Table 4). During the field survey the early blight, root-rot and charcoal rot symptoms of castor was noticed in all visited fields during the kharif-2006-07. The severity of early blight, root-rot and charcoal rot symptoms was more in kharif 2006 than 2007. The present study results revealed that the seeds having infection of *M. phaseolina* showed the transmission of (32.4%) (19.0%) in castor (Average of five seed samples, Table 3, 4).

Table-4. Seed to Seedling transmission *M. phaseolina* in experimental plot during kharif-2006.

Place of collection	% of incidence in SBM *M.pha*	Germ %	% of mortality				Recovery of pathogens
			Pre-emergence	Post-emergence	% of Diseased plants	% of Healthy plants	*M.pha*
Jalahalli	11.0	77.0	23.0	5.0	23.0	31.0	22.0
Hebbal	19.0	76.0	24.0	3.0	33.0	40.0	26.0
Nelamangala	38.0	66.0	36.0	2.0	34.0	30.0	47.0
Mylnalli	24.0	78.0	22.0	3.0	55.0	20.0	21.0
Anekal	30.0	61.0	39.0	1.0	36.0	30.0	46.0
Mean	24.4	71.6	28.8	2.8	36.2	30.2	32.4
SD	9.221714	6.829348	7.194442	1.32665	10.41921	6.337192	11.63787
SE	3.850383	3.414674	3.597221	0.663325	5.209607	3.168596	5.818935

Table-5. Seed to Seedling transmission *M. phaseolina* in experimental plot during kharif-2007.

Place of collection	% of incidence in SBM *M.pha*	Germ %	% of mortality				Recovery of pathogens
			Pre-emergence	Post-emergence	% of Diseased plants	% of Healthy plants	*M.pha*
Jalahalli	22.0	78.0	12.0	5.0	23.0	50.0	13.0
Hebbal	26.0	80.0	20.0	4.0	26.0	60.0	19.0
Nelamangala	47.0	82.0	18.0	6.0	24.0	50.0	24.0
Mylnalli	21.0	78.0	22.0	3.0	55.0	20.0	12.0
Anekal	46.0	71.0	29.0	2.0	19.0	50.0	27.0
Mean	32.4	77.8	20.2	4	29.4	46	19
SD	11.63787	3.709447	5.52810998	1.41421356	13.0015384	13.56466	5.89915248
SE	5.818935	1.854724	2.76405499	0.067876	6.50076919	600,564	2.94957624

Recovery of *M. phaseolina* from diseased plants: Seeds samples were collected from experimental plot and were subjected for seed health test methods (ISTA, 2003) for recovery of disease transmission. The seeds collected from disease transmitted root rot and charcoal rot sick plants. Infection having (11-38%) *M phaseolina* showed the (32.4%) (19, 9%) transmission (Average of five seed samples, Table 3, 4). Reduction of the yield is based on the environmental conditions and the severity of the symptoms.

During the present investigations mode to study the pathogenic *M. phaseolina*, seed borne fungi and other mycoflora of castor and their location, seed to seedling transmission. It is observed that *M. phaseolina*, the pathogenic fungus causing root rot and charcoal rot disease of Castor. It is found to be seed borne as well as soil borne and seed transmitted. This may be the cause for wide spread nature of the root-rot diseases in Karnataka and other states of India. That it is very much necessary to know the seed mycoflora and seed borne pathogen of castor. Detection of *M. Phaseolina* in castor seeds is highly significant due to its destructive nature an castor crop. These fungi were isolated from local variety of castor seeds. *M. phaseolina, Sclerotinia sclerotiorum, F. oxysporum* f sp. *ricini, A. ricini, Cercospora ricini, Botrytis ricini A. alternata, Aspergillus ochraceus, A. flavus* and *A, niger* are most predominant fungi in castor seeds. These samples screened farmers and field samples showed higher incidence of pathogenic and storage fungi. Chitradurga, Davanagere and Chikamagalur seed samples showed higher incidence of fungi then lower in Raichur and Bellary districts. Some of the research workers [2, 4 15,] reported detection and seed borne nature of many pathogenic and saprophytic fungi in different oil seed crops like sunflower, castor, soyabean, niger, safflower, ground nut and sesamum seeds.

Component plating of castor seeds revealed that the pathogenic *M. phaseolina* is present in seed coat, cotyledons and embryonic axis (plumule and radicle) with highest percentage in seed coat than other seed components. Location of the pathogen in the seed is important to control seed borne pathogens, based on location of the pathogen in the seeds. The chemicals are selected to prevent the seed borne pathogens. Majority of the seed borne pathogens are lodged in seed coat, cotyledons, endosperm and embryonic axis of various oil seed crops. This is due to the environmental factors like rain fall, temperature,humidity and in growth stages of the crop. [2,3, 6, 18, 12, 21, 23].

The present study revealed that the transmission of the pathogens were more in kharif-2006 season than kharif 2007 harvested seeds (Table-5). The mode of seed to seedling transmission of the pathogen is depends on the aggressiveness of the pathogen and environmental conditions like rain fall, temperature and relative humidity. These results shows that the kharif-2006 season favors more percentage of pathogens have transmission from seed to seedlings and to seeds. Because, this is environmental factors are induced for the transmission of pathogens. Some research workers have reported in transmission studies of various oilseed crops [2, 3, 6, 12, 13, 17, 21, 23, 24].

CONCLUSIONS

M. phaseolina the causal agent of root-rot and charcoal rot disease of castor. The disease has been reported from Phillipines, India, Ceylon, east Africa, Palestine, West Indies and Eastern United States. In india the disease reported from Andra Pradesh, Gujarat, Maharastra, Bihar and Tamil Nadu and Karnataka Detection of Seed-borne *M. phaseolina* and other mycoflora 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. It suggests that seeds are major agent of fungal transmission. Seeds should be treated with suitable chemical before sowing to reduce the fungal infection. This is due to the environmental factors like rainfall, humidity, temperature, and also in growth stages of the crop.

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