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Seed borne mycoflora of castor beans and their management by botanical extracts

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Abstract
An investigate to detect the seed-borne mycoflora and management by plant extracts were conducted in Seed borne mycoflora of castor beans and their management by botanical extracts the Department of botany, Plant Pathology laboratory, DRM science college Davanagere during 2021. Seven samples of castor beans were collected from Santhebennur, Hirekoglur, Mallapura, Balliganudu, Thanigere, Gollarahalli and Erganalli of different agro climatic regions of Santhebennu hobli, channagiri taluk Davanagere district Karnataka rabi season during-2021. The different plants extracts like garlic and neem leaf extract were used for control of mycoflora and seed germination. These plantextract were treated at different concentrations (1:1, 1:2 and 1:3) and mycoflora were recorded. Total eighteen fungi were recorded in all type of castor beans. Above seed health test methods SBM is superior for detection of seed borne mycoflora than 2,4. D and Santhebennur, Balliganudu, Hirekoglur, Mallapura and Thanigere samples shows higher incidence of mycoflora than others. Among the plant extracts, neem extract were found superior for the inhibition of seed borne pathogens and increase the seed germination in 1:3 percent concentration. So, this study recommends the use of plant extracts which may increase seed germination and inhibit seed-borne fungi without any harmful effect.

Keywords: castor beans, Mycoflora, Balliganudu, SBM, neem extract

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 [19, 20]. The oil content of the seeds ranged from 40-55%. The seed is unique in that its oil contains (85%) ricinoleic acid. The castor bean also contains protein (18-20%) carbohydrate (2%) [11]. It is grown as intercrop, marginal, sub marginal and major crop also. Castor is cultivated over on area of 95 percent hectares with a production 19,50 lakh tones and productivity 2010 kg/ha in Karnataka [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* [9 14]. These diseases reduced the yield, seed germination, seedling vigour and oil quality [18].

In present work, seven local samples of castor beans were collected from Santhebennur, Hirekoglur, Mallapura, Balliganudu, Thanigere, Gollarahalli and Erganalli of different agro climatic regions of santhebennu hobli channagiri taluk Davanagere district Karnataka rabi season during-2021. The different plants extract sike garlic and neem leaf extract were used for control of mycoflora and seed germination. The plant extractwere treated at different concentrations (1:1., 1:2 and 1:3) and mycoflora were recorded.

Materials and methods

Collection of seed samples

Field survey was carried out in Davanagere during rabi season-2021. A total of seven Santhebennur, Hirekoglur, Mallapura, Balliganudu, Thanigere, Gollarahalli and Erganalli samples were collected from retail shopes and farmers. The seed samples were dried in sunlight to bring down the safe storage seed moisture and were stored in cloth bags at room temperature for further use. All the seeds were subjected to standard blotter method (SBM). five samples showing higher incidence of seed borne fungi in SBM method were selected for further studies.

Table 1: Details of castor beans collected in Davanagere during-2021.

Place of collection	Varieties	Sources		Total seeds collected
		Farmers	Retail shops	
Santhebennur	Local	01	--	01
Hirekoglur	Local	01	--	01
Mallapura	Local	01	--	01
Balliganudu	Local	01	--	01
Thanigere	Local	01	--	01
Gollarahalli	Local	01	--	01
Erganalli	Local	00	01	01
Total				07

Analysis of seed bone mycoflora by seed health test methods.

Standard blotter method (ISTA.1993)

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 5 to 10 minutes 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. 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±1ºC) for 3 to 10 days and then examined under labomed vision 2000 compound microscope. The isolated fungi were identified with the help of the keys, monograph and literature [3]. All experiments were carried out in ten replicas.

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 [12].

Identification of seed borne mycoflora on castor beans

The incubated seeds were screened on eighth day using stereo binocular and labomed vision 2000 compound microscope. Incubated seeds were observed under stereomicroscope at 16x and 25x magnification. The incidence of seed borne fungi was detected by observing their growth characters on the incubated seeds on blotter paper following keys outline. Temporary slides were prepared from the fungal colony and observed under compound microscope. The mycoflora were identified with the help of keys suggested by [4, 6, 12, 16, 17,].

Control of seed borne mycoflora by different plant extracts

Five samples having higher incidence of mycoflora in SBM were selected for slurry treatment. The plants like garlic and neem leaf were treated individually at different concentrations (1:1, 1:2 and 1:3).

Preparation of plant extracts

For the investigation antifungal effect of plant extract, plant samples such as bulbs of garlic were city markets, and neem leaves were collected from DRM Science college in and around the campus. The collected plant parts were washed carefully in running tap water, dried and weighed by electric balance. *garlic* and *neem* leaf extracts were prepared by grinding in mortar and pestle. Then 1ml, 2ml and 3ml of distilled water were added, respectively with 1 gram of plant material to prepare plant extracts having 1:1, and 1:3 doses (weight/volume). The crushed materials were filtered through cheese cloth.

Seed treatment with plant extracts

Castor beans were treated in each dilution (1:1) w/v and (1:3) w/v of each two plant extracts - Garlic and Neem. Seed samples of castor beans were dipped in each extract contained one petridishes at different dilution for one hour. Then the plant extract was drained out from the petridish. The treated beans were shade dried on blotting papers for one hour. A set of control was maintained by dipping the seeds in tap water. After incubation, germination and seed borne fungi were observed.

Statistical Analysis

To calculate the means of mediums, ten replicates (independent samples) were taken for each variable (growth of fungi). Homogeneity of the variance was tested by the Levene test and found that the group variances are homogenous. The mean values, Standard deviation (SD), standard error (SE) of the mycoflora and germination in all the mediums were calculated. The statistical analysis was performed using the IBM SPSS software package.

Result and Discussion

The seed borne mycoflora of whole castor beans samples are shown in table 2. The table also shows incidence of infection of these microorganisms on local castor bean verities tested. Among the seven local variety of castor bean showed eighteen fugal species belonging to nonsporulating fungi namely *F. oxysporum. f.sp.ricini* (56-26%) *Alternaria ricini* (58-10%) *A.alternata* (15-10%) *A.tenuissima* (54-13%) *Cercospora ricini*(73-11%) *urvularia lunata*(15-2%) *Cladosporium herbarum*(23-12%) *Botrytis cinarea* (81-8%), *Chaetomium globossum* (23-10%) *Macrophomina phaseolina* (23-10%) *Aspergillus flavus* (76-31%) *A.niger* (18-11%) *A.ochraceus* (81-33%) *A.candida* (14-2%) *A.versicolor* (21-11%) *Penicillium citrinum* (25-17%) *Rhizopus stolonifer* (21-11%) *R.nigricance*(14-2%) *Non sporulating fungi* (NSF) (19-6%) (table-2). Among the local castor bean varieties Santhebennur, Hirekoglur, Thanigere, Balliganudu and Mallapura showed higher incidence of mycoflora than others. Castor beans collected in among the villages farmers samples shows higher incidence of mycoflora than retail shopes. The above seed health tests SBM showed more superior for detecting the seed borne fungi than 2, 4, D method. In several cultivated crops, number of pathogenic and saprophytic seed borne fungi were reported to affect adversely the seed germination, root and shoot length and seedling vigor index as well as optimum plant population per unit area. In this context, results of the present study are in consonance with earlier reports of many workers.

An investigation was conducted to detect the associated seed mycoflora in safflower and its control. Blotter method and agar plate methods were used for detection of seed mycoflora of safflower seeds. Across the two methods adopted, a total of seven fungal genera including Alternaria, Aspergillus, Chaetomium, Rhizopus, Curvularia and Fusarium were detected. The cultivar, Nira showed higher per cent incidence of seed mycoflora. Per cent incidence of seed mycoflora varied across the methods adopted and

cultivars tested. The highest per cent incidence of 46% was observed with the fungus Alternaria carthami on Nari NH1 in blotter method. Out of the two methods tested blotter method was found superior over [1, 5, 15].

Table 2: Incidence of seed borne mycoflora of castor beans during 2021.

Name of the fungi	Methods	Germ (%)	Santhebennur	Hirekoglur	Mallapura	Gollarahalli	Thanigere	Balliganudu	Erganalli
F. oxysporum. f.sp.ricini	SBM	10.0	48.0	40.0	56.0	30.0	26.0	34.0	26.0
	2,4-D	00	18.0	10.0	18.0	4.0	10.0	12.0	8.0
Alternaria ricini	SBM	9.0	36.0	40.0	58.0	10.0	34.0	11.0	12.0
	2,4-D	00	20.0	10.0	14.0	8.0	17.0	19.0	11.0
A.alternata	SBM	6.0	10.0	14.0	15.0	13.0	15.0	12.0	15.0
	2,4-D	00	13.0	8.0	18.0	14.0	19.0	16.0	12.0
A.tenuissima	SBM	9.0	54.0	16.0	13.0	16.0	19.0	16.0	0.0
	2,4-D	0.0	22.0	33.0	12.0	19.0	14.0	12.0	2.0
Cercospora ricini	SBM	13.0	73.0	43.0	28.0	15.0	18.0	22.0	11.0
	2,4-D	1.0	24.0	13.0	23.0	7.0	12.0	10.0	0.0
Curvularia lunata	SBM	18.0	11.0	6.0	8.0	8.0	13.0	15.0	2.0
	2,4-D	00	14.0	100	3.0	2.0	1.0	12.0	00
Cladosporium herbarum	SBM	2.0	12.0	19.0	18.0	21.0	22.0	23.0	12.0
	2,4-D	00	10.0	21.0	9.0	9.0	21.0	13.0	1.0
Botrytis cinarea	SBM	14.0	22.0	22.0	26.0	8.0	81.0	18.0	18.0
	2,4-D	0.0	13.0	0.0	18.0	3.0	3.0	13.0	3.0
Macrophomina phaseolina	SBM	15.0	16.0	10.0	11.0	13.0	14.0	17.0	23.0
	2,4-D	0.0	13.0	12.0	1.0	3.0	2.0	1.0	0.0
Aspergillus flavus	SBM	18.0	76.0	45.0	43.0	33.0	31.0	44.0	40.0
	2,4-D	3.0	10.0	31.0	22.0	11.0	13.0	12.0	11.0
A.niger	SBM	19.0	16.0	15.0	12.0	11.0	18.0	11.0	12.0
	2,4-D	0.0	9.0	8.0	2.0	4.0	5.0	6.0	7.0
A.ochraceus	SBM	12.0	81.0	59.0	61.0	66.0	33.0	34.0	39.0
	2,4-D	0.0	22.0	34.0	0.0	3.0	4.0	5.0	33.0
A.candida	SBM	11.0	9.0	8.0	2.0	4.0	11.0	14.0	12.0
	2,4-D	2.0	0.0	9.0	9.0	8.0	2.0	4.0	1.0
A.versicolor	SBM	18.0	21.0	13.0	12.0	15.0	12.0	11.0	11.0
	2,4-D	0.0	9.0	8.0	2.0	4.0	3,0	3.0	0.0
Penicillium citrinum	SBM	13.0	17.0	19.0	23.0	23.0	25.0	22.0	23.0
	2,4-D	0.0	9.0	8.0	2.0	4.0	3,0	3.0	0.0
Rhizopus stolonifer	SBM	12.0	21.0	13.0	12.0	15.0	12.0	11.0	11.0
	2,4-D	0.0	0.0	0.0	1.0	2.0	3.0	0.0	0.0
R.nigricance	SBM	10.0	11.0	13.0	14.0	0.0	0.0	2.0	3.0
	2,4-D	0.0	0.0	0.0	0.0	0.0	0.0	3.0	3.0
Non sporulating fungi	SBM	00	6.0	11.0	13.0	19.0	12.0	12.0	13.0
	2,4-D	00	0.0	0.0	0.0	0.0	4.0	5.0	6.0
Mean	--	5.690	20.72	19.75	16.08	11.80	15.47	13.37	10.58
SD	--	6.851	20.62	19.08	16.00	12.33	14.92	9.599	10.87
SE	--	3.162	6.928	6.322	7.483	5.472	5.990	5.823	5.099

*Data based on 100 castor beans in each sample and ten Replications

Efficacy of plant extracts on the germination and mycoflora of castor beans

The results of the present study showed that, almost all the treatments significantly showed positive performance in compared to control after treating the seeds with different botanicals. Location of the pathogen in the seed is important to control seed borne pathogens. Based on the location of the pathogen in the seed and biology of the pathogens, plants extracts are selected to prevent the seed borne pathogens. All the plant extracts seed treatments showed reduction in the seed borne fungi. Seeds treated with two plant extracts at different concentrations. In castor the neem extracts gave maximum inhibition mycelia growth of a majority of the seed borne mycoflra and germination percentage was increases in all five samples (Table, 3&4). Where as in case of garlic extract shows maximum reduction of majority of a seed borne fungi (Table, 3) the santhebennur verity, *F. oxysporum f.sp, ricini,* (48-3%) *A.ricini,* (36-2%) *M.phaselina,* (16-2%) *C.ricini,* (73-10%) *B.cineria* (22-2%) and *A.ochraceus* (81-6%). Hirekoglur sample shows *F. oxysporum f.sp, ricini,* (40-9%) *A.ricini,* (40-2%) *M.phaselina,* (10-2%) *C.ricini,* (43-2%) *B.cineria* (22-3%) and *A.ochraceus* (22-3%).Simillarly mallapura sample shows *F. oxysporum f.sp, ricini,* (56-2%) *A.ricini,* (58-5%) *M.phaselina,* (11-1%) *C.ricini,* (28-1%) *B.cineria* (26-2%) balliganudu samples *F. oxysporum f.sp, ricini,* (34-2%) *A.ricini,* (11-1%) *M.phaselina,* (17-1%) *C.ricini,* (22-3%) *B.cineria* (18-2%) and *A.ochraceus* (34-1%) and thanigere shows *F. oxysporum f.sp, ricini,* (26-1%) *A.ricini,* (34-3%) *B.cineria* (81-1%) and *A.ochraceus* (33-3%) increases the germination all five samples respectively.

Some of the workers studied efficacy of plant extracts through control of seed borne mycoflora of oil seeds. Thirtysix rice seed samples of varieties BR6, Pajam and Joya were collected from Parshuram upazila, Feni district of Bangladesh. and nine seed-borne fungi were detected from these seed samples. Five different plants extracts *viz*. garlic, allamanda, neem, chirata and bishkatali with two dilutions

(1:1 & 1:2) were tested for seed treatment. Garlic extract (1:1) dilution found best for three varieties which successfully reduced seed-borne infection and also increased seed germination up to 68.39% over control. Neem (1:1) and chirata (1:1) extracts also increased seed germination up to 66.09% and 67.81%, respectively. Based on the present study, it may be concluded that among the five plant extracts with two dilutions (1:1 & 1:2), garlic (1:1) is most effective in controlling seed-borne fungal flora of rice followed by neem (1:1) and chirata (1:1) extract [8,10 13].

Table 3: Efficacy of garlic extract controlling for predominant seed borne fungi of castor beans 2021

Place of collection	Method	Dilutions	Germ (%)	*F. oxysporum*	*A.ricini*	*M.phaselina*	*C.ricini*	*B.cineria*	*A.ochraceus*
Santhebennur	SBM	Control	10.0	48.0	36.0	16.0	73.0	22.0	81.0
	SBM	1:1	56.0	5.0	3.0	7.0	19.0	15.0	12.0
	SBM	1:2	54.0	5.0	2.0	5.0	8.0	9.0	8.0
	SBM	1:3	50.0	3.0	2.0	2.0	10.0	2.0	6.0
Hirekoglur	SBM	Control	9.0	40.0	40.0	10.0	43.0	22.0	59.0
	SBM	1:1	76.0	21.0	23.0	0.0	11.0	4.0	22.0
	SBM	1:2	70.0	12.0	9.0	1.0	2.0	3.0	9.0
	SBM	1:3	65.0	9.0	2.0	2.0	2.0	3.0	3.0
Mallapura	SBM	Control	14.0	56.0	58.0	11.0	28.0	26.0	61.0
	SBM	1:1	86.0	10.0	8.0	9.0	3.0	11.0	12.0
	SBM	1:2	76.0	9.0	8.0	3.0	3.0	4.0	6.0
	SBM	1:3	68.0	2.0	5.0	1.0	1.0	2.0	0.0
Balliganudu	SBM	Control	13.0	34.0	11.0	17.0	22.0	18.0	34.0
	SBM	1:1	88.0	10.0	1.0	3.0	6.0	6.0	10.0
	SBM	1:2	80.0	3.0	1.0	1.0	6.0	3.0	5.0
	SBM	1:3	74.0	2.0	1.0	1.0	3.0	2.0	1.0
Thanigere	SBM	Control	12.0	26.0	34.0	14.0	18.0	81.0	33.0
	SBM	1:1	86.0	8.0	12.0	1.0	2.0	21.0	22.0
	SBM	1:2	76.0	3.0	3.0	0.0	0.0	3.0	9.0
	SBM	1:3	68.0	1.0	3.0	0.0	0.0	1.0	3.0
Mean	--	--	56.55	15.25	13.10	5.20	13.00	12.90	19.80
SD	--	--	28.46	16.63	16.30	5.653	17.98	18.04	22.78
SE	--	--	3.162	6.928	6.00	4.00	8.544	4.690	9.00

*Data based on 100 castor beans in each sample and ten replications

Table 4: Efficacy of Neem leaf extract controlling for predominant seed borne fungi of castor beans – 2020

Place of collection	Method	Dilutions	Germ (%)	*F. oxysporum*	*A.ricini*	*M.phaselina*	*C.ricini*	*B.cineria*	*A.ochraceus*
Santhebennur	SBM	Control	10.0	48.0	36.0	16.0	73.0	22.0	81.0
	SBM	1:1	56.0	2.0	1.0	0.0	1.0	2.0	11.0
	SBM	1:2	54.0	0.0	0.0	0.0	0.0	0.0	0.0
	SBM	1:3	50.0	0.0	0.0	0.0	0.0	0.0	0.0
Hirekoglur	SBM	Control	9.0	40.0	40.0	10.0	43.0	22.0	59.0
	SBM	1:1	76.0	1.0	1.0	1.0	2.0	1.0	1.0
	SBM	1:2	70.0	0.0	0.0	0.0	0.0	0.0	0.0
	SBM	1:3	65.0	0.0	0.0	0.0	0.0	0.0	0.0
Mallapura	SBM	Control	14.0	56.0	58.0	11.0	28.0	26.0	61.0
	SBM	1:1	86.0	3.0	4.0	1.0	2.0	1.0	1.0
	SBM	1:2	76.0	1.0	0.0	0.0	0.0	0.0	0.0
	SBM	1:3	68.0	0.0	0.0	0.0	0.0	0.0	0.0
Balliganudu	SBM	Control	13.0	34.0	11.0	17.0	22.0	18.0	34.0
	SBM	1:1	88.0	0.0	0.0	1.0	20.	0.0	1.0
	SBM	1:2	80.0	0.0	0.0	0.0	0.0	0.0	0.0
	SBM	1:3	74.0	0.0	0.0	0.0	0.0	0.0	0.0
Thanigere	SBM	Control	12.0	26.0	34.0	14.0	18.0	81.0	33.0
	SBM	1:1	86.0	1.0	1.0	1.0	2.0	1.0	1.0
	SBM	1:2	76.0	0.0	0.0	0.0	0.0	0.0	0.0
	SBM	1:3	68.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	--	--	56.05	10.60	9.30	3.60	10.55	8.70	14.15
SD	--	--	28.46	18.69	17.51	6.099	19.13	19.22	25.26
SE	--	--	3.162	6.928	6.00	4.00	8.445	4.690	9.00

*Data based on 100 castor beans in each sample and ten replications

Conclusion

Thus, the present study highlight the importance of seed borne mycoflora and seed treatment with most effective plant extracts in controlling of mycoflora of castor beans. Detection of seed borne 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 beans which are responsible for deteriorating seed quality, oil 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 % respectively. It was observed that seed treated with the plant extracts effectively increased seed germination and controlled the seed associated mycoflora but it was not ecofriendly, polluted our environment. Neem extract showed promising result in controlling of seed mycoflora of nonedible oil seed castor. So this extract can be used in controlling of seed mycoflora instead of chemical fungicides for safe environment.

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