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Detection and Isolation of seed mycoflora of lablab bean, horse gram and cowpea and control of major seed mycoflora

YK Nirgude, RR Rathod, UR Phondekar, Rohini Gaonkar and NV Chaure

Abstract
Seed is primary most important input in Agriculture. Healthy seeds produce healthy crops and good yields. Most of the plants grown by seeds so it is more important to protect the seeds from pathogens. Seed quality depends on presence or absence of seed borne mycoflora. Lablab bean Horse gram and Cowpea are important legumes in konkan region. They are rich in nutrients content and medicinally important. The seeds are infected by many seed borne fungi which resulted in poor germination, plant growth vigor, seedling mortality and storability. To detect and isolate the seed borne mycoflora various seed health testing methods were attempted i.e. Blotter paper method, Agar plate method and PDA method. All the methods were found effective to detect the seed borne mycoflora. The *in vitro* efficacy of fungicides against major seed borne fungi were tested. All tested fungicides *viz*, Carbendazim 50% WP @ 0.2% (100%), Benomyl 50% WP @ 0.2% (85.18%), Thiophanate methyl 70% WP @ 0.1% (85.18%), Carboxin 75% WP @ 0.25% (68.88%), Mancozeb 75% WP @ 0.25% (67.40%), Thiram 75% WP @ 00.2 (66.66%) and Captan 75% WP @ 0.30% (61.11%) were found effective against mycelial growth inhibition of *Alternaria alternata* and (Carbendazim 50% WP @ 0.2%, (100%), Benomyl 50% WP @ 0.2% (88.88%), followed by Thiophanate methyl 70% WP @ 0.1% (87.03%), Carboxin 75% WP @ 0.25% (83.33%), Captan 75% WP @ 0.30% (62.22%), Thiram 75% WP @ 0.2% (60.37%) and Mancozeb 75% WP @ 0.25% (58.88%) were found effective mycelial growth inhibition of *Fusarium oxysporum*.

Keywords: Detection, isolation, seed mycoflora, lablab bean, horse gram and cowpea

Introduction
Seeds play important role in the production of healthy crops. Healthy seed is the foundation of healthy plant; a necessary condition for good yields. The world's stored grain is damaged mainly by the activity of fungi than other microorganism was stated by Neergard, 1977 [5]. The quality of seed determines by presence or absence of seed borne fungi on seed surface is one of the important aspects.

In India pulses have been considered as the poor man's only source of protein. Increasing their production and keeping their prices within the reach of the poor therefore assumes paramount importance. Pulses, one of the most economical source of protein for human consumption. Pulses contain 18-25% of protein. They are the main source of dietary proteins in a vegetarian country like India. The major pulses crops of the country are red gram or pigeon pea (tur, arhar), chickpea or gram, black gram (urad bean), green gram (moong bean) and lentil (masur). Minor pulses include rajmash and other beans, cowpea, horse gram, moth etc. (Confederation of Indian Industry, 2010).

Cowpea (*Vigna unguiculata* (L.) Walp.), annual legume, originated in Africa. It is an important nutrient supplying pulse in Konkan region. Cowpea grains contain 23-25% protein and 50-67% starch on dry bases (KI Vasava, VR Gohel and KD Vaghela, 2018). Lablab bean (*Lablab purpureus* (L.) Sweet.) is also an important nutrient supplying pulse after cowpea in Konkan region. It contains 23 to 28% crude protiens which is three times more than the cereals. It is reach source of Carbohydrates, Vitamins (Vit-A and Vit-C), calcium and phosphorus (Alam *et al*. 2015) [1]. Horsegram belongs to the Fabaceae family. The botanical name of Horsegram is *Macrotylomauniflorum* formerly known as *Dolichusbiflorus*, it is variously known Kulthi (Marathi), kulthan (Sanskrit), kurti-kalai (Bengali),kollu (Tamil), ullavallu (Telagu), Muthira (Malyalam). It can be grown in drought prone areas and is considered as poor man's pulse.

It is cheapest source of proteins (18.4-25.5%), carbohydrates (51.9-60.9%), iron, calcium and antioxidants. Horse gram is adapted to wide range of soil and climatic conditions. The seeds of horse gram are useful for the cure of disorders in human beings i.e. piles, hiccup, abdominal lump, asthma, kidney stone, pregnancy bleeding (Smita Rana and Vasudha Agnihotri, 2018) [7]. The seeds of these legumes are susceptible to fungal contamination, resulting in seeds deterioration. These fungi are of saprophytic or pathogenic nature which affects seed germination, emergence from soil, plant growth vigor and storability.

Material and Methods

Visual Examination of seed

The collected seeds were observed with naked eyes as well as under stereoscopic binocular microscope, for the presence of abnormal seeds, fungal infested seeds, other crops seed, insect eaten seeds, discoloured seeds, mouldy growth, inert mater, plant debris etc., in the seeds.

Isolation and detection of seed mycoflora

The seed mycoflora of beans were isolated and detected by using seed health testing methods.

Blotter paper method

To detect the seed mycoflora of beans, the ISTA's standard blotter method described by Neergard (1997) [5] was used.

For detection of seed mycoflora, 400 seeds of each beans were selected. Petri dish size three layers of blotter paper were soaked in sterile distilled water and kept at bottom of Petri dish after draining off excess moisture. Seeds were surface sterilized with 1% sodium hypochlorite solution for 1 minute and washed with sterile distilled water in three sequential changes and Fixed numbers of seeds i.e., 10 per plate were placed equidistantly on moisten blotter paper. All Petri dishes were incubated at 27±20 °C temperature for 7 days. Sterile distilled water was added to moisten the blotter paper as and when required. After incubation these plates were examined under microscope. Based on morphological and cultural characteristics various fungi grows in Petri dishes were identify.

Isolation of seed borne mycoflora

The fungal colonies of different fungi associated with seeds of beans were transferred with the help of sterilized fungal inoculation needle onto PDA (Potato Dextrose Agar medium) slants, named and incubated at 27±20 °C for a week. The pathogens were purified and the pure isolates grow on Potato Dextrose Agar slant for further studies.

Agar plate method

As described in blotter paper method 400 seeds of each beans were selected. To detect seed mycoflora these seeds were treated with 1% sodium hypochlorite solution for 1 minute and then washed in sterile distilled water in three sequential changes and placed in Petri dishes (10 seeds / petri dish) containing two percent 20ml water agar. These plates were incubated at 27±20 °C temperature for a week. After a week of incubation, the fungal growth was examined under microscope. As soon as fungal colonies observed on seeds were transferred onto Potato Dextrose Agar (PDA) medium with the help of fungal inoculation needle aseptically under laminar air flow cabinet. Isolates obtained were maintained in pure form onto Potato Dextrose agar slants and kept in refrigerator for further studies.

PDA method

For detection of seed mycoflora by PDA method 400 seeds of each beans were selected. These seeds were placed onto Petri dishes as mentioned above 10 seeds per Petri dish which contained 20ml of autoclaved and cooled PDA. Seeds were treated with 1% sodium hypochlorite solution for 1 minute and then washed in sterile distilled water in three sequential changes. Plates were incubated at 27±20 °C for a week. After incubation fungal colony growth was observed under microscope. The fungal colonies observed on seeds were transferred onto Potato Dextrose Agar (PDA) medium with the help of fungal inoculation needle aseptically under laminar air flow cabinet. Isolates obtained were transferred onto Potato Dextrose agar slants to maintained in pure form and kept in refrigerator for further studies.

observations on growth of various fungi were recorded under stereo-binocular microscope. Based on habit characters, number of seeds showing growth of a particular fungus were counted and their per cent frequency / incidence was calculated by following formula.

$$\% \text{ Frequency (PF)} = \frac{\text{No. of seeds showing growth of a specific fungus}}{\text{Total No. of seeds observed}} \text{X100}$$

Characterization and identification of seed mycoflora

The isolated seed mycoflora were purified by hyphal tip isolation technique, thus pure cultures obtained were maintained on PDA slants. Only two major pathogenic fungi were characterized and identified on the basis of growth habit, cultural and morphological characters. Cultural characteristics *viz*., colony growth, colony morphology, colour, mycelial growth, hypha, conidial colour, size and shape etc. were considered.

Pathogenicity of major seed mycoflora

To prove the pathogenicity the seeds were surface sterilized with 1% sodium hypochlorite solution for five minutes and then washed with sterile distilled water in three sequential changes to remove the traces of solution. Seed were dried with the help of blotter paper. Blot dried seeds soak in spore suspension (10^6 spores/ml) for 12 hrs. These seeds then shade dried and transferred onto sterilized Petri dishes which lined with three layers of moistened blotter paper. For control purpose healthy looking seeds were surface sterilized with sodium hypochlorite solution but not soaked in spores suspension (uninoculated) and kept in another Petri dishes lined with moistened blotter paper. These Petri dishes were incubated at 27+2 °C temperature for a week. Observations were recorded after a week of incubation. Number of seeds showing growth of the test pathogen and pathogen free seeds were recorded. The seeds showing typical growth of the test pathogens were isolated on autoclaved and cooled PDA medium and incubated at 27±20 °C temperature. Test pathogens compared with their pure culture growth based on cultural characteristics.

In vitro evaluation of seed dressing fungicides

For this purpose poison food technique were used. Efficacy of fungicides was studied by applying recommended dose of

fungicides against test pathogens using potato dextrose agar (PDA) as a basal medium. Requisite quantity of test fungicides was calculated based on active ingredients and mixed well in autoclaved and cooled PDA medium in conical flasks. PDA medium amended with fungicides was poured @20 ml per plate aseptically and allowed to solidify at room temperature. After solidification plates were incubated aseptically by putting in the centre a 5mm disc of 7 days old pure culture of test pathogens. The fungicides replicated three times. Another Petri dishes filled with plain PDA i.e. without fungicides and inoculated with the 7 days of old culture of test pathogens for control purpose. Both treated and untreated plates were incubated at 27±20 °C temperature for 7 days.

Treatments details

Tr. No.	Treatments	Conc. (%)	Tr. No.	Treatments	Conc. (%)
T1	Carbendazim 50%	0.1	T5	Thiram 75%	0.2
T2	Mancozeb75%	0.25	T6	Th. Methyl 70%	0.1
T3	Benomyl 50%	0.2	T7	Carboxin 75%	0.25
T4	Captan 75%	0.30	T8	Control (untreated)	-----

Observations were recorded at 24 hrs intervals based on mycelial growth/ colony diameter till the untreated control plates were fully covered with mycelial growth of the test pathogens. Per cent mycelial growth inhibition of test pathogens over untreated control was calculated by applying the formula given by Vincent 1927.

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Results and Discussion

Visual observation

Seeds were examined by naked eyes and also under stereoscopic binocular microscope for various abnormalities i.e., inert matter, discoloration, mouldy growth, other crops seeds, formation of lumps etc. (Plate I) and the maximum number of normal seeds were observed in Cowpea (95%), followed by horse gram (93%) and lablab bean (92%).

Isolation and Detection of seed borne mycoflora of beans

The Seeds of beans (Lablab bean, cowpea and horse gram) collected from the Department of Agronomy, College of Agriculture, Dapoli, were subjected to isolation by applying various seed health testing (SHT) methods. After 7 days of incubation growth of various fungi was observed. Based on growth habit and colony characters, the fungi appeared were distinguished, identified and sub cultured separately on autoclaved, cooled and solidified Potato Dextrose Agar (PDA) medium in sterilized petri plates aseptically and incubated at 27+2 °C. Alam *et al*. (2015) [1] isolated six fungal pathogens such as *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., *Fusarium oxysporum*, *Curvularia lunata* and *Rhizopus stolonifer* from eleven varieties of lablab bean. Parshar R. *et al*. (2019) [10] isolated total 23 fungal species from seeds of four pulses (chickpea, mung bean, pigeon pea and lentil). The majority of species were of genera *Aspergillus* followed by *Fusarium*, *Trichoderma*., *Mucor*, *Curvularia*, *Alternaria*, *Botrytis*, *Rhizopus*, *Cladosporium*, *Drechslera*, *Macrophomina*, *Pythium*, *Chaetomium* and unidentified species. Patil *et al*., (2015) [6] isolated four fungal genera and five fungal species from sunflower seeds Hyb. LSHF-171 and var. Morden. The mycoflora isolated were *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer*. which was substantiated by findings of Alam *et al*. (2015) [1] detected seed mycoflora of lablab bean by blotter paper method. Mandhare (2009) [4] detected seed-borne fungi of green gram by blotter paper and Agar plate method. Similarly, Patil V. B. (2017) [11] studied the seed mycoflora associated with seeds of sunflower and detected *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus* and *Trichoderma harzianum*. He also calculated the per cent frequency of association detected by all seed health testing methods with the seeds of sunflower Hyb. LSFH-171 and found maximum in respect to *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus niger*, *Rhizopus stolonifer* and *Aspergillus flavus*. Among the five seed health testing methods attempted, the most efficient method was blotter paper method followed by 2,4-D method, Agar plate method, modified PDA method and Rolled towel paper method. Ashruti Kesharwani *et al*. (2018) [2] also identified varying degree and type of mycoflora associated with pea seeds. The following mycoflora were found to be associated with seeds of three beans. Their distinguishing morphological and cultural characteristics observed are detailed in following paragraph.

Alternaria alternata

Colony observed on potato dextrose agar (PDA) medium was greenish black in colour. Conidiophores were pale brown to olive brown, straight or flexuous. Conidiophores arise directly from substrate individually. Conidiophore forms bushy heads consisting of 4-8 catenate conidia chains. Conidia were brown in colour, smooth, muriform, short beaked, formed in long chain. Secondary conidiophores were short and single celled.

Aspergillus flavus

The colony colour observed yellow to deep yellow-green on PDA medium. Conidia arranged in long chain on swollen conidiophore, conidia were spherical to sub-spherical and spiny.

Aspergillus niger

Colony appeared on Potato Dextrose Agar (PDA) medium rapidly growing abundantly. Aerial hyphae usually slowly produced. Conidiophores arise directly from substratum. Globose shaped conidia. Conidial heads fuscous, blackish brown. Vesicles were globose shaped.

Fusarium oxysporum

Colony appeared on Potato Dextrose Agar medium was white colour. The mycelium was hyaline. The micro conidia were hyaline, 1-2 septa, ellipsoidal to sub-cylindrical, oval; whereas, macro-conidia were also hyaline, sub-cylindrical, stout, slightly curved and sickle shaped. Chlamydospore were formed singly and in pairs or in clusters in sporodochia.

Rhizopus stolonifer

Rhizopus grow rapidly on potato dextrose agar medium. The fungus produced cottony white colony at initial stage, which turned brownish black in later stage. The colony has wooly texture. It produced stolons and rhizoids. Rhizoids and stolons were hyaline to dark brown, round, oval and elongated. Fungus produces sac like structure called sporangia. Sporangia were globose to sub-globose and black in colour. sporangia content spores.

Trichoderma harzianum

Trichoderma harzianum on potato dextrose agar medium formed 1-2 concentric rings with green conidia. The conidia were denser in centre than towards the margins. *T. viride* appears to be a bit granular on PDA, with green conidia spread throughout the plate. Irregular yellow zone appear without conidia around the inoculum.

Detection of seed mycoflora by various seed health testing methods

The seed mycoflora of beans were detected by using three seed health testing methods *viz*. Blotter paper method, Agar plate method and PDA method as described under Materials and Methods.

Blotter paper method

To study the occurrence of seed-borne mycoflora in laboratory condition, three beans were evaluated by blotter paper method. In this experiment, 10 seeds per plate were kept at equal distance and total 400 seed were evaluated. The per cent frequency of seed-borne fungi was recorded and results are presented in table 1.

Table 1: Per cent frequency of various fungi associated with seeds of beans tested, by blotter paper method

Sr. No.	Fungi detected	% Frequency*		
		Lablab bean	Horse gram	Cowpea
1	A. *alternata*	75.75	73.08	72.66
2	A. *flavus*	69.66	65.33	62.5
3	*A. niger*	72.66	72.75	73.75
4	*F. oxysporum*	70.75	71.58	70.00
5	*R. stolonifer*	60.66	54.91	72.08
6	*T. harzianum*	53.16	61.5	54.66

*: Average of 400 seeds tested of each bean

The results (Table 1) revealed that the incidence of six fungi *viz*., *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Trichoderma harzianum* with the seed of beans *viz*. Lablab bean, cow pea and horsegram. The per cent frequency of association of all the fungi was found maximum with the seeds of lablab bean (53.16-75.75%) followed by cow pea (54.66-73.75%) and horse gram (54.91-73.08%). *Alternaria alternata* was dominant fungi recovered from seeds, followed by *Aspergillus* spp., *Fusarium oxysporum*, *Rhizopus stolonifer* and *Trichoderma harzianum*. Among these six fungi comparatively maximum per cent frequency of *Alternaria alternata* (75.75% in lablab bean followed by 73.08% in horse gram and 72.66% in cowpea) was observed followed by *Aspergillus niger* (73.75% in cowpea followed by 72.75% in horse gram and 72.66% in lablab bean), *Fusarium oxysporum* (71.58% in horse gram, 70.75% in lablab bean and 70.00% in cowpea), *Aspergillus flavus* (69.66% in lablab bean, 65.33% in horse gram and 62.5% in cowpea), *Rhizopus stolonifer* (72.08% in cowpea, 60.66% in lablab bean and 54.91% in horse gram) and *Trichoderma harzianum* (61.5% in horse gram, 54.66% in cowpea and 53.16 in lablab bean).

Agar plate method

To *in vitro* detection of seed mycoflora of three beans were evaluated by agar plate method. As mentioned in blotter paper method 10 seeds per plate were kept at equal distance, total 400 seeds were evaluated. The data on mycoflora observed and its per cent frequency recorded is presented in table 2.

Table 2: Per cent frequency of various fungi associated with beans tested by Agar plate method

Sr. No.	Fungi detected	% Frequency*		
		Lablab bean	Horsegram	Cowpea
1	*A. Alternata*	73.08	73.75	72.66
2	*A. flavus*	64.08	62.33	49.25
3	*A. niger*	50.5	68.58	48.5
4	*F. oxysporum*	70.58	67.83	51.75
5	*R.* stolonifer	47.83	56.08	48.25
6	*T. harzianum*	73.16	54.66	44.16

*: Average of 400 seeds tested of each bean

It is revealed from the data presented in table 2 that, the association of fungi *viz*., *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer* and *Trichoderma harzianum* with the seeds of beans. The per cent frequency of incidence or association of the fungi was found maximum with the seeds of lablab bean (47.83-73.16%) followed by horse gram (54.66-73.75%) and cowpea (44.16-72.66%). Maximum per cent frequency of *Alternaria alternata* (73.75% in horse gram, 73.08% in lablab bean and 72.66% in cowpea) was observed followed by *Fusarium oxysporum* (70.58% in lablab bean, 67.83% in horse gram and 51.75% in cowpea), *Aspergillus flavus* (64.08% in lablab bean, 62.33% in horse gram and 49.25% in cowpea), *Trichoderma harzianum* (73.16% in lablab bean, 54.66% in horse gram and 44.16% in cowpea), *Aspergillus niger* (68.58% in horse gram, 50.5% in lablab bean and 48.5% in cowpea) and *Rhizopus stolonifer* (56.08% in horse gram, 48.25% in cowpea and 47.83% in lablab bean).

PDA method

To study the association of seed-borne mycoflora in laboratory condition, three beans were evaluated by blotter paper method. 10 seeds per plate were kept at equal distance and total 400 seed were evaluated as mentioned in above two methods. The per cent frequency of seed-borne fungi was recorded and results are presented in table 3.

Table 3: Per cent frequency of various fungi associated with beans tested by PDA method

Sr. No.	Fungi detected	% Frequency		
		Lablab bean	Horsegram	Cowpea
1	*A. alternata*	72.83	72.08	72.16
2	*A. flavus*	70.41	62.16	47.33
3	*A. niger*	69.5	73.25	48.16
4	*F. oxysporum*	71.16	69.83	68.91
5	*R.stolonifer*	53.91	50.83	46.5
6	*T. harzianum*	42.5	55.83	41.91

*: Average of 400 seeds tested of each bean

The perusal table 3 reveals that, the association of seed mycoflora *viz*. *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Trichoderma viride*, with the seeds of beans *viz*. lablab bean, horse gram and cowpea. The per cent frequency of association of all the fungi was found maximum with the seeds of cowpea (41.91-72.16%) followed by lablab bean (42.5-72.83%) and horse gram (50.83-72.08%). *Alternaria alternata* was dominant fungi recovered from seeds, followed by *Aspergillus* spp., *Fusarium oxysporum*, *Rhizopus stolonifer* and *Trichoderma harzianum*. Among these six fungi comparatively maximum per cent frequency of *Alternaria alternata* (72.83% in lablab bean, 72.16% in cowpea and

72.08% in horse gram) was observed followed by *Aspergillus niger* (73.25% in horse gram, 69.5% in lablab bean and 48.16% in cowpea), *Fusarium oxysporum* (71.16% in lablab bean, 69.83% in horse gram and 68.91% in cowpea), *Aspergillus flavus* (70.41% in lablab bean, 62.16% in horse gram and 47.33% in cowpea), *Rhizopus stolonifer* (53.91% in lablab bean, 50.83% in horse gram and 46.5% in cowpea) and *Trichoderma harzianum* (55.83% in horse gram, 42.5% in lablab bean and 41.91% in cowpea).

The results revealed that the all three seed health testing methods tested were found to be efficient in performance to detecting the various seed mycoflora associated with seeds of three beans tested. The highest percentage of seed mycoflora detected by the blotter paper method. Among the three seed health testing methods Blotter paper method were most efficient method for detection of seed mycoflora.

Pathogenicity of seed-borne mycoflora

The pathogenicity of tested seed borne mycoflora was proved by seed inoculation technique by blotter paper method, as discussed in 'Materials and Methods chapter. The study revealed that the tested fungi were associate with seeds of beans and pathogenic to seeds and crops. The morphological and cultural characteristics of tested fungi when compared with the pure culture were shows similar characters.

***In vitro* effect of seed dressing fungicides**

The seed dressing fungicides were evaluated *in vitro* at their recommended field dosage based on their active ingredients by applying poison food technique, against test pathogens. The result obtained on per cent inhibition of mycelial growth are given in table 4 and 5.

Table 4: *In vitro* efficiency of various fungicides against *Alternaria alternata* associated with seeds of beans

Tr. No.	Treatments	Conc. %	Mean colony diameter (mm)*	Inhibition* (%)
T_1	Carbendazim 50% WP	0.1	0.00	100 (90.00)
T_2	Mancozeb 75%WP	0.25	29.33	67.40 (67.35)
T_3	Benomyl 50% WP	0.2	13.33	85.18 (55.18)
T_4	Captan 75% WP	0.30	35.00	61.11 (51.41)
T_5	Thiram	0.2	30.00	66.66 (54.73)
T_6	Thiophanate methyl 70% WP	0.1	13.33	85.18 (55.18)
T_7	Carboxin	0.25	28.00	68.58 (56.09)
T_8	Control	-	90.00	00.00 (00.00)
SE±		1.22		
CD (P=0.01)		3.58		

*: Mean of three Replication

The result (table 4) revealed that all of the test fungicides significantly inhibit the mycelial growth of *Alternaria alternata*, over the untreated control. The fungicides *viz*, Carbendazim 50% WP @ 0.2% resulted in 100 per cent inhibition of mycelial growth, Other fungicides also have significant effect on mycelial growth inhibition of *Alternaria alternata* were Benomyl 50% WP @ 0.2% (85.18%) followed by Thiophanate methyl 70% WP @ 0.1% (85.18%), Carboxin 75% WP @ 0.25% (68.88%), Mancozeb 75% WP @ 0.25% (67.40%), Thiram 75% WP @ 00.2 (66.66%) and Captan 75% WP @ 0.30% (61.11%) respectively

Thus, all the fungicides at their recommended dosage, were found effective against *Alternaria alternata*.

Table 5: *In vitro* efficiency of various fungicides against *Fusarium oxysporum* associated with seeds of beans.

Tr. No.	Treatments	Conc. %	Mean colony diameter (mm)*	Inhibition * (%)
T_1	Carbendazim	0.1	0.00	100 (90.00)
T_2	Mancozeb 75% WP	0.25	37.00	58.88 (50.11)
T_3	Benomyl 50% WP	0.2	10.00	88.88 (70.52)
T_4	Captan 75% WP	0.30	34.00	62.22 (52.07)
T_5	Thiram 75% WP	0.2	35.67	60.37 (50.98)
T_6	Thiophanate methyl 70% WP	0.1	11.67	87.03 (68.89)
T_7	Carboxin 75% WP	0.25	15.00	83.33 (65.00)
T_8	Control	-	90.00	00.00 (00.00)
SE±		1.14		
CD (P=0.01)		3.34		

*: Mean of three Replication

The result (table 5) revealed that all of the test fungicides significantly inhibit the mycelial growth of *Fusarium oxysporum*, over the untreated control. The fungicide, Carbendazim 50% WP @ 0.2%, resulted in 100 per cent inhibition of mycelial growth. Other fungicides also has significant effect on mycelial growth inhibition of *Fusarium oxysporum* were Benomyl 50% WP @ 0.2% (88.88%), followed by Thiophanate methyl 70% WP @ 0.1% (87.03%), Carboxin 75% WP @ 0.25% (83.33%), Captan 75% WP @ 0.30% (62.22%), Thiram 75% WP @ 0.2% (60.37%) and Mancozeb 75% WP @ 0.25% (58.88%) respectively. Thus, all the fungicides were found effective against *Fusarium oxysporum* at their recommended doses.

Similarly the mycelial growth of *Fusarium oxysporum* completely inhibited by Carbendazim 50% WP @ 0.2% followed by benomyl 50% WP @ 0.2%, Thiophanate methyl 70% @ 0.1%, Carboxin 75% WP @ 0.25%, Captan 75% @ 0.30%, thiram 75% @ 0.2% and Mancozeb 75% WP @ 0.25% respectively. The seed dressing fungicides carbendazim 50% WP, Benomyl 50% WP, Thiophanate methyl 70%, Carboxin 75% WP, Captan 75%, Thiram 75% and Mancozeb 75% WP were effective against many seed-borne pathogens at their recommended dosage as well as various concentrations reported in earlier studies by several researchers (Somu *et al*. (2014) [8], Patil V. B. *et al*. (2015) [6], Patra, S. and Biswas, M. K. (2016) [9] and Jakatimath *et al*. (2017) [3].The present findings are in conformity with Patil *et al*. (2015) [6], reported that all the fungicides were found to be significantly superior over control in checking the radial growth and sporulation of *Fusarium oxysporum* f. sp. ciceri. Patra, S. and Biswas, M. K. (2016) [9] ten fungicides evaluated, Carbendazim, Propiconazole, and two combination product i.e. (Carbendazim +Mancozeb) and (Tebuconazole + Trifloxystrobin) exhibits completely inhibited (100% inhibition) the mycelia growth of fungus at 1500 ppm concentration followed by Thiophanate Methyl (96.67% inhibition) and least inhibition by Copper-Oxy Chloride of 76.67% inhibition.

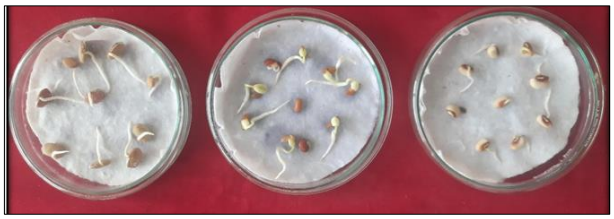

Fig 1: Detection of seed mycoflora by Blotter Paper Method

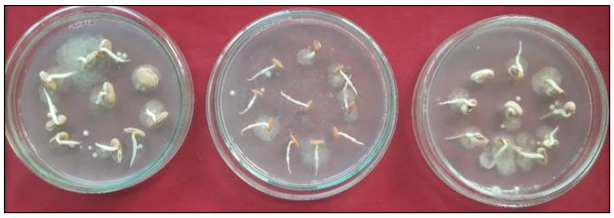

Fig 2: Detection of seed mycoflora by Agar plate method

Fig 3: Detection of seed mycoflora by PDA Method

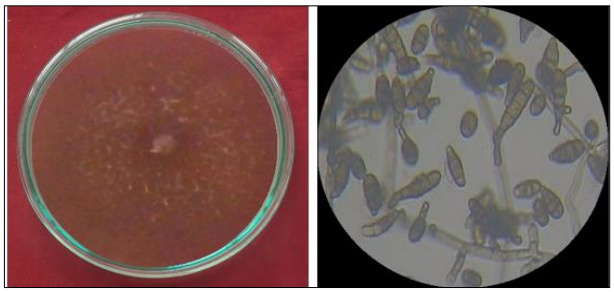

Fig 4: *Alternaria alternata*

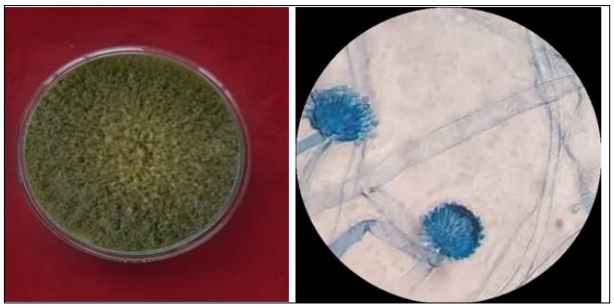

Fig 5: *Aspergillus flavus*

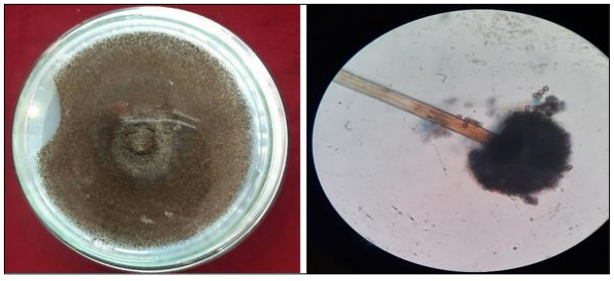

Fig 6: *Aspergillus niger*

Fig 7: *Fusarium oxysporum*

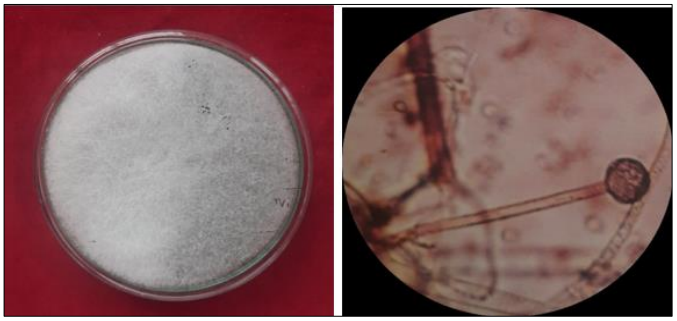

Fig 8: *Rhizopus stolonifer*

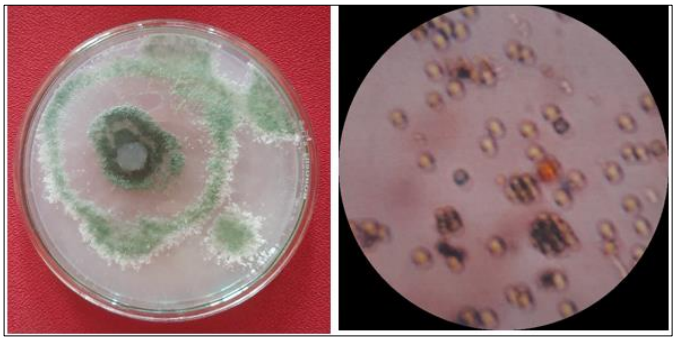

Fig 9: *Trichoderma harzianum*

Fig 10: Efficacy of fungicides against *Alternaria alternata*

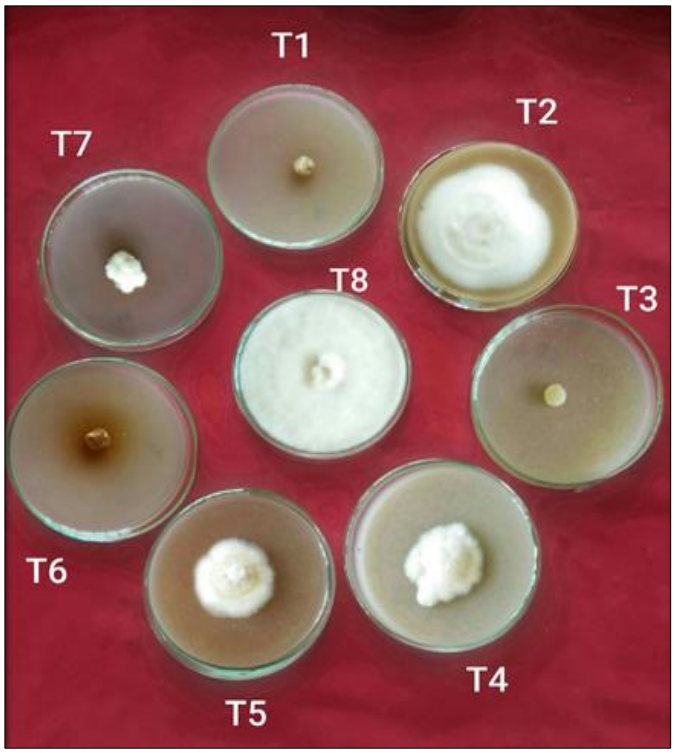

Fig 11: Efficacy of fungicides against *Fusarium oxysporum*

Conclusion

From the present study concluded that the mycoflora associated with the seeds of Lablab bean, Horse gram and Cowpea were *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer* and *Trichoderma harzianum*. Blotter paper method was found most efficient in detection of seed mycoflora associated with beans followed by Agar plate method and PDA method. Among the fungicides evaluated *in vitro* all fungicides were found effective against *Alternaria alternata* and *Fusarium oxysporum*. Carbendazim 50% WP were found most effective.

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