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Seed-borne mycoflora of pulses in Nagaland

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The present investigation was carried out to identify infection of pulse seeds via different seed health testing methods and to determine the effect of some chemical fungicides, bio-agents and botanicals on the seed-borne mycoflora and seed germination. A total of five seed- borne fungi viz., *Aspergillus* sp., *Pencillium* sp., *Rhizopus* sp., *Rhizoctonia* sp. and Trichoderma sp. were detected from five pulse seeds collected from local market of Medziphema, Nagaland. Amongst the seed health testing methods employed, PDA plate method proved to be superior followed by blotter paper method and water-agar method. The fungal occurrence was more in PDA method with mean incidence 14.54%. The predominant fungus was observed to be *Rhizopus* sp. with the incidence of 20.6% and the least fungal incidence was of *Rhizoctonia* msp. (11.99%). Soybean seeds showed the highest mycoflora incidence of 18.89% and the lowest was recorded from French bean (10.22%). Among chemicals, mancozeb @ 0.2% was observed to be best in reducing the fungal incidence of *Penicillium* sp., *Aspergillus* sp., *Rhizopus* sp., *Rhizoctonia* sp. to zero. All the bio-agents in reducing fungal incidence were relatively at par. All the leaf extracts were found to be less effective compared to the other treatments. The bio-agents *T. harzianum* recorded highest germination percentage of pulse seeds at 70%.

Key words: Pulse seeds, seed-borne fungi, fungicides, bio-agents, botanicals

INTRODUCTION

Microorganisms play an important role in affecting the quality of seed, that is the basic input and starting point of agriculture. Among the micro-organisms, fungi are the largest group, which affect the seed health and cause seed-borne diseases. Seeds of many crops are known to carry various types of pathogenic and non- pathogenic fungi which are commonly known as seed mycoflora or seed-borne fungi. Fungi decrease seed germinability, cause seed discolouration, produce toxins that may be injurious to man and domestic animals and may reduce seed weight also. Seed health testing for the presence of seed-borne pathogens is an important step in the management of crop diseases (Zaidi and Pathak, 2014). Several fungi have been reported as internally and externally seed-borne by many workers which cause discolouration, spoilage of seed and also cause number of diseases in field such as root rot (*Rhizoctonia solani*), white rot (*Sclerotinia sclerotiorum*), anthracnose (*Ascochyta pinodella*), powdery mildew (*Erysiphe pisi*),wilt (*Fusarium oxysporum* f.sp. *pisi*) and seed rot (*Aspergillus niger*). More attention is being paid to study the seed mycoflora in view of their importance as deteriorating agents and also as toxin producers (Marak, 2015). Seed health information reveals the organism carried by the seed and the level of infection or infestation that will be introduced to another region or country. Such information comes from experiments or survey under field conditions where the seed is grown (Habib *et al*. 2012).

Seed treatment reduces the host proneness to pathogens by increasing vigour and imparting resistance to the plants. Pulses are the second most important group of food plants after cereals belonging to the family Leguminosae. Pulses are important sources of protein and essential amino acids for major vegetarians (Kandhare, 2014). In India, the major pulses are chickpea, pigeon pea, green gram, black gram and lentil (Narayan and Kumar, 2014). Seed health testing in pulses has been done by many researchers Agarwal *et al*. 2011; Mahamune and Kakde, 2011; Ramesh *et al.* 2013; Saleem and Ebrahim, 2013). Some researchers have worked on managing the seed-borne mycoflora of pulses by fungicides Pan *et al*. 2010; Ashwini and Giri, 2014) in combination with hot water treatment bioagents (Amin *et al*.

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2014; Ashwini and Giri, 2014) and plant extracts (Bhardwaj *et al*. 2013; Dhole and Gurme, 2013; Patil and Madane, 2014; Singh *et al*. 2014). Considering the above-mentioned developments and lack of information on seed mycoflora of pulses in Nagaland, this research work was carried out with the objectives of isolating and identifying mycoflora from important pulse seeds of Nagaland and studying the efficacy of seed treating agents for management of the seed mycoflora.

MATERIALS AND METHODS

This study was conducted in the Department of Plant Pathology, SASRD, Nagaland University. The seed samples of five pulses viz., French bean, red kidney bean, chickpea, soybean and blackgram were collected from local market at Medziphema, Nagaland. The seeds were collected in sterilized polythene bags with proper labeling and brought to the laboratory for further studies. A total of thirty seeds from all the five samples were examined under stereo- binocular microscope to determine the presence of mycoflora. Fungi from different pulses were isolated by placing 10 seeds on moist sterilized filter paper (Whatman No. 1) in Petri plates and incubated at room temperature (25±2°C). The fungal colonies thus observed on the seed surface were directly identified. All the fungi were isolated on PDA plates. The fungal colonies per plate were counted, purified and maintained in refrigerator. Infection percentage of fungi were recorded on the basis of the following formula :

$$\text{Infection \%} = \frac{\text{Number of seeds on which fungal species occurs}}{\text{Total number of seeds}} \times 100$$

The typical identifying characters of each of the seed mycoflora were photographed using a digital microscope. All identifications were made on the basis of morphological characteristics and photographic descriptions of fungi in accordance to and with the help of relevant literatures.

Isolation of seed-borne mycoflora

Three different methods were followed viz., PDA plate, blotter and water-agar method. In PDA plate method, a total of 30 seeds from each sample of five pulses were treated with mercuric chloride 0.1% for two minutes followed by four washings with sterilized water. Surface sterilized seeds were placed equidistantly in circles in Petri plates (nine cm diam.) containing PDA.

In the blotter method, similar surface sterilization was followed as mentioned above and the seeds were placed equidistantly on Petri plates containing three layer sterile filter paper (Whatman no. 1) beds. Whereas, in water-agar method surface sterilized seeds were placed equidistantly in circles in Petri plates containing water- agar medium. Seed-washing method was performed by taking two grams of each seed sample in separate test with 10 ml of sterile distilled water and shaking for 10 minutes on a mechanical shaker. Two hundred fifty microlitre of this suspension was taken through a micropipette and spread to PDA medium.

Each pulse sample was replicated thrice. After placement of seeds, the plates were incubated at 28±1°C. After seven days, infection percentage was calculated using the formula as given above.

Seed treatment with fungicides, bioagent and plant extracts

Five fungicides namely Bavistin 0.1% (carbendazim), Blitox 0.3% (copper oxychloride), Indofil M-45 0.2% (mancozeb), Ridomil 0.1% (metalaxyl+mancozeb) and Captan 0.1% were used to investigate their effect on seed-borne mycoflora of the pulses under study. Thirty seeds of each pulse sample were soaked in solutions of each of the five chemical fungicides for 20 minutes, while that of control were soaked in sterile distilled water. Seeds were then incubated at 28±1°C on PDA medium in Petri plate with three replicates. Fungal colonies appearing from seeds were isolated, purified and identified.

Three bio-agents viz., *Trichoderma viride*, T. *harzianum* and *T. koningii* were used to investigate their effect on seed-borne mycoflora of five pulses under study. Thirty seeds of each sample were treated with talc powder formulations of each bio-agent @ 8 g/kg seeds in the form of slurry for two minutes, while that of control were soaked in sterile distilled water. Treated seeds were incubated at 28±1°C on PDA medium in Petri plate with three replicates. Fungal colonies appearing from seeds were isolated, purified and identified.

Plant samples of eucalyptus (leaf), ginger (rhizome) and garlic (bulb) were collected, washed and shade dried. They were ground with sterile

mortar and pestle using sterile distilled water (1:1 w/v). The resultant plant extract was treated as 100% concentration. Seeds of pulses were dipped in 10% concentration of plant extract separately for 20 minutes, washed with sterile water and placed on PDA medium in three replicates. After incubation at 28±1°C, plates were examined for fungal growth, which, if observed, were isolated, purified and identified.

The data recorded during the course of this investigation were analyzed by ANOVA method and analysis was done using Microsoft Excel software at 5% level of significance.

RESULTS AND DISCUSSION

Seed mycoflora from five different pulse species viz., French bean, red kidneybean, chickpea, black gram and soybean collected from local markets of Medziphema, Nagaland were isolated using three different methods.

It is evident from the data presented in the Table 1 that in PDA method the fungal occurrence was more with mean incidence of 14.54% followed by blotter paper method (12.66%) and water agar method (11.99%). This may be due to the nutrients present in the medium which might have played an important role in initiation of growth of fungi of pulses than blotter method (Shaker *et al*. 2010). Seed-borne fungi of pulse seeds were usually detected by PDA plate and blotter method as recommended by ISTA (Dhole and Gurme, 2013; Singh, 2014). Altogether, five different fungal species viz., Penicillium sp., *Aspergillus* sp., *Rhizopus* sp., *Rhizoctonia* sp. and *Trichoderma* sp. were isolated from the pulse seeds using three different isolation methods under study. The predominant fungus on the pulse seeds was observed to be *Rhizopus* sp. with the mean incidence of 19.77% followed by *Aspergillus* sp. (17.55%) while the least incidence was recorded for *Rhizoctonia* sp. (3.11%).

Soybean seeds were found to harbor the highest incidence (18.89%) of various seed mycoflora (Table 1) except for *Rhizoctonia* sp. which was isolated only from the seeds of black gram with an incidence of 15.56%. The lowest mean incidence of mycoflora was reported from French bean (10.22%) followed by black gram (10.44%). Agarwal *et al*. (2011) while working on pulse seeds collected from Jaipur, Rajasthan, India reported six fungal species from pulse seeds, of which a few are common to our study viz., *Penicillium* sp., *Aspergillus* sp. and *Rhizopus* sp.

Among the seed mycoflora the highest incidence of *Penicillium* sp. was recorded (Table 1) in

Table 1: Percent incidence of different fungi associated with pulse seeds under different isolation methods

Methods	*Penicillium* sp.	*Aspergillus* sp.	*Rhizopus* sp.	*Rhizoctonia* sp.	*Trichoderma* sp.	MEAN
PDA	18.00(25.10)	18.67(25.60)	20.67(27.04)	4.67(12.48)	10.67(19.06)	14.54
Blotter	17.33(24.60)	16.67(24.09)	19.33(26.08)	3.33(10.52)	6.67(14.96)	12.66
Water-agar	14.67(25.52)	17.33(24.60)	19.33(26.08)	1.33(6.63)	7.33(15.71)	11.99
Mean	16.66	17.55	19.77	3.11	8.22	
SEm±	2.82	3.56	3.26	1.20	2.85	
CD(p=0.05)	8.16	10.27	9.42	3.46	8.22	
Pulses	*Penicillium* sp.	*Aspergillus* sp.	*Rhizopus* sp.	*Rhizoctonia* sp.	*Trichoderma* sp.	MEAN
French bean	14.44(22.34)	14.44(22.34)	14.44(22.34)	0.00(0.05)	7.78(16.19)	10.22
Red kidneybean	18.89(25.76)	25.56(30.37)	18.89(25.76)	0.00(0.05)	2.22(8.57)	13.11
Chickpea	21.11(27.35)	11.11(19.47)	20.00(26.57)	0.00(0.05)	11.11(19.47)	12.66
Black gram	6.67(14.96)	4.44(12.17)	16.67(24.09)	15.56(22.23)	8.89(17.35)	10.44
Soybean	22.22(28.13)	32.22(34.59)	28.89(32.51)	0.00(0.05)	11.13(19.49)	18.89
SEm ±	3.65	4.59	4.21	1.55	3.67	
CD(p=0.05)	10.53	13.26	12.16	4.47	10.61	

Note Figures in the parentheses are angular transformed values

soybean seeds (22.22%) that is significantly different from the incidence recorded from black gram seeds (6.67%). With respect to Aspergillus sp., its highest incidence was recorded from soybean seeds (32.22%) followed by red kidney bean (25.56%) and French bean (14.44%) and the lowest from black gram seeds (4.44%). Similarly, for *Rhizopus* sp. the highest incidence was reported

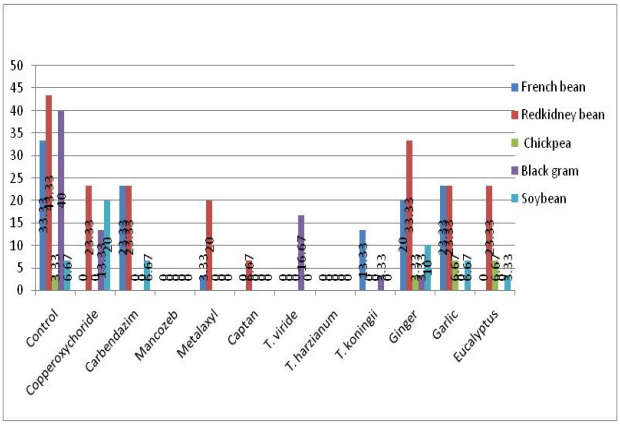

Fig. 1 : Effect of seed treatment on occurrence of *Penicillium* sp. on pulse seeds

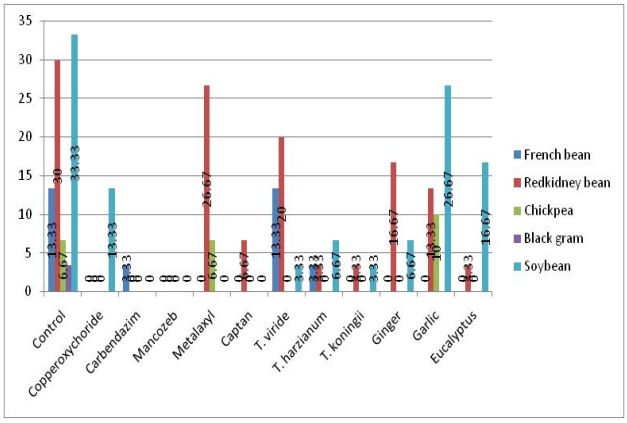

Fig. 2 : Effect of seed treatment on occurrence of *Aspergillus* sp. on pulse seeds

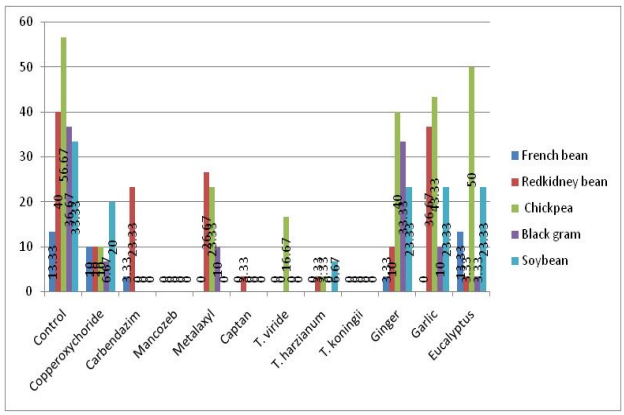

Fig. 3 : Effect of seed treatment on occurrence of *Rhizopus* sp. on pulse seed

from soybean (28.89%) seeds followed by chickpea (20.00%). *Rhizoctonia* sp. was isolated as seed mycoflora from black gram seeds only whereas, Trichoderma sp. shown its highest incidence on soybean (11.13%) closely followed by chickpea (11.11%). Overall, Rhizopus sp. and *Aspergillus* sp. were the predominant fungi in all the pulses. Similar findings regarding mycoflora associated with different pulse seeds have been reported by previous workers Shaker et al., 2010).

Different seed treating agents viz., fungicides, bioagents and plant extracts were tested with the aim of reducing mycoflora of pulse seeds and the

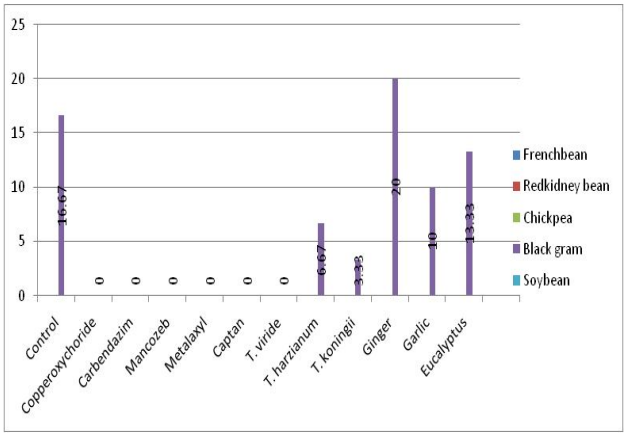

Fig. 4 : Effect of seed treatment on occurrence of *Rhizoctonia* sp. on pulse seed

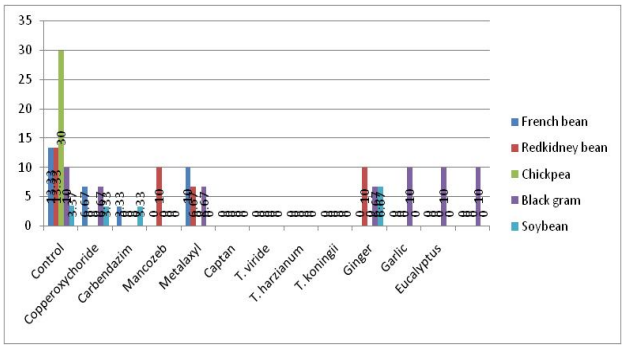

Fig. 5 : Effect of seed treatment on occurrence of fungal species *Trichoderma* sp. on pulse seeds

data pertaining to this study are presented in Figs. 1-5. Among fungicides, mancozeb completely eliminated Penicillium sp. from the pulse seeds (Fig. 1). Among the bioagents *T. harzianum* also completely checked the growth of Penicillium sp. from seeds of all pulses. Among plant extracts, eucalyptus leaf extract showed the minimum incidence of Penicillium sp. In contrast, garlic and eucalyptus extracts promoted the growth of *Penicillium* sp. on chickpea seeds recording an

incidence of 6.67% compared to 3.33% in control. The effect of mancozeb on management of seed mycoflora of pulses has been reported earlier . They reported that Dithane M-45 had effect on *Penicillium* sp. In the present experiment carbendazim was found to be less effective in reducing the *Penicillium* sp. Mahamune and Kakde (2011) have reported antifungal activity of *T. harzianum* on seed mycoflora of French bean. Their finding is in corroboration with the result of our experiment. The effectiveness of *T. viride* in controlling seed-borne mycoflora has been reported by Ashwini and Giri (2014) on green gram and black gram seeds.

With respect to seed mycoflora Aspergillus sp. mancozeb treatment completely eliminated the fungus from all pulse seeds (Fig. 2). It has been reported that all species of *Aspergillus* were eliminated when French bean seeds were treated with mancozeb. Among bioagents *T. koningii* reported the highest inhibition of *Aspergillus* sp. Though all the treatments significantly reduced the *Aspergillus* sp. compared to control, the plant extracts in general were least effective. All the treatments significantly reduced *Rhizopus* sp. on the pulse seeds however seed treatment with mancozeb and *T. koningii* completely eliminated *Rhizopus* sp. (Fig. 3). Though all the plant extracts had reduced the incidence of *Rhizopus* sp. from all the pulse seeds compared to control, they still record a high incidence of the fungus ranging from 18.66-21.99%. The seed mycoflora *Rhizoctonia* sp. was reported from the black gram seeds only in the present investigation (Table 1). All the fungicides had completely eliminated *Rhizoctonia* sp. from black gram seeds at par with *T. viride* (Fig. 4). Plant extracts were least effective among all the treatments in controlling *Rhizoctonia* sp. Ginger treatment increased the incidence of *Rhizoctonia* sp. on black gram seeds to 20.00% from 16.67% in control. Though plant extracts are reported to have inhibitory effect on incidence of seed mycoflora, at times they have been reported to stimulate incidence of some seed mycoflora as well (Telang, 2010).

Trichoderma sp. as seed mycoflora has been reported from all the pulses in study. In the present investigation incidence of *Trichoderma* sp. was reduced to zero by treating the seeds with captan (Fig 5). All the other treatments had significantly reduced the incidence of *Trichoderma* sp. However, garlic and eucalyptus had no effect on *Trichoderma* sp. of black gram seeds. *Trichoderma* spp. are reported to reduce the incidence of seed mycoflora and this can be attributed to the various mechanisms of action including production of chitinase, glucanase, antibiotics etc. In the present investigation the effect of different seed treating agents on germination percentage of pulse seeds was recorded (Table 2). It is evident that all the treatments had significantly increased the germination percentage of pulse seeds except for eucalyptus seed treatment. Notably, the treatment with *T. harzianum* recorded the highest mean germination percentage of 70.66% compared to 46.66% in control. This is followed by treatment with mancozeb (70.00%), captan (68.66%) and *T. koningii* (67.33%). The effect of bioagents on seed germination is at par with the fungicide mancozeb. Increase in seed germination of pulse seeds like green gram and black gram due to seed treatment with *T. viride* was reported by Ashwini and Giri (2014). Fungicide was also reported to improve seed germination. Improved germination of seeds after fungicidal treatment, on the whole, is due to the elimination of fungi because they secrete mycotoxins which are respon sible for the reduction of seed germination .

Leaf extract of *Acacia nilotica* was reported to reduce the incidence of seed mycoflora of legumes and increase seed germination percentage (Dhole and Gurme, 2013). The highest mean percentage of pulse seed germination in response to seed treating agents is 70.66% with the treatment of *T. harzianum* (Table 2). The lowest mean percentage is 52.00% with *Eucalyptus odoratum*. The highest germination percentage of French bean seeds is 100% with *E. odoratum*. There is no effect of any seed treating agents on germination percentage of red kidney bean. For chickpea, the highest germination percentage can be seen with (100%) in the treatments with mancozeb, metalaxyl, captan, *T. harzianum* and garlic. For black gram, the highest percentage can be seen (83.33%) in the treatment with *T. harzianum* and the lowest can be seen (56.67%) in the treatment with metalaxyl. For soybean, the highest germination percentage is 96.67% with the treatment captan compared to 46.67% in control.

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