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Seed-borne Mycoflora of important Oilseeds of Nagaland

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ABSTRACT

The present investigation was carried out to identify fungal infection of five oilseeds viz., rapeseed, mustard, soybean, sesame and perilla oilseeds via different seed health testing methods and seed germination. A total of six seed-borne fungi Aspergillus sp., Colletotrichum sp., Rhizopus sp., Penicillium sp., Nigrospora sp. and Trichoderma sp. were detected from the five oilseeds collected from local market of Medziphema, Nagaland. Amongst the seed health testing methods employed, PDA method was proved to be superior to blotter paper method and water-agar method. The fungal occurrence is more in PDA method with mean incidence of seed mycoflora 15.77%. The predominant fungus was observed to be Trichoderma sp. with 40.67% incidence and the least fungal incidence was of Nigrospora sp. (1.33%). Among the oilseeds, soybean seeds showed highest incidence of seed mycoflora (14.81%) and the lowest incidence of 4.24% was recorded from perilla seeds. The highest germination of oilseeds was recorded in PDA method (50.33%) followed by water-agar method (45.67%).

KEYWORDS

mycoflora, Nagaland, oilseeds

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INTRODUCTION

Seed is the vital input in agriculture (Hassan *et al*, 2015). Pathogen-free seeds ensures good germination and a healthy crop to obtain desirable yield (Diaz *et al*, 1998). About 90% of the crop all over the world including oilseeds are produced by using seeds as the starting material.

Oil seeds are primarily rich in proteins, carbohydrates, and good source calcium, potassium, phosphorus, magnesium and vitamin E. In India, a wide variety of oilseeds are grown and India occupies fourth position in the world in production of edible oilseeds (Chavan, 2011).

The total area, production and productivity of oilseed cultivation in Nagaland are 23740.00 hectares, 20810.00 Metric Ton; and 60.2 q/ ha respectively. The area under oilseeds such as groundnut, soybean, sesame, sunflower, mustard, linseed, etc., in the district of Dimapur under Nagaland is about 5800 ha (Anonymous, 2010). The total area, production and productivity of oilseed cultivation in Dimapur during 2013-2014 were 8850 hectares, 8710 MT and 0.98 t/ ha respectively (Bhalerao *et al*, 2015).

Seed quality gets deteriorated during storage due to biotic factors including microorganisms like fungi, bacteria etc. (Mehrotra and Aggarwal 2003). Infected seeds show poor germination and seedling vigour leading to poor crop yield (Naqvi *et al*, 2013) and also serve as carrier of pathogens to other geographical areas or countries (Waller, 2002). The infections on the seeds may come from the field, during post-harvest handling of the seed lots or during storage (Manimurugan, 2003). Fungi of various taxonomic classes which are mostly pathogenic for example Alternaria, Aspergillus, Cercospora, Curvularia, Drechslera, Fusarium, Penicillium, Rhizoctonia and Trichoderma that is a biocontrol agent including oomycete pathogens like Pythium have been reported from seeds all over the world by various researchers (Kavitha *et al*, 2005).

Oilseeds are attacked by a number of diseases caused by fungi, bacteria, nematodes, virus and other plant pathogenic microorganisms which results into heavy losses in India. Pathogens present in any oilseed lot of economically important crops may be disastrous. Therefore, oilseeds must be substantially free from inoculum with high level of germination and purity before sowing. Most of the fungal pathogens of oilseeds are reported to be seed-borne.

Fungi like Aspergillus niger, A. flavus, Alternaria dianthicola, Curvularia lunata, C. pellescens, Fusarium oxysporum, F. equiseti, Macrophomina phaseolina, Rhizopus stolonifera etc. reduce quality of oilseeds (Chavan, 2011).

It is important to know the presence of and identify the seed mycoflora that might have potentially damaging effect on the oilseeds during crop production or storage to devise appropriate management strategy. Seed health testing (ISTA, 1993) assures safe use of seeds for research, consumption and trade. Therefore, the present work has been undertaken to study the occurrence of the mycoflora of important oilseeds crops of Nagaland.

MATERIALS AND METHODS

Source of seeds

The seed samples of five oilseeds viz. rapessed, mustard, soybean, sesame, perilla were collected from local market in Medziphema, Nagaland. The seeds were collected in sterilized polythene bags with proper labeling, brought to the lab-

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oratory of the Department of Plant Pathology, SASRD, Nagaland University, Medziphema and kept in the refrigerator at 5±1°C until used for subsequent studies.

Isolation method

Agar plate (PDA) method

A total of thirty seeds from each sample of five oilseeds were treated with sodium hypochlorite (NaOCl) 1.0% for 2 minutes followed by four washings with sterilized water. Surface sterilized seeds were placed equidistantly in circles in Petri plates (9cm diam.) containing PDA media. Each sample was replicated three times. Seeds were incubated at 28±1°C for 12 hours of alternating cycles of v day/night under fluorescent light (Bhajbhuje, 2013). After 7 days, the infection % of seed mycoflora was calculated.

Blotter paper method

In the blotter method, a total of thirty seeds from each sample were surface- sterilized with sodium hypochlorite (NaOCl) 1.0% for 2 minutes followed by four washings. The seeds were placed equidistantly on Petri plates containing three-layer sterilized filter paper (Whatman No. 1) beds. Each treatment was replicated three times. Filter papers were kept moist with sterilized distilled water. Incubation details and conditions were the same as for the agar plate method. Data were collected on the incidence of seed mycoflora of five oilseeds (Bhajbhuje, 2013).

Water-agar method

A total of thirty seeds from each sample of five cultivars were treated with sodium hypochlorite (NaOCl) 1.0% for 2 minutes followed by four washings with sterilized water. Surface sterilized seeds were placed equidistantly in circles in Petri plates (9 cm diam.) containing ware-agar media. Each sample was replicated three times. Incubation conditions were the same as for the agar plate method and blotter paper methods. Data were collected on the incidence of seed mycoflora of five oilseeds (ISTA, 1966).

Identification of the seed mycoflora

Isolated fungi were then studied under compound microscope (Laboscope BD-05 77530) and identified up to genus level whenever possible based on their morphological characters in the Department of Plant Pathology, Nagaland University, SASRD, Medziphema Campus, Nagaland. 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 (Nagamani *et al*, 2006).

RESULTS AND DISCUSSION

Per cent incidence of different fungi associated with oilseeds under different isolation methods

Seed mycoflora from five different oilseeds species viz., rapeseed, mustard, soybean, sesame and perilla collected from local markets of Medziphema, Nagaland were isolated using three different isolation methods viz., PDA method, blotter paper method and water-agar method. The data obtained from this experiment are presented in Table 1 .

It is evident from the data presented in the Table 1 that in PDA method the fungal occurrence is more with mean incidence of 15.77% followed by blotter paper method (5.22%) and water-agar method (4.78%). Altogether, six fungal species Aspergillus sp., Colletotrichum sp., Rhizopus sp., Penicillium sp., Nigrospora sp., and Trichoderma sp. were isolated as seed mycoflora from five different oilseeds. The PDA method recorded the highest incidence (40.67) of Trichoderma sp., as seed borne fungus. The other seed-borne mycoflora like Penicillium sp., Rhizopus sp. and Aspergillus sp. were recorded with the incidence of 14.67%, 13.33% and 12.67% respectively. Though PDA and water- agar method could enumerate six mycoflora each, blotter paper method did not yield Nigrospora sp. as seed mycoflora. Solanke et al. (1997) reported that agar plate (PDA) method yielded more seed mycoflora than blotter paper method in soybean. Lalit *et al* (2001) reported that agar plate method was found slightly superior over modified standard blotter paper method for isolation of seed mycoflora of sesame. These finding are in corroboration with the result of our experiment that more incidence and more number of seed mycoflora were obtained from agar plate (PDA) method (Alemu, 2014).

Table 1: Per cent incidence of different fungi associated with oilseeds under different isolation methods

Methods	Aspergillus sp.	Colletotrichum sp.	Rhizopus sp.	Penicillium sp.	Nigrospora sp.	Trichoderma sp.	Mean
PDA	12.67 (20.85)	10.00 (18.43)	13.33 (21.42)	14.67 (22.52)	3.33 (10.52)	40.67 (39.62)	15.77
Blotter paper	12.00 (20.27)	3.33 (10.52)	8.67 (17.12)	4.67 (12.48)	0.00 (0.05)	2.67 (9.40)	5.22
Water- agar	6.00 (14.18)	2.71 (9.48)	9.33 (17.79)	4.00 (11.54)	1.33 (6.63)	5.33 (13.35)	4.78
SEm±	0.29	0.20	0.28	0.32	0.20	0.30	
CD (p=0.05)	0.84	0.57	0.81	0.93	0.57	0.87	

Figures in the tables are mean values and those in parentheses are square root transformed values

In agar- plate method PDA is used; it serves as a source of nutrient that can support growth of many fungi and even traces of fungal infection can be detected using PDA method (Alemu, 2014).

In the contrary Ramesh and Avitha (2005) reported that by blotter technique more fungi were isolated as compared to agar plate method. Some workers observed that both the methods were equally valuable and supplementary to each other (Kumhar *et al*, 1987).

Per cent incidence of different fungi associated with oilseeds

As per the data presented in Table 2 soybean seeds were reported to harbor highest mean incidence (14.81%) of various seed mycoflora followed by sesame (10.37%). The lowest mean incidence of mycoflora was reported from perilla (4.27%). Among the oilseeds, only soybean has recorded six fungal species from the seeds with the highest incidence of Trichoderma sp. (32.22%) followed by Colletotrichum sp. (17.78%). Seeds of rapeseed recorded only four fungal species viz., Aspergillus sp., Rhizopus sp., Penicillium sp. and Trichoderma sp. with a mean incidence of 4.44%. Though sesame has recorded 14.81% incidence of seed mycoflora, it didn't show the presence of Nigrospora sp. Similarly, mustard and perilla also did not record Nigrospora sp. as seed-borne mycoflora.

All six seed-borne mycoflora isolated from seeds of five oilseeds are not pathogenic to oilseeds under study except for Colletotrichum sp. These mycoflora are commonly reported from oilseeds by various workers. Nik (1980) isolated species of pathogenic fungi from soybean which were found to be Colletotrichum dematium, Nigrospora, Penicillium, Rhizopus and Trichoderma.

Tripathi and Singh (1991) tested three soybean genotypes for presence of seed mycoflora and recorded Aspergillus spp., Penicillium oxalicum, Rhizopus sp. and Nigrospora oryzae among many other seed mycoflora.

Altaf *et al* (2004) reported various species of Aspergillus and Penicillium of sesame seeds. Sesame seeds were reported to harbor Aspergillus sp., Rhizopus sp. and Penicillum sp. (Lemture *et al*, 2010). Thus, our finding on various seed mycoflora of oilseeds is in consonance with other workers.

Table 2: Per cent incidence of different fungi associated with oilseeds

Oilseeds	Aspergillus sp.	Colletotrichum sp.	Rhizopus sp.	Penicillium sp.	Nigrospora sp.	Trichoderma sp.	Mean
Rapeseed	6.67 (14.96)	0.00 (0.05)	10.00 (18.43)	6.67 (14.96)	0.00 (0.05)	3.33 (10.52)	4.44
Mustard	18.89 (25.76)	5.56 (13.63)	15.56 (23.23)	5.56 (13.63)	0.00 (0.05)	8.89 (17.35)	9.07
Soybean	10.00 (18.43)	17.78 (24.94)	11.11 (19.47)	10.00 (18.43)	7.78 (16.19)	32.22 (34.59)	14.81
Sesame	11.11 (19.47)	3.33 (10.52)	10.00 (18.43)	11.11 (19.47)	0.00 (0.05)	26.67 (31.09)	10.37
Perilla	4.44 (12.17)	0.08 (1.61)	5.56 (13.63)	5.56 (13.63)	0.00 (0.05)	10.00 (18.43)	4.27
SEm ±	0.38	0.26	0.36	0.42	0.25	0.39	
CD(p=0.05)	1.09	0.74	1.04	1.20	0.73	1.12	

Figures in the tables are mean values and those in parentheses are square root transformed values

Further among the seed mycoflora the highest incidence of Aspergillus sp. was recorded in mustard seeds (18.89%) and lowest in perilla (4.44%). With regards to Colletotrichum sp. soybean seeds were recorded to have the highest incidence (17.78%) and the lowest incidence was recorded from perilla seeds (0.08%). With the respect to Rhizopus sp. its highest incidence was recorded from mustard seeds (15.56%) followed by soybean (11.11%) and sesame and rapeseed (10.00% each) and the lowest from perilla (5.56%). Similarly, for Penicillium sp. the highest incidence was reported from sesame seeds (11.11%) followed by soybean (10.00%). In Trichoderma sp. was isolated as seed mycoflora from all the oilseeds under study with the incidence varying from 3.33 to 32.22%.

Though other workers reported many fungal species from oilseeds on seed mycoflora, our work could yield only six of them. It may be due to the difference in geographical conditions, storage conditions and cultivation practices and post-harvest processing of oilseeds. Mycoflora of seed varied from place to place due to change in condition prevailing during seed development, harvesting and storage (Dwivedi and Gopal, 2014).

Among the isolated seed-borne fungi Aspergillus sp. are known to produce mycotoxins (Reddy *et al*, 2014). In our experiment all the oilseeds were found to contain the fungus at various levels of incidence, 4.44% in perilla to 18.89% in mustard making the oilseeds of this region vulnerable to mycotoxins contamination.

Abdel-Mallek *et al* (1994) also isolated nine Aspergillus sp. from sunflower seed samples and all of them are reported to produce different groups of aflatoxins which are natural toxins and hazardous to animal and man. Penicillium spp. have been associated with mycotoxicoses in animal fed on contam-

inated grain (Brook and White, 1996).

Germination percentage of different oilseeds on PDA, Blotter paper and Water-agar method

The germination percentage of oilseeds under present investigation was studied and the data are presented in Table 3 . The germination percentage of rapeseed in PDA, blotter paper and water-agar treatment was found to be 83.33%, 53.33% and 73.33% respectively. Similarly, for mustard seeds, the germination percentage in PDA was found to be 83.33%, in blotter paper method 56.67% and in water-agar method 60.00%. The germination percentage of soybean was 90.00% in both blotter paper and water agar method and 80.00% in PDA method. Germination percentage recorded in sesame and perilla following the three methods under study was all found to be 2.50 %.

Table 3: Germination (%)

Methods	Rapeseed	Mustard	Soybean	Sesame	Perilla	MEAN
PDA	83.33 (65.91)	83.33 (65.91)	80.00 (63.43)	2.50 (9.10)	2.50 (9.10)	50.33
Blotter paper	53.33 (46.91)	56.67 (48.83)	90.00 (71.57)	2.50 (9.10)	2.50 (9.10)	41.00
Water-agar	73.33 (58.91)	60.00 (50.77)	90.00 (71.57)	2.50 (9.10)	2.50 (9.10)	45.67
	C.D(p=0.05)		**SEm±**			
Method	5.36		1.86			
Oilseed	6.92		2.40			
O x M	11.99		4.15			

Figures in the tables are mean values and those in parentheses are angular transformed values

CONCLUSION

This study reveals that there are six important seed-borne disease causing fungi namely Aspergillus sp., Colletotrichum sp., Rhizopus sp., Penicillium sp., Nigrospora sp. and Trichoderma sp. of five oilseeds crops viz., rapeseed, mustard, soybean, sesame and perilla oilseeds.

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