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Detection of Fungal Load from Groundnut Seeds and their Pathogenic Nature Regards to Pre and Post Emergence Mortality

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Seed infecting fungi are important constraints for the groundnut crop prior harvesting to storage. Many fungal pathogens attack on the seeds and hamper seed health. Isolation of seed-infecting fungi was done by standard blotter method and Agar plate method. Isolation by both the methods revealed the predominantly association of *Sclerotium rolfsii*, *Fusarium moniliforme*, *Fusarium equiseti*, *Helminthosporium* sp., *Aspergillus niger* and *Aspergillus flavus*. In pathogenecity test, highest per cent of pre and post-emergence mortality was observed in seeds of Local variety inoculated with *Aspergillus flavus i.e.* 40.00 and 48.89 per cent respectively.

Key words: Fungal Load, Pre and Post Emergence Mortality, Groundnut.

Groundnut (*Arachis hypogea* L.) is commercially important oilseed crop grown in about 100 countries covering 26.4 million hectares with a total production of 36.1 million tones of nuts in shell. It is important in the diets of rural people because it is rich in 45-47 per cent oil, 26-30 per cent proteins, fats 41-52 per cent and 11-27 per cent carbohydrates which are highly digestible (Waliyar, 2006) . Good and viable seed is essential for establishing desired plant population, good plant growth and better yield. The quality of seeds depends upon the condition prevailing during the development of pods and kernels and methods of curing and storage. Quality seed reduce seed rate and per hectare cost of production. The high relative humidity favors the pod and seed infection by various species of fungi like *Aspergillus, Fusarium, Sclerotium, Rhizopus, Penicillium, Helminthosporium* etc (Chavan and Kakde, 2008).. leading to loss of viability of seeds within short time. Microbial seed deterioration is one of the most serious biotic constraints to the quality production of groundnut seeds as well as many fungal pathogens caused the various abnormality like seed rot, necrosis and pre as well as post emergence mortality (Bhattacharya and Raha, 2002).. Keeping this in point of view following study was conducted to find out pathogenic nature of isolated fungi on pre and post emergence mortality.

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MATERIALS AND METHODS

Isolation

Isolation of fungi associated with groundnut seeds was carried out from randomly taken 400 seeds from the composite grain samples by standard blotter method (Neergaard, 1977 and Bhale *et al*., 2001). Ten seeds of each non surface sterilized and surface sterilization by 1 per cent

sodium hypochlorite solution for one minute, were placed at equi distance on three layers of properly moistened sterilized blotters, and Petri plates were incubated under 12/12 hr alternating light and dark period at 25 ± 2º C. Developing fungal growth on each of the grains after seven days was observed under stereoscopic binocular microscope and recorded accordingly.

Purification and Identification

Each fungal species isolated from seed samples was purified by single spore or hyphal tip method and fungal growth was critically observed under microscope for cultural and morphological characters. Finally, fungal characteristics observed were compared with the characteristics described in earlier literature. The same pure cultures grown in slants were sent to Indian Type Culture Collection (I. T. C. C.), Division of Mycology and Plant Pathology, Indian Agricultural Research Institute (I. A. R. I.), New Delhi for identification and confirmation of isolated fungus.

Impact on pre and post emergence mortality

The test was carried out for *Fusarium moniliforme*, *F. equiseti*, *Sclerotium rolfsii*, *Helminthosporium* sp., *Aspergilus niger* and *A. flavus* Apparently healthy seeds of local variety were used for testing the pathogenecity of different isolates**.**

For proving pathogenecity test of various isolated pathogens, healthy seeds of variety local were surface sterilized for 2 minutes with 0.1per cent mercuric chloride ($Hgcl_2$) solution followed by three subsequent washings in sterilized distilled water to remove $HgCl_2$ from seeds. These seeds were then soaked for 24 hours in sterilized distilled water and were inoculated by rolling on 10-15 days old actively sporulating culture of each test fungus.

The germination paper sheet was wetted by sterilized distilled water. Twenty five seeds of respective treatment were placed on first sheet evenly. Second sheet of germination paper was placed on first sheet followed by wetting it carefully. Both sheets were rolled along with wax coated paper. The rolled towel papers were incubated at 25^0C for 7 days. At the end of incubation period, rolled towel papers were carefully opened. Germinated and ungerminated seeds were counted from each of the treatments. Emergence of seedling from the seeds was considered as successful germination. Three repetitions each of 75 seeds were maintained for each of the treatments.

The observations on germination were recorded 8th days after sowing and seedling mortality on 15th days after sowing. Pre emergence and post emergence mortality were calculated by following formulae:

Pre emergence mortality (8th DAS)

$$\text{Pre emergence mortality (\%)} = \frac{\text{Total No. of ungerminated seeds}}{\text{Total No. of sown seeds}} \times 100$$

Post emergence mortality (15th DAS)

$$\text{Post emergence mortality (\%)} = \frac{\text{Total no. of died seedling}}{\text{Total no. of germinated seeds}} \times 100$$

RESULTS AND DISCUSSION

Isolation of pathogens

Isolation of seed borne fungi from composite sample of groundnut seeds was carried out by standard blotter method and PDA plate method by surface and without surface sterilized seeds revealed the association of six different predominant fungi.

Pathogenecity test *in vitro*

The pathogenic nature of *Fusarium moniliforme*, *Sclerotium rolfsii*, *Helminthosporium* sp., *F. equiseti*, *Aspergillus niger* and *A. flavus* were tested on cv. Local under *in vitro* conditions. The apparently healthy seeds of cv. Local were inoculated with 10-15 days old culture of each pathogen by following methods.

Table 1. Fungal association with naturally infected groundnut seeds of composite samples.

Isolate No.	Fungi	Fungal species Standard blotter method	
		SS	US
1	*Sclerotium rolfsii*	-	*
2	*Helminthosporium* sp.	-	*
3	*Fusarium moniliforme*	*	*
4	*Fusarium equiseti*	-	*
5	*Aspergillus niger*	-	*
6	*Aspergillus flavus*	*	*

* Present - Absent SS – Surface sterilized US- Un sterilized

Table 2. Effect of seeds inoculated with different fungi on pre-emergence and post-emergence mortality *in vitro*

Treatment no.	Name of fungi	Pre-emergence mortality (%)*	Post-emergence mortality (%)*
1	*Sclerotium rolfsii*	22.67	20.69
2	*Helminthosporium* sp.	16.00	25.40
3	*Fusarium moniliforme*	28.00	31.48
4	*Fusarium equiseti*	25.33	23.21
5	*Aspergillus niger*	33.33	40.00
6	*Aspergillus flavus*	40.00	48.89
7	Control (Uninoculated seeds)	6.67	11.43

*Average of three repetitions

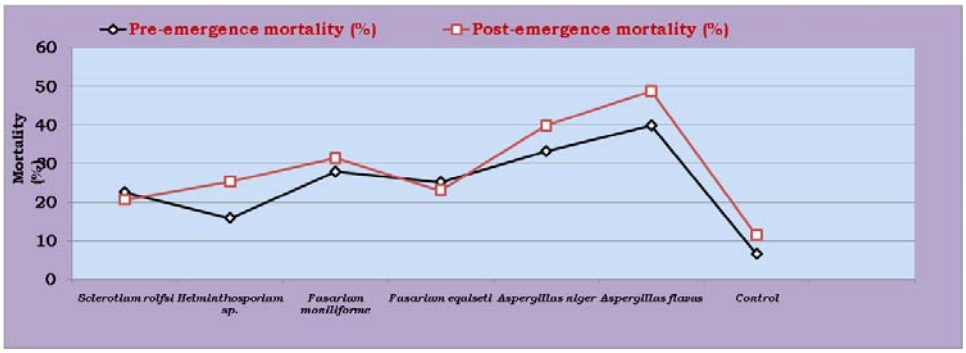

Fig. 1. Effect of different seed borne fungi on pre and post emergence mortality of groundnut *in vitro*

The surface sterilized apparently healthy seeds of cv. Local were inoculated with 10-15 days old culture of each test fungus and 75 seeds for each fungus were placed on paper towel, whereas, uninoculated seeds were served as control.

The data presented in (Table 2 and Fig.1) revealed that inoculation of all fungi isolated from seeds of groundnut had reduced germination per cent and caused pre-emergence and post-emergence mortality as compared to uninoculated seeds. In control, 6.67 per cent pre-emergence mortality and 11.43 per cent post emergence mortality observed. The highest pre-emergence and post-emergence mortality observed in seeds inoculated with *Aspergillus flavus* were 40.00 and 48.89 per cent respectively, followed by *Aspergillus niger* 33.33 and 40.00 per cent respectively. The rest of inoculated fungi also caused considerable pre-emergence and post-emergence mortality which ranged from 16.00 to 28.00 per cent and 20.69 to 31.48 per cent respectively. Reduction in germination in each treatment was caused due to rotting of seeds by inoculated fungi.

The present result confirms the findings of Raj and Sharan (1994) they reported that lowest per cent germination of sunflower seeds caused by *A. flavus* and highest per cent of abnormal seedlings in seeds inoculated with *F. moniliforme*. Similarly, Rasheed *et al*. (2004) noted that *Aspergillus flavus* caused 15 per cent pre emergence and 2 per cent post emergence mortality of groundnut seeds.

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