

Original Research Article

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Seed Borne Mycoflora of *Macrotyloma uniflorum* L (Horse gram)

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Horse gram (*Macrotyloma uniflorum*), belongs to the family *Fabaceae* is one of the minor or lesser known neglected legume mainly cultivated due to high levels of protein. It is considered as poor man's pulse. Horse gram has an excellent nutritional, therapeutic and medicinal properties. Field variety of horse gram seeds were collected from the Indurthi village, Karimnagar district, Telangana, India. The legume seed borne fungi was screened by employing blotter paper method, agar plate method and dilution plate method. The untreated seeds were found to be associated with the highest number of seed borne fungi. 64 species and 32 genera were isolated. Among them *Alternaria spp*, *Aspergillus spp*, *Cheatomium spp*, *Cladosporium spp*, *Curvularia spp*, *Drechslera spp*, *Fusarium spp*, *Mucor spp*, *Pencillium spp*, *Rhizopus spp*, *Trichodroma spp* were dominant species isolated from the three methods. In all the three methods blotter method had recorded most of the fungal species than the other two methods.

Introduction

Food legumes are second most important group of crops after cereals which have been a vital ingredient of balanced human diet since millennia (Bhadana *et al.*, 2013) and recognized as second most valuable plant source for human and animal nutrition (Bhatt and Karim, 2009; Bhartiya *et al.*, 2015). The world's stored grain is damaged mainly by the activity of fungi than other microorganism was stated by Neergard, (1977). Seeds play a vital role in the production of healthy crops. Healthy seed is the foundation of healthy plant; a necessary condition for good yields (Diaz *et al.*, 1998). Seeds that were infected or contaminated with pathogens (Agarwal, 1976). Seed-borne diseases have been found to affect the growth and productivity of crop

plants (Kubiak and Korbas, 1999; Weber *et al.*, 2001; Dawson and Bateman, 2001). Presence or absence of seed borne fungi on seed surface is one of the important aspects that determine the quality of seed.

Horsegram belongs to family *Fabaceae* is a potential grain legume having excellent nutritional and remedial properties with better climate resilience to adapt harsh environmental conditions (Kumar, 2006). It is one of the most important unexploited food legume being grown in almost all over the world including temperate and sub-tropical regions encompassing the countries in East and Northeast Africa, Asian countries particularly, India, China, Philippines,

Bhutan, Pakistan, Sri Lanka and Queensland in Australia (Durga, 2012; Krishna, 2010). Horsegram [*Macrotyloma uniflorum* (L.) Verdc] has great significance in the nutritional security of rural, tribal and underprivileged masses (Tontisirin, 2014). Horse gram is one of the highly nutritious vegetable pulse crop with ethno-medicinal values in India, which is commonly known as *Kulattha* (Sanskrit), *Kurti-kalai* (Bengali), *Kollu* (Tamil), *Ullavallu* (Telgu), *Muthira* (Malyalam), *Gahot* (Kumaon and Garhwal) and etymologically, *Gahot* means “which destroys stone in initial stage” (Pati and Bhattacharjee, 2013; Pande, 1999).

Horsegram is a short day, twining, succulent, annual climbing herb which has trifoliate leaves, white coloured flowers, long linear pubescent pods with curved beak, flattened small seeds with light red, brown, grey, black or mottled testa (Singh, 1991) with photo and thermo sensitive nature (Kumar, 2006). It is mostly grown as catch crop especially under late summer (*Kharif*) or with the rains after a prolonged drought condition (Prakash *et al.*, 2002).

In the present study an attempt was made to find out the fungi which is associated with the seeds of horse gram.

Materials and Methods

The field variety of horse gram seeds were collected from the Indurthi village, Kharimnagar district, Telangana, India. The collected seeds were stored in cloth bags for six months at room temperature for the study of storage fungi. Isolation of seed mycoflora was done by blotter Method (De Tempe, 1953). Agar plate method by Muskett, (1948) and Dilution plate method by Peterson, (1959). These investigations were carried out in freshly harvested seeds samples as well as during their subsequent storage at an interval of 30 days for six months.

Results and Discussion

64 fungal species with 32 genera were isolated from the table.1. *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus flaviceps*, *Aspergillus fumigatus*, *Aspergillus candidus*, *Cheatomium globosum*, *Cheatomium herbarum*, *Cheatomium murorum*, *Cladosporium spp*, *Curvularia lunta*, *Drechslera halodes*, *Fusarium moniliforme*, *Fusarium roseum*, *Macrophominaphaseolina*, *Mucor varians*, *Mucor racemosus*, *Nigrospora sphaerica*, *Pencillium chrysogenum*, *Pencillium citrinum*, *Phomaspp*, *Rhizopus stolonifera*, *Rhizopus nigricans*, *Trichoderma hamatum*, *Trichoderma viridae*, *Trichoderma album*, *Verticillium sps*. Were the dominant species.

Among the 3 methods in Agar method most of the fungi were isolated followed by Blotter method and dilution plate technique.

Seed is the most important input for crop production. Pathogen free healthy seed is urgently needed for desired plant populations and good harvest (Narayan *et al.*, 2013). Many plant pathogens are seed-borne, which can cause enormous crop losses; reduction in plant growth and productivity of crops (Kubiak and Korbas, 1999; Dawson and Bateman, 2001; Islam *et al.*, 2009).

Neetisaxena *et al.*, 2015 reported that Fungi have assumed great economic significance, as they not only cause spoilage of grains, during pre and post-harvest stages of production, but also produce various toxins (mycotoxins).

Delouch *et al.*, (1973) stated that fungi were the major deteriorating agents which are known to affect the capacity of seeds to germinate, besides discoloring the parts, loss in luster, heating, mustiness and changes in oil content, free acids and protein content etc.

Table.1

S. No	Mycoflora	Horse gram seeds					
		B.M		AGP.M		DP.M	
		U	S	U	S	U	S
1.	<i>Alternaria alternata</i>	+	+	+	+	+	+
2.	<i>Alternaria humicola</i>	+	+	+	-	+	+
3.	<i>Alternaria tenuis</i>	+	+	+	-	+	-
4.	<i>Alternaria pori</i>	+	-	+	+	+	+
5.	<i>Aspergillus flavus</i>	+	+	+	+	+	+
6.	<i>Aspergillus flavipes</i>	+	+	+	+	+	+
7.	<i>Aspergillus fumigatus</i>	+	+	+	+	+	+
8.	<i>Aspergillus candidus</i>	+	+	+	+	+	+
9.	<i>Aspergillus niger</i>	+	-	+	+	+	-
10.	<i>Aspergillus nidulans</i>	+	-	+	+	+	-
11.	<i>Aspergillus ochraceus</i>	+	-	+	+	-	-
12.	<i>Aspergillus sulphureus</i>	-	-	+	+	-	-
13.	<i>Aspergillus sydowii</i>	-	-	+	+	-	-
14.	<i>Aspergillus tamarii</i>	-	-	-	+	+	+
15.	<i>Aspergillus terreus</i>	+	-	+	+	-	-
16.	<i>Aspergillus versicolor</i>	+	-	+	-	+	-
17.	<i>Aspergillus carbonarius</i>	+	-	-	-	+	-
18.	<i>Botrytis sp.</i>	+	-	+	+	-	-
19.	<i>Cephalosporium sp</i>	+	+	+	-	+	-
20.	<i>Cercospora spp</i>	+	-	+	-	-	-
21.	<i>Chaetomium cochloides</i>	+	-	+	-	+	-
22.	<i>Chaetomium globosum</i>	+	+	+	+	+	+
23.	<i>Chaetomium herbarum</i>	+	+	+	+	+	+
24.	<i>Chaetomium murorum</i>	+	+	+	+	+	+
25.	<i>Cladosporium sp</i>	+	+	+	+	+	+
26.	<i>Curvularia lunata</i>	+	+	+	+	+	+
27.	<i>Colletotrichum truncatum</i>	-	-	+	-	+	-
28.	<i>Drechslera hawaiiensis</i>	+	-	-	-	+	-
29.	<i>Drechslera holodes</i>	+	+	+	+	+	+
30.	<i>Drechslera tetramera</i>	+	-	+	-	+	-
31.	<i>Fusarium moniliforme</i>	+	+	+	+	+	+
32.	<i>Fusarium Oxysporum</i>	-	+	-	-	+	+
33.	<i>Fusarium roseum</i>	+	+	+	+	+	+
34.	<i>Fusarium solani</i>	+	+	+	-	+	-
35.	<i>Fusarium equiseti</i>	-	-	+	-	+	-
36.	<i>Fusarium semitecum</i>	-	-	-	+	-	-
37.	<i>Helminthosporium sp.</i>	-	-	+	-	+	+
38.	<i>Humicola sp.</i>	-	-	+	-	-	-
39.	<i>Macrophomina phaseolina</i>	-	+	+	+	+	+
40.	<i>Monilia sitophila</i>	+	-	-	-	+	-

41.	<i>Mucor varians</i>	+	+	+	+	+	+
42.	<i>Mucor hiemalis</i>	-	-	+	-	+	-
43.	<i>Mucor racemosus</i>	+	+	+	+	+	+
44.	<i>Myrothecium roridum</i>	+	-	-	-	+	-
45.	<i>Nigrospora sphaerica</i>	+	+	+	+	+	+
46.	<i>Penicillium chrysogenum</i>	+	+	+	+	+	+
47.	<i>Penicillium citrinum</i>	+	+	+	+	+	+
48.	<i>Penicillium notatum</i>	+	-	+	+	+	-
49.	<i>Periconia spp</i>	+	-	+	-	+	-
50.	<i>Phoma sp</i>	+	+	+	+	+	+
51.	<i>Pythium spp</i>	-	-	-	-	+	-
52.	<i>Rhizoctonia bataticola</i>	+	-	+	-	+	-
53.	<i>Rhizopus nigricans</i>	+	+	+	+	+	+
54.	<i>Rhizopus stolonifera</i>	+	+	+	+	+	+
55.	<i>Rhizopus nodosus</i>	+	-	+	-	+	-
56.	<i>Stachybotrysatra</i>	+	-	+	-	-	-
57.	<i>Syncephalastrum sp</i>	+	-	+	-	+	-
58.	<i>Sclerotiumsp</i>	+	-	+	-	+	-
59.	<i>Scopularopsis sp.</i>	+	-	+	-	-	-
60.	<i>Trichoderma hamatum</i>	+	+	+	+	+	+
61.	<i>Trichoderma viridae</i>	+	+	+	+	+	+
62.	<i>Trichothecium roseum</i>	+	+	+	-	-	-
63.	<i>Trichoderma album</i>	+	+	+	+	+	+
64.	<i>Verticillium sp</i>	+	+	+	+	+	+

U- Unsterilized, S- Surface sterilized, B.M- Blotter, AGP.M- Agar plate and DP.M- Dilution Plate Method.

Seed borne Mycoflora plays an important role in determining the quality and longevity of seeds. Microbial invasions can lead to the rotting and loss of seed viability, vigour, germination and oil quality Nagaraja and Krishnappa, (2009); Neetisaxena *et al.*, 2015) The variation was observed in seed mycoflora from freshly stage to atthe end of the storage period. Storage fungi were present in low percentage in freshly harvest seed samples and became dominant as the storage period increased. Storage fungi require high osmotic pressure and no water (Manoharachary and Kunwar, 2006).

In conclusion, in the present investigation seed associated mycoflora were isolated by the three methods blotter method, agar plate

method and dilution plate method. Among them Agar plate method had recorded more fungi than the other two methods. *Alternaria spp*, *Aspergillus spp*, *Cheatomium spp*, *Cladosporium spp*, *Curvularia spp*, *Drechslera spp*, *Fusarium spp*, *Mucor spp*, *Pencillium spp*, *Rhizopus spp*, *Trichodrma spp* were dominant species isolated from the three methods. Storage fungi is in low percentage than the field fungi.

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