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INCIDENCE OF *ASPERGILLUS SPECIES* ON SEEDS OF PULSES FROM MARATHWADA REGIONPangrikar P. P., Solanke S. N. and Bandewar S. T.¹

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ABSTRACT

In agriculture, pulses have great importance in Indian agriculture as they are rich source of protein (17 to 25 percent) as compared to cereals. Pulses have ability to fix atmospheric nitrogen and improve soil quality. Pulses like Green gram (*Vigna radiate* (L.) Wilczek), Black gram or Urid Bean (*Vigna mungo* (L.) Hepper.), Chickpea or Gram (*Cicer arietinum* L.) and Pigeon pea or Tuver (*Cajanus cajan*(L.) Millsp., are mainly cultivated in Marathwada region. The damage to pulse seeds caused by storage fungi due to their growth and metabolic activities. During storage, several microbes including bacteria, nematodes, fungi etc damage seeds by production of extra cellular enzymes and secondary metabolites like toxins. So study of such fungi were carried out and their percent incidence were observed.

Keywords: Pulses, storage fungi, *Aspergillus* spp.

INTRODUCTION

In agriculture, pulses have great importance in Indian agriculture as they are rich source of protein (17 to 25 percent) as compared to cereals. Pulses have ability to fix atmospheric nitrogen and improve soil quality. Pulses are good sources of protein which is cheaper than other protein rich food like meat and fish.

In Marathwada region of Maharashtra State, pulses like Green gram (*Vigna radiate* (L.) Wilczek), Black gram or Urid Bean (*Vigna mungo* (L.) Hepper.), Chickpea or Gram (*Cicer arietinum* L.) and Pigeon pea or Tuver (*Cajanus cajan*(L.) Millsp., are mainly cultivated in both Rabi and Kharif seasons. The seeds of these crops are used for consumption as well as the residue is used as cattle feed.

It is reported in literatures that, during storage, several microbes including bacteria, nematodes, fungi etc damage seeds by production of extra cellular enzymes and secondary metabolites like toxins. The species of *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus* and *Alternaria* were found to be more commonly occurring as post harvest molds in storage condition. Out of which *Aspergillus* are dominant and play vital role in the seed biodeterioration.

The damage to pulse seeds caused by storage fungi due to their growth and metabolic activities can be categorized in 8 categories: 1) decrease in germinability, 2) fat acidity, 3) discolouration, 4) heating, 5) production of mycotoxins, 6) mustiness, 7) caking and 8) Total decay.

Enzymes (lipase) produced by seed born fungi were also involved in seed biodeterioration. The species of *Aspergillus* are reported to release secondary metabolites such as mycotoxins, that makes the product unsafe for human and animal consumptions. Mainly *Aspergillus parasiticus* and *A. flavus* are reported to produce aflatoxin which are carcinogenic in nature. Considering the importance of the fact present research work was carried out.

MATERIAL METHODS**Collection of pulse seed samples**

Different pulse seeds samples were collected by the method described by Neergaard (1973). Different pulse seed varieties were collected from fields, store houses, market places, research institutions and seed companies. For different variety of pulse seed collection, different region of Maharashtra, i.e. Aurangabad, Jalna, Beed, Latur, Osmanabad, Parbhani, Nanded etc. were explored. All the seed samples of different variety were sorted according to different abnormal category, healthy seeds and stored in cloth bags, plastic bags in laboratory conditions at room temperature during the studies.

Detection of seed mycoflora

The standard methods recommended by International Seed Testing Association (ISTA 1996); Neergaard (1973) and Agarwal (1976) were followed for the isolation of seed mycoflora, moist blotter method (SBM) and Agar plate method (APM) were followed.

a) Standard blotter method (SBM)

White blotter papers of 8.5cm diameter was soaked in sterile distilled water and placed in pre-sterilized petriplates of 10cm diameter. Ten pulse seeds of each variety and abnormal category were placed at equal distance on the moist blotters. The plates were incubated at 25±2°C under diurnal conditions for 7 days.

b) Agar plate method (APM)

In order to isolate internal mycoflora, seeds were pre-treated with 0.1% solution of mercuric chloride for two minutes and subsequently thoroughly washed thrice with sterile distilled water and placed on agar plates. Seeds without any such pre-treatment were employed for the total seed mycoflora.

Pre-sterilized glass petriplates of 10cm diameter were poured with 15ml of autoclaved medium. On cooling of medium, ten seeds per petriplates of the test sample were placed at equal distance aseptically. Incubation conditions and other details were same as described for the blotter method.

MEDIA USED IN ISOLATION

I) Potato Dextrose Agar (PDA)

II) Czapek Dox Agar (CZA)

III) Martin's Rose Bengal Agar (RBA)

IDENTIFICATION OF SEED-BORNE FUNGI

The fungi isolated from different pulse seeds, were identified preliminary on the basis of sporulation characters like sexual or asexual spores with the help of stereoscopic binocular microscope. The identification and further confirmation of seed-borne fungi was made by preparing slides of the fungal growth and observing them under compound microscope. The identification was made with the help of manuals. Pure cultures of these fungi were prepared and maintained on potato dextrose agar (PDA) slants.

EXPERIMENTAL RESULTS**1) Incidence of *Aspergillus species* on different pulse seeds**

To study the incidence of different *Aspergillus* species associated with commonly grown varieties of different pulse seeds like Green gram, Black gram, Chickpea and Pigeon pea were collected from different regions of Marathwada of Maharashtra state. These pulse seed were then surface sterilized and inoculated on different media and incubated at 30 °C for 7 days.

As the results observed in the table.1 total ten *Aspergillus* species were isolated. The fungi like *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. niger*, *A. terreus*, *A. ustus* and *A. versicolor* showed their incidence on all selected pulse seeds. *A. oryzae* and *A. parasiticus* were found to be totally absent on Black gram seeds, similarly *A. nidulance* on Chickpea and *A. oryzae* on Pigeon pea were found to be absent. It is clear from the table 1 that the percent incidence of *A. flavus* and *A. niger* were more dominated on all selected five pulse seeds as compare to other isolated fungi.

2. Percent incidence of *Aspergillus* on different media

It is clear from the result observed in table 3 that, blotter paper method yield less *Aspergillus species* from pulse seeds than that of agar plate method. Totally six *Aspergillus* species viz. *Aspergillus flavus*, *A. fumigatus*, *A. nidulance*, *A. niger*, *A. terreus* and *A. ustus* were isolated on blotter paper. The *Aspergillus* species like *A. flavus*, *A. fumigatus*, *A. niger* and *A. ustus* were found to be dominant on blotter paper as compare to other *Aspergillus* species.

It was interesting to note that the fungi like *A. glaucus*, *A. oryzae*, *A. parasiticus* and *A. versicolor* were totally absent on blotter paper but they were recovered from agar plate method. PDA was found to more favorable for isolation of fungi as compared to other media.

On PDA and RBA medium, ten *Aspergillus* species viz. *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. nidulans*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. terreus*, *A. ustus* and *A. versicolor* were isolated from Green gram. On the other hand, seven *Aspergillus* species were isolated viz. *A. flavus*, *A. glaucus*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. ustus* and *A. versicolor* from Green gram on GNA media.

In case of Black gram, eight *Aspergillus* species i.e. *Aspergillus flavus*, *A. fumigatus*, *A. glaucus*, *A. niger*, *A. terreus*, *A. ustus*, *A. versicolor* were isolated on PDA and seven species viz. *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. niger*, *A. terreus*, *A. ustus* and *A. versicolor* were isolated on RBA. On GNA only five *Aspergillus* species were isolated namely *A. flavus*, *A. niger*, *A. ustus* and *A. versicolor*.

Eight species of *Aspergillus* were found to be isolated on Chickpea on PDA namely *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. terreus*, *A. ustus* and *A. versicolor* where as on RBA number of species isolated to six viz. *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. niger*, *A. terreus* and *A. versicolor*. Number of *Aspergillus* species isolated on Chickpea on GNA found to be reduced to five namely *A. flavus*, *A. fumigatus*, *A. niger*, *A. parasiticus* and *A. ustus*.

In case of Pigeon pea, eight *Aspergillus* species were i.e. *Aspergillus flavus*, *A. fumigatus*, *A. glaucus*, *A. nidulans*, *A. niger*, *A. terreus*, *A. ustus* and *A. versicolor* were isolated on PDA, on RBA medium seven species viz. *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. nidulans*, *A. niger*, *A. terreus* and *A. ustus* where isolated and on GNA, only six *Aspergillus* species namely *A. flavus*, *A. fumigatus*, *A. niger*, *A. parasiticus*, *A. ustus* and *A. versicolor* were isolated.

Ten species of *Aspergillus* were isolated in case of Safflower on PDA i.e. *Aspergillus flavus*, *A. fumigatus*, *A. glaucus*, *A. nidulans*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. terreus*, *A. ustus* and *A. versicolor* and nine species on RBA viz. *Aspergillus flavus*, *A. fumigatus*, *A. glaucus*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. terreus*, *A. ustus* and *A. versicolor* where as on GNA only six species *Aspergillus* species i.e. *A. flavus*, *A. fumigatus*, *A. niger*, *A. parasiticus*, *A. terreus* and *A. versicolor*.

3. Percent incidence of *Aspergillus* species on abnormal pulse seeds:

To study the relationship between seed abnormality and associated fungi, all the collected pulse seeds were categorized in four types of abnormalities viz. Shrunken (Sh), Undersized (Us), Discolored (Dc) and Cracked (Cr). These seeds were plated separately on agar media and results were recorded in table 2.

It is clear from the result in the table no.2, that the undersized and discolored seeds yield maximum number of *Aspergillus* species. The percent incidence of *A. flavus*, *A. fumigatus*, *A. oryzae* and *A. terreus* was found to be maximum on discolored seeds i.e. 41.90%, 4.47%, 5.11 and 5.81% respectively. Similarly, *A. glaucus* (4.37%), *A. nidulans* (4.56%) and *A. versicolor* (7.75%) shows maximum incidence on undersized seeds. On the other hand *A. niger* (49.5), *A. parasiticus* (6.25) and *A. ustus* (3.81) showed maximum incidence on cracked seeds.

It was interesting to observe that *A. oryzae* on cracked seeds and *Aspergillus parasiticus* on shrunken seeds were totally absent in all the varieties of pulse seeds.

Table-1: Incidence of *Aspergillus* species on different pulse seeds

<i>Aspergillus</i> species	Pulse seeds (% incidence)			
	Green gram	Black gram	Chickpea	Pigeon pea
<i>A. flavus</i>	31.5	16.0	44.0	27.0
<i>A. fumigates</i>	3.0	1.0	3.5	3.0
<i>A. glaucus</i>	1.5	1.5	0.5	1.0
<i>A. nidulance</i>	0.5	0.5	-	1.0
<i>A. niger</i>	38.0	12.0	38.5	35.0
<i>A. oryzae</i>	5.0	-	0.5	-
<i>A. parasiticus</i>	0.5	-	0.5	2.0
<i>A. terreus</i>	0.5	5.5	1.0	3.0
<i>A. ustus</i>	4.5	2.0	1.0	2.5
<i>A. versicolor</i>	2.5	1.5	3.0	1.0

Table-2: Incidence of *Aspergillus* species on abnormal pulse seeds

<i>Aspergillus</i> species	Pulse seeds (% incidence)			
	Sh	Us	Dc	Cr
<i>A. flavus</i>	27.78	18.84	41.90	39.00
<i>A. fumigates</i>	04.15	03.25	04.47	03.18
<i>A. glaucus</i>	00.87	04.37	01.59	01.33
<i>A. nidulance</i>	00.31	04.56	00.95	02.75
<i>A. niger</i>	29.37	24.62	39.88	49.5
<i>A. oryzae</i>	01.87	01.25	05.11	-
<i>A. parasiticus</i>	-	00.53	00.52	06.25
<i>A. terreus</i>	03.993	01.62	05.81	00.31
<i>A. ustus</i>	02.84	03.18	03.56	03.81
<i>A. versicolor</i>	01.96	07.75	02.29	03.75

Sh = Shrunken, Us = Under sized, Dc = Discoloured, Cr = cracked

Table-3: Incidence of *Aspergillus* species on different media (% incidence)

<i>Aspergillus</i> species	PDA				RBA				GNA				Blotter			
	Gg	Bg	Cp	Pp	Gg	Bg	Cp	Pp	Gg	Bg	Cp	Pp	Gg	Bg	Cp	Pp
A. 1	32.0	10.0	51.0	30.0	31.0	22.0	37.0	25.0	25.0	25.0	30.0	10.5	40.5	30.5	55.5	45.0
A.2	3.0	01.0	3.0	3.0	3.0	1.0	3.5	2.5	-	-	10.0	20.5	10.5	70.5	10.0	80.5
A.3	1.5	1.5	-	0.5	1.0	1.5	0.5	1.5	0.5	-	-	-	-	-	-	-
A.4	0.5	0.5	-	1.0	0.5	-	-	1.0	-	-	-	-	1.0	1.0	-	-
A.5	39.0	11.0	25.5	38.0	38.0	13.0	51.0	32.0	10.0	10.5	25.5	30.5	60.5	20.0	60.0	56.0
A.6	4.5	-	0.5	-	6.0	-	-	-	20.5	-	-	-	-	-	-	-
A.7	0.5	-	1.0	-	0.5	-	-	-	1.0	-	10.5	0.5	-	-	-	-
A.8	0.5	2.5	1.0	3.5	0.5	8.0	1.0	2.5	-	-	-	-	2.0	-	-	10.0
A.9	5.0	0.5	2.0	3.0	4.0	3.0	-	2.0	15.0	1.5	1.5	0.5	20.0	4.5	2.5	10.0
A.10	3.0	0.5	4.0	1.0	2.5	3.0	2.0	-	10.0	1.0	-	1.0	-	-	-	-

PDA= Potato Dextrose agar, RBA= Rose Bengal agar, GNA= Glucose nitrate agar, Gg= Green gram, Bg= Black gram, Cp= Chickpea, Pp= Pigeon pea.

A.1= *A. flavus*, A.2= *A. fumigatus*, A.3= *A. glaucus*, A.4= *A. nidulans*, A.5= *A. niger*, A.6= *A. oryzae*, A.7= *A. parasiticus*, A.8= *A. terreus*, A.9= *A. ustus*, A.10= *A. versicolor*,

CONCLUSION

Ten dominant *Aspergillus* species like *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. nidulans*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. terreus*, *A. ustus* and *A. versicolor* were isolated from pulse seeds.

Blotter paper method yields less *Aspergillus* species from pulse seeds as compared to Agar plate method. On PDA medium maximum *Aspergillus* species were isolated as compared to RBA and GNA media.

Eight species of *Aspergillus* species i.e. *A. flavus*, *A. fumigatus*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. terreus*, *A. ustus* and *A. versicolor* were isolated on PDA medium from Chickpea seeds. Whereas on RBA only six *Aspergillus* species viz. *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. niger*, *A. terreus* and *A. versicolor* from Chickpea seeds.

Only five *Aspergillus* species viz. *A. flavus*, *A. fumigatus*, *A. niger*, *A. parasiticus* and *A. ustus* were isolated on GNA medium from Chickpea seeds.

In Black gram, eight *Aspergillus* species viz. *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. nidulans*, *A. niger*, *A. terreus*, *A. ustus* and *A. versicolor* were isolated on PDA. Where as on RBA and GNA media *Aspergillus* species number was reduced.

From Pigeon pea seeds, ten *Aspergillus* species viz. like *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. nidulans*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. terreus*, *A. ustus* and *A. versicolor* were isolated on PDA.

Among seed abnormalities, discolored and undersized seeds showed maximum association of *Aspergillus* species qualitatively and quantitatively.

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