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ISOLATION METHODS FOR *ASPERGILLUS S*PECIES OCCURRING ON PLANT SEEDS

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ABSTRACT

The aim of the present work is to study the effect of various methods on isolation of*Aspergillus* species associated with plant seeds. The blotter methods, potato dextrose agar method and water agar methods suggested by International Seed Testing Association (ISTA) were used in isolation of the seed borne *Aspergillus* species from plant seeds.

The *Aspergillus* species were isolated by blotter method, potato dextrose agar method and water agar method. *Aspergillus* species were identified on the basis of morphological characters. It has clearly indicated that different fungi along *Aspergillus* species were associated with plant seeds under different condition. It is required to identify *Aspergillus* species and suggestion of promising treatments for fungal biodeterioration and disease management strategy. Further, this investigation helps to control other plant seed diseases effectively. Blotter method proved the best method to detect *Aspergillus* species in biodeterioration process than any other methods. However, the lowest fungal infestation was noted on water agar method.

Key Words: *Aspergillus* species, Plant seeds, Moist blotter plate method, Potato Dextrose Agar plate method, Water agar plate method

INTRODUCTION

Plant seeds are subjected to various operations of contamination by fungal species during growth, after harvesting and during storing. Fungi are the major cause of deterioration and spoilage of seeds and food grains. They attack food and feed crops both in field and after harvest in storage. Hence, they have been found to be active biodeteriorats under a given set of environmental conditions. Many *Aspergillus* species on the seeds showed their association with the other moulds like species of *Alternaria, Dreschleria, Fusarium, Curvularia* and *Cladosporium* while, poor incidence of *Aspergillus* was noted on the seeds which showed of *Rhizoctonia, Syncephalostrum, Penicillum, Trichoderma* and Chaetomium.

Among such mould the species of *Aspergillus* have been reported by different workers on majority of crop seeds.*Aspergillus* plays an important role in their enzymatic nature and decolourization in the process of biodeterioration.

The studies regarding the composition of seed mycoflora of maize carried out by various author. Bilgrami and Choudhary (1990) reported *A. niger, A. sydowi, A. candies* and *A. ochraceus*. Arvinder and Rai (1991) recorded *A. flavus, A. niger, A. fumigatus, A. terreus* and *A. sydowi* on

maize seeds collected from different tribal areas in India.*Aspergillus niger, Aspergillus flavus, Fusarium oxysporum and Botryodiplodiatheobromae* were isolated from the bean (*Phaseolus vulgaris* L.) by Kator et. al., (2016).

MATERIALS AND METHODS

1) Collection of Seed Samples

The method described by Neergaard (1973) has been adopted for the collection of seed samples. Accordingly seed samples were collected from field, store houses and market places and from farmers. A composite sample was prepared by mixing the individual sample together, preserved in cloth bags at room temperature during the studies.

II) Detection and Identification of Seed Borne Fungi from Stored Plant Seeds by Blotter Plate Method

A pair of white blotter paper of 8.5 cm diameter was jointly soaked in sterile distilled water, placed in pre-sterilized corning Petriplates of 10 cm diameter. Ten seeds per plate were placed at equal distance on the moist blotters. One hundred seeds were tested for each treatment. The plates were incubated at 25 ± 2^0C under diurnal condition. On 7^{th} day the seeds were examined under stereoscopic microscope for the preliminary determination of *Aspergillus.* Identification and further fungi occurred on seeds was made by preparing slides of the fungal growth and observing under compound microscope.

III) Detection and Identification of Seed Borne Fungi from Stored Plant Seeds by Agar Plate Method

In this method, pre-sterilized corning glass Petri-plates of 10 cm diameter were poured with 25 ml of autoclaved water Agar (WA) medium. On cooling the medium, 10 seeds per plate were equispaced aseptically. One hundred seeds were tested for each treatment. The plates were incubated at 25 ± 2^0C under diurnal condition. On 7^{th} day the seeds were examined under stereoscopic microscope for the preliminary determination of *Aspergillus.* Identification and further fungi occurred on seeds was made by preparing slides of the fungal growth and observing under compound microscope.

IV) Detection and Identification of Seed Borne Fungi from Stored Plant Seeds by Potato Dextrose Agar Method

In this method, pre-sterilized corning glass Petri-plates of 10 cm diameter were poured with 25 ml of autoclaved Potato Dextrose Agar (PDA) medium. On cooling the medium, 10 seeds per plate were equispaced aseptically; incubation condition.

One hundred seeds were tested for each treatment. The plates were incubated at 25 ± 2^0C under diurnal condition. On 7^{th} day the seeds were examined under stereoscopic microscope for the preliminary determination of *Aspergillus.* Identification and further fungi occurred on seeds was made by preparing slides of the fungal growth and observing under compound microscope.

In order to isolate only internal seed mycoflora, seeds were pre-sterilized with 0.1% solution of mercuric chloride for 1 minute. Subsequently, thoroughly washed twice with sterile distilled water and placed on agar plates, blotter plate and water agar plates. Seeds without any such pre-treatments were employed for the total seed mycoflora (control). For blotter method, potato

dextrose agar method and water agar methods was followed as described by International Seed Testing Association, ISTA (1966) De Tempe (1970), Neergaard (1973) and Agarwal (1976).

RESULTS

Table 1: Effect of Different Methods on Isolation of *Aspergillus* species

Sr. No.	*Aspergillus* Species	Jowar (CSH-1)			Maize (Ganga)		
		Blotter	Water Agar	PDA	Blotter	Water Agar	PDA
% Incidence							
1	*A. flavus*	40	20	30	30	-	30
2	*A. niger*	30	10	20	10	10	30
3	*A. fumigatus*	20	-	20	-	-	20
4	*A. terreus*	30	10	-	30	10	-
5	*A. ruber*	-	-	-	10	-	20
6	*A. glaucus*	-	-	-	-	-	20
7	*A. sulphureus*	-	-	-	-	-	-
8	*A. japonicus*	-	-	-	-	-	-
9	*A. sydowi*	5	-	20	10	-	20
10	*A. versicolor*	20	20	20	-	-	-
11	*A. nidulans*	-	-	-	-	-	-
12	*A. carbonarius*	-	-	-	-	-	-
13	*A. chevalieri*	-	-	-	-	-	-
14	*A. ornatus*	30	10	20	30	10	-

Table 2: Effect of Different Methods on Isolation of *Aspergillus* species

Sr. No.	*Aspergillus* Species	Green Gram (PB)			Black Gram (Chaffa)		
		Blotter	Water Agar	PDA	Blotter	Water Agar	PDA
% Incidence							
1	*A. flavus*	20	20	10	30	-	30
2	*A. niger*	10	10	20	10	10	30
3	*A. fumigatus*	10	-	20	-	-	20
4	*A. terreus*	40	-	-	30	10	-
5	*A. ruber*	-	-	-	30	10	-
6	*A. glaucus*	-	-	-	-	-	20
7	*A. sulphureus*	-	-	-	-	-	-
8	*A. japonicus*	-	-	20			
9	*A. sydowi*	5	-	20	10	-	20
10	*A. versicolor*	20	20	20	-	-	-
11	*A. nidulans*	-	-	-	-	-	-
12	*A. carbonarius*	-	-	-	-	-	-
13	*A. chevalieri*	-	-	-	-	-	-
14	*A. ornatus*	30	10	20	30	10	-

Table 3: Effect of Different Methods on Isolation of *Aspergillus* Species

Sr. No	*Aspergillus* Species	Safflower (Tara)			Groundnut (SB-11)		
		Blotter	Water Agar	PDA	Blotter	Water Agar	PDA
1	*A. flavus*	20	20	30	40	20	20
2	*A. niger*	30	10	30	30	10	20
3	*A. fumigatus*	20	-	20	10	10	30
4	*A. terreus*	30	-	-	-	-	-
5	*A. ruber*	-	-	-	20	-	10
6	*A. glaucus*	-	-	-	-	-	-
7	*A. sulphureus*	-	-	-	-	-	-
8	*A. japonicus*	-	-	-	-	-	20
9	*A. sydowi*	-	-	-	-	-	-
10	*A. versicolor*	20	-	10	10	-	20
11	*A. nidulans*	-	-	-	-	-	-
12	*A. carbonarius*	-	-	-	-	-	-
13	A. *chevalieri*	-	-	-	10	-	20
14	*A. ornatus*	-	-	-	40	10	30

DISCUSSION

In order to isolate species of *Aspergillus* the three methods (moist blotter, PDA method and water agar method) were employed for plating the seeds of jowar, maize, green gram, gram, safflower and groundnut. Results are summarised in Table (1, 2 &3) and Graph (1, 2, 3, 4, 5 & 6).

It is clear from the results that the seeds of above crops yielded totally 14 species of *Aspergillus* (Plate-1, 2, 3 & 4).

Table 1 shows the results that water agar shows presence of the *A. flavus*and *A. versicolor* only on jowar whereas,*A. niger, A. terreus and A. ornatus* isolated from both jowar and maize. Potato Dextrose Agar plate method (PDA) also recorded the maximum number of *Aspergillus* species on jowar and maize. *A. terreus* and *A. chevalieri* could get only by blotter methods, while it was completely absent in all the crops on water agar media. However, in case of jowar, maize and gram only *A. terreus* could grow on water agar and blotter paper. PDA was found to be useful for appearance of some new species like *A. japonicus* and *A. versicolor* in maize, *A. glaucus* and *A, fumigatus* in green gram and *A. versicolor* in groundnut. Similar, results were recorded by (Panchal and Dhale 2011) that PDA method was found to be favorable for the maximum counts of saprophytic fungi and also for detection of some specific fungi.

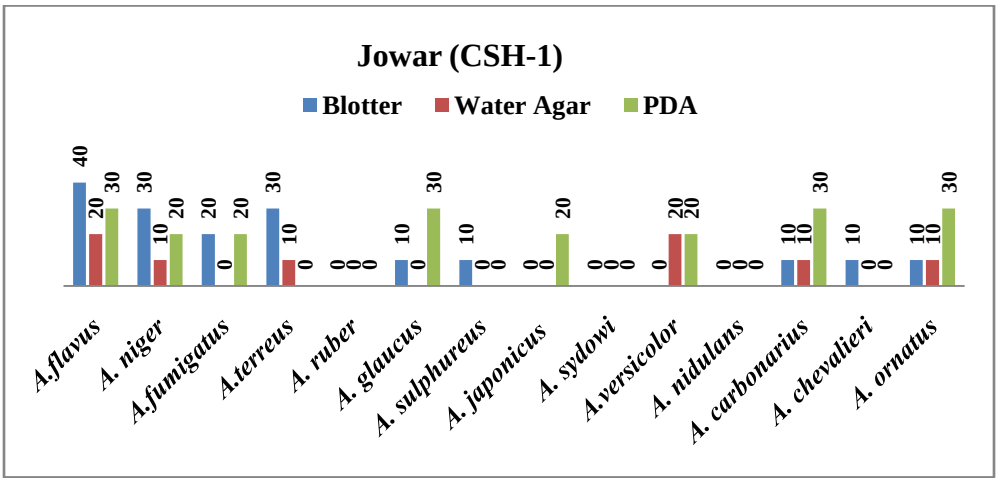
It was interesting to note that blotter method supported for appearance of more species of *Aspergillus* than the PDA method. *A. ruber, A. sydowi* and *A. nidulans* are shown their presence on both seeds of jowar and maize seen in case of *A. glaucus, A. carbonarius, A. ornatus* from jowar seeds, *A. glaucus, A. ornatus and A. ruber* from maize, *A. sydowi, A. ruber* from gram and

A. chevalieri A. fumigatus, A. versicolor from groundnut. Present results are also supported by (Alemu and Nega 2014) different fungal species was isolated and identified both in moist blotter plate and potato dextrose agar plate method. It can be seen that the number of identified fungus genus and species was close to each other for both methods (Dinler and Günay 2018).

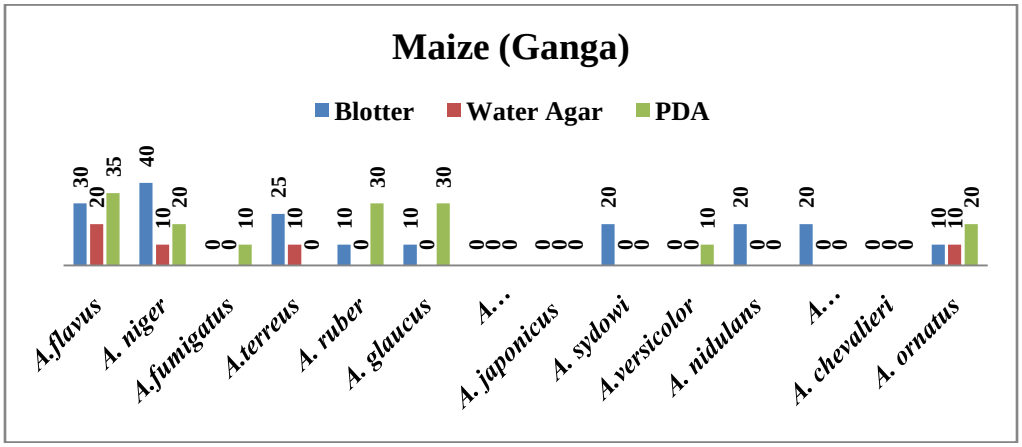
Results in Table 2, shows presence of *A. flavus, A. niger, A. versicolor* and *A. ornatus* on green gram isolated by water ager plate methods. Whereas, black gram shows the presence of *A. niger, A. ruber, A. terreus* and *A. ornatus* on water agar plate. Moist blotter paper plate method supports the growth of *A. terreus, A. niger, A. fumigatus, A. terreus, A. sydowi, A. versicolor* and *A. ornatus* on green gram while as *A. flavus, A. niger, A. terreus, A. ruber, A. sydowi*and *A. ornatus* were found on black gram. Potato Dextrose Agar plate methods shows the presence of *A. flavus, A. niger, A. fumigatus, A. japonicus, A. sydowi, A. versicolor* and*A. terreus* on green gram whereas, blackgram shows the presence of *A. flavus, A. niger, A. fumigatus, A. glaucus* and *A. sydowi*.

Results in Table 3, explains that on safflower and groundnut *A. flavus* and *A. niger*are reported whereas, *A. fumigatus* and *A. ornatus* only on groundnut by water agar plate method. Moist blotter plate method shows the presence of *A. flavus, A. niger, A. terreus, A. ruber* and*A. versicolor*on safflower whereas, groundnut reported *A. flavus, A. niger, A. fumigatus, A. ruber A. versicolor, A. chevalieri* and *A. ornatus.*Potato dextrose agar plate method isolated the presence of *A. flavus, A. niger*and *A. fumigatus*both on safflower and groundnut. *A. terreus* and *A. versicolor were reported* on safflower. *A. ruber, A. japonicus, A. versicolor, A. chevalieri* and *A. ornatus* were isolated from groundnut by potato dextrose agar plate method.

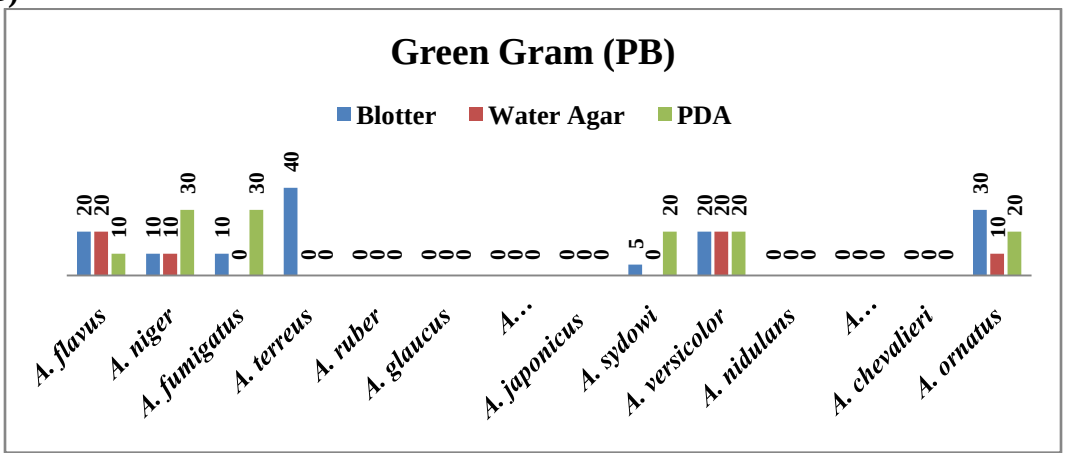
Graph 1: Effect of Different Methods on Isolation of *Aspergillus* Species from Jowar (CSH-1)



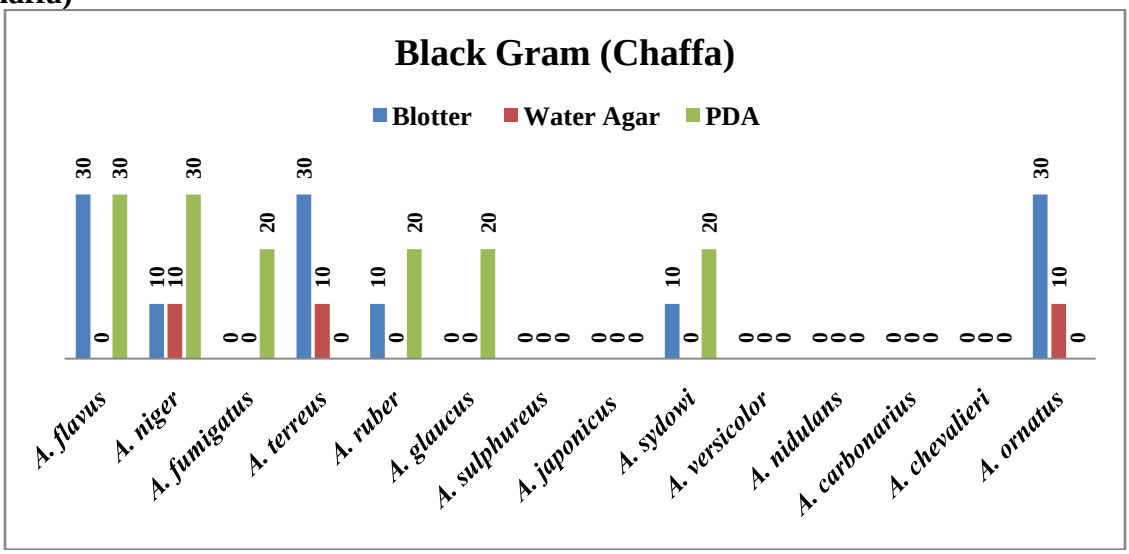
Graph 2: Effect of Different Methods on Isolation of *Aspergillus* Species from Maize (Ganga)



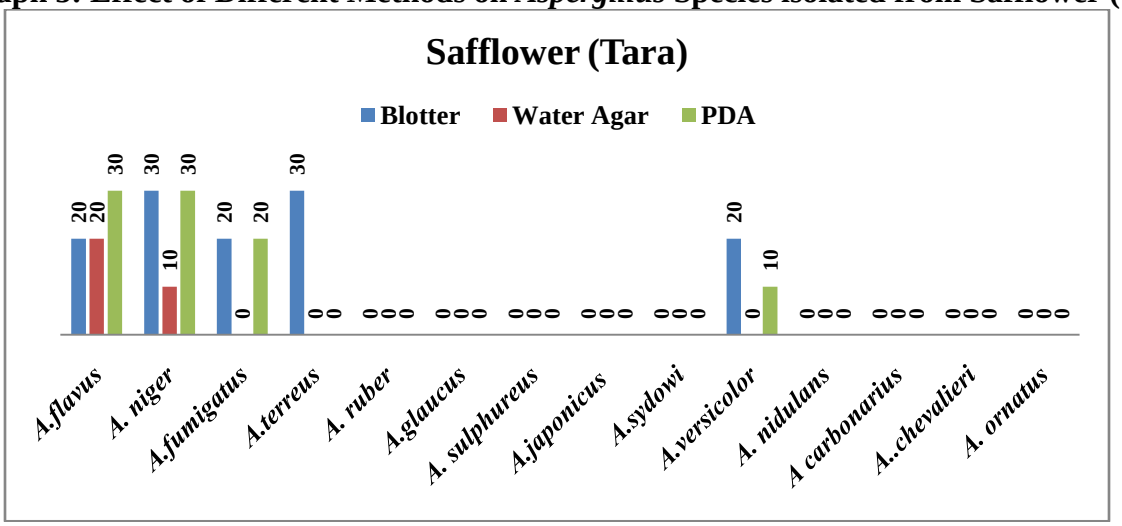
Graph 3: Effect of Different Methods on Isolation of *Aspergillus* Species from Green gram (PB)



Graph 4: Effect of Different Methods on Isolation of *Aspergillus* Species from Black Gram (Chaffa)



Graph 5: Effect of Different Methods on *Aspergillus* Species isolated from Safflower (Tara)



Graph 6: Effect Different Methods on *Aspergillus* Species isolated from Groundnut (SB-11)

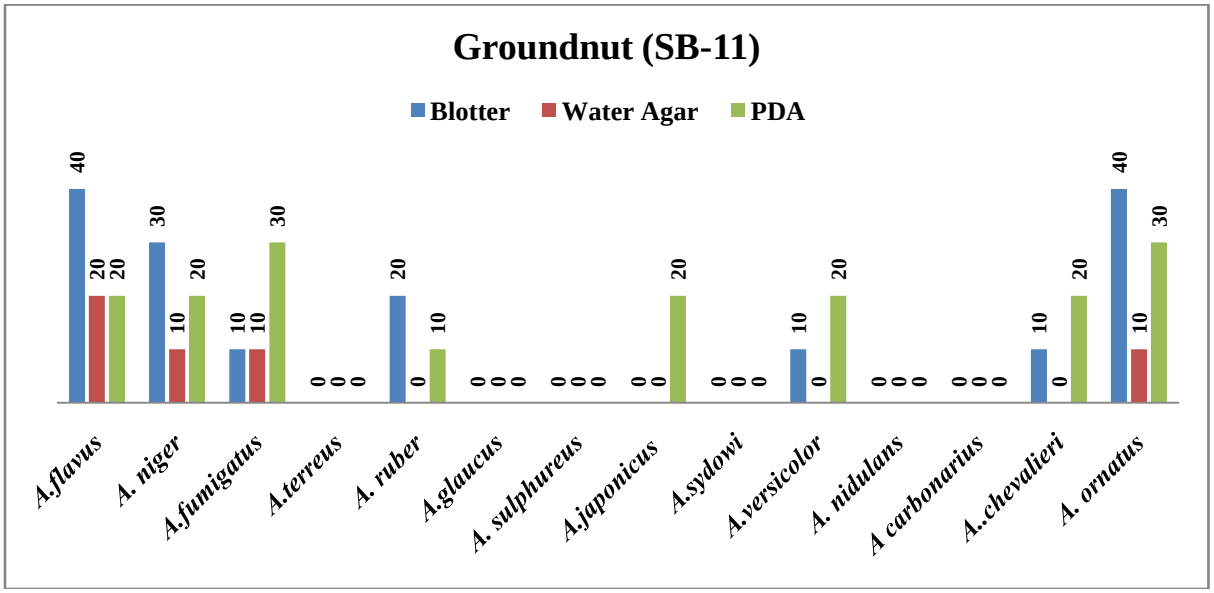


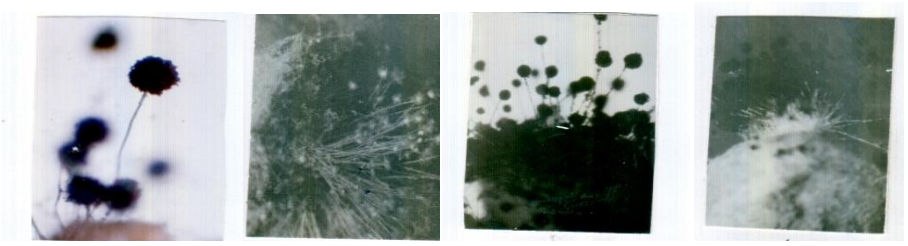
Figure 7: ***Growth of Aspergillus Species Occurring on Moist Blotter Paper***

Photoplate-1 ***Photoplate-2***

Figure 8: ***Growth of Aspergillus Species Occurring on Agar Medium***

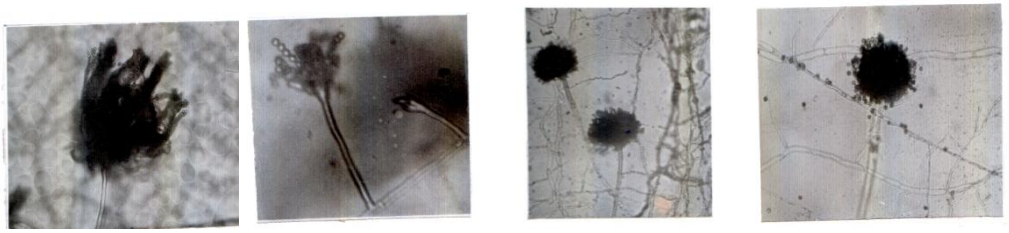
Photoplate-1 ***Photoplate-2***

Figure 9: ***Micrographs ofGrowth of A. niger on different crop seeds 1. Maize 2. Groundnut 3. Gram 4. Tur***



Photoplate-1 ***Photoplate-2*** ***Photoplate-3*** ***Photoplate-4***

Figure 10: Micrographs of sporulating structure in different species of *Aspergillus* *1.A. chevalieri 2. A. terreus 3.A. flavus 4. A. niger*



Photoplate-1 ***Photoplate-2*** ***Photoplate-3*** ***Photoplate-4***

During isolation studies it has been observed that blotter method supported to grow more species of *Aspergillus* with minimum contaminations while, in agar plates there was always crowding species of *Alternaria, Curvularia, Cladosporium, Helminthosporium, Fusarium*. The blotter method yielded higher no of fungi as compare to agar plate (Singh et al 2011; Shrivastava et al 2011).The members of phycomycetes which ultimately made difficult to isolate *Aspergillus* in pure forms by agar plate method. Hence it can be concluded that the blotter method proved to be superior over agar plates for isolation of maximum number of *Aspergillus* species.

CONCLUSION

From the above results and discussion it has clearly indicated that different fungi were associated with plant seeds under different condition, especially *Aspergillus* species. Hence, it is concluded that water agar shows presence of the *A. flavus* on jowar where *A. niger* isolated from both jowar and maize. PDA also recorded the maximum number of *Aspergillus* species on jowar and maize. The result of present study shows the most common *Aspergillus* species are found on agar plate than blotter and water agar method.*Aspergillus* species may survive on different substrates and environmental conditions and their complete elimination is difficult. However, the use of good hygiene practices and good storage management can minimize mycoflora association with plant seeds. To manage not only fungal biodeterioration but also other plant seed diseases and also further, investigation of storage fungi can be done appropriately by above method. Blotter plate method proved the best method to make a detail survey of biodeteriorating fungi especially *Aspergillus* species than any other methods.

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REFERENCES

1. Agarwal, V. K. (1976). Seed-borne fungi and viruses of some important crops. Research Bulletin 108 G. B. Pant University of Agricul.& Technol, Pantnagar.

2. Al-Askar, A. A., Ghonem K.M., Rashad Y. M., Abdulkhair W.M., Hafez E.E., Shabana Y. M. &Baka Z. A. (2014). Occurrence and Distribution of Tomato Seed-borne Mycoflora İn Saudi Arabia and İts Correlation with the Climatic Variables.Microbial Biotechnol. 7(6), 556–569.

3. AlemuNega (2014). Isolation and Identification of Fungal Pathogens Associated with Cold Storage Type of (Coffee Arabica.)Seed, at Jimma.Journal of Biol., Agriculture and Healthcare, Vol.4, No.25, 20-26.

4. Arvinder, Rai and Rai,B.K. (1991). Seed mycoflora of Zea mays in tribal area.Indian Phytopathology, Vol. 44, pp.526.

5. Bilgrami, K. S and A. K. Choudhary (1990). Effect of climatological factors on the incidence to co-existing mycoflora on maize kernels. Indian Phytopathology, Vol. 44: 527-529.

6. De, Tempe, J. (1970). Testing cereal seeds for Fusarium infection in the Netherlands Pro.Int. Seed Test. Ass.35: 193-206.

7. Dinler,Havvaand GünayMerve (2018). Determination of Fungal Agents in Some Vegetables Seeds in Greenhouse Production Areas in Uşak Province. International Journal of Agriculture and Forestry, 8 (2): 83-91.

8. ISTA (1966). International Seed Testing Association.Rules for seed health testing. 31: 1-152.

9. Kator L., Aondo, T. O., Akinyemi, B. K.(2016). Isolation and Identification of Seed Borne Fungi of Common Bean (Phaseolus vulgaris L.) from Selected Markets in Makurdi.International Journal of Applied Agricultural Sciences, Volume 2, Issue 5, September Pages: 75-78

10. Mathur, S.B. and Kongsdal, O. (2003). Common Laboratory Seed Health Testing Methods for Detecting Fungi, 1st edn. Bassersdorf, Switzerland: International Seed Testing Association.

11. Neergaard, P. (1973). Deterioration of seed borne pathogens by culture tests. Seed Science & Technology, 1: 217-254.

12. Panchal, V. H. and Dhale D. A. (2011). Isolation of seed-borne fungi of sorghum (Sorghum vulgarepers.). Journal of Phytology, 3 (12): 45-48

13. Singh, J., Srivastva, S.,Sinha,A.and Bose,B.(2011. Studies on Seed Mycoflora of Wheat (Triticum vulgare L.) treated with Potassium Nitrate & its Effect on Germination During Storage. Research Journal of Seed Sciences, Vol 4, Issue 3, 148-56.

14. Srivastava, S., Sinha,A. and Srivastava,C.P. (2011). Screening of Seed-borne Mycoflora of Jatropha curcas L. Research Journal of Seed Science, 4: 94-105.