Int. Journal of Universal Science and Technology
ISSN: 2454-7263, Volume: 05 No: 03 Published: Jan., 2019
Paper ID: IJUP3126, Web: www.universalprint.org
Title Key: Occurrence and Control of Seed borne…

Occurrence and control of seed borne pathogenic fungi of black gram (Vigna mungo L.)

[*1]Warkhade B.B., [2]Bhutekar S.S, [3]Ghuge G. K., [4]Gujar R.K.

[*1,3,4]Department of Biotechnology, Model College Ghansawangi.Dist.Jalna.Maharashtra, India, 431209.
[2]Department of Biochemistry, Model College Ghansawangi.Dist.Jalna.Maharashtra, India, 431209.
*corresponding author:warkhade.bala@gmail.com

Abstract
Seed-borne pathogens causes enormous losses to crop in the world as well as in India. The presence of pathogenic propagules in a seed lot is pivotal because infected seed may fail to germinate, causes infection to seedlings and growing plants. In Present study fungi associated with seed of black gram was investigated andfour species were isolated by two different methods i.e. Blotter method and agar plate method. Pathogenic fungi frequently isolated were *Fusarium sp., Alternaria sp*. *Macrophomina sp., Aspergillus sp.*40% to 50% of fungi isolated was of *Fusarium* and hence control of this fungus was performed by using *Catharanthusroseus* plant extracts in different concentration. It was observed that 15% concentration of extract shows 100% inhibition of *fusarium*.
Keywords: Black Gram,BlotterMethod, Agar plate Method, Pathogenic Fungi, Plant Extract

Introduction
Fungi are significant demolishers of grains during storage, rendering them unfit for human consumption by hindering their nutritive value and often by producing mycotoxins (Marin *et al.*, 1999; Janardhana*et al.*, 1998).Grain legumes are attacked by a wide range of diseases many of which are seed-borne. Black gram (Vigna mungo (L.) Hepper) is one of the most important legumes of the arid and semiarid tropics (Chen et al., 1987) and source of easily digestible protein. Several factors are responsible for low production of Blackgram. Among them, diseases play an important role (Nine, 1980; Pal, 1996). Many fungal pathogens, some of which are seed transmitted, often reduce the germination ability or kill the infected plants or substantially reduce the productive capacity. These pathogens also causepost-emergence damping off, death of emerging radicle, and discoloration of roots, hypocotyles and cotyledons.Seed health testing methods like blotter paper method, deep freeze blotter, 2, 4 - D blotter paper method and agar plate methods have been employed for detection of internal and external seed borne mycoflora (Solanke RB *et al.*, 1997; Paul YS, 1989; Rajeswari B *et al.*, 2009). The main objective of this study was to isolate, and control seed borne fungi of blackgram.

Materials and Methods

Collection of samples
Samples were collected from three different industries i.e. Mahamandal seeds, Nirmal seeds and Ankur seeds. These three different samples were collected and stored in laboratory for further studies.

Int. Journal of Universal Science and Technology
ISSN: 2454-7263, Volume: 05 No: 03 Published: Jan., 2019
Paper ID: IJUP3126, Web: www.universalprint.org
Title Key: Occurrence and Control of Seed borne…

Isolationof Fungi
Two generalized isolation procedures were employed for the isolation of pathogenic fungi (Neergaard, 1977; Wan Zamun*et al.*, 1978). The two methods were the moist blotter and the potato dextrose agar (PDA) method.

Standard blotter method
The standard blotter method was developed by Doyer in 1938, which was later included in the International Seed TestingAssociation Rules of 1966.Hundred seed of each variety weretested by employing standard blotter method in 3 replications. Fourpieces of blotting paper of 90 mm size were moistened with distilledwater and placed in 90 mm sterilized Petri plates after drainingexcess water. Untreated seeds were placed at the rate of 25 seedsper Petri plate at equal distance. The plates were incubated at roomtemperature under alternate cycles of 12 hours NUV lightand darkness. After eight day of incubation the seeds wereexamined under stereoscopic –binocular microscope for theassociated fungi and they were identified based on habit and colonycharacters (Anonymous,1996)

Agar plate method.
In this method, pre-sterilized petri plates were pouredwith 15 ml of autoclave potato dextrose agar (PDA). On cooling themedium, the seeds per plate of the sample to be studied wereequidistantly placed aseptically. The plates were incubated at roomtemperature under alternate cycles of 12 hours NUV lightand darkness. After eight day of incubation the seeds wereexamined under stereoscopic –binocular microscope for theassociated fungi and they were identified based on "habit and colonycharacters

Identification of fungi
Pure cultures of individual fungal isolates were critically examined and identified. Fungi were identified were based on gross colony morphology and microscopic characters. Colony identification was based on colony characteristics such as color and the texture of mycelia and type of pigmentation. Microscopic characteristics of spores such as shape and color also used to identify the pathogens associated with the seeds.

Efficacy of selected *Catharanthusroseus*plant extract against*Fusarium*.

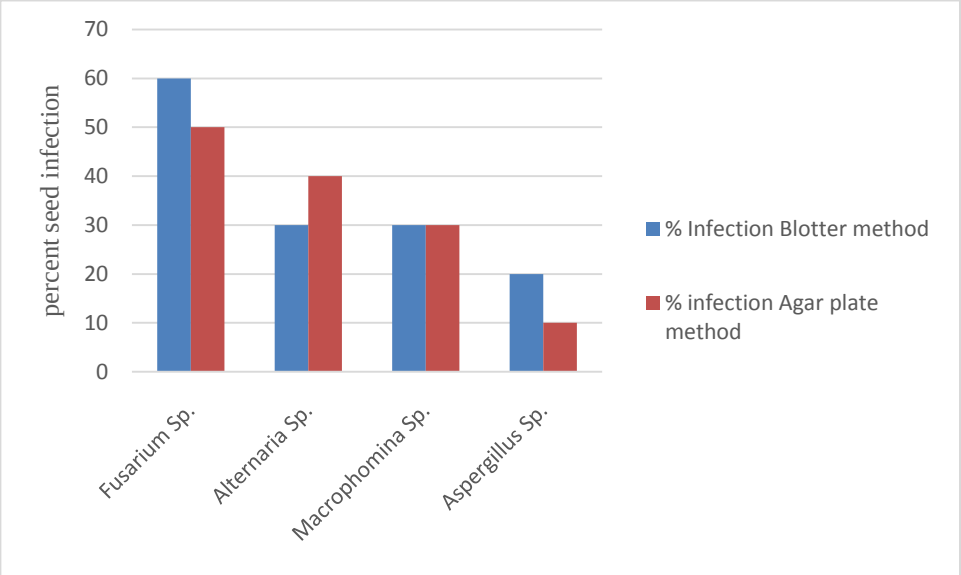
Preparation of plant extract and control
Catharanthusroseus plant extract was prepared in acetone. Fresh leaves of *Catharanthusroseus* were collected and washed with distilled water to remove surface dust. 10gm of leaves were chopped into fine pieces. These chopped leaves were added to 100ml of acetone and placed for overnight. Next day filtered and filtrate was used as plant extract and stored at 4^0C for further use.
Antifungalactivity of leaf extract was tested by poisoned food technique.Control of *Fusarium* was performed using different concentration agar plate method. Concentration used were 5%, 10%, and 15%, of extract. To this plate Loopful of *Fusarium* was placed and growth was observed after 3 days. Diameter of *Fusarium*colony was measured and compared to the control. Using this data,the percent inhibition of mycelial growth was calculated using the formula: -
%I = [(C-T)/C] x100).
I= percentage inhibition.

C=radial growth in control.
T=radial growth in the treatment.

Resultand Discussion

Significant differences in occurrence of seed mycoflora were observed and the results indicated that irrespective of the locations and sources, a total of 4 fungal species viz., *Fusarium sp.*, *Alternaria sp*. *Macrophomina sp.*, *Aspergillus sp*. were detected. Total percent incidence of seed mycoflora in Blotter method is 60%, 30%, 30%, 20% of *Fusarium sp.*, *Alternaria sp*. *Macrophomina sp.*, *Aspergillus sp.* respectively.Total per cent incidence of seed mycoflora in agar plate method is 50%, 40%, 30%, 10% of *Fusarium sp.*, *Alternaria sp*. *Macrophomina sp.*, *Aspergillussp.*respectively. (Graph.1)

Graph 1. Percent of seed infection by blotter method and agar plate method



Efficacy of selected *Catharanthusroseus* plant extract against*Fusarium.*

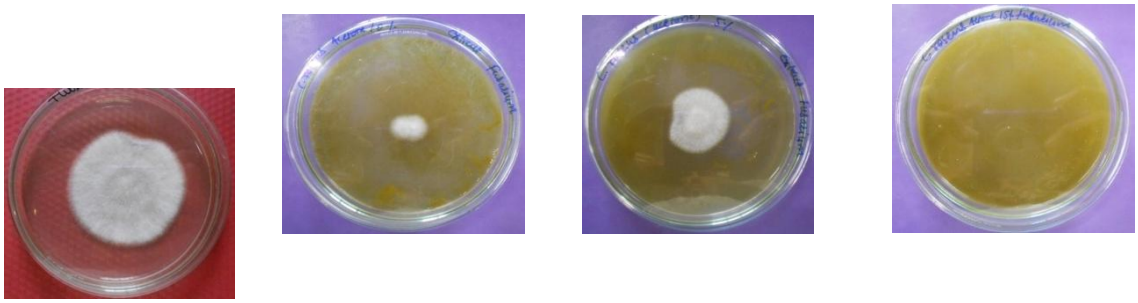
Catharanthusroseus plant acetone extract was used for control of the *Fusarium sp*. as this fungal genus observed high prevalence. Concentration used were 5%, 10%, and 15%, of *Catharanthusroseus* extract. The antifungal activity of the extract was enhanced by increase in the concentration of the extract. Extract concentration 15% was showing 100% inhibition of *Fusarium sp.* which was calculated by measuring mycelial growth after 3 days of incubation. (Table 2. Figure 1.)

Int. Journal of Universal Science and Technology
ISSN: 2454-7263, Volume: 05 No: 03 Published: Jan., 2019
Paper ID: IJUP3126, Web: www.universalprint.org
Title Key: Occurrence and Control of Seed borne…

Table 2. Percent inhibition of *Fusarium sp.*

Extract concentration	Fungal colony diameter after 3 days	% Inhibition
control	30mm	__
5%	20mm	33.33
10%	10mm	66.66
15%	00mm	100

Figure 1. Efficacy of selected *Catharanthusroseus* plant extract against*Fusarium sp.*



(A) ***(B)*** ***(C)*** ***(D)***

A: control, B: 5% extract, C: 10% Extract, D: 15% Extract

Mycoflora of seed varied from place to place due to change in conditions prevailing during seed development, harvesting and storage. The detection of seed-borne pathogenic fungi and seeddiseases is an important aspect of disease management. Determining the presence of seed-borne pathogens allows managers to apply the appropriate controls or modify management practices to avoid the problem in the future. All the fungal isolates observed in this study were listed among the eight species isolated from ten soybean cultivars with agar (PDA) plate and blotter methods in India (Khayum*et al*., 2006) and the thirty-nine species isolated fromone cultivar of soybean using four methods of isolation in Pakistan (Nasreen, 2003). Both studies also found the agar plate method of isolation to perform better than the blotter method in isolating the fungi.
Antifungal properties and phytochemical screening of extracts of *Ocimumgratissimum L*. (Amadi*et al.*,2010). Thus, there is a need to search for alternative approaches to considering these as first step in the present investigation.

Conclusion

Out of total fungal species recorded, *Fusarium sp.*were found predominant in the samples. The seed can be used by treating with the 15% extract concentration to prevent seed infection with plant pathogens. The present investigation is an important step in developing plant-based pesticides which are ecofriendly for the management of the seed borne fungi and development of commercial formulation of botanicals. Further investigation will be done for developing commercial formulation based on field trail and toxicological experiment.

References

1. Amadi, J.E, Salami, S.O and Eze, C.S (2010) Antifungal properties and phytochemical screening of extracts of African Basil (*Ocimumgratissimum L.*) *Agric. Biol. J. N. Am.*, 1(2): 163-166
2. Anonymous. (1996), International rules of seed testing. *SeedSci.Tech*.24: 1-335.
3. Chen, C.Y., Tsou S.C.S. and Wang, H.H., (1987). Utilization patterns of mungbean in the Chienese diet. In Mungbean- Proceedings of the Second International Symposium, Bangkok, Thailand. AVRDC tropical vegetable information service.
4. Doyer, D.C. (1938). Manual for the determination of seed borne diseases. *Int. Seed. Test. Assoc*. 28:133-151.
5. Janardhana, G.R., Raveesha, K.A. and Shetty, H.S. (1999). Mycotoxin contamination of maize grains grown in Karnataka (India). *Food Chemical Toxicology* 37: 863 – 868.
6. Khayum, Ahammed, S., Anandam, R. J., Prasad, Babu, G., Munikrishnaiah, M. Gopal, K. (2006): Studies on seed mycoflora of soybean and its effect on seed and seedling quality characteristics. *Legume Research*, 29(3):186-190.
7. Marin. S., Homedes, V., Sanchis, V., Ramos, A.J. and Magan, N. (1999). Impact of Fusariummoniliforme and F. proliferatumcolonisation of maize on calorific losses and fumonisin production under different environmental conditions. *Journal of Stored Product Research* 35: 15 – 26.
8. Nasreen, N. (2003): Detecting seed-borne fungi of soybean by different incubation methods. *Pakistan Journal of Plant Pathology* 2(2):114-118.
9. Neergaard, P. (1977): "Seed Pathology". *The Macmillan Press*. London.
10. Nine, Y.L., (1986). Opportunities for research on diseases of pulse crops. *Indian Phytopathology*. 39 (3), pp.333-342
11. Pal, M., (1996). Pulse disease scenario. *Indian Phytopathology*. 49 (2), pp.129-131
12. Paul YS. (1989). Seed borne mycoflora of soybean and its control in Himachal Pradesh. *J. Mycology & Pl. Path.* 19 (3): 253-257
13. Rajeswari B and Meenakumari KVS. (2009). Bioagents and fungicides for the management of seed and seedling diseases of soybean. *Indian J. Pl. Prot*. 37.1&2 12131.
14. Solanke RB, Kore, SS and Sudewad, SM. (1997). Detection of soybean seed borne pathogens and effect of fungicides. *J. Agri. Univ*. 22(2): 168-170
15. Wan ZainunNik and D.G. Parbery (1978): The isolation of pathogenic fungi from seed of tropical pasture legume species. *Malays. Appl. BioI*. 7(2), 121-130.