SCREENING OF BIOCONTROL AGENT AGAINST YELLOW MOLD DISEASE OF *Arachis hypogaea* L.SEED

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Abstract

The threat of aflatoxin contamination in food commodities and its association with health risks in both animals and humans continues to raise increasing concern over years. In this report, fungal species found in association with peanuts in storage and their potential to produce aflatoxin in collected samples was determined. About 60 to 70% of peanut were infected with various moulds including *Aspergillus flavus, A.terreus, A.niger, A.octraceus, A.spinulorsus, A.fumigutus, Trichoderma* sp, *Fusarium solani, Curvularia geniculata, Curvularia lunata, Fusarium moniliforme, Penicillium granulatum, Cephalosporium* sp., *F.oxysporum, Helminthosporium oryae, Memnomilla* sp. and *Penicillium notatum* were found to be the most patent *Aspergillus flavus* strains. This level of toxicity is more than five times higher than the acceptable dosage in edible peanuts. This report points out the health risks associated with aflatoxin contamination in edible food commodities despite enormous efforts to control this mycotoxin. Current research efforts to control or minimize the intake of aflatoxins especially in warmer regions of the world are hereby included.

Keywords: Groundnut, fungi diversity, antagonistic activity, *Aspergillus flavus*

INTRODUCTION

Soil is the major component of earth s ecosystem which comprises of organic matter, minerals, gases and large numbers of macro and microorganisms. The soil ecosystem is supported by several interactions among its physical, chemical and biological components (Buscot, 2005). Many biological processes take place in soil and determine functions that provide various services within ecosystems: turnover of organic matter, symbiotic and non symbiotic atmospheric nitrogen fixation, denitrification, aggregation etc. (Chenu and Stotzky, 2002). Rhizosphere is the narrow zone of soil surrounding the root where microbe populations are stimulated by root activities. Rhizosphere is known to be a hotspot of microbial activities. Soil microflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth (Kiran Singh *et al*., 1999). Microorganisms in the soil and rhizosphere are beneficial in

increasing soil fertility and plant growth as they are involved in several biochemical transformations and mineralization activities in soils. Type of cultivation and crop management practices found to have greater influence on the activity of soil microflora. Fungi are an important component of soil microbiota more in abundance than bacteria, depending on soil depth and nutrient conditions. Different soils have specific fungal flora, but the majority of species found in them are cosmopolitan (Ainsworth and Sussman, 1968). Fungi are fundamental for soil ecosystem functioning (Warcup, 1951), especially in forest and agricultural soils, they play a key role in many essential processes such as organic matter decomposition and elemental release by mineralization (Christensen *et al*., 1989). It was estimated 1.5million fungal species are present in natural ecosystems, but only 5-10% has been described formally (Hawksworth 2001). The aim of the present investigation is to isolate mycoflora from ground nut field and control the yellow mold disease of seed.

Materials and Methods

Collection of soil samples

Rhizosphere soil samples were collected from different groundnut field of various Districts in Tamil Nadu (S1, S2, S3, S4, S5 and S6) (Table-1). In each field 1kg of soil sample was collected from the surface area reaching about 10-15 cm depth and near the rhizosphere region of plants. Soils were collected in sterile polythene bags and sealed on the spot. Samples were stored in laboratory at 4°C until further analysis.

Isolation of Mycoflora

Dilution plate technique described by Warcup (1951) was used for the isolation of fungi from various rhizosphere soil samples. 10 grams of soil samples were suspended in 90 ml of distilled water. The flasks were shaken thoroughly in order to get uniform distribution of the soil particles. The soil suspensions were diluted in 10 fold increment from 10^{-3} to 10^{-5}. The Volume of 10 ml of soil sample suspension from each serial dilution was pipette onto different melted, cooled culture media Potato Dextrose Agar (PDA) supplemented with 1% Streptomycin. The pH of the culture media was maintained at 5.5 being optimal for the growth and sporulation in a majority of fungi. Each culture media was prepared in a liter of distilled water and autoclaved at 120°C at 15 psi for 20 min. 1% Streptomycin was used as an antibiotic for the restrain of bacterial growth. Each colony was sub cultured and maintained on potato dextrose agar slants.

The inoculated plates were incubated at room temperature 28±2°C in an inverted position for 5-7 days. Three replicates were maintained for each sample**.**

Data Analysis

Population density is expressed in terms of Colony Forming Unit (CFU) per gram of soil with dilution factors. The percentage contribution of each isolate was calculated by using the following formula (Gaddeyya *et al.,* 2012).

Identification of Soil Fungi

Fungal morphology were studied macroscopically by observing colony features (Texture and Color) and microscopically by staining with Lacto phenol cotton blue and observed under compound microscope for conidiophores, conidia and arrangement of spores. The fungi were identified with the help of literature (Nagamani *et al*., 2006).

Effect of antagonistic fungi on the suppression of pathogenic fungi of yellow mold disease

The antagonistic activity of various soil fungi against the test pathogen were studied with dual culture plate technique (Morton and Strouble, 1955) under *invitro* condition. The test pathogen *Aspergillus flavus* and the soil fungi viz., *Aspergillus terreus, A. niger, A. fumigatus, F.oxysporum, P.notatum, P.granulatu* and *Trichoderma viride* were grown separately on PDA medium. The agar block cut from actively growing margin of individual species of soil fungi and test organism were inoculated opposite to each other approximately 3 cm apart on potato dextrose agar medium in petriplates. Three replicates were maintained for each set. Controls were set in single and dual inoculated cultures at the fungus. The position of the colony margin on the back of the disc was recorded daily. The assessment was made for both organisms. The percentage of inhibition of growth was calculated as follows.

$$\text{percentage of inhibition of growth} = \frac{r\text{-}r'}{r} \times 100$$

Where,

r = growth of the fungus from the centre of the colony towards the centre of the plate in the absence of antagonistic fungus.

r ' = growth of the fungus from the centre of the colony towards the antagonistic fungus.

The colony interaction between test pathogen and soil fungi was proposed methods (Skidmore and Dickinson 1976).

Results and Discussion

Isolation and identification

The Present investigation was conducted to find out the fungal diversity in six different groundnut fields. In this study, 136 fungal colonies of 16 fungal species belonging to 7 genera were isolated and identified. Sixteen fungal isolates were identified (Table-2). *Aspergillus flavus*, *A.terreus, A.niger, A.octraceus, A.spinulorsus, A.fumigutus*, *Trichoderma* sp., *Fusarium solani, Curvularia geniculata, Penicillium granulatum, F.oxysporum, Helminthosporium oryae, Memnomilla* sp. and *Penicillium notatum* based on cultural characters and sporulation structure. The colonies of *Aspergillus* and *Fusarium* belonging to were predominant in all soil samples in groundnut fields. The similar and significant report has already been made in all soil samples of crop fields such as sunflower, sesame, capsicum, rice, green gram, sugarcane, ground nut and black gram by Shiny Niharika *et al*., (2013).

Gaddeyya *et al.,* (2012) determined the fungal population and their diversity in agricultural fields of Salur. During the investigation period 173 fungal colonies of 15 fungal species were observed. The maximum fungal species belongs to Deuteromycotina (169 colonies) and Zygomycotina (4 colonies) were observed. Among the isolates the genera *Aspergillus* and *Penicillium* were dominant. Rohilla and Salar (2012) twenty three soil samples were characterized for the incidence of fungal strains from pesticides contaminated agricultural soils. A total of 59 fungal isolates were obtained from the analyses of 23 soil samples taken from pesticide contaminated soils through soil dilution agar plating method.

Effect of antagonistic fungi on the suppression of pathogenic fungi of yellow mold disease

In the present study, antagonistic activity of *Aspergillus terreus, A. niger, A. fumigatus, F.oxysporum, P.notatum, P.granulatum* and *Trichoderma viride* against *Aspergillus flavus* were studied by *in vitro* dual culture experiment. The species of *Trichoderma* and *A. niger* showed the ability to inhibit the pathogen. But these species showed variability in the percentage of inhibition. The percentage inhibition of growth of pathogen against *Trichoderma viride, Aspergillus niger* and *A. fumigatus* were 45, 42 and 39 percentage with respectively. Growth inhibition decreased as follows; *Aspergillus terreus* (31), *A. niger* (42), *A. fumigatus* (39), *F.oxysporum* (34), *P.notatum* (33), *P.granulatum* (31) and *Trichoderma viride* (45) Table-3.

Swati *et al*. (2018) in dual culture *invitro* antagonistic activity of *Trichoderma harzianum* was studied against soil borne fungal pathogens isolated from rhizosphere soil of groundnut viz.,

Aspergillus flavus, Aspergillus niger, Fusarium roseum, Macrophomina phaseolina, Phythium myriotylum, Rhizoctonia solani and *Sclerotium rolfsii. Trichoderma harzianum* had been marked a significant inhibition of selected fungal pathogens as compared to their respective controls. The different workers checked these pathogens against *Trichoderma harzianum* from different hosts. During the present investigation all these pathogens were firstly tested against *Trichoderma harzianum* isolated from rhizosphere soil of groundnut from Marathwada region. The maximum percentage of inhibition were revealed by *Trichoderma harzianum* against *Fusarium roseum* (62.18%), followed by *Phythium* (51.85%), *Aspergillus flavus* (50.37%), *Aspergillus niger* (46.55%), *Rhizoctonia solani* (41.78%), *Macrophomina phaseolina* (30.58 %) and minimum inhibition was shown against *Sclerotium rolfsii* (27.73%).

Dhotre and Wanmare (2017) were got the equal interaction of *Trichoderma* against root rot causing fungus of soybean i.e. *Fusarium moniliforme* (85 mm) followed by *Rhizoctonia solani* (81.5 mm), *Fusarium oxysporum* (80 mm), *Fusarium solani* (79 mm), *Fusarium roseum* (79 mm), *Phytopthora sojae* (76 mm), *Macrophomina phaseolina* (73.5 mm). Also the were found that the *Trichoderma harzianum* inhibit the growth of *Macrophomina phaseolina* followed by *Aspergillus niger* and *Fusarium oxysporum* pathogens isolated from seeds. The *Trichoderma* reached the pathogen within 3- 4 days and overgrew them in 8-10 days. The occurrence of an inhibition zone in dual culture characterizes the secretion of some diffusible non-volatile substances. The zone of inhibition was found against two pathogens i.e. *Macrophomina phaseolina* and *Rhizoctonia solani.* This zone of inhibition lasts for 3 to 7 days and then this antagonist was overgrowing on them. Similar kind of results was found by Hajigharari *et al*., (2008) for different species of *Trichoderma* including *Trichoderma harzianum* against *Rhizoctonia solani* and *Macrophomina phaseolina*.

Conclusion

In this study, 136 fungal colonies of 16 fungal species belonging to 7 genera were isolated and identified from six different rhizosphere soil samples. *Aspergillus flavus* is the causative organisms of yellow mold disease. The fungus colonies are white in color and produce three types of spores, microconidia, macroconidia and chlamydospores. In antagonistic activity, *Trichoderma viride, A.niger* had a marked significantly inhibitory effect on the growth of selected phytopathogens compared to their. The use of these bio-agents are not only safe for the farmers and consumers but also eco-friendly, close effective, easy to produce and easy to apply

the formulation. These studies form the base for eco-friendly management of plant disease which will minimize the usage of pesticide and chemical fungicide.

Table 1: Soil samples of three different districts

Sample	Districts	Places
S1	Pudukkottai	Narangiyapattu
S2		Regunathapuram
S3	Thanjavur	Nambivayal
S4		Perayurani
S5	Thiruvarur	Edamalaiyur
S6		Vaduvur

Table 2: Occurrence of soil mycoflora in different Groundnut field from various Districts of Tamilnadu

Name of the fungi	Average No. of individual colonies (CFU/ml)						Total No. of colonies
	Pudukkottai		Thanjavur		Thiruvarur		
	S1	S2	S3	S4	S5	S6	
A.flavus	2	1	3	2	4	2	14
A.niger	1	1	2	1	1	1	7
A.terreus	3	2	3	2	2	1	13
A.octraceus	1	1	1	-	1	1	5
A.spinulorsus	1	-	-	2	-	3	6
A.fumigatus	1	1	2	2	1	2	9
C.lunata	1	-	4	-	-	-	8
C.geniculata	1	-	1	2	2	-	4
Cepalosporium sp.	2	2	1	2	3	-	12
F.moniliforme	2	2	2	2	-	3	12
F.oxysporum	2	2	1	1	2	2	10
H oryzae	1	1	1	1	1	2	7
Memnomilla sp.	1	1	1	1	-	1	5
Penicillum sp.	1	1	1	-	1	1	5
P. granulatum	2	2	2	1	3	1	11
Trichoderma sp.	2	2	1	2	1	1	8
Total no. of colonies	24	19	26	22	22	23	136

Table 3: Effect of antagonistic fungi on the suppression of pathogenic fungi

S. No	Growth response of the antagonistic and test fungus	Antagonistic fungi tested						
		A. terreus	***A niger***	***A. fumigatus***	***F. oxysporum***	***P. notatum***	***P. granulatum***	***T. vidire***
1	Colony growth of the pathogen towards antagonist(mm)	20	23	21	24	26	19	17
2	Colony growth of the pathogen away from the antagonist (mm)	19	22	21	21	23	22	24
3	% growth inhibition of the pathogen in the zone of the interaction (mm)	24	20	28	31	30	25	26
4	% colony growth of the antagonist in control i.e. Growth towards the centre of plate in the absence of the pathogen (mm)	31	42	39	26	33	31	45
5	Control growth of the antagonist away from the pathogen (mm)	24	17	31	20	22	26	40
6	% growth of inhibition in the zone of interaction	20	22	19	34	24	27	20

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