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In vitro efficacy of Fungicides, Plant Extracts, and Biocontrol Agents against the Toxic Strain of *Aspergillus flavus* (OK606055)

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ABSTRACT: Groundnut is an important oil seed crop for domestic markets as well as for foreign trade in several developing and developed countries. However, its production is limited by *Aspergillu*s species, which cause quantitative losses and generate highly toxic and carcinogenic chemical substances called aflatoxins. To minimize the risk of aflatoxin contamination in groundnut investigated to determine the efficacy of 19 fungicides, 7 botanicals and 7 biocontrol agents. The fungicides, Azoxystrobin + Tebuconazole, Fluopicolide + Propamocarb hcl, Propiconazole and Azoxystrobin showed 100% inhibition of the fungus *Aspergillus flavus* (OK606055) over the control @500ppm, 1000ppm and 1500 ppm concentration. Among botanicals Neem leaf extract@ 5000, 10000 ppm concentration maximum reduced the growth of test pathogen with 50.59%, 100% growth inhibition compared with control. Among 7 biocontrol agents tested *Trichoderma viride* isolate 4 recorded maximum growth inhibition (79.17%) followed by *Trichoderma viride* isolate 2 (78.08%) and *Trichoderma viride* isolate 1 (76.53%) against *Aspergillus flavus* (OK606055).

Keywords: *Aspergillus flavus*, Azoxystrobin, fungicides, botanicals.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the world's most important oilseed crops, ranking the 13th most important food crop and 4th most important oilseed crop of the world. *Aspergillus flavus* and *Aspergillus parasiticus* are one of the primary causes of aflatoxin contamination in groundnut, which hinders export. They are the most potent hepatocarcinogens among all the known natural and synthetic compounds. It is possible for groundnuts to be contaminated with aflatoxin during the pre-harvest and post-harvest phases. Due to *A. flavus*, groundnut seed quality deteriorates, rendering the product unsuitable for marketing and consumption. The *A. flavus* pathogen caused seed and seedling deterioration and aflarot disease in groundnut. The presence of aflatoxin in groundnut kernels poses a significant hazard to human and animal health, as well as international trade. *A. flavus* airborne, soil-inhabitant fungi. The present investigation aimed to evaluate various fungicides, botanicals and biocontrol agents to manage the *Aspergillus flavus.*

MATERIALS AND METHODS

A. In vitro evaluation of fungicide

On a pre-sterilized potato dextrose agar medium, the necessary quantity of fungicides was added. 20 ml of a tainted medium were put into sterile Petri dishes. 5 ml discs of mycelia from the actively growing zone of a ten-day-old culture were inoculated into the center of each Petri plate. The control was maintained without the use of a fungicide. Each treatment was performed three

times. The plates were incubated at a temperature of 27°C, and the radial growth of fungal mycelium was measured in both directions. The data were statistically analyzed, and fungicide efficacy was expressed as a percentage of mycelia growth inhibition relative to the control. The formula for calculation (Vincent, 1947) was as follows:

$$I = \frac{C - T}{C} \times 100$$

I = Percent inhibition
C= Radial growth in control
T = Radial growth in treatment

Table 1: List of fungicides used in the management of the causal fungus.

Treatments	Chemical Name	Trade Name
T_1	Tricyclazole	Beam
T_2	Cymoxanil + mancozeb	Curzate M8
T_3	Thiram 75 WS	Thivron
T_4	Pyraclostrobin 10% CS	Defender CS
T_5	Metalaxyl	Ridomil
T_6	Mancozeb	Saaf
T_7	Thiophanate methyl 70% WP	Rook
T_8	Carboxin + thiram	Vitvaxpower
T_9	Kasugamycin + copper oxychloride	Conika
T_{10}	Pyraclostrobin + metiram	Cabrio top
T_{11}	Propineb	Anthracol
T_{12}	Hexaconazole	Contest
T_{13}	Difenoconazole	Score
T_{14}	Azoxystrobin + Tebuconazole	Nativo
T_{15}	Validamycin	Sheathmar
T_{16}	Propiconazole 25 EC	Tilt
T_{17}	Azoxystrobin 23 SC	Amistar
T_{18}	Fluopicolide + Propamocarb Hydrochloride	Infinito
T_{19}	Tebuconazole 25.9% EC	Folicur

B. Preparation of crude extract

Fresh plant material was collected from the field and rinsed twice with tap water and then distilled water. The plant parts were dry for a brief period of time. Each plant part was weighed and grounded in a grinder by adding an equal amount of sterilized distilled water. This extract was then filtered through two layers of cotton fabric and utilized as a stock solution. Except for garlic, which was centrifuged at 4000 rpm for 15 minutes, all extracts were centrifuged at 10000 rpm for 15 minutes, and the extracts were regarded as 100 percent and adjusted to varied concentrations as required.

Table 2: List of botanicals used in the management of the causal fungus.

Treatments	Common name	Botanical name	Plant parts used
T_1	Garlic	*Allium sativum*	Clove
T_2	Ginger	*Zinger officinalis*	Rhizome
T_3	Thuja	*Thuja compacta*	Leaf
T_4	Neem	*Azadirachta indica*	Leaf
T_5	Onion	*Allium cepa*	Bulb
T_6	Ocimum	*Ocimum sanctum*	Leaf
T_7	Eucalyptus	*Eucalyptus globus*	Leaf

C. Poisoned food technique

The anti-fungal properties of plant extracts were evaluated using the poisoned food technique (Nene and Thapliyal 1973). 5, 10 ml of pure extracts were combined with 95, 90 ml of melted potato dextrose agar medium to achieve concentrations of 5000ppm and 10,000ppm, respectively. Without adding plant extract, the control was maintained. In a petri dish, 20 ml of media was poured and allowed to solidify. Five ml culture discs were placed in the center of the solidified Petri plates. Each dish was incubated at ambient temperature. When maximal growth was observed in the control plate, the mycelia growth was measured. The effectiveness was expressed as a percentage of mycelia growth inhibition relative to the untreated control.

D. In vitro evaluation of biocontrol agents

The effectiveness of biocontrol agents against *Aspergillus flavus* was evaluated using the dual culture technique. The fungus was tested against fungal biocontrol agents such as *Trichoderma viride*, *Trichoderma hamatum*, and *Trichoderma harzianum*, as well as bacterial biocontrol agent *Pseudomonas fluorescens*. For the experiment, the fungal antagonist

was grown in potato dextrose agar media, and the bacterial antagonist was grown in nutrient agar media.

E. Dual culture technique

In Petri dishes, approximately 20ml of potato dextrose medium for fungi antagonist and nutrient agar medium for bacteria antagonists were poured and allowed to settle. The fungal mycelial disc (5 mm) was transferred to one end of the plate and the fungal antagonist culture disc was deposited at the opposite end, leaving 5-6 mm between the two discs. In the case of a bacterial antagonist, the bacterium was streaked on one side of the plate, while the fungal culture disc was placed on the opposite side. Each treatment was performed three times. The data was statistically analysed. The efficacy of biocontrol agents was expressed as a percentage of mycelia growth inhibition relative to the control and calculated as Vincent (1947).

Table 3: List of biocontrol agents used in the management of the causal fungus.

Treatments	Biocontrol agents	Place of Collection
T_1	*Trichoderma viride* isolate1	Department of Agriculture Entomology, College of Agriculture, OUAT, Bhubaneswar.
T_2	*Trichoderma viride* isolate 2	Department of Plant Pathology, ANGRU, Bapatala
T_3	*Trichoderma viride* isolate 3	Department of Plant Pathology, IIHR, Bengaluru
T_4	*Trichoderma viride* isolate 4	Department of Plant Pathology, ANGRU, Tirupati
T_5	*Trichoderma hamatum*	ICRISAT, Hyderabad
T_6	*Trichoderma harzianum*	Department of Plant Pathology, ANGRU, Tirupati
T_7	*Pseudomonas fluorescens*	CHES, Bhubaneswar.

RESULTS AND DISCUSSION

A. In vitro efficacy of fungicides against the toxic strain of Aspergillus flavus (OK606055)

All compounds significantly differed from the control in terms of their ability to suppress growth. For the *Aspergillus flavus* pathogen, every treatment exhibited a noticeably different inhibitory pattern. @ 500 ppm Azoxystrobin, Tebuconazole, Propiconazole and combination fungicides like Azoxystrobin + Tebuconazole, Fluopicolide + Propamocarbhcl completely reduce the growth of *Aspergillus flavus* inducing 100% growth inhibition followed by Tricyclazole and Hexaconazole with 82.38% and 76.45% inhibition respectively when compared to untreated control. @1000 ppm Tricyclazole, Azoxystrobin, Tebuconazole, Propiconazole and combination fungicides like Azoxystrobin + Tebuconazole, Fluopicolide + Propamocarbhcl and Kasugamycin + COC completely reduce the growth of *Aspergillus flavus* inducing 100% growth inhibition followed by Hexaconazole, Carboxin + Thiram and Thiophinate-methyl with 89.45%, 83.90% and 82.65% inhibition respectively against the growth of test pathogen. @1500 ppm complete reduction with 100% growth inhibition observed with Tricyclazole, Azoxystrobin, Tebuconazole, Propiconazole, Propineb and combination fungicides like kasuigamycin + COC Azoxystrobin + Tebuconazole and Fluopicolide + Propamocarbhcl observed against test pathogen followed by Carboxin + Thiram, Thiphanate-methyl with 91.22% and 86.39% inhibition respectively. Validamyicn recorded least percentage inhibition @ 500, 1000 and 1500 ppm with 31.34%, 36.6% and 52.46% respectively compare to untreated control (Table 4, Plate:1 a, b & c). These results are similar to the findings of several scientists Nandeesha *et al*. (2013); Futane *et al*. (2018); Raju and Naik (2006).

Table 4: Efficacy of various chemicals against radial growth of *Aspergillus flavus* (OK606055).

Treatments	500 PPM		1000 PPM		1500 PPM	
	Mean radial growth(mm)	Percent inhibition	Mean radial growth(mm)	Percent inhibition	Mean radial growth(mm)	Percent inhibition
Tricyclazole	13.27(3.71)	82.38	0.00(0.71)	100.00	0.00(0.71)	100.00
cymoxanil + mancozeb	37.80(6.19)	49.80	27.13(5.26	64.78	21.20(4.66)	73.03
Thiram	28.43(5.38)	62.24	19.23(4.44)	75.03	12.43(3.60)	84.18
Pyraclostrobin	40.57(6.41)	46.13	31.70(5.67)	58.85	24.23(4.97)	69.17
Metalaxyl	27.07(5.25)	64.05	25.10(5.06)	67.42	15.77(4.03)	79.94
Mancozeb	31.70(5.67)	57.90	22.57(4.80)	70.70	0.00(0.71)	100.00
Thiophanate-methyl	21.23(4.66)	71.80	13.37(3.72)	82.65	10.70(3.35)	86.39
Carboxin + thiram	19.63(4.49)	73.93	12.40(3.59)	83.90	6.90(2.72)	91.22
Kasugamycin + copper oxychloride	18.53(4.36)	75.39	0.00(0.71)	100.00	0.00(0.71)	100.00
Pyraclostrobin + metiram 5+55	22.60(4.81)	69.99	18.63(4.37)	75.81	15.33(3.97)	80.49
Propineb	29.33(5.46)	61.04	22.03(4.75)	71.40	0.00(0.71)	100.00
Hexaconazole	17.73(4.27)	76.45	8.10(2.93)	89.48	0.00(0.71)	100.00
Difenoconazole	31.43(5.65)	58.26	24.50(5.00)	68.19	17.90(4.29)	77.23
azoxystrobin tebuconazole	0.00(0.71)	100.00	0.00(0.71)	100.00	0.00(0.71)	100.00
Validamycin	51.70(7.22)	31.34	49.07(7.04)	36.30	37.37(6.15)	52.46

Propiconazole	0.00(0.71)	100.00	0.00(0.71)	100.00	0.00(0.71)	100.00
Azoxystrobin	0.00(0.71)	100.00	0.00(0.71)	100.00	0.00(0.71)	100.00
fluopicolide + propamocarb hcl	0.00(0.71)	100.00	0.00(0.71)	100.00	0.00(0.71)	100.00
Tebuconazole	0.00(0.71)	100.00	0.00(0.71)	100.00	0.00(0.71)	100.00
Control	75.30(8.70)		77.03		78.60(8.89)	
CD at 5%	0.1134		0.1714		0.1746	
SEM	0.0397**		0.0600**		0.0611 **	

*Figures in the parentheses indicate $\sqrt{(x+0.5)}$ transformed values

B. In vitro plant extracts against the toxic strain of Aspergillus flavus(OK606055)

All of the studied plant extracts against the growth of *Aspergillus flavus* at concentrations of 5000 ppm and 10000 ppm showed a significant difference in growth inhibition. Neem leaf extract at both concentrations 5000, 10000 ppm was superior then all other treatments with maximum reduced the growth of test pathogen with 50.59%and 100% growth inhibition respectively compared with the control. After to neem leaf extract @5000 ppm onion bulb extract showing 34.40 % inhibition. At the 10000-ppm concentration, eucalyptus oil showed 78.86% inhibition followed by Thuja leaf extract showed 76.02% inhibition (Table 5, Plate 2). The results of the study are confirmed by findings reported by Mahmoud *et al*. (2011).

Table 5: Efficacy of various botanicals against radial growth of *Aspergillus flavus* (OK606055).

Treatments	5000 ppm		10000 ppm	
	Mean radial growth(mm)	Per cent inhibition	Mean radial growth(mm)	Per cent inhibition
Garlic cloves (*Allium sativum*)	5.59 (2.56)	31.20	2.20 (1.78)	73.17
Ginger (*Zinger officinalis*)	5.51 (2.54)	32.27	2.25 (1.80)	72.52
Thuja (*Thuja compacta*)	6.20 (2.68)	23.79	1.97 (1.72)	76.02
Neem (*Azadiracta indica*)	4.02 (2.23)	50.59	0.00 (1.00)	100.00
Onion bulb (*Allium cepa*)	5.33 (2.51)	34.40	2.37 (1.83)	71.14
Ocimum (*Ocimum sanctum*)	6.14 (2.66)	24.44	2.17 (1.77)	73.58
Eucalyptus (*Eucalyptus globus*)	5.88 (2.61)	27.63	1.73 (1.85)	78.86
Control(untreated)	8.13 (3.01)		8.20 (3.03)	
CD at 5%	0.26		0.165	
SEM	0.086		0.084	

*Figures in the parentheses indicate $\sqrt{(x+0.5)}$ transformed values

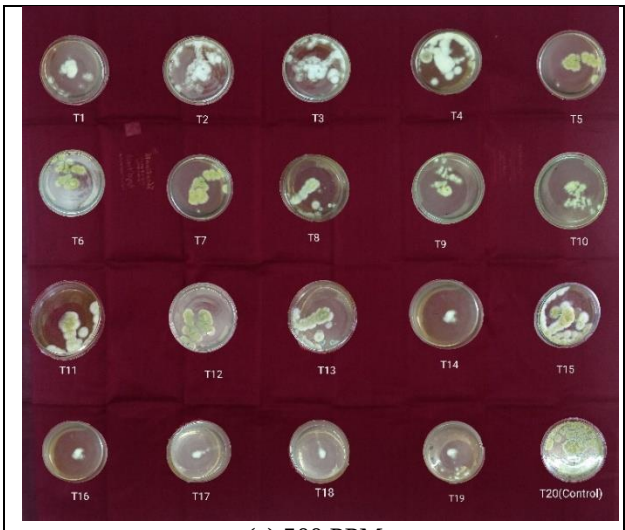
C. In vitro efficacy of biocontrol agents against the toxic strain of Aspergillus flavus (OK606055)

In the dual culture approach, bacterial and fungal bioagents were evaluated for their ability to prevent *Aspergillus flavus* from radial growth. When the growth in the control plate reached its maximum, the radial growth (mm) was measured, and the results are shown in Table 6. Among 7 biocontrol agents tested *Trichoderma viride* isolate 4recorded maximum growth inhibition (79.17%) followed by *Trichoderma viride* isolate 2 (78.08%) and *Trichoderma viride* isolate 1 (76.53%) against *Aspergillus flavus*. Among all treatments minimal percent inhibition (64.50%) was observed for *Pseudomonas fluorescens* against growth of *Aspergillus flavus* (Plate 3). The findings of the study are supported by Baig *et al.* (2012); Sudha *et al.* (2013); Spadaro *et al.* (2005).

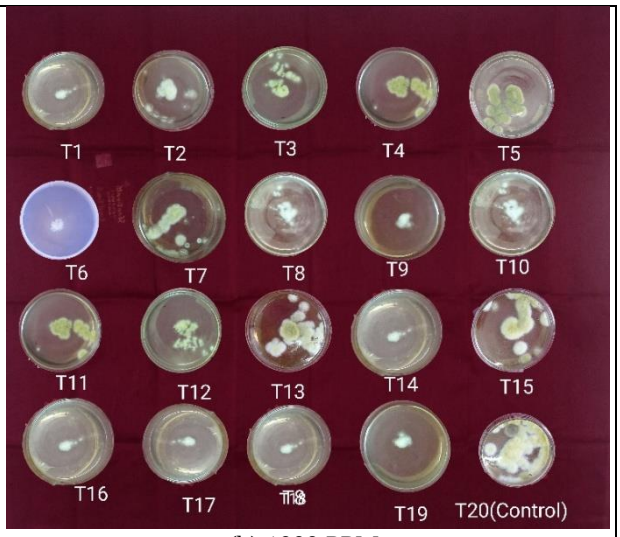
Table 6: Efficacy of various Biocontrol agents against radial growth of *Aspergillus flavus* (OK606055).

Treatment details	Mean radial growth(mm)	Per cent inhibition
Trichoderma viride isolate 1	18.67(4.37)	76.52
Trichoderma viride isolate 2	17.43(4.23)	78.08
Trichoderma viride isolate 3	21.23(4.65)	73.31
Trichoderma viride isolate 4	16.57(4.13)	79.17
Trichoderma harzianum	19.6(4.48)	75.36
Trichoderma hamatum	23.34(4.88)	70.65
Pseudomonas fluorescens	28.23(5.35)	64.50
Control	79.53(8.93)	
CD at 5%	0.605	
SEM	0.202**	

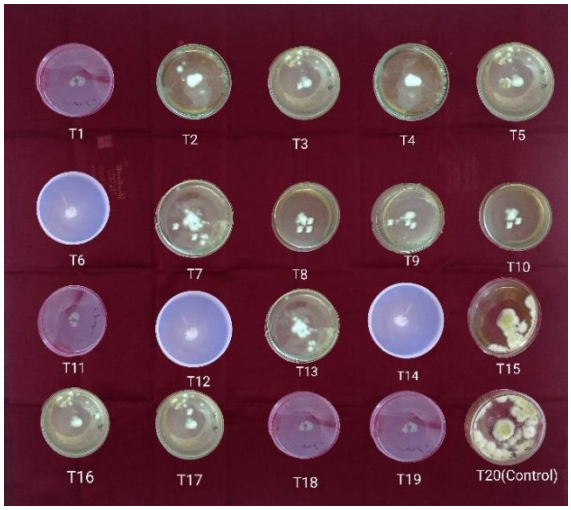
*Figures in the parentheses indicate $\sqrt{(x+0.5)}$ transformed values



(a) 500 PPM



(b) 1000 PPM



(c) 1500 PPM

Plate 1: Efficacy of various chemicals against radial growth of *Aspergillus flavus* (OK606055).

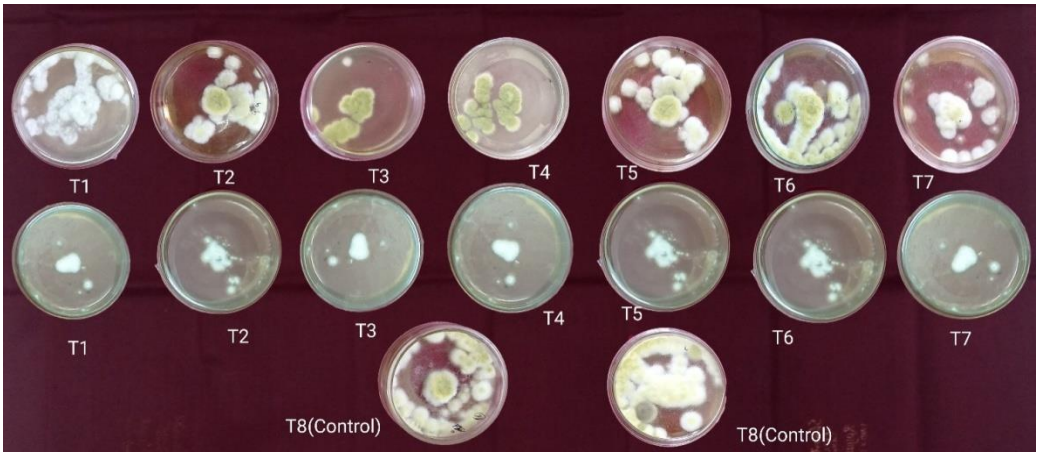


Plate 2: Efficacy of various botanicals against radial growth of *Aspergillus flavus* (OK606055).

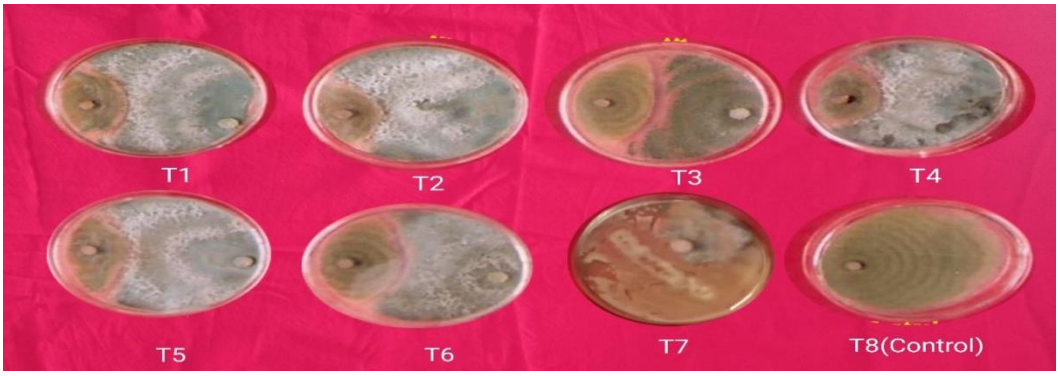


Plate 3: Efficacy of various Biocontrol agents against radial growth of *Aspergillus flavus* (OK606055).

CONCLUSION

The following conclusions are being taken as a result of the findings from the investigation on the treatment of *Aspergillus flavus*. All of the *in vitro* tested test fungicides, botanicals, and biocontrol agents were fungistatic and antifungal to the test pathogens. However, it was discovered that fungicides like Azoxystrobin, Tebuconazole, and Propiconazole, as well as combination fungicides like Fluopicolide and Propamocarb hcl, were more effective against *Aspergillus flavus*.

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