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In Vitro Evaluation of Botanicals and Bioagents against *Aspergillus flavus* Link Ex. Fries in Groundnut

Chaudhari V. J., Nakrani B. R.

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is an important food crop for vegetable oil production. Groundnut suffers from many diseases among them *Aspergillus flavus* has the potential to infect in the field, preharvest, postharvest, storage and during transit. They produce a potent toxin and carcinogenic substance called aflatoxin which has a great impact on human health. The experiment was conducted to the evaluation of five botanical extracts with suitable control at 5, 10 and 15 % concentrations by poison food technique *in vitro*, the most effective botanical of garlic clove extract, which inhibited fungal growth by 53.88, 74.16 and 80.19 % at 5, 10 and 15 % concentrations, respectively, whereas tulsi leaf and ginger rhizome extract were found to be the least effective. To evaluate *in vitro* bioagents by dual culture method, the maximum growth inhibition was found in isolates T_3 (*Trichoderma viride*, Mahadeviya) which was followed by T_5 (*Trichoderma harzianum*, Chandisar), whereas bacterial bio-agents T_{15} (*Bacillus subtillis*) and T_{14} (*Pseudomonas fluorescens* II) considered as potential bacterial antagonists. In both T_{10} (*Trichoderma viride*, Jambusar) and T_{17} (*Pseudomonas stutzeri*) have least effective.

Keywords *Aspergillus flavus*, Groundnut, Botanicals, Bioagents.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an annual legume and also known as peanut. It is the thirteenth most important food crop of the world and third most important oil seed crop used for vegetable oil production. Groundnut is cultivated in the tropical and subtropical regions of the world. India stands first in area with 3.9 million hectares, second in production (6.7 million ton) and productivity of 1422 $kgha^{-1}$. In Gujarat, groundnut is grown on about 20.72 lakh hectares with a production of 54.64 lakh ton and an average productivity of 2637 $kgha^{-1}$ (Anon 2021). Groundnut seeds (kernels) contain 35.8- 54.2 % oil (Jambunathan *et al*. 1985), 16.2-36.0 % protein (Dwivedi *et al*. 1990) and 10-20 % carbohydrate (Salunkhe *et al*. 1992). Groundnut crop suffers from many diseases, among the soil borne diseases, afla rot caused by *Aspergillus flavus* is an important disease in groundnut growing areas of the world (Klich 2007). *A. flavus* is most common in warm temperate zones and environment with low water level and higher temperature. It is known to infect both in pre and

Chaudhari V. J. [1]*, Nakrani B. R. [2]
[2] Assistant Professor
Dept. of Plant Pathology, C. P. College of Agriculture,
S. D. Agricultural University, Sardarkrushinagar,
Dantiwada, Gujarat, India

Email: chaudharivrushank0@gmail.com
*Corresponding author

post-harvest situations in the field/ storage, so there is a need to manage pathogen under both conditions. So bioagents and natural products would help to minimize ill effects of fungicide use. Thus, study is required to screen various botanical fungicides and biocontrol agents under *in vitro* conditions before they are taken to field level.

MATERIALS AND METHODS

Evaluation of different botanical fungicides against *A. flavus in vitro*

The botanical fungicides of different plants belonging to different families were evaluated on the growth of most virulent species of *A. flavus in vitro* by poison food technique (Table 1).

Fresh and healthy, 100 g plant parts of each plant species as listed were collected and washed thoroughly with tap water followed by sterilized distilled water. Respective plant parts were crushed in a mixer grinder by adding 100 ml sterilized distilled water to obtain 1:1 extracts separately, then centrifuged and filtered through double layered sterilized muslin cloth in conical flasks and plugged. Sterilized 100 ml PDA was taken in 250 ml conical flasks, after cooling to about 45°C temperatures, 10 ml of respective concentration of extracts were mixed thoroughly. From the 100 ml PDA mixed with extracts, 20 ml was poured aseptically into sterilized petri plates with three replications. The PDA Petri plate was inoculated with a 5 mm mycelial disc cut from the periphery of the *A. flavus*. The culture was grown on a PDA medium in the center with the help of a sterilized cork borer. The Petri plates containing PDA media without extract served as a control. The plates were incubated at 27±2°C temperature in an incubator for seven days.

Details of experiment

Sl. no.	Content	Descriptions
1	Location	Dept. of Plant Pathology, C.P.C.A., S.D.A.U., S.K., Nagar
2	Experimental design	FCRD
3	Factor A (Treatment)	6
4	Factor B (Concentration)	3
5	Replication	3
6	Technique	Poison food technique

Observations recorded

After seven days of incubation, colony diameter and per cent inhibition were calculated by using the formula given by Vincent (1947).

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

Where,

PGI= Per cent growth inhibition
C= Colony diameter in control (mm)
T= Colony diameter in treatment (mm)

Evaluation of different bio-agents against *A. flavus in vitro*

Different antagonists (Table 2) were evaluated for their antagonistic activity against most virulent species of *A. flavus in vitro* by dual culture technique. The test organism and pathogen were grown separately on a PDA medium. From seven days old culture, 5 mm mycelial disc of the test organism and pathogen were cut aseptically and placed opposite to each other approximately 60 mm apart on to Petri plate containing 20 ml PDA, while in bacterial antagonists, streaked at one end of each Petri plate poured aseptically with 20 ml PDA medium 24 hrs prior to the pathogen inoculation. The plates with only pathogens served as a control. The plates were incubated at 27±2°C temperature for seven days and the radial growth was

Table 1. List of different botanical fungicides were used against *A. flavus in vitro*.

Treat. No.	Common name	Botanical name	Plant parts use	Concentration (%)		
T_1	Neem	*Azadirachta indica* L.	Leaves	5	10	15
T_2	Garlic	*Allium sativum* L.	Clove	5	10	15
T_3	Onion	*Allium cepa* L.	Bulb	5	10	15
T_4	Ginger	*Zingiber officinalis* Rosa	Rhizome	5	10	15
T_5	Tulsi	*Ocimum sanctum* L.	Leaves	5	10	15
T_6	Control	-	-	-	-	-

measured (Dennis and Webster 1971).

Observation recorded

Inhibition zone were measured at 24 hrs interval till the colony in the control plate covered with mycelium of pathogen. The Per cent Growth Inhibition (PGI) were calculated by using the formula suggested by Vincent (1947).

$$PGI = \frac{C\text{-}T}{C} \times 100$$

Where,

PGI= Per cent growth inhibition
C= Colony diameter in control (mm)
T= Colony diameter in treatment (mm)

Details of experiment

Sl. no.	Content	Descriptions
1	Location	Dept. of Plant Pathology, C.P.C.A., S.D.A.U., S.K. Nagar
2	Experimental design	CRD
3	Treatment	19
4	Replication	4
5	Technique	Dual culture technique

Table 2. List of different bio-agents were used against *A. flavus in vitro*.

Treat. No.	Bio-agents	Source
T_1	*Trichoderma viride*	*Trichoderma* spp. isolated from groundnut growing farmer's field.
T_2	*Trichoderma viride*	
T_3	*Trichoderma viride*	
T_4	*Trichoderma harzianum*	
T_5	*Trichoderma harzianum*	
T_6	*Trichoderma viride*	
T_7	*Trichoderma viride*	
T_8	*Trichoderma viride*	
T_9	*Trichoderma harzianum*	
T_{10}	*Trichoderma viride*	
T_{11}	*Trichoderma viride*	
T_{12}	*Trichoderma harzianum*	
T_{13}	*Pseudomonas fluorescens* I	Department of plant pathology
T_{14}	*Pseudomonas fluorescens* II	
T_{15}	*Bacillus subtilis*	
T_{16}	*Bacillus amyloliquefaciens*	
T_{17}	*Pseudomonas stutzeri*	
T_{18}	*Pseudomonas putida*	
T_{19}	*Control*	-

RESULTS AND DISCUSSION

Evaluation of different botanical fungicides against *A. flavus in vitro*

Many botanicals are known to have an inhibitory effect on the growth and reproduction of various fungi. This information is certainly useful in exploiting the inhibitory principle for developing botanical fungicides in plant disease management. In the present investigation, a total of five botanical neem (leaves), garlic (clove), onion (bulb), ginger (rhizome) and tulsi (leaves) extract with suitable control were evaluated at 5, 10 and 15% concentrations by poison food technique *in vitro* to know their inhibitory effects on the growth of most aflatoxigenic strains of *A. flavus* (AF-6).

The results (Table 3) revealed that all the plant extracts were inhibitory to the growth of *A. flavus in vitro*. The highest mean growth inhibition of 69.41% was recorded with garlic clove extract, followed by neem leaves (64.86%) and onion bulb (60.24%) extract, whereas the lowest in ginger rhizome (11.83%).

The most effective botanical garlic clove extract, which inhibited fungal growth by 53.88, 74.16 and 80.19% at 5, 10 and 15% concentrations, respectively. In neem leaves per cent inhibition was recorded as 49.07% at 5% concentration, 67.53% at 10% concentration and 77.97% at 15% concentration. Whereas, the per cent growth inhibition of 47.26, 63.52 and 69.94% at 5, 10 and 15% concentrations respectively were recorded with onion bulb extract.

The inhibition of fungal growth was increase with increase in concentration in all the tested botanicals.

The interaction effect of botanical × concentration on growth inhibition of *A. flavus* indicating that maximum growth inhibition (80.19%) was recorded with garlic clove extracts at 15% concentration which was at par with neem leaves extracts (77.97%) at 15% concentration followed by garlic clove (74.16%) at 10% concentration and least growth inhibition was observed in ginger rhizomes extracts (2.65%) at 5% concentration.

Table 3. Evaluation of different botanical fungicides against *A. flavus in vitro.* Figures in parentheses are retransformed values of arc sine transformed values. Treatment means with the letter(s) in common are not significant by DNMRT at 5% level of significance.

Tr. No.	Botanicals	Plant Parts use	Growth inhibition (%) Concentration (%)			Mean
			5	10	15	
T_1	Neem	Leaves	44.46^{f} (49.07)	55.26^{c} (67.53)	62.01^{a} (77.97)	53.91^{b} (64.86)
T_2	Garlic	Clove	47.22^{e} (53.88)	59.45^{b} (74.16)	63.57^{a} (80.19)	56.75^{a} (69.41)
T_3	Onion	Bulb	43.43^{f} (47.26)	52.84^{d} (63.52)	56.75^{c} (69.94)	51.01^{c} (60.24)
T_4	Ginger	Rhizome	9.37^{k} (2.65)	20.72^{j} (12.52)	26.80^{h} (20.33)	18.96^{e} (11.83)
T_5	Tulsi	Leaves	19.62^{j} (11.28)	22.69^{i} (14.88)	36.03^{g} (34.61)	26.12^{d} (20.26)
T_6	Control		4.05^{l} (0.50)	4.05^{l} (0.50)	4.05^{l} (0.50)	4.05^{f} (0.50)
	Mean		28.03^{c} (27.44)	35.84^{b} (35.85)	41.54^{a} (47.26)	-
			Botanical	Concentration	Botanical × Concentration	
	SEm ±		0.33	0.23	0.57	
	CD at 5%		0.94	0.67	1.63	
	CV%			2.80		

Different botanicals contain different chemical constituents, which are responsible for various activities in one or the other way. The effectiveness of different botanicals against *A. flavus* might be due to the presence of some compounds which may have antifungal property. Garlic contains allicin which might have played vital role in inhibiting the mycelial growth of *A. flavus*.

The present investigation is more or less similar to the work done by earlier workers. Bora (2008) reported that the extract of *Allium sativum* L. was effective with the inhibition of 79.22% followed by *Allium cepa* (78.71%) and *Azadirachta indica* (74.07%). Kakad *et al*. (2019) revealed that highest mycelial growth inhibition of *A. flavus* was recorded at 10% concentration with *Allium sativum* (85.32%), followed by *Curcuma longa* (57.94%), *Zingiber officinalis* (54.76%), *Azadirachta indica* (51.98%) and *Ocimum sanctum* (22.61%). Shricharan *et al*. (2020) found that the maximum inhibition in garlic (80.00%) at 20% followed by tulsi (71.87%), green chilli (69.23%) and ginger (62.68%). Vineela *et al*. (2020) reported that the *Allium sativum* proved the most effective botanical and recorded cent per cent reduction in growth of *Aspergillus* spp. *in vitro* followed by *Annona squamosa* (55.13%), *Lantana camara* (43.47%), *Allium cepa* (40.70%), *Zingiber officinale* (27.73%), *Azadirachta indica* (20.70%) and *Curcuma longa* (18.46%) at 20% concentration.

Evaluation of different bio-agents against *A. flavus in vitro*

The present investigation was carried out to evaluate the *in vitro* efficacy of twelve isolates of *Trichoderma* spp. obtained from groundnut growing farmer's field and six bacterial bio-agents (Dept. of plant pathology) against most aflatoxigenic (AF-6) strains of *A. flavus* by dual culture method.

The results presented in Table 4 revealed the significant difference in the growth inhibition of all the antagonist. Among the tested fungal and bacterial antagonist, the fungal antagonist found superior over bacterial antagonist. It was evident from these studies that among all the *Trichoderma* spp. evaluated, the maximum growth inhibition (77.72%) was found in isolates T_3 (*Trichoderma viride*, Mahadeviya) which was at par with T_5 (*Trichoderma harzianum*, Chandisar) (74.47%). Next effective isolate was T_6 (*Trichoderma viride*, Laxmipur) (73.75%) which was statistically at par with T_9 (*Trichoderma harzianum*, Aanjna) (73.59%) and T_1 (*Trichoderma viride*, Dangi-

ya) (72.97%) consistently showed strong antagonistic activity against *A. flavus* which was significantly superior over the rest. Whereas least in T_{10} (*Trichoderma viride*, Jambusar) (47.53%) which was at par with T_7 (*Trichoderma viride*, Himatpur) (49.73%). In case of bacterial bio-agents T_{15} (*Bacillus subtilis*) (39.88%) was potential antagonists followed by T_{14} (*Pseudomonas fluorescens* II) (27.94%) which was at par with T_{13} (*Pseudomonas fluorescens* I) (27.36%) and T_{16} (*Bacillus amyloliquefaciens*) (27.08%), whereas least in T_{17} (*Pseudomonas stutzeri*) (15.43%) which was at par with T_{18} (*Pseudomonas putida*) (19.16%).

Table 4. Evaluation of different bio-agents against *A. flavus in vitro*. Figures in parentheses are retransformed values of arc sine transformed values. Treatment means with the letter(s) in common are not significant by DNMRT at 5% level of significance.

Tr. No.	Bio-agents	% Growth inhibition over control
T_1	*Trichoderma viride* (Dangiya)	58.68^{bc} (72.97)
T_2	*Trichoderma viride* (Navi Bhildi)	52.90^{ef} (63.61)
T_3	*Trichoderma viride* (Mahadeviya)	61.84^{a} (77.72)
T_4	*Trichoderma harzianum* (Bhabar Nava)	46.53^{g} (52.68)
T_5	*Trichoderma harzianum* (Chandisar)	59.65^{ab} (74.47)
T_6	*Trichoderma viride* (Laxmipur)	59.18^{bc} (73.75)
T_7	*Trichoderma viride* (Himatpur)	44.85^{gh} (49.73)
T_8	*Trichoderma viride* (Himatnagar)	50.61^{f} (59.74)
T_9	*Trichoderma harzianum* (Aanjna)	59.08^{bc} (73.59)
T_{10}	*Trichoderma viride* (Jambusar)	43.58^{h} (47.53)
T_{11}	*Trichoderma viride* (Bayad)	56.81^{cd} (70.04)
T_{12}	*Trichoderma harzianum* (Geendava)	54.46^{de} (66.22)
T_{13}	*Pseudomonas fluorescens* I	31.54^{j} (27.36)
T_{14}	*Pseudomonas fluorescens* II	31.91^{j} (27.94)
T_{15}	*Bacillus subtilis*	39.16^{i} (39.88)
T_{16}	*Bacillus amyloliquefaciens*	31.36^{j} (27.08)
T_{17}	*Pseudomonas stutzeri*	23.13^{k} (15.43)
T_{18}	*Pseudomonas putida*	25.96^{k} (19.16)
T_{19}	Control	4.05^{l} (0.50)
SEm +		0.84
CD at 5%		2.37
CV%		3.80

Such differential efficacy of bioagents were reported by various research worker. Anjaiah *et al*. (2006) reported that inoculation of selected antagonistic strains fluorescent *Pseudomonads, Bacillus* and *Trichoderma* spp. on groundnut have shown significant reduction of seed infection by *A. flavus*. Bagwan *et al*. (2011) reported that the combination of *T. viride, B. subtilis* and *P. fluorescens* were found effective in reducing *A. flavus* rhizospheric population, per cent incidence of afla root infection and colonization of kernels and aflatoxin B_1 content. Bhushan *et al*. (2013) evidenced that *B. subtilis* was highly effective in reducing the growth of *A. flavus* than *P. fluorescens*. Ranganathswamy *et al*. (2017) found *Trichoderma harzianum* inhibited maximum mycelial growth of *A. flavus*, whereas least inhibition was observed in *Pseudomonas fluorescens*.

CONCLUSION

It is concluded from the present investigation that the garlic clove extract was most effective among the botanical fungicides tested followed by neem leaves and onion bulb extracts, among the different bio-agents tested *Trichoderma viride* was most effective as fungal bioagents, whereas bacterial bio-agents *Bacillus Subtills* was most effectively inhibited the growth of *A. flavus in vitro*.

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