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# Study of antifungal activity of leaf extract of some wild plants of Asteraceae on *Aspergillus flavus*

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**ABSTRACT**

Aflatoxins have great significance due to health hazards to men and other useful animals. The aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus*. Recently, the use of natural products like cinnamon and clove oil has also been suggested for the inhibition of aflatoxin production as well as growth of *A. parasiticus*. In view of the known antifungal properties of plant extracts, an attempt has been made in the present investigation to explore the Antifungal Activity of Plant extracts against growth of *A. flavus* by some wild plants of family Asteraceae, *viz., Ageratum conyzoides, Carthamus oxycantha, Eclipta alba, Echinops echinatus, Launea nudicaulis, Lactuca dissecta, Parthenium hysterophorus, Sonchus aspera, Vernonia cinerea* and *Xanthium strumarium* have been studied against growth of toxigenic strain of *A. flavus* by standard cup method on solid medium. The diameter of zone of inhibition was measured in mm and percent inhibition in growth was calculated. Out of three concentrations *viz.,* 10, 15 and 20 per cent of leaf extracts, the 20 per cent concentration was found most effective. Further, hot water extract of leaf was adjudged more inhibitory as compared to cold water extract of plants. The maximum inhibition (70%) in growth of test fungus was caused by hot water leaf extract of *A. conyzoides*. It also can be concluded that leaf extract of different experimental plants have antifungal activity to varying extents.

**Keywords:** Asteraceae, Aflatoxin, Antifungal, Wild plants, Leaf extract.

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## INTRODUCTION

In the recent past, study of mycotoxins has gained a great significance as most of them are known to pose health hazards in men and domestic animals. More than a dozen important mycotoxins with their derivatives have been reported to contaminate different types of food materials (Bennett and Klich, 2003).

Among these, aflatoxins are extremely important, owing to the highest degree of carcinogenic potency (Sarma *et al.*, 2017). The aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus* but the occurrence of later is quite less in Indian sub continent. Due to deleterious effects of aflatoxin, attention is now, therefore, directed towards achieving effective control measures against aflatoxin production on food commodities. Several physical, chemical and biological methods of control have been proposed (Kumar *et al*. 2017).

Several plants are known for their medicinal and antifungal properties since ancient times. Their use in controlling many fungal and bacterial diseases have been reported by various workers (Sharma, 2002; Singh, 2009).Recently, the use of natural products like cinnamon and clove oil has also been suggested for the inhibition of aflatoxin production as well as growth of *Aspergillus* species (Alpsoy, 2010). Considering these facts, in the present investigation, effect of extract of 10 wild plants of family Asteraceae on growth of toxigenic strain of *A. flavus* was studied by standard cup method.

## MATERIALS AND METHODS

Ten wild plants of family Asteraceae *viz., Ageratum conyzoides, Carthamus oxycantha, Eclipta alba, Echinops echinatus, Launea*

*nudicaulis, Lactuca dissecta, Parthenium hysterophorus, Sonchus aspera, Vernonia cinerea* and *Xanthium strumarium* have been screened in the present investigation for their anti-aflatoxigenic nature. Leaves of above mentioned plants have been collected from fields of District Agra in sterilized polythene bags and stored at 4°C in refrigerator till processed. In most of the cases, samples were processed within two days but never later than a week of their collection.

**Preparation of plant extracts**

In each case, 100 g of fresh plant sample of above mentioned plants were washed twice with tap water and sterilized distilled water. Subsequently, cold and hot water extracts were prepared according to the method of Gerard Ezhilon *et al*. (1994). In this method for cold water extraction, the surface sterilized 100 g. leaf of plant were crushed in 500 mL distilled water (2/10, w/v) with mixie. Later, macerates were squeezed through double layered cheese cloth and then centrifuged at 5000 r.p.m. for 5 minutes. The supernatant was filtered through Whatman No.1 filter paper and then sterilized by passing through the Seitz filter (G-5). The extract (20%) thus obtained was used for the in vitro experiments. The clear supernatants were diluted with sterile distilled water to arrive at the required concentrations (10% and 15%). The plant part extracts obtained in this way were stored at 4°C for further use.

For hot water extraction, 100 g of sterilized leaf sample of each plant was chopped and plunged in required quantity of water (2/10, w/v) taken in a beaker and heated over a water bath at 80°C for 10 minutes. The materials were then processed with music and strained through cheese cloth. It gave the standard plant extract solution (20%). The extracts were subjected to low speed centrifugation (5000 rpm for 5 min) and the clear supernatants were diluted with sterile distilled water to arrive at the concentration of 10% and 15%. The plant extracts obtained in this way were stored at 4°C for further use.

**Preparation of spore suspension**

Highly aflatoxigenic strain of *A. flavus* (ATCC-15517) was sub-cultured on potato dextrose agar medium from the stock culture. The slants were incubated for seven days at 28±1°C. The harvesting of the spores and preparation of spore suspension @ 10 spores/mL was carried out according to the method of Hesseltine *et al*. (1966).

**Antiaflatoxigenic activity of plant extracts**

It was studied by the Standard Cup Assay Method. In this method, 1 ml spore suspension of toxigenic strain of *A. flavus* was transferred by separate sterile pipettes under aseptic conditions to seed the assay plates. The sterilized and luke warm PDA medium was poured into Petri-plates at the rate of 20 ml/ plate, The plates were gently shaken to disperse the spore suspension uniformly in the medium. After allowing the medium to solidify, cups were prepared by using 4 mm cork borer. The various plant extracts (10%, 15% and 20%) were added to cups in triplicate and then incubated at 28±1°C for four days. After incubation period, the diameter of zone of inhibition was measured in mm and percent inhibition in growth was calculated as suggested by Misra and Dixit (1977).

**Statistical analysis**

Data analysis of results was performed through one way ANOVA (Analysis of Variance) at value $p<0.05$ followed by Tukey's Post Hoc test with $p\leq0.05$ was used to determine the significant differences between the results obtained in each experiment.

**RESULTS AND DISCUSSION**

The leaf extracts of these plants were tested on growth of toxigenic strain of *A. flavus* (ATCC 15517) by standard cup method on solid medium. Three concentrations *viz.*, 10, 15 and 20 percent of leaf extracts in cold and hot water were used.

The perusal of Tables 1 and 2 indicates that leaf extracts of different plants inhibited growth of *A. flavus* to varying extent. The hot water extract of leaf was found to be more inhibitory as compared to cold water extract. The maximum inhibition in growth of *A. flavus* was 70 per cent and it was noted due to 20 per cent concentration of hot water leaf extract of *Ageratum conyzoides*. The cold water leaf extract of this plant at 20 per cent

concentration could reduce the growth of *A. flavus* to the extent of 66 per cent. The next effective plant was *V. cinerea*, which inhibited the growth of test fungus to the extent of 66 per cent in case of hot water leaf extract, while cold water extract of this plant inhibited the growth up to 64.4 per cent.

Table 1. Effect of cold water leaf extract of plants on growth of *A. flavus* (Standard cup method)

| Name of plant | Zone of inhibition (in mm) | | | F – value | Inhibition Percentage | | |
|---|---|---|---|---|---|---|---|
| | 10% | 15% | 20% | | 10% | 15% | 20% |
| *Ageratum conyzoides* | 38.7±0.3512$^{a}$ | 45.0±0.2645$^{b}$ | 60.2±0.5291$^{c}$ | 774.2528 | 43.0 | 50.0 | 66.0 |
| *Carthamus oxycantha* | 21.2±0.4163$^{a}$ | 23.2±0.4358$^{ab}$ | 25.2±0.5291$^{b}$ | 18.653 | 23.5 | 25.7 | 28.0 |
| *Eclipta alba* | 34.2±0.4163$^{a}$ | 36.0±0.2645$^{b}$ | 38.7±0.3605$^{c}$ | 41.2223 | 38.0 | 40.0 | 43.0 |
| *Echinops echinatus* | 12.5±0.2886$^{a}$ | 14.4±0.3000$^{b}$ | 18.9±0.4582$^{c}$ | 84.552 | 14.0 | 16.0 | 21.0 |
| *Launea nudicaulis* | 10.0±0.2886$^{a}$ | 13.0±0.2645$^{b}$ | 15.2±0.5291$^{c}$ | 47.1688 | 11.0 | 14.4 | 17.0 |
| *Lactuca dissecta* | 15.2±0.4163$^{a}$ | 16.2±0.4358$^{a}$ | 18.9±0.4582$^{b}$ | 19.1689 | 17.0 | 18.0 | 21.0 |
| *Parthenium hysterophorus* | 20.5±0.2886$^{a}$ | 23.4±0.3000$^{b}$ | 25.2±0.5291$^{c}$ | 37.2144 | 22.7 | 26.0 | 28.0 |
| *Sonchus aspera* | 35.2±0.4163$^{a}$ | 38.7±0.3000$^{b}$ | 46.5±0.3605$^{c}$ | 255.2354 | 39.1 | 43.0 | 52.0 |
| *Vernonia cinerea* | 38.7±0.3512$^{a}$ | 40.5±0.7767$^{a}$ | 58.0±0.1154$^{b}$ | 460.7703 | 43.0 | 45.0 | 64.4 |
| *Xanthium strumarium* | 32.4±0.3055$^{a}$ | 38.6±0.2645$^{b}$ | 43.2±0.5291$^{c}$ | 198.7574 | 36.0 | 43.0 | 48.0 |

Control growth – 90±0.00 mm in diameter; Note: The values represent the mean ± SEM of triplet experiments. Statistical analysis through one way ANOVA at value $p<0.05$ followed by Tukey's Post Hoc test with $p\leq0.05$ was used. The means with different alphabets are significantly different with each other as indicated by Tukey's Post Hoc test (row by row analysis) at alpha = 0.05.

This study revealed that four plants *viz., A. conyzoides, V. cinerea, S. aspera* and *X. strumarium* showed more than 50 per cent inhibition in growth of *A. flavus*. The remaining plants *viz., C. oxycantha, E. alba, E. echinatus, L. nudicaulis, L. dissecta and P. hysterophorus* showed 30%, 45%, 21%, 16%, 26% and 30% inhibition respectively in case of hot water leaf extract at 20% conc. Among these, *E. alba* was found to be little better than others in inhibiting Growth of *A. flavus*.

It is noteworthy to mention here that on the basis of hot water leaf extract the antifungal activity of the plants studied can be graded in descending order as *A. conyzoides* > *V. cinerea* > *S. aspera* > *X. strumarium* > *E. alba* > *P. hysterophorus* > *L. dissecta* > *C. oxycantha* > *L. nudicaulis* > *E. echinatus.*

It is interesting to note that leaf extracts of all experimental plants showed inhibitory effect on growth of *A. flavus* in solid media. Several workers like Varshney *et al.* (2001), Gautam *et al.* (2003). Reddy *et al.* (2007) and Suurbaar *et al.* (2017) noted antifungal properties of leaf extracts of many flowering plants. *A.conyzoides* was found to be most effective followed by *V. cinerea* and *S. aspera*. Kamboj and Saluja (2008) reported that extract of *Ageratum* species was most effective for inhibition in growth of fungus. Likewise, Fiori *et al.* (2000) reported that crude extract of *A. conyzoides* was more effective in inhibiting the mycelial growth of *Didymella bryoniae*, whereas the essential oil of *A. conyzoides* provided 100 per cent inhibition in the mycelial growth and germination of spores of *D. bryoniae*, which supports the findings of present investigation in relation to *A. flavus*. Ilondu (2013) also described phytochemical composition and antifungal activity of leaf extracts of *V. cinerea* against phytopathogenic fungi. Another plant of family Asteraceae viz., *P. hysterophorus* showed about 40 per cent inhibition in growth of *A. flavus*. Kurucheve *et al.* (1997) also reported that cold water leaf extract of *P. hysterophorus* inhibited the growth of *Rhizoctonia solani* up 45 per cent. Rangarajulu *et al.* (2003) studied fungitoxic effects of certain plants on *Colletotrichum capsici* and noted cent per cent inhibition of conidial germination by extract of *Argemone mexicana* and *P. hysterophorus*. Likewise, Dhaliwal *et al.* (2002) studied effect of essential oils obtained from 10 different plants on spore germination of *Uncinula* nectar.

Table 2. Effect of hot water leaf extract of plants on growth of *A. flavus* (Standard cup method)

| Name of plant | Zone of inhibition (in mm) | Zone of inhibition (in mm) | Zone of inhibition (in mm) | F - value | Inhibition Percentage | Inhibition Percentage | Inhibition Percentage |
|---|---|---|---|---|---|---|---|
| | Conc. 10% | Conc. 15% | Conc. 20% | | Conc. 10% | Conc. 15% | Conc. 20% |
| *Ageratum conyzoides* | 40.5±0.2887[a] | 46.6±0.3055[b] | 63.0±1.0263[c] | 330.2597 | 45 | 52 | 70 |
| *Carthamus oxycantha* | 23.4±0.3055[a] | 25.2±0.4163[ab] | 27.0±0.5292[b] | 17.7804 | 26 | 28 | 30 |
| *Eclipta alba* | 36.0±0.5774[a] | 38.7±0.3512[b] | 40.5±0.2887[b] | 28.4995 | 40 | 43 | 45 |
| *Echinops echinatus* | 12.6±0.3055[a] | 16.2±0.7572[b] | 18.9±0.4933[c] | 32.933 | 14 | 18 | 21 |
| *Launea nudicaulis* | 9.9±0.6658[a] | 12.6±0.2082[b] | 14.4±0.611[b] | 17.8944 | 11 | 14 | 16 |
| *Lactuca dissecta* | 16.0±0.5[a] | 18.9±0.4933[b] | 23.4±0.7024[c] | 42.2726 | 17.7 | 21 | 26 |
| *Parthenium hysterophorus* | 23.4±0.3055[a] | 25.0±0.5774[ab] | 27.0±1.0408[b] | 6.4634 | 26 | 28 | 30 |
| *Sonchus aspera* | 36.0±0.7311[a] | 40.5±0.2887[b] | 46.6±0.3055[c] | 121.8773 | 40 | 45 | 52 |
| *Vernonia cinerea* | 40.5±0.2887[a] | 45.0±0.5[b] | 60.2±0.9866[c] | 244.6636 | 45 | 50 | 66 |
| *Xanthium strumarium* | 34.2±0.4163[a] | 39.6±1.2288[b] | 45.0±0.5292[c] | 44.5565 | 38 | 44 | 50 |

Control growth – 90±0.00 mm in diameter; Note: The values represent the mean ± SEM of triplet experiments. Statistical analysis through one way ANOVA at value $p<0.05$ followed by Tukey's Post Hoc test with $p\leq0.05$ was used. The means with different alphabets are significantly different with each other as indicated by Tukey's Post Hoc test (row by row analysis) at alpha = 0.05.

Among these plants, oil of *P.* hysterophorus was also found inhibitory to spore germination of *Uncinula* nectar and the extract of this plant was also found inhibitory to growth of *A. flavus* in the present investigation. Sharma (2002) indicated that growth of toxigenic strain of A. flavus in terms of dry weight of mycelium was decreased by all the plant extracts tested and maximum inhibition (44.2%) was caused by extract of *Allium sativum* and the minimum inhibition (23.5%) was shown by extract of *Euphorbia hirta*. Reddy *et al.* (2007) reported 46 per cent control of leaf spot of Mulberry caused by *Cercospora moricola* by leaf extract of *P. hysterophorus*. It is suggested that 'parthenin' a constituent of *P. hysterophorus* is having fungitoxic and herbicidal activity.

The results of present study indicated that leaf extracts of all the ten tested plants possessed antiaflatoxigenic potential under laboratory conditions and could be used as botanical fungicides for the management of *A. flavus*. However, this was a preliminary investigation and in further study, active compounds present in these leaf extracts as well as effectiveness of combinations of these plant extracts could be investigated.

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**REFERENCES**

Alpsoy, L. 2010. Inhibitory effect of essential oil on aflatoxin activities. *African Journal of Biotechnology* **9**: 2474-2481.

Bennett, J. W., and Klich, M. 2003. Mycotoxins. *Clinical Microbiology Reviews*, **16**(3): 497–516. https://doi.org/10.1128/cmr.16.3.497-516.2003

Dhaliwal, H. S., Thind, T. S., Chander, M., and Chhabra, B. R. 2002. Activity of some essential oils against *Uncinula* nectar causing powdery mildew of grapevine. *Indian Phytopathology* **55**(4):529-531.

Ezhilan, G. J., Chandraekar, V., and Kurucheve, V. 1994. Effect of six selected plant products and oilcakes on the sclerotial production and germination of *Rhizoctonia solani*. *Indian Phytopath* **47**: 183-185.

Fiori, A. C. G., Schwan-Estrada, K. R. F., Stangarlin, J. R., Vida, J. B., Scapim, C. A., Cruz, M. E. S., and Pascholati, S. F. 2000. Antifungal activity of leaf extracts and essential oils of some medicinal plants against *Didymella bryoniae*. *Journal of Phytopathology* **148**: 483-487.

Gautam, A. K., and Bhadauria, R. 2008. Occurrence of Toxigenic moulds and mycotoxins in Ayurvedic medicine Triphala churn. *Journal of Mycology and Plant Pathology* **38**(3): 664-666.

Hesseltine, C. W., Shatwell, O. L., Ellis, J. J., and Stubblefield, R. D. 1966. Aflatoxin formation by Aspergillus flavus. *Bacteriological Reviews* **30**: 395-805.

Ilondu, E. M. 2013. Phytochemical composition and efficacy of ethanolic leaf extracts of some *Vernonia* species against two phytopathogenic fungi. *Journal of Biopesticides* **6**(2):165-172.

Kamboj, A., and Saluja, A. K. 2008. *Ageratum conyzoides* L.: A review on its phytochemical and pharmacological profile. *International Journal of Green Pharmacy* 59 – 68.

Kumar, P., Mahato, D. K., Kamle, M., Mohanta, T. K., and Kang, S. G. 2017. Aflatoxins: A Global Concern for Food Safety, Human Health and Their Management. *Frontiers in Microbiology* **7**: 2170. https://doi.org/10.3389/fmicb.2016.02170

Kurucheve V., Gerard E. J., and Jayaraj J. 1997. Screening of higher plants for fungitoxicity against *Rhizoctonia solani* in vitro. Indian Phytopythology **50**(2): 235-241.

Misra, S. B., and Dixit, S. N. 1977. Fungicidal properties of *Clematis gouriana*. *Indian Phytopathology* **30**: 577-579.

Rangarajulu, S. K., Gomathi, V., and Kannabiran, B. 2003. Fungitoxic effects of root extracts of certain plant species on *Colletotrichum capsici* causing anthracnose in *Capsicum annuum*. *Indian Phytopath.* **56** (1): 114-116.

Reddy, C. S., Reddy, K. R. N., Prameela, M., Mangala, U. N., and Muralidharan, K. 2007. Identification of antifungal component in clove that inhibits *Aspergillus* spp. Colonizing rice grains. *Journal of Mycology and Plant Pathology* **37**(1): 87-94.

Sarma, U. P., Bhetaria, P. J., Devi, P. and Varma, A. 2017. Aflatoxins: Implications on Health. *Indian Journal of Clinical Bio Chemistry* **32**(2):124–133.DOI 10.1007/s12291-017-0649-2.

Sharma, A. 2002. Studies on effect of some medicinal plant extracts on aflatoxin production and growth of *Aspergillus flavus*. Ph D. Thesis, Dr. B.R.A. University of Agra.

Singh, U. 2009. Studies on antiaflatoxigenic potential of some wild plants of Asteraceae. Ph. D. Thesis, Dr. B. R. Ambedkar University Agra.

Suurbaar, J., Mosobil, R., and Donkor, A. 2017. Antibacterial and antifungal activities and phytochemical profile of leaf extract from different extractants of *Ricinus communis* against selected pathogens. *BMC Res Notes,* **10**:660. https://doi.org/10.1186/s13104-017-3001-2.

Varshney, V. 2001. Effect of plant extracts on *Drechslera graminea*, the causal agent of stripe disease of barley. *Indian Phytopathology* **54**(1): 88-90.

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