
Antifungal potential of some essential oils as a fumigant against a stored grain fungus, *Aspergillus flavus*

Singh, M. P.¹, Mishra, A. K.² and Singh, R.^{3*}

¹House no-E-30, Mahadevpuram-Jharkhandi, PostKunraghat-Gorakhpur-273008, India; ²Regional Educational Officer, Gorakhpur, U.P., India; ³Department of Botany, Smt. Indira Gandhi Govt. PG College, Lalganj, Mirzapur, U.P., India.

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Abstract In the present investigation, 20 essential oils extracted from different angiospermic plant parts were screened against *Aspergillus flavus*. Among them, maximum percent mycelial inhibition was recorded with *Chenopodium* and *Trachyspermum* oil. Further physico-chemical properties of these two essential oils were also identified by using GLC. When MIC of these essential oil were tested, it was noted that at 100ppm act as fungistat and above 200- 400 ppm act as fungicide.

Keywords: *Aspergillus*, *Chenopodium*, *Trachyspermum*, Essential oil, GLC, MIC

Introduction

Storage fungi are the dominant type of moulds associated with stored food commodities. These fungi principally include species of genera *Aspergillus* and *Penicillium*. The serious economic nature of post-harvest diseases is evident from the fact that the cost of processing and marketing of most foods and vegetables greatly exceeds the value of raw commodity itself.

Fungal colonization can lead to undesirable effects on organoleptic quality of the grains through the production of volatile metabolites affecting the taste and smell. Production of mycotoxins by several fungi has added a new dimension to gravity of the problem (Richard *et al.*, 1989; Miri *et al.*, 2019). The mycotoxin problem is more acute in tropical countries like India where the high temperature and humid conditions prevail during major part of the year. *A. flavus* is able to produce aflatoxins in foods and feedstuffs (Rojas *et al.*, 2005). The post-harvest diseases have been carried out by different physical and chemical treatments. Physical treatments are capital intensive while chemical treatments create pesticidal pollution and wholesale mortality of many animal

*Corresponding Author: Singh, R.; Email: rashmiknpg@gmail.com

and plant species due to their non-biodegradable nature. To minimize the hazardous effects, attempts are being made to develop plant based pesticides. Essential oils extracted from plants have shown antimicrobial property (Bosquez-Molina *et al.*, 2010), low mammalian toxicity, and less environmental effects (Burt, 2004), eco-friendly and biodegradable properties (Tzortzakis and Economakis, 2007, Bomfim *et al.*, 2020). The research finding was investigated the use of essential oils extracted from different plants and plant parts to control *Aspergillus flavus*.

Material and methods

Isolation of essential oils

500gm of fresh parts of each plant were cut separately into small pieces and then thoroughly washed with sterilized water. The volatile fraction (essential oil) was isolated by hydrodistillation by Clevenger's apparatus. In case of essential oil bearing plants, the collecting funnel of the Clevenger apparatus showed two distinct layers-an upper oily layer and the lower aqueous layer. Both the layers were separated and the essential oils were stored in clean glass vials after removing water traces with the help of capillary tubes and anhydrous sodium sulphate. The percent recovery (w/v) of each oil was determined following Mishra and Dubey (1994) by the following formula:

$$\text{Percent recovery of oil} = \frac{\text{Volume of essential oil (ml)}}{\text{Weight of plant part (gm)}} \times 100$$

Antifungal activity of essential oil against Aspergillus flavus

The volatile antifungal activity of essential oil was tested by fumigation technique. Experiments were done in triplicates. Ten ml of PDA medium was pipetted to each petri dish. Open small plastic cup filled with cotton soaked test oils was put in the centre of petri dish containing medium separately to get requisite fumigation concentration. For control sets requisite amount of sterilized water in place of oil was kept in plastic cups. Fungal discs (4m in diameter) cut from the periphery of a seven day old culture of *Aspergillus flavus* were placed aseptically on both sides of the plastic cup into each petri dish of treatment and control sets. The percentage mycelial inhibition was calculated by the following formula:

$$\text{Percentage of mycelia inhibition} = \frac{dc-dt}{dc} \times 100$$

where,

dc = Average diameter of fungal colony in control sets
dt = Average diameter of fungal colony in treatment sets

Characterization of oils from *Chenopodium* and *Trachyspermum*

The volatile oils were analyzed by Gas liquid chromatography (GLC) for their chemical heterogeneity. The GLC of oils was done at the Regional Sophisticated Instrumentation Centre, Central Drug Research Institute, Lucknow.

Minimum inhibitory concentration (MIC) and nature of fungi toxicity

Minimum inhibitory concentration at which oils showed absolute fungi toxicity was determined by the fumigation technique as described previously. The fumigant concentrations of 50, 100, 150, 200, 300, and 400 ppm (v/v) were used. The observation was recorded on sixth day and percentage mycelia inhibition was calculated. Nature of toxicity (fungistatic/fungicidal effects) of the oils against the tested fungus was determined by following Thompson (1989). On the sixth day the inhibited discs were taken out of the petri dishes and re-inoculated to another sets of plates containing PDA medium. The growth of the inhibited fungal discs on fresh medium was observed.

Results

During screening of 20 essential oils of angiospermic plants against tested fungus (Table 1), most of the oils showed either poor (below 50%) or moderate (above 50% and below 100%) activity. However, essential oils from leaves of *Chenopodium ambrosioides* and seeds of *Trachyspermum ammi* (Figure 1) inhibited the growth of tested fungi completely. Therefore, they were selected for further studies. It was also evident as shown in Table 1 that the fungi toxicity of the essential oils is depended on the parts of plant tested.

The quality of fungi toxic oil isolated from leaves of *C. ambrosioides* and seeds of *T. ammi* were standardized by their various physicochemical properties (Table 2) and GLC (Figure 2). MIC of essential oils from leaves of *C. ambrosioides* and seeds of *T. ammi* were determined to find out their potential as post-harvest fumigants. In the present investigation, the MIC of *Chenopodium* and *Trachyspermum*'s oils was 100ppm (Table 3). At 100ppm, these oil showed fungistatic effect, but at above concentrations of 200, 300 and 400 ppm showed their fungicidal effect (Figure 3).

Table 1. Screening of essential oils of Angiospermic plants for their fungi toxicity against *Aspergillus flavus*

Angiospermic Plants	Family of plant	plant part from which essential oil isolated	percent recovery of oil	percent mycelia inhibition of test fungus
<i>Agelemarmelos (L) Correa</i>	Rutaceae	Leaf	0.2 ± 0.02	31.11 ± 8.31
<i>Ageratum conyzoides L.</i>	Asteraceae	Leaf	0.08 ± 0.016	25.78 ± 4.23
<i>Allium sativus L.</i>	Liliaceae	Clove	0.17 ± 0.25	47.77 ± 4.16
<i>Ammomum subulatum Roxb.</i>	Zingiberaceae	Leaf	0.51 ± 0.19	62.78 ± 6.98
<i>Anethum graveolens L.</i>	Apiaceae	Leaf	0.41 ± 0.076	53.89 ± 9.26
<i>Caesulia axillaris Roxb.</i>	Asteraceae	Leaf	0.2 ± 0.045	79.22 ± 7.23
<i>Callistemon lanceolatus DC.</i>	Myrtaceae	Leaf	0.63 ± 0.085	32.22 ± 1.57
<i>Chenopodium ambrosioides L</i>	Chenopodiaceae	Leaf	0.36 ± 0.04	100
<i>Cinnamomum camphara L</i>	Lauraceae	Leaf	0.04 ± 0.025	60.33 ± 7.52
<i>Citrus reticulata Blanco.</i>	Rutaceae	Leaf	0.19 ± 0.02	52.22 ± 5.67
<i>C. sinensis (L)</i>	Rutaceae	Leaf	0.14 ± 0.017	27.78 ± 5.67
<i>Curcuma longa (L) Koenig</i>	Zingiberaceae	Rhizome	0.15 ± 0.029	46.11 ± 5.49
<i>Cymbopogon citrates (DC)</i>	Poaceae	Leaf	0.5 ± 0.061	30 ± 10
<i>Elettaria cardamomum Maton</i>	Zingiberaceae	Leaf	0.1 ± 0.12	63.89 ± 2.83
<i>Eucalyptus citriodora Hook.</i>	Myrtaceae	Leaf	0.62 ± 0.085	69.44 ± 3.42
<i>Eupatorium cannabinum L.</i>	Asteraceae	Leaf	0.1 ± 0.061	13.33 ± 2.72
<i>Foeniculum vulgare Mill.</i>	Apiaceae	Leaf	0.32 ± 0.179	56.11 ± 10.39
<i>Foeniculum vulgare Mill.</i>	Apiaceae	Seed	1.60 ± 0.30	90.6 ± 6.71
<i>Trachyspermum ammi L.</i>	Apiaceae	Leaf	0.16 ± 0.03	22.22 ± 2.07
<i>Trachyspermum ammi L.</i>	Apiaceae	Seed	1.30 ± 0.04	100
<i>Zingiber officinale</i>	Zingiberaceae	Leaf	0.018 ± 0.01	71.67 ± 6.24
<i>Zingiber officinale</i>	Zingiberaceae	Rhizome	0.17 ± 0.015	50.56 ± 6.71

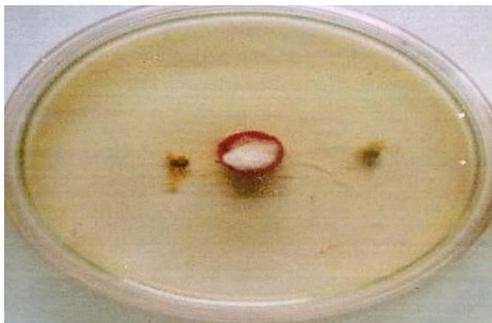


Figure 1. Volatile antifungal activity of essential oil by fumigation technique

Table 2. Physico-chemical properties of essential oils of *Chenopodium ambrosioides* and *Trachyspermum ammi*

Parameters	<i>Chenopodium oil</i>	<i>Trachyspermum oil</i>
Colour	Light pale yellow after storage turned raddish yellow	Light yellow after storage turned in brownish yellow
Odour	Pungent	Pungent
Specific Gravity	0.9890 at 25 ⁰ C	0.9360 at 25 ⁰ C
Optical rotation	-4 ⁰ 20' at 20 ⁰ C	-6 ⁰ at 20 ⁰ C
Refractive index	1.246 at 20 ⁰ C	1.420 at 20 ⁰ C
Solubility		
Acetone	Soluble (1:1 V/V)	Soluble (1:1 V/V)
Absolute	Soluble (1:1 V/V)	Soluble (1:1 V/V)
Alcohol	Soluble (1:1 V/V)	Soluble (1:1 V/V)
90% Alcohol	Soluble (1:1 V/V)	Soluble (1:1 V/V)
Benzene	Soluble (1:1 V/V)	Soluble (1:1 V/V)
Chloroform	Soluble (1:1 V/V)	Soluble (1:1 V/V)
Petroleum ether		
Acid number	5.2	6.5
Saponification Value	44.2	85
Ester Value	39	78.5
Phenolic Content	Absent	Present
pH	4.5	4.5

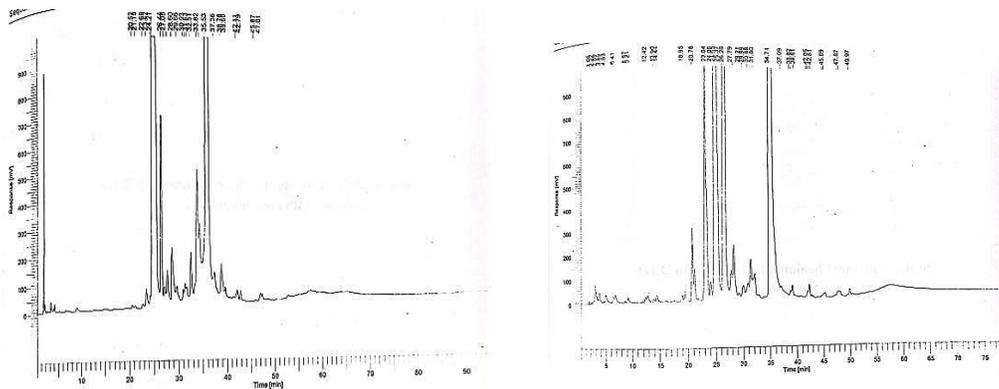


Figure 2. GLC of essential oils obtained from [A] *Chenopodium* [B] *Trachyspermum*

Table 3. Minimum inhibitory concentrations and fungi toxicity of oils of *Chenopodium* and *Trachyspermum* against *Aspergillus flavus*

Concentration in ppm	Percent mycelial inhibition of tested fungus \pm SD	
	<i>Chenopodium</i> oil	<i>Trachyspermum</i> oil
50	68.05 \pm 15.34	79.16 \pm 9.0
100	100*	100*
150	100*	100*
200	100**	100**
300	100**	100**
400	100**	100**

*Fungistatic effect, **Fungicidal effect

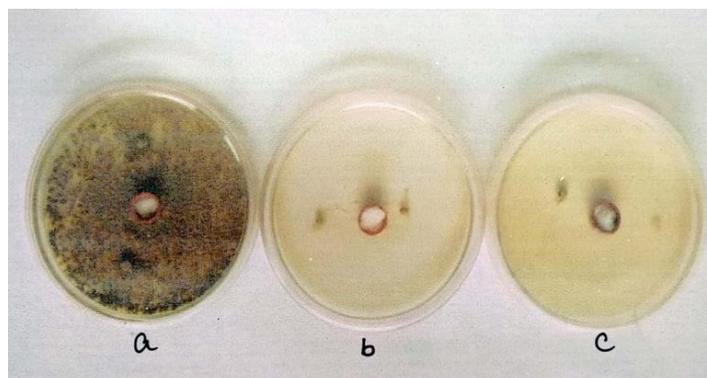


Figure 3. MIC experiment a. Control, b. *Chenopodium* oil, c. *Trachyspermum* oil

Discussion

Traditionally, the postharvest diseases have been controlled by spray of synthetic fungicides such as thiabendazole, imazalil and sodium ortho-phenyl phenate (Poppe *et al.*, 2003). The alternative control methods are needed because of negative public perceptions about the use of pesticides, development of resistance to fungicides and high development cost new chemicals (Bull *et al.*, 1997). In this respect, vapour emitting chemicals have been used with success against post-harvest disease of food commodities to be a better future over non-volatile chemicals (Dubey *et al.*, 2000). Antifungal property of the extracts or essential oils obtained from some plants against *A. flavus* has been evaluated (Kumar *et al.*, 2007). These authors reported that some plant oils and/or extracts could effectively inhibit the growth of *A. flavus*. Therefore, the research investigation, the volatile fraction of higher plants (essential oils) were screened for their toxicities to exploit as natural fumigants to control *A. flavus*. Essential oils can be qualitatively standardized by their various physicochemical properties (Guenther, 1972). It has been well demonstrated that concentration of different ingredients in the essential oils varies with growth stages, ecological conditions and the technique used for isolation of the oil from the plant (Mishra and Dubey, 1994). Therefore, the quality of an essential oil exhibiting biological properties must be standardized on the basis of its physicochemical properties (Dube *et al.*, 1989). It is found that oils of *Chenopodium* and *Trachyspermum* with identical physicochemical properties and GLC should be employed to control *A. flavus*.

References

- Bomfim, N. d. S., Kohiyama, C. Y., Nakasugi, L. P., Nerilo, S. B., Mossini, S. A. G., Romoli, J. C. Z., Mikcha, J. M.G., Filho, B. A. d. A. and Machinski Jr. M. (2020). Antifungal and antiaflatoxigenic activity of rosemary essential oil (*Rosmarinus officinalis* L.) against *Aspergillus flavus*. Food Additives and Contaminants: Part A, 37:153-161.
- Bosquez-Molina, E., Ronquillo-de Jesús, E., BautistaBanos, S., Verde-Calvoa, J. R. and Morales-López, J. (2010). Inhibitory effect of essential oils against *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* in stored papaya fruit and their possible application in coatings. Postharvest Biology and Technology, 57:132-137.
- Bull, C. T., Stack, J. P. and Smilanick, J.L. (1997). *Pseudomonas syringae* strains ESC-10 and ESC-11 survive in wound on citrus and control green and blue molds of citrus. Biological Control, 8:81-88.
- Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology, 94:223-253.
- Dube, S., Upadhyay, P. D. and Tripathi, S. C. (1989). Antifungal, physico-chemical and insect repellent activity of essential oil of *Ocimum basilicum*. Canadian Journal of Botany, 67:2085-2087.

- Dubey, N. K. (2000). Bioprospecting-option for India. *Current Science*, 78:369-370.
- Guenther, E. (1972). The production of essential oils, p. 87: In: E. Guenther (ed.). *The essential oils*. Krieger Publ. Co., Malabar, Fla.
- Kumar, R., Mishra, A. K., Dubey, N. K. and Tripathi, Y. B. (2007). Evaluation of *Chenopodiumambrosioides* oil as a potential source of antifungal, antiaflatoxic and antioxidant activity. *International Journal of Food Microbiology*, 115:159-164.
- Miri, Y. B., Belasli, A., Djenane, D. and Ariño, A. (2019). Prevention by essential oils of the occurrence and growth of *Aspergillusflavus* and Aflatoxin B1 production in food systems: Review. DOI: <http://dx.doi.org/10.5772/intechopen.88247>.
- Mishra, A. K. and Dubey, N. K. (1994). Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied and Environmental Microbiology*, 60:1101-1105.
- Poppe, L., Vanhoutte, S. and Höfte, M. (2003). Modes of action of *Pantoeaagglomerans* CPA-2, an antagonist of postharvest pathogens on fruits. *European Journal of Plant Pathology*, 109:963-973.
- Richard, J. L., Cole, R. J. and Archibalol, S. O. (1989). Mycotoxins, economic and health risks. Council Agriculture Science Technology Report, 116.
- Rojas, T. R., Sampayo, C. A. F., Vázquez, B. I., Franco, C. M. and Cepada, A. (2005). Study of interferences by several metabolites from *Aspergillus* spp. in the detection of aflatoxic strains in media added with cyclodextrin. *Food Control*, 16:445-450.
- Thompson, D. P. (1989). Fungitoxic activity of essential oil components on food storage fungi. *Mycologia*, 81:151-153.
- Tzortzakis, N. G. and Economakis, C. D. (2007). Antifungal activity of lemongrass (*Cymbopogoncitratu*s L.) essential oil against key postharvest pathogens. *Innovative Food Science and Emerging Technologies*, 8:253-258.

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