International Journal of Agricultural Technology 2021 Vol. 17(1):317-324
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

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*]

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

Singh, M. P., Mishra, A. K. and Singh, R. (2021). Antifungal potential of some essential oils as a fumigant against a stored grain fungus, *Aspergillus flavus.* International Journal of Agricultural Technology 17(1):317-324.

Abstract The 20 essential oils extracted from different angiospermic plants, *Agele marmelos* (L) Correa, *Ageratum conyzoides* L., *Allium sativus*,. *Ammomum subulatum* Roxb. *Anethum graveolens* L., *Caesuliaaxillaris* Roxb,. *Callistemon lanceolatus* DC., *Chenopodium ambrosioides* L., *Cinnamomumcamphara* L., *Cinnamomumcamphara* L., *Citrus reticulate* Blanc,. *Citrus sinensis* L., *Curcuma longa* L. Koenig, *Cymbopogan citrates* DC., *Elettaria cardamomum* Maton., *Eucalyptus citriodera* Hook., *Eupatorium cannabinum* L., *Foeniculum vulgare* Mill., *Trachyspermum ammi* L., *Zingiber officinale* were tested to screen against *Aspergillus flavus*. Among them, the maximum percent mycelial inhibition was recorded with *Chenopodium* and *Trachyspermum* oils. Further physico-chemical properties of these two essential oils were identified by using GLC. MIC of *Chenopodium* and *Trachyspermum* essential oils at 100-400 ppm actively against *A. flavus* (post harvest fungus) which proved to be fungistatic and fungicidal effects as control mechanism. It would be developed to be potential as post-harvest fumigants.

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).

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

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

Material and methods

Isolation of essential oils

The 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 aquous layer. Both 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 oils were determined following Mishra & 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 potato dextrose agar (PDA) medium was pipetted to each petriplate. Open small plastic cup filled with cotton soaked test oils was put in the centre of petriplates containing medium separately to get requisite fumigation concentration. For control sets requisited amount of sterilized water in place of oil was kept in plastic cups. Fungal discs of 4m in diameter were cut from the periphery of a seven day old

culture of *A. flavus*(a storage fungus) were placed aseptically on both sides of the plastic cup into each petri plate in 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 Chenopodium and Trachyspermum oil

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

Minimum inhibitory concentration (MIC) and nature of fungitoxicity

Minimum inhibitory concentration at which oils showed absolute fungitoxicity was determined by the fumigation technique as described previously. The fumigant concentration 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) of the oils against the test fungus was determined by following Thompson (1989). On the sixth day the inhibited discs were taken out of the petriplates 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 the tested fungus, *A. flavus* (Table 1). The most of tested essential oils from angiospermic plants, *Agelemarmelos* (L.) *Correa,Ageratum conyzoides* L., *Allium sativus,*. *Ammomum subulatum* Roxb. *Anethum graveolens* L., *Caesuliaaxillaris* Roxb,. *Callistemon lanceolatus* DC., *Chenopodium ambrosioides* L., *Cinnamomum camphara* L., *Cinnamomum camphara* L., *Citrus reticulate* Blanc,. *Citrus sinensis* L., *Curcuma longa* L. *Koenig, Cymbopogan citrates* DC., *Elettaria cardamomum* Maton. *Eucalyptus citriodera* Hook., *Eupatorium cannabinum* L., *Foeniculum vulgare* Mill.,

Trachyspermum ammi L., *Zingiber officinale* showed either poor (below 50%) or moderate (above 50% and below 100%) activities. However, essential oils from leaves of *Chenopodium ambrosioides* and seeds of *Trachyspermum ammi* (Figure 1) completely inhibited the growth of tested fungus. Therefore, they were selected for further studies. It is also showed an evident from Table1 that the fungitoxicity of the essential oils is depended on the parts of tested plants. The quality of fungitoxic oil isolated from leaves of *C.ambrosioides* and seeds of *T.ammi* was 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 in order to find out their potential as post-harvest fumigants. In the present investigation the MIC of *Chenopodium* and *Trachyspermum* oil was 100 ppm (Table 3). At 100 ppm of these oils showed fungistatic effect and concentrations of 200, 300 and 400 ppm expressed their fungicidal effect (Figure 3).

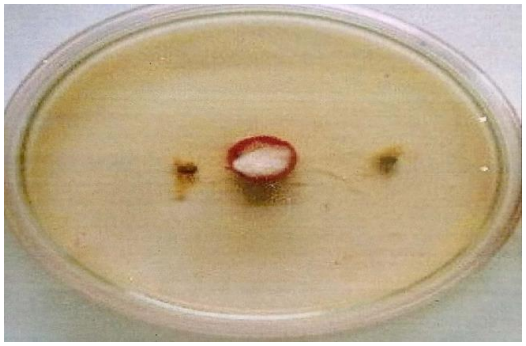

Figure 1. Volatile antifungal activity of essential oil by fumigation technique

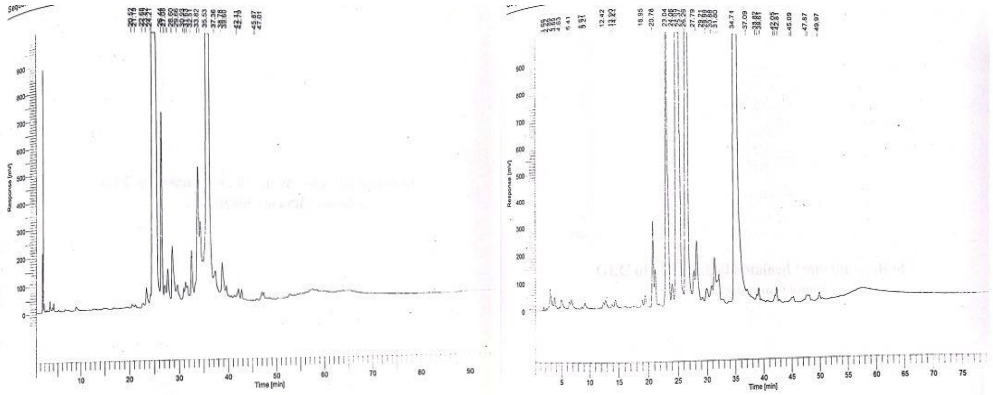

Figure 2. GLC of essential oils obtained from [**A**] *Chenopodium* [**B**] *Trachyspermum*

Table 1. Screening of essential oils of Angiospermic plants for their fungitoxicity 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**
Agelemarmelos (L) Correa	*Rutaceae*	*Leaf*	0.2 ± 0.02	*31.11* ± 8.31
Ageratum conyzoides L.	*Asteraceae*	*Leaf*	*0.08* ± 0.016	*25.78* ±4.23
Allium sativus L.	*Liliaceae*	*Clove*	*0.17* ± 0.25	*47.77* ± 4.16
AmmomumsubulatumRoxb.	*Zingiberaceae*	*Leaf*	*0.51* ± 0.19	*62.78* ± *6.98*
Anethum graveolens L.	*Apiaceae*	*Leaf*	*0.41* ± 0.076	*53.89* ±9.26
CaesuliaaxillarisRoxb.	*Asteraceae*	*Leaf*	*0.2* ± 0.045	*79.22* ± 7.23
Callistemon lanceolatus DC.	*Myrtaceae*	*Leaf*	*0.63* ± 0.085	*32.22* ± 1.57
Chenopodium ambrosioides L	*Chenopodiaceae*	*Leaf*	*0.36* ± 0.04	*100*
Cinnamomumcamphara L	*Lauraceae*	*Leaf*	*0.04* ± 0.025	*60.33* ±7.52
Citrus reticulate Blanco.	*Rutaceae*	*Leaf*	*0.19* ± 0.02	*52.22* ± 5.67
C.sinensis (L)	*Rutaceae*	*Leaf*	*0.14* ± 0.017	*27.78* ± 5.67
Curcuma longa (L)Koenig	*Zingiberaceae*	*Rhizome*	*0.15* ± 0.029	*46.11* ±5.49
Cymbopogan citrates (DC)	*Poaceae*	*Leaf*	*0.5* ±0.061	*30* ± 10
Elettaria cardamomum Maton	*Zingiberaceae*	*Leaf*	*0.1* ± 0.12	*63.89* ± 2.83
Eucalyptus citriodera Hook.	*Myrtaceae*	*Leaf*	*0.62* ± 0.085	*69.44* ± 3.42
Eupatorium cannabinum L.	*Asteraceae*	*Leaf*	*0.1* ± 0.061	*13.33* ± 2.72
Foeniculumvulgare Mill.	*Apiaceae*	*Leaf*	*0.32* ± 0.179	*56.11* ± 10.39
Foeniculumvulgare Mill.	*Apiaceae*	*Seed*	*1.60* ± 0.30	*90.6* ± 6.71
Trachyspermumammi L.	*Apiaceae*	*Leaf*	*0.16* ± 0.03	*22.22* ± 2.07
Trachyspermumammi L.	*Apiaceae*	*Seed*	*1.30* ± 0.04	*100*
Zingiber officinale	*Zingiberaceae*	*Leaf*	*0.018* ± 0.01	*71.67* ± 6.24
Zingiber officinale	*Zingiberaceae*	*Rhizome*	*0.17* ± 0.015	*50.56* ± 6.71

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

Parameters	***Chenopodium oil***	***Trachyspermum oil***
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 25^0C	0.9360 at 25^0C
Optical rotation	$-4^0$20' at 20^0C	-6^0 at 20^0C
Refrective index	1.246 at 20^0C	1.420 at 20^0C
Solubility		
Acetone	Soluble (1:1 V/V)	Soluble (1:1 V/V)
Absolute 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	Soluble (1:1 V/V)	Soluble (1:1 V/V)
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

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

Concentration (ppm)	**Percent Mycelial inhibition of test fungus ±SD**	
	Chenopodium oil	*Trachyspermum* oil
50	68.05 ± 15.34	79.16 ± 9.0
100	100*	100*
150	100*	100*
200	100**	100**
300	100**	100**
400	100**	100**

*Fungistatic effect, **Fungicidal effect

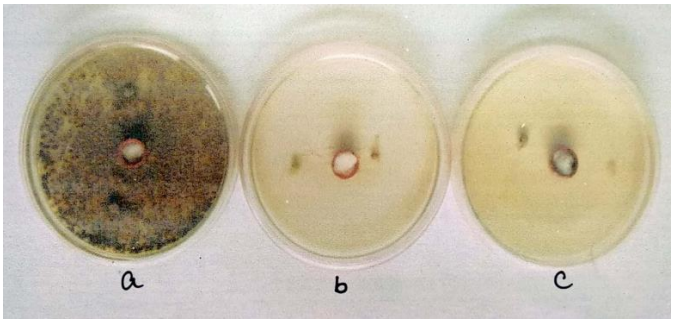

Figure 3. MIC experiment a. Control, b. *Chenopodium* oil, c. *Trachyspermum* oil

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 pathogen 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, and have a better future over non-volatile chemicals (Dubey *et al.,* 2000).Antifungal property of the extracts or essential oils obtained from some plants against *Aspergillus flavus*is evaluated (Kumar *et al*., growth of this pathogen fungus.

Therefore, in the present investigation the volatile fractions of essential oilsfrom plants were screened for their toxicity to be exploited as natural fumigants to control *A. flavus.* The essential oils were isolated from those parts of the plants, which were easily available for large-scale exploitation desired amount of oil may be isolated. 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 (Dubey *et al*., 1989). It is also suggested that for faithful reproduction of the result, oils of *Chenopodium* and *Trachyspermum* with identical physicochemical properties and GLC should be employed.

Oils of *Chenopodium* and *Trachyspermumwere* evaluated for their potency in terms of minimum inhibitory concentration (MIC). This aspect of investigation is necessary for any fungicide in order to prescribe appropriate dosage as the higher dosages may cause deleterious effects. The determination of fungitoxicant (fungistatic/ fungicidal) is of great practical value as it helps to regulate the time intervals of treatment. The result showed that the MIC of *Chenopodium* and *Trachyspermum* oils were 100ppm. At 100ppm these oil showed fungistatic effect ,and concentrationsof 200, 300 and 400 ppm showed their fungicidal effect. These findings would benefit to control a stored grain storage fungus, *A. flavus*.

References

Bomfim, N. d. S., Kohiyama, C. Y., Nakasugi, L. P., Nerilo, S. B., Mossini, S. A. G., Romoli, J. C. Z. R., Mikcha, J. M. G., Filho, B. A. de 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 Colletotrichumgloeosporioides and Rhizopusstolonifer 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, physic-chemical and insect repellent activity of essential oil of *Ocimumbasilicum*. Canadian Journal of Botany, 67:2085-2087.

Dubey, N. K. (2000). Bioprospecting-option for India. Current Science, 78:369-370.

Kumar, R., Mishra, A. K., Dubey, N. K. and Tripathi, Y. B. (2007). Evaluation of Chenopodium ambrosioides oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. International Journal of Food Microbiology, 115:159-164.

Kumari, A., Kumar, R., Maurya, S., Chowdhary, J. S. and Kumar, S. (2013). Antifungal efficacy of aquous extracts of Neem cake, karanj cake and vermicompost against some phytopathogenic fungi. The Bioscan, 8:671-674.

Miri, Y. B., Belasli, A., Djenane, D. and Ariño, A. (2019). Prevention by Essential Oils of the Occurrence and Growth of *Aspergillus flavus* 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, pp.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 aflatoxigenic 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 (*Cympopogoncitratus* L.) essential oil against key postharvest pathogens. Innovative Food Science and Emerging Technologies, 8:253-258.

,

(Received: 22 April 2020, accepted: 30 December 2020)