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Antifungal Activity of Aroma Chemicals against Graminicolous Seed Borne Fungi by Poisoned Food Method

A. R. Gulhane, G. K. Giri and Shilpa V. Khambalkar*

Sorghum Research Unit, Dr. PDKV, Akola - 444 104, Maharashtra, India

**Corresponding author*

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A B S T R A C T

In vitro inhibition of the five different aroma chemicals were studies against three seed borne fungal pathogen species in the concentration range of 500 and 1000 ppm. All aroma chemicals were found effective, the eugenol inhibited the maximum mycelial growth significantly which is followed by terpineol. The phenyl acetate showed least mycelial inhibition of the fungi. Over all in the present investigation, eugenol was found most effective aroma chemical against the species of *Curvularia, Drechslera* and *Bipolaris.*

Introduction

Gramineae is an important family which consist of all cereals i.e. rice, wheat, sorghum and pearl millet. These are used as a staple food all among the world. Rice is the most perfect staple food crop of the world. India is the largest rice growing country in the world and it's greatest consumer. In India, paddy is grown on about 42.75 million hectares which accounts for nearly 30 per cent of total area under cereals. Wheat is one of the major widely cultivated cereals food crops in India and world. It is one of the most important staple food of man. In India area production and productivity of wheat 30.00 million hectare and 93.50 million tonnes. Sorghum is the fifth major cereal crop in the World (Anonymous, 2014). Few fungi insect the cereal crops during grain development and seed borne. These are *Curvularia* species, *Drechslera* species, *Bipolaris* species, *Fusarium* species and *Aspergillius niger* (Islam *et al.,* 2009; Panchal and Dhale, 2011). Evaluated 16 aroma chemicals against *Drechslera sorokiniana, Phomopsis sojae, Fusarium solani, Colletotrichum graminicola* and *Macrophomia phaseolina* seed borne fungal pathogen at the concentration of 100 to 8000 ppm. Eugenol, methyl eagenol, citral, Z. phenethyl acetate and Z. phenethyl propinate were found to be highly effective against *Drechslera sorkiniana, Phomompsis sojae, Fusarium soloni, Colletotrichum graminicola* and *Microphomina phaseolina* with minimum inhibitory. Concentration value to be between

270 to 1704 ppm in poisoned food method. Eugenol is very effective against pathogenic seed borne fungl of rice viz., *F. moniliformi, A. solani, Bipolaris oryzae, Rhizoctonia solani, A. flavus* and *Curvularia lunata*. (Pitipong Thobunluepop *et al.,* 2007; Pitipong Thobunluepop *et al.,* 2009; Lalita *et al.,* 2010). Antifungal compound of EOS (Plant part) essential oil containing euqenol and terpineol were effective against *Curvularia lunata* in suppressing mycelial growth. Kocic tanackov and Dimic (2013).

The main objective of the present research were designed to study the efficacy of aroma chemical against graminicolous seed borne fungi viz., *Curvularia* spp., *Drechslera* spp. and *Bipolaris* spp.

Materials and Methods

Potato dextrose agar medium was prepared, and distributed in 100 and 250 ml conical flask and sterilized at 1.04 kg /m^2 for 15 min in autoclave. Requisite quantity of each of the aroma chemicals was added in sterilized melted (45^0C) PDA separately so as to obtain 500 and 1000 ppm concentration. Flask containing poisoned medium was shaked well to have even and uniform distribution of aroma chemical in to the medium. About 20 ml of melted poisoned PDA was poured in each sterilized petriplate and allowed to solidify. These petriplates were inoculated by test fungus separately. Five mm disc of one week old fungus lawn culture was cut with a sterilized cork borer, lifted and transferred aseptically at the centre of a petriplate containing the poisoned medium. The control plates were prepared same way without aroma chemicals. The edges of petriplates were sealed with parafilm and treated plates were incubated at 27 ± 2^0C for a period of seven days. Colony diameter was recorded in mm after seven days and per cent of mycelial growth inhibition was calculated. The data of mycelial growth was also subjected to statistical analysis and conclusions were drawn (Gomej and Gomej, 1984).

Results and Discussion

The significant differences were recorded due to aroma chemicals and isolates. Among the aroma chemicals, minimum mycelial growth was observed in eugenol (38.40 and 29.73 mm) at 500 and 1000 ppm respectively with maximum mycelial growth inhibition (57.14 and 66.81%) followed by terpineol (43.58 and 53.34%). Phenyl acetate showed least mycelial inhibition (13.23 and 17.80%). (Table 1 and Fig. 1)

Isolates of *Drechslera tetramera* obtained from various graminicolous crops was also evaluated against five aroma chemicals. (Table 2 and Fig. 2). Maximum mycelial growth was arrested in eugenol (33.66 and 24.55 mm) with highest inhibition i.e. 62.60 and 72.71% at 500 and 1000 ppm respectively followed by terpineol (53.56 and 62.84%).

Evaluation of different aroma chemicals against *Bipolaris sorghicola.* (Table 3 and Fig. 3)

The data presented in Table 3 showed highest mycelial inhibition of *Bipolaris* spp. in eugenol 62.74% (500 ppm) and 75.31% (1000 ppm), followed by terpineol 53.43 and 64.22%.

Results of poisoned food method indicates that, among all aroma chemicals, eugenol (500 and 1000 ppm) were found most effective for arresting the mycelial growth of all *Curvularia* spp., *Drechslera tetramera and Bipolaris* spp. followed by terpineol.

Least mycelial inhibition of all these fungi was recorded in phenyl acetate at 500 and 1000 ppm concentration.

Table.1 Mean of mycelial growth inhibition due to different aroma chemicals against various *Curvularia* spp.

Treatments (Aroma chemicals)	Radial mycelial growth (mm)	Mycelial growth inhibition (%)	Radial mycelial growth (mm)	Mycelial growth inhibition (%)	Mean mycelial growth inhibition (%)
	500 ppm	500 ppm	1000 ppm	1000 ppm	
Linalyl acetate	59.06	34.08	52.86	41.00	37.54
Benzyl benzoate	71.00	20.75	64.46	27.90	24.32
Phenyl acetate	77.24	13.23	73.66	17.80	15.51
Eugenol	38.40	57.14	29.73	66.81	61.97
Terpineol	50.55	43.58	41.8	53.34	48.46
Control	89.60	-	89.60	-	-

Table.2 Mean of mycelial growth inhibition due to different aroma chemicals against *Drechslera tetramera*

Treatments (Aroma chemicals)	Radial mycelial growth (mm)	Mycelial growth inhibition (%)	Radial mycelial growth (mm)	Mycelial growth inhibition (%)	Mean mycelial growth inhibition (%)
	500 ppm	500 ppm	1000 ppm	1000 ppm	
Linalyl acetate	46.22	48.64	39.77	55.80	52.22
Benzyl benzoate	52.89	41.23	45.66	49.26	45.24
Phenyl acetate	73.22	18.64	69.11	23.20	20.92
Eugenol	33.66	62.60	24.55	72.71	67.65
Terpineol	39.99	53.56	33.44	62.84	58.20
Control	90.00	-	90.00	-	-

Table.3 Mean of mycelial growth inhibition due to different aroma chemicals against various *Bipolaris* spp.

Treatments (Aroma chemicals)	Radial mycelial growth (mm)	Mycelial growth inhibition (%)	Radial mycelial growth (mm)	Mycelial growth inhibition (%)	Mean mycelial growth inhibition (%)
	500 ppm	500 ppm	1000 ppm	1000 ppm	
Linalyl acetate	50.25	43.38	43.65	50.81	47.09
Benzyl benzoate	62.75	29.29	56.49	36.34	32.81
Phenyl acetate	73.66	17.00	67.33	24.12	20.56
Eugenol	33.08	62.74	21.89	75.31	69.02
Terpineol	41.33	53.43	31.75	64.22	58.82
Control	88.75	-	88.75	-	-

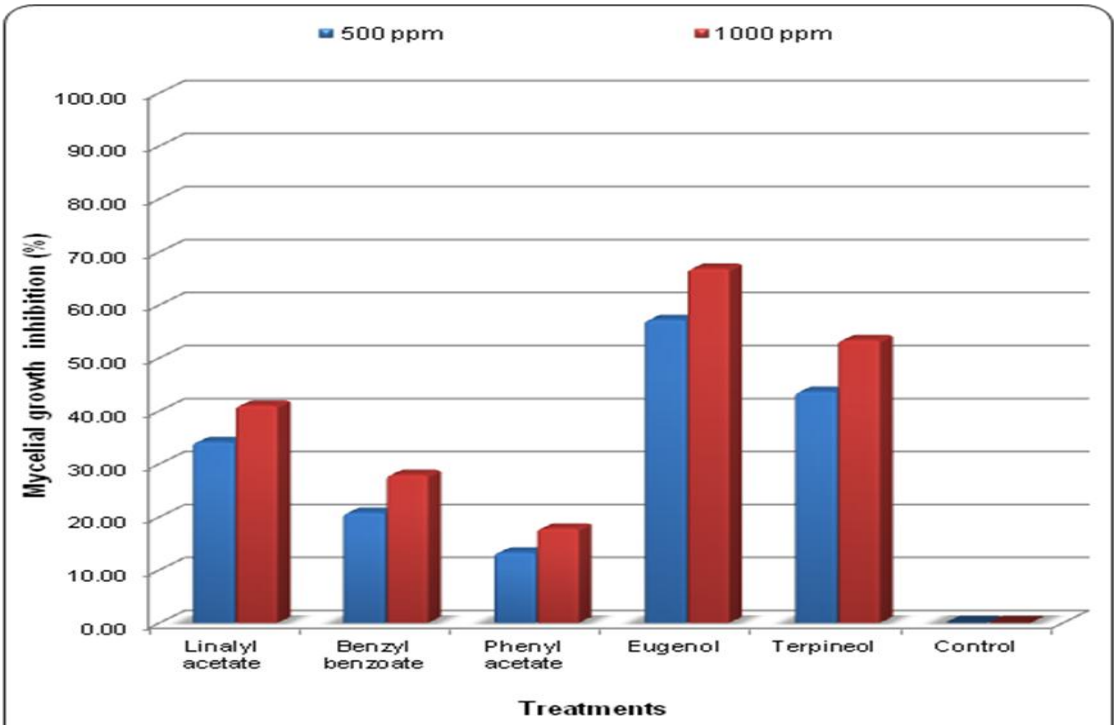

Fig. 1. Mean of mycelial growth inhibition due to different aroma chemicals against various *Curvularia* spp.

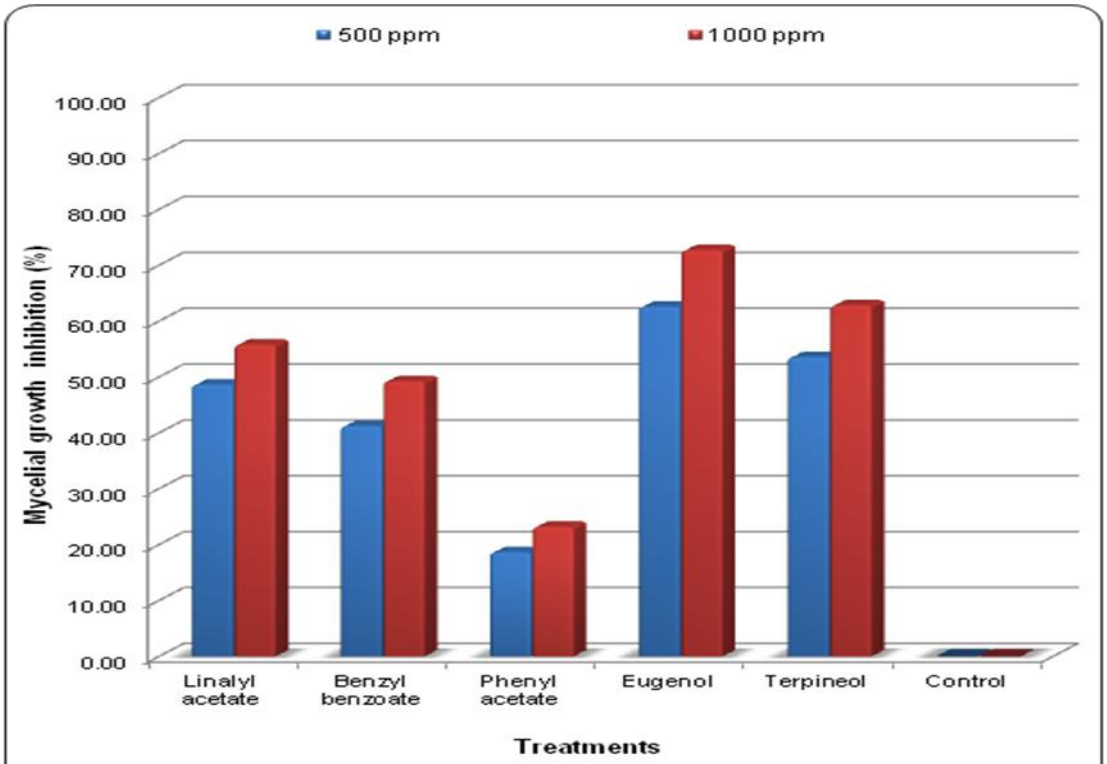

Fig. 2. Mean of mycelial growth inhibition due to different aroma chemicals against *Drechslera tetramera*

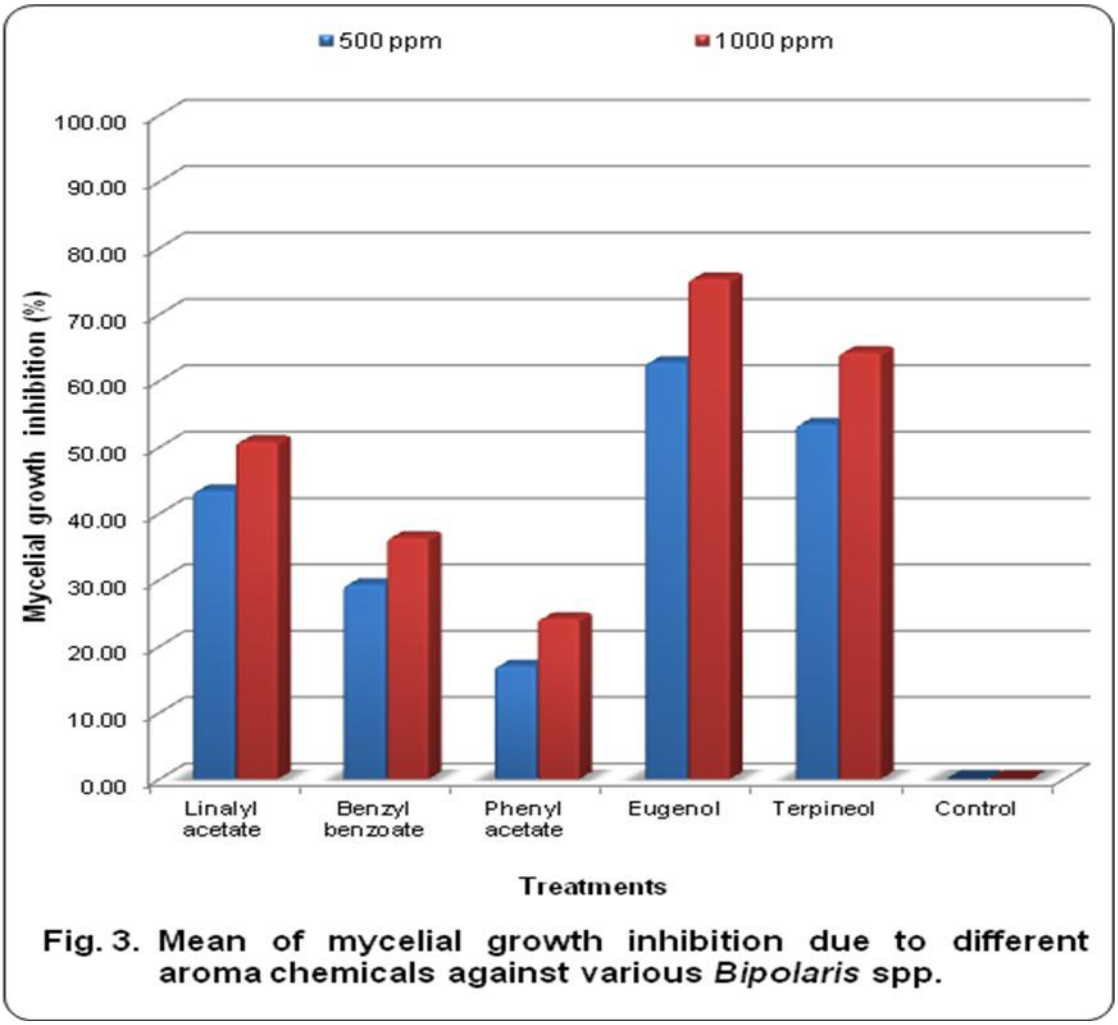

Fig. 3. Mean of mycelial growth inhibition due to different aroma chemicals against various *Bipolaris* spp.

The test aroma chemicals had significantly inhibited the radial mycelial growth of *Curvularia* spp., *Drechslera tetramera and Bipolaris* spp. except phenyl acetate and benzyl benzoate. These finding are similar with those of Shabana *et al.,* (2008) who reported antifungal activity against *Bipolaris oryzae* at different concentration, benzoic acid or salicylic acid at 9 mM completely inhibited the mycelial growth of *Bipolaris oryzae* tested by poisoned food method. The similar results were recorded by Patimaporn plodpai *et al.,* (2013) who reported that benzyl benzoate and benzyl hydroxybenzoate obtained from *Desmos chinensis* were antifungal against *Rhizoctonia solani* in rice. The present findings are in close accordance with those of Nguefack (2007) who observed eathanol extract containg phenolic and oxygenated terpenoids significantly reduced the incidence of *Bipolaris oryzae* in rice. The present results are in conformation with earlier workers, Janki Kandhari *et al.,* (2010) also observed that the aroma compound eugenol followed by terpineol were most effective against *Rhizoctonia solani* in rice.

The poisoned food method indicates that, among all aroma chemicals, eugenol (500 and 1000 ppm) were found most effective for arresting the mycelial growth of all *Curvularia* spp., *Drechslera tetramera and Bipolaris* spp. followed by terpineol. Least mycelial inhibition of all these fungi was recorded in phenyl acetate at 500 and 1000 ppm concentration. The test aroma chemicals

had significantly inhibited the radial mycelial growth of *Curvularia* spp., *Drechslera tetramera and Bipolaris* spp. except phenyl acetate and benzyl benzoate.

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