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Assessment of *Mentha piperita* L. Essential Oil as Green Pesticide against Fungal and Insect Infestation of Chickpea

Ashok Kumar[1*] and Nawal Kishore Dubey[2]

[1]*Department of Botany, Dr. Bhimrao Ambedkar Government Degree College, Maharajganj-273303, Uttar Pradesh, India*
[2]*Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi-221005, India*

**Corresponding author*

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A B S T R A C T

The study assessed *Mentha piperita* essential oil (MpEO) for its pesticidal efficacy against fungal and insect pests. The chemical profile of MpEO exhibited menthol (46.82%) as major components. The MIC of MpEO against toxigenic fungal isolate *Aspergillus flavus* LHPCA-08 was recorded at 0.5 mg/ml concentration and also checked aflatoxin B_1 production at 0.3 mg/ml concentration as well as showed broad spectrum fungitoxicity (1.0 mg/ml) against all the tested moulds. MpEO also exhibited significant antioxidant potential (IC_{50} 11.4 µg/ml) would be helpful to minimize lipid peroxidation. A noteworthy reduction in ergosterol content with increasing MpEO concentration indicates plasma membrane as the possible target site of antifungal action. The insecticidal activity of MpEO against pulse beetle (*Callosobruchus chinensis* L.) was directly related to concentration and exposure period. The 100% insect mortality of MpEO was found within two hours at 200 µg/L concentration, and also absolute mortality was recorded when exposed for ten hours at 1 µg/L concentration. The oviposition by *C. chinensis* was completely checked at 10 µl/L while F_1 emergence was completely inhibited at 200 µl/L. During *in situ* experiments, 92.08% protection of the chickpea from *C. chinensis* by MpEO showed superiority over malathion, where 89.34% protection was recorded. The MpEO showing potent antifungal, antiaflatoxigenic, antioxidant and insecticidal efficacy hence, may be recommended as plant based preservative in the management of postharvest losses by fungal and insects pests during storage.

Introduction

The production as well protection of food commodities is burning issue in developing countries to nourish such a huge population and about 70% extra food production will be required till 2050 to feed them (Parfitt *et al.*, 2010). Nearly one-third of the food commodities were lost every year after harvesting by various pests especially moulds and insects (Gustavsson *et al.*, 2011). The conducive climatic conditions as well as lack of proper storage infrastructure in postharvest operations stimulate losses of stored

chickpeas to insects and storage moulds (Peace, 2020).

During storage, the insect damage usually exacerbates fungal colonization. Among storage moulds, *Aspergillus flavus* produces aflatoxins, which harmfully affect the human health and other livestock (Omotayo *et al.,* 2019). Among insects, bruchids are most hazardous pests of stored pulses. The genus *Callosobruchus* of the family bruchidae, has several species, which cause great damage to many economically important legumes in the tropics (Shukla et al, 2009).

The application of various synthetic pesticides since last few decades have made a great contribution in management of such storage losses but, on the other hand, also raised a number of issues related to environment and wellbeing (Rajkumar *et al.,* 2019). Hence, there is need to develop some safer alternatives of synthetic chemicals, which can provide protection of stored commodities from quantitative losses due to insects and fungi as well as their mycotoxins. Now days, western society also relying on 'green consumerism' therefore, plant based products are promoted as safe alternatives of synthetic pesticides and placed under 'generally recognized as safe' (GRAS) category (Food and Drug Administration, 2013). Several higher plant essential oils (EOs) exhibited activity against bacteria, moulds, mycotoxins and insects (Pandey *et al.,* 2017; Basak, 2018; Lasram *et al.,* 2019; Pavela *et al.,* 2019). Some EO based formulations viz. TALENT, EcoSMART and EcoPCOR have been used on large scale in food and agriculture industries (Singh *et al.,* 2019).

Therefore, in the present study, the efficacy of *Mentha piperita* L. (Lamiaceae) essential oil (MpEO) against *Aspergillus flavus* Link and pulse beetle *Callosobruchus chinensis* L., which cause severe deterioration of chickpea (*Cicer arietinum* L.), has been studied. In addition, antiaflatoxigenic and antioxidant activity of MpEO was also assessed so as to recommend MpEO as green pesticide to protect food commodities from fungal and insect infestation during storage to prolong their shelf life.

Materials and Methods

Sampling and test organisms

Chickpea seeds (variety- Radha) of about 4-6 months of storage were procured and collected in sterilized polythene bags to avoid further fungal and insect invasion.

A toxigenic strain *Aspergillus flavus* LHPCA-08 was selected because of its high aflatoxigenicity among recovered isolates of *A. flavus* from chickpea seeds by direct plating method. The aflatoxigenicity of *A. flavus* isolates were determined by Sinha *et al.,* (1993).

The pulse beetles, *C. chinensis* adults were obtained from naturally infested chickpea seeds from local market retailer. The obtained beetles were reared on clean and uninfested chickpea. The hundred adult insects were released in 500 g chickpea seeds in a plastic jar covered with muslin cloth to ensure ventilation. The jar was kept under controlled temperature (30±2°C) and relative humidity (70±5%) (Shukla *et al.,* 2009).

Procurement and GC-MS analysis of MpEO

The MpEO was locally procured from Shiv Shakti Trading Co., Maharajganj (U.P.), India and stored in dark glass vials to protect its composition from light. The composition of OcEO was analyzed through gas chromatography (Perkin Elmer Auto XL GC) equipped with a flame ionization detector.

The GC conditions were as follows: column, EQUITY–5 (60m x 0.32mm x 0.25µm); H_2 was the carrier gas; column Head pressure 10 psi; oven temperature program isotherm 2 min. at 70ºC, 3ºC/min. gradient to 250ºC, isotherm 10 min; injection temperature, 250ºC; detector temperature 280ºC. The GC-MS analysis was also performed using Perkin Elmer Turbomass GC-MS. The effluent of the GC column was introduced directly into the source of MS. Spectra were obtained in the EI mode with 70ev ionization energy. The compounds were identified by comparison of their relative retention times and the mass spectra with those of authentic reference compounds shown in the literature (Adams, 2007).

Antifungal and antiaflatoxigenic efficacy of MpEO

Fungitoxic and aflatoxin inhibitory efficacy of MpEO was tested against the toxigenic isolate of *A. flavus* LHPCA-08 using SMKY (Sucrose, 200g; $MgSO_4.7H_2O$, 0.5g; KNO_3, 0.3g; Yeast extract, 7.0g; Distilled water, 1000ml) broth as nutrient medium following Sinha *et al.*, (1993). Requisite amount of MpEO was dissolved separately in 0.5 ml of 5% tween-20 were pipetted aseptically to different presterilised Erlenmeyer flasks (150 ml) containing 49.5 ml of SMKY broth to procure the final concentrations viz. 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml. The control sets were kept parallel to the treatment sets without OcEO. Then, flasks were inoculated aseptically with 50 µl spore suspension ($\approx 10^6$ spores ml^{-1}) of toxigenic isolate of *A. flavus* LHPCA-08 prepared in 0.1 % Tween-80 (Rosengaus *et al.*, 2000) and incubated at 27±2 °C for 10 days. The content of each flask was filtered (Whatman no. 1) and mycelium was oven dried at 100°C till their weight remained constant for biomass determination. Mycelial biomass of treatment and control sets was measured and per cent mycelial inhibition was calculated (Dwivedy *et al.*, 2018). The filtrates of control and treated sets were extracted separately with 50 ml chloroform in a separating funnel to dissolve AFB_1 in chloroform. The separated chloroform extract was evaporated near dryness on water bath at 60-70°C. After evaporation, chloroform extract residue was re-dissolved in 1 ml chloroform and 50 µl of it was spotted on TLC plate (20×20 cm^2 of silica gel). The TLC plates were developed in toluene: isoamyl alcohol: methanol (TIM) mobile phase (90:32:2; v/v/v) and intensities of AFB_1 in the form of fluorescent blue spots were observed under ultra violate fluorescence analysis cabinet at 360 nm (Dwivedi *et al.*, 2018). The fluorescent blue spots of AFB_1 on developed TLC plate were scraped out and suspended in 5 ml cold methanol (4±2°C) followed by centrifugation at 3000 rpm for 5 min. The optical density of supernatant was recorded at wavelength of 360 nm and AFB_1 content was determined following Tian *et al.*, (2012).

$$AFB_1 \text{ content } (\mu g/L) = \frac{\text{Absorbance} \times \text{Mol.wt.of AFB1}(312)}{\text{Molar extinction coefficient of AFB1}(21{,}800) \times \text{Path length } (1\,\text{cm})} \times 1000$$

Fungitoxic spectrum of MpEO against some common storage fungi

Fungitoxic spectrum of MpEO was also determined against 12 storage moulds viz. *Alternaria alternata*, *Aspergillus candidus*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus*, *Cladosporium cladosporioides*, *Curvularia lunata*, *F. oxysporum*, *Penicillium italicum* and *Trichoderma viride* recovered from chickpea seeds by direct plating technique on PDA medium. Requisite amount of MpEO was dissolved separately in 0.5 ml of 5% tween-20 mixed with 9.5 ml PDA medium in different presterilized Petri dishes to attain final concentrations i.e. 0.25, 0.50, 0.75, 1.0, 1.25, 1.50 and 2.0 mg/ml. The negative control sets were kept parallel to the

treatment sets without MpEO along with positive control sets having bavistin (Carbendazim 50% WP), a prevalent synthetic fungicide. A fungal disc (5 mm) of each test fungus was inoculated separately and incubated at 27±2 °C for 7 days. After incubation, minimum inhibitory concentration (MIC) was recorded (Kumar *et al.*, 2011).

Effect of MpEO on ergosterol content

Effect of MpEO on ergosterol content in plasma membrane of *A. flavus* LHPCA-08 was assessed following Tian *et al.*, (2012). Requisite amount of the MpEO was dissolved separately in 0.5 ml of 5% tween-20 were pipetted aseptically to different presterilised Erlenmeyer flasks (150 ml) containing 49.5 ml of SMKY broth to procure the final concentrations from 0.1 to 0.6 mg/ml. The flask without MpEO was treated as control. Each flask was inoculated with 100 µl spore suspension of *A. flavus* LHPCA-08 followed by incubation at 27±2 °C for 5 days. Recovered mycelia from treated and control sets were subjected to extraction and quantification of ergosterol.

Free radical scavenging activity of MpEO

The antioxidant activity of the MpEO was determined by DPPH radical scavenging assay on TLC as well as its free radical scavenging activity was measured through spectrophotometery following Tepe *et al.*, (2005). Free radical scavenging activity of the MpEO was measured by recording the extent of bleaching of the purple-coloured DPPH solution to yellow. Different graded concentrations (1.0 to 20.00 µg/ml) of the samples were added separately to 4% DPPH solution in methanol (5 ml). After a 30 min of incubation at room temperature, the absorbance was taken against a blank at 517 nm using spectrophotometer. Scavenging of DPPH free radical with reduction in absorbance of the sample was taken as a measure of their antioxidant activity following Sharififar *et al.*, (2007). Ascorbic acid was used as positive control. Per cent free radical scavenging activity (FRSA) was calculated using the following formula –

$$\%FRSA = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

where, A_{blank} is the absorbance of the control (without test compound), and A_{sample} is the absorbance of the test compound.

Insect repellent activity of MpEO

The insect repellency of MpEO was tested against *C. chinensis* following Upadhyay *et al.*, (2019) using 'Y' shaped Olfactometer. The repellency experiment was carried out at room temperature (27±2ºC). A cotton swab soaked separately with requisite amount of MpEO to find out the desired concentration viz. 10, 20, 50, 100, 150 and 200 µg/L with respect to the aerial volume of Olfactometer was plugged in treatment arm whereas in control arm cotton swab without MpEO was plugged. Thirty adult insects were inserted separately from the base of the Olfactometer. After 30 min the number of insects in the treatment and control arm was recorded.

Insecticidal activity of MpEO

The insecticidal activity of MpEO against *C. chinensis* was assessed by direct contact method. The requisite amount of MpEO was soaked on two layered Whatman no. 1 filter paper placed in a Petri dish (90 mm) to find out the final concentrations i.e. 0.1, 0.5, 1, 5, 10, 50, 100 and 200 µg/L as per aerial volume of Petri plate. Thirty insects of equal age (5-7 days) were inserted separately in Petri dishes fumigated with MpEO. Control sets were kept parallel to treatment sets without MpEO. Percent mortality of insects in each set was recorded on 2, 4, 6, 8 and 10 hour exposure (Kumar *et al.*, 2009).

Oviposition deterrent activity of MpEO

The MpEO was tested for its effect on oviposition of *C. chinensis*. Healthy seeds (50 g) of chickpea were taken in plastic containers (250 ml) and fumigated separately with requisite concentrations viz. 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 µg/L of MpEO. After an hour, 30 bruchids of almost same age were introduced in each container separately for egg laying. The control sets run along with treatment set without MpEO. Insects were removed after 24 hours and laid eggs were counted after 3-4 day of release. The effect of MpEO in reduction of egg laying was calculated following Sabbour (2019).

Ovicidal activity of MpEO

The MpEO was tested for its inhibitory effect on F_1 adult emergence of *C. chinensis*. Chickpea seeds containing eggs were taken in plastic containers (250 ml) and fumigated separately with requisite concentrations viz. 5, 10, 20, 50, 100, 200 and 500 µg/L of MpEO. The fumigated sets along with control were incubated at 27±2°C and RH 70±5%. The number of pulse beetles of F_1 generation emerging from chickpea seeds from fumigated and control sets was recorded up to 30 days. The effect of MpEO on adult development (F_1 generation) was determined following Kumar *et al.,* (2009).

Determination of feeding deterrence index

To determine its practical applicability, 5.0 kg of chickpea seeds were fumigated with 1.0 g MpEO in plastic containers (10 L) for six months. Along with treatment sets, one positive control fumigated with malathion, an organophosphate insecticide at 1.0 g/10 L and one negative control without fumigation was also kept. All the containers were infested with 25.0 g of previously infested chickpea seeds. The level of insect infestation and grain damage was monitored after six months by quantifying the feeding deterrence index (Brari and Kumar, 2019).

Seed germination test

The viability of fumigated chickpea seeds was tested by seed germination test. After six months of storage, 200 uninfested seeds were taken from each treated group and soaked in distilled water for 3 h. Thereafter, seeds were aseptically transferred to Petri dishes of 15 cm diameter (20 seeds per Petri plate) containing moist filter paper and incubated at 25±2ºC. Two hundred healthy and uninfested seeds were taken from the market as control for comparison. The number of seeds germinated within a week was recorded as viable (Kumar *et al.,* 2009).

Statistical analysis

All the experiments were accomplished in triplicate and data were expressed as Mean±standard error (SE) followed by one way ANOVA ($P < 0.05$) and Tukey's multiple range tests. The software SPSS (version 16.0) was used for statistical analysis of data.

Results and Discussion

Chemical description of MpEO

M. piperita plants are abundantly cultivated in Maharajganj district of Uttar Pradesh for its EO, highly demanded in various industries. The chemical composition of EOs varies with age of the plant, season of collection, geographical area and soil characteristics (Heikal, 2017; Rawat *et al.,* 2020). Hence, procured MpEO was standardized to determine its chemical profile through GC–MS analysis. The GC-MS analysis of MpEO showed 38 considerable peaks in which Menthol (46.82%) was found as major

component followed by Menthone (16.14%), β-Caryophyllene (4.14%), Germacrene D (2.89%), 1,8-Cineole (2.71%), Menthofuran (2.29%), Menthol acetate (2.16%) and Terpinen-4-ol (2.04%). Rest other identified components were found in small or in trace amount (Table 1). The major component of MpEO is different from earlier findings where, p-mentha-6,8-dien-2-one (Afridi *et al.,* 2016), menthone (Moghaddam *et al.,* 2013), carvone (Rezende *et al.,* 2017), limonene oxide (Mehani *et al.,* 2015), etc. were reported as major components.

Antifungal and antiaflatoxigenic efficacy of MpEO

The MpEO exhibited remarkable broad fungitoxic spectrum at 1.0 mg/ml concentration against all the tested (12) storage fungi and also showed superiority over synthetic fungicide bavistin (Figure 1). The broad spectrum fungitoxicity and superiority over bavistin would be suitable to provide complete protection from a range of fungal pathogens. The MIC of MpEO against toxigenic isolate *A. flavus* LHPCA-08 was found to be 0.5 mg/ml whereas it completely checked the AFB_1 production at 0.3 mg/ml concentration (Table 2). A direct relationship has been observed between fungal biomass and aflatoxin production. Fungal biomass and AFB_1 production exhibited a significant declining trend with increasing MpEO concentration. Therefore, to check AFB_1 production, mycelial growth must be below the threshold limit so that aflatoxin could not be produced (Kumar *et al.,* 2008). The MpEO completely checked the AFB_1 production at lower concentration than earlier reported EOs viz. *Coriandrum sativum* (Das *et al.,* 2019), *Pimenta dioica* (Chaudhari et. al., 2020), *Artemisia nilagirica* (Kumar *et al.,* 2020) etc. The antifungal and antiaflatoxigenic efficacy of MpEO may be due to bioactivity of its major constituent or synergism among several components. Menthol, a well known antifungal agent (Moghaddam *et al.,* 2013; Reddy *et al.,* 2019) is major component (46.82%) of MpEO, may also played promising role in its fungitoxicity.

Effect of MpEO on ergosterol content of fungal cell membrane

Ergosterol is specific sterol in fungal cell membrane providing membrane integrity and flexibility as well as stability of membrane associated enzymes (Chellappandian *et al.,* 2018) and significant alteration of its biosynthesis adversely affect fungal growth (Bhattacharya *et al.,* 2020). Ergosterol content of cell membrane was found decreasing with increasing MpEO concentration. The per cent inhibition of ergosterol contents by MpEO treatment at 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml concentration was 36.11%, 70.02%, 81.67%, 92.22% and 100% respectively (Figure 2). The decrease in ergosterol level with increasing MpEO concentration clearly denotes that the bioactive components of MpEO targeted the cell membrane and rendering them more permeable to leakage of ion and other cell content (Ultee *et al.,* 2002).

Antioxidant activity of MpEO

Food commodities are also deteriorated by free radical mediated oxidation of unsaturated lipids during storage (Ahmed *et al.,* 2016). Oxidative stress stimulates *A. flavus* to produce more AFB_1 during storage (Grintzalis *et al.,* 2014) which results quantitative as well as qualitative losses to stored commodities and reduces their shelf life. The MpEO exhibited significant radical scavenging activity (IC_{50} values 11.4 µg/ml) in concentration dependent manner (Figure 3), which was found comparatively higher than ascorbic acid (5.3 µg/ml). The IC_{50} value of MpEO was found quite lower than some earlier reported EOs (Mishra *et al.,* 2016) and

also comparable to synthetic antioxidants (Dwivedi *et al.*, 2018). The presence of various phenolic compounds and/or synergistic effect among compounds may also play significant role in antioxidant activity of EOs (Fadel *et al.*, 2020; do Nascimento *et al.*, 2020). Because of to free radical scavenging activity, the MpEO may serve as plant based antioxidants in shelf life enhancement as well as protection from oxidative stress by decelerating oxidative rancidity of lipids.

Table.1 Chemical composition of MpEO

RT	Compounds	Percentage
7.175	3-Hexen-1-ol	**0.06**
8.275	α-Thujene	**0.03**
9.618	α-Pinene	**1.21**
10.375	Camphene	**Tr***
10.875	Myrcene	**Tr***
11.125	β-Pinene	**0.96**
11.500	3-Octanal	**0.02**
11.675	Sabinene	**0.08**
11.775	α-Terpinene	**0.23**
12.885	p-Cymene	**0.83**
13.041	DL-Limonene	**0.56**
13.075	1,8-Cineole	**2.71**
13.941	β-Ocimene	**0.88**
14.585	γ-Terpinene	**0.26**
16.075	Linalool	**0.16**
19.526	Menthone	**16.14**
20.001	Menthofuran	**2.29**
22.446	**Menthol**	**46.82**
28.401	Terpinen-4-ol	**2.04**
28.493	Eugenol	**1.30**
29.501	Pulegone	**Tr***
29.601	Menthol acetate	**2.16**
29.801	Menthyl acetate	**1.34**
30.851	β- Bourbonene	**0.17**
31.126	β- Elemene	**0.37**
31.692	β- Caryophyllene	**4.14**
31.926	Camphenol	**Tr***
32.351	Germacrene D	**2.89**
33.576	α- Terpinolene	**0.24**
33.876	Ethyl linoleolate	**Tr***
34.501	α- Humulene	**0.12**
34.951	α- Cubebene	**0.54**
35.576	β- Costol	**0.22**
35.876	δ- Cadinene	**0.28**
38.301	α- Furnesene	**Tr***
41.026	Valencene	**Tr***
41.201	Germacrene B	**Tr***
41.776	Caryophyllene oxide	**0.82**
	Total	**89.87%**

RT= Retention time; Tr*- Trace amount (<0.01%)

Table.2 Effect of MpEO concentrations on *A. flavus* LHPCA-08 biomass and AFB_1 production

Concentration (mg/ml)	OcEO	
	Fungal Biomass (g)	AFB_1 production (µg/L)
Control	0.393 ± 0.014^{a}	2163.963 ± 106.388^{a}
0.1	0.228 ± 0.008^{b}	652.624 ± 39.663^{b}
0.2	0.127 ± 0.008^{c}	204.184 ± 36.258^{c}
0.3	0.063 ± 0.006^{d}	0^{d}
0.4	0.023 ± 0.004^{d}	0^{d}
0.5	0^{e}	0^{d}

Values are mean (n=3) ± Standard Error; P < 0.05. The means followed by same letter in the column are not significantly different according to ANOVA and Tukey's multiple comparison tests

Table.3 Effect of concentration and exposure duration of MpEO on mortality (%) of *C. chinensis*

Treatment (µg/L)	% Mortality with exposure period (hrs)				
	2 hr	4 hr	6 hr	8 hr	10 hr
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
0.1	8.26 ± 1.06^{b}	12.16 ± 2.14^{b}	27.18 ± 3.16^{b}	48.12 ± 3.14^{b}	78.24 ± 6.28^{b}
0.5	10.33 ± 2.14^{b}	15.28 ± 1.86^{b}	38.42 ± 4.32^{c}	68.62 ± 6.38^{c}	92.18 ± 7.46^{c}
1.0	13.18 ± 2.16^{b}	24.32 ± 3.22^{c}	49.38 ± 6.18^{d}	82.18 ± 5.32^{d}	100 ± 0.00^{d}
5.0	22.24 ± 4.32^{c}	38.18 ± 4.32^{d}	66.32 ± 5.28^{e}	96.32 ± 6.38^{e}	100 ± 0.00^{d}
10.0	38.58 ± 6.18^{d}	54.58 ± 6.18^{e}	82.84 ± 7.42^{f}	100 ± 0.00^{f}	100 ± 0.00^{d}
50.0	64.14 ± 5.22^{e}	77.26 ± 7.14^{f}	100 ± 0.00^{g}	100 ± 0.00^{f}	100 ± 0.00^{d}
100.0	86.33 ± 6.38^{f}	100 ± 0.00^{g}	100 ± 0.00^{g}	100 ± 0.00^{f}	100 ± 0.00^{d}
200.0	100 ± 0.00^{g}	100 ± 0.00^{g}	100 ± 0.00^{g}	100 ± 0.00^{f}	100 ± 0.00^{d}

Values are mean (n=3) ± Standard Error; P < 0.05. The means followed by same letter in the column are not significantly different according to ANOVA and Tukey's multiple comparison tests

Table.4 Comparative efficacy of MpEO and malathion on feeding behaviour of *C. chinensis*

Fumigants	Dose	FDI (%)
MpEO	100 mg/L	**92.08 ± 2.68**
Malathion	**100 mg/L**	**89.34 ± 4.22**

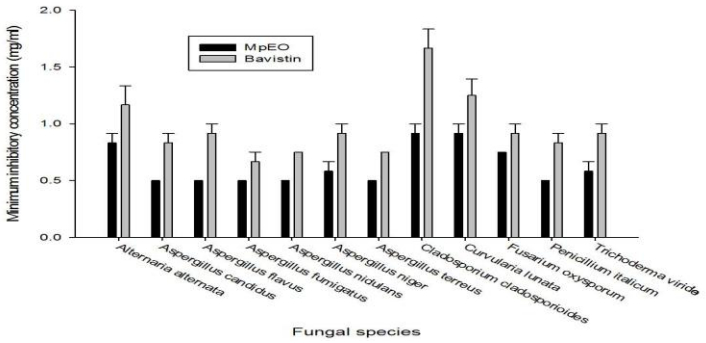
Values are mean (n = 3) ± standard error

Table.5 Comparative efficacy of MpEO and malathion on germination of chickpea seeds

Treatment	Germination (%)
Control	**86.80 ± 3.44**
***Mentha* EO**	**74.67 ± 3.12**
Malathion	**71.33 ± 4.68**

Values are mean (n = 3) ± standard error

Fig.1 Fungitoxic spectrum of MpEO against some storage moulds and its comparison with Bavistin



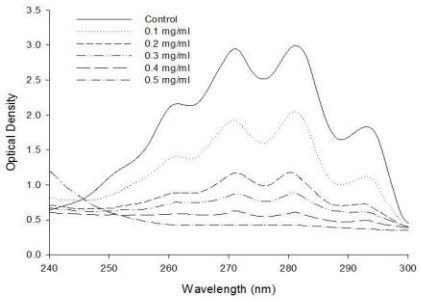


Fig. 2 Effect of MpEO concentration on ergosterol content of fungal cell membrane

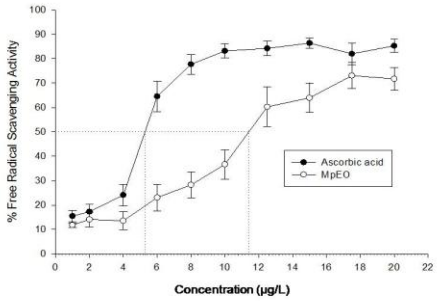


Fig. 3 Free radical scavenging activity of MpEO

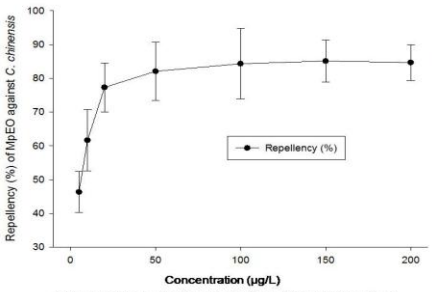


Fig. 4 Insect repellent activity of MpEO against *C. chinensis*

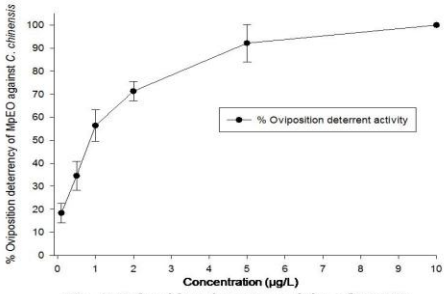
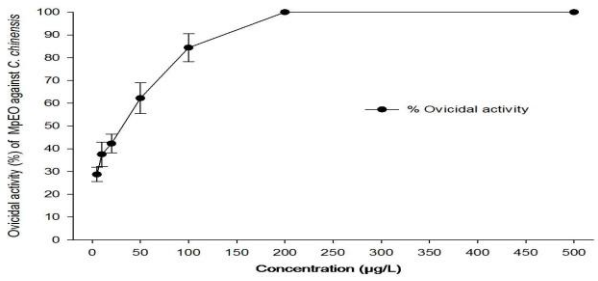


Fig. 5 Oviposition deterrent activity of MpEO against *C. chinensis*

Fig.6 Ovicidal activity of MpEO against *C. chinensis*



Bio-activity (repellency, insecticidal, oviposition deterrency & ovicidal) of MpEO against pulse beetle C. chinensis

An effort has been made to evaluate the efficacy of MpEO against pulse beetle *C. chinensis* severely infested stored chickpea seeds. The percent insect repellency of MpEO against *C. chinensis* increased with increasing concentration. The percent repellency increased upto 100 µg/L and beyond this became almost constant (84.6%) (Figure 4). Similarly, MpEO exhibited potent insecticidal activity which was found directly proportional to concentration and exposure period. During the study, 100% mortality of the insect was recorded at 200 µg/L concentration of MpEO when exposed for two hours, while complete mortality was also noted at 1 µg/L concentration when exposure period was increased to 10 hour (Table 3). In addition, oviposition deterrency and ovicidal activity of MpEO was also evaluated. MpEO altered the egg laying behavior of *C. chinensis*. The oviposition deterrency increased with increasing MpEO concentration and egg laying was completely checked at 10 µg/L (Figure 5). The MpEO potentially inhibited the adult emergence from the eggs. The F_1 emergence decreased with increasing EO concentration and completely checked at 200 µg/L (Figure 6).

Some plant products like azadirachtin (Chaudhary *et al.,* 2017), pyrethrum (Sun *et al.,* 2020), rotenone (Huang *et al.,* 2018), sabadilla (Isman, 2006), termeron (Leyva *et al.,* 2020) etc. are in large scale use in control of different insect pests. The volatiles present in the MpEO may be altering the egg laying behaviour of the insects as reported by Autran *et al.,* (2009). Such products in pest management are termed as 'behavior altering chemicals or "semiochemicals" and are recommended in integrated pest management. The use of such 'behavior altering chemicals' would solve the problem of resistance development in target pests which is frequently reported by use of different prevalent synthetic chemicals which act through cidal (lethal) mode of action (Isman, 2006). Likewise, the reduction in F_1 emergence from the treated eggs may be due to mortality of developing embryos in the eggs exposed to the MpEO. Such a virtue of MpEO would increase its market value if formulated as botanical pesticide in protection of stored food commodities.

Feeding deterrency and seed germinability during in situ study

The *in situ* experiments was conducted to observe the feeding deterrent efficacy of MpEO and its feeding deterrence index (FDI) was recorded as 92.08%, showing percent protection of the chickpea from *C. chinensis* infestation and also found superior over the prevalent organophosphate insecticide malathion where 89.34% FDI was recorded (Table 4). The treated chickpea seeds having no significant losses in their viability even after six months of fumigation as compared to control. MpEO treated chickpea seeds showed 74.67% germination while malathion exhibited 71.33% (Table 5) again showing superiority over malathion strengthens its non-phytotoxic nature.

The plant *M. Piperita* is also used as phytomedicine in different diseases (Trevisan *et al.,* 2017; Rita and Animesh, 2011) showing its non-mammalian toxic nature. Thus, non-phytotoxic and non-mammalian toxic nature of the MpEO strengthens its possible exploitation as a safer green pesticide for stored food commodities. The findings may draw the attention of food industries to conduct further experiments regarding large scale exploitation of MpEO as botanical pesticide to protect stored food commodities from fungi and their mycotoxins as well as

insect pests during storage. The attraction of modern society in 'green consumarism' (Smid and Gorris, 1999) desiring fewer synthetic ingredients in foods and recommendation of herbal products as 'generally recognized as safe' (GRAS) in the developed countries may lead scientific interest in MpEO as food preservative.

In conclusion the findings of present study reveals that, MpEO exhibited antifungal, antiaflatoxigenicity, broad fungitoxic spectrum, free radical scavenging activity, insect repellent, insecticidal, oviposition deterrent, ovicidal and non-phytotoxicity which strengthening its exploitation as green pesticide in place of synthetic chemicals for enhancing the shelf life of stored food commodities and other edible products during storage.

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