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Natural Plant Oils and Terpenes as Protector for the Potato Tubers against Phthorimaea operculella Infestation by Different Application Methods

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Abstract. For protecting potato tubers from the potato tuber moth (PTM) infestation during storage, different concentrations of ten natural plant oils and three commercial monoterpnes were tested, some as fumigants or dusts against adults or dusts against neonate larvae, while others as sprays on the gunny sacks in which potato tubers were stored. Tuber damage indices as well as persistence indices for tested materials were assessed. Vapors of *Cymbopogon citratus, Myristica fragrans* (nutmag), *Mentha citrata and a-Ionone* (monoterpene) caused a highly significant reductions in the life span of exposed moths as well as in new adult offsprings. Other tested oils as *Cinnamonium zeylanicum, Myristica. fragrans* (Mace) and *Pelargonium graveolens* caused a insignificant effect. There was no significant effect of the tested vapors on egg hatchability, except in case of oils of *C. citratus, M. fragrans* (nutmag) and *M. tragrans*(Mace oil) which caused high reduction in egg hatchability. According to the values of damage indices, the most effective oil vapors were arranged ascendingly as follows: *Myristica* (nutmag) < *Cymbopogon* < *Mentha* < *a - Ionone.* Dusting potato tubers with 1% conc., (mixed with talcum powder) of *Myristica, Mentha, Cymbopogons* oils and a-Ionone (monoterpene) caused high reduction in egg deposition, adult emergence as well as percentage of penetrated larvae of PTM. According to their damage indices, *Cymbopogon* and ά-Ionone were the most protective oils, followed by *Myristica* and *Mentha.* Spraying gunnysacks with 1% conc., of the aforementioned natural oils separately elicited high reduction in PTM progeny; while their combinations did not elicit any significant synergistic effect. According to their tuber damage indices, it was found that *Cymbopogon* oil alone or mixed with *Myristica* oil showed the best protective effect, followed by *Myristica* oil alone and *Mentha* oil mixed with *Cymbopogon* oil. Assessment of the persistence index of various tested materials during storage indicated that: Vapors of *Cymbopogon* and *Myristica* (nutmag) gave the best protection from PTM infestation during storage (for 20 days). Dusting potato tubers with plant oil of *Cymbopogon* (mixed with talcum powder) gave the best protection during storage (for 15 days). Spraying gunnysacks with natural plant oils of *Cymbopogon* and *Myristica,* separately or mixed elicited the best protection from infestation by PTM during storage up to 20 days.

Key words: Potato tuber moth, *Phthorimaea operculella*, Natural oiles, Terpenes, Different application treatments.

Introduction

There is a renewed interest amongst scientists to study the bioactivity of plant essential oils and their constituents of terpenes against phytophagous insect pests (BAKKALI *et al.*, 2008, ADORJAN & BUCHBAUER, 2010). The antifeedant and repellent effects of various plant essential oils have been reported on weevils *Sitophilus granarius* L. and *Sitophelus zeamaiz* Mostschulsky (Coleoptera Dryophthoridae), both widespread stored grain pests (CONTI *et al.*, 2010, 2011; MOSSI *et al.,* 2011).

In developing countries, most of the potato farmers cannot afford the increasing cost of storing potatoes in cold stores, so the tubers are often heaped under a tree or in rustic shelter and covered with a thick layer of straw, potato foliage or another handy materials (ESSAMET *et al.,* 1988). Other farmers rely heavily on insecticides, which are dusted or sprayed on the tubers at the beginning of the storage period. However, the prophylactic use of insecticides, especially for stored potato tubers, causes serious problems. These include the development of insect resistance to insecticides, persistence of residues in tubers, destruction of beneficial organisms, human intoxication and contamination of the environment. Recently, several programs for pest control have been developed, including the use of powders and oils of natural plant origin, resistant potato strains (varieties), or using intercropping system, etc. SHELKE *et al.* (1985) studied the effect of seven vegetable oils against adults of the PTM on potato tubers in the laboratory. They found that 0.05 and 0.1 % neem oil *(Azadirachta indica)* had Oviposition deterrent effect. SHARABY (1988) mentioned that reproduction in the potato tuber moth was significantly reduced when either males or females were exposed to the vapor of orange peel oil and such effect increased with increasing oil concentration and exposure time. In addition, a pronounced reduction in egg production and egg viability occurred when the moths were exposed to the vapors arising from oil treated paper discs. EL-NAHAL *et al.* (1989) tested the toxic effect of the vapors of the essential oil *Acorus calamas* (L.) rhizomes on the adults of several stored grains. HOOSHANG *et al*. (2013) investigated the fumigant toxicity of essential oils of Basil, European pennyroyal, Lavender, Mint and Savory, on potato tuber moth. Treated Potato tubers by methanoilc extract of Lavender elicited the lowest percentage of first larval penetration. Probit analysis of essential oils showed that the most effective oil was Savory oil. SHARABY *et al.* (2014), recorded that Dusting potato tubers with bulb powder of *Allium cepa* (50% cone. mixed with talcum powder) displayed a highly effective role in the reduction of deposited eggs as well as adult emergence there from. *Allium cepa*, *Pelargonium graveolens* and *Cymbopogon citratus* oils caused high reduction in larval penetration into treated tubers. Mixture of *Pelargonium*or *Allium* mixed with talcum powder gave good protection for a long storage period (30-40 days). The aim of the present work is to studying the effect of various plant oils and commercial monoterpenes, for protecting the potato tubers from PTM infestation during storage period.

Materials and Methods

A stock laboratory colony of the PTM has been raised on potato tubers, which are the main natural host. The culture was maintained under laboratory conditions at 26 ± 2°C and 70 ± 5 %R.H.; following the technique mentioned by EL-SHERIF (1966).

Protection of potato tubers from PTM infestation during storage

A - Fumigation.

a- Treatment of adults

Virgin male and female moths were continuously exposed to vapors of ten natural oils and three commercial monoterpenes. The tested oils were Bergamote, Lemongrass, Mace, Nutmag, Clove, Eucalyptus, Ginger grass, Cinnamone, Cedar wood and Geranium (Table 1). They were obtained in pure state from the "Sugar and Complete Industrial Company" El Ahram Street - Giza. The commercial monoterpenes were Geraniol, a-Ionone and IsoeugenoL. They were obtained from Sigma Aldrich Corporation, U.S.A. A quantity of 0.05 ml of the tested oil was introduced into a small plastic tube (1 x 3 cm) that was suspended by a thread from the lid of a test container (500 ml). Each container was provided with 2-3 clean tubers weighing about100gm. The following aspects were determined: (1) Longevity of treated male and female moths. (2) Number of emerged offspring. (3) Damage index of tuber: The assessment of the mean index of damage was determined according to the following categories based on the degree of larval tunneling visible from external examination (FENEMORE, 1980):.

Infestation category	Damage category	Weighing factor
1. Clean.	No visible- sign of infestation.	0
2. Slight.	One or two mines, which could be removed readily on peeling.	X_1
3. Moderate.	More than two mines present, and up to one-third of surface showing damage.	x_2
4. Severe.	More than one-third of the tuber surface showing damage.	x_3

The mean damage index of tuber (D.I.) for each experiment could be determined by the formula given by FENEMORE (1980).

The maximum possible index is, thus, 30 tunnels/tuber if all tubers fall into the severe category according to FENEMORE

$$D.L = \frac{(\text{no. slight}X_1) + (\text{no. moderate } X_2) + (\text{no. severe } X_3)}{\text{Total number of tubers}} \times 10$$

(1980). Each test was repeated five times.

Another experiment was carried out to compare the effectiveness of lower oil volumes (0.0125 and 0.025 ml /500 ml air of the tested container) of the most effective oils from the aforementioned survey by the higher concentration (0.05ml oil / 500 ml air). The same aspects were determined during the experimental period.

All tests were replicated ten times under laboratory conditions 28 ±2 C°.

b - Treatment of eggs

Deposited eggs of PTM on potato tuber surface were counted by using magnifying lens. They were placed in the container (500 ml) continuously exposed to vapors of different volumes (0.0125, 0.025 and 0.05ml/500ml) of plant oils or commercial monoterpenes as mentioned before in treatment of adults. Control experiments were used without treatment. Each treatment was replicated five times in separate containers. The percentage of egg hatchability was determined. The treated eggs were examined under a magnifying lens and compared with untreated ones. If the exposed eggs failed to hatch but showed development beyond the stage when tested oils were applied, it was assumed that failure to hatch was due to inhibition of embryonic development rather than acute toxicity of the oils vapors according to Saxena and Sharma (1972).

B - Dusting potato tubers

Two experiments were carried out: Different concentrations (0.25, 0.5 and 1%) of Bergamote (*Mewtfza citrata),* lemongrass *(C. citratus\\-* Nutmag *(Myristica fragrans)* and ά-Ionone were prepared and mixed with a carrier material (Talcum powder). They were left for ten minutes at room temperature to dry, and used as dust.

In first experiment, 2-3 potato tubers (about 200gm) were dusted with one of the prepared dusting materials. The dusted tubers were introduced into containers (20 x 20 x 10 cm) where five pairs of virgin adults were confined. The following biological aspects were studied: (1) Average number of deposited eggs. (2) Percentage of emerged offspring, (3) Duration of life span (egg to adult (4) Damage index of potato tubers.

In the second experiment, 50 neonate larvae were introduced in a test chamber (20x20x10 cm) containing 1-2 dusted potato tubers (about 100 g). The percentage of penetrating larvae and percentage of pupation were determined. Each test was replicated five times.

C . Spraying gunny sacks

To protect sound potatoes kept at gunny sacks from PTM infestation, gunnysacks (14 x 22 cm) were treated as follows:

Different concentrations (0.25, 0.5 and 1%) of the tested plant oils alone or combined were mixed with water. Two drops of Tween 80% were added as emulsifier to ensure homogenous water emulsion. The gunny sacks were sprayed and left for dryness before potato tubers were kept in them. Each treatment contained 2-3 (about 200gm) potato tubers , previously exposed to artificial infestation by three pairs of virgin male and female moths. The following aspects were studied: (1) Number of progeny (full grown larvae, pupae and emerged adults) after 25 days of infestation; (2) Damage index of potato tubers; (3) Persistence index of the residual materials.

Residual effectiveness experiments were conducted to estimate the biological persistence of the tested natural plant oils, commercial monoterpenes when being applied at highly effective concentration (or the maximum possible concentration on potato tubers (dusting), on gunnysacks (spraying) or in the storage chamber (fumigation) according to MANSOUR *et al.* (1997). The residual effectiveness was bioassayed using various stages of PTM according to the method of protection; thus: In case of treatment of PTM adult stage by natural plant oils and commercial monoterpenes (Concentration 0.05 ml/500 ml air exposure in glass jar) for fumigation or 1% conc., for spraying and dusting application) were used. Dusted potato tubers, sprayed sacks and fumigated containers were kept at room temperature, alongside with untreated samples. At different time intervals, samples of dusted tubers, treated gunnysacks or potato kept at fumigated container were taken for residual effectiveness assessment by subjecting them to neonate larvae or adult stage of PTM. According to the methods of protection, the following parameters were recorded: (1) Mean damage index, for all methods of application mentioned before; (2) Reduction percent in life span of adult stage exposed to vapors of plant oils or commercial monoterpenes (Reduction = %x-y, where x= length of life span of untreated moth, y= length of life span of treated moth); (3) Reduction percent of progeny found in the tubers kept in treated gunny sacks; (4) Percentage of larval mortality; when neonate larvae fed directly on the treated tubers. Tests for residual effectiveness were continued till reaching a degree of protection to > 5 0 %value.

Statistical analysis. All data were statistically subjected to analysis of variance (ANOVA) through "SPSS" Computer program.

Results and Discussion

1. Effect of vapors of natural plant oils and commercial monoterpenes.

a - On adult life spane and fertility.

Results on the effects of vapors of ten natural plant oils and three commercial monoterpenes (at 0.05 m oil/500 ml air) on adult life (virgin male and female moths) and the produced offspring are given in Table 1. It was clear that, there was variable reductions in the life span of both virgin male and female moths continuously exposed to vapors of tested plant oils comparable to control moths. Thus, the average life span of female PTM was highly reduced when exposed to fumes of *C. citratus, M. fragrans* (Nutmag and Mace oil) and *M. citrate* to reach 0.8, 1.2, 3.3 and 2.8, while that of male moths was reduced to reach 1.7, 1.3 0.5, 2.5, 0.9 and 3.8 days, respectively; compared to untreated female and male moths (control); being 10.8 and 10.0 days, respectively at (P>0.01).

The remaining natural plant oils and commercial monoterpenes (except ά-ionone) show low significant effect on the life span of exposed moths comparable to the unexposed ones ($P > 0.05$). On the other hand, most of the tested oils were highly effective on the PTM fertility. Thus, the emerged adult offspring of female moths continuously exposed to fumes of *Cymbopogon, Myristica (nutmag* oil), *Mentha* and ά-Ionone were reduced to reach 0.5, 0.5, 1.5and 2.3 offspring/female, respectively. The difference from that of the untreated female moths 34.3 offspring/female was statistically extremely significant ($P < 0.01$).

The other tested oils caused a moderate reducing effect on the fertility of adults exposed to fumes of included *Cinnamonium zeylanicum, M. fragrans* (Mace oil) and *P. graveolens* as their average numbers of emerged offspring were 11.8, 11.8and 24.4 offspring/female, respectively. The remaining tested oils showed insignificant effects on the fertility of tested moths as compared with the control (P > 0.05). Although, only few of the tested oils (at 0.05 ml / 500 ml air) succeeded to reduce the life span of exposed adults, many of them affected their fertility. Therefore, the most effective components were selected and tested at lower vapor emanating from oil concentrations (0.025 ml, and 0.0125 ml/500ml air).

Table 1. Effect of natural plant oils vapors and commercial monoterpenes at 0.05 m1/500m1 on the life span and emerged offspring of continuously exposed PTM.

Tested oils (at 0.05ml/500ml)	Life span (days)		No. emerged adult offspring/female mean+ S.D.
	Female mean +S.D.	Male mean +S.D.	
Cinnamonium Zeylanicum	11.5+0.6[a]	11.3+2.9[a]	11.8+3.7[c**]
Cymbopogon citratus.	0.8+0.2[b] **	1.7+0.5[b**]	0.5+0.2[d**]
Eucalyptus rostratits.	13.8+1.8[a]	6.5+4.9[a]	27.0+5.6[a]
Janiperus virginiana	8.3+0.6[a]	9.5+1.1[a]	24.3+2.8[b*]
Mentha citrata	2.8+0.6[b] **	3.8+1.1[b**]	1.5+0.5[d**]
Myristica fragrans (Mace)	3.3+1.1[b] **	2.5+0.9[b**]	11.8+1.9[c**]
Myristica fragrans (Nutmag)	1.2+0.7[b] **	1.3+0.5[b] **	0.5+0.2[d**]
Pelargonium graveolens	8.3+1.4[a]	11+1.6[a]	24.4+3.4[b*]
Syzygium aromaticum	9.3+0.6[a]	6.5+0.4[a]	26.0+5.9[a]
Zingiber officinale	11.3+0.7[a]	11.5+1.3[a]	15.5+4.3[c**]
B-Cmmercial monoterpenes.			
Geraniol	9.3+1.5[a]	8.8+2.1[a]	27.3+3.03[a]
Isoeugenol	10.3+0.8[a]	10.8+0.5[a]	34.3+3.8[a]
α - lonone	4.6+0.9[b] *	5.8+1.5[a]	2.3+1.4[d] **
Control	10.8+1.1[a]	10.3+1.2[a]	34.3+2.8[a]

$L.S.D_{0.05}$ =4.6 $L.S.D_{0.05}$ = 9.9

$L.S.D_{0.01}$ = 6.09 $L.S.D_{0.01}$ = 13.6

Means with the same letters in vertical columns are not significantly different (P >0.05).

** significant at (p>0.05 , ** highly significant at (p> 0.01).*

Our results in agreement with recorded by Kordail et al, (2005) that the essential oils such as lemon grass (*C. winteriana*), *E. globulus*, rosmary (*R. officinalis*), vetiver (*Vetivera zizanoides*), Clove (*Eugenia caryophyllus*) and thyme (*Thumus vulgaris*) are known for their pest control properties. While peppermint (*M. piperita*) repels ants, flies, lice and moths, pennyroyal (*M. pulegium*) ward off fleas, ant, lice, mosquitoes, ticks and moths. Spearmint (*M. spicata*) and basil (*O. basilicum*) are also effective in warding off flies.

Results given in Table 2 indicate, that the effectiveness was concentration dependence; i.e. higher concentration caused higher reduction in the life span and fertility of exposed adult moths. Thus, in case of treatment by *C.citratus* oil, it was found that the average life span of exposed female and male moths decreased from 5.8 and 5.8 days to 3.6and 4.5 days, till it reached 0.8 and 1.7 days, respectively, at concentrations 0.0125, 0.025 and 0.05 ml / 500 ml air, respectively. Treatment of PTM by *M.fragrans* (nutmag) oil, also caused a reduction in the life span of exposed female & male moths from 6.3 and 4.8 days at 0.0125 ml to 4.0and 3.5 days at 0.025 ml, till it reached 1.3and 1.6 days at 0.05 ml / 500 ml air for female and male moths, respectively. An almost similar trend was reached, in case *of M. citrata* oil and ά-Ionone (commercial monoterpene). On the other hand, the average number of emerged offspring was severely affected by the different concentrations of the tested oils comparable to the control. However, *Cymbopogon* and *Myristica* oils did not elicit potent variation (P > 0.05) between 0.0125 and 0.025 ml / 500 ml air; but the effectiveness became highly significant (P < 0.01) when comparing these two concentrations with the higher concentration (0.05 ml / 500 ml air). Thus, the average emerged offspring was 11.5, 10.5 and 0.5 in case of *Cymbopogon* oil, and 15.8, 12.5 and 0.5 in case of *Myristica* oil at concentrations 0.0125, 0.025 and 0.05 ml/ 500 ml air, respectively. Treatments by ά-Ionone (terpene) and *Mentha* (oil) exhibited significant potent difference (P < 0.05) between 0.0125 and 0.025 ml; but highly significant difference (P < 0.01) with higher concentration (0.05 ml / 500 ml air). Thus, the average emerged offspring was 29.0, 21.8and 2.3 in case of a-Ionone, and 14.7, 9.3 and 1.5 in case *oil M. citrata* oil at concentrations 0.0125, 0.025 and 0.05 ml / 500 ml air, respectively. Based on the present findings, it can be concluded that *C. citratus, M. fragrans* (nutmag), *M. citrata* and a-Ionone at a high concentration (0.05 ml / 500 ml air) can successfully reduce potato infestation by the potato tuber moth, through shortening the life span of adults and reducing their fertility. As the majority of exposed females died before laying eggs; and failure of others to lay their full load of eggs. In this respect, Sharaby (1988) showed that the fecundity and fertility of the potato tuber moth were significantly reduced when moths of either sex were exposed to vapors of orange peel oil emanating from 160 uLoil in 250 ml glass jars. Oviposition and egg hatching were totally inhibited when female moths were exposed to 220 uLof the oil. EL-NAHAL *et al* (1989) found that vapors of the essential oils of *Acorus calamus* (L.) rhizomes had toxic effect against adults of several stored product insect species; and also confirmed that the period of exposure appeared to be the most important factor affecting the efficiency of these vapors rather than the dosage. Stamopoulos (1991) tested four essential oils (geranium, cypress, eucalyptus and bitter almond) in their vapor form against *Acanthoscelides obtecutus* (Say). He found that eucalyptus vapor strongly reduced fecundity, decreased egg hatchability and increased neonate larval mortality. schmidt *et al* (1991) showed that the numbers of produced offspring of *Sitophilus granarius* (L.), *S. oryzae* (L) and *Callosobruchus chinensis* (L.) emerging from food, on which adults were placed during and after treatment with *A, calamus* oil vapours were lower than in the respective control. The author correlated that to the increase of exposure time rather than to increase in dose. It has also been shown that aromatic plants (as *Labiatae sp.)* were the most active in the protection of *Phaseolus vulgaris* (L.) from *A. obtecutus* infestation. These plants are rich in essential oils, which show vapor toxicity toward adults, and inhibit reproduction by ovicidal and larvicidal effects (REGNAULT-ROGER & HAMRAOUI, 1993, 1994). REGNAULT-ROGER & HAMRAOUI (1995) showed that most of the volatile monoterpenes as carvacol, Linalool eugenol and others exhibited the highest fumigant toxic effect on the adult stage of *A. obtecutus,* beside inhibiting reproduction. In addition, they found that monoterpenes cumulatively affected the survival of the beetle but no compounds acted with the same intensity at each developmental stage. However, as far as the writer is aware, the mode of action of the tested oils is not exactly known, and further studies have to be carried out especially to clarify how they are involved in the physiology of reproduction. The rapid action against some pests is indicative of a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine (KOSTYKOVSKY *et al.*, 2002)

Table 2. Effect of different concentrations of vapors of natural plant oils and commercial monoterpenes on the life span and emerged offspring of PTM.

Tested oils	Conc. Ml/500ml	Life span (days)		No. emerged adult offspring/female mean ± S.D.	% of egg hatching
		Female mea ±S.D.	Male mean±S.D.		
A- Natrual oils.					
Cymbopogon citratus	0.0125	5.8±0.6^{ca*}	5.8±0.6^{ca*}	11.5±2.5^{ca*}	75.2
	0.025	3.6±0.6^{db*}	4.5±1.04^{ca*}	10.5±3.1^{ca*}	67.9
	0.05	0.8±0.2^{dc*}	1.7±0.5^{dc*}	0.5±0.2^{dc*}	41.3
Mentha citrata	0.0125	6.8±1.1^{ca*}	6.0±0.5^{ca*}	14.7±2.4^{ca*}	85.7
	0.025	5.8±0.6^{ca*}	4.5±0.6^{ca*}	9.3±1.1^{cb*}	76.8
	0.05	2.8±0.6^{cb*}	3.8±1.1^{cb*}	1.5±0.5^{dc*}	62.2
Myristica fragrans (Nutmag)	0.0125	6.3±1.5^{ca**}	4.8±0.8^{ca**}	15.8±2.6^{ca**}	73.6
	0.025	4.0±0.5^{ca**}	3.5±0.6^{ca**}	12.5±2.9^{ca**}	70.96
	0.05	1.3±0.5^{cb**}	1.6±0.8^{dc**}	0.5±0.2^{dc**}	43.3
B-Cmmercial monoterpenes.					
α- lonone	0.0125	7.8±1.03^{ba**}	8.8±1.1^{ca***}	29.0±1.8^{aa**}	79.8
	0.025	7.3±1.03^{ca**}	7.8±0.6^{ca**}	21.8±1.3^{cb**}	77.5
	0.05	6.4±0.9^{ca**}	5.8±1.5^{cb**}	2.3±1.4^{dc**}	60.2
Control	---	10.8±1.1^{a}	10.3±1.9^{a}	34.3±2.8^{a}	96.5

$L.S.D_{0.05}$ =2.43 *$L.S.D_{0.05}$ = 5.8*
$L.S.D_{0.01}$ = 3.22 *$L.S.D_{0.01}$ = 8.1*
Means with the same letters in vertical columns are not significantly different (P>0.05).
** significant atp> 0.05.,** highly significant at p>0.01.*

b - On egg hatchability

The effectiveness of vapors of different concentrations of four tested oils on egg hatchability of the potato tuber moth is given in Table 2. Results obtained indicated that the higher the concentration of the oil, the lower the percentage of egg hatchability. Thus, all tested oils could reduce the hatchability of eggs of PTM and the percentage of egg hatchability was oil dose dependent. Moreover, vapors of *Cymbopogon* and *Myristica* oils appeared to be the most effective as they caused the highest reduction in the percentage of egg hatchability at all tested concentrations. The obtained results concerning the role of vapors of volatile oils in reducing egg hatchability are in agreement with SCHMIDT et al. (1991) who recorded that egg hatchability of *Callosobrusus chinensis* (L.) was reduced after 96hr. exposure to vapors of *Acorus calamus* (L.). They attributed that to the toxicity of the vapors to eggs. *Citronella* (*C. nardus*) essential oil has been used as an insect repellent and an animal repellent. Combining few drops each of citronella, lemon (*Citrus limon)*, rose (*Rosa damascene*), lavender and basil essential oils with one liter of distilled water is effective to ward off indoor insect pests. The larvicidal activity of, citronella oil has been attributed to its major monoterpenic constituent citronellal (ZARIDAH *et al.*, 3003). In addition, PATHAK & KRISHNA (1992, 1993) observed high mortality following exposure of eggs of *Corcyra cephalonica* (Stainton) and eggs of *Earias vitella* (F.) to vapors of eucalyptus and clove oils, respectively. GURUSUBRAMANIAN & KRISHNA (1996) found that severe reduction in egg hatchability occurred in *Earias vitalla* (Fabricius) and *Dysderus koenigii* (F.) when their eggs were exposed to the vapour of *Alluim sativum.* They attributed that to some chemical ingredients present in the volatiles of *A. sativum* (bulbs) which probably diffused into eggs and affected the vital physiological and biochemical processes associated with embryonic development. The embryonic development in these eggs was not relatively complete; and the egg color did not change from crystal – transparent to dark color as in the control eggs.

c - The damage assessment of potato tubers exposed to vapors of natural plant oils and commercial monoterpenes.

Data on protection of stored potato tubers by using vapors of ten oils and three commercial monoterpenes (at 0.05 ml/500 ml air) are given in Table 3. By screening a large number of natural plant oils and commercial monoterpenes, it was clear that few numbers of the tested materials had the ability to protect potato tubers from PTM infestation. The most effective oils which gave the lowest tuber damage index, could be arranged in an ascending order as follows: *M. fragrans* (nutmag) < *C. citratus* < *M. citrata* < a-Ionone; showing 2.4 ± 0.8, 2.5 ± 1.5, 4.1 ± 1.6 and 6.6 ± 1.4 tunnels/tuber, respectively, compared to 30.0 ± 0.0 tunnels / tuber in the control. The difference is highly significant ($P < 0.01$).

Table 3. Damage index of potato tubers treated with vapors of natural plant oils and commercial monoterpenes.

Tested oils (at 0.05 ml / 500 ml)	Tuber damage index (tunnels / tuber) mean ± S. D.
A-Natural plant oils :	
Cinnamonium zeylanicum	27.5± 0.9 a
Cymbopogon citratus	2.5± 1.5 c **
Eucalyptus rostratus	25.8± 1.6 a
Janiperus virginiana	26.6± 1.4 a
Mentha citrata	4.1± 1.6 c **
Myristica ragrans (Mace oil)	21.3± 3.3 b **
Myristica fragrans (Nutmag oil)	2.4± 0.8 c **
Pelargonium graveolens	23.3± 1.3 b**
Syzygium aromaticum	24.1± 1.6 b **
Zingiber officinale	22.5± 1.2 b**
B- Commercial monoterpenes:	
Geraniol	23.3± 1.3 b **
Isoeugenol	28.3± 1.02a
x-Ionone	6.6± 1.4 c **
Control	30.0± 0.0 a

$L.S.D_{0.05}=5.04$ $L.S.D_{0.01}=6..9$

Means with the same letters have no significant difference ($P > 0.05$).

* significant at p>0.05., ** *highly significant at p>0.01.*

On the other hand, a moderate damage was recorded in tubers exposed to vapours of Geraniol (monoterpene), *P. graveolens, M. fragrans* (Mace oil), and *Z. officinale* (natural oil), where the average damage indices were 23.3, 23.3, 21.3and 22.5 tunnels /tuber, respectively. The difference from the control is statistically significant ($P < 0.05$). The remaining oils and monoterpenes did not record any significant difference ($P > 0.05$) from the control.

Damage index of potato tuber treated with natural oils and commercial monoterpene shows the role of lower concentrations of the most effective of the aforementioned oils (0.025 ml and 0.0125 ml / 500 ml air) in protecting potato tubers against PTM damage are given in Table 4. The obtained data showed highly significant difference ($P < 0.01$) in tuber damage indices among different concentrations of each oil. The effect was dose dependent, as higher doses showed the least tuber damage index; indicating their efficacy in tuber protection. In case of treatment by using *M. citrata* oil, the mean damage index of tubers decreased from 25.8 ± 1.6 tunnels/ tuber at 0.0125ml/ 500ml., till it reached 4.1 ± 1.6 tunnels / tuber 0.05 ml/500 ml concentrations. An almost similar trend was reached in case of *C.citratus* and *M. fragrans* (nutmag oil) which appeared the most effective, while a-Ionone (monoterpene) appeared the least effective, particularly at lower concentrations (0.0125 and 0.025 ml / 500 ml). Similarly, BEKELE *et al.* (1997) found that exposure of adults of *Sitotroga cerealella* (Oliv.) to higher dosages of ground leaves and essential oil extract of *Ocimum kenyense* (Ayobangira) induced 100% mortality within 24hr., and reduced feeding, indicating high protecting potency against the insect damage. SHAAYA *et al.* (1997) used vapors of a large number of essential oils extracted from various spice and herb plants against several major stored product insects. They found that *Labiatae sp.* oil (ZP_5j) at higher concentrations (1.5 - 4.5$\mu L/\ l$ air) caused 90% mortality of all insects in space tests after 24 hr. of exposure. They suggested that most of plant oils can play an important role in stored grain protection and reduce the need for and risks associated with the use of insecticides. Essential oil constituents are primarily lipophilic compounds that act as toxins, feeding detterents and oviposition deterrents to a wide variety if insect pests, it was also effective as a fumigant (KOUL *et al.*, 2008).

B -Effect of dusting potato tubers with natural plant materials on certain biological aspects of PTM.

Potato tubers were treated by different concentrations of the tested materials (natural plant oils and commercial monoterpene) using the dusting technique materials. Treated tubers were exposed to the potato tuber moth for eggs laying. All the biological aspects of the pest were followed up. Results on the persistence index of dusting potato tubers with plant oil and monoterpene (at 1% cone, with talcum powder) against larvae of PTM are presented in Table 5. It was clear that, all tested materials caused initially high larval mortality and low damage index. They, however, showed variable degardative periods. Thus, treatment of potato tubers with *Cymbopogon* and *Mentha* oils (1% in talcum powder) initiated (after one day) high percentages of larval mortality; being 88.8 and 87.0%, respectively, and low damage index; being 2.4 tunnels / tuber for each of them. After 20 days of storage in cas *of Cymbopogon* and 15 days of storage in case of *Mentha,* the final larval mortality decreased to reach 23.5 and 28.05%, with damage indices of 19.2and 22.4 tunnels/ tuber, respectively. This led to 35.1 and 24.3% protection, with 5.29 and 4.84 persistence index, respectively. On the other hand, treatment of potato tubers with *Myristica* and α-Ionone caused initially 82.6 and 83% larval mortality; showing damage indices 5.2 and 4.4 1.1 tunnels / tuber, respectively. After 10 days in case *of Myristica* and 15 days in case of ά- lonone, the final effect was reached, where the larval mortality was 39.0 and 24.7%, respectively, with a damage index of 18.4 tunnels/ tuber and 37.8% protection for each of them. This led to 4.72 and 4.46 persistence index, respectively.

Table 4. Damage index of potato tubers treated with different concentrations of natural plant oils and a commercial monoterpene

Tested oils	Conc. ml/500 ml	Tuber damage index (tunnels / tuber) mean-± S. D.
A- Natural plant oils :		
Cymbopogon citratus	0.0125	17.5 ± 2.5 bc**
	0.025	5.0 ± 2.1dc**
	0.05	3.3 ± 2.3 d c**
Mentha citrata	0.0125	25.8 ± 1. 6b*
	0.025	12.5 ± 3.8 cb**
	0.05	4.1 ± 1.6 dc**
Myristica fragrans (nutmag oil)	0.0125	13.3 ± 2.7 cb**
	0.025	7.5 + 2.1 cb**
	0.05	2.7 ± 0.3 dc**
B- Commercial monoterpene:		
a-Ionone	0.0125	23.3 ± 1.3 ba*
	0.025	19.9 ± 2.4 da*
	0.05	4.1 ± 1.6 dc*
Control	---	30.0 ± 0.0 a

$L.S.D_{0.05}$ 5.04 $L.S.D_{0.01}$ = 6.9

Means with the same letters in vertical columns for each oil concentration have no significant difference (P > 0.05). * significant at p>0.05., ** highly significant at p> 0.01.

C - Persistence index of plant oils sprayed on gunnysacks.

The results obtained in Table(6)shows that, spraying natural plant oils separately or mixed together to gunny sacks resulted in various levels of reduction of PTM progeny and damage indices, throughout the storage period. Thus, the highest initial percentages of reduction of PTM progeny were 95.7, 95.7 and 94.3% after one day of storage in gunny sacks sprayed with 1% *Myristica, Cymbopogon* + *Myristica* and *Cymbopogon,* respectively. These gradually decreased to reach 47.1, 49.3 and 48.4% after 20 days of storage with respective damage indices of 18.4, 18.4 and 19.9 tunnels/ tuber, respectively. So their persistence indices were 9.84, 10.30 and 7.14, respectively. Treatment of the gunny sacks with *Mentha* + *Cymbopogon* and *Mentha* + *Myristica,* on the other hand, exhibited a moderate initial reduction in PTM progeny after one day of storage, showing 89.95 and 78%, respectively. These gradually decreased till reached 48.9 and 40.7% after 15 days of spraying gunny sacks; with damage indices of 18.4 and 19.2 tunnels / tuber, respectively. This led to 8.15 and 7.83 persistence index, respectively. *Mentha* oil or the three oils when mixed together caused relatively less reduction in the percentage of PTM progeny after one day of spraying the gunny sacks; being 51.2 and 60.8%, respectively. Their potency highly decreased after 10 days of storage causing *32,6%* progeny reduction in case of *Mentha;* and 42.7% in case of mixed oils after 5 days of spraying gunny sacks; so their persistence indices were 7.14 and 3.43, respectively. It was noticed that, the best protection occurred after one day of storage for all tested oils and then gradually decreased till it reached a lower level after variable periods according to theoil used. Accordingly, their persistence indices varied, and was highest in case of oils of *Myristica, Cymbopogon* and their mixture together; as they remained potent for more than 20 days after spraying. These observations agree with Sharma *et al* (1983) who found that treatments of the Jute or tarpaulin cover spread over stored potatoes with 10% neemrich oil extract provided prophylactic measure against the potato tuber moth. In this respect, Abdalla and Matter (1994) also recorded that spraying covers of potato heaps with lemongrass or neem extract significantly reduced moth infestation up to 2 and 3 weeks for storing, respectively; while mixing both extracts did not significantly increase their action. From the present results, it could be concluded that most plant oils could exhibit good protection for long periods when used as vapours against PTM, sprayed in gunny sacks or used as powder dusted on potato tubers. However, dried plant powders gave much longer protection of the potato tubers from PTM infestation. Raman *et al* (1987) mentioned that *E.globulus, Lantana camara* and *Minthostachys* sp., both in the dried shredded and powder form, were effective in controlling PTM damage in potatoes for 4 months; while treatment of potato tubers with vegetable oils or neem extract did not reduce PTM damage or tuber rot for long storage period. Similarly, Khashyap *et al* (1992) tested the efficacy of *Cannabis sativa* dry leaves powder in controlling PTM in stored potatoes in India. They found that 2cm. thick layer of *C. sativa* protected potatoes for up to 120 days. Clove oil and their role in protecting stored products from target insects were studied. For example, Sighamony *et al* (1986) studied the efficiency of Clove, Ceder wood and Karanja oils at doses of 25 -100 ppm against *Sitophilus oryzae* and *Rhyzopertha dominica.* Their results indicated that all tested oils had the ability to protect wheat grain from insect infestation up to 60 and 30 days,respectively, after exposure. The persistence of toxicity ranked Clove oil > Karanja oil > Ceder wood oil against *S. Oryzae;* while in case of *R. dominica,* both toxicity and persistence were lower and ranked Karanja oil > Ceder wood oil > Clove oil. Bhaduri *et al* (1990) found that treatment of bean seeds with *C. nardus* and *C. citratus* oils exhibited protection up to 90 days from *Callosobruchns maculatus* (F.) infestation.

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Table 5. Persistence index of dusting potato tubers with natural plant oils and a commercial monoterpene against PTM larvae

Duration of storage period / Tested oils (1%)	One day		5 days		10 days		15 days		20 days		Residual efficiency (F/I)	*Persistence* index (F/I X days)
	Larval mortality (%)	Average D. L	Larval mortality (%)	Average D. I.	Larval mortality (%)	Average D. I.	Larval mortality (%)	Average D. I.	Larval mortality (%)	Average D. I.		
A. Natural plant oils												
Mentha citrate	I 87	2.4 ± 1.2 (91.5)	86.5	6.2±2.03 (79.1)	51.3	14.8±1.5 (50)	F 28.05	22.4±1.5 (24.3)	16.7	27.6±0.6 (6.8)	0.322	4.84
Cymbopogon citratus	I 88.8	2.4±1.2 (91.5)	73.8	4.0±0.9 (86.5)	60.5	10.4±1.4 (64.9)	41.8	11.6±1.8 (60.8)	F 23.5	19.2±0.7 (35.1)	0.265	5.29
Myristica fragrans	I 82.6	5.2±1. 4 (81.7)	64.3	12±2.2 (59.5)	F 39.0	18.4±2.1 (37.8)	21.6	17.2±1.5 (41.9)	19.5	26±1.1 (12.2)	0.472	4.72
B- Commercial monoterpe e												
ά- Ionone	I 83	4.4±1.1 (84.5)	77.6	7.8±0.7 (73.6)	50	11.6±1.2 (60. 8)	F 24.7	18.4±2.1 (37.8)	11.8	26±1.I (12.2)	0.298	4.46
Control (Talcum powder)	I 1.0	28.4±0.7	11.0	29.6±0.5	12.6	29.6±0.5	10.8 .	29.6±0.8 .	11.0	29.6±0.5	-	-

$L.S.D_{0.05}$ = 4.06 $L.S.D_{0.05}$= 10.5

I= initial effect; F = final effect, D.I. = damage index

I= initial effect; F = final effect, D.I. = damage index

- The values between brackets indicate % of protection (i.e, % of reduction in D.I.).
- Deviations in the table indicate the standard deviations of means.

Table 6. Persistence index of tested natural plant oils sprayed on gunny sacks against PTM infestation.

Duration of storage	One day		5 days		10 days		15 days		20 days		Residual efficiency (F/I)	Persistence index (F/I X days)
Tested oils (1%)	Average Emerged progeny	Average D. I.	Average Emerged progeny	Average D. I.	Average Emerged progeny	Average D. I.	Average Emerged progeny	Average D. I.	Average Emerged progeny	Average D. I.		
Cympopogo n Citratus	2.4 ± 1.6 *i* (94.3)	0.5t0.5 (98.3)	9.8±2.4 (75.3)	1.2±0.8 (96)	13.4±2.7 (71.6)	4.8±0.8 (84)	14.6±2.2 (68.4)	11.6±1.8 (61.3)	22.8±2.2 F (48.4)	19.9±2.4 c33.7)	0.357	7.14
Mentha Citrata	20.4t 1.8	12.8±1.9 (5 7 . 3)	22±2. I (44.4)	14.4t1.7 (52)	31.4±3.03 F (32.6)	7.2±2.2 (42.7)	38.0±2.02 07.7)	24.8±1.5 (18.7)	38±2.1 (14.02)	24.8±1.5 (18.7)	0.637 0.637	6.37
Myristica fragrans	1.8±1.2 i(95.7)	0.5±0.5 (98.3)	8.2±1.9 (79.3)	4.4±1.1 (85.3)	11.8±2.8 (74.7)	8.4±2.0 (72)	18.2±2.7 (60.6)	11.6±1.8 (61.3)	23.4±3.1 F (47.1))	18.4±2.1 (38.0	0.492	9.84
Menthe + *Myristica*	9.2±1.9 i (78)	5.6±2.2 (81.3)	15.4±1.6 (61.1)	6.2±2.5 (79.3)	20.2±2.1 (56.7)	14.8±1.5 (50.7)	27.4±2.01 F (40.7)	19.2±0.7 (36.0) ..-	30.4±2.9 (31.2)	21 2±1.6 (29.3)	0.521	7.83
Menthe + *Cyinbopogon*	4.2±2.2 i (89.95)	1.21-0.8 (98)	17.4±2.1 (56.06)	6.2±2.1 (79.3)	17.9±3.7 (61.6)	6.2±1.9 (79.3)	23.6±2.5 *F* (48.9)	18.4±1.9 (38.7)	28.6±2.7 (35.3)	22.4±1.8 (25.3)	0.544	8.15
Cymbopogon + *Myristica*	1.8±1.2 1(95.1)st	0.5±0.5 *(98.3)*	7.4±1.97 (81.3)	4.0±0.9 (86.7)	12.8±2.03 (72.5)	6.2±2.1 (79.3)	18±2.4 (61.04)	11.6±1.8 (61.3)	22.4±2.1 F (49.3)	18 4±1.9 (38.7)	0.515	10.30
Cymbopogon + *Myristica* + *Mentha*	16.4t3.8 (60.8)	7 8+0 7 (74)	23.1±1.9 F (41.7)	19±0.8 (36.6)	27.6±2.5 (40.8)	19.2±0.7 (36)	38±0.9 (17.7)	22 4+1 5 (49.3)	40.2±1.7 (9.05)	26 0±1.1 (13.3)	0.686	3.43
Control	41.8±2.3	30±0.0	39.6±3.9	30±0.0	46.6±2.9	30·1·0.0	46.2±3.1	30±0.0	44.2±3.7	30±0.0	-	-

1= initial effect; F = fmal effect D.I. = damage index

- For each storage period, the values between brackets in the left columns represent the % of reduction in PTM progeny; while those in the right columns the % of protection (i. e., % of reduction in D. I.)
- Deviations in the table indicate the standard deviations of means.

L.S.D 0.05 = 4.9 L.S.D 0.01= 6.7

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