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Preparation of some safe synthetic botanical materials as fumigant tablets as alternatives for hazard phostoxin for controlling cowpea beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) infesting leguminaceous seeds

Nilly, A. H. Abdelfattah¹ and Mai, M. A. Gnedey²

¹Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

²Central Agriculture Pesticides Laboratory, Agricultural Research Center, Dokki, Giza, Egypt.

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Abstract:

Although phostoxin fumigant tablets are considered the best material for controlling cowpea beetle for their ease of use and the most successful material against this pest it has undesirable and hazardous effects against man, beneficial animals and insects, this research was completed for preparation of the two safe botanical materials, thymol and camphor as fumigant tablets and evaluation their effect against cowpea beetle as a solution of damage effect of phostoxin. Both botanical materials were prepared as pure and mixed with the anti-releasing agent, talc or bee wax at ratios 80:20 and 65:35 (botanical: anti-releasing agent) as fumigant tablet which their diameter was 13 mm and 0.3 mm height. The initial effect was determined by exposure cowpea beetles *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) adults, three days and determination % mortality and the residual effect was examined after 1, 2 and 3 months only for the lowest concentration which succeeded in initial effect, i.e. which after three days of exposure, adults died in 100 percent. Results obtained indicated that camphor was very highly effective than thymol in initial effect since it gave 100 % mortality at 0.03 gm/120 ml (wt./v.) while thymol gave 100 % mortality rate at 0.4 gm/120 ml (wt./v.). Results of residual effect indicated that the succeeded concentration in initial effect gave 100 % mortality in their residual effect up to three months storage. Results indicated also that treatment with botanical tablets protected cowpea seeds germination which was very high than untreated infested seeds since the larvae burrow in seeds and destroy endosperms then reduce germination. For economic consideration, it could be recommended camphor at 0.03 gm/120 ml for controlling cowpea beetle infesting leguminaceous seeds.

Introduction

Stored grain insects are a worldwide concern because they decrease grain quantity and quality. Their harm to stored grains and their products may be 5–10% in the temperate environment and 20–30% in

a tropical environment (Haque *et al.*, 2000). While tropical areas, insect pests cause the loss of stored food grains also semitropical settings (Tripathi *et al.*, 2001). As a result, there is a requirement for a variety of acceptable pesticides or repellents for use on food

grains. In general, synthetic pesticides and fumigation are the primary ways of pest management in stored products. Cowpea *Vigna unguiculata* (L.) is among the most well-known cost-effective legume seeds, having a high protein, vitamin, and mineral content (Singh *et al.*, 2003).

Cowpea beetle *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) is regarded as one among the most popular damaging brushed species of various legumes, including cowpea, in the world's tropic and subtropical areas (Babarinde *et al.*, 2016). Inside the seeds, the larval and pupal stages grow. The larvae burrow into the seed, destroying the endosperm (Adenekan *et al.*, 2018). It destroys 25% of seeds, reducing germination by up to 13% and lowering the market value after six months of storage (Haile, 2006). Phostoxin fumigant tablets are used at a large scale for controlling leguminous beetles, stored grain insects as it easy for application and very effective against this pest.

Unfortunately, phostoxin is very toxic to man, therefore efforts should be directed towards finding more safe materials to be used instead of phostoxin for controlling cowpea beetle. Previous researches indicated that both thymol and camphor have fumigant effect against stored grain insects (Rozman *et al.* (2006), Negahban *et al.* (2007), Abdelgaleil *et al.* (2009), Chu *et al.* (2010), Liska *et al.* (2010), Hossain *et al.* (2014) and Wahba *et al.* (2018).

The goal of this research is the preparation of those safe botanical materials thymol and camphor alone or mixed with anti-releasing agents (Bee wax and talc) as fumigant tablets as alternatives to hazard phostoxin for controlling cowpea beetle.

Materials and methods

1. Botanical materials:

1.1. Thymol (99%)

- IUPAC name: 2-Isopropyl-5-methylphenol

Thymol (2-isopropyl-5-methylphenol) is a natural monoterpene phenol derivative of cymene, C₁₀H₁₄O₁. The melting point is 51°C boiling point is 232 °C and solubility in water (Insoluble in water). Produced by: Alpha Chemika, India. Supplied by El-Goumouria Co. for Trading Medicines, Chemicals and Medical Appliances Importation Department 32 El Sawah Str., Cairo, Egypt.

1.2. Camphor (99%):

- IUPAC name: (1, 7, 7-Tri methyl bicycle [2.2.1] heptan-2-one). Camphor is a waxy, white or transparent solid with a strong, aromatic odor. It is a terpenoid with the chemical formula C₁₀H₁₆O. Melting point is 175-181°C and Boiling point is 204°C. Produced by: S.D. (Fine.Chem. limited), India. Supplied by: El-Goumouria Co. for trading Medicines, Chemicals and Medical Appliances Importation Department 32 El Sawah Str., Cairo, Egypt.

1.3. Anti-releasing agent:

1.3.1. Talc powder: (Magnesium meta silicate): it was supplied by El-Nasr Co. for Phosphate, Cairo, Egypt. Talc is a mineral composed of hydrated magnesium silicate with the chemical formula H₂Mg₃ (SiO₃)₄ or Mg₃Si₄O₁₀ (OH)₂.

1.3.2. Bee wax: It was supplied from Bee Pest's Research Department, Plant Protection Research Institute.

2. Cowpea beetle culture:

The rearing technique for stock culture and experimental insects was, according to Kalifa and Badawy (1961), preparing and conditioning of seeds was, according to Selim (1963) and Abdel-Kawy (1979). For starting a culture of testing, insect, adult cowpea beetle, *Callosobruchus maculatus* reared on cowpea seeds in a glass jar (Each of approximately 500 ml) and each jar was covered with

muslin cloths and fixed with rubber bands. Approximately 500 adults were placed in jars containing seeds for egg-laying and then housed in an incubator at $30\pm 1^{\circ}\text{C}$ and $65\pm 5\%$ RH. to create an initial population of insect adults that were all the same age. All insects were removed from the media after three days, and the jars were returned to controlled conditions.

The seeds were regularly observed for lay eggs and subsequent adult emergence. Hatched eggs were known by the presence of larval frass which causes the egg to turn milky–white as larvae tunnel into the seeds for larvae and pupae age calculated. The insect was reared in the Department of Stored Products and Grains Pests of the Plant Protection Research Institute of the Agricultural Research Centre in Dokki, Giza, Egypt.

3. Preparation of thymol and camphor as fumigant tablets:

Each botanical material was used alone (100%) and mixed with bee wax and talc as an anti-releasing agent at two concentrations 80% and 65% active ingredient. Active ingredient alone or mixed with an anti-releasing agent more melted using heat, mixed well then poured in circle plastic vessel has 13 mm diameter and 0.3 cm high, after $\frac{1}{2}$ hr. formed tablets were turned off from the plastic vessel and stored in plastic sheet up to biological tests. Five different fumigant tablets were prepared for each botanical material:

3.1. Botanical alone (100%).

3.2. Botanical+ wax (80%).

3.3. Botanical+ wax (65%).

3.4. Botanical+ talc (80%).

3.5. Botanical+ talc (65%).

4. Initial the efficacy of the prepared fumigant tablets against cowpea beetle adults:

To study the initial effect of the tested materials, 20g of cowpea seeds were translated to small container 120 ml capacity seeds and immediately

infested with 20 adult insects of *C. maculatus* then different weights of the prepared fumigant tablets were added. Three replicates for each weight compared with untreated. The jars were covered with caps, dead and alive adults were counted after 3 days of exposure and mortality percentages were calculated according to (Abbott, 1925).

5. Determination of the insecticidal effect against immature stages of cowpea beetle:

The effect of treating immature stages of the cowpea beetle with fumigant tablets on adult emerging was carried out according to Shemais *et al.* (2002) by placing adults on cowpea seeds for one day to lay eggs. Fumigant tablets were added after 1 day of laying eggs for 3 days to study the latent effect on the egg stage also, fumigant tablets were added after 12 (As a larva) and 24 days (As pupa) from laying of the eggs for 7 days to study the latent effect on larvae and pupae, both of hatching egg and emerged adults were counted.

6. Determination of the residual effect of the prepared fumigant tablets of cowpea beetle:

It was determined for cowpea seeds stored with different weights of tablets after 1, 2 and 3 months of treatments by the same test mentioned materials according (Abdelfattah and Zein, 2019).

7. Determination of the residual effect on the viability of cowpea seeds (Germination test):

The germination test was carried out under laboratory conditions to evaluate the effect of thymol and camphor on cowpea seeds after 1, 2 and 3 months of storage. 20 seeds were randomly picked from treated and untreated groups and placed on a surface layer of cotton wool in a petri dish (6×1 cm) which was wetted careful with tap water. Seeds were set up in a three-replicate, entirely random design. After 7 days later the germination

percentage was calculated (AL-Akhdar *et al.*, 2019).

Results and discussion

1. Initial effect on adults:

Results in Table (1) about the initial effect of treatment with fumigant tablets of thymol for 3 days on cowpea adults indicated that concentration 0.4 gm gave 100% for all thymol tablets while at 0.3 gm, a mixture of thymol with talc showed the highest toxic effect than a mixture with wax, therefore mixing thymol with talc is preferred than mixing with wax. On the other

hand, results presented in Table (2) about the effect of camphor fumigant tablets against cowpea beetle adults, indicated that all tested concentrations up to 0.03 gm for all camphor mixture succeeded in their initial toxicity since they gave 100% mortality. As general results of Tables (1 and 2), it could be said that camphor fumigant tablets are more active than thymol.

Table (1): Initial toxicity of fumigation with different concentration of thymol prepared as fumigant tablets against adult of *Callosobruchus maculatus*.

Treatment	% mortality					
	1 gm	0.5 gm	0.4 gm	0.3gm	0.2gm	0.1 gm
Wax 80%	100	100	100	77.5	95	65
Wax 65%	100	100	100	87.5	41.5	40
Talc 80%	100	100	100	100	95	65
Talc 65%	100	100	100	95	65	45
Pure	100	100	100	100	100	100
Control	0.0	0.0	0.0	0.0	0.0	0.0

Table (2): Initial toxicity of fumigation with different concentration of camphor prepared as fumigant tablets against adult of *Callosobruchus maculatus*.

Treatment	% mortality					
	0.25gm	0.12 gm	0.06 gm	0.03 gm	0.02 gm	0.01 gm
Wax 80%	100	100	100	100	75	0.0
Wax 65%	100	100	100	100	25	0.0
Talc 80%	100	100	100	100	100	0.0
Talc 65%	100	100	100	100	17.5	0.0
Pure	100	100	100	100	100	25
Control	0.0	0.0	0.0	0.0	0.0	0.0

2. Latent effect against immature stages:

2.1. First experiment:

The lowest concentrations which gave 100% mortality against adults were tested against immature stages eggs, larvae and pupae by the methods mentioned before. Results in the Table (3) indicated that all tested materials succeeded against the egg stage since no adults emerged in egg treatments except in camphor wax 65% and camphor talc 65%, but all treatments failed against larvae and

pupae stages since they did not prevent an adult emergency, therefore the second experiment was carried at more higher concentrations 1g thymol and 0.25g for camphor.

2.2. Second experiment:

Results in Table (4) about the latent effect of cowpea beetle egg with fumigant tablets for 3 days with thymol at 1 gm and camphor at 0.25 gm /120 ml, results obtained indicated that no adult emerged in all treating with thymol and camphor.

Table (3): The effect of treatments against immature stages of cowpea beetle fumigated until adult emerged.

Treatment		Egg		Larva		Pupa	
		Emerged adult no	Reduction%	Emerged adult no	Reduction %	Emerged adult no	Reduction %
Thymol	Wax 80% 0.4 g	0.0	100	46.5	43.6	65	21.2
	Wax 65% 0.4 g	0.0	100	43.5	47.3	82	0.6
	Talc 80% 0.3 g	0.0	100	49	40.6	82	0.6
	Talc 65% 0.4 g	0.0	100	63.5	23	54	34.5
	Pure 0.1	0.0	100	64	22.4	80.5	2.4
Camphor	Wax 80% 0.03 g	0.0	100	14	83.5	22.5	72.7
	Wax 65% 0.03 g	7.0	77.8	23.5	71.5	41	50.3
	Talc 80% 0.02 g	0.0	100	11	86.6	44	46.6
	Talc 65% 0.04 g	0.3	99	46	44.2	13	84.2
	Pure 0.02 g	0.0	100	4	95	15	82
Control		31.5		82.5	43.6	82.5	

Table (4): Latent effect of treatment of *Callosobruchus maculatus* eggs with fumigation tablets for 3 days.

Treatment		Mean No. of eggs	Hatched %	Pupation %	Adult emergence %	Reduction %
Thymol 1 gm /120 ml	Wax 80%	111.33	7.41	0	0	100
	Wax 65%	98.67	2.1	0	0	100
	Talc 80%	106.67	7.27	0	0	100
	Talc 65%	88	3.68	0.75	0	100
	Pure	106.33	13.33	0.31	0	100
Champhor 0.25 gm /120 ml	Wax 80%	158.33	18.45	5.89	0	100
	Wax 65%	125	10.25	5.06	0	100
	Talc 80%	89.33	25.46	2.98	0	100
	Talc 65%	125	7.64	2.66	0	100
	Pure	113	1.29	0	0	100
Control		115.33	100	87.58	79.63	20.37

As shown in Table (5) about the treatment of cowpea beetle larva and pupa with fumigant tablets for 7 days with thymol at 1 gm and camphor at 0.25 gm., results obtained indicated that no adult emerged in case of treating

larva with thymol and camphor, while in case of pupa treatment all concentration except mixing thymol with wax 80% and camphor with talc 65% gave 100% reduction in an adult emergency.

Table (5): Latent effect of thymol and camphor prepared as fumigant tablets against larva and pupa stages of *Callosobruchus maculatus* for 7 days.

Treatment		Larva		Pupa	
		% adult emergence	Reduction %	% adult emergence	Reduction %
Thymol 1 gm /120 ml	Wax 80%	0	100	50	50
	Wax 65%	0	100	0	100
	Talc 80%	0	100	0	100
	Talc 65%	0	100	0	100
	Pure	0	100	0	100
Champhor 0.25 gm/120 ml	Wax 80%	0	100	0	100
	Wax 65%	0	100	0	100
	Talc` 80%	0	100	0	100
	Talc 65%	0	100	12	88
	Pure	0	100	0	100
Control		100		100	

3. Residual effect of botanical materials against cowpea beetle:

This test was carried out by concentrations that only succeeded in initial effecting against adults and immature stages to determine its ability to protect cowpea seeds from *C. maculatus*

for long periods. As shown in Table (6) all tested concentrations of thymol or camphor fumigant tablets gave complete protection of cowpea seeds up to 3 months of a treatment since they gave 100% mortality against adults.

Table (6): Residual effect of thymol and camphor prepared as fumigant tablets against adults of *Callosobruchus maculatus* on cowpea seeds.

Treatment		1 month (%)	2 months (%)	3 months (%)
Thymol 1gm / 120 ml	Wax 80%	100	100	100
	Wax 65%	100	100	100
	Talc 80%	100	100	100
	Talc 65%	100	100	100
	Pure	100	100	100
Champhor 0.25 gm / 120 ml	Wax 80%	100	100	100
	Wax 65%	100	100	100
	Talc` 80%	100	100	100
	Talc 65%	100	100	100
	Pure	100	100	100
Control		0	0	0

Results obtained well agree with Rozman *et al.* (2006) about the fumigant effect of camphor against rusty grain beetle and *S. oryzae*, also with Abdelgaleil *et al.* (2009) against rice weevil *S.oryzae*, Chu *et al.* (2010) about camphor maize weevil and Hossain *et al.* (2014) about camphor

against *S. oryzae*. The results are compiled also with Wahba *et al.* (2018) about the fumigation effect of thymol against the cowpea beetle.

4. Residual effect on cowpea seeds:

Results in the Table (7) about the effect of all treatments on cowpea germination in case botanical materials

were very high than untreated infested control seeds since larvae burrow into the seed and destroy its endosperm then reducing germination. Germination in case camphor was more than thymol, camphor + talc showed the highest

value followed by camphor + wax, while thymol + wax (80:20) showed the lowest value of germination followed by thymol + wax (65:35) while in case of thymol + talc showed the highest value of all thymol treatments.

Table (7): Residual effect of botanical materials on cowpea seeds germination percentage.

Treatment		1 month (%)	2 months (%)	3 months (%)
Thymol 1 gm / 120 ml	Wax 80%	46.66	46.66	31
	Wax 65%	76.66	76.66	66.5
	Talc 80%	93.3	93.3	88.5
	Talc 65%	95	95	90
	Pure	53.3	53.3	40
Champhor 0.25 gm / 120 ml	Wax 80%	96.7	96.7	95
	Wax 65%	97.66	97.66	95
	Talc 80%	100	100	96
	Talc 65%	100	100	97
	Pure	100	100	96.7
Infested Control		38	33.3	0
Uninfested Control		100	100	100

5. Mode of action:

The mode of action of the tested materials may be due to one or more of the following: -Respiration effect as mentioned by De-Oliveira *et al.* (1997) and Lee *et al.* (2001 b). -Fumigation effect as mentioned by Lee *et al.* (2001a), Negahban *et al.* (2007) and Jayakumar *et al.* (2017). -Inhibition of acetyl cholinesterase as mentioned by Lee *et al.* (2001a). -Repellent effect as mentioned by Nerio *et al.* (2010).

It could be concluded that both thymol and camphor prepared as fumigant tablets were active against cowpea beetle and showed complete protective effect up to 3 months after treatment, the study proved also that camphor was more active than thymol on the other hand the anti-releasing agent talc was better than wax.

As a general recommendation, it could be recommended with camphor prepared as fumigant tablet at 0.25gm/120 ml as an alternative to the danger phostoxin for controlling cowpea beetle, also more experiments at large scale with this recommended

concentration and lower concentration should be carried out.

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