Efficacy of phyto-oils and speedbox for the control of *Callosobrochus maculatus*

Shaaya, E.*#, Kostyukovsky, M.
Dept. of Food Quality and Safety, The Volcani Center, Israel

*Corresponding author, Email: elines@smile.net.il
#Presenting author, Email: elines@smile.net.il

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Abstract

Currently, the measures to control pest infestation in grain and dry stored food rely heavily on toxic fumigants and contact insecticides. During centuries, traditional agriculture in developing countries has developed effective means for insect pest control using botanicals. Their efficacy and optimal use still need to be assessed, in order to make them cheap and simple means for insect control available to users. The aim of the current study is to evaluate the potential use of edible and essential oils and their active constituents for the control of *Callosobrochus maculatus.* The most active contact insecticides were edible crude oils of rice, maize, cotton seeds and palm. In field studies, using rice and palm oils at a rate of 1.5 and 3 g/kg, chick peas were completely protected from insect infestion for a period of 4-5 months. The fatty acids capric and undecanoic were also found very potent. The essential oils and their constituents were found to have higher activity as fumigans than contact insecticides, mainly on eggs and pupae. By using speedbox, the phosphine treatment was reduced from 5-7 days to 2-3 days to control all developmental stages of *C. maculatus.*

Keywords: fumigation, contact insecticides, edible oils, essential oils, speedbox

1. Introduction

The pulse beetle, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) is one of the major pests of stored cowpeas, lentils, and green and black gram in the tropics (Sharma, 1984; Raja et al., 2007). The infestation often begins in the field on the dry ripe seeds before harvest. The damage to the seeds can reach up to 50% after six months of storage (Caswell, 1980) and up to 90% in the storage annually, according to the International Institute of Tropical Agriculture (1989).

At present, only two fumigants are still in use: methyl bromide and phosphine. The first one is being mostly phased out in developing countries due to its ozone depletion effects (WMO, 1995; Shaaya and Kostyukovsky, 2006). The second fumigant, phosphine, is the only one widely used today, but there have been repeated indications that certain insects have developed resistance to it. It should be mentioned that although effective fumigants and contact synthesized insecticides are available, there is global concern on their negative effects to non-targeted organisms, pest resistance and pesticides residues (Kostyukovsky et al., 2003). In recent years, an attention has been focused on the use of botanicals as possible alternatives to toxic insecticides, for the protection of agricultural products, due to their low mammalian toxicity and low persistence in the environment (Raja et al., 2001; Papachristos and Stampoulos, 2002). Numerous plant species have been reported to have insecticidal properties capable of controlling insects (Amason et al., 1989; Grainge and Ahmed, 1988).

Our earlier investigations on the effectiveness of the essential oils extracted from aromatic plants, showed great promise for the control of the major stored product insects. Several of them were found to be active fumigants at low concentrations against these insects (Shaaya et al., 1991, 1993, 1994).

The use of edible oils as contact insecticides to protect grains, especially legumes, against storage insects, is traditional practice in many countries in Asia and Africa. The method is convenient and inexpensive for the protection of stored seeds in households and in the small farms. The present investigation was intended to evaluate the efficacy of essential and edible oils as fumigants and contact insecticides to suppress populations of the legume pest insect *C. maculatus*.

Phosphine is mainly in use today for stored product insect pest control. However, some limitations, such as low temperatures and relatively long exposure time, limit its use. In order to overcome these difficulties, a special devise, called ‘speedbox’ has been developed. Our previous studies showed a high efficacy and advantage of the Phosphine fumigation by speedbox against stored product insects, compared with the common’ Phostoxin tablets (Kostyukovsky et al., 2010; Kostyukovsky and Shaya, 2012). In the current study the toxicity of Magtoxin tablets and plates was also compared with the treatment using Speedbox.

2. Studies with edible oils and fatty acids as contact insecticides

The biological activity of the edible oils and straight chain fatty acids, which contain carbon atoms from C5 to C18, was evaluated in laboratory tests against *C. maculatus*. The insects were reared on chickpeas seeds at 25°C and 70% r.h. The required amount of the test material was mixed with the appropriate amount of acetone (50 ml/kg seeds) and the mixture was applied to the seeds in droplets with continuous hand mixing. The acetone was evaporated under a hood for several hours. Control seeds were treated with acetone alone. Five males and five females one day old were introduced to 5 g seeds for each test.

The biological activity of a number of crude and distilled edible oils, and a number of strait chain fatty acids, which contain from C5 to C18, were evaluated in laboratory tests against the common legume pest *C. maculatus*. All the edible oils tested were found to have different degrees of activity at a concentration of 1 g/kg (1 kg/ton) chickpeas seeds (Table 1). The most active oils were crude oils from rice, maize, cottonseeds and palm. 90 – 96% of the eggs laid did not develop to larvae and only 0 - 1% developed to F_1 adults in the treated seeds with these oils (Table 1). In addition, some of the oils tested were found to prevent oviposition. The most active oils in this regards was the rice crude oil. Only 50 eggs were laid on seeds treated with 1 g/kg oil compared to 287 eggs on the control seeds (Table 1).

In field studies, crude rice and palm oils protected chick peas completely from insect infestation at a rate of 3 g/kg (3 kg/ton) for a period of 4 – 5 months; and partially for up to 15 months (Fig. 1). Also 1.5 g/kg protected the seeds mostly for the same period.

In order to obtain insight into the nature of the activity of the oils, the activity of strait chain fatty acids ranging from C5 to C18 carbon atoms at a concentration of 4g/kg – was studied against *C. maculatus* (Table 2). The results showed that C9 to C11 acids were the most active in preventing oviposition at this concentration. Only 13 eggs were found on seeds treated with C9 and C10, but none on seeds treated with C11 (Table 2). C12 to C16 fatty acids were less effective, and the activity remarkably decreased by the lower C5 to C7 and the higher C17 to C18 acids. At lower concentrations of 1.6, 0.8 and 0.4 g/kg, C11 acid was found the most active; lesser number of eggs laid compared to C9 and C10 and no eggs developed to adults. The data presented in Table 3 shows clearly that C9 - C11 acids are strong repellents to *C. maculatus*, but have no high lethal effect on the adults (Table 3).

Table 1 Biological activity of various edible oils against *Callosobruchus maculatus* (1 g/kg).

Oil	No of eggs laid	Eggs mortality (%)	Adult emergence	
			No	%
Crude rice	50	100	0	0
Crude palm	375	99	4	1
Refined palm	237	84	38	16
Crude maize	137	96	1	1
Refined maize	150	97	3	2
Crude soya bean	212	85	21	10
Refined soya bean	262	96	10	4
Crude cotton seed	212	99	2	1
Refined cotton seed	275	89	22	8
Crude coconut	125	95	6	5
Distilled peanut	150	95	8	5
Distilled safflower	287	97	9	3
Crude olive	175	74	46	26
Refined olive	300	86	18	6
Refined sunflower	187	82	30	16
Distilled kapok	387	88	39	10
Control	287	5	270	94

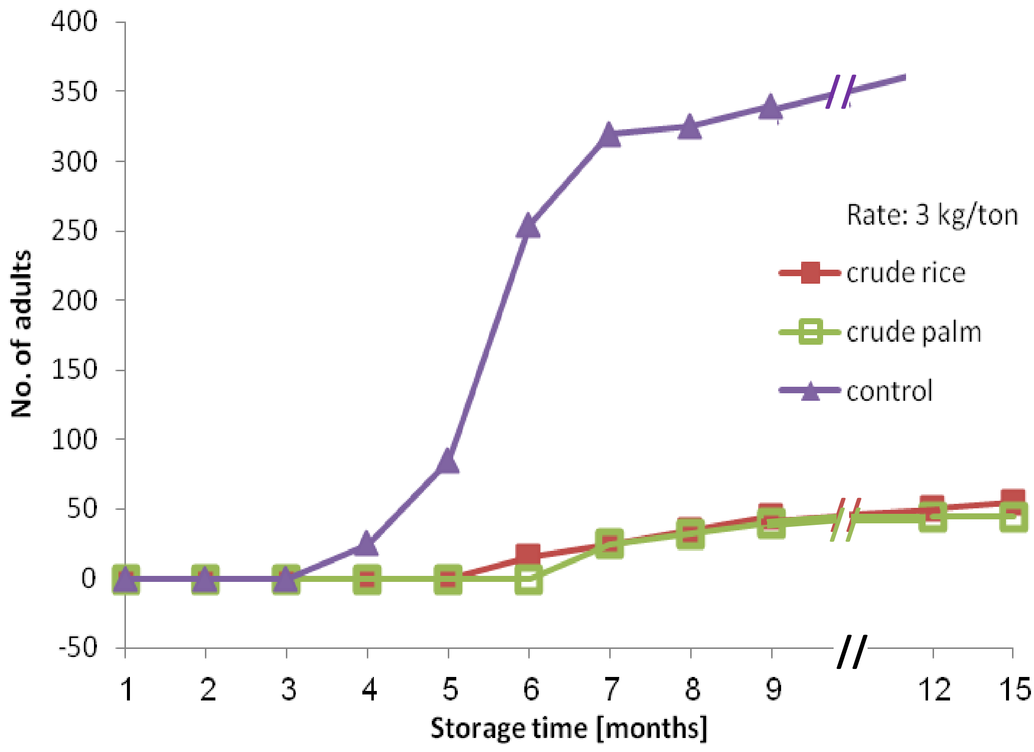

Figure 1 Field experiments using crude rice and crude palm oils to protect chickpeas seeds from infestation by *Callosobruchus maculatus.*

Table 2 Biological activity of straight chain fatty acids C5-C18 against *Callosobruchus maculatus* (4 g/kg).

Fatty acid	No of eggs laid	Adult emergence	
		No	%
C5	185	110	59
C6	130	92	71
C7	112	66	59
C8	105	22	21
Nanonoic-C9	13	0	0
Decanoic-C10	13	0	0
Undecanoic-C11	0	0	0
C12	32	22	69
C13	32	20	62
C14	62	45	73
C15	72	56	77
C16	65	37	57
C17	120	88	73
C18	140	115	82
Control	287	272	95

Table 3 Toxicity and repellency of the fatty acids C9-C11 tested against *Callosobruchus maculatus.*

Fatty acid	Concentration kg/t	No. of eggs laid	Undeveloped eggs, %	Adult emergence	
				No.	%
	1.6	60	100	0	0
C9	0.8	67	95	3	4
	0.4	100	72	18	18
Control	0	360	3	345	96
	1.6	65	100	0	0
C10	0.8	27	92	1	4
	0.4	62	78	7	11
Control	0	300	5	280	93
	1.6	20	100	0	0
C11	0.8	20	100	0	0
	0.4	22	100	0	0
Control	0	300	3	287	96

3. Studies with essential oils as contact and fumigant insecticides

The toxicity of essential oils as contact insecticides was evaluated using the same methods as edible oils.

The fumigant activity of essential oils was performed in space fumigation, in chambers, which were developed in our laboratory (Shaaya et al., 1991). The chamber comprises 3.4 l glass flasks with flat bottom, closed with glass stopper, fitted with a hook and septum for taking gas samples for measuring the fumigant concentration. The test insects were applied to a small piece of filter paper (Whatman number 1) and suspended together with the test insects by the hook in the fumigation chambers. Treatments were carried out for 24 hours as a standard test. The final adult mortality was calculated 72 hours from the ending of the treatment period. Correction for control mortality was done using the Abbot's (1925) formula.

3.1. Essential oils as contact insecticides

The efficacy of a large number of essential oils as contact insecticides was evaluated for the control of *C. maculatus* at a concentration of 400 ppm. The various oils tested were found to have toxic effect on the eggs, and on the development of the eggs to adults (Table 4). In the case of seeds treated with the oil of Syrian marjoram, the number of eggs laid on the treated seeds was 83% of control, but 0% of the eggs developed to adults. Other oils were found to cause reduction in the number of eggs laid on the treated seeds but less effective on eggs to adult development. In the case of clary sage oil, only 40 eggs were laid on the treated seeds (22% of control) but 55% of the eggs were developed to adults (Table 4).

Table 4 Contact activity of essential oils on egg laying, egg development and F_1 of *Callosobruchus maculatus.* 400 ppm X 24 h.

Essential oil	Eggs laid		Eggs developed to larvae (%)	No. of adults F_1	Eggs developed to adults (%)
	No.	From control (%)			
Syrian marjoram	171	83	87	0	0
Lemon grass	144	70	88	5	3.5
Geranium	119	34	14	8	6.7
Vistria	157	57	77	15	9.6
Basil	51	78	93	23	45
Clary sage	40	22	75	22	55
Orange	67	34	84	45	67
Grapefruit	120	64	77	49	41
Lemon	172	88	91	50	29
Caraway	97	51	79	62	64
Cumin	148	59	74	55	37
Thyme	120	69	68	46	38
Celery	239	179	79	169	71
Thyme leaved savory	98	68	89	75	77
Ruta	109	75	76	51	47
Rosemary	123	68	79	64	32
Peppermint	159	92	83	54	34
SEM76 oil	67	28	93	27	17
Control	150-350	-	85-95	120-280	80-85

3.2. Essential oils as fumigants

In order to isolate active essential oils we firstly screened a large number of essential oils extracted from aromatic plants and isolated their main constituents, using space fumigation. (Shaaya et al., 1991, 1993, 1994). SEM76, an essential oil obtained from Labiate plant species, was found to be the most potent fumigant. A concentration of 0.5 – 1.2 µl/l air was enough to cause 100% kill of all major stored-product insects. Space fumigation studies with SEM76 against various developmental stages of *C. maculatus*, showed that eggs and young larvae before they penetrated into the seeds, were the most susceptible to the compound. A concentration of 0.5 µl/l air was enough to cause 100% mortality of the eggs and the first instar larvae. After the larvae penetrated into the seeds, they became more tolerant (Table 5). Only pupae 1-2 days before adult emergence became again sensitive to the compound.

Table 5 Fumigant activity of the essential oil SEM76 extracted from Labiatae species on various developmental stages of *Callosobruchus maculatus.*

Stage treated	Age	Concentration (g/m^3)	No. of eggs	F_1 No. of adults	Eggs developed to adults (%)
Egg	20-24 hours	0.5	20	0	0
Larvae outside the seed	0-1 day	0.5	20	0	0
Larvae inside the seed	2 days	1.0	20	3.5	17
		1.5	20	4.5	22
Larvae	3 days	1.5	20	10.5	53
		3.0	20	8	40
Larvae	7 days	1.5	20	14	70
		3.0	20	13	63
Larvae	11 days	1.5	20	17.5	88
		3.0	20	16.5	83
Pupae	young	1.5	20	12	60
		3.0	20	11	55
Pupae	1-2 days before emergence	0.5	20	4	20
		1.5	20	1	5
		3.0	20	0	0
Control		0	20	17	85

4. Studies with phosphine fumigation by speedbox

The Speedbox is a small waterproof aluminium box containing a heater and a ventilator for injection and recirculation of the phosphine gas into the treated space. It has been developed by Detia Degesch GmbH Germany and can be used only with Phosphine Degesch Plates® (Jakob et al., 2006). The phosphine concentration was monitored by Bedfont device model 415 and by Draeger tubes. The field test was done in the 15 m^3 fumigation room filled with grain bags in 30% of the space. This technology was compaered with traditinal Phosphine formulations, such as Phostoxin tablets (Aluminium Phosphide) and Magtoxin tablets and plates (Magnesium Phosphide). The phosphine application rates were 4-8 g of gas per m^3. The range of exposure time was 36 to 96 h. The plates were heated to 36°C into the Speedbox. The tested insects at all developmental stages were placed between the bags in three replicates. The control insects were kept outside the fumigation room under the same temperature conditions. The mortality of internal stages was counted one week, two weeks and a month after treatment. The tested insects were maintained after treatment under laboratory conditions at 28±0.5°C and 65±5% r.h.

The dynamics of phosphine concentrations after various times following fumigations using Speedbox and compared with different types of phosphine formulations in use at the rate of 4 g/m^3 is shown in Table 6. In the case of using the Speedbox, the phosphine concentrations of 200 ppm and 830 ppm were reached 2 and 8 h respectively following the fumigation compared with 35-102 ppm and 200-520 ppm with the other formulations.

Table 6 Phosphine concentration 1-72 hous following fumigation using different types of formulations, 4 g/m^3 in the 15 m^3 fumigation room.

Treatment	**Phosphine concentration, ppm hrs**						
	1	**2**	**4**	**8**	**24**	**48**	**72**
Al tablets	20	35	90	200	610	960	910
Mg tablets	30	102	185	300	590	730	650
Mg plates	40	85	210	520	1150	920	700
Mg plates by speedbox	80	200	470	830	980	830	630

Al: Phostoxin, Mg: Magtoxin

The accumulative phosphine concentrations by using Speedbox were much higher during 72 h of the fumigation compared with the non Speedbox technologies (Table 7).

Table 7 Accumulative concentration of Phosphine 1-72 hous following fumigation using different types of formulations, 4 g/m^3 in the 15 m^3 fumigation room.

Treatment	**Phosphine concentration, ppm hrs**						
	1	**2**	**4**	**8**	**24**	**48**	**72**
Al tablets	20	55	205	865	3185	12345	23775
Mg tablets	30	132	467	1522	3692	13127	19167
Mg plates	40	125	475	2155	6545	18665	28325
Mg plates by speedbox	80	280	1070	3840	7600	19070	28620

Al: Phostoxin, Mg: Magtoxin

As a result of high Phosphine concentration reached practically at the beginning of fumigation using Speedbox, 100% mortality of all developmental stages were recorded after only 36 h treatment at the rate of 6 g/m^3(Table 8).

Table 8 Toxicity of phosphine at various concentrations using SPEEDBOX against *Callosobruchus maculatus* in the 15 m^3 fumigation room.

Insect stage	Mortality (%) after exposure to phosphine hours for obtain 2,000 ppm					
	6g/m^3x36h	6g/m^3x48h	8g/m^3x48h	8g/m^3x60h	4g/m^3x72h	4g/m^3x96h
	6 h	6 h	4 h	3 h	9 h	9 h
Adult	100	100	100	100	100	100
Egg	99.8	100	100	100	100	100
Larvae	100	100	100	100	100	100
Pupae	99.8	100	100	100	100	100

4. Conclusions

Studies with edible oils and fatty acids showed that crude oils of rice, maize, cotton seeds and palms were found the most potent against *C. maculatus*, compared to the other oils tested. The purified forms of these oils were found less effective. In field studies, rice and palm crude oils at rates of 1.5 and 3 g/kg protected chickpeas completely from insect infestation for a period of 4 – 5 months, and partially for up to 15 months. Studies by Khaire et al. (1992) showed that adult emergence of *C. chinensis* was completely prevented for up to 100 days in pigeonpea treated with 1% of neem oil or karanj oil. In addition, these oils were found to have no adverse effect on seed germination. Boeke et al. (2004) showed that a number of botanical products might provide effective control of *C. maculatus* in cowpea.

Among the strait chain fatty acids ranging from C5 – C18 carbon atoms, it was found that C9 – C11 acids were the most effective in preventing ovipostion of *C. maculatus* on the treated seeds, but have no pronounced lethal effect on adults. In earlier studies (Shaaya et al., 1976) showed that wheat seeds treated with C10 fatty acid at a rate of 4g/kg repelled *S. oryzae*, but that forced contact with the beetles with the treated seeds was found to have no effect on mortality rates of the beetles (Shaaya et al., 1976). Essential oils as contact insecticides were found to have low toxicity. A high concentration of 400 ppm of the most active essential oil from Syrian marjoram was needed to prevent the development from eggs to adult. On the contrary, essential oils and their constituents as fumigants showed high activity in controlling young larvae and pupae prior to emergence of *C. maculatus*. In space fumigation, at a concentration of 1 µl/l air, the *Ocimum gratissimum* oils and its constituent, eugenol, caused 100% mortality of *C. chinensis* 24 h after treatment (Ogendo et al., 2008).

The essential oils of citrodora and lemnon grass were found to have insecticidal and ovicidal activities agains adults and eggs of *C. maculatus* (Raja and William, 2008). Keita et al. (2001) reported that kaolim powder aromatized with pure oil of *Ocimum* spp. at 6.7 µl/g of pea seeds provided complete protection over 3 months of storage.

By using Speedbox, it was possible to reduce the time of phosphine treatment from 5-7 days to 2-3 days to control all developmental stages of *C. maculatus.*

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