Evaluation of Orange Peel *Citrus Sinensis* (L) as a Source of Repellent, Toxicant and Protectant against *Zabrotes Subfasciatus* (Coleoptera: Bruchidae)

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ABSTRACT

Continued application of synthetic insecticides arise development of resistance and pollution of the environment. Laboratory experiments were conducted to test the efficacy of products of orange (*Citrus sinensis*) peels in the control of the stored products beetle *Zabrotes subfasciatus* (L) in stored haricot beans (latin name). Different levels of the extracts and essential oil of Citrus sinensis was tested. Conventional synthetic insecticide, Pirimiphos-methyl, was used as a standard check. Toxicity potential of different extracts of *C. sinensis* was tested against *Z. subfasciatus*. Extracts prepared using different solvents against the beetles were not toxic. However, essential oils at highest rate of 750mg applied at 3ml per filter paper gave 100 % mortality after 24 h. Beans treated with 15g of sun dried powder of orange peel and 750mg of essential oil killed 65% and 67% of Z. *subfaciatus* after 96 hours respectively. Powders from ground peels caused significant reduction in progeny emergence of *Z. subfasciatus* ($P< 0.05$). There was no progeny produced when essential oil was used, even at lower dosage levels of 30mg. All the treatments were repellent to *Z. subfasciatus.* The essential oil of orange peel had a high level of toxicity in the fumigation bioassay against *Z. subfasiatus*.

Key words: *Citrus sinensis*, essential oil, *Zabrotes subfasciatus*, Pirimiphos-methyl, Percent weight loss, Damage assessment.

1 INTRODUCTION

Globally a minimum of 10% of cereals and legumes are lost after harvest (Boxall et al., 2002). Insect pests cause heavy economic losses to stored grains throughout the world and their impacts are more devastating in poor countries (Boxall et al., 2002). The two most universal and potent[important] pests in pulse storage are the Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) and the common bean weevil *Acanthoscelides obtectus* (Say) in Africa (Slim, 1993). Ferede and Tsedeke (1995) reported that these two species are the major pests of stored beans in Ethiopia. The study conducted in Bako recorded 14% of total loss by *Z. Subfasciatus* in haricot been stored for 12 month (Adane and Abrham, 1996). Infestation by *Z. subfasciatus* can cause a loss of about 12% of the available protein (Mc Farlance, 1988).

Control of stored products insect pests rely heavily on hazardous synthetic productd. The increasing problems associated with the use of synthetic chemicals for the control of stored

products insects necessitates the development of safe repellent agents against stored products pests. Many workers, including developing countries have taken the lead in this quest (Jilani et al., 1988; Cobbinhah and Appiah-Kwarteng, 1989; Jembere et al., 1995; Mekuria, 1995; Adane and Abrham, 1996; Bayeh and Tadesse, 1996; Bekele et al., 1997; Firdissa and Abrham, 1998; Emana, 1999; Glob et al., 1999).Use of plant products as insecticide is one of the important approaches of insect pest management and it has many advantages over synthetic insecticides (Weinzierl and Henn, 1992). Plant materials with insecticidal properties are one of the most important locally available, biodegradable and inexpensive methods for the biological control of pests. provide small-scale farmers with locally available, biodegradable and inexpensive method for the control of pests of stored products. Considering the importance of plant insecticides, the [F]farmers of [in] Ethioipia treat their stored products with local herbs to reduce storage losses due to pests (Yemane and Yilma, 1998). Firdissa and Abrham (1999) reported that *Chenopodium sp.* performed very well and resulted in high percentage of adult mortality, reduced progeny emergence and low percent grain damage. Bayeh and Tadesse (1996) found that neem (*Azadirachta indica*), birbira (*Milletia sp.*) oil and the powder of pyrethrum flower are toxic against *Callosobruchus chinensis*. Cow pea treated with the powder of orange peels is associated with LD_{50} of 4% (w/w) for *Callosobruchus maculates* (F.) exposed to it (Don Pedro, 1985). Essential oil derived from orange peels is known to have toxic, feeding deterrent, and poor development effects on lesser grain borer, *Rhyzoperta domonica* (F.), rice weevils, *Sitophilus oryzae* (L.) and red floor beetle, *Tribolum castaneum* (Herbst) (Tripathi et al., 2003). The peel oil was also reported to have toxicity toward *Culex pipiens* (Mwaiko and Savaeli, 1992); and cow pea weevils, *Callosobruchus maculates* (F.) (El-sayed and Abdel-Razik, 1991). Further more, the peel oil has fumigant action against fleas (Weinzierl and Henn, 1992) and house hold insects *Blatella germanica* (L.) and *Musca domestica* (L.) and stored product *Sitophilus oryzae* (Karr and Coats, 1988). Other plants that have been reported to repel pests of stored products include *Croton macrostachyus, Ricinus communis, Datura stramonium, Cpsicum frutescens, Azadirchata indica, Ocimum sp.* and *Eucalypyus sp.* (Jembere et al., 1995; Emana, 1999; EARO, 1999; El Altta and Ahmed, 2002). Considering the importance of plant insecticides in the pest management, the present work is designed to investigate the efficacy of the products of orange peels in the control of the pulse grains insect *Zabrotes subfasciatus.*

2 MATERIALS AND METHODS

2.1 Insects culture

Adults of *Z. subfasciatus* (Boheman) brought from Melkassa Agricultural Research Center were cultured at Department of Biology, University of in Addis Ababa, Insect Science insectory at 27 ± 3^{o}C and 55-70% RH (Schoonven, 1978). Whole haricot bean seeds bought from local farmers from Melkassa south of Addis Ababa were kept in an oven at 60^{0}C for 6 hrs to disinfest the seeds from any prior infestation before using them as a substrate for insect rearing (Jembere, 2002). Fifty pairs of the adult of *Z. subfasciatus* were placed in 1-litre glass jars containing 250g seeds. The jars were then covered with nylon mesh that was held in place with rubber bands. The parent bruchids were sieved out after an oviposition time of 13 days. Then the seeds were kept under laboratory condition until F1 progeny emergence. The F1 progeny, which emerged after 30 days, were sieved out and used for the experiment.

2.2 Plant material collection and extraction

2.2.1 Solvent extract of plant materials

Fresh orange fruit (Valencia variety) brought from the Awash agro industry, were peeled and chopped with a knife and then soaked in distilled water, petroleum ether, ethanol and acetone at the rate of 10g/100ml, 20g/100ml and 30g/100ml of each solvent for extraction (Jembere, 2002). After 24 hours the mixtures were filtered with cheese cloth and filter paper (Watman No 9). Then the filtrates were ready to be used for the different treatments.

2.2.2 Dried and ground materials

The fresh orange peels of *C. sinensis* were dried under shade. Ground materials were obtained by grinding the dry peels into a fine powder using mortal and pestle. The rates used were 5g (2%), 10g (4%) and 15g (6%)/250g of grains.

2.2.3 Isolation of essential oil extract

Essential oil of *C. sinensis* was isolated by hydrodistillation of fresh orange peels using a Clevenger type apparatus. An average yield of 7.4ml oil was collected from 1kg of orange peel. The oil was kept in refrigerator for later on use. At the time of use the oil was weighed in three doses of 30mg, 150mg and 750mg and dissolved in 10ml of acetone.

2.3 Assessment of Toxicity, Progeny and Damage

Different levels of the extracts and essential oil were applied to a filter paper of 9mm diameter at the rate of 1ml, 2ml and 3ml per filter and placed in a Petri dish of 10cm diameter. In the case of

organic solvent extracts, the treated filter was exposed to the open air to allow the organic solvent to evaporate. Then, 1ml of distilled water was added to the entire surface of the each treated filter papers, as a carrier of the extracts. Variable exposure times were considered, which was based on the nature of the solvent. In case of acetone and petroleum ether the exposure time was 30 minutes, while it was 60 minutes for ethanol (Jembere, 2002). Other filter papers were also treated with three levels of different solvents as control. After treatment, 5pairs of 3-7 day-old adults of both sex of *Z. subfasciatus* were introduced into the treated and control filter papers in the petri dishes. The treatments were replicated three times. Mortality of the adult insects were counted after 24, 48, 72 and 96hrs.

For powder treatment, 250g of disinfected haricot bean seeds were introduced into three 1 L glass jars that were treated differently with the powdered orange peels (i.e. 5, 10 and 15g of the powder). The grains of Pirimiphos-methyl treated and untreated were included as standard check and control, respectively. After treatment, 20, three to seven day old *Z. subfsciatus* of mixed sex were introduced to the treated and untreated seeds in the glass jars. The jars were covered with nylon mesh and held in place with rubber bands. The number of dead insects in each jar was sieved and counted after 24, 48, 72 and 96 hrs.

The treated jars were kept for additional 10 days of oviposition time after mortality assessment. All live and dead insects were sieved and discarded after 13 days of introduction. The treated and control grains were then kept until emergence of F1 progeny. Then the number of F1 progeny produced by *Z. subfasciatus* was counted. Counting was stopped after 45 days from the day of introduction to avoid overlapping of generation.

Damage assessment was carried out on treated and untreated grains. Samples of 100 grains were taken from treated and untreated grains and the number of damaged (grains with characteristic holes) and undamaged grains were counted and weighed. Percent weight loss was calculated by count and weight method cited in FAO (1985) as:

$$\% \text{ Weight loss} = \frac{(UaN - (U+D) \times 100}{UaN}$$

Where U = weight of undamaged fraction in n the sample, N.
N = total number of grains in sample.
Ua = average weight of one undamaged kernel.
D = weight of damaged fraction in n the sample

The assessment was replicated five times for each treatment.

2.4 Repellency bioassay

The repellent action of the ground peel and essential oil of *C. sinensis* against *Z. subfasciatus* were assessed in a choice and with no choice bioassay system in a 'Y' olfactometer. In choice test, hundred grams of untreated disinfected haricot bean for *Z. subfasiatus* were put into one of the gas washing bottle, while differently treated seeds were put in the other gas washing bottle. For no choice test, one of the gases washing bottle was left empty. Then air was pumped with regulated air pump at a rate of 1.2 l/minute into a gas washing bottle containing activated charcoal for filtration through rubber tubing. The filtrated air then passes to the two washing gas bottles containing untreated and treated seeds or with no seeds. Finally the air reaches the two arms of the "Y" tube glass which is attached to the stem where the insects are released. After this set up following the method of Jembere et al. (1995), twenty five adults of each *Z. subfasiatus* of mixed sex and age were released into the "Y" olfactometer glass. After 30minutes, the numbers of insects which moved into the untreated (Nc) and treated bottle (Nt) were counted. After each test the "Y" glass tube and the gas washing bottles were washed with water, rinsed with acetone and dried at 80^{o}C for 1h. Each treatment was replicated four times and percentage repellency (PR) values were computed using the methods of Jilani et al. (1988) as:

$$PR\ (\%) = \frac{Nc - Nt}{N} \times 100$$

Where, Nc is the individuals in the control bottle (untreated seeds).

Nt is the individuals on the treated bottle.

N is the total number of insects tested.

2.5 Fumigation toxicity

The fumigation toxicity of the essential oil was tested following the method of Wang *et al.* (2001) with some modification. Wide mouth bottles of 1-liter capacity with lids were used as exposure chamber. Filter papers of 9mm diameter were treated with 1, 2 and 3ml of essential oil at the rate of 12mg, 60mg, 300mg dissolved in 10ml of acetone; the same amount of acetone alone was applied as control. The solvent was allowed to evaporate for 20minutes and then the filter paper was placed at the bottom of a 1-liter glass bottle. Twenty insects in small nylon mesh bag with 100g food substrate were hung at the center of the glass bottle (7cm high) above the

filter paper. The bottles were then closed tightly with a lid. Each treatment with respective control was replicated five times. Mortality was checked after 24hrs.

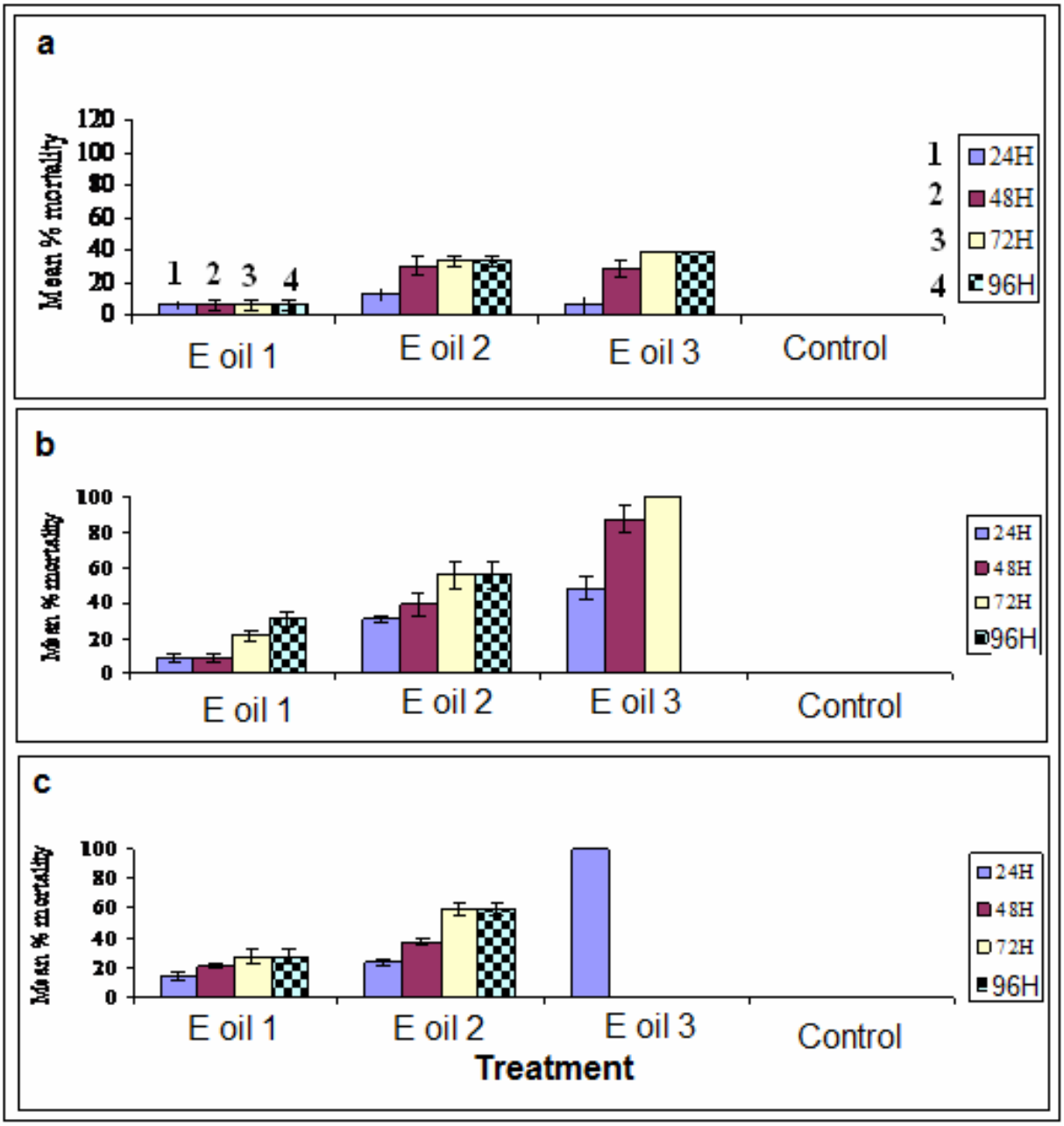

Figure 1. Mean % mortality of *Z. Subfasciatus* due to essential oil of *C. sinensis* applied at rate of 1ml (a), 2ml (b) and 3ml (c) after different times of exposure (E oil1 = Essential oil at 0.03g; E oil 2 = Essential oil at 0.15g; and E oil 3 = Essential oil at 0.75g).

3 RESULTS

3.1 Toxicity of the Plant Material

Acetone, ethanol, petroleum ether and water extracts of *Citrus sinensis* at all rates was not toxic. It was only the essential oil of *C. sinensis* that showed significant effect (P<0.05) at all levels. At the highest rate (750mg/filter) applied at 3ml gave 100% mortality of *Z. subfasciatus* after 24 hour exposure time (Fig. 1).

3.2 Adult Mortality in the Grain

The essential oil of *C. sinensis* at the highest dose of 0.75g/250g of haricot bean gave 67.4% mortality after 96 hours of exposure (Table 1). *C. sinensis* applied as powder were also toxic at higher dosage of 15g/250g of grain causing 65.95% mortality after 96 hour of exposure (Table 1). The result of this experiment showed that all treatments of the essential oil of orange peel were relatively toxic to *Z. subfasciatus*.

Table 1. Mean % mortality of Z. subfasciatus due to C. sinensis powder and essential oil of treated seeds.

Treatment	***Dosage (g/250g)***	***Mean % adult mortality, h after exposure***			
		24	***48***	***72***	***96***
Orange powder 5g	5	21.34±1.45b	26.45±2.08bc	36.23±1.73b	45.00±3.33b
10g	10	27.70±1.14bc	34.18±2.09cd	44.04±3.46bc	54.83±2.72bc
15g	15	35.25±1.02c	47.91±3.34de	57.98±2.86cd	65.95± 1.25de
Orange essential oil 0.03g	0.03	21.14±2.70b	39.15±2.97cd	53.81±2.97cd	56.79±0.00cd
0.15g	0.15	26.07±4.27bc	45.96±2.54de	61.33±3.08d	61.33±3.08cde
0.75g	0.75	47.89±2.88d	57.00±3.65e	63.55±2.08d	67.40±2.34e
Pirimiphos-methyl 0.125g	0.125	100±0.00e	Nob	Nob	Nob
Control 0.0g	0	0.00 ± 0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

Mean with in a column followed by different letters are significantly different, P<0.05%, Tukey student test (HSD). Nob = No observation

3.3. Progeny emergence

The numbers of F1 progeny produced in each treatment were significantly low compared to the number of progeny produced in control. All levels of the powder of *C. sinensis* peels caused significant reduction in progeny emergence of *Z. subfasciatus* (P< 0.05)(Table 2). No emergence

of adult progeny was observed from pirimiphos-methyl treated haricot beans. A similar result was obtained with haricot beans treated with 750mg (0.3%) of essential oil, but there was no significant difference (P>0.05) between the mean number of F_1 adult progeny that emerged in beans treated with 30, 150 and 750mg (Table 2). The result of this experiment showed that all treatments of essential oil were effective as the standard insecticide, pirimiphos-methyl, by significantly reducing F_1 progeny emergence.

3.4 Damage assessment

Weight loss assessments result of treated and untreated grains are shown in table 2. All the treatments significantly reduced weight loss compared to the untreated check 45 days after introduction of *Z. subfasciatus* into treated and untreated beans. No weight loss of stored haricot beans was observed in seeds that were treated with pirimiphos-methyl at a recommended rate. The highest dosage of the powder peel protected the haricot beans against feeding by *Z. subfasciatus* which resulted in no noticeable feeding damage on seeds. However this result was achieved at a minimum dose (30mg) for essential oil of *C. sinensis.* The present finding showed that essential oil of orange peels effectively reduced the grain damage weight loss even at lower dose than the standard check pirimiphos-methyl at recommended rate.

Table 2. Mean number of F1 progeny produced and weight loss caused by *Z. subfasciatus* on seeds treated with powder and essential oil of *C. sinensis*.

Treatments	***Dosage (g/250g)***	***Mean number of F1 progeny***	***Mean %weight loss***
Orange powder	5	31.66 ± 5.81^{b}	0.53 ± 0.09^{b}
	10	17.66 ± 0.88^{ab}	0.38 ± 0.08^{ab}
	15	13.66 ± 1.45^{ab}	0.00 ± 0.00^{a}
Orange essential oil	0.03	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}
	0.15	0.66 ± 0.66^{a}	0.00 ± 0.00^{a}
	0.75	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Pirimiphos-methyl	0.125	$0.00 \pm .00^{a}$	0.00 ± 0.00^{a}
Control (untreated)	0	123.33 ± 6.88^{e}	2.33 ± 0.09^{e}

Mean with in a column followed by different letters are significantly different, P<0.05%, Tukey student test (HSD).

3.5 Fumigation toxicity

The result on fumigant toxicity of essential oil of *C. sinensis* towards *Z. subfasciates* is presented in table 3. No dead insects were observed at lower dose of 0.012 mg/10ml of acetone applied at all levels 1, 2 & 3ml/filter paper. The highest dose of essential oil showed significantly higher

(P< 0.05) mortality. Particularly essential oil of 0.3g applied at the rate of 2 and 3ml per filter paper induced 100% mortality of *Z. subfasciatus*. Acetone treated controls did not cause any significant mortality of the bruchids at 1, 2 and 3ml level of application.

Table 3. Fumigant toxicity of *C. sinensis* essential oil treated seeds to *Z. subfasciates*.

Treatments (g/100g of seed)	***Dose (ml/filter)***	***% mortality after 24h exposure***
0.06 gram	1ml	0.25 ±0.25a
	2ml	0±0.00a
	3ml	18.75±2.39b
0.3gram	1ml	26.25±1.25c
	2ml	100.00±0.00d
	3ml	100.00±0.00d
Control (acetone treated)	3ml	0.00±0.00a

Mean with in a column followed by different letters are significantly different, P<0.05%, Tukey student test (HSD).

3.6 Repellency

Table 4 shows the mean repellency values for the test materials at different doses. The percent repellency obtained with out choice was less than with choice test. All the treatments showed significant repellent effect against *Z. subfasciatus* (P< 0.05).

Table 4. Mean percentage repellency of different plant materials and dose levels for Z. *subfasiatus*.

Treatments (g/100gm seeds)		***Mean % repellency (PR)***	
		No choice	***With choice***
Orange powder	2g	25.00±4.04de	25.00±1.73de
	4g	33.25±.85e	33.80±1.97f
	6g	32.50±1.44e	38.45±1.65f
Orange oil	0.012g	25.75±2.56de	33.27±2.03ef
	0.06g	33.50±2.39e	37.60±0.90f
	0.3g	37.87±0.96f	53.75±1.37f
Control		0.00±0.00a	0.00±0.00a

Mean with in a column followed by different letters are significantly different, P<0.05%, Tukey student test (HSD).

4. DISCUSSION

Orange peel oil at high concentration level of 750mg applied at 3ml caused 100% mortality of *Z. subfasciatus* exposed to treated beans at all concentrations used. Four days after introduction,

adult mortality of the insect was 67% (orange peel oil) and 66% (orange peel powder)., where, as pirimiphos-methyl caused 100% mortality with-in 24 hour.

The toxicity of *C. sinensis* peel oil may be attributed to d-limonene (Sharaby, 1988). Tripathi et al. (2003) reported the contact toxicity of d-limonene with LD_{50} 74.73, 85.37 and 79.78 for *R. dominica, S. oryzae* and *T. castaneum*. An over-all test of efficacy between the treatments has shown that mortality was directly related to the dosage and time. This indicates that higher dosage is more efficient in management of pests. In case of the sun dried powder, the orange peel seems very promising, though significant result is gained at higher dosage than the standard rate of 5% suggested for most botanicals for storage pest management. Belmain and Stevenson (2001) also reported effective use of *C. sinesis* powder against legume pests. The effectiveness of the orange peel powder is probably due to silica or silica like component, which are abrasive and the ability of the particles to adhere to the grain.

All the treatments caused significant reduction of F1 adult emergence compared to control. The extent to which the orange peel products affected the survival of the subsequent progeny was found to vary among them. In the progeny count, only those newly emerged adults were considered which were alive. This indicated that the active ingredients of botanicals which are responsible for the toxicity of the plant kill the insects gradually. *Citrus sinesis* peel oil was superior to untreated and powder causing 100.00, 99.44 and 99.00% reduction in adult emergence at 0.75, 0.15 and 0.03g/250g of haricot bean. The study showed that orange peel oil is even better than pirimiphos-methyl causing 100% reduction of F1 emergence at lower dose. The current findings are similar to the results of Tripathi et al. (2003) who has also reported oviposition reduction effect of orange peel oil against *T. castaneum* by 94.5%. Similarly, Sharaby (1988) also reported reduced oviposition and egg hatching of potato tuber moth, *Phthorimaea operculella* exposed to 220µl of the oil. It is also reported by Levinson et al. (2003) that orange peel oil at 1ml suppressed oviposition of Mediterianin fruit fly, *Ceratitis capitata*. The present study also shows that orange peel oil has strong oviposition deterrent effect against *Z. subfasciatus*. Powdered sun dried orange powder were also effective in reducing F1 adult emergence though not effective as orange peel oil and pirimiphos-methyl.

Significant reductions in feeding damage which indicates the higher protectant potential of these materials against insect damage were observed. The plant materials were highly effective significantly reduced damage to haricot bean when compared to control. It appears therefore that

orange peel oil has insecticidal properties which accounts for much higher levels of effectiveness. Furthermore, orange peel powder at 15g/250g of haricot bean seed also protected the grain from damage by hundred percent, strongly suggesting the presence of physical interfering agents in orange peel powder.

Orange peel oil showed highest fumigant toxicity causing 100% mortality at 0.3g/100g of haricot bean applied at 3ml adjusted to 24h exposure. The fumigant toxicity decreased with decreasing concentration. The orange peel oil has been reported to have fumigant toxicity 13 times more than that of methyl bromide (Tripathi et al., 2003). The present study also showed that orange peel has strong fumigant toxicity effect against the *Z. subfasciatus.* Keita *et al.* (2001) reported that the mode of the action of fumigant toxicity of essential oil against insects might be the inhibition of acetylcholinesterase.

Using essential oils as a fumigant for stored grain and legumes could be particularly relevant as methyl-bromide is removed from use. However, there is only very limited evidence demonstrating their ability to penetrate through grain bulks which must occur if plant extracts are to emulate fumigant gases. In addition, the understanding of sorption and residue on target grain are important issue to use essential oils as a potential fumigant (Lee et al., 2003).

The repellency effect of *Citrus sinensis* peels oil was relatively lower. This suggests that the active compounds which acted as repellent and fumigant might be chemically different.

The result of the current study suggested that materials derived from *Citrus sinensi* , may be used as pulse protectant against *Z. subfasciatus* for small scale farmers. Therefore, investigation on incorporating, improving and adopting for the control of stored product insects need to be investigated.

5. CONCLUSION

This study indicated *Citrus sinensis* posses toxicity, feeding and ovipositional deterrent effect on *Z. subfasciatus*. The *C. sinensis* oil and its sun dried powder significantly reduced the F1 progeny and weight loss. Furthermore, the study showed the peel oil possessed contact and fumigant toxicity on the *Z.subfasciatus*. Hence all the result of this study indicated that orange peel may be used as a potential stored grain protectant.

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