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Original Research Article

Grain health protectant activity of essential oils against infestation and damage of haricot bean by *Zabrotes subfasciatus* (Boheman)

Abstract: Experiments were conducted to evaluate the efficacy of essential oils extracted from *Chenopodium ambrosioides* (L.), *Rosmarinus officinalis* (L.), *Eucalyptus globulus* (Labill), *Trachyspermum ammi* (Sprague) and *Cymbopogon citratus* (Stapf) against *Zabrotes subfasciatus* (Boheman) under laboratory condition (27±3 ^{0}C, 50-70% RH). The test insects were reared in glass jar and investigated on whole Awash-1 haricot bean variety grains. A standard insecticide, actellic super dust 5% and untreated control were included for comparison. The experiment was arranged in a completely randomized design in three replications. Essential oils from the above mentioned plants were admixed with the bean grains at the rate of 750 mg/150 g of seeds. The results showed that there was complete mortality (100%) of *Z. subfasciatus* adults within 24 hours after treatment due to *C. ambrosioides*, *E. globulus*, *T. ammi* and *C. citratus*. Application of essential oils to bean grains significantly reduced progeny production of *Z. subfasciatus*. Over 97% inhibition of F_1 progeny production by the pest was recorded for all essential oil treatments at the highest dose of 750 mg. Application of essential oil extracts also significantly reduced the rates of haricot bean grain infestation (up to 100%) by *Z. subfasciatus*. Furthermore, essential oil extracts admixed to the bean grain proved to have no significant effects on the germination capacity of the treated haricot bean grains. From the current experiment it can be concluded that the tested botanicals are as effective as the standard insecticide in controlling Z. *subfasciatus and* can be used for the management of the pest.

Key words: Botanicals, essential oils, ~~mortality, reduced progeny, weight loss~~, *Zabrotes subfasciatus*

1. INTRODUCTION

The common bean (*Phaseolus vulgaris* L.), is one of the principal food and cash crop legume grown in the tropical world (Abate and Ampofo, 1996; Schmale *et al*., 2002). It constitutes the essential source of cheap, high-quality dietary proteins for resource-poor farmers. A major problem in attempting to increase the supply of beans in rural and urban households is high losses during storage caused by two species of bruchids: *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) (Nchimbi-Msollaand, 2002). These

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species are the most destructive storage pests of beans throughout the world, particularly in the tropical belt (Nchimbi-Msollaand, 2002; Schmale *et al*., 2002; Mulungu *et al*., 2007). In Ethiopia, *Z. subfasciatus* and *A. obtectus* are the major pests of stored beans causing an average grain loss of 60% within 3-6 months of storage period (Emana *et al*., 2003). Damaged beans characteristically appeared with multiple emergence holes of bruchid beetles and emitting a pungent odor which results in grains that are not fit for consumption and market (Giga *et al*., 1992). To offset and/or minimize the losses, farmers use different methods of control which ranges from synthetic insecticides to different cultural practices.

When properly used, synthetic pesticides may play a significant role in reducing storage losses due to insect pests (Tapondjou *et al*., 2002). However, their current applications for the control of storage insect pests is limited because of resistance development by insect pest, pest resurgence, consumers concern, widespread environmental hazards and increasing costs of application (Bekele *et al*., 1997; Rahman and Talukder, 2006). These pitfalls called for looking into alternative pest control options such as botanical insecticides (Shaaya *et al*., 1997). Plant products have been used for many years by the small scale farmers in different parts of Africa. In the past two decades, many efforts have been undertaken to investigate plants with better botanical insecticides which can be used as an alternative to synthetic insecticide (Emana *et al*., 2003). Mixing of essential oil extracts of various plants with stored-grains, usually result in the reduction of oviposition rate, suppression of adult emergence, and mortality of adults, which ultimately lead to low infestation and yield losses (Shaaya *et al*., 1997; Keita *et al*., 2001; Tapondjou *et al*., 2002). The present study was conducted to determine the bioactivities of different essential oil extracts against the infestation of *Z. subfasciatus* on common bean grains to find candidate sources for future integrated pest management applications.

2. MATERIALS AND METHODS

2.1. Insect rearing

Adults of *Z. subfasciatus* were obtained from Melkasa Agricultural Research Center and reared in the insectary of Addis Ababa University, Biology Department. The experiment was conducted at 27 ± 3°C and 50-70% RH. Haricot bean seeds of the variety Awash-1 used for the rearing were bought from Melkassa local market and were kept in an oven at 40°C for 4 h to disinfest the seeds from the internal infestation and allowed to cool for 2 hours before use (Bekele, 2002).

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Hundred adults from the same cohort, comprising 50 males and 50 females of *Z. subfasciatus* were placed in 1lt volume glass jars containing 250 g of haricot bean seeds. The parent bruchids were removed by sieving 13 days after completion of their oviposition period. Seeds were kept under laboratory condition until the emergence of F_1 progeny.

2.2. Plant material collection and extraction

Plant materials (i.e. leaf, inflorescence, and seed) used for the study were collected from natural habitats around and in Addis Ababa and the botanical garden of Essential Oil Research Center (EORC) in Addis Ababa and Wendogenet Agricultural Research Center (WARC) (Table1). The identity of the test plants was confirmed at the National Herbarium of Addis Ababa University, where type specimens were deposited.

2.3 Distillation of Essential oils

Each essential oil from each botanical specimen was extracted by hydro-distillation using Clevenger apparatus (Winzer®) by the trained staffs of EORC in Addis Ababa. The dried parts of the test plants were cut into small pieces and were placed in a distillation flask with approximately 5 times water in volume and 10 glass beads (100gm sample in 200ml distilled water). The distillation flask contained each sample was placed in heating mantle (> 1500C) to complete the cycle for duration of 2.5 hours. The distillate was collected in a separating funnel in which the aqueous portion is separated from the volatile oil. The water (lower) layer was slowly drowned off until only the oil layer remains. The percentage of oil content was calculated based on dry matter bases (ml/g biomass). The oil was collected in vials and stored at about 4°C in refrigerator until use.

2.4. Insecticidal bioassay

Essential oil extracts were admixed with the test grains sample at the rate of 0.02, 0.1 and 0.5% (30, 150 and 750 mg/ 150 gm of grain) dissolved in 10 ml of acetone and shaken thoroughly to ensure uniform distribution over grain surface. One blank control was run concurrently consisting of acetone treated grain. Primiphos-methyl dust at the rate of 0.08% was used as a standard check. Treated grains were kept for 24 hour to allow for the complete evaporation of acetone before running the bioassays. After the treatment application, 20, 3-5 days-old unsexed

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adult *Z. subfasciatus* was introduced to the treated and untreated seeds in the glass jar. The jars were covered with nylon mesh and held in place with rubber bands. Each treatment was replicated three times. The experiment was arranged in completely randomized design and conducted in the laboratory. Number of dead insects in each jar was sieved and counted after 24, 48, 72 and 96 hour after treatment application. Percentage mortality was corrected using Abbott's (1925) formula:

$$PT = (Po - Pc) / (100 - Pc),$$

Where PT = corrected mortality (%), Po= observed mortality (%), Pc = Mortality in the control glass jar (%).

2.5. F_1 Progeny Assessment

The treated jars were kept for additional 10 days for oviposition after mortality assessment. All live and dead insects were sieved and discarded after 13 days of introduction. Then the grains were kept until emergence of F_1 progeny. The number of F_1 progeny was counted upon emergence until 45 days from the date of introduction to avoid overlapping generation.

2.6. Damage Assessment

Damage assessment was done on treated and untreated grains. To determine the rate of seed, samples of 100 grains were taken randomly from each jar of the treatment. The number of damaged (grains with characteristic hole) and undamaged grains were counted and weighed. Percentage seed weight loss = [UNd DNu/U (Nd + Nu)] x 100 (where, U = Weight of undamaged grain, D = Weight of damaged grain, Nd = Number of damaged seeds and Nu = Number of undamaged seeds).

2.7. Germination test assay

For germination test, 100 seeds were taken randomly from each treatment jar. Then, 20 seeds from each treated and control group were placed separately in petri dishes containing moistened filter paper (Whatman No. 1). Each treatment was replicated five times. Healthy untreated seeds were used as a control. The number of emerged seedlings from each petri dish was counted and recorded after 7 days. The percent germination was computed using the following formula:

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Viability index (%) = (NG*100)/TG (where, Where NG = number of seeds germinated and TG = total number of seeds tested in each Petri dish.

2.8. Statistical analysis

Data were analyzed using SPSS Ins., Version 13, 2004. Percentage mortality data were transformed using arcsine transformation before the analysis and analyzed with one-way analysis of variance. Homogeneity of variance was determined using Levene's test. Mean values were separated using Tukey's studentized range test (HSD). Significance levels are given for $P < 0.05$ (Gomez and Gomez, 1984).

3. RESULTS

3.1. The effect of essential oil treatments on mortality of parent bruchids

Application of essential oil extracts significantly ($P < 0.05$) increased the adult mortality of *Z. subfasciatus* at the highest dose (0.5%) 24 hour after treatment application. Results of mortality of adult *Z. subfasciatus* 24-96 hour after treatment application are given in Table 2. Essential oil extracts of *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* induced 100% mortality of *Z. subfasciatus* at 0.5% in 24 hour after treatment application. Similar trends were also observed for seeds treated with *C. ambrosioides* and *T. ammi* at 0.02 and 0.1% at 24 hour after treatment application. Significantly ($P<0.05$), lower mortality was obtained with seeds treated with essential oils of *R. officinalis*, *E. globulus* and *C. citratus* at 0.02%, 24 hour after treatment application.

3.2. Effect of essential oil extracts on F_1 progeny production, percent inhibition and percent grain weight loss.

The trial evidenced that significantly ($P< 0.05$) lower number of F_1 progeny was produced in most of the treatments compared to the untreated grains (Table 3). No progeny was produced by *Z. subfasciatus* in grains treated with essential oil extracts of *C. ambrosioides*, *E. globulus*, *T. ammi* and *C. citratus* at the highest dose of 0.5. The highest oviposition inhibition rate was recorded in bean seeds treated with *T. ammi* essential oil extract at all levels of application (Table 3).

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Significantly (P< 0.05) higher number of F_1 progeny was produced in bean seeds treated with *C. citratus* essential oil extract at 0.02%. Similarly, this essential oil extract was less effective in inhibition of oviposition and subsequent progeny emergence of *Z. subfasciatus*.

Grain weight loss caused by *Z. subfasciatus* was significantly (P < 0.05) higher in the control compared to grains treated with essential oil extracts of *C. ambrosioides*, *E. globulus*, *T. ammi*, *R. officinalis* and *C. citratus* 45 days after infestation (Table 3). Grains treated with essential oil extracts at 0.1 and 0.5% gave better protection against the attack by *Z. subfasciatus*, without noticeable feeding damages compared with the untreated grains. Significantly, lower percent protection of bean seeds was recorded with essential oil extracts of *C. ambrosioides*, *E. globulus* and *C. citratus* at the lower concentrations. There was no weight loss recorded on grains treated with primiphos-methyl.

3.4. The effect of essential oil extracts on germination of haricot bean seeds

The trial demonstrated that the percent germination of bean seeds was ranged from 89% to 96%. There was no significant (P > 0.05) difference in the germination capacity of haricot bean seeds treated with essential oil extracts and the untreated seeds (Table 4).

4. DISCUSSION

Different parts of essential oil extract treatments showed various insecticidal effects on *Z. subfasciatus* in 96 hour exposure period. The essential oil extracts of *C. ambrosioides* and *T. ammi* were the most effective treatments in the control of *Z. subfasciatus* since the lower concentration induced a high mortality. These treatments gave 83-93% mortality of the adult bruchids, which is not significantly different from the effects of primiphos-methyl which also induced 100% mortality of the tested insect at similar exposure time of 24 hour after treatment application. *R. officinalis*, *E. globulus* and *C. citratus* also had a toxicity effect, but their essential oils were less effective than that of *C. ambrosioides* and *T. ammi* at the lower application doses. At the highest concentrations, all essential oils tested depicted significantly higher toxicity to *Z. subfasciatus* since they caused, each one at least 93% of mortality 96 hour after treatment application. Activity of essential oils against adult bruchids appeared to be related to level of application and exposure periods.

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Toxicity is one of the broad effects of bioactive compounds present in plants to insects. The toxic effect of plant essential oils against various storage insect pests have been pointed out by several authors (Srivastava *et al*., 1988; Odeyemi, 1993; Tapondjou *et al*., 2002; Rahman and Talukder, 2006; Dawit and Bekele, 2010), who attributed their toxic effect to various terpene constituents of the essential oils. Therefore, the insecticidal properties of the current investigated essential oils against *Z. subfasciatus* might also be attributed to their essential oil constituents.

This study also demonstrated that the all essential oil extracts tested reduce significantly the F_1 adult emergence of *Z. subfasciatus* compared to the untreated check though the plant materials vary among themselves. Treatment of bean seeds with essential oil extracts of *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* at the highest dose (750 mg/ 150 g of grain) resulted in significant reduction of F_1 progeny production. The treatments manifested up to 96-100% reduction in adult emergence. Likewise, complete inhibition in emergence of adult bruchids was obtained with the lowest concentration (30 mg) treatment of *T. ammi* essential oil. This reduction in F_1 progeny emergence in the treated grains could be the fact of the early death of the adult *Z. subfasciatus* due to bioactivities of the essential oils tested. Aboua *et al*. (2010) studied the effect of three essential oils on *C. maculatus* oviposition and reported that application of oils occlude seed funnels leading to the death of the developing insect by asphyxia.

In related studies, all these tested plant materials showed oviposition inhibition effect on different insect pests. *E. globulus* essential oil extract completely inhibited F_1 progeny emergence of *C. maculatus* (Rahman and Talukder, 2006). Similar result was documented for rosemary essential oil against *A. obtectus* (Papachouristos *et al*., 2004). Paranagama *et al*. (2003) also studied the deleterious effect of the essential oils of *C. citratus* and *C. nardus* on oviposition and F_1 adult emergence of *C. maculatus*. Hence, the results of this study are consistent with observation of the earlier findings.

The cause for a considerable protection of haricot bean seeds against the attack by *Z. subfasciatus* by the essential oil extracts of some of the botanical plants in the current investigation could be due to the presence of different chemicals which interfere with the feeding

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habit of the pest. Identification of the chemicals responsible should be an immediate research agenda.

The germination test demonstrated that the plant materials tested against *Z. subfasciatus* did not show any visible adverse effects on germination capacity of the seeds. Some of the treatments were infected by moulds which resulted in a reduced germination percentage ranging from 89-96%. Similar results were reported by Keita *et al*. (2001) and Rahman and Talukder (2006).

In conclusion, the findings of the current study indicated that plant materials tested possess bio-insecticidal properties that can be used in the management of insect pests on stored bean. In line with this, there is a possible scientific rationale for the incorporation of essential oil extracts of *C. ambrosioides*, *R. officinalis*, *E. globulus*, *T. ammi* and *C. citratus* into the bean grain protection practices of resource-poor farmers. They are also safe, cheap, easily biodegradable, and technologically and environmentally friendly since they are used in ethno-botany for the treatment of various ailments. It is also essential that further work to isolate, improve their efficacy and reliability, and appropriate technological systems need to get priority concern.

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Table 1: List of essential oil plant materials tested against *Z. subfasciatus* on haricot bean seed.

Treatments	Common name	Parts used for oil extraction
Chenopodium ambrosioides	Mexican tea	fresh leaves and inflorescences
Eucalyptus globulus	Eucalyptus	leaves
Rosmarinus officinalis	Rosemary	fresh leaves and inflorescences
Cymbopogon citratus	Lemmon grass	leaves
Trachyspermum ammi	Bishop's weed	seeds

Table 2: Cumulative mean % mortality of adult *Z. subfasciatus* due to essential oils treated to bean seeds after different exposure hours.

Treatments	Dosage (g/150 g grain)	Mean % adult mortality, h after exposure			
		24	48	72	96
C. ambrosioides	0.03	83.33±8.82a	96.67±3.33a	100.00±00a	100.00±00a
	0.15	96.67±3.33a	100.00±00a	100.00±00a	100.00±00a
	0.75	100.00±00a	100.00±00a	100.00±00a	100.00±00a
R. officinalis	0.03	33.33±5.39bc	66.67±5.39b	83.33±3.33ab	90.00±5.77ab

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	0.15	53.33±0.33b	70.00±3.65b	88.33±0.60ab	93.33±0.29ab
	0.75	90.00±5.73a	96.67±3.33a	96.67±3.33ab	100.00±00a
E. globulus	0.03	43.33±1.92bc	63.33±3.93b	80.00±6.93b	83.33±3.33b
	0.15	43.33±3.33bc	73.33±6.71b	83.33±3.33ab	93.33±0.29ab
	0.75	100.00±00a	100.00±00a	100.00±00a	100.00±00a
T. ammi	0.03	93.33±6.67a	100.00±00a	100.00±00a	100.00±00a
	0.15	96.67±3.33a	100.00±00a	100.00±00a	100.00±00a
	0.75	100.00±00a	100.00±00	100.00±00a	100.00±00a
C. citratus	0.03	23.33±6.05cd	35.00±4.46	43.33±3.47c	45.00±2.88c
	0.15	96.67±5.77a	98.33±1.67	98.33±1.67ab	98.33±0.19a
	0.75	100.00±00a	100.00±00a	100.00±00a	100.00±00a
Primiphos-methyl	0.125	100.00±00a	100.00±00a	100.00±00a	100.00±00a
Control (acetone treated)	0	0±00d	0±00d	0±00d	0±00d

296 Means within a column followed by different letters are significantly different (P< 0.05), Tukey student test (HSD).

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299 Table 3**:** Mean numbers of F_1 progeny produced and weight loss caused by *Z. subfasciatus* on

300 bean seeds treated with different plant materials of essential oil at different rates.

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Treatments	Dosage (g / 150 grain)	Mean± SE number of F_1 progeny	% Mean± SE weight loss
C. ambrosioides	0.03	12.33±0.67ab	0.13±0.09ab
	0.15	0.67±0.05a	0.00±00a
	0.75	0.00±0.00a	0.00±00a
R. officinalis	0.03	7.67±1.13ab	0.02±0.01a
	0.15	5.00±0.54ab	0.01±0.00a
	0.75	1.00±00a	0.00±00a
E. globulus	0.03	10.67±1.50ab	0.08±0.05ab
	0.15	8.33±1.26ab	0.02±0.01a
	0.75	0.00±00a	0.00±00a
T. ammi	0.03	0.00±00a	0.00±00a
	0.15	0.00±00a	0.00±00a

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	0.75	0.00±00a	0.00±00a
C. citratus	0.03	26.33±1.23bc	0.24±0.07bc
	0.15	2.67±0.94a	0.00±0.00a
	0.75	0.00±00a	0.00±00a
Primiphos-methyl	0.125	0.00±00a	0.00±00a
Control (acetone treated)	0	37.66±2.03c	0.36±0.04c

Means within a column followed by different letters are significantly different (P< 0.05), Tukey student test (HSD).

Table 4: Effect of different essential oil extracts on percent germination of haricot bean seeds

Treatments	Dosage (g/150 g grain)	% mean ±SE germination of seeds
C. ambrosioides	0.03	91.33±1.20a
	0.15	92.00±1.53a
	0.75	92.00±0.58a
R. officinalis	0.03	90.00±1.53a
	0.15	90.67±0.67a
	0.75	90.67±0.88a
E. globules	0.03	89.67±1.67a
	0.15	91.33±1.45a
	0.75	90.00±2.00a
T. ammi	0.03	90.67±1.53a
	0.15	90.33±0.57a
	0.75	93.00±1.00a
C. citratus	0.03	90.00±1.00a
	0.15	90.00±1.00a
	0.75	92.00±1.00a
Primiphos-methyl	0.125	90.67±1.76a
Control (acetone treated)	0	95.67±0.88a

Means within a column followed by the same letter are not significantly different by Tukey student test (HSD) (P > 0.05).