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TOXICITY AND BIOEFFICACY OF WEED ESSENTIAL OILS AGAINST COWPEA BRUCHIDS AND THEIR EFFECT ON MUNGBEAN SEEDS

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ABSTRACT: These investigations aimed to determine toxicity and bioefficacy of weed essential oils Bitter bush (*Eupatorium odoratum* L.), Finger grass (*Limnophila aromatica* (Lamk.) Merr.), and Vietnamese mint (*Polygonum odoratum*) derived by hydrodistillation against cowpea bruchids, *Callosobruchus maculatus* (Fabricius) on stored mungbean. Experiments were assessed under laboratory conditions (30±2°C, 70-80%RH and 16L:8D photoperiods). Results showed that weed essential oils from Bitter bush, Finger grass and Vietamese mint leaves have contact toxicity on cowpea bruchids as shown by the impregnated filter paper test. LC_{50} values were 137.15, 225.17 and 99.12 ppm at 48 h after exposure, respectively. Fumigant toxicity by the fumigation method on cowpea bruchids showed all weed essential oils had high efficiency against the cowpea bruchids (100% of mortality). Repellency toxicity test showed that weed essential oils from Bitter bush, Finger grass and Vietnamese mint leaves have repellent toxicity on cowpea bruchids as LC_{50} values were 607.23, 141.93 and 109.81 ppm at 6 h after exposure, respectively. The potential of weed essential oils on reproduction of the cowpea bruchids female adults had strong repellent activity for egg laid on mungbean seeds, were 100% at 48 h. Three weed essential oils did not affect the seed germinating. These results suggested that essential oils from three weed plants could be used as potential control agents for cowpea bruchids, and the database can be used for active ingredient studies to develop commercial products in the future.

Keywords: Lethal concentration, Toxicity, Mungbean insect pests, Oviposition behavior, Seeds viability

1. INTRODUCTION

Mungbean, *Vigna radiata* L. Wilczek is one of the most important legume crops in Thailand. Mungbean grown as short rotation crops interspersed with rice and vegetable crops. It provides an inexpensive source of dietary protein to the people and use as protein supplement for meat and fish in animal feed industries. It contains digestible carbohydrate, dietary fiber, calories and lysine [1], [2]. Growing mungbean need to keep seeds for used as seed and consumption. The production of mungbean (*V. radiate*) was restricted by biotic and abiotic factors both in the field and the seed in storage. Among the constraining biotic factors are insect pest. While crops may be infested in the field, infestations are often too low to detect at harvest. Bruchids are most often not detected until seed has been stored for over long periods (e.g. for longer than three months), especially at small scale farming levels. Bruchids are a major and growing problem in stored mungbean in all regions. Bruchids breed rapidly in storage and by the time they are detected, the infested grain is usually unmarketable. The bruchids responsible for most infestations in mungbean is the cowpea bruchids, *Callosobruchus maculatus* (Fabricius). Larvae developing within the grain do the largest damage. Their damage caused loss of weight, nutritional value and viability of stored grains [3].

Control of cowpea bruchids in the field and store has to be considered in relation to the economic importance of the crop, since it is obvious that these weevils are capable of attacking cowpea both in the field and in storage. In the latter, where feasible, the use of synthetic insecticides is one of the methods used to control cowpea bruchids [4]. Insecticides may be applied as liquid or fumigant formulations. However, continuous use of chemical insecticides may lead to serious problems such as insecticide resistance. Non-chemical methods of bruchids control offer an attractive alternative because the neither leave chemical residues in the commodity no could their use give rise to resistance in the pest. Such methods include periodic exposure of the grains to the sun, coating seeds with cooking oils, or mixing them with ash or sand [5]. Some plant materials have insecticidal properties that could help to control the invading pests [6]. Alternative options for protection cowpea bruchids are using of nature local plant extracts. They can be found and annually growth, such as using of essential oils extracted from the local weeds. Therefore, the most people see weeds and no useful. The objective of this study was to evaluate the lethal toxicity, fumigant toxicity, repellent toxicity and oviposition behavior of weed essential oils on cowpea bruchids, including their effect on mungbean seed viability.

2. MATERIALS AND METHODS

2.1 Insect Stock Culture

Cowpea bruchids, *Callosobruchus maculatus* (Fabricius) was collected from the previously infested mungbean seeds in the grain storage of Department of Agriculture Technology, Faculty of Technology, Mahasarakham University. Insect rearing was carried out inside a glass bottles (∅ 15 cm and height 30 cm) covered with mesh netting and kept under laboratory conditions (30±2°C, 70-80%RH and 16L: 8D photoperiods). Fifty pairs of male and female of *C. maculatus* adults were isolated and released in a glass bottle having 500 g of mungbean seeds, removed infested seeds and sterile by kept under freezing for 2 weeks and left for 24 h under ambient conditions [7], covered by mesh netting. After 4 weeks, they were separated and used female adults (3 days old) in the investigation. Toxicity and bioefficacy bioassays were performed on the adults.

2.2 Stored Product

Mungbean obtained from the local market healthy and fresh seeds were used to avoid any pre storage infestation seeds or egg laying of cowpea bruchids and stored followed Ojanwana and Umoru [7]. Mungbean was used in experiments as effect of weed essential oils on the egg laying behavior and mungbean seed germination.

2.3 Weed Essential Oils Preparation

Three weed essential oils extracted from Bitter bush (*Eupatorium odoratum* L.), Finger grass (*Limnophila aromatica* (Lamk.) Merr.) and Vietnamese mint (*Polygonum odoratum*), were collected around the Mahasarakham local in the Northeast of Thailand. Bring fresh leave of each weed was washed and air dried in the shade. Using a steam distillation, the extraction of the essential oils was performed from 1,000 g of fresh weed leaves and 1 liter of distilled water with a rotary evaporator, at the Department of Agricultural Technology, Faculty of Technology, Maha-sarakham University. The weed essential oils were desiccated with anhydrous sodium and kept into a vial with the lid closed in the dark at 4°C until use.

2.4 Experimental Procedure

The experiment was conducted at laboratory conditions (30±2°C, 70-80%RH and 16L:8D photoperiod). Experimental design was Completely Randomized Design (CRD) with 5 replicates in 5 laboratory experiments.

2.4.1 Contact toxicity bioassay of EOs

Contact toxicity of three weed essential oils against cowpea bruchids were evaluated by impregnated filter paper test, a modified method after Fournet *et al*. [8]. Bioassay was conducted at 30±2°C, 70-80%RH and 16L:8D photoperiod. The serial solutions of weed essential oils were prepared by dissolving in acetone to achieve the desired concentrations: 5-6 concentrations. For each preparation was dropped and flowed on a disk of filter paper (∅ 9 cm) placed in a Petri dish (∅ 9 cm and height 1.5 cm) using micropipette. The treated filter paper was air dried and allowed to evaporate the solvent completely before cover petri dish was placed on the petri dish. Ten female adults of cowpea bruchids taken from insect stock culture were released in a petri dish and five replicates were set for each concentration. Acetone was used as controls. The dead cowpea bruchids, no response to blunted needle poking, after 24, 48 and 72 h were recorded. The data were analyzed for the Median Lethal Concentration (LC_{50}).

2.4.2 Fumigant toxicity bioassay of EOs

Fumigant toxicity of three weed essential oils was tested against female adults of the cowpea bruchids *C. maculatus* modified method of Keita *et al.* [9]. Ten female adults taken from the insect stock culture were placed in a glass bottle (∅ 5 cm and height 6 cm) and covered with mesh netting and rubber band. Using micropipette dropped weed essential oil inside the center of filter paper (∅ 2 cm) in another a glass bottle with various concentrations. Bring a glass bottle contained cowpea bruchids to overlap with a glass bottle dropped of weed essential oil into it. Use clear tape wrapped around a joint of both glass bottles and carried out under laboratory conditions (30±2°C, 70-80%RH and 16L:8D photoperiods). Cowpea bruchids *C. maculatus* cannot be directly contact oil because it had a mesh barrier. Dead of adult cowpea bruchids *C. maculatus* were counted after 12, 24 and 48 h.

2.4.3 Repellent toxicity bioassay of EOs

Repellency toxicity bioassay was modified method [10] by weighing 10 g of sterile mungbean placed in flask and treated with the solvents of three weed essential oils. Let until mungbean seeds dried to evaporate. Pour the mungbean into plastic cup (∅ 5 cm and height 2 cm), had a hole in the bottom so that insect can get through but mungbean cannot, placed on the top a glass bottle (∅ 5 cm and height 6 cm). Ten female adults of cowpea mungbean *C.* maculatus (3-5 days old) were released the middle of plastic cup within contain treated mungbean seeds. It closed with mesh net (size 6 × 12 cm) to prevented insect evasion. Each treatment was replicated five times and the numbers of cowpea bruchids *C. maculatus* settled on each glass bottle were counted and recorded at hourly intervals for 1, 3, 6 and 12 h. Treated mungbean with acetone was used as control**.**

2.4.4 Impact of EOs on the oviposition behavior

Weighing 10 g of sterile mungbean placed in flask and treated with various the solvents of three weed essential oils. Let until mungbean seeds dried to evaporate. One pair of adults cowpea bruchids placed in a glass bottle within contains treated mungbean seeds for 48 h. Adults were separated and counted the number of eggs on surface of mungbean in each treatment, compared the control with acetone.

2.4.5 Effect of EOs on the mungbean seed viability

Weighing 10 g of sterile mungbean placed in flask and treated with various concentrations (LC_{20} and LC_{40}) of three weed essential oils. Let until mungbean seeds dried to evaporate. Bring mungbean seeds tested seed germination by each treated mungbean seed was counted and divided into 5 replicates (50 seeds per replicate). Germination test was conducted in a plastic box (size 22 × 33 cm and height 7 cm) within contain 1,000 g of fine sand, was incubated at 150°C for 10 h and separated the other contamination with mesh net, and distilled water 132 ml. Put 50 seeds in a plastic box by a row of five rows along the length of the row of 10 seeds off the plastic covered and kept on the shelf under laboratory conditions. When seed germination was opened the plastic box and keep moisture, add water. Until after 7 days of seed germination were counted seedling in each treatment for checking percentage of seed germination.

2.5 Data Analysis

Toxicity bioassay, linear regression analysis was performed from the data obtained to estimate adult mortality for each concentration of three weed essential oils. The mortality was calculated using the Abbott formula [11]. The resulting concentration-mortality data was subjected to probit analysis [12]. Data recorded for percentage of mortality in all toxicity experiments and percentage of seed viability of different treatments were subjected to statistical analysis using CRD design by one-way analysis of variance (ANOVA). Means were compared by using Duncan's Multiple Range Test (DMRT).

3. RESULTS

3.1 Contact Toxicity Bioassay of EOs

Weed essential oil of Vietnamese mint (*P. odoratum*) had the most contact toxicity to cowpea bruchids within 48 h after treatment with the LC_{50} of 99.12 ppm compare with 137.15 ppm of Bitter bush (*E. odoratum*) and 225.17 ppm of Finger grass (*L. aromatic*), respectively (Table 1). Mortality value exposed with three weed essential oils to cowpea bruchids after treatment at 72 h resulted in adult mortality values between 84-100% and it cause the highest significant different ($P<0.01$) (Table 2).

3.2 Fumigant Toxicity of EOs

It is reported that all weed essential oils had efficiency against the cowpea bruchids, 100% of adult mortality when comparison with distilled water and acetone treatments and also was high significant different.

3.3 Repellent Toxicity of EOs

Result showed that essential oil of Vietnamese mint (*P. odoratum*) has highest against the cowpea bruchids. And then Finger grass (*L. aromatic*) oil and Bitter bush (*E. odoratum*) oil had against the cowpea bruchids LC_{50} at 6 h were 109.81, 141.93 and 607.23 ppm, respectively (Table 3). Repellent value exposed with three weed essential oils to cowpea bruchids after treatment at 12 h resulted in adult mortality values between 64-98% and it cause the highest significant different ($P<0.01$) (Table 4).

3.4 Effect of EOs on the Oviposition Behavior

The effect of tested three weeds essential oils on the reproduction of the cowpea bruchids female adults were studied using no-choice test. All weed essential oils acted as oviposition deterrent. Egg laid by female on treated seeds with weed essential oils had strong repellent activity (100%) at 48 h.

3.5 Effect of EOs on the Mungbean Seed Viability

The findings of the present study indicated that mungbean seeds treated with all weed essential oils did not lose their viability and also did not show significant effect on the seed germination rate within 7 days (Table 5).

Table 1 Contact toxicity of three weed essential oils against the cowpea bruchids *C. maculatus* at 48 h.

Weed essential oils	LC_{50}[a] (ppm)	95%CL[b] (ppm)	χ^2	*P*-value
Bitter bush (*E. odoratum*)	137.15 ± 87.63	42.15 – 446.26	0.0086	0.9263
Finger grass (*L. aromatic*)	225.17 ± 99.43	97.24 – 521.38	0.0245	0.8756
Vietnamese mint (*P. odoratum*)	99.12 ± 132.90	11.40 – 861.24	0.0003	0.9862

[a] LC_{50} represent the median concentration. [b] 95%CL represent the lower and upper fiducially limits.

Table 2 Mortality of cowpea bruchids *C. maculatus* with contact toxicity treated three weed essential oils at 24, 48 and 72 h.

Treatments	Conc. (ppm)	Mean (±SE) mortality (%)		
		24 h	48 h	72 h
Distilled water		0.0 ± 0.00 g	0.0 ± 0.00 i	2.0 ± 0.45 g
Acetone		2.0 ± 0.45 f	2.0 ± 0.40 h	12.0 ± 1.10 f
Bitter bush	100	28.0 ± 0.84 c	50.0 ± 0.63 f	84.0 ± 0.55 e
(*E. odoratum*)	200	30.0 ± 1.22 b	50.0 ± 0.89 f	84.0 ± 1.14 e
	300	34.0 ± 0.89 a	56.0 ± 1.02 c	86.0 ± 1.14 d
Finger grass	100	24.0 ± 0.55 e	42.0 ± 0.75 g	84.0 ± 1.14 e
(*L. aromatic*)	200	34.0 ± 1.34 a	54.0 ± 1.02 d	86.0 ± 0.55 d
	300	34.0 ± 1.14 a	62.0 ± 2.04 b	90.0 ± 1.41 c
Vietnamese mint	100	26.0 ± 0.89 d	52.0 ± 0.40 e	90.0 ± 0.71 c
(*P. odoratum*)	200	28.0 ± 1.30 c	52.0 ± 0.98 e	98.0 ± 0.45 b
	300	34.0 ± 1.14 a	66.0 ± 1.74 a	100.0 ± 0.00 a

Means within the same column followed by the same letter are not significantly different (DMRT: P>0.05).

Table 3 Repellent toxicity of three weed essential oils against cowpea bruchids *C. maculatus* at 6 h.

Weed essential oils	LC_{50}[a] (ppm)	95%CL[b] (ppm)	χ^2	*P*-value
Bitter bush (*E. odoratum*)	607.23 ± 145.44	319.82 – 894.64	0.0005	0.9815
Finger grass (*L. aromatic*)	141.93 ± 42.85	61.61 – 197.77	0.1546	0.6942
Vietnamese mint (*P. odoratum*)	109.81 ± 51.64	45.06 – 267.61	0.0525	0.8189

[a] LC_{50} represent the median concentration. [b] 95%CL represent the lower and upper fiducial limits.

Table 4 Repellent Percentage of cowpea bruchids *C. maculatus* with repellent toxicity treated three weed essential oils at 1, 3, 6 and 12 h.

Treatments	Conc. (ppm)	Mean (±SE) Repellent Percentage (%)			
		1 h	3 h	6 h	12 h
Distilled water		0.0 ± 0.00 g	0.0 ± 0.00 j	0.0 ± 0.00 i	0.0 ± 0.00 i
Acetone		2.0 ± 0.45 f	4.0 ± 0.55 i	6.0 ± 0.55 h	6.0 ± 0.55 h
Bitter bush	100	10.0 ± 0.71 e	14.0 ± 0.55 h	34.0 ± 0.55 g	68.0 ± 0.84 f
(*E. odoratum*)	200	18.0 ± 0.45 c	20.0 ± 0.00g	38.0 ± 0.84 f	74.0 ± 1.14 e
	300	20.0 ± 0.71 b	30.0 ± 0.71 e	40.0 ± 1.00 e	94.0 ± 0.89 b
Finger grass	100	14.0 ± 0.55 d	22.0 ± 0.45 f	40.0 ± 1.22 e	64.0 ± 1.14 g
(*L. aromatic*)	200	20.0 ± 0.71 b	40.0 ± 1.00 b	62.0 ± 1.30 b	86.0 ± 0.89 c
	300	24.0 ± 0.89 a	48.0 ± 0.45 a	66.0 ± 1.14 a	94.0 ± 0.89 b
Vietnamese mint	100	18.0 ± 0.84 c	32.0 ± 1.10 d	46.0 ± 0.89 d	78.0 ± 0.84 d
(*P. odoratum*)	200	24.0 ± 1.14 a	38.8 ± 1.82 c	60.0 ± 2.00 c	94.0 ± 0.55 b
	300	24.0 ± 1.14 a	40.0 ± 1.58 b	62.0 ± 0.84 b	98.0 ± 0.45 a

Means within the same column followed by the same letter are not significantly different (DMRT: P>0.05).

Table 5 Effect of three weed essential oils in various concentrations on mungbean seeds viability.

Treatments	Conc. (ppm)	Mean (±SE) Germination Percentage (%)	
		5 days	7 days
Distilled water		92.0 ± 1.00 d	92.0 ± 1.00 d
Acetone		87.6 ± 3.56 k	87.6 ± 3.56 h
Bitter bush	100	88.4 ± 0.84 i	89.2 ± 1.52 g
(*E. odoratum*)	200	89.2 ± 0.55 h	89.2 ± 0.55 g
	300	91.2 ± 2.30 e	91.2 ± 2.30 e
Finger grass	100	90.4 ± 3.77 f	90.4 ± 3.77 f
(*L. aromatic*)	200	95.2 ± 1.52 a	95.2 ± 1.52 a
	300	94.2 ± 1.67 b	94.8 ± 1.67 b
Vietnamese mint	100	92.0 ± 1.58 c	93.2 ± 2.07 c
(*P. odoratum*)	200	88.0 ± 2.55 j	89.2 ± 2.70 g
	300	89.6 ± 1.92 g	93.2 ± 1.14 c

Means within the same column followed by the same letter are not significantly different (DMRT: P>0.05).

3.6 Copyright Form

Copyright form signed by all authors is necessary for GEOMATE. It should be submitted along with the paper submission. Copyright form can be downloaded from geomate web site.

4. DISCUSSIONS

Different spice of plant products in the form essential oils (EOs, powders, pellets, extracts or distillates could be harnessed as potential toxicants, deterrents, antifeedants, repellents and fumigants for exclusion of stored-product pests from grain, and have been used, but low toxicity has obtained much attention for alternative control measures of stored-product pest. Diverse essential oils (EOs) and other plant products have been used. Three weed essential oils investigated showed sufficient protection of cowpea grains from damage by cowpea bruchids and these oils acted oviposition deterrent by adult female. Also, we observed not to affect seed germination which confirms non-adverse effect on grain chemistry. This demonstrated the ability of the oils to act as suffocation materials with the possibility of preventing respiration [13]. However, plant natural products that constitute effective safer alternatives to synthetic insecticides without producing adverse effects on the ecosystem have been tested in the management of stored-product pests [14]-[15]. In this study, we evaluated the insecticidal and repellent properties of EOs of Bitter bush (*E. odoratum* L.), Finger grass (*L. aromatica* (Lamk.) Merr.), and Vietnamese mint (*P. odoratum*) against cowpea bruchids, under laboratory conditions.

5. CONCLUSIONS

Based on the results obtained in the current study, it may be conclude that Bitter bush (*E. odoratum* L.), Finger grass (*L. aromatica* (Lamk.) Merr.), and Vietnamese mint (*P. odoratum*) weed plant materials have a broad spectrum of activity against cowpea bruchids *C. maculatus*, and the essential oils could have potential as bioinsecticides in stored product protection. However, since plant products volatilize quickly in the environment and do not persist for longer duration unlike synthetic pesticides, there could be a need for re-application to obtain the desired results. The efficacy of plant-based pesticides could also be enhanced when dissolved or mixed with a slow release fixative material or carrier such as starch or liquid paraffin, and incorporated as an integral part of integrated pest management system especially at a small-scale farmer level. The essential oil from these plants could become a viable alternative to conventional chemical control strategies. However, further studies need to be conducted in order to evaluate the safety of these oils before practical use in stored-product insect control.

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