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EFFICIENCY OF INDIAN BORAGE ESSENTIAL OIL AGAINST COWPEA BRUCHIDS

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ABSTRACT: Cowpea bruchids is one of the most important insect pests in stored products, especially of whole grains used for breeding or consumption. Currently, synthetic insecticides cause adverse effects to human health and the environment, thus new alternative biopesticides from herb or plant are needed for cowpea bruchids control. The aim of this research was to evaluate insecticidal activities of essential oil from Indian borage, *Plectranthus amboinicus*. Efficacy of this essential oil against *C. maculatus* was investigated by impregnated filter paper and vapor-phase tests. Experiments were conducted under Completely Randomized Design (CRD) with 4 replications. Contact activity was considered on essential oil at 6 concentrations of 0 300 600 900 1,200 and 1,500 ppm and fumigant activity was performed at 6 concentrations including 0 3 6 9 12 and 15 µL/L air. All experiments were assessed under laboratory conditions (30±2ºC, 70-80%RH and 16L:8D photoperiods). Data of deaths cowpea bruchids were recorded after treated at 24 to 168 h. Contact activity showed 1,200 ppm of *P. amboinicus* essential oil at 168 h was the best performance in the contact toxicity on cowpea bruchids and fumigation activity presented 12 µL/L air of *P. amboinicus* essential oil at 72 h had the highest effectiveness in the fumigation toxicity on cowpea bruchids, with 100% adult mortality. The data pointed that *P. amboinicus* essential oil showed the high potential for insecticidal activity for cowpea bruchids control. Hence, the essential oil of *P. amboinicus* might be used as an alternative for grain protection against stored-product insects.

Keywords: Insecticidal activity, Essential oil, Plectranthus amboinicus, Callosobruchus maculatus

1. INTRODUCTION

Callosobruchus maculatus (Fabricius) (Coleoptera: Chrysomelidae: Bruchinae), the cowpea bruchids which is the most important pest of during the storage period [1], has involved the great attention because it is widely distributed throughout the tropical and sub-tropical regions. Generally, the infestation starts in the field where the adults lay eggs on green or drying pods. Infestation in the field has no serious implications as the damage in the field is low. However, once infested seeds are stored, enormous damage occurs due to rapid multiplication of insects in a very short time. This species can cause damage of legume seeds up to 100 % during storage [2], resulting in maximum damage of 2 to 5 kg seeds within 45 to 90 days in optimum temperature (30±1ºC) and moisture conditions (75±3 %). The adult female lays eggs on the seed surface and the hatching larvae bore into the seed. The neonate larvae penetrate the grains causing serious damage, such as grain weight loss, and reductions in germination and nutritional value [3], [4]. The whole development takes place inside a single seed and the adults emerge out by leaving behind holed seed [5]. More than one larva can develop within a single grain. Damaged legume seeds have thus reduced weight, become unsuitable for human or animal consumption and have the poor germinating ability [6].

Synthetic pesticides are currently the method of choice to protect stored grain from insect damage. However, continuous or excessive uses of synthetic pesticides have created serious problems arising from factors such as ozone depletion, environmental pollution, increasing costs of application, pesticide residue in food, insects develop insecticidal resistance and toxicity hazards on non-target organisms in addition to direct toxicity to users [7], [8]. Therefore, the environment needs some other alternatives of chemical pesticides. These problems have highlighted the need to develop new types of selective insect-control alternatives. One alternative to synthetic insecticides is the botanical pesticides i.e. insecticidal plants or plant compound and the use of natural compounds. Plants may provide a potential alternative to currently used insect-control agents because they constitute a rich source of bioactive chemicals [9]. Aromatic plants are among the most efficient insecticides of botanical origin and essential oils often constitute the bioactive fraction of plant extracts [10]. Currently, essential oil, which results from secondary metabolism in plants, is in use for insect management [7].

Essential oils and their constituents in relation to contact and fumigant insecticidal actions have been well demonstrated to be a potent source of the botanical pesticide against stored grain product pests [11], [12]. Especially their main compounds monoterpenoids, offer promising alternatives to classical fumigants [13] and also have some effects on the physiological, biological and behavior of insects such as growth rate, life span, reproduction and can cause their death [14], [15]. Studies on essential oils have stimulated research on stored grain pest control, with very promising results. Mahmoudvand et al. [16] demonstrated the contact insecticidal activity of essential oils of *Lippia citrodora* Kunth., *Rosmarinus officinalis* L., *Mentha piperita* L. and *Juniperus sabina* L. presented activity against *C. maculatus* (F.). According to Raja et al. [17], the essential oil of *Mentha spicata* L. caused increased mortality, decreased oviposition and emergence of insects in cowpeas. Some chemical components of essential oils, extracted from plants of the Labiatae family, exhibit low toxicity to mammals but can interfere in specific regions of the nervous system of pest insects [18] causing the insect death. In the present investigation the essential oil of Indian borage, *Plectranthus amboinicus* (Lour.), from fresh leaves was studied for their contact and fumigant activities on the adult *C. maculatus*. The results may be used for *C. maculatus* control in storage units, providing direct and indirect benefits for small and medium cowpea producers.

2. MATERIALS AND METHODS

2.1 Insect Rearing

Cowpea bruchids, *Callosobruchus maculatus* (Fabricius) was used for the present experiment. A small population of C. maculatus was obtained from the seed store located in Maha Sarakham province, Thailand. Fifteen pairs of cowpea bruchids adults were maintained in a plastic box (diameter 22 cm, height 10 cm). The cultures were reared on a diet of 500 g of mungbean seeds, *Vigna radiata* (L) Wilczek, They were reared and allowed for mating and oviposition under laboratory conditions, inside a growth chamber at 30±5°C, 70±5% relative humidity, and a photoperiod of 16 h: 8 h (L:D). Adult insects of cowpea bruchids used for tests were 3 days old.

2.2 Extraction of Essential Oil

The essential oil was extracted from fresh leaves of Indian borage, *Plectranthus amboinicus* (Lour.) following by using hydrodistillation in a modified Clevenger-type apparatus. In carrying out the steam distillation process, 200 g of an air-dried sample of *P. amboinicus* was weighed into the distillation flask and 600 ml of distilled water added. The apparatus was set up using a clamp on a heating mantle and heated for a period of 3 h. The essential oil deposited on water was then collected through the attached graduated measuring tube by opening the tap. The essential oils treated with anhydrous sodium sulfate to remove the remaining water after the extraction. The essential oil was preserved in sealed glass containers and refrigerated in the dark at 4 ºC until its use.

2.3 Experimental Procedure

The essential oils were tested separately in a completely randomized design, consisting of six treatments (essential oils and control) with four replicates.

2.3.1 Contact activity

The insecticidal contact activity of essential oil of Indian borage *P. amboinicus* against adults of cowpea bruchids *C. maculatus* was evaluated by the impregnated filter paper test. Essential oil prepared in acetone (1mL) at different concentrations (0 300 600 900 1,200 and 1,500 ppm) were applied on filter papers (Whatman No. 1, diameter 9 cm). The solvent was allowed to evaporate for 2 min and place at the bottom of each petri-dish (diameter 9 cm). Ten adults (unsex) of cowpea bruchids *C. maculatus* (3-day olds) were introduced into each petri dish and covered with a lid, in a room condition at 30±5°C, 70±5% relative humidity, and a photoperiod of 16 h: 8 h (L:D). Control was maintained in which treatment was made with 1 mL of acetone alone. Adult mortality was assessed after treatment 24 h exposure to 168 h. The insects were considered to be dead as no leg or antennal movements were observed.

2.3.2 Fumigant activity

The insecticidal fumigant activity of Indian borage *P. amboinicus* was assessed by vapour phase test. In brief, each filter paper strip (Whatman No. 1, cut into 1.5x5 cm) treated with each test oils (0 3 6 9 12 and 15 µL/L air) previously dissolved in acetone (100 µL), it was placed into the hanging of glass vials (diameter 2.5 cm x height 5 cm) from the center of screw cap of fumigation bottle (diameter 5.5 cm x height 10.5 cm) to avoid contact effect of insects with paper strip. Ten adults (unsex) of cowpea bruchids *C. maculatus* (3-day olds) were placed in fumigation bottles and covered with a lid, in condition at 30±5°C, 70±5% relative humidity, and a photoperiod of 16 h: 8 h (L:D). Controls received 100 µL of acetone only. Adult mortality was observed after 24 h exposure to 168 h. The insects were considered to be dead as no leg or antennal movements were detected.

3. RESULTS

3.1 Contact Toxicity

Mortality of cowpea bruchids *C. maculatus* at 168 h after treatment was highest at the concentration of 1,200 ppm of essential oil from Indian borage *P. amboinicus* with cumulative values of 100% and there was a significant difference (P<0.01) in relation to the other concentrations, However, it did not show any differences compared with 600 900 and 1,500 ppm (Table 1 and Fig. 1). The number that died increased as the concentration of the essential oil and time of exposure increased for the insect. At all concentrations treated of essential oil for 120 h caused more than 50% adult mortality of cowpea bruchids *C. maculatus* compared with the control.

Table 1 Mortality of cowpea bruchids *C. maculatus* with contact activity treated essential oil of Indian borage *P. amboinicus*

Conc. (ppm)	Mean (±SE) of adult mortality (%) of cowpea bruchids *C. maculatus*						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
0	0.0±0.0[b]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[b]	0.0±0.0[c]
300	5.0±5.8[ab]	17.5±9.6[b]	22.5±5.0[b]	35.0±5.8[b]	50.0±11.5[b]	70.0±11.5[c]	80.0±11.5[b]
600	10.0±14.1[ab]	20.0±21.6[ab]	27.5±29.9[b]	47.5±35.9[ab]	62.5±26.3[ab]	77.5±17.1[bc]	90.0±8.2[ab]
900	12.5±9.6[a]	22.5±9.6[ab]	35.0±5.8[ab]	60.0±8.2[a]	62.5±9.6[ab]	82.5±5.0[abc]	92.5±9.6[a]
1200	15.0±5.8[a]	32.5±9.6[ab]	45.0±12.9[ab]	62.5±18.9[a]	70.0±8.3[ab]	92.5±9.6[ab]	100.0±0.0[a]
1500	15.0±12.9[a]	40.0±11.5[a]	52.5±9.6[a]	65.0±10.0[a]	77.5±9.6[a]	97.5±5.0[a]	100.0±0.0[a]

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

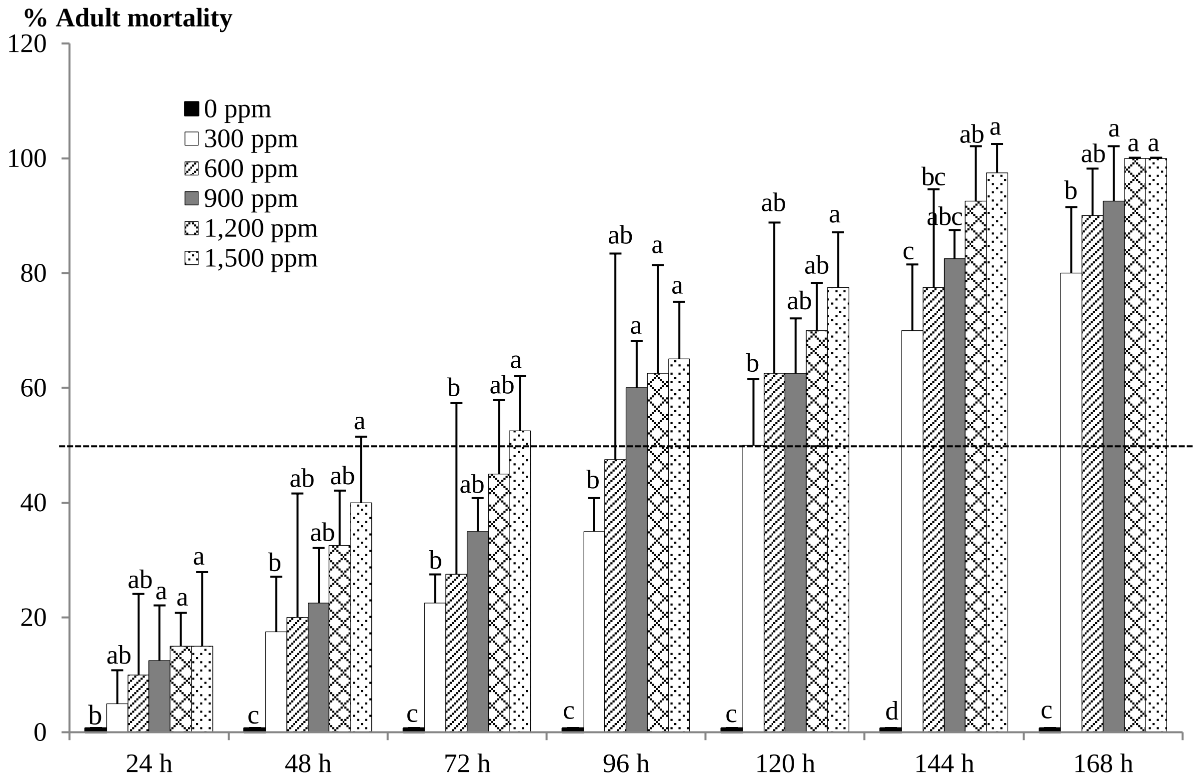

Fig. 1 Contact activity of essential oil of Indian borage *P. amboinicus* on adult mortality of cowpea bruchids *C. maculatus* within 168 h

3.2 Fumigant Toxicity

Mortality value exposed with 12 μL/L air of essential oil from Indian borage *P. amboinicus* leaves to adults of cowpea bruchids *C. maculatus* after treatment at 72 h resulted in 100%mortality and it caused the highest significant difference (P<0.01). However, it did not show differences compared with 9 and 15 μL/L air (Table 2 and Fig 2). All concentrations of essential oil were significantly different efficiency against cowpea bruchids *C. maculatus* with adult mortality when comparison with 0 μL/L air, acetone treatments and at 120 h showed >50% adult mortality of cowpea bruchids *C. maculatus*. Cowpea bruchids *C. maculatus* was more sensitive to the essential oil of Indian borage *P. amboinicus* by insecticide fumigant than contact activities at greater mortality during the first 72 h.

3.3 Copyright Form

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Table 2 Mortality of cowpea bruchids *C. maculatus* with fumigant activity treated essential oil of Indian borage *P. amboinicus*

Conc.	Mean (±SE) of adult mortality (%) of cowpea bruchids *C. maculatus*						
(μL/L air)	24 h	48 h	72 h	96 h	120 h	144 h	168 h
0	0.0±0.0[b]	0.0±0.0[c]	0.0±0.0[d]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]
3	32.5±12.6[a]	37.5±17.1[b]	52.5±20.6[c]	65.0±23.8[b]	65.0±14.1[b]	82.5±20.6[b]	92.5±9.6[b]
6	35.0±25.2[a]	50.0±28.3[b]	60.0±28.3[bc]	72.5±23.6[b]	77.5±28.7[b]	95.0±5.8[ab]	100.0±0.0[a]
9	37.5±9.6[a]	52.5±17.1[b]	80.0±18.3[ab]	80.0±18.3[b]	92.5±9.6[ab]	97.5±5.0[a]	100.0±0.0[a]
12	40.0±14.1[a]	85.0±10.0[a]	100.0±0.0[a]	100.0±0.0[a]	100.0±0.0[a]	100.0±0.0[a]	100.0±0.0[a]
15	65.0±30.0[a]	97.5±5.0[a]	100.0±0.0[a]	100.0±0.0[a]	100.0±0.0[a]	100.0±0.0[a]	100.0±0.0[a]

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

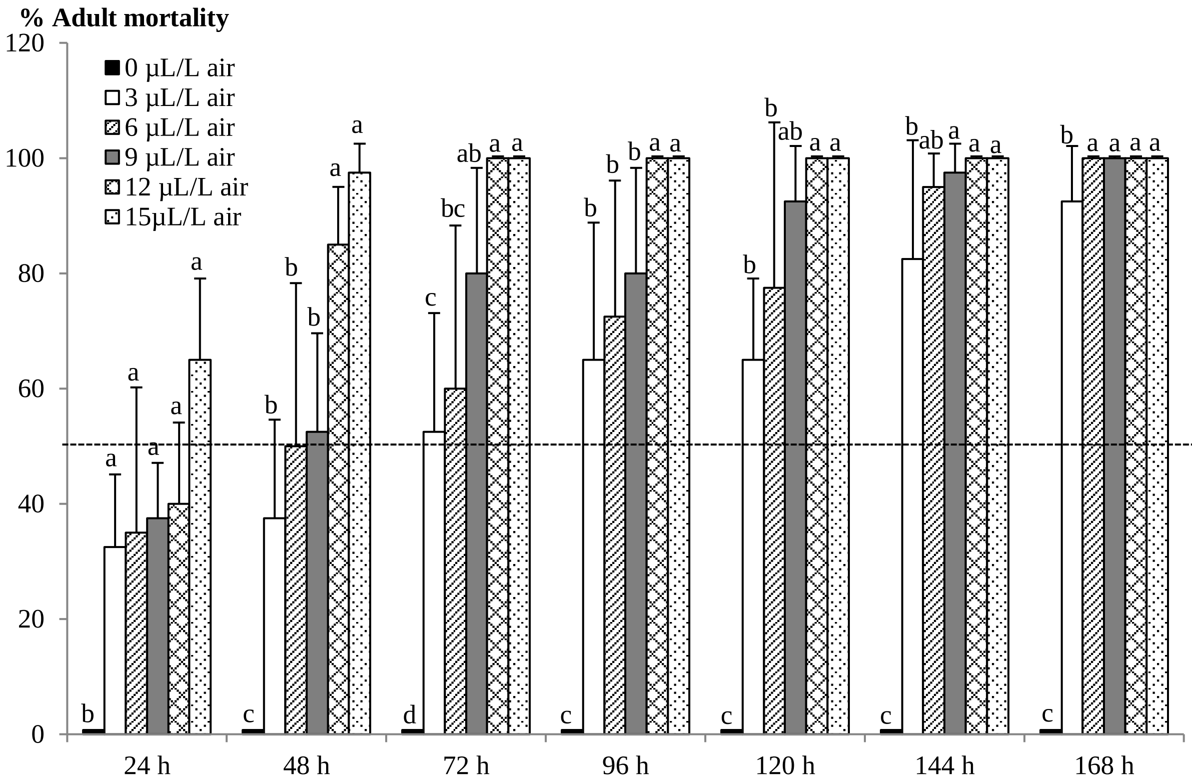

Fig. 2 Fumigant activity of essential oil of Indian borage *P. amboinicus* on adult mortality of cowpea bruchids *C. maculatus* within 168 h

4. DISCUSSIONS

In the current study, the essential oils obtained from leaves of Indian borage *P. amboinicus* demonstrated fumigant and contact activity to cowpea bruchids *C. maculatus*. In contact method, tested essential oil showed a significant increase of mortality (P< 0.001) to the test insects at higher dosage (1,200 ppm) after 120 h of treatment with 100% adult mortality, whereas in fumigation method insecticidal properties were more rapid to test insects within 72 h of treatment. In fumigation method, cowpea bruchids *C. maculatus* showed high susceptibility to the essential oil of Indian borage *P. amboinicus* oil, even at low concentrations (12 μL/L air) after 72 h of treatment with 100% adult mortality.

The insecticidal activity of many plant essential oils might be attributed to monoterpenoids [19]. Due to the high volatility, they have fumigant activity that might be of importance for controlling stored product insects. Monoterpenoids were reported earlier as fumigants and contact toxicants on various insect pests [20]. Many studies have demonstrated differential susceptibility of stored product beetle species to the essential oils. *Callosobruchus* species were more susceptible to essential oils or their components than those of other insect species [21], [22].

5. CONCLUSIONS

The results obtained suggest good potential for the use of essential oils of Indian borage *P. amboinicus* as both fumigant and contact toxic agents against cowpea bruchids *C. maculatus*. Though the examined essential oils had contact as well as fumigant activity, the fumigant toxicity of the essential oils was much more potential in a shorter period (72 h). From this study, it is concluded that Indian borage *P. amboinicus* (Lour.) essential oil could be used as botanical insecticides postharvest or fumigant since it is a potential alternative control method for cowpea bruchids *C. maculatus*.

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