

THE TOXICITY AND BIOACTIVITY OF CLIMBING WEDELIA POWDER AND ESSENTIAL OIL AGAINST MAIZE WEEVIL

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Abstract: Many types of powders and essential oils derived from plants are used as traditional protection for stored products against insect pests. They are relatively non-toxic to mammals and they meet the criteria for reduced risk pesticides. The toxicity and bioactivity of the powder and essential oil of the climbing wedelia, *Wedelia trilobata* (L.) A.S. Hitchcock, were determined against maize weevil, *Sitophilus zeamais* Motschulsky. The powder and essential oil were obtained from the aerial parts of *W. trilobata* and tested for their toxicity, as well as insecticidal and residual activities, against *S. zeamais*. The LC_{50} value of the *W. trilobata* powder to *S. zeamais* was 582.86 g/kg grain within 72 hours, while the essential oil was 1,146.19 μ L/L air within 24 hours. Mixing powder with grain at a dosage of 80 g/kg grain killed *S. zeamais* at 7.50% after 12 days of exposure and reduced the progeny of *S. zeamais* by 89.96%. After fumigation, concentrations of 150, 200 and 250 μ L/L air of *W. trilobata* essential oil showed 2.50%, 5.00% and 12.50% of adult mortalities in *S. zeamais* after 24 hours of exposure, with 81.97%, 83.07% and 97.65% reductions in progeny, respectively. The *W. trilobata* powder and essential oil showed efficacy in the protection against the newly emerged progeny of *S. zeamais* (>80%). The *W. trilobata* essential oil exhibited moderate to strong residual activities. The results suggest that the powder and essential oil of *W. trilobata* may be effectively used in the management of *S. zeamais* populations in grain storage.

Keywords: Plant products, toxicity, insect pests, insecticidal activity, residual activity.

Introduction

Insect pests cause quantitative and qualitative losses to stored grain products at the farmers' level (Kadjo *et al.*, 2016). *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is a serious pest of stored grain in Thailand (Hayashi *et al.*, 2004). The damage caused by *S. zeamais* on stored rice grain is extremely high in tropical and subtropical regions (Ribeiro *et al.*, 2014), decreasing the weight of agricultural products and consequently its market and nutritional values (Temesgen and Waktole, 2013). The management of *S. zeamais* populations is generally controlled with insecticides. Traditional control methods are based on the

use of chemical compounds, such as phosphine, methyl bromide, chlorpyrifos-methyl and deltamethrin (Tadesse and Subramanyam, 2018). Nerio *et al.* (2010) reported that synthetic insecticides adversely affect human health and cause environmental pollution. Research efforts are currently focused on finding alternative non-chemical, ecologically friendly strategies that can be used to reduce or eliminate *S. zeamais* in storage systems. Alternative treatments, such as modified atmospheres, low temperatures, microbial insecticides, plant extracts, and inert dusts, are currently of interest for the control of stored product insect pests, in agreement with the principles of the Integrated Pest Management (Athanasidou *et al.*, 2007; Riudavets *et al.*,

2014; Campolo *et al.*, 2013) with low toxicity to mammals (Regnault-Roger *et al.*, 2012), rapid degradability and minimal impacts on the germination of plants (Cloyd, 2004).

Natural pesticides made from secondary plant substances can also be used as alternatives for synthetic insecticides. Plants with insecticidal properties are currently one of the most studied alternative methods to control stored product insect pests. This method is widely used throughout the world due to ease of application and the nature of the substrate to be protected. The use of powders is preferred to other plant derivatives (Procópio *et al.*, 2003). A large number of plant substances have physiological and behavioral effects on stored product pests, and are used as alternatives to synthetic insecticides (Rajedran and Sriranjini, 2008). Recent research has turned toward selective biorational pesticides that are safer, cheaper and easier to produce than synthetic insecticides. The application of selected plant products as powders or active isolates, such as essential oils, represents a simple, effective and relatively safe control method that is available to manage the populations of stored product insect pests (Koul *et al.*, 2008; Jindal *et al.*, 2013). Several plant species applied as powders or essential oils showed protective effects against stored grain insects, including toxicity, as well as antifeeding, repellence and growth inhibitory activities (Kéita *et al.*, 2001; Tapondjou *et al.*, 2002; Kim *et al.*, 2010; Nenaah & Ibrahim, 2011; Kim & Lee, 2014; Wanna & Krasaetep, 2019; Wanna & Kwang-Ngoen, 2019).

Wedelia trilobata (L.) A.S. Hitchcock is a member of the Asteraceae (formerly Compositae), the sunflower or daisy family. It is a notoriously invasive weed that occurs in a wide range of tropical and subtropical areas (IUCN, 2001). The major chemical constituents of *W. trilobata* are *ent*-kaurane diterpenes, sesquiterpene lactones and triterpenes. These have a variety of biological activities, such as antibacterial, antitumor, hepatoprotective and central nervous system depressant properties (Wu & Zhang, 2008; Li *et al.*, 2016). This plant species is well known for its natural properties

in Thai traditional medicine, and has been reported to possess several types of biological activities. Numerous anti-insect properties of the plant involve toxic effects against many insects (Yooboon *et al.*, 2019). *W. trilobata* crude extracts have a larvicidal effect on *Spodoptera litura*, *S. exigua* and *Plutella xylostella* larvae after topical application (Junhirun *et al.*, 2018). The insecticidal activity of the essential oils of many plants might be attributed to monoterpenoids (Tong & Coats, 2010). Monoterpenoids has been reported as fumigants and contact toxicants for various insect pests (Rice & Coats, 1994). The major volatile constituents of the *W. trilobata* essential oil obtained from leaves were alpha-pinene, germacrene D, d-limonene (Nirmal *et al.*, 2005) and phellandrene (Khater & Shafeiy, 2015). Here, the toxicity and bioactivity of the powder and essential oil of the climbing wedelia, *W. trilobata*, were evaluated against the adult stage of *S. zeamais* under laboratory conditions.

Materials and Methods

Insect Rearing

Maize weevil, *S. zeamais*, from grain storages located in Kantharawichai district, Maha Sarakham Province, Thailand, was used throughout this study. Fifteen pairs of adults were maintained in a square plastic box with a width of 15 cm and height of 30 cm. The cultures were reared on 1 kg of jasmine rice, *Oryza sativa* L. at 30 ± 5 °C and $70 \pm 5\%$ relative humidity, with 12:12 h light/dark cycle, and they were allowed to mate and oviposit. The maize weevil adults used for the tests were 7 days old.

Collection and Preparation of *W. trilobata* Powder

The aerial parts of *W. trilobata* were collected from local gardens at Mahasarakham University, Thailand. Fresh samples were dried in a hot air oven at 30 °C for 3 days. The dried parts were turned into powder using an electric blender and sieved through mesh size 0.5 mm. The resulting fine powder was maintained in a tightly sealed dry bag until required for use in further bioassays.

Extraction of W. trilobata Essential Oil

Powdered samples of *W. trilobata* were subjected to hydrodistillation using a modified Clevenger-type apparatus to obtain the essential oil. The extraction conditions were 150 g powders: 1,500 ml distilled water with distillation for 6 hours. The essential oil was removed from the remaining water after extraction by centrifugation at 8,000 rpm for 10 minutes. Oil yield (% v/w) was calculated on a dry weight basis. The extracted oil was preserved in a sealed amber glass bottle and refrigerated in the dark at 4 °C until required for use.

Contact Toxicity of the Dry Ground Powder Admixed with Grains

This experiment was conducted according to Nenaah and Ibrahim (2011). The fine powder of *W. trilobata* was mixed with 50 g of jasmine rice grain in 250 mL glass bottles at concentrations of 20, 40, 60 and 80 g/kg grain. Powder/grain admixtures were thoroughly hand-mixed with a rotary shaker for 15 minutes to ensure complete mixing homogenization. The *S. zeamais* adults were transferred in groups of five pairs (7 days old) into 250 mL glass bottles containing treated or untreated (control) grain. The glass bottles were covered with fixed cotton cloths and maintained at the same laboratory conditions described for rearing. Four replicates were performed for each treatment along with the control. The number of deaths of *S. zeamais* adults was counted daily and the mortality percentages were calculated after 3, 6 and 12 days of exposure. Another experiment was conducted to determine the LC₅₀ and LC₉₅ values of the *W. trilobata* powder. Maize weevils were exposed to treated grain in the same manner and mortality counts were recorded 72 hours post-treatment. The mortality data were corrected for control mortality according to Abbott's formula (Abbott, 1925) when mortality in the control ranged between 5% and 20%. Probit analysis was used to analyze the dose-mortality response (Finney, 1971) and LC₅₀ and LC₉₅ values were estimated.

Fumigant Toxicity of W. trilobata Essential Oil

The fumigant toxicity of the essential oil was investigated as previously described by Wanna and Krasaetep (2019). Whatman (no.1) filter paper strips (1.5 ´ 5 cm) were impregnated with 100 µL of 50, 100, 150, 200 and 250 µL/L air dilution of *W. trilobata* essential oil as prepared earlier. After evaporating the solvent for 2 minutes at room temperature, filter paper strips were hung in the glass vials (diameter 2.5 cm ´ height 5 cm) from the center of the screw-capped fumigation bottles (diameter 5.5 cm ´ height 10.5 cm) to avoid contact with the insects. Each fumigation bottle contained 50 g of grain. Five pairs of adult maize weevil (7 days) were transferred into each fumigation bottle for the vapor-phase test. The cap of each bottle was screwed tightly shut and the bottles were kept at 30 ± 5 °C and 70 ± 5% relative humidity and 12:12 h light/dark cycle. In the control sets, insects were fumigated with 100% hexane without any of the tested materials. Adult mortality was observed after 1 and 24 hours of exposure. The insects were considered to be dead if no leg or antennal movements were detected. Each set of treatments was repeated four times and the percentage of adult mortality was calculated using the Abbott formula. Fumigant toxicity was expressed as µL/L air. Another experiment was conducted to determine the LC₅₀ and LC₉₅ values of the *W. trilobata* essential oil. Maize weevils were exposed to the treated fumigation bioassay and mortality counts were recorded 24 hours after treatment. Mortality data were corrected for control mortality according to Abbott's formula (Abbott, 1925) when mortalities in the control ranged between 5% and 20%. Probit analysis was used to analyze the dose-mortality response (Finney, 1971), and LC₅₀ and LC₉₅ values were assessed.

Effect on F₁ Progeny Production of S. zeamais

In previous experiments, after counting parental mortality, the grain was sieved and the remaining living adults were removed. The bottles were kept under the same experimental conditions

until the emergence of F₁ progeny of *S. zeamais*. Based on the life cycle of the untreated control, the counting period of F₁ progeny of *S. zeamais* was established so as to avoid an overlap of generations. The percentage reduction in adult emergence or inhibition rate (% IR) was calculated by the following equation: %IR = (Cn - Tn) ´ 100 / Cn (Taponjdjou et al., 2002), where Cn is the number of newly emerged insects in the untreated (control) grain and Tn is the number of newly emerged insects in the grain treatment.

Results

Contact Toxicity

The effect of the *W. trilobata* powder on the percentage mortality and progeny production of *S. zeamais* was dependent on exposure time and dose. The LC₅₀ and LC₉₅ values of the *W. trilobata* powder admixed with grain on adults of *S. zeamais* were 582.86 and 1,097.14 g/kg grain, respectively (Table 1). The adult mortality of *S. zeamais* on grain admixed with *W. trilobata* powder varied with dosage and exposure time. The *W. trilobata* powder gave low activity to

S. Zeamais, with resistance for all treatments at less than 8% adult mortality. A dosage of 80 g/kg grain killed *S. zeamais* adults at 7.50 ± 0.96% after 12 days of exposure, with results not significantly different (p > 0.05) for all exposure periods (Table 2).

Fumigant Toxicity

The effect of the *W. trilobata* essential oil on percentage mortality and progeny production of *S. zeamais* was also dependent on exposure time and concentration. The LC₅₀ and LC₉₅ values of the *W. trilobata* essential oil on adults of *S. zeamais* by vapor phase test were 1,146.19 and 2,130.88 µL/L air, respectively (Table 3). Analysis of variance demonstrated that the *W. trilobata* essential oil on adult mortality rate of *S. zeamais* gave the highest significant difference (p £ 0.01) on the basis of concentration rate after 24 hours of exposure. *S. zeamais* also showed resistance to *W. trilobata* essential oil, especially at the high dosage rate of 250 µL/L air and 24 hours exposure period, when adult mortality reached 12.50 ± 1.31% (Table 4).

Table 1: Contact toxicity of *Wedelia trilobata* powder against adult *Sitophilus zeamais* at 24 hours

n	Linear regression y = ax + b	LC ₅₀ (g/kg grain)	LC ₉₅ (g/kg grain)	r ² value
200	y = 0.0875x - 1	582.86	1,097.14	0.82

n presents 200 insects of all adult *S. zeamais* tested. LC₅₀ represents the lethal median concentration.

Table 2: Mortality of *Sitophilus zeamais* exposed to *Wedelia trilobata* powder mixed with grains at different concentrations

Dose (g/kg grain)	Mortality (% mean ± SE) after exposure period		
	3 days	6 days	12 days
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
40	2.50 ± 0.50	2.50 ± 0.50	2.50 ± 0.50
60	2.50 ± 0.50	2.50 ± 0.50	2.50 ± 0.50
80	7.50 ± 0.96	7.50 ± 0.96	7.50 ± 0.96
F-test	ns	ns	ns

ns represents non-significant difference (p > 0.05).

Table 3: Fumigant toxicity of *Wedelia trilobata* essential oil against adult *Sitophilus zeamais* at 24 hours

n	Linear regression $y = ax + b$	LC ₅₀ ($\mu\text{L/L air}$)	LC ₉₅ ($\mu\text{L/L air}$)	r ² value
200	$y = 0.0457x - 2.381$	1,146.19	2,130.88	0.76

n presents 200 insects of all adult *S. zeamais* tested. LC₅₀ represents the lethal median concentration.

Table 4: Mortality of *Sitophilus zeamais* exposed to *Wedelia trilobata* essential oil using fumigation bioassay

Concentration ($\mu\text{L/L air}$)	Mortality (% mean \pm SE) after exposure period	
	1 hour	24 hours
Control	0.00 \pm 0.00	0.00 \pm 0.00 c
50	0.00 \pm 0.00	0.00 \pm 0.00 c
100	0.00 \pm 0.00	0.00 \pm 0.00 c
150	0.00 \pm 0.00	2.50 \pm 0.31 bc
200	0.00 \pm 0.00	5.00 \pm 0.62 b
250	0.00 \pm 0.00	12.50 \pm 1.31 a
F-test	N/A	**

N/A represents not applicable. ** represents significant difference at $p \leq 0.01$.

Means within the same column followed by the same letter are not significantly different (LSD: $p > 0.05$).

Effect on F₁ Progeny Production

In all cases, significant differences ($p \leq 0.01$) were observed in the numbers of newly emerged *S. zeamais* adults with the treatment of *W. trilobata* powder and essential oil at different doses, concentrations and exposure times. The F₁ progeny of *S. zeamais* decreased with the increase of dose rates and concentrations of *W. trilobata*. Fewer numbers of newly emerged *S. zeamais* adults were observed for all *W. trilobata* powders and essential oils when compared with

the control (without *W. trilobata* treatment). The *W. trilobata* powder at the highest dose (80 g/kg grain) gave the lowest number of newly emerged adults (31.00 \pm 3.24) and the highest reduction in adult F₁ progeny, reaching 89.96% (Table 5). The highest concentration (250 $\mu\text{L/L air}$) of *W. trilobata* essential oil showed the lowest number of newly emerged *S. zeamais* adults (3.75 \pm 0.44), with 97.65% reduction in adult F₁ progeny (Table 6).

Table 5: F₁ progeny of *Sitophilus zeamais* exposed to *Wedelia trilobata* powder mixed with grains at different concentrations

Dose (g/kg grain)	F ₁ progeny after exposure period	
	New emerged adults (mean \pm SE)	(%) Reduction (IR)
Control	308.75 \pm 17.49 a	-
20	206.25 \pm 12.38 ab	33.20
40	146.75 \pm 9.49 bc	52.47
60	101.75 \pm 7.25 c	67.04
80	31.00 \pm 3.24 d	89.96
F-test	**	-

** represents significant difference at $p \leq 0.01$.

Means within the same column followed by the same letter are not significantly different (LSD: $p > 0.05$).

Table 6: F₁ progeny of *Sitophilus zeamais* exposed to *Wedelia trilobata* essential oil by fumigation bioassay

Concentration ($\mu\text{L/L}$ air)	F ₁ progeny after exposure period	
	New emerged adults (mean \pm SE)	(%) Reduction (IR)
Control	159.50 \pm 5.73 a	-
50	62.50 \pm 2.71 b	60.82
100	58.50 \pm 2.59 b	63.32
150	28.75 \pm 1.89 c	81.97
200	27.00 \pm 1.87 c	83.07
250	3.75 \pm 0.44 d	97.65
F-test	**	-

** represents significant difference at $p \leq 0.01$.

Means within the same column followed by the same letter are not significantly different (LSD: $p > 0.05$).

Discussion

One of the most valued properties of natural plant products is their toxic activity against insects. They can be successfully used to control pests in storage systems to minimize or even replace the application of harmful chemical pesticides. *W. trilobata* applied as powder or essential oil showed residual effect of contact and fumigant activities against *S. zeamais*. The toxicity of the powder and essential oil varied with the dosage of the plant products and time of exposure. In Thailand, plant powders have been mixed with stored grain since ancient times. This method can be used as a natural, safe, and less expensive strategy to protect grain from insect infestations. Most previous experiments focused on testing the activity of plant products over short periods of time. However, for practical utilization of plant materials to protect stored grain products, further information is required on the residual effects of these biorationals over longer time durations against key insect species (Ilboudo *et al.*, 2010). The efficiency of botanicals also needs to be assessed in more realistic conditions to determine their activity at the farm level, as well as at the storage level. Unlike other botanicals, such as essential oils and volatile components that undergo thermal and/or photodegradation, plant powders retain some of their properties over time and, hence, could be used as grain protectants when admixed in stores. Our results revealed significant oviposition deterrence and strong inhibition of *S. zeamais* adult emergence

(F₁ offspring) in jasmine rice grain treated with *W. trilobata* powder at 40-80 g/kg grain and 50-250 $\mu\text{L/L}$ air for *W. trilobata* essential oil. Major constituents of crude oil delayed the metamorphosis of larvae with the production of sterile, moribund and dwarfish adults (Fagoonee and Umrit, 1981). Nowadays, more research has been done to discover alternative effective methods to manage pests without environmental contamination from the use of conventional pesticides. Essential oils are regarded as a new class of ecological natural products for controlling insect pests. The development of safe and scientifically proven herbal products should be given priority in an increasingly regulated world. Concerns for essential oil residues on food crops are alleviated by growing evidence that many essential oil constituents acquired through diets are beneficial for human health (Huang *et al.*, 1994).

Conclusion

The *W. trilobata* essential oil exhibited moderate to strong residual activity against *S. zeamais*. The *W. trilobata* powder and essential oil showed efficacy in the protection against newly emerged progeny (>80%) of *S. zeamais*. Our results suggest that *W. trilobata* can be used effectively for the management of *S. zeamais* in grain storage when applied as powder or essential oil. The use of botanicals should be encouraged in small farm storage

due to their low cost and abundant availability to ameliorate losses incurred in untreated seed. The application of plant products on grain seed during storage is an inexpensive and effective technique, and a technology that can be readily accepted by farmers. Assessments of appropriate formulations for utilization in grain storage should be extended to evaluate mammalian safety and insecticidal mode of action.

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