

COMPARATIVE BIOACTIVITY OF PLANT EXTRACTS AND SYNTHETIC INSECT GROWTH REGULATORS AGAINST *Spodoptera litura* (F.) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Comparative bioactivity of plant extracts and synthetic insect growth regulators against *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Laboratory bioassays were conducted to compare the effects of the leaf extract of *Acalypha indica* L. (Euphorbiaceae) with synthetic insect growth regulators (IGRs) triflumuron and buprofezin against *Spodoptera litura* (F.). The experiment was set up as a randomized complete block design (RCBD). The treatments were two concentrations of *A. indica* extracts 1000 and 2000 ppm, two concentrations of buprofezin 100 and 200 ppm, two concentrations of triflumuron 120 and 240 ppm and control. Each treatment was replicated three times. Second instar larva of *S. litura* were used for the bioassays. Mortality and biological variables of treated and control larvae were recorded daily. The results indicated that the application of *A. indica* extracts and synthetic IGRs (buprofezin & triflumuron) significantly caused the *S. litura* mortality throughout the experimental period. At first, the toxicity of triflumuron on larval *S. litura* was significantly higher compared to those of buprofezin and *Acalypha indica* leaf extract. However, at the end of experimental period all treatments caused high mortality on *S. litura*, and those all were significantly different from control. The treatments also caused abnormal growth in larval, pupal, and adult stages. While in the control, larvae molted into normal adults. The results indicated that the use of biorational control agents such as synthetic insect growth regulators (IGRs) and those based on naturally derived products such as botanical insecticides show promise as a potential tool in *S. litura* management programs.

Key words: abnormal growth, *Acalypha indica*, buprofezin, mortality, *Spodoptera litura*, triflumuron

INTRODUCTION

Spodoptera litura (F.) (Lepidoptera: Noctuidae) is one of the most economically important insect pests in many countries, including in Indonesia, Korea, India, Japan, China, and other countries of Southeast Asia (Yang *et al.*, 2019; Garad *et al.*, 1985; Bae & Park, 1999; Aitkenhead *et al.*, 1974). Rao *et al.* (1993) reported *S. litura* as a polyphagous pest that has about 150 host species. Larvae feed gregariously and may completely devour the leaves and causing considerable economic loss to many vegetables and field crops, such as corn, peanut, soybean, tomato, cabbage, potato, and tobacco (Brown & Dewhurst, 1975; Kalshoven, 1981; Ghumare & Mukherjee, 2003; Ahmad *et al.*, 2013). This insect pest has high reproductive potential and can lay hundreds of eggs in egg batches (Shahout *et al.*, 2011; Chen & Hsiao, 1984). The economic importance of *S. litura* is owing to its high increase rate and wide host

spectrum. In Indonesia, the yield loss in soybean due to *S. litura* attack varies between 23 and 45% (Fattah & Ilyas, 2016). Moreover, Marwato & Suharso (2008) reported that the yield loss in soybean can be as high as 80–100%.

Currently, control of *S. litura* relies mainly on the application of chemical insecticides, including carbamates, pyrethroids and organophosphates (Liburd *et al.*, 2000). It has been known that widespread and continuous use of these chemical insecticides cause environmental problems, negative impact on beneficial insects, and leads to the development of insect resistance (Ahmad *et al.*, 2007; Perry *et al.*, 1998; Ecobichon, 1996). Management of *S. litura* using conventional insecticides has failed mainly because of insecticide resistance (Onstad *et al.*, 2018; Jepson *et al.*, 2014; Farrar *et al.*, 2018). Finding environmentally safe of pest management strategies is a necessity.

The alternative control tactics that show promise for *S. litura* resistant management programs is the use of biorational control agents such as synthetic insect growth regulators (IGRs) and botanical insecticides. IGRs differ widely from the commonly used insecticides, as they exert their insecticidal effects through their influence on development, metamorphosis and reproduction of the target insects by disrupting the normal activity of the endocrine system (Khan, 2016; Dhadialla et al., 1998; Retnakaran et al., 1985; Khatun et al., 2017; Khatter, 2015). These compounds have been tested successfully against several insect species namely, the papaya mealybug *Paracoccus marginatus* (Khan, 2016); the cotton leaf worm *Spodoptera littoralis* (Khatter, 2015; Nasr et al., 2010); the brown planthopper *Nilaparvata lugens* (Kanaoka et al., 1996; Uchida et al., 1985; Izawa et al., 1985); the rice weevil *Sitophilus oryzae* (Das, 2013; Merzendorfer & Zimoch, 2003); the earthworm *Aporrectodea caliginosa* (Badawy et al., 2013); brinjal shoot and fruit borer *Leucinodes orbonalis* (Das & Islam, 2014); the sweet potato whitefly *Bemisia tabaci* (Ishaaya et al., 1998); and the Colorado potato beetle *Leptinotarsa decemlineata* (Karimzadeh et al., 2007).

An example of insect growth regulators that has been studied is chitin synthesis inhibitors (CSIs). This insecticide inhibit chitin synthesis in insects that results in a disruption of the molting process (Retnakaran et al., 1985; Karimzadeh et al., 2007). Moreover, Merzendorfer & Zimoch (2003) found that CSIs prevent the formation of chitin, a carbohydrate that is an important structural component of the insect's exoskeleton that is required before a larval molt. Triflumuron and buprofezin are the example of CSIs. In other case, it has been reported that various natural insecticides (derived from plants) can also work as IGRs. One of the plants species that can act as IGRs is the indian nettle, *Acalypha indica* L. (Euphorbiaceae) (Kamalakaran et al., 2015). Some studies on *A. indica* proved that the content of plant extracts can inhibit the growth and development of insect pest, *Aedes albopictus* mosquito (Ashwini et al., 2017); *Anopheles stephensi* (Govindarajan et al., 2008); *Aedes aegypti* (Kamalakaran et al., 2015; Pratiwi et al., 2015); *Dysdercus cingulatus* (Sahayaraj & Shoba, 2012); *Leucinodes orbonalis* (Manibala & Praveena, 2017). Based on information above, the objective of this study was to compare the effect of two synthetic IGRs triflumuron and buprofezin with plant-derived insect growth regulators (*A. indica* extract) against, *S. litura* under laboratory conditions.

MATERIALS AND METHODS

Research Site. All experiments were conducted in the Laboratory of Arthropod Pests, Faculty of Agriculture, University of Lampung from February to Juni 2018.

Insect Rearing. *S. litura* were initiated from freshly collected larvae from the corn field at the Agricultural Experimental Field of University of Lampung. The insect colonies were confined in transparent plastic cage (14 cm in diameter; 30 cm in height) with its top and sides cut off and covered with fine mesh nylon net for air ventilation. Larvae were provided daily with fresh castor bean leaves (*Jatropha curcas*). The emerged adults were provided with 10% honey solution on a cotton wick as a food source. Moths were allowed to lay eggs on the cage, then the egg culsters were collected daily, and transferred into Petri dishes until hatching. All rearing cages were maintained under laboratory conditions of 27 ± 2 °C and RH 70 ± 5 %.

Preparation of Plant Extract. The *A. indica* leaves were collected from the area around Bandar Lampung, washed with tap water and rinsed with distilled water to remove the sand particles. The leaves were air dried for a week at room temperature (27 °C). The dried leaves were grounded into fine powder using a blender machine. An amount of 100 g powder of the leaf material was mixed with 1000 mL ethanol on 2L Erlenmeyer flasks to make suspension. Plant suspension was continuously shaken with magnetic stirrer at 180 rpm/min for 24h at room temperature. The suspensions were filtered through Whatman No. 1 paper via a Buchner funnel. The residue was reextracted again twice under the same procedure as before. All the crude plant extracts were evaporated to dryness using a Rotary Evaporator at 50 mm Hg pressure and 50 °C to get thick and viscous materials and stored in air tight bottles. Suspensions of two concentrations of plant extracts, 1000 and 2000 ppm were prepared by dissolving 0.1 g and 0.2 of the evaporated leave extracts in 1000 mL distilled water. To this mixture, 0.1 mL of Tween 80 (0.05%) was added as an emulsifier.

Preparation of Chemicals (IGRs). The IGRs tested were Lugen 100 EC (10% buprofezin) from PT Nufarm, Indonesia and Destello 480 SC (12% triflumuron) from PT Bayer, Indonesia. Two concentrations, 100 and 200 ppm of buprofezin (as the recommendation of doses range of each product) were prepared by dissolving 1 mL and 2 mL of Lugen 100 EC in 1000 mL distilled

water. While, two concentrations 120 and 240 ppm of triflumuron were provided by dissolving 1 mL and 2 mL of Destello 480 SC in 1000 mL distilled water.

Toxicity and Growth-Inhibitory Bioassay. A total of 7 treatments was made by preparing: two concentrations of *A. indica* extracts 1000 and 2000 ppm, two concentrations of buprofezin 100 and 200 ppm, two concentrations of triflumuron 120 and 240 ppm, and control (C). Control larvae were treated with distilled water only. Three replicates (each of 10 larvae per concentration) were used. All treatments were arranged in randomized complete block design (RCBD). Second instar larvae of *S. litura* were placed in sterile plastic Petri dishes, 10 individuals in each dish. All individual insects were treated with each suspension and distilled water by using modified sprayer. The treated larvae were feed with fresh castor bean leaves and maintained until pupation. Mortality and biological variables of treated and control larvae were recorded daily. These biological aspects include; number of abnormal larvae, number of normal and abnormal pupa, and number of normal and abnormal adults.

Data Analysis. Data were subjected to analysis of variance (ANOVA) and means were separated by Tukey's HSD (honestly significant difference) at $\alpha = 0.05$. Values were represented as mean \pm SE. Features of impaired development were recorded in pictures.

RESULTS AND DISCUSSION

The results of one way ANOVA showed that the application of synthetic IGRs (buprofezin and triflumuron) and extract of *A. indica* significantly affected the mortality of *S. litura* at 1, 4, 6, and 8 days after treatment (Table 1). Moreover, it was reveal that the mortality of *S. litura* was gradually increased in each of consecutive observations. The larvae were considered dead if they did not move when slightly touched with a fine camel brush.

After 1 d exposure, mortality began to occur for triflumuron 120 ppm ($43.3 \pm 3.3\%$) and 240 ppm ($73.3 \pm 3.3\%$) treatments. However, no mortality was observed at the treatment of buprofezin 100 ppm and 200 ppm and *A. indica* extract at 1000 ppm. According to Das (2013), buprofezin had no direct effect on the mortality of rice weevils *Sitophilus oryzae* L. (Coleoptera: Curculionidae) regardless the concentrations. In addition, Khatun *et al.* (2017) and Merzendorfer & Zimoch (2003) stated that buprofezin

was not directly toxic to insects but potentially reduce pest populations by disrupting normal growth and development through disruption of chitin synthesis. Moreover, multiple comparison means by Tukey test revealed that the mortality of *S. litura* treated with triflumuron 240 ppm ($73.3 \pm 3.3\%$) was significantly higher than that of with *A. indica* extract 2000 ppm ($16.7 \pm 3.3\%$).

At 4th day after, treatment maximum mortality (100%) was found to occur in the larvae treated with triflumuron 240 ppm followed by those treated with triflumuron 120 ppm ($73.3 \pm 3.3\%$), and *A. indica* extract at 2000 ppm ($50.0 \pm 5.8\%$). These results agree with findings of Khatter (2015) who noted that triflumuron exhibited the highest toxic action against the second instar of *S. littoralis* larvae. Moreover, at 8th day of observation, we found that all treatments caused an equally high mortality of on *S. litura* and those were significantly different with the control.

In addition to mortality, we also evaluated the effects of synthetic IGRs (buprofezin, triflumuron) and *A. indica* extracts on growth and development of *S. litura*. Several morphological abnormalities were observed including deformation of larva, pupa, and adult of *S. litura*. The results clearly indicate that the larval deaths of *S. litura* (Table 1) were closely related with the molting abnormalities of larva (Table 2). Moreover, our data showed that application of *A. indica* extract and synthetic IGR insecticides (buprofezin and triflumuron), significantly cause abnormal growth of *S. litura* larvae (Table 2). Most of the larvae treated with these compounds showed symptoms of molting failure before larval death. Several morphological abnormalities were observed in larval development (Figure 1). Our result indicated that treated larvae with triflumuron became pale and discolored in the posterior end of the abdomen indicated that this insecticide inhibits chitin biosynthesis and cuticle formation (Figure 1A). The new cuticle was weakened, leads to an unsuccessful molt and eventual death. Similar results were observed by Khatter (2015) who found that the second larval instar of *S. littoralis* (Lepidoptera: Noctuidae) treated with triflumuron showed incomplete chitin synthesis. The similar symptom was also observed on *Manduca sexta* treated with chitin synthesis inhibitors (Abdel-Monem *et al.*, 1980). In addition, our result also indicated that the application of buprofezin on *S. litura* larvae caused failure in shedding off the larval exuviae (Figure 1B). In this case, the newly molted *S. litura* larvae died while shedding the old cuticle and head capsule. Similar findings were also reported by Izawa *et al.* (1985) who found that the death of *Nilaparvata lugens* nymphs

treated with buprofezin was attributed to the chitin synthesis inhibitors act during metamorphosis which causes abortive molting. Badawy *et al.* (2013) noted that a significant growth inhibition on the earthworm, *Aporrectodea caliginosa*, was also seen when exposed to buprofezin and triflumuron. Buprofezin and triflumuron are chitin synthesis inhibitors, act by

interfering with chitin synthesis. Therefore, most of the larvae treated with these compounds showed symptoms at molting. In other words, treated larvae fail to complete the molting process due to inhibition of the synthesis of new cuticle, specifically, chitin biosynthesis. The dead larvae showed shrunken body compared to the untreated larvae (Figure 1C). The results showed that the action

Table 1. Larval mortality (mean ± SE) of *S. litura* treated synthetic IGRs (buprofezin & triflumuron) and *A. indica* extract

Treatment	Mortality (%) at different days after treatment			
	1	4	6	8
Triflumuron 120 ppm	43.3 ± 3.3b	73.3 ± 3.3a	83.3 ± 0.0a	93.3 ± 3.3a
Triflumuron 240 ppm	73.3 ± 3.3a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
Buprofezin 100 ppm	0.0 ± 0.0b	20.0 ± 5.8c	63.3 ± 5.8b	80.0 ± 5.8a
Buprofezin 200 ppm	0.0 ± 0.0b	3.3 ± 2.1c	76.7 ± 8.8a	93.3 ± 3.3a
<i>A.indica</i> extracts 1000 ppm	0.0 ± 0.0b	3.3 ± 2.1c	56.7 ± 8.8b	83.3 ± 16.7a
<i>A.indica</i> extracts 2000 ppm	16.7 ± 3.3b	50.0 ± 5.8b	76.7 ± 3.3a	90.0 ± 3.3a
Control	0.0 ± 0.0b	3.3 ± 5.38c	3.3 ± 3.3c	3.3 ± 3.3b
F Value	174.78	115.20	40.33	21.58
Pr > F	0.000	0.000	0.000	0.000

Means followed by the same letter within the same columns are not significantly different (Tukey’s HSD test; α= 0.05).

Table 2. Effects of synthetic IGRs (buprofezin & triflumuron) and *A. indica* extracts on the development* aspects of the *S.litura*

Treatment	Larval stage (%)		Pupal stage (%)		Adult stage (%)	
	Death larvae	Malformed larvae	Pupation	Malformed pupae	Adults emergence	Malformed adults
Triflumuron 120 ppm	93.3 ± 3.3a**	93.3 ± 3.3a**	6.7 ± 2.8b**	6.7 ± 2.8	0.0 ± 0.0b**	0.0 ± 0.0
Triflumuron 240 ppm	100.0 ± 0.0a	100.0 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0	0.0 ± 0.0b	0.0 ± 0.0
Buprofezin 100 ppm	80.0 ± 5.8a	80.0 ± 5.8a	20.0 ± 0.0b	10.0 ± 3.3	10.0 ± 3.3b	10.0 ± 3.3
Buprofezin 200 ppm	93.3 ± 3.3a	93.3 ± 3.3a	6.7 ± 2.8b	3.3 ± 2.8	3.3 ± 2.8b	3.3 ± 2.8
<i>A.indica</i> extracts 1000 ppm	83.3 ± 16.7a	83.3 ± 16.7a	16.7 ± 5.8b	10.0 ± 3.3	6.7 ± 2.8b	6.7 ± 2.8
<i>A.indica</i> extracts 2000 ppm	90.0 ± 3.3a	90.0 ± 3.3a	10.0 ± 2.3b	6.7 ± 2.8	3.3 ± 2.8b	3.3 ± 2.8
Control	3.3 ± 3.3b	3.3 ± 3.3b	96.7 ± 5.8a	0.0 ± 0.0	96.7 ± 5.8a	0.0 ± 0.0
F Value	21.58	23.16	19.22	1.64	13.43	1.13
Pr > F	0.000	0.000	0.000	0.208	0.000	0.395

* insect development was calculated from larvae till adult emergence; ** Means followed by the same letter within the same columns are not significantly different (Tukey’s HSD test; α = 0.05).

of this botanical insecticide caused larval-pupal intermediate appearance with anterior half (head and thorax) as pupa whereas abdomen as larva forms (Figure 1C). Similar findings were also reported by others. Manibala & Praveena (2017) found that the *A. indica* extract act as an antifeedant and insect growth regulator against fruit borer, *Leucinodes orbonalis* (Lepidoptera: Pyralidae). This abnormal growth may be related to the fact that extract of *A. indica* may act as a growth regulator inhibitor on *S. litura*. Moreover, Sharma & Gupta (2009) found that *A. indica* extract had antifeedant effect on the cabbage white butterfly, *Pieris brassicae* (L) (Lepidoptera: Pieridae). Nazri *et al.* (2016) studied the phytochemical constituents of *A. indica* and reported the presence of various secondary metabolites, namely tannins, phenolics, flavonoids, terpenoids, and saponins. Relatively similar results were found by Mohan *et al.* (2012), phytochemical screening of *A. indica* leaves extract revealed the presence of alkaloids, tannins, steroids, saponins, flavanoids, glycosides and phenolic compounds. Other study by Sahayaraj & Shoba (2012) showed that *A. indica* extracts offer a wide array of bioactive compounds which are sufficiently toxic and alters the growth and development of *Dysdercus cingulatus*.

Our results showed that all treated *S. litura* with triflumuron died at larval stage. Therefore, the abnormalities in pupation were only observed on *S. litura* treated with buprofezin and *A. indica* leaves extract (Table 2; Figure 2A & B). Malformed pupae were not able to complete pupation, while in the control, all *S. litura* succeeded to form a normal pupa (Figure 2C). In buprofezin case, treated *S. litura* showed larval-pupal intermediate, consisting of larval head and thorax, whereas abdomen of some pupae were rudimentary in appearance with larval prolegs still present (Figure 2A). On the other hand, treatment of *A. indica* leaves extract caused abnormal green unsclerotized pupal integument with larval exuviae attached to dorsal region of anterior part of the body (Figure 2B). Similar results were reported by Das & Islam (2014) whose works showed that in fruitborer (*L. orbonalis* Guen.) (Lepidoptera: Pyralidae) treated with buprofezin caused developmental disruption.

Xavier *et al.* (2016) observed larval-pupal intermediate with improperly hardened pupal case and retained larval appendages in *S. litura* treated with the ferns *Cyclosorous interruptus*, *Christella dentata* and *Nephrolepis cordifolia*. Similarly, Ashwini *et al.* (2017) reported the pupal emergence inhibition activity of *A. indica* leaf extract against dengue vector,



Figure 1. Various forms of larval abnormalities of *S. litura* growth due to larval feeding of leaf treated. (A) Triflumuron; (B) Buprofezin; (C) *A. indica* extracts; (D) Normal untreated larvae.



Figure 2. Abnormal of pupae of *S. litura* developed from exposed larvae fed on treated leaf. (A) Buprofezin; (B) *A. indica* leaf extracts; (C) Normal pupae developed from untreated larvae.



Figure 3. Adult *S. litura* emerged from pupae. (A) Normal adult which the larvae were fed with untreated (control) leaf; (B) Crumbled, deformed one emerged from pupae developed from larvae that were fed with leaf treated with extract of *Acalypha indica* leaf.

Aedes albopictus mosquito. Moreover, their study showed that the detrimental effect of *A. indica* was possibly because of alkaloids, flavanoids, tannin, steroids, terpenoids and phenolic compounds. Similar findings also reported by Sahayaraj & Shoba (2012) who found *A. indica* extracts offer a wide array of bioactive compounds that disrupts normal insect development.

Our results indicated that *S. litura* survived to adult stage on those treated with *A. indica* leaves extract (Table 2; Figure 3). However, they showed pupal-adult intermediates with crumbled and deformed wings (Figure 3B), while all *S. litura* pupae of control molted into normal adults (Figure 3A). The results have demonstrated that the action of these botanical insecticides cause physiological disturbances leading to growth abnormalities like deformed adults. According to Ahmed *et al.* (1981), insecticidal potentials and biological activity *A. indica* extract caused deformation of emerged adults in *Musca domestica*. Such kind of detrimental effect has also been observed by Gaur & Kumar (2019) in *S. litura* treated with medicinal plant, Ashwagandha (*Withania somnifera*).

CONCLUSION

Our results indicate that the mortality of *S. litura* showed significant difference between control and *A. indica* leaf extract and synthetic insect growth regulators triflumuron & buprofezin at 1, 4, 8 d after treatments. The highest mortality (100%) was found on

larval *S. litura* treated with triflumuron at 4th days after treatment and those mortality was significantly higher compared those with buprofezin and *A. indica* leaf extract. However, at the end of experimental period, all treatments caused high mortality of *S. litura* and those was significantly different compared with control. The application of all compound treatments caused not only mortality in larval stage, but also caused defects in pupal and adult stages, and in some cases produced larval-pupal and pupal-adult intermediates.

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