

Efficacy of the Extract from Pongam Leaves (*Pongamia pinnata* L.) Against *Spodoptera exigua* (Hübner) and *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)

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The beet armyworm *Spodoptera exigua* (Hübner) and the tobacco armyworm *S. litura* Fabricius (Lepidoptera: Noctuidae) are economically important insect pests of a variety of crops primarily in tropical and subtropical regions. Conventional control of these pests usually depends on synthetic chemical insecticides. Botanical pesticides have recently been paid considerable attention as an environmentally friendly measure to manage various insect pests. The present study was carried out to determine the efficacy of a leaf extract from pongam tree *Pongamia pinnata* L. against the two armyworms. The results showed that the acute lethal toxicity of pongam leaf extract was high against the two armyworm species; the LC₅₀ values were 1.94%, 1.52% and 1.10% at 24, 48 and 72 hours, respectively for *S. litura* whereas the values for *S. exigua* larvae were 3.18%, 2.57% and 1.89% at 24, 48 and 72 hours, respectively. Our study also indicated that low concentrations of pongam leaf extract caused significant reductions of vitality of the armyworms; they took more time to mature during the larval stage. Taken together, the treatment with a pongam leaf extract can negatively affect the armyworm populations directly and indirectly, and cause a reduction of overall pest numbers in the next generation. Thus, the pongam leaf extract is a recommendable bio-pesticide to control the armyworms.

Key words: herbal pesticide, sub-lethal toxicity, integrated pest management, chemical control

INTRODUCTION

The beet armyworm *Spodoptera exigua* (Hübner) and the tobacco armyworm *S. litura* Fabricius (Lepidoptera: Noctuidae) are economically important polyphagous insect pests of various crops, in particular, in tropical and subtropical regions (Brown and Dewhurst, 1975). Conventional control of these pests is dependent on synthetic chemical insecticides. However, excessive reliance on insecticides can cause environmental pollution and development of pesticide resistance while it should have strong negative impacts on non-target organisms including natural enemies that are beneficial to crop production (van Driesche and Bellows, 1996; Tran *et al.*, 2004; Tran and Ueno, 2012). In Vietnam, the two armyworms are also serious pests of various crops but armyworm control with synthetic insecticides does not always work well (Ueno, 2006; 2015). Thus, alternative measures that are costly reasonable are on demand in the country.

Plants commonly produce chemical compounds to protect themselves from herbivores like insects. There are considerable evidence that materials or extracts from several plant species are useful as botanical or organic pesticides (for review; Prakash and Rao, 1996). Botanical pesticides are potentially an alternative to conventional

synthetic pesticides, because it is usually believed that the natural products would have lesser negative impacts on environments and human health (Isman *et al.*, 2011) while botanical pesticides are reasonably costly and may be effective to control target pests; in fact, a number of medicinal plants have been used as pest control materials (Lale, 1992; Isman, 1995; Pavela, 2009; Roy *et al.*, 2010; Erler *et al.*, 2010).

Pongam *Pongamia pinnata* L. is a forest tree that belongs to the family Leguminosae and has widely been used for biodiesel production (Krishnamurthi, 1969; Meera *et al.*, 2003). It is widely distributed throughout tropical Asia including South East Asia and India as far as the Seychelles islands and Australia (Arote and Yeole, 2010). Pongam has long been used as crude drugs to treat wounds, painful rheumatic joints, skin disease, abscesses, tumor, ulcers, diarrhea, and so on (Shoba and Thomas, 2001; Meera *et al.*, 2003). In addition, pongam has widely been tested for their insecticidal, nematocidal, antifungal, antibacterial and antiviral activities (Simin *et al.*, 2002; Kerasi *et al.*, 2010).

More recently, pongam has been recognized as a botanical pesticide; several studies have confirmed the pesticidal properties against insect pests (Samuel *et al.*, 2009; Yankanchi and Lendi, 2009; Mamun *et al.*, 2009; Lale and Kulkarni, 2010; Verma *et al.*, 2011). For example, toxicity of pongam is shown for the human head louse *Pediculus humanus capitis* (Samuel *et al.*, 2009), the bean beetle *Callosobruchus chinensis* (Yankanchi and Lendi, 2009), the termite *Odototermes obesus* (Verma *et al.*, 2011), and the red flour beetle *Tribolium castaneum* (Mamun *et al.*, 2009). Also, a deterrence or repellent effect has been demonstrated against mosqui-

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toes (Lale and Kulkarni, 2010).

The previous studies, however, have focused on toxic activity of materials prepared from pongam seeds, stems and roots whereas only a few reports have been addressed pesticidal effects of the extract from pongam leaves (Tran *et al.*, 2016). However, pongam leaves are an abundantly available raw material, and, if the leaves are usable for pest control, manufacturing botanical pesticides in large quantities is possible by using pongam leaves. We have previously demonstrated that pongam leaf extracts are usable to control the turnip aphid (Tran *et al.*, 2016). Accordingly, in the present study, we focus on a methanol extract from pongam leaves and examine its potential to control the beet armyworm and the tobacco armyworm. We will show the efficacy of armyworm control with a pongam leaf extract and discuss the usefulness of pongam as a botanical pesticide.

MATERIALS AND METHODS

Insect rearing

Seeds of pak choi *Brassica chinensis* were sown in a tray (20 cm × 60 cm × 15 cm) in a mixture soil (40% water content, pH=5.5–6.5, 0.035%N, 0.123%P₂O₅, 0.018%K₂O). Two weeks after germination, each seedling plant was transplanted to a plastic pot (9 cm in diameter). Leaves from grown plants were used for feeding the armyworms.

Larvae of *S. exigua* and *S. litura* were collected from severely infested plants grown at the experimental farm of Hue University, Vietnam. Both insects were reared individually in plastic boxes (10 cm×5 cm×5 cm) and reared on pak choi leaves. The boxes were maintained in an environmental chamber at a constant temperature of 25°C under a photoperiod of 16L: 8D until the larvae pupated. Sterilized soil was provided for pupation. The pupae were collected from the soil and were placed in a shelf (200 cm × 60 cm × 50 cm) covered with a fine nylon mesh. The self was maintained at the same condition mentioned above and was used for rearing the adult moths for oviposition. Potted pak choi plants were placed inside the shelf to obtain eggs of the moths. Plants were removed from the shelf to collect newly hatching larvae, and larvae were reared on pak choi leaves.

Preparation of plant extracts

We collected the leaves of *P. pinnata* from Phu Vang District, Thua Thien Hue Province, Vietnam. The pongam leaves were washed under tap water to remove dirt and debris. The leaves were kept in shade for air-drying and then were dried in the oven at 60°C to gain constant weight. The leaves were then powdered with an electric grinder. The powdered leaves were packed in Soxhlet apparatus. Upon use, the pongam leaf powder was used to extract with methanol. The extract was concentrated in a vacuum evaporator. The extract was then stored at 4°C in amber colored airtight bottles. Different concentrations of the plant extract were prepared by dissolving the stock solution in Acetone 300, 99.5+% (GC) before

the bioassays mentioned below.

Bioassays

Bioassays were done as follows. The diluted extract was evenly applied on pak choi leaves (approximately 5 ml per potted plant) with a power-pack aerosol hand sprayer (Hand Spray Nozzle, Takeda Engei Co., Japan). No surfactants were added to the extract. Two hours after spraying, 5 freshly hatched first instars (6 hours old) of *S. litura* or *S. exigua* each were gently transferred on a potted plant (10–15 cm in height with 2–3 leaves). Then, the potted plant was put in a plastic cage (45 cm × 30 cm × 25 cm) covered with a fine nylon mesh. As a control, distilled Acetone was applied to pak choi plants, and then test armyworms were placed on them in the same way as mentioned above. The cages were kept at 25°C, 60–70% humidity under a 16L: 8D light period.

First, the approximate LC₅₀ was determined using the two armyworm species. The stock solution was diluted with Acetone 300, 99.5+% (GC) to prepare a 5% solution. The concentrations of test solutions were adjusted by adding acetone to the 5% solution. The doses tested were from 0.0 to 3% for both *S. exigua* and *S. litura*. The mortality was determined at 24 h after spraying. Test with a given concentration was conducted with one potted plant with 15 first instars. For each concentration, tests were replicated three times; thus, 45 armyworms were in all used for each concentration.

Second, sublethal effects on armyworms were examined. For this purpose, we examined the development (measured as time in days) of the armyworms treated with low concentrations of the extract. A serial time-dose response bioassay was used to determine response of the insects to different doses lower than LC₅₀ values. The ranges of doses were prepared by diluting the extract with acetone; doses equivalent to 0.5, 1.0 and 1.5% were tested. Mortality was determined for each larval instar. Alive insects were maintained under the above conditions, and were monitored daily until all insects had pupated.

Data analyses

Statistical treatments were carried out with the aid of JMP 11.2 (SAS, 2014). Probit analysis was used to determine LC₅₀ and LC₉₅ values. The development time was analyzed with one-way ANOVA, and the means were separated by Fisher's PLSD tests.

THE RESULTS

Results of the probit analysis of dose-response data (LC₅₀, slopes and intercepts of the dosage-mortality lines) for *S. exigua* and *S. litura* are summarized in Table 1. The pongam leaf extract was toxic to *S. litura* with the LC₅₀ found to be 1.934%, 1.517% and 1.097% at 24, 48 and 72 hours, respectively. The LC₉₅ was 4.431%, 3.465% and 2.473% at 24, 48 and 72 hours, respectively. Against *S. exigua*, the LC₅₀ of the pongam leaf extract

Table 1. Median and 95% lethal concentrations of pongam leaf extract to *Spodoptera litura* and *S. exigua*

Time after treatment (hours)	LC ₅₀ (%)	95% fiducial limits of LC ₅₀	LC ₉₅ (%)	Regression equation (Y=a+bx)	x ² (df, P)	R ²
<i>Spodoptera litura</i>						
24	1.943	1.714 – 2.207	4.431	Y = - 1.284+0.661 X	20.120 (19, 0.387)	0.837
48	1.517	1.327 – 1.705	3.465	Y = -1.281+0.844 X	21.453 (19, 0.312)	0.884
72	1.097	0.940 – 1.244	2.473	Y = -1.312+1.195 X	24.485 (19, 0.178)	0.893
<i>Spodoptera exigua</i>						
24	3.175	2.688 – 4.101	6.778	Y = - 1.450+0.457 X	12.153 (19, 0.879)	0.728
48	2.574	2.172 – 3.261	6.487	Y = - 1.082+0.420 X	14.513 (19, 0.753)	0.716
72	1.891	1.563 – 2.305	5.639	Y = - 0.830+0.439 X	12.504 (19, 0.863)	0.755

Table 2. Effects of different doses of pongam leaf extract lower than LC₅₀ on developmental time in days of *Spodoptera litura* and *S. exigua*

Concentration (%)	<i>Spodoptera litura</i>		<i>Spodoptera exigua</i>	
	Larva	Pupa	Larva	Pupa
0.0	22.4±0.36a	7.8±0.14a	13.8±0.07a	7.9±0.19a
0.5	24.0±0.52ab	8.0±0.30a	14.9±0.05b	8.4±0.31a
1.0	24.0±1.18ab	8.7±0.33a	15.3±0.13b	8.1±0.46a
1.5	26.3±1.93b	8.3±0.33a	16.0±0.21c	8.3±0.49a
df	67	59	115	68
F-value	4.64	2.09	79.61	0.69
P-value	0.005	0.112	<0.0001	0.601

Means with the same letters within a column are not significantly different by Fisher's PLSD after one-way ANOVA ($\alpha = 0.05$). Data are shown as mean±SE.

was found to be 3.175%, 2.574% and 1.891% at 24, 48 and 72 hours, respectively. The LC₉₅ was 6.778%, 6.487% and 5.639% at 24, 48 and 72 hours, respectively.

When the first instars of *S. litura* were exposed to extracts with different concentrations lower than LC₅₀, the mean total larval developmental times were 24.0, 24.0 and 26.3 days at concentration of 0.5, 1.0 and 1.5%, respectively, and the values were larger than that of control (acetone) (22.4 days) ($F=4.637$; $df=67$; $P<0.01$). There was a clear trend that the larval developmental time increased with increasing doses (Table 2). There was no significant difference among the mean developmental times of the pupae ($P>0.05$) (Table 2).

Development time of *S. exigua* exposed to extracts lower than LC₅₀ is summarized in Table 2. The total larval development time increased significantly as the extract concentration increased ($F=79.607$; $df=115$; $P<0.0001$). The mean developmental times in day of total larval stages were 14.9, 15.3 and 16.0 at concentration of 0.5, 1.0 and 1.5%, respectively, the values of which were larger than that of control (13.8 days). There was no significant difference in the mean developmental times of the pupae ($P>0.05$), suggesting that the extract did not affect pupal development.

DISCUSSION

The present experiments have shown that the extract of pongam leaves has sufficient larvicidal activity against the beet armyworm and the tobacco armyworm. Insecticidal properties of pongam leaf extracts can be attributed to karanjin (3-methoxy furano-2,3,7,8-flavone) and pongapin (2-(1,3-Benzodioxol-5-yl)-3-methoxy-4H-furo[2,3-h]-1-benzopyran-4-one), and these are the major flavonoids in pongam (Asolkar *et al.*, 1992; Katekhaye *et al.*, 2012). Each of karanjin and pongapin has been shown to possess pesticidal properties (Kumar *et al.*, 2006, Verma *et al.*, 2011). Because pongam contains both flavonoids, there may be a significant synergistic effect that enhances the toxicity of pongam (Poonia and Kaushik, 2013). In fact, the present study showed that the two armyworms were highly susceptible to pongam leaf extract, and its LC₅₀ values were low (Table 1). Thus, the acute lethal toxicity of pongam leaf extract is sufficiently high usable for the control of the two armyworms.

The leaf extract of pongam tree was also reported to be effective against some insect pests (Sridhar and Chetty, 1989; Kulat *et al.*, 1997; Samuel *et al.*, 2009;

Tran *et al.*, 2016). Samuel *et al.* (2009) demonstrated that a methanol extract of pongam leaves showed excellent anti-lice activity with values ranging between 32.6 and 82.9%. Similarly, high toxicity of pongam leaf extract has also been reported for the second instars of *S. litura* (LC₅₀ 72h: 5.44%), the larvae of *Trogoderma granarium* (LC₅₀ 72h: 19.9 μ /insect) and the adult of *T. granarium* (LC₅₀ 72h: 65.9 μ /insect) (Kumar *et al.*, 2006).

The extracts of pongam bark, leaf and seed oil have antifeedant and/or repellent effects on insect pests. For example, Kumar *et al.* (2006) showed an antifeedant effect of the extracts on *S. litura* and repellent effects on stored product pests *Trogoderma granarium* and *Tribolium castaneum*. Likewise, a repellent effect was confirmed for blood sucking mosquitos (Lale and Kulkarni, 2010). Although we did not examine the presence of antifeedant effect on the two armyworms, our study did provide evidence that low concentrations of a pongam leaf extract caused a significant increase in larval developmental time of the two armyworms (Table 2). The longer developmental time detected in our study may result from an antifeedant function of the pongam leaf extract. Alternatively, physiologically sublethal effects may be present on the extract, which can result in slowed growth rate of the armyworms.

Sub-lethal effects are often chronic and are expressed as some change in the insect's life history attributes. Consequently, such effects may have a long-term impact on the reproduction over generations or on the population level. Sub-lethal residues can negatively affect insects that survive pesticide applications, e.g., those that emerge as adult from protected situations, or those that disperse into areas where residues exist. Biological parameters to detect such sublethal effects include daily fecundity, total progeny production, longevity, developmental time, egg viability, consumption rates and insect behavior (Ruberson *et al.*, 1998).

In addition, the indirect negative effects of plant extracts on insect pests expressed as deterrents and repellents may have potentially important behavioral consequences that further reduce pest severity. More than six decades ago when the use of chemical pesticides was not common, farmers used different part of pongam to protect paddy crop in India (Lale and Kulkarni, 2010). The presence of pongam tree near paddy field helped in repelling harmful insects. Also, dried leaves of *P. pinnata* are used as an insect repellent in stored grains. Recently, the repellent effect of pongam on mosquito was also reported (Lale and Kulkarni, 2010).

Available literatures have suggested that natural extracts from plants should be noticed for its safety for the environment and human health while the emergence of resistance is minimal due to its different mode of action (Isman, 1995; Breuer *et al.*, 2003). Moreover, some extracts (containing similar substances to those present also in *P. pinnata* leaves) may attract natural enemies and thus increase natural parasitization levels (Charleston *et al.*, 2006). Botanical insecticides based on pongam

leaves thus seem to possess the potential for being used together with biological control agents (Tabone *et al.*, 2010). Therefore, a trial is recommended to test pongam leaf extracts for the armyworm management.

Although the toxicity of *P. pinnata* to the beet armyworm and the tobacco armyworm has been confirmed under a laboratory condition in the present study, the efficacy as a crop protectant should also be assessed under the field condition. The recent field trials of the other botanical insecticides to control pest insects and mites have demonstrated that botanical insecticides can be as effective as conventional insecticides and are compatible with conventional products (Dadang *et al.*, 2009; Erler *et al.*, 2010). These studies suggest that the botanical pesticides are usable as a stand-alone product in certain pest/crop contexts, but may be even more effective as a tank mix with conventional insecticides (Isman *et al.*, 2011). Also, it is not examined whether the effects of such botanical insecticides on target pests are due to the contact toxicity, or residual toxicity or deterrence (i.e., sublethal behavioral effects). Further laboratory, greenhouse and field experiments with *P. pinnata* are needed to address this question.

AUTHOR CONTRIBUTIONS

D. H. Tran designed and conducted the laboratory experiments, analyzed the data, and prepared the first draft of the manuscript. M. Takagi contributed in molding the research concept. T. Ueno discussed the results and polished up the manuscript.

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