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Control Efficacy of Pongam (*Pongamia pinnata* L.) Leaf Extract against the Turnip Aphid *Lipaphis pseudobrassicae* (Davis) (Hemiptera: Aphididae)

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The turnip aphid *Lipaphis pseudobrassicae* (Davis) is considered a cosmopolitan pest of cruciferous crops. It removes photoassimilates from the crops and is responsible for transmitting a number of plans viruses. Control of the turnip aphid is important in stable production of cruciferous crops. The present study was carried out to determine the efficacy of leaf extract from the pongam tree *Pongamia pinnata* L. against the turnip aphid. In laboratory tests, pongam leaf extract showed acute toxicity to the turnip aphid; the LC_{50} values were 0.585%, 0.151% and 0.113% at 24, 48 and 72 hours, respectively. Laboratory observations also indicated that low concentrations of pongam leaf extract caused significant reduction of vitality and fertility of the turnip aphids of the subsequent generation and thus caused an indirect reduction of overall pest numbers in the next generation. Pongam leaf extract has thus nonlethal chronic toxicity. Therefore, pongam leaf extract can be recommended as an organic–based pesticide to manage the turnip aphid.

Key words: Botanical extracts, cabbage, IPM, *Millettia*, organic pesticides, pest control

INTRODUCTION

The use of plants, plant materials or crude plant extracts as botanical pesticides have long been touted as an alternative to conventional synthetic pesticides, presumably because the natural products would have lesser environmental and human health impacts than conventional synthetic pesticides, which have adverse effects on non–target organisms and ecosystems (Isman *et al*., 2011). Many medicinal plants have been used as pest control tools (Lale, 1992; Isman, 1995; Pavela, 2009; Roy *et al*., 2010; Erler *et al*., 2010). Farmers and researchers often claim the successful use of plant materials in pest control including ash (Ajayi *et al*., 1987), oil (Ahmed *et al*., 1999, Isman *et al*., 2011), extracts (Devanand and Rani, 2008; Mamun *et al*., 2009), and botanical powders (Shukla *et al*., 2007; Gupta and Srivastav, 2008).

Pongam, *Pongamia pinnata* L., is a forest tree belonging to the family Leguminosae and is commonly used for biodiesel production (Krishnamurthi, 1969; Merra *et al*., 2003). It is widely distributed throughout tropical Asia including South East Asia and India as far as Australia and the Seychelles Islands (Arote and Yeole, 2010). Pongam has been used in those areas for agricultural and environmental management because it can be cultivated on any type of soil with low moisture demand and because it is a suitable plant species for controlling soil erosion and binding sand dunes due to its dense network of lateral root (Meera *et al*., 2003; Verma *et al*., 2011).

Also, the various parts of *P. pinnata* tree have been used as crude drug for treating tumor, skin disease, abscesses, painful rheumatic joints, wounds, ulcers, and diarrhea (Shoba and Thomas, 2001; Meera *et al*., 2003). In addition, a great interest has recently been put in studying the insecticidal, nematicidal, antifungal, antibacterial and antiviral activities of *P. pinnata* (Simin *et al*., 2002; Kerasi *et al*., 2010). A number of recent studies have demonstrated that pongam contains pesticidal properties against pests such as aphids, houseflies, louse, termites, mosquito, and beetles; toxicity and deterrence of pongam to pests were confirmed in the laboratory for the human head louse *Pediculus humanus capitis* (Samuel *et al*., 2009), the red flour beetle *Tribolium castaneum* (Mamun *et al*., 2009), the pulse beetle *Callosobruchus chinensis* (Yankanchi and Lendi, 2009), mosquitos (Lale and Kulkarni, 2010) and the termite *Odototermes obesus* (Verma *et al*., 2011). Thus, it is revealed that pongam also contains chemicals that should be useful for pest management.

However, the previous studies have focused on toxic activity of extracts mainly from pongam fruits, stems and roots, whereas only a few reports have tested the usefulness of pongam leaves. Given that leaf parts are most abundant raw materials from pongam trees, it is of our particular interest to investigate whether pongam leaf also contains chemicals that are useful for pest control. In the present study, we investigated the efficacy of extract from pongam leaves against an aphid pest.

Here, we use the turnip aphid *Lipaphis pseudobrassicae* (Davis) (Hemiptera: Aphididae) as a target pest. The turnip aphid is a serious pest of cruciferous crops. It is native in Asia where it has a wide distribution. In addition, the aphid has apparently been introduced into many other countries outside Asia (Essig, 1948). The turnip aphid can seriously damage the crops by remov-

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ing photoassimilates and by transmitting at least 16 plant viruses, and is often difficult to manage (Chan *et al*., 1991). In many Asian countries, thus, effective but costly reasonable and environmentally safe measures to combat the turnip aphid is on strong demand. We report here the usefulness and effectiveness of pongam leaf extract in controlling the turnip aphid, which is often difficult to manage with chemical pesticides.

MATERIALS AND METHODS

Insect rearing

The colonies of the turnip aphid *L. pseudobrassicae* were collected from severely infested plants grown at the experimental farm of Hue University, Vietnam. The turnip aphid was reared on pak choi *Brassica chinensis*. Seeds of the plant were sown in a tray ($20\,cm \times 60\,cm \times 15\,cm$) in a mixture soil (40% water content, pH = 5.5 – 6.5, 0.035% N, 0.123% P_2O_5, 0.018% K_2O). Two weeks after germination, a single plant was transplanted in a plastic pot ($9\,cm$ in diameter). A tray ($32\,cm \times 44\,cm \times 6\,cm$) containing 10 potted plants was placed in a small greenhouse until use. Aphid culture was maintained under laboratory conditions of 25 ± 0.5°C, 60 – 70% humidity and 16L: 8D on potted plants for 6 months before using in the following experiments.

Preparation of plant extracts

The leaves of *P. pinnata* were collected from Phu Vang District, Thua Thien Hue Province, Vietnam. Afterwards they were washed under tap water to remove debris. The plant materials were kept in shade for air–drying and then were dried in the oven at 60°C to gain constant weight. The dried leaves were powdered using an electric grinder. The powdered leaves were evenly packed in Soxhlet's apparatus, and the extraction was done with methanol. The extract was dried with a vacuum evaporator, and was then labeled and stored at 4oC in amber colored airtight bottles. Different concentration of plant extracts were prepared by dissolving the stock solutions in Acetone 300, 99.5 +% (GC) to use in the bioassay.

Bioassays

Bioassays were conducted in the Laboratory of Entomology, Hue University, Vietnam. The diluted extract was sprayed on the leaves until runoff (approximately $5\,ml$/ potted plant) using a power–pack aerosol hand sprayer (Hand Spray Nozzle, Takeda Engei Co., Japan). No surfactants were added to either extract. Two hours after spraying, each potted plant (10–15 cm in height with 2–3 leaves) was exposed to 15 first instars of the aphid and was placed in a plastic cage ($45\,cm \times 30\,cm \times 25\,cm$) covered with a fine nylon mesh. As a control, distilled Acetone was applied to infested plants in the same way as extract above. The cages were kept under the condition of 25°C, 60–70% humidity and a 16L: 8D light period.

A range of doses was tested to obtain the approximate LC_{50}. A $50\,ml$ stock solution was prepared with a concentration of 5%. The stock solution was made by diluting with Acetone 300, 99.5 +% (GC). The concentrations for testing were changed by adding acetone to the stock solution; the doses tested were from 0.2 to 1.2%. The mortality was determined at 24 h after spraying. A test with a given concentration was made with one potted plant with 15 first instar aphids. For each concentration, tests were replicated three times, thus, data with 45 aphids in all were obtained for each concentration.

Serial time–dose response bioassay was used to determine response of the aphids to different doses lower than LC_{50} doses obtained from the dose–response bioassays. The range of doses tested for each insecticide were prepared by diluting extract with acetone until being dose equivalent to 0.1, 0.2, 0.3, 0.4 and 0.5%. Mortality was determined at 24 h, 48 h, 72 h, 96 h and 120 h after initial exposure. Each concentration was carried out in 3 replications with 45 first instar aphids.

Alive aphids were maintained under the above conditions, and monitored daily until all aphids had died to determine developmental time, longevity and fecundity. Based the data obtained from the above experiments, the net reproduction rate (Ro), mean generation time (T) and intrinsic rate of natural increase (r_m) were calculated according to the equations given by Birch (1948).

$$Ro = \sum l_x m_x \,;\; T = \sum x l_x m_x \Big/ \sum l_x m_x \,;$$

$$\sum (\exp(-r_m x) l_x m_x) = 1$$

where; x is female age, lx is the proportion of females surviving to age x, and mx is the expected number of daughters produced per female alive at age x.

Data analysis

Dose–response data were analyzed with the probit analysis. The development time, longevity and fecundity were analyzed with one way ANOVAs, and mean were separated by Fisher's PLSD tests. All statistical procedures were carried out using the SPSS 12.0 and JMP 10.0.

RESULTS

The results of the probit analysis for dose–response data (LC_{50}, slopes and intercepts of the dosage–mortality lines) for *L. pseudobrassicae* are given in Table 1. The LC_{50} of pongam leaf extracts were found to be 0.585%, 0.151% and 0.113% at 24, 48 and 72 hours, respectively. The LC_{95} was 2.651%, 1.817% and 1.129% at 24, 48 and

Table 1. Median lethal concentrations of pongam leaf extract to *Lipaphis pseudobrassicae*

Time after treatment (hours)	LC_{50} (%)	95% fiducial limits of LC_{50}	LC_{95}
24	0.585	0.427 – 0.736	2.651
48	0.151	0.126 – 0.305	1.871
72	0.113	0.044 – 0.219	1.129

72 hours, respectively.

When the aphids were exposed to treated extracts with different concentrations lower than LC_{50}, the mean developmental times were 6.4, 6.8, 7.1 and 7.4 days at concentration of 0.2, 0.3, 0.4 and 0.5%, respectively, and the times were shorter than that of control (5.8 days) (F = 26.52; df = 71; P<0.0001). There were no significant differences among the mean developmental times of the aphids exposed to the concentrations of 0.3, 0.4 and 0.5 % (P>0.05) (Table 2).

The mean longevities of aphids exposed to the different concentrations were shorter than the longevity in control (F =11.53; df = 71; P<0.0001). The longevities were 4.1, 3.3, 3.2, 2.6, 2.5 days at the concentrations of 0.0, 0.2, 0.3, 0.4 and 0.5%, respectively. There were no significant differences among the mean longevities of aphids exposed to the concentrations of 0.3, 0.4 and 0.5% (P>0. 05) (Table 2).

The mean fecundities of the aphids exposed to the different concentrations were smaller than the fecundity of control aphids (F = 16.55; df = 71; P<0.0001). The fecundities were 15.1, 11.7, 8.8, 6.8, 6.0 (i.e., the number of aphid progeny produced) at the concentrations of 0, 0.2, 0.3, 0.4 and 0.5%, respectively. There were no significant differences among the mean fecundities of aphids exposed to the concentrations of 0.3, 0.4 and 0.5% (P>0. 05) (Table 2).

The extract with different concentrations was negative impact on population increase of *L. pseudobrassicae*. There was a difference in net reproduction (Ro), generation time (T) and intrinsic rate of natural increase (r_m) of the aphids exposed to the different concentrations or acetone. The intrinsic rates of increase value were 2.7, 2.6, 2.3, 2.1 and 2.1 day^{-1} at the concentrations of 0, 0.2, 0.3, 0.4 and 0.5%, respectively. Mean generation times were 0.64, 0.66, 0.71, 0.72 and 0.74 days at the concentrations of 0, 0.2, 0.3, 0.4 and 0.5%, respectively. Mean net reproductive rates were 19.71, 14.97, 11.76, 8.12 and 7.97 at the concentration of 0, 0.2, 0.3, 0.4 and 0.5%, respectively (Table 3).

Table 2. Effects of different doses of pongam leaf extract on developmental time, longevity and fecundity of *Lipaphis pseudobrassicae*

Concentration (%)	Developmental time (day)	Longevity (day)	Fecundity (aphid)
0.0	5.8 ± 0.5d	4.2 ± 1.0a	15.1 ± 4.8a
0.2	6.4 ± 0.4c	3.3 ± 0.9b	11.7 ± 3.8b
0.3	6.8 ± 0.5b	3.2 ± 0.6bc	8.8 ± 3.0bc
0.4	7.1 ± 0.3ab	2.6 ± 0.6c	6.8 ± 1.5c
0.5	7.4 ± 0.4a	2.5 ± 0.4c	6.0 ± 0.5c
df	71	71	71
F–value	26.5	11.5	16.6
P–value	<0.0001	<0.0001	<0.0001

Means with the same letters within a column are not significantly different by Fisher's PLSD after one–way ANOVA (P<0.05). Data are shown as mean ± SE.

Table 3. Effects of different doses of the extract on net reproduction (Ro), generation time (T, days) and intrinsic rate of natural increase (r_m, day^{-1}) of *Lipaphis pseudobrassicae*

Concentration (%)	Ro	T	r_m
0.0	19.71	0.64	2.74
0.2	14.97	0.66	2.59
0.3	11.76	0.71	2.29
0.4	8.12	0.72	2.09
0.5	7.97	0.74	2.11

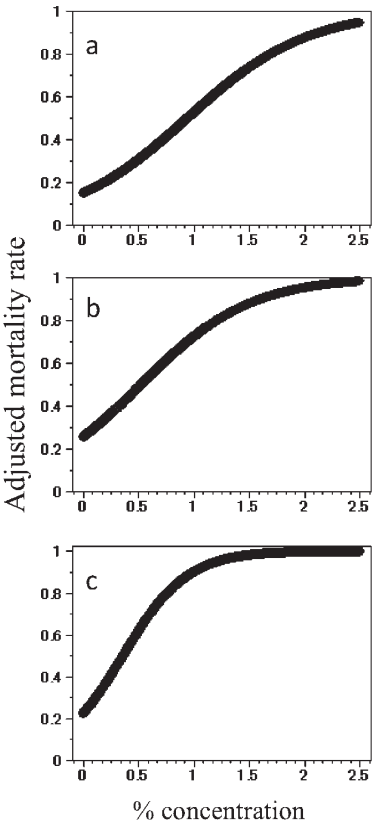

Fig. 1. The dosage–mortality curves for mortality rates of the turnip aphid exposed to pongam extracts with different concentrations (a: 24 h; b: 48 h; c: 72 h). The curves were obtained with probit models. The mortality rates were adjusted with Abbott's formula.

DISCUSSION

Leaf extract of pongam tree was reported to be effective against some insect pests such as *Euproctis fraterna* (Sridhar and Chetty, 1989), *Aphid gossypii, Amrasca devastans* (Kulat *et al*., 1997), and *Pediculus humanus capitis* (Samuel *et al*., 2009). The results of our study additionally provides evidence that pongam leaf extracts have a toxic effect against the turnip aphid *L. pseudobrassicae* causing high level of the mortality. Pongam leaf extracts contain two major flavonoids, *i.e*., karanjin and pongapin (Asolkar *et al*., 1992; Katekhaye *et al*.,

2012), and these two flavonoids have been shown to possess pesticidal properties (Kumar *et al*., 2006, Verma *et al*., 2011; Poonia and Kaushik, 2013). In the present study, the turnip aphids are shown to be highly susceptible to pongam leaf extracts; the LC_{50} values are low (Table 1). Kulat *et al*. (1997) have also indicated that pongam leaf extract was highly toxic to the aphid *A. gossypii*. Samuel *et al*. (2009) indicated that methanol extracts of pongam leaves processed excellent anti–lice *P. humanus capitis* activity with values ranging between 32.6 and 82.9%. Similarly, high toxicity of pongam leaf extracts has been reported for the second instar larvae of *Spodoptera litura* (LC_{50} 72 h: 5.44%), the larvae of *Trogoderma granarium* (LC_{50} 72 h: 19.9 μg/insect) and the adult of *T. granarium* (LC_{50} 72 h: 65.9 μg/insect). Thus, pongam leaf extracts are a promising tool to combat insect pests that are often difficult to control solely with synthetic pesticides.

Apart from acute lethal toxicity of pongam extracts on insects, the extracts are known to have nonlethal, negative effects against insects. For example, two recent studies have demonstrated the presence of antifeedant and/or repellent effects on many insect pests such as *S. litura*, *T. granarium* and blood sucking mosquitos (Kumar *et al*., 2006; Lale and Kulkarni, 2010). Although such antifeedant effects were not examined in our study, we did provide evidence that low concentrations of pongam leaf extracts caused significant reduction of vitality and fertility of the turnip aphids of the subsequent generation (Table 2) and demonstrated an indirect reduction of overall pest numbers in the next generation (Table 3). Sub–lethal effects are often chronic and are expressed as some changes in the insect's life history attributes but can have long–term impacts on the pest population. Sub–lethal residues may negatively affect pest insects that survive pesticide applications, those that emerge as adult from protected situations, or those that disperse into previously treated areas where residues exist. Biological parameters that are negatively affected can include daily fecundity, total progeny production, longevity, developmental time, egg viability, consumption rates and behavioral response (Ruberson *et al*., 1998; Johnson and Tabashnik, 1999). Thus, pongam leaf extracts can negatively impact target pests via acute lethal and delayed sublethal toxicities.

Natural extracts from plants or botanical extracts have been noticed for their safety to the environment and human health as well as their effective function to kill pest insects (Isman, 1995; Breuer *et al*., 2003). Further, appearance of resistance strains of the pests appears to be minimal due to different mode of action when compared with synthetic pesticides. Moreover, some extracts (containing similar substances to those present also in *P. pinnata* leave) may attract natural enemies (Charleston *et al*., 2006) and hence increase parasitism levels under field conditions. Botanical insecticides based on pongam leaves are thus usable concurrently with biological control agents (Tabone *et al*., 2010). Therefore, pongam leaf extract can be recommended for protection of cruciferous crops from the turnip aphid *L. pseudobrassicae*.

In Asia, there are numerous insect pest species that are difficult to control solely by synthetic pesticides. Use of multiple measures, such as biological and cultural control will be essential to effectively managing the pests (Ueno, 2006; Tran and Ueno, 2012). Additionally, botanical or organic pesticides can be a promising measure that is costly reasonable. The present study highlights usefulness of botanical pesticides as a promising measure to control crop pests.

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