INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
10–500/ZIP/2011/13–4–517–522
http://www.fspublishers.org

Full Length Article

Insecticidal Properties of *Piper nigrum* Fruit Extracts and Essential Oils against *Spodoptera litura*

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ABSTRACT

Chemical analysis by GC and GC-MS revealed presence of 39 compounds in the essential oil fraction of *Piper nigrum* fresh fruits. Limonene was the major compound present with 35.06% of total oil followed by beta-pinene (12.95%) and linalool (9.55%). Insecticidal properties of *Piper nigrum* fruit extracts and essential oils were investigated against tobacco army worm, *Spodoptera litura* using topical application bioassay on uniform weighted second instar larvae in the laboratory. The hexane extract was most effective in killing the larvae and showed the highest toxicity at 48 h after treatment. Toxicity of extracts decreased in the order of hexane (LD50: 1.8 mg/g) > acetone (LD50: 18.8 mg/g) > chloroform (LD50: NA, the toxicity was very low) > essential oil (no mortality). Insect development and growth index observations showed that the hexane extract had antifeedant properties resulting in severe growth inhibition of *Spodoptera litura*. 2011 Friends Science Publishers

Key Words: Botanical insecticide; *Piper nigrum*; *Spodoptera litura*; Topical bioassay

INTRODUCTION

Insect pests are a major constraint on crop production, especially in developing countries. Natural plant extracts play an increasingly prominent role as alternatives to synthetic pesticides due to the increasing concern on health hazards, environmental pollution and negative effects on non target organisms (Sharma *et al*., 2006). Many floral volatiles have anti-microbial or anti-herbivore activity (DeMoraes *et al*., 2001; Friedman *et al*., 2002; Hammer *et al*., 2003) and hence act to protect valuable reproductive parts of plants from damage (Dudareva *et al*., 2004). However, intensive screening is necessary to select compounds with pesticidal properties, but harmless to the environment and ecosystem. Researches on potential botanical extracts which are safe with little or no residues and naturally derived with minimal technology are urgently needed. There are more than 2400 plant species belonging to 189 plant families which are said to be rich sources of bioactive organic compounds (Rao *et al*., 2005). Among the families of plants investigated to date, one showing enormous potential is the pepper family, otherwise known as *Piperaceae* (Dodson *et al*., 2000). The phytochemical screening of black pepper fruit shows that it contains 4% alkaloids in the berry (Dev & Koul, 1997; Awoyinka *et al*., 2006). Although information on the compounds responsible for the insecticidal activities is scarce, it has been documented that the amide olefinic or alkyl isobutylamides compounds such as piperine, piperettine, tricostacine, peepuloidin, piplartin and trichonine contribute in no small measure (Adgeh, 1989; Awoyinka *et al*., 2006). These compounds have been demonstrated to be toxic to fruit flies, adzuki bean weevils, cockroaches and several other insect species (Su & Hovart, 1981; Gbenwonyo *et al*., 1993; Awoyinka *et al*., 2006). Hence, the present study was conducted to: (i) evaluate the toxicity of *P. nigrum* extracts against the *S. litura* larvae and (ii) study the effects of *P. nigrum* hexane extract on development and antifeedant reaction in *Spodoptera litura*.

MATERIALS AND METHODS

Plant materials and preparation of extracts: Fresh and dried fruits of *P. nigrum* were obtained from Sibu, Sarawak, East Malaysia. The essential oil fraction was extracted from fresh fruits by hydrodistillation method (Maisonneune & Saint-Ruffine, 1975). The essential oils of *P. nigrum* was stored in glass vial and put in refrigerator (-4º) for further experiment. Dried fruits were ground into powder and soaked in hexane, chloroform and acetone to yield sequentially the non-polar, intermediate and polar compounds. The solvents used were based on the elutropic series. Three hundred grams of black pepper powder was soaked in 1 L solvent and incubated for 48 h. The mixture of solvent and plant material was filtered by Whatman No. 1 filter paper and the solution was collected. The plant material was soaked in 1 L of hexane for another 48 h. Final filtered solution was accumulated and evaporated by rotary

To cite this paper: Fan, L.S., R. Muhamad, D. Omar and M. Rahmani, 2011. Insecticidal properties of *Piper nigrum* fruit extracts and essential oils against *Spodoptera litura*. *Int. J. Agric. Biol*., 13: 517–522

evaporator to yield dark viscous extracts. Same extraction methods were repeated by soaking the black pepper powder individually by chloroform and acetone.

Identification of chemical constituents: Qualitative and quantitative data were determined by GC and GC-MS, respectively. The identification of the chemical constituents was assigned on the basis of comparison of their mass spectra using Shimadzu NIST/EPA/NIH_+ mass spectral database (225-04793-92), which produces standard bar graphs for direct comparison with published spectra.

Gas chromatography (GC): The oil was injected onto a Shimadzu GC-17A system, equipped with an AOC-20i autosampler and a split/splitless injector. The column used was BPX 5, 30 m, 250 µm i.d., 0.25 µm df, coated with 5% phenymethylsilane, operated with the following oven temperature programme: 60ºC, held for 3 min, rising at 20°C/min to 100ºC, rising at 7°C/min to 240ºC, held for 5 min; injection temperature and volume, 250°C and 1.0 µL, respectively; injection mode, split; split ratio, 28:1; carrier gas, helium at 29.6 cm/s linear velocity and inlet pressure 27.0 kPa; detector temperature, 280°C; column flow rate, 0.7 mL/min; sampling rate, 0.50 s. Data was acquired by means of GC solution software (Shimadzu).

GC-MS: Shimadzu QP 5050 A mass spectrometer system equipped with a capillary column BPX 5 (30 m x 250 µm, 0.25 µm film thickness) was used. Helium was used as the carrier gas. The MS operating conditions were: ionization was induced by an electron impact (EI) at 70 eV, ion source 250ºC. The compounds present were identified by conducting a library search using Shimadzu NIST/EPA/NIH_+ mass spectral database. The operating parameters were identical with those of GC analysis described above.

Bioassay to evaluate toxicity of extracts: The experiments were conducted using topical application bioassay. Armyworm larvae were obtained from laboratory colonies maintained by Malaysia Agriculture Research and Development Institute (MARDI), Serdang. Five doses of solvent extracts (ranging from 0 to 40 mg/g) and essential oils (ranging from 0 to 40 µL/g) were tested on one hundred larvae per dose with acetone used as a control. A 0.5-µL droplet of pesticide-acetone solution or acetone was administered to the thoracic tergum using a micro syringe (5 µL, Hamilton Series 600/700 Standard microliter syringe, Fisher Scientific). After treatment, treated larvae were individually introduced into each Petri dish. Organic mustard leaves were applied to consume by the treated larvae. Mortality was assessed at 24 h and 48 h. Larva was considered dead if it was unable to make a coordinated movement when gently prodded. Percentage of mortality was recorded for each treatment. Control (acetone only), individual chloroform and acetone extract were used as comparison, therefore a total of 6 treatments were evaluated. Mortality of larvae was recorded at 24 and 48 h after treatment. Data were evaluated by probit analysis (PoloPlus program) to determine the LD_{50} (representing the dosage in µg/g insect that caused 50% mortality) along with 95% confidence intervals.

Effect of hexane extract on development of *Spodoptera litura*: Based on the results of the toxicity study, the hexane extract was selected to further evaluate its effects on growth and development of *S. litura* until adult emergence. The experiment was conducted by topical bioassay on 2nd instar larvae as previously described. After application, treated larvae were placed singly in disposable Petri dishes for observation until adult emergence. Larvae mortality (%), larvae head capsule length (mm), larvae weight gain (g), percentage of pupation (%), pupa weight (g), malformed pupae (%), adult emergence (%) and sex ratio were recorded. Corrected efficacy (%) of larvae mortality was calculated using the formula proposed by Abbott (1925), malformed pupae and pupae mortality were calculated by Sun-Shepard's formula (Püntener, 1981) and adult emergence was calculated by Henderson-Tilton's formula (Henderson & Tilton, 1955). Larval growth index and total developmental growth index were calculated as per Gupta and Girah (2001). All data were analyzed using the ANOVA procedure in SAS Statistical package and means were compared by Tukey's Studentized Range (HSD) Test.

Antifeedant test: The experiment was conducted using the leaf dip method. *P. nigrum* hexane extract was selected to test on the 2nd instar of *S. litura*. The hexane crude extract (0.3883 g) was dissolved in 1.5 mL xylene (solvent) and 0.1 g Triton X-100 (surfactant) as emulsifiable concentrate and finally dissolved in 20 mL distilled water to get a clear solution. The percentages of formulation were assigned based on experimentation including trial and errors. Leaf discs, 60 mm diameter, were cut and soaked in the prepared extract, air dried and singly placed into disposable petri dishes. Feeding tests were conducted using second instar larvae of *Spodoptera litura*. One larva per dish and a total of 50 larvae were used per treatment. Leaf disc areas were measured using a leaf area meter (LI-3100, LI-COL, USA) at 24 and 48 h after exposure. Antifeedant index (AI) was calculated as per Gupta and Girah (2001).

RESULTS AND DISCUSSION

Chemical constituents of black pepper essential oil: Distilled oil production from the fresh fruits of *P. nigrum* was 0.8% (v/w), yielding a colourless liquid rich in pungent fragrance with many volatile components (Table I). Thirty-nine components were identified. The monoterpene compounds consisted of approximately 64.05% of the total oil. Oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes were present in relatively lower amounts, representing 13.06%, 8.14% and 7.32%, respectively. Limonene was the major compound present with 35.06% of total oil from the fruits of black pepper, followed by beta-pinene (12.95%) and linalool (9.55%). Singh *et al*. (2004) reported 49 compounds representing 99.4% of total oil. The major components identified were

Table I: Composition of essential oil components in fresh leaves of *Piper nigrum* (Compounds listed in order of elution from GC-MS column and identification was based on comparison with pure standards)

No.	Component	Formula	Mol. Weight	Retention time (min)	% in total oil
1.	Unknown	C_7H_8	92	3.513	5.97
2.	2-Methyl-5-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene	$C_{10}H_{16}$	136	5.798	0.21
3.	α-Pinene	$C_{10}H_{16}$	136	5.963	4.31
4.	Sabinene	$C_{10}H_{16}$	136	6.635	2.42
5.	β-Pinene	$C_{10}H_{16}$	136	6.773	12.95
6.	β-Myrcene	$C_{10}H_{16}$	136	6.829	2.21
7.	3-Carene	$C_{10}H_{16}$	136	7.267	5.51
8.	o-Cymene	$C_{10}H_{14}$	134	7.585	1.38
9.	Limonene	$C_{10}H_{16}$	136	7.653	35.06
10.	Sabinene hydrate	$C_{10}H_{18}O$	154	7.717	0.65
11.	Trans-Sabinenehydrate	$C_{10}H_{18}O$	154	8.440	0.11
12.	Linalool	$C_{10}H_{18}O$	154	8.883	9.55
13.	Plinol	$C_{10}H_{18}O$	154	9.053	0.26
14.	Terpinen-4-ol	$C_{10}H_{18}O$	154	10.637	1.40
15.	α-α-4-Timethyl-benzenemethanol	$C_{10}H_{14}O$	150	10.775	0.17
16.	α-Terpineol	$C_{10}H_{18}O$	154	10.928	0.80
17.	Linalool	$C_{10}H_{18}O$	154	11.358	0.12
18	2-Undecanone	$C_{11}H_{22}O$	170	12.643	0.10
19.	δ-Elemene	$C_{15}H_{24}$	204	13.565	0.15
20.	Copaene	$C_{15}H_{24}$	204	14.473	0.38
21.	B-Elemene	$C_{15}H_{24}$	204	14.696	0.37
22.	Caryophyllene	$C_{15}H_{24}$	204	15.442	3.98
23.	α-Caryophyllene	$C_{15}H_{24}$	204	16.163	0.52
24.	Eudesma-4(14),11-diene	$C_{15}H_{24}$	204	16.849	1.15
25.	α-Selinene	$C_{15}H_{24}$	204	16.954	0.57
26.	Cubenol	$C_{15}H_{26}O$	222	17.289	0.69
27.	Calamenene	$C_{15}H_{22}$	202	17.417	0.06
28.	Spathulenol	$C_{15}H_{24}O$	220	18.576	0.59
29.	Caryophyllene oxide	$C_{15}H_{24}O$	220	18.715	2.62
30.	Ledol	$C_{15}H_{26}O$	222	18.942	0.11
31.	3-Ethyl-2,5-dimethyl-1,3-hexadiene	$C_{10}H_{18}$	138	19.248	0.15
32.	Eudesm-7(11)-en-4-ol	$C_{15}H_{26}O$	222	19.359	0.26
33.	Spathulenol	$C_{15}H_{24}O$	220	19.482	1.31
34.	δ-Cadinol	$C_{15}H_{26}O$	222	19.814	1.42
35.	α-Cadinol	$C_{15}H_{26}O$	222	20.002	0.19
36.	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	$C_{15}H_{24}O$	220	20.291	0.67
37.	4-Methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-cycloheptane	$C_{15}H_{24}$	204	21.552	1.02
38.	7-Bromo-bicyclo[4.1.0]heptane	$C_7H_{11}Br$	174	22.559	0.19
39	Decahydro-2,2-dimethyl-naphthalene	$C_{12}H_{22}$	166	24.650	0.40

Table II: LD_{50} of *Piper nigrum* fruit extracts on *S. litura* 2nd instar larvae

Treatment	Hours	Extract	Slope ± SE	x^2	df	LD_{50}	95% confidence interval
		Essential oil	-	-	-	-	-
	24	Hexane	3.471±0.482	2.7858	2	1.824mg/g	1.120 to 2.857
P. nigrum		Chloroform	1.151±0.303	3.4902	3	NA	NA
(fruits)		acetone	1.168±0.228	1.448	3	17.813mg/g	12.484 to 32.485
		Essential oil	-	-	-	-	-
	48	Hexane	3.447±0.480	2.3273	2	1.806mg/g	1.186 to 2.642
		Chloroform	1.261±0.300	3.5687	3	NA	NA
		acetone	1.186±0.225	0.679	3	15.714mg/g	11.229 to 26.742
P. nigrum	24	Chloroform	1.405±0.225	3.8607	3	10.312mg/g	6.098 to 22.013
(fruits)	48	Chloroform	1.466±0.225	3.0474	3	8.998mg/g	5.705 to 15.291
Comparison	24	acetone	2.048±0.277	2.091	3	10.479mg/g	8.343 to 14.234
	48	acetone	1.862±0.251	1.174	3	9.405 mg/g	7.414 to 12.891

NA = Not available

beta-caryophyllene (24.2%), a sesquiterpene, and limonene (16.9%) and sabinene (13%), both monoterpenes. On the other hand, Jirovetz *et at*. (2002) had observed that the main compounds (concentration >3.0%) from essential oil of dried fruits of black pepper from Cameroon were germacrene D (11.01%), limonene (10.26%), β-pinene (10.02%), α-phellandrene (8.56%), β-caryophyllene (7.29%), α-pinene (6.40%) and *cis*-β-ocimene (3.19%). These results were different to the present study as the analysis was based on dried fruit. The components of essential oils from fresh fruits would be different to the dried fruits of black pepper. The present investigation

showed that fresh fruit oil contained high concentration of limonene (35.06%), whereas the limonene concentration in dried fruit oil as reported by Jirovetz *et al*. (2002) (10.26%) and Singh *et al.* (2004) (16.9%) were relatively much lower.

Toxicity test: Table II shows the mortality of *S. litura* with respect to the doses of essential oils and solvent extracts of black pepper fruits. Total insect mortality rate was recorded after 24 and 48 h of treatment. Results showed that the hexane extract was the most effective against 2nd instar larvae of *S. litura* with the lowest LD_{50} of 1.824 mg/g insect followed by the acetone extract (17.813 mg/g), chloroform extract (LD_{50} was not available in statistical analysis, because of low toxicity, please refer to Table II) and essential oil (non toxic) at 24 h after treatment. At 48 h after treatment, LD_{50} of the hexane and acetone extracts were 1.806 mg/g and 15.714 mg/g, respectively. The chloroform extract showed relatively very low toxicity as compared to the hexane and acetone extracts.

The Piperaceae family has yielded many promising phytochemicals with insecticidal activity (Arnason *et al*., 2002). Results of the present study showed that the hexane extract and acetone extract of dried black pepper seeds, which contain combinations of piperamides can be used to kill *S. litura*, especially at the 2nd instar larval stage. The chemistry of black pepper fruits has been extensively investigated and reveals a number of piperidine and pyrrolidine amides. The amide, piperine is the most recognized compound and present in the highest concentration of all secondary compounds in seeds of *P. nigrum*, but 2 to 10-fold differences can occur between samples (Semler & Gross, 1988). Earlier investigations with *P. nigrum* dried seed extracts indicated that piperamides were responsible for the toxicity of the extracts to the adzuki bean weevil *Callosobruchus chinensis* L. (Miyakado *et al*., 1979, 1980). Piperamides singly, or more importantly in combination, could still replace contact insecticides, specifically neurotoxic compounds such as carbamates, organophosphates and pyrethroids, for which resistance has developed. A combination of these amides within a botanical formulation would thus provide the advantage of all of the previously mentioned attributes: novel target site, enzyme inhibition and low mammalian toxicity (Scott *et al*., 2008).

Plant terpenoids have been known to be toxic to insects (Metcalf & Metcalf, 1992). The toxic effects of plant materials can be attributed to their essential oil composition as insecticidal properties of limonene, beta-pinene and linalool had been reported (EPA, 1994; Raguso & Pichersky, 1999; Lewinshon *et al*., 2001; Jung *et al*., 2007). However, in the present study, the essential oil doses of fresh *P. nigrum* fruits of upto 40 µL/g, insect did not show any toxic effects on the larvae tested. Hence, with fresh fruit extracts, a higher dose might be needed to kill the larvae.

Effect of hexane extract on growth and development of *Spodoptera litura*: Results showed that black pepper hexane extract possessed strong growth inhibiting properties against *Spodoptera litura* (Table III). Effect of hexane extract on insect development was evaluated daily until adult emergence. Corrected efficacy of larvae mortality (%) treated with hexane extract was 48% compared to those treated with acetone only. Efficacy of the hexane extract on

Table III: Corrected efficacy (%) of black pepper, *P. nigrum* hexane extract on larval mortality, malformed pupae, pupal mortality and adult emergence

Parameter	Treated (%)	Control (%)	Corrected efficacy (%)
Larvae mortality	54	11	48.31
Malformed pupae	15.22	13.48	25.29
Pupae mortality	33.33	16.88	42.96
Adult emergence	66.67	83.12	19.79

Table IV: Effect of hexane extract of *Piper nigrum* fruits on development period and growth index of *Spodoptera litura*

Parameter	*P. nigrum* (hexane)	Control
Larval period (days)	14.5	16.0
Pupation (days)	7.2	9.3
Total development period (days)	21.7	25.3
Larval Growth Index, LGI	3.15	5.54
Total Development Growth Index, TDGI	1.20	2.53

Table V: Effect of hexane extract of *Piper nigrum* fruits on larvae weight gain

Observation day	Weight gained, g	
	Hexane extract	Untreated
1	0.0142 [b]	0.0175 [a]
2	0.0750 [a]	0.0519 [b]
3	0.1255 [a]	0.1409 [a]
4	0.1985 [b]	0.2391 [a]
5	0.2254 [b]	0.2587 [a]
6	0.1546 [a]	0.1713 [a]
7	0.0979 [a]	0.0619 [b]
8	0.0492 [b]	0.0268 [b]

Tukey's HSD (Honestly Significant Difference) Test for pupae weight
*Means within rows with the same letters are not significantly different ($p<0.05$)

Table VI: Effect of hexane extract of *Piper nigrum* fruits on *Spodoptera litura* sex ratio

Treatment	Sex ratio (male: female)
Control	1: 0.77
P. nigrum (hexane)	1: 0.86

Table VII: Antifeedant Index of hexane extract of *Piper nigrum* fruits against second instar larvae of *Spodoptera litura*

P. nigrum (hexane extract)		
Concentration (g/L)	Antifeedant Index, AI	
	24 h	48 h
17.66	45.25	13.38
8.83	49.61	12.88
4.41	16.02	6.70
2.21	14.81	11.45

malformed pupae, pupae mortality and adult emergence were 25.29, 42.96 and 19.79%, respectively.

The results on physical development showed growth of the head capsule in 4th, 5th and 6th instar larvae was 1.076, 1.761 and 2.620 mm, respectively. The life span for 4th instar larvae treated by control treatment was significantly different compared to the hexane extract, which were 2.087 and 1.1087 day, respectively. Life span of 5th instar larvae treated with *P. nigrum* extract was significantly longer (1.7826 days) than in the control treatment (1.4783 day). However, the longevity of 6th instar larvae in the control treatment was significantly longer (4.5217 days) compared to the *P. nigrum* treatment (3.413 days) (Fig. 1). Total larval period (1st to 6th instar) for the control treatment was 16 days while it was 14.5 days in the hexane extract treatment, which was 1.5 days shorter than the control (Table IV).

Pupation periods in the *P. nigrum* extract and control treatments were 7.2 and 9.3 days, respectively. Total development period of *S. litura* (1st instar until adult emergence) in contact with *P. nigrum* hexane extract and control treatments were 21.7 and 25.3 days, respectively. The hexane extract exhibited a lower larval growth index (LGI) and a lower total developmental growth index (TDGI) of 3.15 and 1.20, respectively as compared to the control treatment (which were 5.54 & 2.53, respectively).

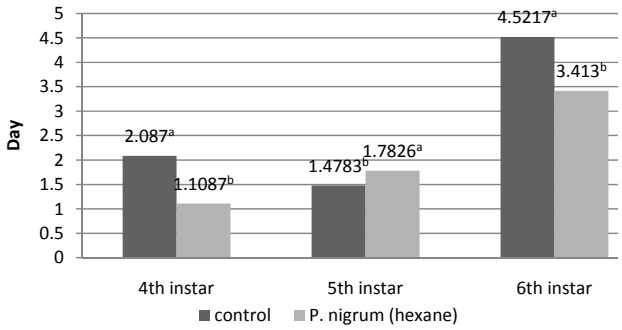
Average weight gains of larvae in *P. nigrum* hexane extract and control treatments are presented in Table V. Results showed that most daily weight gains of larvae treated with hexane extract were significantly lower compared to the control, except on the 2nd, 7th and 8th day. The mature larvae stopped feeding and pupated in a small cell in the disposable Petri dishes. After emergence, the gentle of adult moths were identified through their morphology and external genitalia. The results showed that the sex ratio (male: female) for adults emerging in contact with *P. nigrum* hexane extract and control treatments were 1: 0.86 and 1: 0.77, respectively (Table VI).

Xue *et al.* (2009) had studied the development of *S. litura* under laboratory conditions (26°C, 60-80% RH) fed on different host plants. Their result showed that overall larval development of *S. litura* was significantly affected by the host plant from shortest to longest in the following order: Chinese cabbage (13.3 days), cowpea (15.8 days), sweet potato (17.5 days), and tobacco (23.2 days). The study showed that pupal development times ranged from 10.9-9.5 days when fed on different host plants. In the present study, the larval duration found in the control and *P. nigrum* treatment ranged from 14.5-16.0 days, which was still within the range of the 4 different hosts. But the pupal duration found in *P. nigrum* treatments were relatively lower compared to the study by Xue *et al.* (2009). Observation by Xue *et al*. (2009) showed that the survival rate of *S. litura* larvae on Chinese cabbage was 75.4% and the pupa weight for male and female *S. litura* were 0.354 and 0.362 g, respectively. The sex ratio (female: male) was 1: 0.64, which was different in the present study, where the

Fig. 1: Effect of hexane extract of *Piper nigrum* fruits on life span and head capsule measurement of *Spodoptera litura* larvae

Tukey's HSD (Honestly Significant Difference) Test for larvae longevity

*Means with the same letter are not significantly different within the same instar ($p<0.05$)



number of males was higher compared to females The sex ratio (male: female) in *P. nigrum* hexane extract treatment was 1: 0.86, while it was 1: 0.77 in the control. The present study demonstrates that the difference in development of *S. litura* could be influenced by others physical and biological factors.

Antifeedant test: Results of the growth and development study showed that weight gained by larvae treated with *P. nigrum* hexane extract were lower as compared to control treatments (Table VII). The antifeedant test (leaf dip method) conducted to evaluate the antifeedant properties of hexane extract against the second instar larvae showed that the antifeedant effect of hexane extract on second instar larvae of *S. litura* ranged from 14.81 to 49.61% compared to the untreated control at 24 h after treatment. However, the antifeedant properties decreased at 48 h after treatment, with protection ranging from 6.70 to 13.38% over the control treatment.

Behaviour modification (antifeedant & repellent effects) effects of *Piper* spp. extracts have been examined in greenhouse trials and results showed that pepper seed extracts deterred Lily leaf beetles *Lilioceris lilii* Scopoli and *Acalymma vittatum* from damaging leaves of lily and cucumber plants at concentrations of 0.1–0.5% (Scott *et al*., 2004). The repellent activity was observed to benefit the plants for up to 4 days post-spraying. However, the residual repellent effect of *P. nigrum* was much less under full sunlight, and herbivore damage resumed shortly after application (Scott *et al*., 2003). Similar loss in activity was observed in the present study, where the activity of black pepper hexane extract degraded relatively faster compared to the observations by Scott *et al*. (2003).

CONCLUSION

Limonene was the major compound in the essential oil of *P. nigrum* fresh fruit. The hexane extract of *Piper nigrum* fruits was more effective in controlling the armyworm, *S.*

litura. However, the essential oil fraction was found to be non-toxic. Further investigations need to be focused on identification of chemical constituents in the hexane extract and enhancement of efficacy through suitable formulation.

Acknowledgement: The financial support of the School of Graduate Studies (SGS), Universiti Putra Malaysia, Malaysia under the Graduate Research Fellowship Program to the first author is gratefully acknowledged.

REFERENCES

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*., 18: 265–267

Adgeh, B.J., 1989. Residual toxicity of three plant material against three storage insect pests. *Laguna* 10: 84

Arnason, J.T., T. Durst and B.J.R. Philoge`ne, 2002. Prospection d'insecticides phytochimiques de plantes tempe´re´es et tropicales communes ou rares. *In*: Regnault-Roger, C., B.J.R. Philoge`ne and C. Vincent (eds.), *Biopesticides D'origine Ve´ge´ Tale*, pp: 37–51. Editions TEC and DOC, Paris

Awoyinka, O.A., I.O. Oyewole, B.M.W. Amos and O.F. Onasoga1, 2006. Comparative pesticidal activity of dichloromethane extracts of *Piper nigrum* against *Sitophilus zeamais* and *Callosobruchus maculates*. *African J. Biotechnol*., 5: 2446–2449

De Moraes, C.M., M.C. Mescheer and J.H. Tumlinson, 2001. Caterpillar-induced nocturnal plant volatiles repel nonspecific females. *Nature*, 410: 577–580

Dev, S. and O. Koul, 1997. *Insecticides of Natural Origin Hardwoodv*, p: 365. Academic Publishers Amsterdam

Dodson, C.D., L.A. Dyer, J. Searcy, Z. Wright and D.K. Letourneau, 2000. Cenocladamide, a diydropyridone alkaloid from *Piper cenocladum*. *Phytochemistry*, 53: 51–54

Dudareva, N., E. Pichersky and J. Gershenzon, 2004. Biochemistry of Plant Volatiles. *Plant Physiol*., 135: 1893–1902

EPA, 1994. *R.E.D. Facts: Limonene*. Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency

Friedman, M., P.R. Henika and R.E. Mandrell, 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enteric*. *J. Food Prot*., 65: 1545–1560

Hammer, K.A., C.F. Carson and T.V. Riley, 2003. Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. *J. Appl. Microbiol*., 95: 853–860

Henderson, C.F. and E.W. Tilton, 1955. Tests with acaricides against the brown wheat mite. *J. Econ. Entomol*., 48: 157–161

Jirovetz, L., G. Buchbauer, M.B. Nagassoum and M. Geissler, 2002. Aroma compound analysis of *Piper nigrum* and *Piper guineese* essential oils from Cameroon using solid phase microextraction gas chromatography, solid phase micoextraction gas chromatography mass spectrometry and olfactometry. *J. Chromatogr*., 976: 265–275

Jung, W.C., Y.S. Jang, T.T. Hieu, C.K. Lee and Y.J. Ahn, 2007. Toxicity of *Myristica fagrans* seed compounds against *Blattella germanica* (Dictyoptera: Blattellidae). *J. Med. Entomol*., 44: 524–529

Lewinshon, E., F. Schalechet, J. Wilkinson, K. Matsui, Y. Tadmor, K. Nam, O. Amar, E. Lastochkin, O. Larkov, U. Ravid, W. Hiatt, S. Gepstein and E. Pichersky, 2001. Enhanced levels of the aroma and flavor compound S-linalool by metabolic engineering of the terpenoid pathway in tomato fruits. *Plant Physiol*., 127: 1256–1265

Maisonneune, S.A. and Saint-Ruffine, 1975. Volatile constituents of the leaves of *Ziziphus spinachristi* (L.) Willd. from Bushehr, Iran. *Eur. Ph*., 3: 68

Metcalf, R.L. and E.R. Metcalf, 1992. *Plant Kairomones in Insect Ecology and Control*, p: 168. Chapman and Hall Inc., USA

Miyakado, M., I. Nakayama, H. Yoshioka and N. Nakatani, 1979. The Piperaceae amides I: Structure of pipercide, a new insecticidal amide from *Piper nigrum* L. *Agric. Biol. Chem*., 43: 1609–1611

Miyakado, M., I. Nakayama and H. Yoshioka, 1980. Insecticidal joint action of pipercide and co-occurring compounds isolated from *Piper nigrum* L. *Agric Biol Chem*., 44: 1701–1703

Püntener, W., 1981. *Manual for Field Trials in Plant Protection*, 2nd edition. Agricultural Division, Ciba-Geigy Limited

Raguso, R.A. and E. Pichersky, 1999. A day in the life of a linalool molecule: chemical communication in a plant-pollinator system. Part 1: linalool biosynthesis in flowering plants. *Plant Species Biol*., 14: 95–120

Rao, N.V., T.U. Maheswari and K. Manjula, 2005. *Review on Botanical Pesticides as Tools of Pest Management*, pp: 1–16. Narosa Publishing House Pvt., Ltd

Scott, I.M., H. Jensen, J.G. Scott, M.B. Isman, J.T. Arnason and B.J.R. Philoge`ne, 2003. Botanical insecticides for controlling agricultural pests: piperamides and the Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). *Arch. Insect Biochem. Physiol*., 54: 212–225

Scott, I.M., H. Jensen, R. Nicol, L. Lesage, R. Bradbury, P. Sa´nchez-Vindas, L. Poveda, J.T. Arnason and B.J.R. Philoge`ne, 2004. Efficacy of Piper (Piperaceae) extracts for control of common home and garden insect pests. *J. Econ. Entomol*., 97: 1390–1403

Scott, I.M., H.R. Jensen, B.J. Philogene and J.T. Arnason, 2008. A review of *Piper* spp. (Piperaceae) phytochemistry, insecticidal activity and mode of action. *Phytochem. Rev*., 7: 65–75

Semler, U. and G.G. Gross, 1988. Distribution of piperine in vegetative parts of *Piper nigrum*. *Phytochem*., 27: 1566–1567

Sharma, A., P. Kaushal, K.C. Sharma and R. Kumar, 2006. Bioefficacy of some plant products against Diamondback moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). *J. Entomo. Res. Soc*., 30: 213–217

Singh, G., P. Marimuthu, C. Catalan and M.P. deLampasona, 2004. Chemical, antioxidant and antifungal activities of volatile oil of black pepper and its acetone extract. *J. Sci. Food Agric*., 84: 1878–1884

Su, C.F. and R. Hovart, 1981. Isolation, Identification and Insecticidal properties of *Piper nigrum* amides. *J. Agric. Food Chem*., 29: 15–118

Xue, M., Y.H. Pang, H.T. Wang, Q.L. Li and T.X. Liu, 2009. Effects of four host plants on biology and food utilization of the cutworm, *Spodoptera litura*. *J. Insect Sci*., 10: 1–14

(Received 05 October 2010: Accepted 10 January 2011)