International Journal of Entomology Research
ISSN: 2455-4758; Impact Factor: RJIF 5.24
Received: 12-04-2019; Accepted: 14-05-2019
www.entomologyjournals.com
Volume 4; Issue 4; July 2019; Page No. 53-58

Efficacy of black pepper (*Piper nigrum)* extract against pepper whitefly, *Aleurodicus dispersus* (Hemiptera: Aleyrodidae)

Yap Chin Ann
Research and Development Division, Malaysian Pepper Board, Kuching, Sarawak, Malaysia

Abstract
Three extracted fractions from black pepper berries, *Piper nigrum* in three concentrations were examine for their insecticidal bioactivity against pepper whitefly, *Aleurodicus dispersus* (Hemiptera: Aleyrodidae), adults and second nymphal instars under controlled conditions. Obtained results showed that pepper whiteflies adults and nymphs were the most susceptible to methanol extract. The survival rate reduced to 17.1% and 50% for whitefly adult and nymphs respectively at 10% (w/w) after 72 hours of treatment as compared to control treatment. Extracts of leaves and stems was found to be not effective against the adult and nymphal instars, respectively. GC/MS analysis reveals the present of various type of piperamide compounds and secondary metabolites in pepper berries extract that were responsible for insecticide activity through neurotoxic effect. The main components of piperamides were piperine, piperidine, pyrrolidine and pyridine which contribute to 12.91% of all secondary compounds in berries. Other active compounds that may cause mortality of whitefly include monoterpenes, sesquiterpenes, fatty acids and alkaloids, and some other constituents include α-pinene, linalool, copaene, and aniline. Thus, the bioactive extracts of black pepper berries extract have the potential to lower whitefly populations in a comprehensive pest management program in pepper cultivation activity.

Keywords: *Piper nigrum*, whitefly, pepper extract, piperamides

1. Introduction

Pest infestations assume alarming proportions leading to yield loss and slow decline in pepper plantation. To overcome this problem, chemical control seems to be the best option which enables to thoroughly eliminate the pest infestation. However, the widespread use of synthetic insecticide has led to many negative effects including toxic residue accumulation, development of resistant strains, toxicity to environment and increase cost of operation.

Different approach has been developed to prevent, mitigate and control pest infestation in pepper cultivation. Beyond good culture practice and chemical control, the utilization of plant derived material or metabolite has been considered as a potential alternative way to tackle this problems. Today, a number of scientists have focused their interested in the control of pest infestation with alternative control agents as extract of plant leaves, flowers and seeds (Satya *et al*., 1996, Santos *et al*., 1990, Matthews, 1993 and, Ngamo *et al*., 2007) [1, 2, 3, 4].

Among the plant investigated to date, plant from the family piperaceae constituted a promising source of biological active compounds with insecticide activity. The most recognized species is black pepper, *Piper nigrum* L. is the perennial climbing vines extensively grown for its berries as spice. Black Pepper is considered as one of the most important ingredient in human food and ayuvedic medicine since ancient time. Pepper berries contain a number of active compounds especially piperamides compounds which are responsible for pharmacological activities. Steam distillations of pepper berries produce many active compounds containing lignans, flavonoids, expoxides, piperine and etc (Srinivasan, 2009) [5]. These components contribute to antibacterial, fungicidal and insecticidal properties of pepper.

The pepper whitefly, *Aleurodicus dispersus*, is a major pest of pepper in humid tropical areas around the world where pepper were grown (Yap, 2016) [6]. *The* Whiteflies are quite active and are commonly found feeding on the lower leaf surfaces (Fig 1). The whitefly has also been implicated in contributing to the pepper vine decline syndrome in many pepper producing countries (Yap, 2018) [7]. The adult insects may cause defoliation of whole pepper plants causing losses in vine vigor and yields. Heavy colonization of A. *disperses* in pepper vines encourage the growth of sooty mould and led to reduction in photosynthesis rate (Byrnee *et al*., 1990, Kajita and Alam, 1996) [8, 9]. In addition, severe infestation of this pest also reduce the yield quantitative and qualitatively through the formation of large quantity of wax and honeydew which promote the transmission of viral pathogen to the vines. Apart from that, this pest has also been reported infested many type of tropical crop e.g. guava, passion fruit, orchard (Kajita & Alam 1996; Aiswariaya *et al*. 2007) [9, 10]. Besides, serious infestations of *A. dispersus* have also been reported affect various type of ornamental plants, including poinsettia (*Euphorbia pulcherrima* Willd) and madhobilota (*Hiptage benghalensis*) (Alim *et al*. 2014) [11]. In the present study, the objective is to determine the efficacy of pepper extract against *A. disperses* and to determine the main active compounds responsible for this insecticide activity by using GCMS analysis.

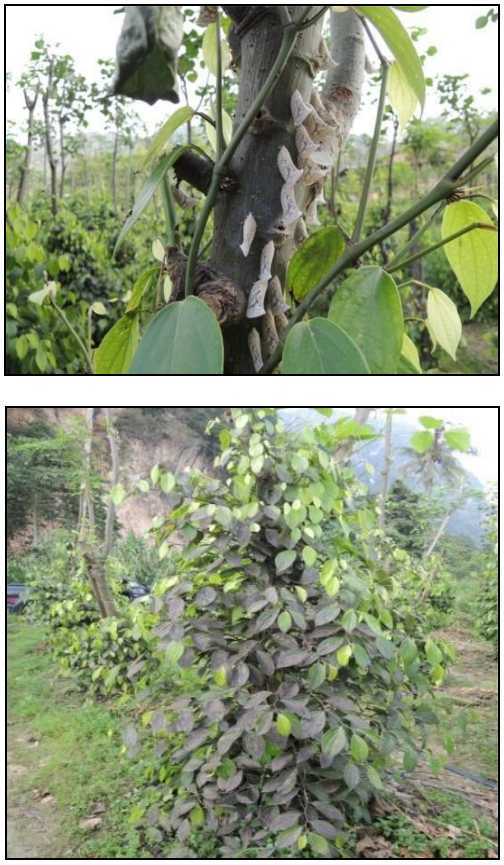

Fig 1: White flies (A) adult; (B) Formation of sooty mold on pepper leaves

2. Material and Methods

2.1 Crude extraction

The clean dried black pepper berries were grinded with an electric grinder to obtain the fine powder. 500 g of the fine powder were soaked in one liter of methylene chloride put in a 2-liter dark glass jar for 3 days with continuous shaking (Su, 1985) [12]. The solution was filtered with a piece of gauze and then, followed by a filter paper for three times till the clear solution. The obtained solution was evaporated in a rotary evaporator at 50°C and the residue was weighed and kept in a refrigerator at 4°C till use. The extracted crude was dissolved in the same solvent to get a 10% (w/w) stock solution, which diluted to obtain 1.25%, 2.5%, and 5% solutions. The same procedure was repeated for leaf and stem with ethanol and methanol as ascending arrangement of polarity.

2.2. Screening bioassays with plant extracts against *A. disperses*

The experiments were conducted in the greenhouse under control condition at Malaysian Pepper Board with temperature of 25 ± 2°C and relative humidity of 80 ± 10%. Treatments included 3 concentration levels with 3 extraction solvents and 1 control via Methylene chloride, ethanol, methanol and distilled water. 3 replicated per treatment were arranged in a randomized design and the experiment was conducted twice.

2.3. Whitefly colony

Aleurodicus disperses colony were reared on tomato plants, in the specially designed whitefly cage (150cm x 90cm x 140cm) covered completely with 40 mesh insect netting. The tomato plants were allowed to grow in 15 cm plastic pot to ensure sufficient food supplied to whitefly for subsequent bioassay test.

2.4. Repellent activity on whitefly adults

Repellent activity of the three fraction extracts of black pepper berries against adults whitefly was carried out using modified method of Cruz-Estrada *et al* (2013) [13]. Repellent activity was performed in whitefly cage as mentioned above. Test solutions with different concentration were prepared using methanol solvent (1.25%, 2.5%, and 5%). The tomato plants with 7 new leaves were used for experiment in the whitefly cage located in the greenhouse. Approximately 9 ml of black pepper extract with different concentration was applied twice on whole tomato plants at 3 hours interval by using 10 ml plastic sprayer. The tomato plants were then allowed to air dry under open condition in the whitefly cage. Subsequently, 10 adult whiteflies were introduced into whitefly cage containing treated tomato plants. The adult whiteflies were allowed to survive in the cage for 72 hours. The assessment was conducted by calculating the total number of whiteflies that remain alive 72 hours after treatment. Repeat the same procedure for black pepper leaf and stem extracts for determination of repellent activity against whitefly adults.

2.5. Repellent activity on whitefly nymphs

Prior to whitefly nymphs bioassay, 10 adult's whiteflies were reared on tomato plants in whitefly cage for approximately 10 days to allow it to lay eggs. After 10 days of growth in whitefly cage, instar nymphs of whitely were collected from the lower leaf surface and counted using stereomicroscope. Black pepper extracts were prepared in a similar manner to the above mentioned procedure. The whole tomato plants were sprayed with pepper extract and kept to dry before introducing whitefly nymphs into it. The whitefly nymphs were allowed to grow for 10 days. The efficacy of black pepper extract against whitefly nymphs was determined by calculating the total number of nymphs status under the stereomicroscope analysis: dead or healthy

2.6. Data analysis

Insecticide bioassays data were reported as total number of alive adult and nymphs. The analysis of data was performed using analysis of variance and Means in column with different letters are significantly different at 0.05 level using Duncan Multiple Range Test. (SAS) [14] version 8.1 for Windows. Probit analyses were used to determine LC_{50} and LC_{90} of all pepper extracts with different solvents (Stevens, 1999) [15].

2.7. GC/MS analysis

The chemical constituents of the black pepper berries crude extracts were identified by gas chromatography-mass spectrometry (GC/MS) at the Central Pesticides Laboratory, Malaysian Pepper Board. The compounds identification was done by comparing NIST, WILEY libraries and with the authentic spectra (Adams, 1995) [16] and the data of peaks with those reported in literature. The constituent percent were computed using GC peak areas on BP-I column without applying correction factors.

3. Results and Discussion

Bioassay of three pepper berries extract against *A. disperses* indicated that after 72 hours of application, pepper

whiteflies was the most susceptible to methanol extract followed by methylene chloride and ethanol extract. The later extraction solvents were 2 fold less sensitive, respectively. The repellent effect of 3 different *P. nigrum* extract against *A. disperses* was assessed by observing the number of insect that were still alive after application. Methanol extracts of black pepper, significant insecticidal effect and could be a potential organic insecticide against *A. disperses*. This might probably be due to present of sufficient quantity of secondary metabolites with insecticide activity. This finding was supported by research reported by Padalkar and Gaikar., 2008 [17] who stated that the recovery of piperine and its constituents was the highest when methanol solvent was used. Besides, earlier research also showed that black pepper extracts not only carrying insecticide properties, but also has been widely used for their antimicrobial properties in integrated disease management against several type of insect pest in agriculture industry (Kehinde *et al*., 2018) [18].

The toxicity effects of black pepper berries extract on the adult and nymphal whiteflies were recorded in Table 1, which showed that the methanol extract cause the most high mortality percentage. The maximum number of adult and nymphal whiteflies that remain alive after the treatment was 95.1% and 82.6% respectively at the concentration of 2.5% (w/w) after 72 hours of treatment as compared to control treatment, 56.2% and 73.1% at 5% (w/w) after 72 hours of treatment and 17.1% and 50% at 10% (w/w) after 72 hours of treatment as compared to control treatment. The LC_{50} value was 1.485 for methanol with toxicity index 74.64% as in Table 2. On the other hand, the mortality percentages resulted from ethanol and methylene chloride fraction demonstrated almost similar efficacy pattern with the effective concentration was at 10% (w/w) with maximum mortality for adult and nymphal whiteflies after 72 hours of treatment as compared to control treatment were 82.9% and 50.0% respectively. The LC_{50} was 2.142 and 2.215 for methylene chloride and ethanol extract with toxicity index of 86.42 and 83.54 respectively (Table 2). This indicated that the efficacy of *P. nigrum* extract were dependent on concentration of active compound present in the extract. The higher the concentration of active compounds, the higher the repellent effect against *A. disperses.* The mortality cause by methanol extract were higher than other solvent indicated that this extraction protocol manage to extract more active compounds with insecticide activity e.g. piperine, piperidine, analine, linalool and etc. These observations were nearly agreed with those study reported by Su and Horvat (1981) [19] and Parmar *et al*., 1997 [20]. They reported that species of the family Piperaceae particularly black pepper are rich in amides as the major secondary metabolites and responsible for the insecticidal properties of these plants such as piperine ([(E,E)-1[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine]). This finding was further supported by the result of GCMS below. Also, similar results were observed previously (de Paula *et al*., 2000 and Scott *et al*., 2004) [21, 22] when *P. nigrum* extracts were tested on Lepidoptera insects.

Table 1: Number of *Aleurodicus disperses* adult and nymphs encountered live on treated cucumber plants with 3 chemical extracts of black pepper berries under controlled glasshouse condition

Extract	Conc. (%) w/w	No of live adults	No of live Nymphs
Methylene chloride	2.5	6.33±0.21b	32.77±0.52
	5	3.33±0.58e	27.66±2.31b
	10	2.22±0.60f	19.33±3.41c
Ethanol	2.5	5.11±0.72c	29.88±2.42b
	5	4.22±0.95d	28.00±2.41b
	10	2.22±0.48f	20.77±2.32c
Methanol	2.5	6.77±0.21b	29.00±3.25b
	5	4.00±0.68d	25.66±2.49ab
	10	1.22±0.31g	17.55±3.10d
Water	/	7.12±0.32a	35.11±3.05a

Means in column with different letters are significantly different at 0.05 level using Duncan Multiple Range Test.

Table 2: LC_{50} value of black pepper berries extract against the *Aleurodicus disperses* adults

Extract	LC_{50}	LC_{90}	Slope	Toxicity index (%)
Methylene chloride	2.142	17.954	1.751	86.42
Ethanol	2.215	18.523	1.689	83.54
Methanol	1.485	15.642	2.91	74.65

In the second treatment experiment, the results shown that the 3 chemical extracts of black pepper leaf and stem were not significant different in the number of live adults or nymphs from the control. The number of alive adults and nymphs is almost similar or slight higher than control (Table 3). This mean that these 3 chemical extracts of black pepper leaf and stem did not cause mortality to adults and nymphal whiteflies encountered after treatment compared to controls. The number of live adults and nymphal whiteflies were ranged between 6.88 – 7.33 and 35.11-36.22 indicated that black pepper leaf and stem extract did not carried any repellent effect against *A. disperses.* This finding was further supported by research reported by Gupta *et al*., 2013 [23] who stated that the recovery rate for piperine extracted from pepper leaf and stem was < 0.05%. The concentration of this piperamide compounds were considered redundant and will not bring any insecticide activity to any insects.

Table 3: Number of *Aleurodicus disperses* adult and nymphs encountered live on treated cucumber plants with 3 chemical extracts of black pepper leaf and stem under controlled glasshouse condition

Extract	Conc. (%) w/w	No of live adults	No of live Nymphs
Methylene chloride	2.5	7.33±0.66a	36.22±1.88a
	5	7.33±1.05a	35.11±2.98ab
	10	6.88±0.51ab	35.11±2.33ab
Ethanol	2.5	7.66 ±0.82a	36.33±3.62a
	5	7.12±0.98 b	36.22±3.98a
	10	7.88±0.66b	35.11±2.33ab
Methanol	2.5	7.12±0.23 b	35.11±2.26ab
	5	7.33±0.22a	35.11±2.23ab
	10	6.88±0.53ab	36.22±0.58a
Water	/	7.12±0.32a	36.11±3.05a

Means in column with different letters are significantly different at 0.05 level using Duncan Multiple Range Test.

The analysis by GC/MS chromatography revealed that the chemical constituents of the methanol extract of *P. nigrum* berries were characterized and identified from the mass spectral library and Kovats' retention indices. The Identification of the methanol fraction of black pepper berries extracts by GCMS analysis are shown in Fig 2. Chromatogram GC-MS analysis of the methanol extract of *P. nigrum* showed the presence of twenty five major peaks and the components corresponding to the peaks were determined and listed in Table 4. The obtained results showed that *P. nigrum* extract contain many functional groups and can be classified to monoterpenes, sesquiterpenes, scetogenins (fatty acids or derivatives), alkaloids, and miscellaneous components. Of the 25 compounds identified in *P. nigrum*, the component with the highest content was piperine (4.98%). Other major compounds included copaene (4.31%), α-pinene (4.01%), β-phellandrene (3.81%), Limonene (3.68%), pyrrolidine (2.98%), piperidine (2.85%), pyridine (2.1%) and aniline (1.32%). All of these compounds play an important role in providing repellent effect to *A. disperses* infestation. The present of these insecticide properties of black pepper further strengthen the hypothesis that black pepper poses a repellent effect against insect pest. However, Ferreira *et al* (1999) [24] in analyzed the *P. nigrum* extract had reported the main components of black pepper extract were sesquiterpene β-caryophyllene (11.8%) and the monoterpenes limonene (9.8%) and 3-σ-carene (14.3%). Similar report has also been reported on the different concentration of active compounds present in *P. nigrum* extract (Scott *et al*., 2008, Aziz *et al*., 2012, Liu *et al*., 2007) [25, 26, 27]. The explanation for this different was the constituents of different active compounds can vary with environmental conditions, such as climate, soil type and brightness.

The present of phytochemicals with insecticidal activity in black pepper has extensively studied. Among these phytochemicals, there is an increasing interest in the introduction of black pepper secondary metabolites for managing pest infestation (Jirovetza *et al*., 2002) [28]. For Example the detection of linalool, limonene, pipene and copaene in *Piper* species have been proven to be effective against insect pest (Hussein *et al*., 2017) [29]. Obtained data showed that the present of piperamides and secondary metabolites in methanol extract of dried black pepper berries can cause mortality of *A. disperses* adult and nymphs after 72 hours of treatment. Among piperamide compounds that are responsible for repellent effect include piperine, pyrrolidine, pyridine, and piperidine. The piperamide compounds almost contribute to 12.91% of the active compounds presented in pepper berries. This observation agreed with the work reported by Scott *et al* (2008) [25] which identified the piperamide compounds act as neurotoxic to insects. A similar result was reported by Singh *et al.*, 2004) [30] which mentioned that amide are the most important compounds and present in the highest concentration of all secondary compounds in berries of *P. nigrum*, but 2 to 10-fold differences can occur between samples. This research finding are also in accordance to Miyakado *et al*., 1980) [31] who stated that piperamides compounds extracted from black pepper berries were responsible for the toxicity of bean weevil *Callosobruchus chinensis* in legume crop. In conclusion, the piperamides and it components extracted from black pepper berries significantly increase the mortality of *A. disperses* adult and nymphs by different mechanism of action, being neurotoxin one of these mechanisms, at least *in vivo*. Therefore, Black Pepper extract could be a potential control mechanism for replacing contact insecticide particularly neurotoxic compounds such as carbamates, pyrethroid and organophosphate. On the basis of aforementioned results, the present piperamides compounds and secondary metabolites in black pepper berries extract provide the advantage of all of the previously mentioned attributes: novel target site, enzyme inhibition and low mammalian toxicity (Scott *et al*., 2008). The sheer abundance of piperamides and secondary metabolites suggested that black pepper extract is a promising ingredient for the production of insect repellent.

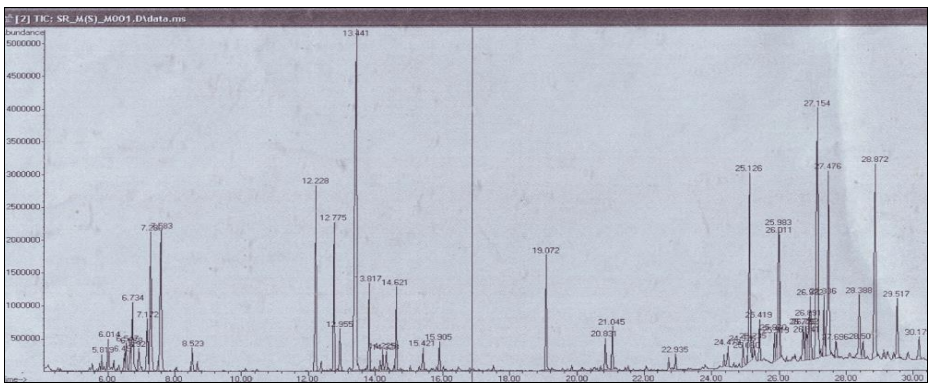

Fig 2: GCMS analysis of black pepper berries extract

Table 4: Main component identified by GCMS analysis of methanol black pepper berries extract

No	Compounds	Retention time (min)	Area,%
1	L-oxirane	5.818	1.35
2	α-pipene	6.012	4.01
3	β-phellandrene	6.666	3.81
4	β -pipene	6.732	1.22
5	β – Myrcene	6.921	1.21
6	D-Limonene	7.584	3.68
7	3-carene	8.522	0.87
8	linalool	12.228	2.58
9	copaene	12.773	4.31
10	α -terpinene	12.955	0.50
11	caryophyllene	13.440	1.00
12	naphtalene	14.226	1.02
13	carvone	15.421	0.66
14	cubebene	17.108	0.26
15	zingiberene	19.072	NC
16	piperidine	20.832	2.85
17	pyrrolidine	21.045	2.98
18	α- Elemene	21.602	NC
19	cadinene	22.935	NC
20	pyridine	24.926	2.10
21	benzamide	25.124	0.54
22	dodemorph	25.235	0.59
23	piperine	25.918	4.98
24	aniline	26.840	1.32
25	decadienanmide	30.172	1.32

NC: Not calculated

3. Conclusion

This study demonstrates that the methanol extract of *P. nigrum* berries has a high insecticide activity against the *A. disperses* adult and nymphs. Thus, it can be a possible candidate for biocontrol against this whitefly infestation particularly at pepper industry.

4. Acknowledgments

The authors wish to thank the Government of Malaysia and Malaysian Pepper Board for financing and making the research possible. We thank Sang ak Jam and Wan Ambi are also thanked for excellent technical assistance.

5. References

1. Satya V, Jindal S, Vir S. Field infestation of *Caryedon serratus* Olivier (*Coleoptera: Bruchidae*) on the pods and seeds of *Acacia niloticain* the Thar desert of India. Journal of Tropical Forest. Science. 1996; 9(2):189-193.
2. Santos JP, Maia JDG, Cruz I. Damage to germination of seed corn caused by maize weevil (*Sitophilus zeamais*) and Angoumois grain moth (*Sitotroga cerealella*). Pesquisa agropecuaria brasileira. 1990; 25(12):1687-1692.
3. Matthews GA. Insecticide Application in the Stores. In Matthews, G.A. and Hislop, E.C. (eds.). Application Technology for Crop Protection. CAB, London, 1993, 305.
4. Ngamo TLS, Goudoum A, Ngassoum MB, Mapongmetsen PM, Lognay G, Malaisse F, *et al.* Chronic toxicity of essential oils of 3 local aromatic plants towards Sitophilus zeamais Motsch (Coleoptera: Curculionidae). African Journal of Agriculture Research. 2007; 2:164-167.
5. Srinivasan K. Black Pepper (*Piper nigrum*) and its bioactive compound, piperine. Molecular Targets and Therapeutic Uses of Spices, India, 2009, 25.
6. Yap CA, Zehnder JAR. Residue and dissipation of fipronil and itsm in black pepper (*Piper nigrum* L.). OSR Journal of Environmental Science Toxicology and Food Technology. 2016; 10(6):28-37.
7. Yap CA. Quality Attributes of Sarawak Pepper (*Piper nigrum* L.) – A Review. Focus on Pepper, 2018; 10(2):1-18.
8. Byrnee DN, Houck MA. Morphometric identification of wing polymorphism in Bemisia tabaci (Homoptera: Aleyrodidae). Annals of the Entomology Society of America. 1990; 83:487-493.
9. Kajita H, Alam MZ. Whiteflies on guava and vegetables in Bangladesh and their aphelinid parasitoids. Applied Entomology and Zoology. 1996; 31:159-162.
10. Aiswariaya KK, Manjunatha M, Naik M. Biology and host range of spiraling whitefly. Karnataka Journal of Agricultural Science. 2007; 20:149-152.
11. Alim MA, Hossain AA, Rahman F. Population dynamics of spiraling whitefly on ornamental plants and its natural enemies in Bangladesh. Bangladesh Journal of Entomology. 2014; 24:47-55.
12. Su HCF. Laboratory study on effects of Anethum graveolens seeds on four species of stored product insects. Journal of Economical Entomology. 1985; 78:451-453.
13. Cruz-Estrada A, Marcela GA, Rocío BA, Esaú RS. An insecticidal effect of plant extracts on immature whitefly Bemisia tabaci Genn. (Hemiptera: Aleyroideae). Journal of Biotechnology. 2013; 6(1):16-25.
14. SAS. SAS User's Guide: Statistics. 5th edition. SAS Institute, Inc, 1992.

15. Stevens J. Intermediate Statistics: A Modern Approach. Lawrence Erlbaum Associates, Publishers, United Kindom, 1999, 424.
16. Adams RP. Text book of identification of essential oil components. Gas chromatography/ mass spectroscopy, United State, 1995, 46.
17. Padalkar KV, Gaikar VG. Extraction of piperine from Piper nigrum (black pepper) by aqueous solutions of surfactant and surfactant + hydrotrope mixtures. Separation Science and Technology. 2018; 43:11-12.
18. Kehinde AL, Olusola AI, Kafayat OA. The insecticidal effect of piper nigrum (Black Pepper) and Curcuma Longa (Turmeric) in the control of Anopheles gambiea Giles (Dip., Culicidae). Jordan Journal of Biological Science. 2018; 11(2):195-200.
19. Su HCF, Horvat R. Isolation, identification and insecticidal properties of Piper nigrum amides, Journal of Agriculture and Food Chemistry. 1981; 29:115-118.
20. Parmar VS, Jain SC, Bisht SK, Jain R, Taneja P, Tyagi OD. Photochemistry of the genus Piper. Photochemistry. 1997; 46:591-673.
21. De Paula VF, De A Barbosa LC, Demuner AJ, Pilo´-Veloso D, Picanco MC. Synthesis and insecticidal activity of new amide derivatives of piperine. Pest Manage Science. 2000; 56:168-174.
22. Scott IM, Jensen B, Nicol R, Lesage L, Bradbury R, Sa´nchez-Vindas P, Poveda L, Arnason JT, Philoge`ne BJR. Efficacy of Piper (Piperaceae) extracts for control of common home and garden insect pests. Journal of Economy Entomology. 2004; 97:1390-1403.
23. Gupta A, Monika G, Sudhakar G. Isolation of piperine and few sesquiterpenes from the cold petroleum ether extract of Piper nigrum (black pepper) and its antibacterial activity. International Journal of Pharmacy and Phytochemical Research. 2013; 5(2):101-105.
24. Ferreira SRS, Nikolov ZL, Doraiswamy LK, Meireles MAA, Petenate AJ. Supercritical fluid extraction of black pepper (*Piper nigrum* L.) essential oil. Journal of Supercritical Fluids. 1999; 14:235-245.
25. Scott IH, Jensen B, Philogène J, Arnason H. A review of Piper spp. (Piperaceae) phytochemistry, insecticidal activity and mode of action. Photochemistry Review. 2008; 7(1):65-75.
26. Aziz S, Naher S, Abukawsar MD, Roy SK. Comparative studies on physicochemical properties and GC-MS analysis of essential oil of the two varieties of the black pepper (*Piper nigrum Linn.*). International Journal of Pharmacy Phytopharmacology Research. 2012; 2(2):67-70.
27. Liu L, Song G, Hu Y. GC–MS Analysis of the Essential Oils of *Piper nigrum* L. and *Piper longum* L. Chromatographia. 2007; 66:785-790.
28. Jirovetza L, Buchbauera G, Ngassoumb MB, Geisslerc M. Aroma compound analysis of Piper nigrum and Piper guineense essential oils from Cameroon using solid-phase micro extraction–gas chromatography, solid-phase micro extraction–gas chromatography-mass spectrometry and olfactometry. Journal of Chromatography A. 2002; 9(76):265-275.
29. Hussein AE, Elhaseeb A, Mohamed RA. Toxicity of three chemical extracts of black pepper fruits against two stored grain insect pests. International Journal of Pharmacology Science Invention. 2017; 6(10):20-28.
30. Singh GP, Marimuthu C, Catalan F, de Lampasona MP. Chemical, antioxidant and antifungal activities of volatile oil of black pepper and its acetone extract. Journal of Science Food and Agriculture. 2004; 84:1878-1884.
31. Miyakado MI, Nakayama H, Yoshioka T. Insecticidal joint action of pipercide and co-occurring compounds isolated from Piper nigrum L. Agriculture Biological Chemistry. 1980; 44(7):1701-1703.