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Persistence of Imidacloprid and *β*-Cyfluthrin Residue in Black Pepper (*Piper Nigrum L.)* under Malaysia Climatic Condition

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Abstract: Persistence behavior of imidacloprid and beta-cyfluthrin in black pepper was studied following application of formulation of Solomon at 0.6 (recommended dose) and 1.2 (double recommended dose) g a.i./ha for one pepper production cycle. The results also showed that the Solomon at 0.6 mL/L concentration significantly suppressed the pest infestation in pepper vines. Pepper vine treated with Solomon produce more berries compared to control and showed no phytotoxicity. Analytical procedure of Solomon active ingredient was validated prior to actual analysis. Satisfactory recoveries ranging between 87.6%-106.7% were obtained for the fortified pepper berries samples. Results showed low level of imidacloprid and beta-cyfluthrin residues in dried pepper berries ranging between < 0.01 mg/kg to 1.43 mg/kg and < 0.01 mg/kg to 1.612 mg/kg respectively. The half-life value period for imidacloprid were found to be 2.06 and 2.30 days and for beta-cyfluthrin, these values were observed to be 1.57 and 1.49 days respectively at recommended and double recommended dose. Imidacloprid and beta-cyfluthrin residue dissipated below the limit of quantification (LOQ) of 0.01 mg/kg after 7 and 9 days respectively at recommended and double recommended dose. Soil sample collected 15 days after the last spray did not show the present of Imidacloprid and beta-cyfluthrin residue at their detection limit of 0.01 mg/kg.

Key words: Black pepper, imidacloprid, beta-cyfluthrin, dissipation, residue.

1. Introduction

Pepper (*Piper nigrum L.*), the king of spices, with its varied uses and dominance in the global spice trade, is the oldest and widely used spice in the world. It has occupied a position that is supreme and unique and is today a foreign exchange earner for several countries, including Malaysia. Pepper has secured a pivotal position in food, pharmaceuticals, perfumery and cosmetic industries. With the development of modern science and technology and greater awareness and demand among people for use of natural products, particularly in food and pharmaceuticals, pepper has indeed secured a better position and has a better prospect in the year to come. Trade in pepper can promote sustainable pepper industry, to reduce the number of poor pepper farmers in Malaysia.

Sarawak is well known for its consistency and reliable quality in the international market, Nevertheless, pepper crop is affects by several pest and diseases throughout the year. The most severe pest such as pepper weevil (*Lophobaris piperis*), Pepper tingid bug (*Diconocoris hewetii*), Green pepper bug (*Dasynus piperis*) and Mealy bug (*Planococcus citri*) cause heavy crop losses or plant death while others are less severe with minor economic significance [1]. Hot and humid tropical and subtropical climates aggravate this situation. Various fungicides have been used to control and to avoid severe crop losses. The massive and indiscriminate use of pesticide not only poses health hazards to farm worker, but also leaves harmful pesticide residue on crop and soil, and can lead to the development of pest resistance strains [2, 3].

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Recently, new interest for pepper production improvement came in the agenda of researcher due to high pepper demand as well as high pepper prices. This study is in line of such new interest and aimed to evaluate the dissipation pattern of newly developed Solomon and insecticides to pepper vine. Solomon is a newly developed solvent insecticide with very strong repellent effects. This insecticide contains two active ingredients namely imidacloprid and beta-cyfluthrin and technical grade contain more than 98% of the materials. It is a broad spectrum insecticidal active ingredient registered for application of various food commodities including fruits, vegetable and stored product. It kills insects on contact and through digestion by disrupting their nervous system and is effective against both sucking and chewing insects and has been widely used to control pests of various vegetables and fruits. Even though this product has been registered with Malaysian Pesticide Board for application of various food commodities including fruits, vegetable, but the effect and maximum residue level of the said insecticide on black pepper is still unknown. Therefore, the objective of this study was to determine the residue level of Solomon in black pepper grown at the supervised field trial. Another important mandate was to determine the MRL and Pre-harvest interval of Solomon after the application.

2. Material and Methods

2.1 Chemical and Reagents

Beta-cyfluthrin (purity 99.0%) and imidacloprid (purity 99.0%) were purchased from Sigma-Aldrich, Malaysia. The imidacloprid and beta-cyfluthrin formulation (Solomon) was obtained from Bayer (Co) Sdn Bhd., Malaysia. All the solvent, namely acetonitrile, ethyl acetate and ethanol were HPLC grade. Primary secondary amine (PSA) sorbent was purchased from Agilent Technologies, USA. The other reagents namely acetic acid, ammonium formate, anhydrous sodium sulphate and magnesium sulfate were analytical reagent grade and purchase from the Merch Malaysia Sdn Bhd. All common solvents were redistilled in all-glass apparatus before use.

2.2 Field Trial

The residue trials were conducted for one pepper production cycle (flowering stage to harvesting stage) at main commercial grower's plots in Kuching Sarawak from 2015-2016. The chemical characteristic of experimental site soil were organic matter = 0.21%; pH = 5.6; sand = 71%; silt = 14.2%; clay = 12.2% and Electrical Conductivity (EC) = 0.28 dsm^{-1}. The plots having 30 mature pepper vines (5 years old vines) each were selected and treated with Solomon as described below. Pepper vine of the variety "Kuching" was planted with spacing of 2.1 m between the row and 2.1 m within the row, with a population of 2,000 plants per ha. The treatment consisted of 3 doses of Solomon (22.5, 45 and 90 g active ingredient per ha), 1 dose of Decis (0.225 mL/L active ingredient per ha) and untreated control. The pesticide was applied with a motorized sprayer at monthly intervals up to a maximum of 7 applications (complete fruiting cycles) before harvesting. The development of the pepper berries to maturity took 8 months from flowering to full ripeness. Details of the experimental treatment are given in Table 2. The first spray was performed 2 week after flower formation, followed by a spray of monthly interval for 7 months (harvesting stage) between September 2015 to April 2016.

2.3 Assessing for the Incidence of Pest Infestation

Observations on the pest infestation was recorded at 4 days interval after last spraying by following the score charts as showed in Table 1 below. The percent (%) disease index (PDI) was worked out by using modified Mckinney's (1923) formula [4]:

$$\text{PDI}=\frac{\text{Sum of all number rating}}{\text{Total number of leave observe}}\times\frac{100}{\text{Maximum grade in the score chart}}$$

Table 1 Score chart for pest infestation.

Grade	Disease intensity	Description
0	0	No insect
1	1-20	1-5 insects
2	21-40	6-10 insects
3	41-60	11-15 insects
4	61-80	16-25 insects
5	> 80	> 25 insects

An observation for phytotoxicity effect of Solomon was made in the vine after each spray in the field trials. The leaves, spike and fruit were regularly examined for injury of leaf tip, leaf surface, wilting necrosis, epinasty and hyponasty. During harvesting season, all remaining pepper vine in the cultivation area of each replicate were harvested for determination of yield per vine. All data was statistically analyzed using analysis of variance (ANOVA) as applicable to a split-plot design [5]. The significance of the treatment effect was determined using Duncan's Multiple Range Test (DMRT) and least significant differences (LSD) will be calculated at the 5% probability level.

2.4 Sampling Procedure

The fresh sample of pepper berries collected on 2 hours, 3, 5, 7 and 9 days after the last spray were then undergo series of process to produce black pepper. The pepper berries were separated from pepper spike right after harvesting. The separated green pepper berries were then undergo blanching processing by soaking the pepper berries in boil water for 1 min and drying for 2 days under sunlight following accepted practices before send for laboratory analysis. All dried black pepper was then powdered using blender from which 3 g sample was collected in triplicate for estimation of residues. Soil samples were collected from the periphery of each pepper vine at a depth of 15 cm. From each treatment, 5 kg of soil was collected, pooled, air dried and passed through a 2 mm-sieve. A representative 20 g soil samples in triplicate was analyzed for Imidacloprid and beta-cyfluthrin.

2.5 Method Development and Validation

For recovery study, ground pepper sample were fortified with known amount of imidacloprid and beta-cyfluthrin standards. Appropriate amounts of imidacloprid and beta-cyfluthrin standards were spiked onto pepper samples to obtain the recoveries at 0.01, 0.1 and 0.5 mg/L concentration. Three replicates were prepared for each sample. Recovery of imidacloprid and beta-cyfluthrin were calculated using the following equation:

Recovery of active ingredient = Detected active ingredient (mg/kg)/spiked active ingredient (mg/kg) × 100

2.6 Analysis of Pesticides

Extraction of imidacloprid and beta-cyfluthrin residues from pepper berries was carried out using a multi residue method modified from a published method [6]. Homogenized pepper berries sample (2.5 g) was put into a 250 mL bottle, followed by water (7.5 mL), acetonitril (10 mL), magnesium sulphate (4 g) and sodium chloride (1 g). The mixture was homogenized using homogenizer (IKA Ultra Turrax) for about 1 min. The entire solution was centrifuged for 5 min at 4000 rpm. The supernatant was transferred to a fresh tube, and 1.2 g of magnesium sulphate and 0.4 g of Z-Sept was added. Following centrifugation for 5 min, 6 mL of clean extract was diluted with 600 µL of water. The diluted extract was analyzed by Liquid chromatography mass spectrometry (LCMS).

3. Results and Discussion

3.1 Efficacy of Solomom against Pepper Pest Infestation

Prior to the spray applications, there were very high numbers of pests on the trees and evidence of recent leave and berries drop below the vines. The analysis of variance (ANOVA) on the pest infestation indicated that percentage of pest incidence differed significantly among different treatment from 5 to 30 days after the treatment (Table 2). All the pesticides treated vine had significantly lower percentages of pest incidence than the control vines. During the different dates of application and observation, Solomon at 120 g a.i concentration significantly suppressed the pest incidence with the percentage of PDI value ranged between 0-5.25%, followed by the lower doses, 60 g a.i AI with PDI value ranged between 0-6.26%. The Solomon applied at 30 g a.i AI showed the lowest PDI values (3.21-26.16%) among the Solomon treatment and greatest PDI% was detected in control (16.35%-57.23%). The results also showed that the maximum pest suppression was brought out by the first 25 days after the treatment thereafter the efficacy of the solomon treatment was decline. This phenomenon was expected because Mandal et al., 2010 [7] had reported that imidacloprid and beta-cyfluthrin have a very short half life with the half value of 1.39 and 1.74 days respectively. This effect might be attributing to the fact that pepper is a crop of hot humid tropics and its characteristic climatic requirements are high rainfall and temperature and high relative humidity. The high rainfall ranging between 2000-3500 mm/year reduce the repellent effect of the Solomon and thus reduce the efficacy of Solomon in pepper pest. Besides that, pepper has been planted in Sarawak for more than a decade, therefore this area was considered as a pest and disease pool for black pepper. Once the repellent effect was gone, they will reattack the pepper vine for food and shelter. Moreover, all the doses of Solomon had not caused any phytotoxic effect to the pepper vines.

3.2 Black Pepper Yield

Due to higher pest infestation, controlled vines produced less spike and more light berries (0.69 kg/vine), whereas Solomon treated trees produced more quality berries which found to be statistically significant (Table 2). Pepper vines treated with 0.6 g/L Solomon produce more quality berries with the production per vine was 1.69 kg, followed by vines treated with 1.2 g/L Solomon (1.64 kg). Plot treated with 0.3 g a.i Solomon showed low pepper yield with the production per vine only 0.95 kg/vine. There is no significant different between this two treatment regimens indicated that the efficacy of this two treatment regime can be recommended to be use in pepper industry.

3.3 Efficacy of Analytical Method

The recovery experiments were carried out at different levels to establish the reliability and validity of the analytical method following the principles as per SANCO document (12495/2011). Sample of the pepper berries were spiked with imidacloprid and

Table 2 Yield and efficacy of imidacloprid and beta-cyfluthrin against pepper pest following application at 30, 60 and 120 g a.i./ha.

Dose (g a.i./ha)	PDI% (Days after spray)												Yield	
	5		10		15		20		25		30			
	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
Untransformed value														
30	12.58	1.23	15.52	1.35	18.51	2.52	21.85	3.25	26.16	4.23	32.01	3.21	0.95	0.12
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.23	0.03	6.26	0.02	1.69	0.31
120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.15	0.02	5.25	0.03	1.64	0.25
control	16.35	3.23	22.39	1.25	26.85	2.36	37.56	5.26	42.85	57.23	52.69	56.25	0.69	0.10
P value	0.001		0.001		0.001		0.001		0.001		0.001		0.001	
F value	200.02		1498.52		326.25		759.32		200.26		142.12		652.35	
Transformed value														
60	(6.90)		(7.06)		(7.86)		(8.96)		(10. 56)		(4.52)		7.85	
120	(4.26)		(5.02)		(5.66)		(6.54)		(8.90)		(3.96)		6.05	
control	(13.62)		(14.93)		(17.52)		(25.63)		(22.29)		(11.36)		17.52	

Table 3 Percentage recoveries of Imidacloprid and Beta-cyfluthrin from spiked pepper berries.

Active ingredient	Spike concentration	Percentage recovered (%)				SD	% RSD
		R1	R2	R3	Average		
Imidacloripid	0.01	100.5	103.3	106.7	101.9	2.0	1.9
	0.1	92.1	90.4	97.5	93.3	3.7	3.7
	0.5	87.6	90.5	93.5	90.5	3.0	3.1
Beta-cyfluthrin	0.01	95.2	103.2	99.6	99.3	4.0	4.1
	0.1	100.6	94.2	98.1	97.6	3.2	3.2
	0.5	100.6	104.6	103.0	102.73	2.0	2.1

SD= Standard deviation RSD= Relative standard deviation.

beta-cyfluthrin at level of 0.01, 0.05 and 0.10 mg/kg. From the results obtained, the recoveries rate for imidacloprid ranged between 87.6%-106.7% and beta-cyfluthrin ranged between 95.2%-104.6% with both relative standard deviation (RSD) of < 4.1% were obtained from overall recovery data of 3 level of spiking suggested that the analytical method used for imidacloprid and beta-cyfluthrin were effective (Table 3). Therefore, the results have been presented as such without applying any correction factor. The control plot samples from untreated plots and reagent blanks were also processed in the same was so as to detect interferences. The limit of quantification (LOQ) of the analytical method for imidacloprid and beta-cyfluthrin in pepper berries were 0.01 mg/kg. The LOQ is the lowest level of spiking (0.01) mg/kg that gives acceptable recovery (87.6%-106.7%), precision (relative standard deviation of recoveries < 15%) with good linearity (R^2 = 0.9986 and 0.9958 for Imidacloprid and Beta-cyfluthrin). Examples of chromatogram of imidacloprid and beta-cyfluthrin peak are shown in Fig. 1 and Fig. 2.

3.4 Persistence of Imidacloprid and Beta-cyfluthrin in Pepper Berries

Data on imidacloprid and beta-cyfluthrin residue in dried pepper berries from supervised residue trials are presented in Table 4. Results from the residue trials indicated that imidacloprid applied at the recommendation rate resulted in low residue in pepper berries ranging from < 0.01 mg/kg to 0.607 mg/kg. The highest recorded for imidacloprid residue was detected in trial with application of two time maximum recommended rate of active ingredient (1.20 mL/L) with the residue value of 1.43 mg/kg. The mean initial deposit of imidacloprid were 0.607 and 1.43 mg/kg on pepper berries after 7 application of Solomon at recommended and double recommended doses respectively. After 5 day the corresponding residue came down to 0.121 and 0.326 mg/kg, representing a loss of 80.07% and 77.62%. These deposit dissipated to below determine limit, < 0.01 mg/kg after 7 and 9 days at recommended and double recommended doses respectively. Sharp fall in imidacloprid residue in the first 5 days could be attributed to the physical removal by the weathering in view of their poor contact with plant material [8]. The present of waxy hydrophobic layer on the leaves surface became a barrier to retain the fungicide on the surface and thus reduce the efficacy of the fungicide on pepper vine. This research finding is consistent with the results reported by Mukherjee and Gopal (2000) [9] who report that the safe waiting period for application of imidacloprid on brinjal were 5 and 7 days following application of 42 and 84 g a.i. of imidacloprid. Residue data were subjected to regression equations for half-life values. The half-life equations determination coefficients (R^2) were ranged from 0.818 to 0.8218 (Table 4). The study revealed that imidacloprid dissipation rate in black pepper was independent of initial deposit. The theoretical half live of imidacloprid at the recommended and double recommended dose showed less variation on the trial for both cases. The average half-life values were 2.06 and 2.30 days for both lower

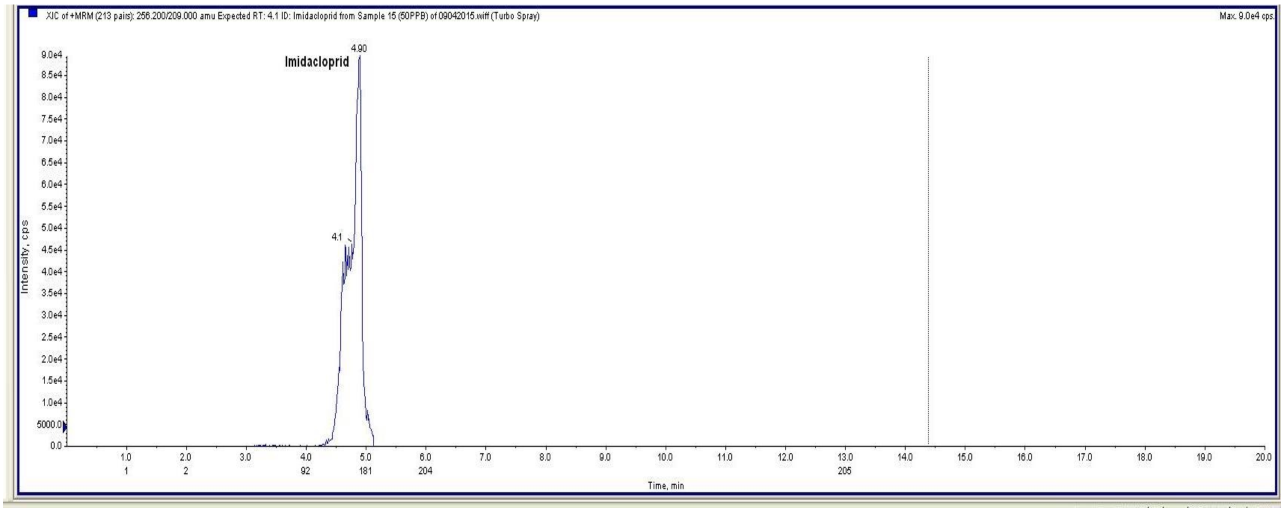

Fig. 1 LCMS chromatogram of imidacloprid standard at retention time of 4.1 min.

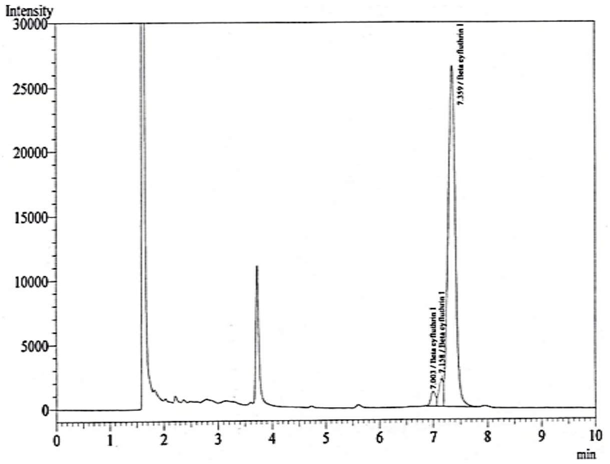

Fig. 2 LCMS chromatogram of beta-cyfluthrin at retention time of 7.158 min.

and higher doses indicated that the initial residue decreased to its half within 2.06 and 2.30 days respectively. The results also indicated that the pre-harvest interval for imidacloprid applied at the recommended and double recommended rates was at the day 7 and day 9 after the last spray.

In term of beta-cyfluthrin residue values, the residue value is slightly higher than imidacloprid residue. The beta-cyfluthrin residue can be detected in almost of the sample collected with the residue value ranging between < 0.01 mg/kg to 1.612 mg/kg. The highest residue value was detected in the farm that has been applied with two time maximum recommended rate with the residue value of 1.612 mg/kg. The initial total residue of beta-cyfluthrin in pepper berries sample were 0.853 mg/kg and 1.612 mg/kg respectively in both standard and double doses. No residues of beta-cyfluthrin on sample collected from control plot. Residue concentration decreased rapidly on the first day of application. These initial residues deposited to 0.141 and 0.244 mg/kg after 5 and 7 days at single and double doses respectively, thereby showing a loss of about 83.5% and 84.9% following application of 0.6 and 1.2 g. a.i of Solomon. The residue concentration of beta-cyfluthrin in pepper berries reach below the detectable limit of < 0.01 mg/kg in 5 and 7 days at recommended and double recommended dose respectively. This indicated that the PHI value for beta-cyfluthrin applied at 0.6g a.i and 1.2 g a.i were on the day 5 and day 7 respectively. Half-life values of beta-cyfluthrin calculated as per Hoskins 1961 [10] were observed to be 1.57 and 1.49 days respectively. Similar results were reported by Disshit et al., 2001. The decline of the residue may be attributed to growth dilution between application and sampling, volatilization that occurs during the first days following application, transfer of imidacloprid and beta-cyfluthrin from plant to soil due to the systemic properties of Solomon, sunlight UV radiation, or other complex condition. In addition, the low level of imidacloprid and beta-cyfluthrin residue might probably also due to the blanching method used in the drying process.

Table 4 Residue of imidacloprid and beta-cyfluthrin (mg/kg) in pepper berries and soil following application at 0.6 and 1.2 g a.i./ha of Solomon.

DAS	Imidacloprid		Beta-cyfluthrin	
	210	420	90	180
0*	0.607	1.43	0.853	1.612
3	0.320	0.78	0.442	0.851
5	0.121	0.32	0.141	0.403
7	< 0.01**	0.04	< 0.01**	0.214
9	< 0.01	< 0.01**	< 0.01	< 0.01**
Soil samples after 15 days	0.01	< 0.01	< 0.01	< 0.01
½ life equation	y = -0.0814x + 0.4701	y = -0.2007x + 1.1761	y = -0.1143x + 0.6731	y = -0.215x + 1.29
R^2	0.818	0.8218	0.8421	0.8587
Half- life	2.06	2.30	1.57	1.49

0* 2 hours after last spray ** Recommended pre-harvest interval R^2, half-life equations determination coefficients.

The results obtained also showed that the soil sample collected after 15 days after treatment did not present any residue of imidacloprid and beta-cyfluthrin at LOQ of 0.01 mg/kg. Though soil was not directly treated with insecticide, it was found to contain trace of imidacloprid and beta-cyfluthrin (Table 4). This may be due to the fact of direct fall of insecticides from stem and foliage to the soil while applying, wash-off by rainfall and other mechanism of physical removal of insecticides.

4. Conclusions

Spraying the Solomon has limited the pest infestation in pepper vines. The disease incidence reduced was greater when Solomon sprayed at 0.6 g a.i ha^{-1} onwards. Treating vines with this concentration provide 100% reduction of pest infestation through fruiting cycles. Besides that, application of this treatment regime do not give any phytotoxicity effect on pepper vine indicating that this is an optimum rate for pest control in black pepper. Results obtained suggested that the use of combination of imidacloprid and beta cyfluthrin at the recommended and double the recommended rate does not seem to pose any hazard to consumers if a waiting period of 9 days is observed before consumption of black pepper.

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