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Residue and Dissipation of Fiproniland Its Metabolites in Black Pepper (*Piper Nigrum L.*)

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Abstract: *Fipronil is a systemic insecticide having persistence in the plant system and is used for the management of the flea beetle, white flies, leaf hopper, green pepper bug and pepper tingid bug in black pepper. In field experiment, Regent 80WG at 45.0 g a.i ha^{-1}– 90.0 g a.i ha^{-1} significantly suppressed the pest infestation. Pepper vine treated with Reagent 80WG produce more berries compared to control and showed no phytotoxicity. Hence, the optimum rate of Regent 80WG were fixed to 45.0 g a.i ha^{-1}. For the residue analysis, satisfactory recoveries ranging between 92.1%-110.1% were obtained for the fortified pepper berries samples. Fipronil dominated as the parent compound and among the metabolites, the sulfone derivative was the most dominant followed by the sulfide, amide and desulfinyl, which were found below determination limit (0.01 mg kg^{-1}). It can be concluded that the pre-harvest interval for Regent 80WG was on day 12 and the proposed MRL value was 0.20 mg/kg.*

Keywords:*Fipronil, black pepper, maximum residue level (MRL), Regent 80WG, phytotoxicity*

I. Introduction

The commercially traded black pepper is produced from the most important species of the genus *Piper* of the family Piperaceae. Pepper has been known from ancient times and has been referred to as the “King of Spices” and “Black Gold” revealing the important attached to the spice (JRG, 2009). The pepper grown in subtropical climatic conditions are threatened by the proliferation of insects resulting in massive loss in yield. It is predicted that about 30-40% losses take place annually due to different insect pests of black pepper(Wong, 2002 and Yap, 2015).Pesticides are an inevitable tool in controlling these pests. For better yield and quality, insecticides are repeatedly applied during the entire period of growth and sometimes even at the fruiting stage. As results of pesticide applications the pepper berries absorb the agrochemicals and on consumption by human beings create health hazards to human population. Since the pepper are often consumed either raw or without much processing or storage, this lead to exert adverse effect on human health in addition to disturbing the ecosystem (Abou-Arab, 1999 and Kumari*et al*., 2003).

There are numerous classes of pesticide, with different modes of action as well as different potential for adverse effect on health and environment. Malaysian Pesticide Board indicated 260 compounds are registered and used as pesticides to control various pepper pest and diseases. Of these, only 5 active ingredient were used in pepper industry namely lambda-cyhalothrin, deltamethrin, carbofuran, acetamipridandchloropyros (Anonymous, 2012). The continuous used of these pesticides will finally lead to the development of resistant strains due to long term utilization. Recently, new interest for pepper production improvement came in the agenda of researcher due to high pepper demand as well as high pepper prices. Fipronilis a phenyl pyrazole insecticide having broad spectrum activity against household and agriculture pests. Fipronil represents the second generation of insecticide that act on the gamma-aminobutyric acid (GABA) receptor system as a non-competitive blocker. It interferes with passage of chloride ions through GABA-regulated chloride ionophore thereby disrupting inhibitory postsynaptic neuron activity, resulting in uncontrolled central nervous system function and eventually causing death (Tingle et al., 2003). It is one of the most persistence, lipophilic and toxic insecticides licensed for use. Degradation of Fipronil takes place either by reduction to sulfide, oxidation to sulfone, hydrolysis to amide and photolysis to desulfinyl (Hainzal and Casida, 1998). Application of this pesticide in agro-ecosystem perpetually leaves their residue in varying quantities and hazards depend on the chemical nature, its rate of metabolism, the toxicity of the metabolites, etc.

A number of scientistshave long been interested in the effect of pesticides onpepper pest and its residue in food. Even though Fipronil 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 objectives of this study were to evaluate the phytotoxicity and the optimal dosage of newly developed Regent 80WG insecticides to pepper vine. At the same time todetermine the residue persistence and maximum residue level (MRL) of Regent 80WG in black pepper after the application.

II. Material And Methods

2.1 Chemical and Reagents

Fipronil (purity 97.5%) and DesulfinylFipronil (purity 97.8%) were purchased from Sigma-Aldrich, Malaysia. Fipronilsulfone MB-46136 (purity 99.7%), Fipronil sulfide MB-45950 (purity 98.8%) and the formulation of Fipronil (Regent 80WG) was obtained from Bayer(Co) Sdn Bhd., Malaysia. All the solvent, namely acetonitrile, ethyl acetate and ethanol wereHPLC 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

Three supervised residue trials were conducted for one pepper production cycle (flowering stage to harvesting stage) at two commercial grower's plots in Kuching and Serian, Sarawak from 2015-2016 namely Ng Chin Leong farm, Serlan farm and PIJ Plantation farm. The plots having 30 mature pepper vines (5 years old vines) each were selected and treated with Fipronil (Regent 80WG) using the manufacturer's recommended rate (45.0 g a.iha^{-1}) and two time maximum recommended rate (90.0 g a.iha^{-1}). 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 Regent 80WG (22.5, 45.0 and 90.0 g active ingredient per ha), 1 dose of Decis (0.225ml/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 month (harvesting stage) betweenSeptember 2015 toApril 2016.

2.3 Assessing for the Incidence of Pest Infestation

The observation on the number of pepper pests were performed on 10 random chosen pepper vines per plot. Counting was done early in the morning between 6-7 am when the insects were less active and the numbers of insect pests were recorded. Specimens were collected for identification of species. For the efficacy study against fruit and shoots infestation, the berries from 20 spikes of 10 tagged vines were harvest 15 days after the last spray and number, weight of berries were recorded.Observations on the disease incidence 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 Mckinney's (1923) formula:

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

An observation for phytotoxicity effect of Regent 80WG 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 (Gomez and Gomez, 1984). 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.

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

2.4 Sampling Procedure

The fresh sample of pepper berries collected on 2 hours, 3, 5, 7, 9 and12 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 minute 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 3g sample was collected in triplicate for estimation of residues.

2.5 Method Development and Validation

For recovery study, ground pepper sample were fortified with known amount of Fipronil standards. Appropriate amounts of Fipronil standards were spiked onto pepper samples to obtain the recoveries at 0.01, 0.1 and 0.5 mg L^{-1} concentration. Three replicates were prepared for each sample. Recovery of Fipronil was calculated using the following equation:

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

2.6 Analysis of Pesticides

Extraction of Fipronil residues from pepper berries was carried out using a multi residue method modified from a published method(Anastassiades et al., 2003). Homogenized pepper berries sample (2.5g) was put into a 250ml bottle, followed by water (7.5ml), acetonitril (10ml), magnesium sulphate (4g) and sodium chloride (1g). The mixture was homogenized using homogenizer (IKA UltraTurrax) for about 1 minute. The entire solution was centrifuged for 5 minutes at 4000rpm. The supernatant was transferred to a fresh tube, and 1.2g of magnesium sulphate and 0.4g of Z-Sept was added. Following centrifugation for 5 minutes, 6ml of clean extract was diluted with 600µl of water. The diluted extract was analyzed by liquid chromatography mass spectrometry (LCMS).

2.7 MRL Estimation

A European Union method was employed for calculation of MRL values. The estimation was based on the equation shown below:

$MRL= R = KS$

R= Mean of HR** (Highest Residue after Pre-Harvest Interval, PHI)

K= One-sided tolerance factor for normal distribution with 95% confidence interval

S= The standard deviation of HR after PHI

III. Results And Discussion

3.1 Identificationof Pepper Pests

The preliminary results showed that the newer insecticide namely Regent 80WG were effective against several black pepper pests as described below:

(i) Flea Beetle

Species name:*Lanka ramkrishnae*

The adult is blushish black and ~2.5mm long (Fig.1). It has well-developed femurs and thus move by jumping than by flying. The adults feed on both young and mature leaves, shoots and spikes. Damaged young leaves and shoots become distorted and in mature leaves, hole are left behind after effect of feeding. The damaged portion of the flowerspikes turn yellow and fail to produce berries.

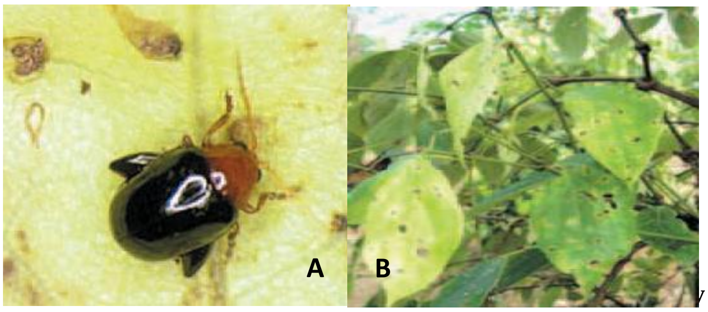

Fig 1: Flea beetle. (A) Adult; (B) Damaged leaves

(ii) Pepper Tingid Bug

Species name:*Diconocorishewetti Distant*

The adult is black and 4.5 mm long, with two humps or protruding “shoulders” on its thoracic region. The front wings have lace-like patterns (Fig 2). The adults feed by sucking the sap from the flower spike and lead to prematurely spike drop and wilt. On less severely damage spikes, the formation of berries in sparse and uneven. These damages results in direct crop loss

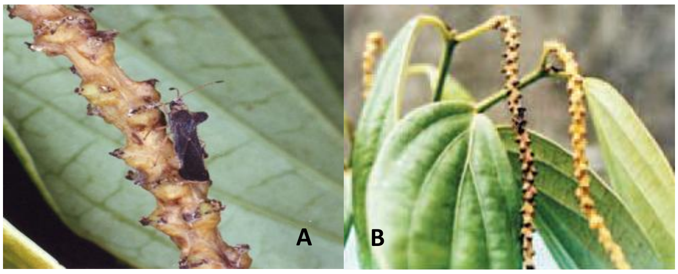

Fig 2: Pepper Tingid Bug. (A) Adult; (B) Damaged fruit spike

(iii) Green Pepper Bug

Species name:*Dasynuspiperis*

The adults are light green and 12-13mm long, with a narrow body (Fig.3). The front wings contain many vines. The adults are easily disturbed and do not fly very far. The adults suck sap from both immature and mature berries. The damaged immature berries turn brown, becoming hollow and fall off from the fruit spikes. On damaged mature berries, brown to black marks on the pericarp are seen but the seeds are not damaged. These berries results in lighter and lower quality pepper corns.

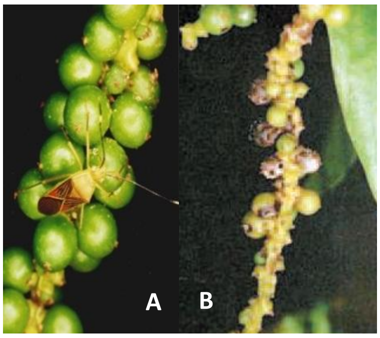

Fig 3: Green Pepper Bug. (A) Adult; (B) Damaged fruit and spike

(iv) Leaf Hopper

Species name:*Kalitaxillasinica*

The adult has clear, membranous lace-like wings and is 3.0 -3.5mm long (Fig. 4). Leaf hopper is a leaf sucking insects. The adult suck sap from young leaves, producing pin-like brown sucking marks with white to yellow irregular shaped halo on the leaves.

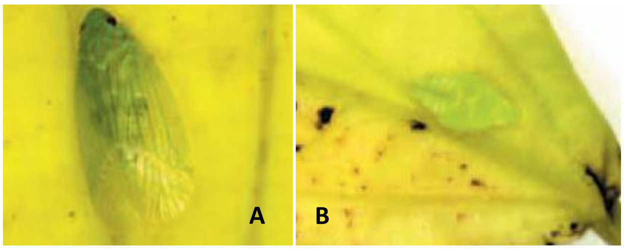

Fig 4: Leaf Hopper. (A) Adult; (B) Damaged leaves

(v) White Flies

Species name:*Aleurodicusdispersus*

The adult is approximately 30 mm long. Like other whiteflies, adults are quite active and are commonly found feeding on the lower leaf surfaces (Fig. 5). The adult reduces plant growth and vitality and in some cases may cause plant death. In addition, extensive feeding produces large quantities of honeydew which promotes the growth of sooty mold and a reduction in photosynthesis.

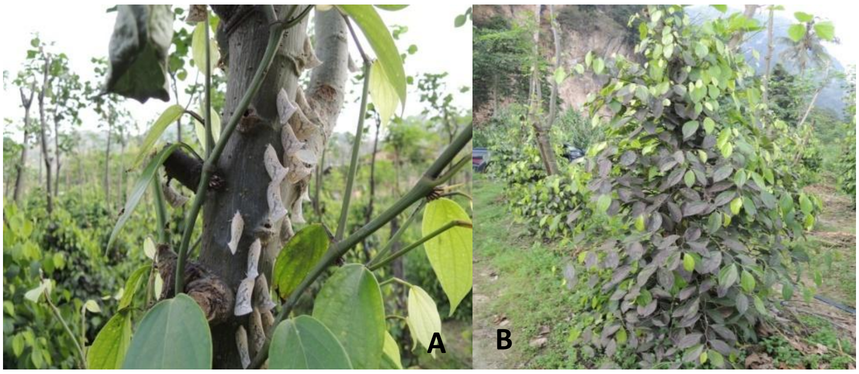

Fig 5: White flies (A) adult; (B) Formation of sooty mold on pepper leaves

(vi) Ants(*Lasiusniger*)

Ants are very common in pepper farms. They build their nests in the fruit spikes, leaves and in the mound and some species can be a source of irritation to the berry pickers during harvesting (Fig.6A). They do not cause direct damage to pepper vines, however, some species at high densities can severely distrupt control programes, particularly those directed towards the control of honeydew-producing insects. Soft scales, mealy bugs and aphids excrete honeydew, a sugar-rich solution derived from the plant sap. This honeydew is a food source for several common ant species and they are attracted to the honeydew (Fig.6B).

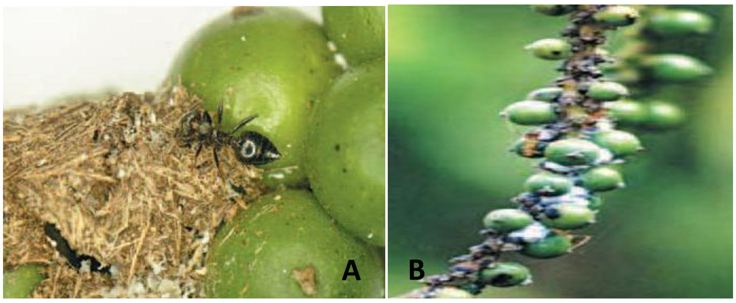

Fig 6: Ants, (A): Ant nest on fruit spike (B): Ants attracted tom honeydew

3.2 PEPPER PESTS CONTROL

3.2.1 Reductionof Fruit and Shoot Damage After Last Spray

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. There were no significant differences between trees in the percentage of infested berries or the mean number of insects per vines as detailed in Table 2. After the last spray, the overall numbers of insects in all treatments had reduced. All the pesticides treatment had significantly lower percentages of berries infested with insects than the control vines (Table 2).

The results were analyzed to account for the insects population decrease in all treatments (Table 2). Regent 80WG at 90.0 g a.i ha^{-1}concentration recorded the lowest percentage berries infestation of 13.02% with the total berries weight of 38.14% as compared to control with the berries infestation and total berries weight percentage of 41.72 % and 10.54% respectively. Similar reading was also recorded on commercialized insecticide (Decis 250) with the berries infestation and total berries weight value of 12.37% and 36.11% respectively. This was followed by the Regent 80WG at 45.0 g a.i ha^{-1}concentration with the fruit infestation and total berries weight percentage of 15.30 % and 35.05%. No significant difference was observed among Regent 80WG treatment 2, Regent 80WG treatment 3 and Decis indicated that the Regent 80WG at concentration between 45.0 g a.i ha^{-1}– 90.0 g a.i ha^{-1} was on par with commercialized product, Decis 250. The Regent 80WG at 22.5 g a.i ha^{-1}concentration recorded the highest percentage of berries infestation with the berries infestation value of 18.36%. Although the efficacy of Regent 80WG at 22.5 g a.i ha^{-1}concentration recorded the lowest efficacy value than commercialized insecticide (Decis 250), but they were significantly superior to control (Table 2).

Table 2: Efficacy of insecticide against pepper pests after the last spray

No	Treatment	Dosage	Pest infestation	
			Number basis	Weight basis
T1	Regent 80WG 1	22.5 g a.i ha^{-1}	18.36^{c} (27.34)	15.03^{b} (18.39)
T2	Regent 80WG 2	45.0 g a.i ha^{-1}	15.30^{ab}	35.05^{c}

			(25.62)	(28.42)
T3	Regent 80WG 3	90.0 g a.i ha^{-1}	13.02^{a} (23.58)	38.14^{c} (36.59)
T4	Decis 250	0.225mL/L	12.37 (20.11)	36.11^{c} (32.75)
T5	Control	-	41.72^{d} (40.51)	10.54^{a} (17.06)
Sem. ±		-	1.02	1.32
C.D at 5%		-	3.27	3.75
C.V.%		-	7.28	6.52

3.2.2Effiacy of Fungicide Against Pepper Pests

The present study was undertaken to evaluate the efficacy of Regent 80WG against pepper pests under field condition. It was evident from Table 3 that the efficacy of Regent 80WG differ significantly in managing pepper pests as described above. All the fungicide significantly reduce disease incidence as compared to control. In this study, the incidence of pepper pest infestation was observed at 4 days interval after last spray till completion of the harvesting stage. From the results obtained, the control vines showed higher number of insects with typical symptoms like blighted flower, elongated dark black spot of stalk, and berries and leaves drop. Whereas Regent 80WG treated vines showed lesser symptom than control. The reduction of pepper pests varied between doses of Regent 80WG. Among doses, 22.5g a.i ha^{-1} slightly reduces the pest infestation with the PDI value of 15.35% on days 28 after the last spray. The disease reduction over control was only 71.40%. For Regent 80WG treatment 2 (45.0 g a.i ha^{-1}) and treatment 3 (90.0 g a.i ha^{-1}), the bio efficacy was very promising with the disease reduction over control was 100%. This indicated that the bio efficacy of Regent 80WG increased with increase in the concentration (Table 3). Similar reading was also recorded on commercialized fungicide (Decis 250) with disease reduction over control was 100% as well.

Spraying of Regent 80WG has limited the pest infestation. The pest infestation incidence reduced was greater when Regent 80WG sprayed at 45.0 g a.i ha^{-1} and 90.0 g a.i ha^{-1}. Treating vines with this concentration provided 100% reduction of pest infestation compared to untreated vines for which disease incidence was 53.68 PDI. The controlling effect was mainly due to translaminar and systemic movement of Fipronil inside the tissue. Regent 80 WG at said concentration not only suppressed the pest infestation but also increase the production of pepper berries per vine basis. Several other studies demonstrated the efficacy of Fipronil in reducing disease severity. For examples, Kadam et al., (2014) and Rajinder et al., (2013) reported that Fipronil effectively controlled thrips (*Scirtothripsdorsalis H*) of Pomegranate fruits and yellow stem borer (*Scipophagaincertukas*) in rice as against control. The pesticide rate of application experiment was useful for determining the optimum rate of pesticide application. Prior to commencement of this study no work has been undertaken on optimum rates of application of Fipronil for pest control in black pepper. The results of this experiment indicated that the response to rate for completely controlling pests infestation was ranged between 45.0 g a.i ha^{-1} to 90.0 g a.i ha^{-1}. Moreover, all the doses of Regent 80WG had not caused any phytotoxic effect to the pepper vines.

Table 3: Efficacy of Insecticides on pepper pests

Pesticide	Con g/ha^{-1}	Disease Index (PDI) (%)							Disease reduction over control	Yield (kg/ vine)
		4 DAS	8 DAS	12 DAS	16 DAS	20 DAS	24 DAS	28 DAS		
Regent 1	22.5	11.87^{b} (19.46)	12.15^{b} (20.02)	11.48^{b} (19.04)	12.77 (20.87)	13.36^{b} (21.42)	16.79^{b} (23.78)	15.35^{b} (23.08)	71.40%	0.95^{b}
Regent 2	45.0	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	100%	1.59^{c}
Regent 3	90.0	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	100%	1.61^{c}
Decis	0.225	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	0.00^{a} (0.50)	100%	1.54^{c}
Control		49.52^{c} (45.12)	51.20^{c} (46.64)	48.61^{c} (42.28)	48.20^{c} (42.85)	52.60^{c} (47.52)	49.23^{c} (44.56)	53.68^{c} (49.02)		0.74^{a}

DAS- Days after spray * Mean of three replications; In a column, means followed by a common letter are not significantly different at the 5% level by DMRT, Values in parentheses are arcsine-transformed value.

3.3 Pesticide Response to Rate of Application

The pesticide rate of application experiment was useful in determining the optimum rate of Regent 50WG for pest control. The lower concentration (22.5g a.i ha^{-1}) had shown higher infection rates, more disease than the higher concentration of 45.0 g a.i ha^{-1} and 90.0 g a.i ha^{-1}. The bio efficacies of both higher concentration of Regent 80WG are similar with commercialized fungicide, Decis 250. The response of Regent

80WG pesticide to rate applied is illustrated graphically. From the graph (Fig. 7), it was clearly indicated that the pest infestation incidence decline drastically from 22.5g a.i ha^{-1} to 45.0 g a.i ha^{-1} and 90.0 g a.i ha^{-1}. The graph also showed that the optimum rate was arrived at 45.0 g a.i $ha^{-1}$by considering the flattening of disease curve between rates (Fig. 7). Similar optimum yield was also achieved at 45.0 g a.i ha^{-1} where yield response continuously to climb with increasing rates (Fig. 7).

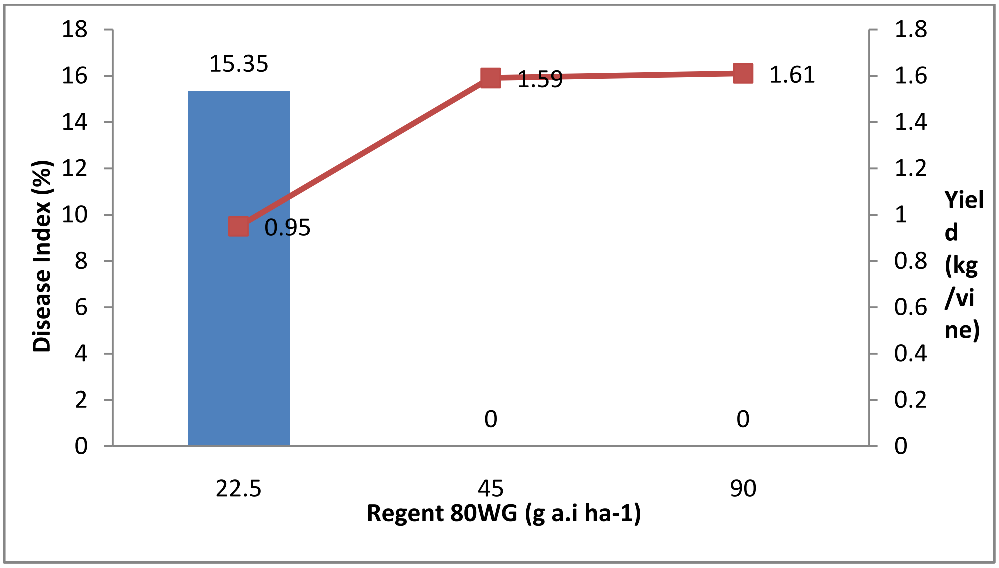

Fig 7: The effect of rate of application of Regent 80WG on disease severity (PDI) and yield (kg/vine)

3.4 Method Validation

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). From the results obtained, the mean percent recovery for driedpepper berries were ranged from 92.1%-110.1% with a relative standard deviation (RSD) of <5.4 % wereobtained from overall recovery data of 3 level of spiking (0.01, 0.1 and 0.5 mg/kg). These indicated that the analytical method used were effective for further analysis (Holland et al., 2011). The limit of quantification (LOQ) of the analytical method for Fipronil in pepper berries is 0.01 mg/kg. The LOQ is the lowest level of spiking (0.01) mg/kg that gives acceptable recovery (92.1%-110.1%) and precision (relative standard deviation of recoveries <15%). One example of calibration curve (for quantification of detected residue) with good linearity (R^2 = 0.9647) within 0.02 -0.1µl/ml is shown in Fig. 8. Chromatogram example of Fipronil peaks in standard solution is shown in Fig.9

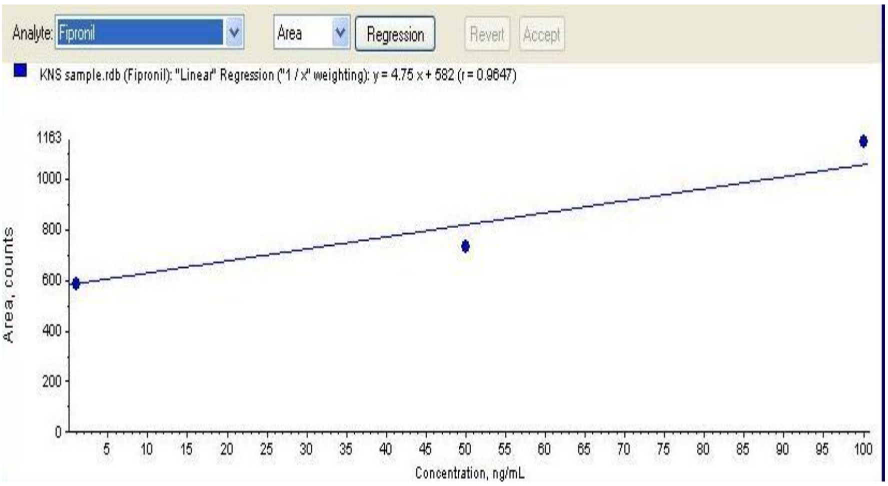

Fig 8: Calibration curve of Fipronil

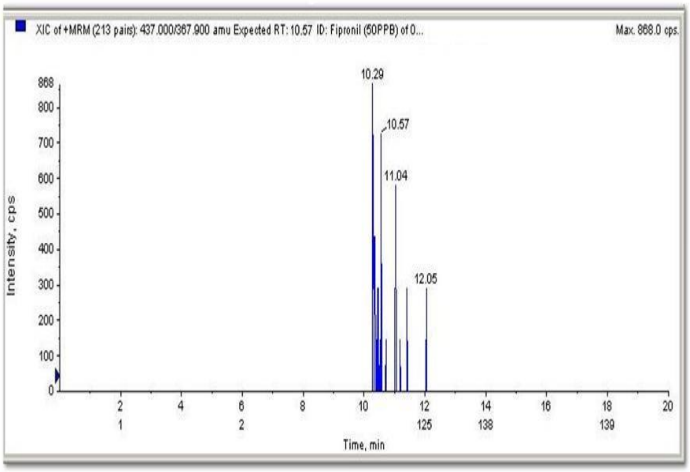

Fig. 9: The LC-MS/MS MRM chromatogram of fipronil and its metabolites. RT:10.29 – Fipronildesulfinyl; RT: 10.57 – Fipronil; RT: 11.04 – Fipronil sulfide; RT:12.05 – Fipronil sulfone

3.5 Persistence of Fiproniland Metabolites in Fresh and Dried Sample of Black Pepper

Results on the Fipronil residue in pepper berries from three (3) supervised residue trials are presented in Table 4. From the results obtained, it showed that the Fipronil applied at the manufacturer's recommendation rates (45.0 g a.i ha^{-1}) resulted in low residue value in pepper berries ranging from 0.01 to 0.17 mg/kg. The highest residue value was detected in trial located at Serlan farm with residue value of 0.17 mg/kg. The major components was the parent compound Fipronil along with a small amount of the other three metabolites viz. Fipronildesulfinyl, Fipronil sulfide and Fipronil sulfone. It constituted the parent compound Fipronil(92.9%), Fipronil sulfone (64.3%), Fipronildesulfiny (71.40%) and Fipronil sulfide (14.3%). At normal dose (45.0 g a.i ha^{-1}), initial total residue of Fipronil in pepper berries sample collected from Serlan farm, PIJ Plantation farm,and Ng Chin Leong farm were 0.17 mg/kg, 0.16mg/kg and 0.13mg/kg respectively.No residues of Fipronil on sample collected from control plot.Residue concentration decreased rapidly on the first day of application. The residue concentration of Fipronil in pepper berries were not detected from day 9th onwards. Residue data were subjected to regression equations for half-life ($t_{1/2}$) values. The average half-life (RL_{50}) value from 3 supervised residue trials site was 3.21 days indicated that the initial residue decreased to its half within 3.21 days. The results also indicated that the pre-harvest interval for Fipronil applied at the manufacturer's recommendation rates (45.0 g a.i ha^{-1}) was at the day 9 after the last spray.

For the residue trials that applied with double concentration of Fipronil (90.0 g a.i ha^{-1}), the residue detectedwas higher than recommendation rates with the highest residue value of 0.23 mg/kg was detected in trial located at Serlan farm followed by site located at PIJ plantation (0.18 mg/kg) and Ng Chin Leong farm (0.19mg/kg). It constituted the parent compound Fipronil (93.33%), Fipronil sulfone (73.33%), Fipronildesulfiny (86.67%) and Fipronil sulfide (20%).The total residue decline to below detectable level after day 12 onwards. Among the metabolites, Fipronil derivative had the highest concentration among others metabolites which persisted up to the 12 days in appreciable quantity. This was followed by the Fipronildesulfiny andFipronil sulfone. The metabolites Fipronilsulfide residue was meagre. Therefore, the degradation processes of Fipronil in black pepper seem to occur through oxidation, reduction, hydrolysis and photolysis, respectively. The presence of sulfone and Fipronildesulfiny clearly indicated that the reduction and photolysis process plays a major role in the metabolism of Fipronil. This finding was consistent with the results reported on Fipronil residue in Chili, India (George et al., 2014). The above finding also revealed that higher rate of application resulted in higher initial deposit. The average half-life (RL_{50}) value from 3 supervised residue trials site was 4.41 days indicated that the initial residue decreased to its half within 4.41 days. The pre-harvest interval for Fipronil that applied with double concentration of manufacturer's recommendation rates was on the day 12.

Overall residue data showed that the application of Fipronil ranging from 45.0 g a.i ha^{-1}- 90.0 g a.i ha^{-1}resulted in low level residue value after the last spray. The initial deposit was mainly constituted by the parent compound Fipronil along with other 3 metabolites. The low residue levels of Fipronil in pepper berries might probably due to the low dosage used and the compound's rapid degradation. The rapid degradation could be due to the wetweather at the experimental site. This assumption was supported by the rainfall data collected the study site (Fig. 10). Similar study also being done by other research who reported that the rapid degradation of Fipronil residue in crop was mainly due to the wet weather (Westcot and Reichle, 1987). Based on the overall results obtained, it can be concluded that the pre-harvest interval for application of Regent 80WG pesticide was on day 12 after the last treatment.

Table 4: Dissipation of Fipronil and it metabolite residue in dried pepper berries following application of 45.0 g a.i.ha^{-1} and 90.0 g a.i.ha^{-1} of Regent 80WG

Trial site	Days after spraying (DAS)	45 g a.i.ha^{-1}					90 g a.i.ha^{-1}				
		Fipronil residue±SD (mg/kg)	Fipronil sulfone residue (mg/kg)	Fipronil desulfenyl residue (mg/kg)	Fipronil sulfide residue (mg/kg)	Total residues (mg/kg)	Fipronil residue±SD (mg/kg)	Fipronil sulfone residue (mg/kg)	Fipronil desulfenyl residue (mg/kg)	Fipronil sulfide residue (mg/kg)	Total residues (mg/kg)
Serlan farm	0 (2h after spraying)	0.08	0.03	0.06	BDL	0.17	0.15	0.03	0.05	BDL	0.23
	1	0.06	0.02	0.05	BDL	0.13	0.10	0.02	0.04	BDL	0.16
	3	BDL	0.04	0.03	BDL	0.07	0.07	0.01	0.01	BDL	0.09
	5	BDL	BDL	0.04	0.01	0.05	0.04	BDL	0.03	BDL	0.07
	7	BDL	BDL	0.02	BDL	0.02	BDL	BDL	0.01	0.02	0.03
	9	BDL	BDL	BDL	BDL	0	BDL	0.01	BDL	BDL	0.01
	12	BDL	BDL	BDL	BDL	0	BDL	BDL	BDL	BDL	0
PIJ Plantation farm	0 (2h after spraying)	0.08	0.03	0.05	BDL	0.16	0.08	0.02	0.04	0.01	0.14
	1	0.04	0.01	0.06	BDL	0.11	0.06	0.01	0.03	BDL	0.11
	3	0.01	0.02	0.03	BDL	0.06	0.04	0.01	0.03	BDL	0.08
	5	0.01	BDL	0.02	BDL	0.03	0.02	0.01	0.01	BDL	0.04
	7	BDL	BDL	BDL	BDL	0	0.02	BDL	BDL	BDL	0.02
	9	BDL	BDL	BDL	BDL	0	BDL	BDL	BDL	BDL	0
	12	BDL	BDL	BDL	BDL	0	BDL	BDL	BDL	BDL	0
Ng Chin Leong farm	0 (2h after spraying)	0.05	0.03	0.05	BDL	0.13	0.12	0.03	0.03	BDL	0.18
	1	0.03	0.01	0.05	BDL	0.09	0.07	0.02	0.03	0.01	0.13
	3	0.01	BDL	0.02	0.01	0.04	0.04	0.02	0.01	0.01	0.08
	5	0.02	BDL	BDL	BDL	0.02	0.01	0.01	0.02	0.01	0.05
	7	BDL	BDL	0.01	BDL	0.01	BDL	0.01	BDL	BDL	0.01
	9	BDL	BDL	BDL	BDL	0	BDL	BDL	0.01	BDL	0.01
	12	BDL	BDL	BDL	BDL	0	BDL	BDL	BDL	BDL	0

BDL- Below detectable level; a.i. – Active ingredient; DAS – Days after spray

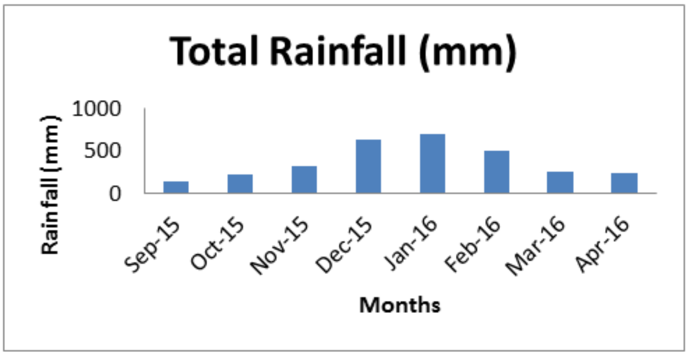

Fig 10: Rainfall data of the experimental site during treatment period

3.6 Maximum Residue Limit (MRL) Estimation

MRL for Fipronil were calculated using a European Union method(Hyder et al., 2003). This method required a minimum of 3 residue trials for a minor crop which is defined as crop which has a mean dietary intake of less than 7.5g/person/day. Black pepper is considered as a minor crop with the food consumption data by United Stated Department of Agriculture (USDA) recorded its mean dietary intake is 6.9g/person/day (USDA reference number: 02030). For MRL calculation, the highest residue from each trial will be chosen as one data point; these data points are group and computed for relevant statistical values. For values that are below the limit of quantification (LOQ), they are assumed to be at the LOQ. The estimation was based in theequation shown below:

MRL for Fipronil =R+KS

$$= 0.1833 + (1.015 \times 0.02)$$
$$= 0.2036 \text{ mg/kg}$$

R is the mean of the highest residue (HR) of very trial after PHI (3 trials with 3 highest residues in ascending order are 0.14, 0.18 and 0.23 mg/kg). S is the standard deviation and K is the one sided tolerance factor for normal distributions with 95% confidence level. It should be noted that HR of each trial will be assumed to be at the LOQ when all residue results after the PHI are < LOQ. The estimated MRL value is 0.2036 mg/kg. This value is rounded up to 0.20 following the rules set by Codex in having the common classes of MRL value such as 0.01, 0.02, 0.05, 0.1, 0.5, 1.0(FAO/WHO, 1997). Therefore, the proposed MRL of Fipronil in black pepper based on residue data is 0.20 mg/kg.

The MRL result for black pepper obtained in this experimental trial was slightly higher than MRL for other fresh crop stated in the Food Act 1983 (Malaysia)(Food Act, 2014). For example MRL for chili, cabbage, brinjal is 0.05. This results is expected cause the Fipronil residue were found to get concentrated during sun drying which could be due to high adsorption of the pesticide to the skin and the stability of the molecule to high temperature and weight reduction consequent to dehydration. The result obtained in this study corroborated the finding of George et al., 2014 wherein the sun light increase the Fipronil residue in dried foodstuffs as compared to fresh dried foodstuffs.

IV. Conclusion

Spraying the Regent 80WG has limited the pest infestation in pepper vines. The disease incidence reduced was greater when Regent 80WG sprayed at 45.0 g a.i ha^{-1}- 90.0 g a.i ha^{-1}. Treating vines with this concentration provide more than 90% 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. The results of the experiments indicated that this product could be registered for use at 45.0 g a.i ha^{-1}.Lack of MRLs for agriculture commodities may give rise to non-tariff trade barrier for the country bounded by WHO trade agreement. This study was part of an effort by Malaysian Pepper Board to establish MRLs of fungicides for export commodities. Based on the overall results obtained, it can be concluded that the pre-harvest interval for application of Regent 80WG pesticide was on day 12 after the last treatment. A proposed MRL of 0.20 mg/kg for Fipronilwas determined for pepper berries (*Piper nigrum L.*) based on residue trials data.

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