Quest Journals
Journal of Research in Business and Management
Volume 7 ~ Issue 3 (2019) pp: 75-81
ISSN(Online):2347-3002
www.questjournals.org

Research Paper

Determination of Proposed Maximum Residue Limit of New Fungicide, Amistartop in Black Pepper (Piper Nigrum L.)

Yap Chin Ann
Research and Development Division, Malaysian Pepper Board, Lot 1115 JalanUtama, Pending Industrial Area, 93916 Kuching, Sarawak , Malaysia

ABSTRACT: *Residue trials of Amistartopin black pepper were conducted to estimate the pre-harvest interval (PHI) and maximum residue limit (MRL) for one fruiting cycles. The experimental trials were conducted at three commercial grower's plot in Sarawak, Malaysia namely Kuching, Padawanand Serian farm. The farmswere divided into 3 plots with each plot contained 30 mature vines. Each plot was further treated with 3 treatments which consist of plot treated with Amistartop fungicide using the manufacturer recommended rate and two time maximum recommended rate. The fungicide was applied at monthly interval throughout production cycle using motorized sprayer with 8 applications. Pepper berries were randomly collected from the plot at 2 hours, 3, 5, 7, 9, 12 and 15 days after the last spray. The extraction procedure was validated prior to actual analysis. Satisfactory recoveries ranging between 84.30% - 109.2% indicated that the method developed was valid for subsequent analysis. Results obtained showed thatazoxystrobin and difenoconazoleresidues were at the low level with the residue value ranging between <10µg/kg to 217.34µg/kg and <10µg/kg to 65.95µg/kg respectively. The results obtained also indicated that the pepper berries could be safely consume after 15 days of application (PHI). The determined value of proposed MRL for azoxystrobin and difenoconazolewere 1.0 and 0.2 mg/kg respectively.*
Keywords: *azoxystrobin, difenoconazole, black pepper, maximum residue limit (MRL)*

Received 06 May, 2019; Accepted 13 May, 2019 © the Author(S) 2019.
Published With Open Access At www.Questjournals.Org

I. INTRODUCTION

Certain contaminants in pepper and pepper products like pesticide residue, aflatoxins, heavy metal when present even in very small quantities can cause health hazards and therefore, are quite undesired. Even their very presence is considered to be indication of poor quality. Since pepper constitute an important raw material for food industry, they are liable to be checked quality of pepper before being consumed by consumer or export. To meet the increasing demand for the better quality products by consumers and importing countries, Malaysia should be critical of it pepper's standard particularly on pesticides residue contents.The pesticide residue issue in pepper becomes even more serious as most of the peppers are often consumed in raw. This scenario finally leads to negative impact to human health besides disturbing the environment (Lambert et al., 2000).Besides, with the continuous use of pesticide in pepper industry, the pepper export will be significantly dropped nearly to half when Codex regulations are fully implemented. Thus, there is a need to determine the MRL of pesticide residues in black pepper particularly for a new crop protection product.

Black pepper is very susceptible to disease. Since the use of pesticides cannot be totally avoided in the production, one cannot produce pepper berries with "zero" pesticide residue level, though "zero" level is considered to be ideal from the health point of view. However, there should be concentrated efforts to reduce the level of pesticide residues to the minimum possible. In other words, establishment of maximum residue limits (MRLs) is necessary to determine the maximum level of pesticide residue that can be detected in foodstuffs. Therefore, a residue trial must be conducted to study the persistence and pre-harvest intervals (PHI) of pesticide residue in plant after treated with a series of agrochemical treatments.

Among the diseases, pepper anthracnose (or black berries diseases) caused by Colletotrichumgloeosporioidesand Colletotrichumcapsici is the most serious diseases (Wong, 2002). It affects both vegetative and reproductive structure. Initial infection starts from leaves and spread to spike and berries causing black berries diseases. Continuous used of these pesticides will finally lead to the development of pest resistant strain. To overcome this challenges, simultaneous administration of two or more active ingredient in ready mix formulation has become popular and are available in the market viztebuconazole + trifloxystrobin, beta cyfluthrin + imidaclorpirid, chlopyrifos+ cypermethrinetc. The study of joint action of fungicide is important for the possible extension of the use of the toxicant and also for minimizing their toxic residues in non-target organisms.

A new readymix fungicide formulation Amistartop, consist of azoxystrobin and difenoconazole used to control a wide range of fungus disease. Azoxystrobin (methyl (E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate), is a synthetic biodegradable strobilurin fungicide that act by inhibition of mitochondrial respiration in fungi. It inhibits spore germination, mycelial growth, and spore production of fungi. Azoxystrobin is reported to be effective against four major groups of plant pathogenic fungi including Ascomcetes (e.g. powdery mildews), Basidiomycetes (e.g. rusts), Deutoromycetes (e.g. rice blast) and Oomycetes (e.g. downy mildew) (Bartlett et al.,2002; Anandet al.,2008). Low use rates, flexible application methods, excellent efficacy and favourable safety profile make this fungicide well suited for modern integrated disease management programs in many cropping systems (Maienfischet al., 2001).

Difenoconazole (cis,trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether) is one of the well-known ergosterol biosynthesis inhibitors. This fungicide is a steroid demethylation inhibitor, leading to morphological and functional changes in the fungal cell membranes (Khalfallahet al.,1998). It gives rapid knockdown activity to control wide range of fungus and provided good control of soil borne disease in public health.

Amistartop is considered as one the effective broad spectrum fungicide used in various food commodities including fruits and vegetable in Malaysia. However, the relative suitability and the persistence of Amistartop residue on black pepper is still unknown. Information on the efficacy and maximum residue level is important to ensure the safety of pepper berries for consumption and export. Therefore, the present study was conducted to determine the efficacy and residue level of Amistartop fungicide in black pepper and to establish MRL for Amistartopfungicide in black pepper.

II. MATERIAL AND METHOD

2.1 Study plot, treatment and sampling

The experiment was conducted in 2016 at three commercial growerslocated at Kuching, Padawan and Serianarea. The particle size analysis showed that this brownish yellow soil is belonged to clay loam of fine sandy clay and had a pH of 5.4. Each experimental plotwas further divided into 3 treatments with each treatment contained 30 mature pepper vinesof 5 years old vines age. The three treatment consist of T1: 0.50 ml/L, T2:1.0 ml/L and T3: Control plot.The pepper vines were sprayed in the morning before 10 a.m. The fungicide was applied at monthly interval using motorized sprayer with8 applicationsbefore harvesting. The complete production cycle for black pepper from flowering to full ripeness is 8 months. Therefore the application of Amistartopwasstarted during flowering stage at the month of September. This is the month where the onset on the raining season in Sarawak. Date of Amistartopapplications are as described follows:

1st application : 11 September 2015
2nd application : 12 October 2015
3rd application : 11 November 2015
4th application : 11 December 2015
5th application : 11 January 2016
6th application : 11 February 2016
7th application :11 March 2016
8th application : 11 April 2016

After fungicide application, approximately 500 g of green pepper berries were randomly collected after 2 hours, 3, 5, 7, 9 and 15 days at post fungicide application of last spray. Overall the three trials represented 3 replicates of the residue decline pattern of applied Amistartop at the recommended rate. The sampling dates are described as follows:

1st sampling : 11 April 2016 (2 hours after last spray)
2nd sampling : 13 April 2016(3 days after last spray)
3rd sampling : 15 April 2016(5 days after last spray)
4th sampling : 17 April 2016(7 days after last spray)
5th sampling : 19 May 2016(9 days after last spray)
6th sampling : 21 May 2016 (9 days after last spray)
7th sampling : 25 May 2016(15days after last spray)

Mature green spike with 1 or 2 berries turned yellow are harvested. The fruit spikes are threshed manually to separate the green berries from spike. The separated pepper berries were then undergo blanching processing by soaking the pepper berries in hot water of about 90°Cfor 1 minute prior to drying for 2-3 days following accepted practices, with the moisture content less than 12% (ASTA 2.0). The samples finally sent to MPB quality laboratory for residue extraction and analysis. The rainfall data throughout the experiment period are shown in Figure 1.

2.2 Analytical procedure

Pesticide standards were obtained from Sigma Aldrich.Analytical grade of acetonitrile, sodium chloride, anhydrous magnesium chloride, dichloromethane and hexane were purchased from J.T. Baker, Philipsburgs, USA. The silica gel was purchase from Merck, Darmstadt, Germany. Pesticide stock solution (50 mgL^{-1}) were prepared by dissolving appropriate amounts of pesticides standards with hexane solvent. Appropriate aliquots of the stock solutions were diluted with hexane solvent to make solutions that contained 0.01, 0.1 and 0.5 $mgL^{-1}$of the pesticides.

2.3Method development and validation

The recovery study was conducted by fortified known amount of fungicide active ingredient standards with ground black pepper sample. Appropriate 10µl of azoxystrobin anddifenoconazolestandards were spiked onto grounded pepper samples to obtain the recoveries at 0.01, 0.1 and 0.5 mgL^{-1} concentration. Each experiment of single concentration was conducted in triplicate. The Recovery percentage of azoxystrobinand difenoconazolewere calculated using the following equation:

Percentage of Recovery (%) = Detected residue (mg/kg) /spiked residue(mg/kg) x 100

Recovery between 70-120% indicated that the method is suitable in determining analytequantitatively (Holland et al., 2000).

2.4 Analysis of pesticides

Extraction and estimation of azoxystrobin and difenoconazoleresidueswere carried out as according to the method of Anastassiadeset al., 2003.Approximately 2.5g of grinded homogenized pepper powder were filled into a 250ml bottle, followed by addition of 7.5 ml of water, 10ml of acetonitrile, 4 gram ofmagnesium sulphate and 1 gram sodium chloride. The mixture was then homogenized and centrifuged for 5 minutes at 4000rpm. The supernatant obtained was added with 1.2gram of magnesium sulphate and 0.4g of Z-Sept.Following centrifugation for 5 minutes, approximately 600µl of water was added to 6ml of clean extract before the extractswere injected into high resolution liquid chromatography (HRLC) for pesticide residue determination.

2.5 MRL estimation

MRL calculation was determined by using European Union method (Hyderet al., 2003). The MRL estimation was calculated using equation as shown below:

MRL= R = KS

R= Mean of highest residue value
K= One-sided tolerance factor for normal distribution with 95% confidence interval
S= standard deviation of highest residue after post-harvest interval

III. RESULTS AND DISCUSSION

Recoveries rate for azoxystrobinranged between 84.3% - 109.2% and difenoconazole ranged between 86.4%-102.2%with both relative standard deviation (RSD)of <4.2 % were obtained from overall recovery data of 3 level of spiking suggested that the analytical method used for azoxystrobin and difenoconazolewere effective (Table 1).

The limit of quantification (LOQ) of the analytical method for azoxystrobin and difenoconazolein pepper berries were10µg/kg. The LOQ is the lowest level of spiking (10µg/kg) that gives acceptable recovery 84.3% -109.2% and precision relative standard deviation of recoveries <15%. The example of calibration curve (for quantification of detected residue) with good linearity (R^2 = 0.998 and 0.9980 for azoxystrobin anddifenoconazolerespectively)within 0.02 -0.1µl/ml is shown in Figure 2 and Figure 3.

Theazoxystrobin and difenoconazoleresidue data presented indried pepper berries from 3 supervised residue trials are shown in Table 2. Results obtained showed that azoxystrobin applied at the manufacturer's recommendation rate having low residue in pepper berries ranging from <10 µg/kg to 95.21µg/kg.Azoxystrobin detected in trial 2 was the highest residue detected among the treatment when the experimental plot was treated with1.0 ml/L of active ingredient (double concentration) with the residue value of 217.34 µg/kg. Also, in general, the concentration of difenoconazole residue detected in pepper berries was comparatively lower than azoxystrobin residue. Similarly, difenoconazole residue can be detected in almost of the sample collected with the residue value ranging between <10 µg/kg to 65.95µg/kg. The results obtained also showed that the residue compound for both azoxytrobin and difenoconazole residue were at a maximum at 2 hours after spraying. Thereafter, both residue levels decreased. The degradation process of both active ingredients in black pepper seems to occurred through oxidation, reduction, hydrolysis and photolysis respectively (Kumar et al., 2013). The post-harvest interval (PHI) for application of 0.50ml/L(recommended rate) and 1.0ml/L(two time maximum recommended rate) of Amistartop fungicide were on the day 12and day 15 respectively after the last treatment. The low residue levels of azoxystrobinand difenoconazoledetected might be due to rapid degradation of pesticide compound and low dosage use. This scenario was further support by the dry field condition on the last 2 application which attribute to rapid degradation of this residue. Similarly, Braun et al., 1992 also reported that both of these residues have rapid degradation characteristic infruit and vegetables.Also, the low level of azoxystrobin and difenoconazole residue in pepper berries might probably be due to the blanching method used in the drying process.In this study, the application of two time maximum recommended rate of Amistartop was conducted to give a worst case scenario of residue or maximum level of residue that could be expected in the crop to ensure the food safety. This protocol is very important cause most of the farmer midset is that the efficacy of certain pesticide is proportional to the volume of pesticide use. The more concentrated the pesticide use, the better the control of pest and diseases. Therefore,based on the detection of pesticide residue in pepper berries, it can be concluded that the post-harvest interval (PHI) for Amistartop is on day 15 after last spray. In other words,the pepper berries could be safely consume after 15 days of application.

3.1 Maximum residue limit (MRL) estimation

A European Union method (Hyderet al., 2003) to determine the MRL for azoxystrobin and difenoconazolewas employed. Based on this method, a minimum of 3 residue trials is needed for MRL establishment with the mean dietary intake of foodstuffs of less than 7.5g/person/day. In this case, Black pepper wasfall into this category with the 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 the equation shown below:

MRL for azoxystrobin = R + KS
= 0.191+ (4.202 x 0.13)
= 0.74 mg/kg

MRL for difenozonazole = R + KS
= 0.05 + (3.215 x 0.04)
= 0.18 mg/kg

Based on European Union method for MRL calculation, The estimated MRL value for azoxystrobinand difenoconazole were 0.74 aand0.18 mg/kg respectively. This value was further rounded up to 1.0 and 0.2 following the rules set by Codex. Therefore, the proposed MRL of azoxystrobinand difenoconazole in black pepper were1.0 mg/kg and 0.2mg/kg. The proposed MRL value obtained in this experimental trial was slightly higher than MRL for other fresh crop stated in the Food Act 1983. For example MRL for azoxystrobin in cucumber is 0.5 and MRL for difenoconazole is cocoa bean and palm oil (Main commodity in Malaysia) is 0.1. This research finding is expected becauseboth of these residues were get concentrated during sun drying process. This phenomenon could be due to dry weight loss consequent to dehydration. Besides, high molecule stability at high temperature also is another factor contributes to high MRL value in dried pepper berries. In addition, this research finding was further supported by George et al., 2014 who reported that sun light increase the pesticide residue in dried foodstuff as compared to fresh foodstuff.

IV. CONCLUSION

The Lack of acceptable of MRLs for agriculture commodities by national governments lead to barriers in trade. In order to assure Malaysian pepper industry prosperity, Malaysian Pepper Board has taken an initiative to conduct a research to establish MRLs of fungicides for export commodities. On the basis of present findings it could be concludedthat the pre-harvest interval for application of Amistartop fungicide was on day 15 after the last application. A proposed MRL of 1.0 mg/kg for azoxystrobin and 0.20 mg/kg for dfenozonazolewere determined for pepper berries (Piper nigrum L.) based on residue trials data.

ACKNOWLEDGMENTS

The authors wish to thank Malaysian Pepper Board for financing and making the research possible. Lawrence akTuah, Sang ak Jam and Wan akAmbi are also thanked for excellent technical assistance.

REFERENCES

[1]. Anand, T.,Chandrasekaran, A., Pillai, A., Kuttalam, S., Senthilraja, G. Raguchander, T. and Samiyappan, R. 2008.Effectiveness of azoxystrobin in the control of Erysiphecichoracearum and Pseudoperonosporacubensis on cucumber. Journal of Plant Protection Research 48: 147-159.

[2]. Anastassiades, M., Lehotay, S.J., Stajnbaher, D. and Schenck, F.J. 2003. Fast and easy multi-residue method for the determination of pesticide residues in produces by acetonitrile extraction/partitioning and dispersive solid-phase extraction. Journal of AOAC International 86: 412-431.

[3]. Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., Hamer, M. and ParrDobrzanski, B. 2002.The strobilurin fungicides. Pest Management Science 58: 649-662.

[4]. Braun, H.E., Ritcey, G., Ripley, B.D., McEwen, F.L. and Frank, R. 1992. Studies of the disappearance of nine pesticides on celery and lettuce grown on muck solid in Ontario. Pesticide Science 13: 119-128.

[5]. George, X., Chandran, M., George, T., Beevi, S.N., Mathew, T.B., Pau, A., Arimboor,R., Vijayasree, V., Pradeepkumar, G.T. and Rakith, R. 2014. Persistence and effect of processing on reduction of fipronil and its metabolites in chillipepper (Capsicum annum L.) fruits. Environment Monitoring Assessment 186: 5429-5437.

[6]. Holland, P.T., Botd, A.J. and Malcolm, C.P. 2011. Performance validation of a multi-residue method for 170 pesticides in kiwifruit.InFajgeli, A. and Ambrus, A. (Eds).Principle and Practices of Method Validation.p. 29-40 Cambridge, United Kingdom: Royal Society of Chemistry

[7]. Hyder, K., Travis, K.Z., Welsh, Z.K. and Pate, I. 2003. Maximum Residue Level: Fact or fiction? Human and Ecological Risk Assessment 9(3): 721-740.

[8]. Khalfallah, S., menkissoqlu-spiroudi, U. and Constantinidou, H.A. 1998.Dissipation study of fungicide tetracanazol on greenhouse grown cucumbers. Journal of Agriculture and Food Chemistry 46: 1614-1617.

[9]. Kumar, V., Singh, S., Chhokar, R.S., Malik, R.K., Brainard, D.C. and Ladha, J.K. 2013. Weed management strategies to reduce herbicide use in zero-till rice-wheat cropping systems in the Indo-Gangetic Plains. Weed Technology 27: 241-254

[10]. Lambert, M., Leven, B.A. and Green, R.M. 2000.New methods of cleaning up heavy metal in soils and water.In Hubert, C. G. and Jacob, H.K. (Eds). Environmental Science andTechnology Briefs for Citizens, p. 74-90. Manhattan: Kansas State University.

[11]. Maienfisch, P., Angst, M., Brandl, F., Fischer, W., Hofer, D., Kayser, H., Kobel, W., Rindlisbacher, A., Senn, R., Steinemann, A. and Widmer, H., 2001. Chemistry and biology of thiamethoxam: A second generation neonicotinoid. Pest Management Science 57: 906–913.

[12]. Wong, M.H. 2002.Fungal Diseases of black pepper and their management in Sarawak, Malaysia.In Sim, S.L. and Lai, K. F. (Eds).Proceeding of Pests and Diseases of Pepper Symposium, p. 265. Kuching:Department of Agriculture Sarawak.

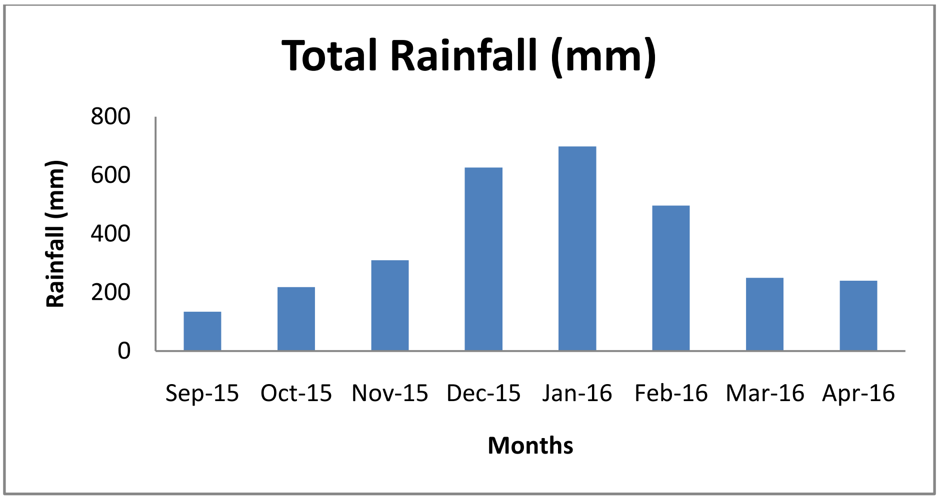

Figure 1: Rainfall of the experimental site during treatment period

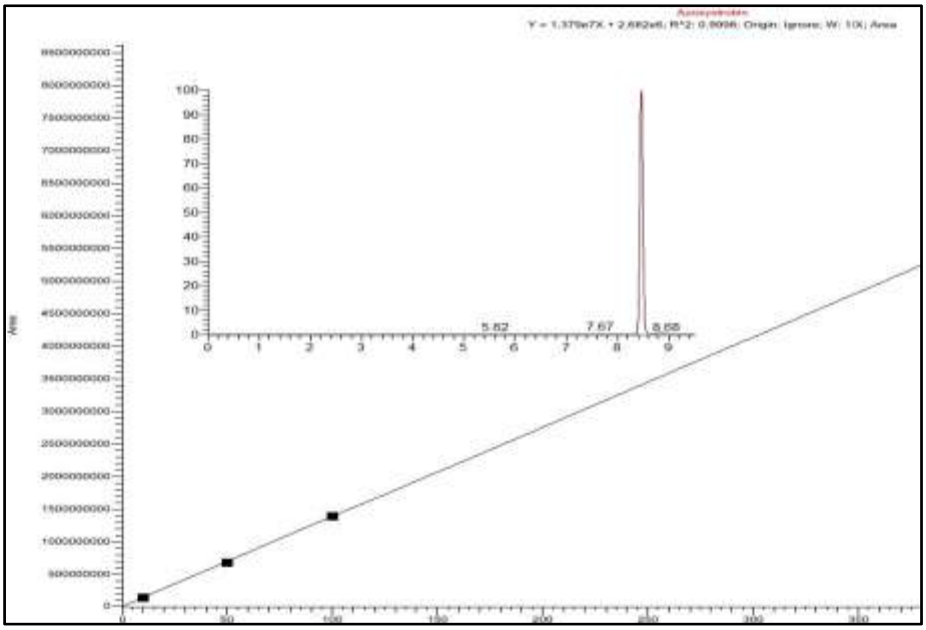

Figure 2: Calibration curve of azoxystrobin

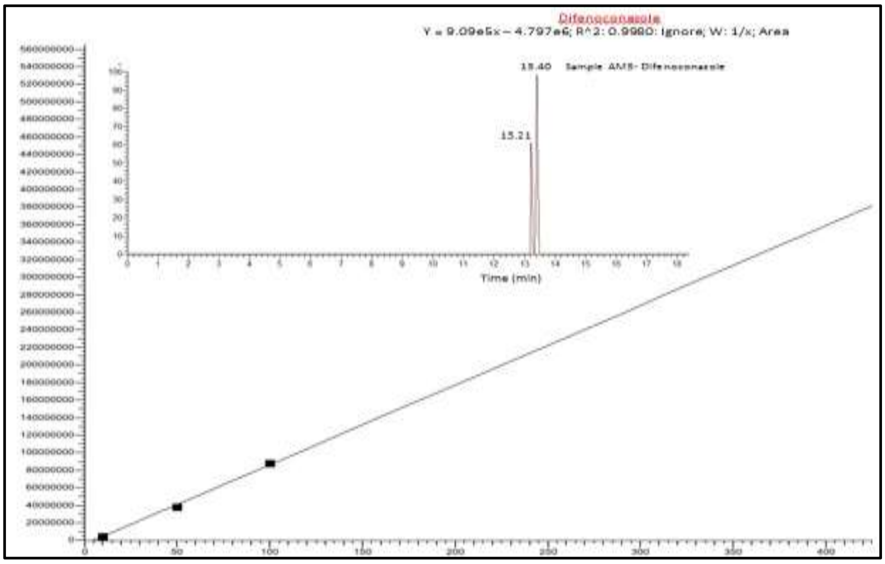

Figure 3: Calibration curve of difenoconazole

Table 1: Percentage recoveries of azoxystrobin and difenoconazole from spiked pepper berries

Active ingredient	Spike concentration	Percentage recovered (%)				SD	% RSD
		R1	R2	R3	Average		
Azoxystrobin	0.01	92.5	102.3	109.2	101.3	3.8	4.2
	0.1	90.1	93.4	96.5	84.3	3.5	3.8
	0.5	84.3	90.3	86.7	87.1	4.5	3.9
Difenoconazole	0.01	86.4	94.3	102.2	94.3	4.3	3.4
	0.1	89.5	93.6	100.1	94.40	4.0	4.1
	0.5	93.7	100.6	99.2	97.8	3.9	4.0

SD= Standard deviation; RSD= Relative standard deviation

Table 2: Residue data summary from supervised trials of azoxystrobinand difenoconazolein pepper berries

Trial	Application rate per treatment			Azoxystrobin		Difenoconazole	
	g/L a.i	Water litre/ha	ml/L water	Means Residue (µg/kg)	Sampling Interval (DAT)	Means Residue (µg/kg)	Sampling Interval (DAT)
1 Kuching	200	450	0.50	89.52	0*	34.16	0*
				57.81	3	22.75	3
				35.26	5	17.92	5
				19.72	7	10.42	7
				11.15	9	<10	9**
				<10.00	12**	<10	12
				<10.00	15	<10	15
			1.0	202.85	0	55.54	0
				157.69	3	41.30	3
				85.86	5	39.70	5
				42.15	7	21.41	7
				23.56	9	13.30	9
				13.44	12	<10	12**
				<10.00	15**	<10	15
2 Padawan	200	450	0.50	95.21	0	28.21	0
				41.21	3	30.12	3
				28.12	5	16.36	5
				15.36	7	10.11	7
				10.42	9	<10	9**
				<10	12**	<10	12
				<10	15	<10	15
			1.0	217.34	0	65.95	0
				179.76	3	52.96	3
				89.84	5	30.15	5
				43.56	7	22.65	7
				24.84	9	13.41	9
				12.45	12	10.01	12
				<1.0	15**	<10	15**
3 Serian	200	450	0.50	68.72	0	25.62	0
				72.61	3	19.72	3
				30.56	5	12.61	5
				15.75	7	<1.0	7**
				<10	9**	<1.0	9
				<10	12	<1.0	12
				<10	15	<1.0	15
			1.0	153.41	0	39.36	0
				95.32	3	32.75	3
				73.75	5	20.6	5
				33.57	7	15.24	7
				23.4	9	11.22	9
				15.9	12	<10	12**
				<10	15**	<10	15

0* 2 hours after last spray; ** Recommended pre-harvest interval;
DAT: Days after treatment

Yap Chin Ann" Determination of Proposed Maximum Residue Limit of New Fungicide, Amistartop in Black Pepper (Piper Nigrum L.)" Quest Journals Journal of Research in Business and Management, vol. 07, no. 03, 2019, pp 75-81