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Persistence of flubendiamide residues in the cabbage field soil under semi-arid region of Rajasthan

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Abstract

Flubendiamide be included in a novel class of insecticide, which used in control of lepidopteran pest complex of cabbage, such as cabbage white butterfly diamondback moth, cluster caterpillar et. A study was undertaken at Rajasthan Agricultural Research Institute, Durgapura, Jaipur during Rabi, 2020 to find outthe residue persistence of flubendiamide residues in the soil of cabbage field, when sprayed at its recommended dose (flubendiamide 18.24 g.a.i. ha^{-1}) and double of the recommended dose (flubendiamide 36.48 g.a.i. ha^{-1}). The samples were extracted with acetonitrile and cleaned up using a modified QuEChERS method and the residues were analyzed by HPLC. The residues level of flubendiamide in cabbage field soil collected at harvest time of cabbage crop were below the detectable level (BDL) at the recommended dose and double of the recommended dose, respectively.

Keywords: Dissipation, residues, flubendiamide, cabbage, persistence, soil.

Introduction

Cabbage (*Brassica oleracea* L. var. capitata) belongs to the family cruciferae is most important among cole crop that is mainly cultivated as the Rabi season crop in India. India rank second in cabbage production in the world next to China, (http: //www.fao. org/faostat/en/#data/QC).Total area of Cabbage in Rajasthan is 1171 hectare with annual production of 11040 MT. (Anonymous, 2018-19). It is being cultivated both in hills and plains and the reason is its wide adaptation to climatic range (Mohan and Gujar, 2003). It is one of the most popular nutritive vegetables in India, consumed both as raw and cooked form. It is grown throughout the year in India.

Cabbage is a very rich source of all nutrients like vitamins *viz*., A, B_1, B_2 and C, minerals and best supporting component for protein, carbohydrates and antioxidants and it is consumed as both cooked and raw as salad. The low production of cabbage in the country could be attributed to several factors, the most important is, damage caused by various insect pests. The low production of cabbage in the country could be attributed to several factors, the most important being the damage caused by various insect pests. Among them Diamond back moth (DBM), *Plutellaxylostella* L. the most destructive pest causes 52.00 per cent yield loss in crucifers. (Tohnishi *et al.* 2005; Anonymous 2009). The pest incidence in cabbage is commonly more during February to September, though it is noticed throughout the year. Flubendiamide represents a novel class of insecticides with extremely high activity against a broad spectrum of lepidopterous insects, (Mohapatra *et al.*, 2010) including diamondback moth, cabbage white butterfly, cluster caterpillar flea beetles (*Phyllotreta sp*.), tobacco caterpillar, (*Spodopteralitura* Fab.); cabbage leaf webber, (*Crocidolomiabionotalis* Zell *etc*. in brassica vegetables (Tohnishi *et al*. 2005; Anonymous 2009). In order to meet the rising demands of veg-

etables for the increasing population, and to counter the impact of these insect pests, different types of pesticides are used. For managing insect pests of cabbage, farmers depend mainly on the application of insecticides like fipronil 05.00% SC (DBM), fipronil 80.00% WG (DBM), flubendiamide 20.00% WG (DBM), acetamiprid 20% SP (Aphids), carbofuran 03.00% CG (Nematode), chlorantraniliprole 18.5% SC (DBM), chlofenapyr 10.00% SC (Dimond back moth, *Plutellaxylostella*), chlorfluazuron 05.40% EC (DBM, Tobacco leaf eating caterpillar), chlorpyrifos 20.00% EC (DBM), cyantraniliprole 10.26% OD (Cabbage Aphid, *Brevicorynebrassicae*) (Mustard Aphid, *Lipaphiserysimi*), (DBM) CIB and RC. (30.11.2021). According to the contemporary ICMR and ICAR (Pesticide Residue Project) report, 8-10% or 12% of food commodities in India have detectable quantities of pesticides, with 2-3% of them exceeding Maximum Residue Limits (MRLs). However, the existence of pesticide residues in food commodities has become a major cause of disquiet. Insecticides applied on the crop ultimately get way into the soil. Pesticides in the soil rapidly act on the soil micro flora and fauna, beneficial microorganism, natural enemies, soil texture, resulting in deficient soil fertility and ultimately affect crop yield. So persistence of these recommended insecticides in soil were also studied out. Flubendiamide did not show any phytotoxicity when applied even at a very high concentration of 400 mg L^{-1} (Hirooka *et al.* 2007a). Latif *et al.* (2009) reported that flubendiamide is safe in comparison to other pesticide for natural enemies and might fit well into the integrated pest management (IPM) programs Shane (2006). It is being used largely by farmers in pest management The present study was carried out to determine persistence of flubendiamide and its metabolite desiodoflubendiamide residues in cabbage field soil following treatment at the recommended dosage and double of the recommended dosage.

Materials and Methods

Reagents and Instruments: Certified Reference Material (CRM) were procured from accu standard and all the solvents used were HPLC grade. The chemicals (Na_2SO_4, primary secondary amine (PSA) and $mgSO_4$ were used analytical regent grade and activated by heating at 30 ^{0}C for 12 hrs. and kept in desiccators. HPLC, Analytical balance, Mixer, Centrifuge and Turbovap-evaporator.

Pesticides and application rate: Used Commercial formulations, of flubendiamide (20% WG) at recommended dose @ 18.36 g. ha^{-1} and double of recommended dose @ 36.48 g. ha^{-1}

Field experimental design: The field experiment was conducted at Rajasthan Agricultural Research Institute, Durgapura, Jaipur, during Rabi, 2020 with four replications including untreated control. The experiment consist of three treatments viz. control, recommended dose offlubendiamide 20 % WG (18.24 ga.i. ha^{-1}) and double of the recommended dose flubendiamide 20 % WG (36.48 g a.i. ha^{-1}). All the essential agronomic practices were also followed regularly. There is no rainfall received during the experimental period. The first spray of insecticide was done at fruit initiation stage using a hand operated knapsack sprayer and second spray at 10 days interval after first spray, and the control plots were sprayed with normal water. At recommended dose (18.24 g a.i. ha^{-1}) and double of recommended dose (36.48 g a.i.ha^{-1}), whereas one plot was left untreated and used for the sampling of soil as control in each treatment. It was ensured that the insecticide which is used for the investigation has not been used earlier in the experimental plot. About 1 kg of soil sample was collected randomly by quadrate method and separately from the control and treated plots of each treatments at the harvest of cabbage crop. Analysis of flubendiamide and des-iodoflubendiamide residues in soil samples were estimated using a High Performance Liquid Chromatograph (HPLC) by Phenomenex Luna C18 column at 254 k (wavelength) and using mixture acetonitrile: water (60:40, v/v) as mobile phase at 1.2 mL min-1. Under these operating conditions. The retention time of flubendiamide were observed to be 4.545. Soil samples were fortified with flubendiamide and desiodoflubendiamide metabolite at different levels and analyzed.

Sampling Soil: Collected (1 kg) of soil samples from the sprayed field of cabbagefrom each replication at harvest time for analysis. Dur-

ing sampling, soil samples were collected from the depth of 0-15 cm from each replication and treatment by quadrate method after removing surface left outs of crop. After it samples were placed into separate plastic containers and allowed to shade dry at room temperature in the laboratory. The air dried samples were desegregated manually using a pestle and a marble mortar, passed through a No. 20 mm brass soil sieve and mixed thoroughly to achieve homogeneity.

Extraction QuEChERS: (Quick, Easy, Cheap, Effective, Rugged and Safe) 10 g of representative soil sample were taken in a 50 ml centrifuge tube and added 20 ml acetonitrile (Asensio Ramos *et al.,* 2010). Shaken test tube vigorously for one minute, 4 g of magnesium sulphate and 1 g of sodium chloride were added. Citrate buffered medium (1g trisodium citrate dehydrate and 0.5 g of disodium hydrogen citrate sesquihydrate was added) to improve the recovery values. Centrifuge at 3,300 rpm for 5 minutes and there is a layer of supernatant. 10 ml of the supernatant were taken into 15 ml centrifuge tube and added 1.5 g of magnesium sulphate and 250 mg of PSA for cleanup. The content was shaken for few seconds and then sonicated for 1 minute; and the tube was centrifuged for 10 minutes at 4,400 rpm. From the above centrifuge tube, 4 ml of aliquot were taken of and evaporated up to dryness using turbovap-evaporator at 40 ^{0}C and n-hexane washing was given two times. The dry residue was redissolved in 1 ml acetonitrile. In case, aqueous phase is noticed, little amount of anhydrous sodium sulphate were added and filtered through 0.22µ PTFE filters and samples was ready for analysis.

Standards: The reference standard of flubendiamide obtained from Pesticide Residues Laboratory, Division of Entomology, RARI, Durgapura, Jaipur, Rajasthan, was used for quantification.

Flubendiamide

a. Standard stock solution: The analytical grade flubendiamide with 98.2% purity was dissolved in 100 ml volumetric flask with acetonitrile to get 1000 mg kg^{-1} standard stock solution.

b. Intermediates stock solution: The standard stock solution was brought at room temperature and 1 ml from the standard stock solution was transfer to 100 ml volumetric flask, made up the volume and shaken well to obtain a homogenous intermediates stock solution of 10 mg kg-^{1}. This was utilized for fortification of samples.

c. Working standard: From the intermediate stock solution, after brining to room temperature, working standard of 0.05 to 1 mg kg^{-1} were prepared by serial dilution techniques and labeled graduated test tubs. The working standards were used to find out retention time of these compounds and for quantitative determination of residues in samples.

Linearity and Recovery study: Linearity studies were performed for flubendiamide and metabolite des-iodoflubendiamide with the concentrations of 0.05, 0.10, 0.25, 0.50, 0.75 and 1 ppm. The soil samples were fortified at 0.01, 0.05 and 0.10 mg kg-1 for flubendiamide by adding required quantity of 10 mg kg^{-1} intermediates stock solution to work out the recovery per cent of analytical methodology.

Instruments parameters: flubendiamide residues were estimated by HPLC by Phenomenex Luna C18 column at 254 k (wavelength) and using mixture acetonitrile: water (60:40, v/v) as mobile phase at 1.2 mL min-1. Under these operating conditions. The retention time of flubendiamide were observed to be 4.545. Soil samples were fortified with flubendiamide and desiodo-flubendiamide metabolite at different levels and analyzed.

Analysis of flubendiamide residues: The detection and quantification of flubendiamide residue in soil was performed by HPLC. Prior to injection of the sample extract for analysis, standard solutions of different concentrations of pesticides were prepared and injected properly in the instrument. Insecticide compound were qualitatively identified by comparing the retention time of peaks and quantitatively estimated on the basis of area of chromatograms obtained in each test sample with that of the analytical standard. Sample results were expressed in mg kg^{-1}. From this value of actual amount of insecticide residue presented in the sample was determined by using the following formula

Residue in analyzed Soil samples

Residues = Peak area (Sample) × Conc.std

(ppm) × µL. Std. injected ×
(ig/g) Final volume of the sample (1mL)
Peak area (Std.) × weight of the sample (2 g)
× µL of sample injected

$$\text{Wt. of sample} = \frac{\text{Sample wt. (10 g)} \times \text{Aliquot taken (4mL)}}{\text{Analyzed (g)Volume of extract (20 ml)}} = 2\text{g}$$

Recovery

$$\text{Percent Recovery} = \frac{\text{Sample peak area} \times 100}{\text{Standard peak area}}$$

Statistical analysis: Statistical analysis was performed on Microsoft Excel-2016 (Microsoft Corporation, USA). All analysis was performed in triplicate and the results were expressed as mean ± SD.

Results and Discussion

Recovery: To ensure the reliability of the results the recovery study was also performed for flubendiamide and its metabolite des-iodoflubendiamide of cabbage field soil samples. The soil samples were spiked with flubendiamide at 0.01, 0.05 and 0.10 mg kg^{-1} fortification levels and analyzed as per the methodology described above. The results of the recovery studies are presented in Table 1. The recovery studies of flubendiamide anddes-iodoflubendiamide was carried out at the fortification level of 0.01, 0.05 and 0.10 mg kg^{-1} in soil. The mean recovery of flubendiamide and des-iodoflubendiamide at 0.01, 0.05 and 0.10 mg kg^{-1} fortification level was 89.2, 93.0 and 89.2 and 87.2, 93.1, and 91.7 percent in soil, respectively. The present recovery experiment are in agreement with those of Sahoo *et al.* (2009) who conducted a recovery experiment at fortification level of 0.20, 0.10, 0.05, and 0.01 µg g^{-1} of flubendiamide in soil and the mean recovery of flubendiamide from soil was 86.0 to 97.50 percent. According to the SANTE (2015) guidelines, any analytical method which records mean recovery in the range of 70-120 per cent is accurate and precise for analysis. Hence, the method applied in the present study for the extraction of flubendiamide, and its metabolites from cabbage field soil was accurate and precise.

Residues: The residues of flubendiamide were confirmed by high performance liquid chromatography (HPLC). This technique was able to identify and quantify 50 ng of flubendiamide. It appears that relatively low doses (18.24 g a.i. $ha^{-1}$and 36.48 g a.i. ha^{-1}. respectively) may play a role in the faster dissipation/degradation of flubendiamide under the cover of cabbage crop and favorable climatic conditions such as high intensity of temperature, and some other factors, e.g. Evaporation, guttation, leaching and crop uptake may be assumed to play some role, leading to rapid dissipation of these pesticides.

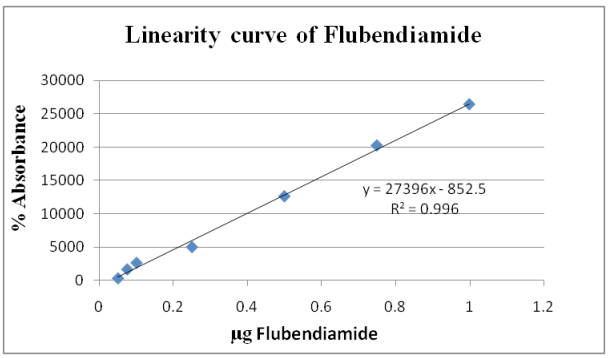
Persistence and dissipation of flubendiamide

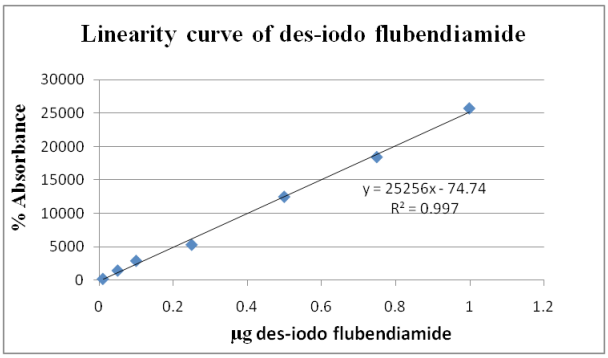
Table 1. Percent recovery of flubendiamidein soil at different fortification levels.

Level of Fortification (mg kg^{-1})	Replications	Percent recovery in Soil			
		Flubendiamide		Des-iodoflubendiamide	
		µg recovered	Recovery(%)	µg recovered	Recovery (%)
0.01	R_1	0.00857	85.7	0.00912	91.2
	R_2	0.00872	87.2	0.00836	83.6
	R_3	0.00914	91.4	0.00868	86.8
	R_4	0.00925	92.5	0.00871	87.1
Mean ± SD		89.2±2.828		87.2±2.698	
0.05	R_1	0.04461	89.2	0.04338	86.8
	R_2	0.04418	88.4	0.04695	93.9
	R_3	0.04515	90.3	0.04769	95.4
	R_4	0.05212	104.2	0.04812	96.2
Mean ± SD		93.0±6.487		93.1±3.716	
0.10	R_1	0.08791	87.9	0.09296	93.0
	R_2	0.08525	85.3	0.09618	96.2
	R_3	0.08992	89.9	0.08681	86.8
	R_4	0.09365	93.7	0.09075	90.8
Mean ± SD		89.2±3.068		91.7±3.419	

residues in soil under cover of cabbage crop have been studied (obtained from three treatments i.e. control, recommended dose (18.24 ga.i. ha^{-1}) and double of the recommended dose (36.48 g a.i. ha^{-1}) are given in Table 2. The soil samples were collected at harvest time of cabbage crop. In case of soil samples the residues at harvest time of cabbage crop was not detected in the samples of recommended dose (18.24g.a.i. ha^{-1}) and double of the recommended dose (36.48g.a.i. ha^{-1}). The control samples of soil did not show the residues also. Present studies are in agreement with Mohapatra *et al*. (2017) who did not found the residues of flubendiamidein soil samples collected after 15 days of following 2 applications of flubendiasmide 480 SC. Similarly, Usha *et al*. (2017) studied the cabbage field soil samples and there is no residue detected after 15 days of spraying four application of flubendiamide 480 SC at 0.25 ml L^{-1} (single dose) and 0.5 ml L^{-1} (double dose) g a.i $ha^{-1.}$

The results are also similar with Mohapatra *et al*. (2014) and Sharma *et al*. (2014) who have reported the residue persistence of flubendiamide and its metabolite, des-iodoflubendiamide, on cabbage field soil following two spray applications of flubendiamide 480 SC of standard and double dose at the rate of 24 and 48 g a.i. ha^{-1}at 15-day interval, and no residues were found in soil samples after 15 days. These results also in accordance with Paramasivam *et al.* (2013), Chawla *et al.* (2011), Mohapatra *et al*. (2011) and Das *et al*. (2012) who reported flubendiamide residues below the detectable levels (BDL, < 0.01 mg kg{1) in soil samples of cabbage, brinjal, tomato, and okra at 10, 15, 20 and 15 days respectively after following single and double doses of different formulations (20 WG, 480 SC, and 39.35% SC) of flubendiamide. The observations in the present studies are in accordance with the findings of all the above researchers.





Limit of quantitation and Limit of detection

Limit of quantitation (LOQ) and Limit of detection (LOD) of flubendiamide and its metabolites in soil was 0.05 and 0.01 mg kg^{-1}. The residue level

Table 2. Residues (mg kg^{-1}) of Flubendiamide and metabolite des-iodoflubendiamide in soil under cabbage crop at recommended dose (18.24g.a.i. ha^{-1}) and double of the recommended dose (36.48 g.a.i. ha^{-1}).

Days	Replications	Recommended dose (18.24g.a.i. ha^{-1})		Double of the recommended dose (36.48 g.a.i. ha^{-1})	
Soil Control		Average* Residues ± SD	% Dissipation	Average *Residues ± SD	% Dissipation
	R_1	ND	-	ND	-
	R_2	ND	-	ND	-
	R_3	ND	-	ND	-
	R_4	ND	-	ND	-
Soil at Harvest time	R_1	ND	-	ND	-
	R_2	ND	-	ND	-
	R_3	ND	-	ND	-
	R_4	ND	-	ND	-

*Average of four replications ND-Not Detected

of pesticide in field soil collected at harvest time of cabbage crops were below detectable level (BDL) at recommended dose and double of the recommended dose.

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