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Dissipation Pattern of Lufenuron 5.4 EC Residues in Soil and Cabbage in Temperate Region

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A field study was conducted to assess the dissipation pattern of lufenuron 5.4 EC in/on cabbage and soil. Lufenuron was sprayed thrice at fifteen days interval @ 30 and 60 g a.i./ha. Soil and cabbage head samples were collected at 0 (1 h after spraying), 1, 3, 5 and 7 days after third spray. The initial deposits of lufenuron were 0.196 and 0.327 µg/g from 30 and 60 g a.i./ha, respectively on cabbage. Lufenuron residues persisted up to 5 days in the cabbage treated with 30 g a.i./ha and 60 g a.i./ha. Whereas in soil, residues were below detectable level at both doses. The half life values for the two doses were 1.49 and 1.51 days, respectively. Decline behaviour of dissoluble pesticide residues was computed following seven transformations and the best fit was first order for both treatments. The waiting period suggested after spraying lufenuron 5.4 EC was 7 days.

Key words: lufenuron, IGR, Chitin Synthesis Inhibitor, Dissipation, Residue, Soil, Cabbage

Vegetables are rich sources of nutrients and vitamins for human health. India is the second largest producer of cabbage and cauliflower after China. The Diamondback moth (DBM) (*Plutella xylostella* : Plutellidae) is the major destructive pest on crucifers causing significant economic losses to the tune of even 92 per cent (Uthamasamy *et. al.*, 2011). Chemical control is the major method of controlling DBM under field conditions. Pesticides, increase crop productivity by saving crop losses, improve quality of produce and thus help in the farmers' income. The role and contribution of pesticides will be much more in the coming years, especially in the country like India as the demands for food continues to grow exponentially due to fast growth of population. Health problems with farmers were also not uncommon in areas, where more pesticides were used against DBM (Weinberger and Srinivasan, 2009). One of the major disadvantages of pesticide use is their residues in foodstuff that should seldom exceed the Maximum Residue Limits (MRLs) set by the food authorities. Hence, proper monitoring of insecticide residues in crop produce is very important for reducing health hazard to consumers.

Chitin synthesis inhibitors interfere with chitin biosynthesis in insects (Gijswijt *et al.*, 1979). Lufenuron is a benzoyl phenyl urea, chitin synthesis inhibitor insecticide [(*RS*)-1-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]-3-(2,6-difluorobenzoyl) urea] with low toxicity to non- target organisms and to the environment. It is a contact and stomach acting Insect Growth Regulator (IGR) to control lepidopteran pests in cruciferous crops. Lufenuron treated larval instars of *Spodoptera littoralis* Boisd. Suffered from inhibited growth and loss of weight with impaired and malformed adults (Adel, 2012) Since, vegetables like cabbage are readily consumable, the level of residues of pesticides retained in its tissues after application is of paramount importance in terms of consumer health. The biochemical pathway of metabolism and dissipation of insecticides in plant body is also highly complex. Hence, studies were conducted to evaluate the dissipation pattern of lufenuron 5.4 EC on cabbage in Nanjanadu, Ooty during December 2012 - March 2013.

Materials and Methods

Lufenuron 5.4 EC was field tested in two doses to evaluate its dissipation in cabbage during 2012 - 2013 at Tamil Nadu Agricultural University Farm at Nanjanadu, Ooty. The experiments were conducted in randomized block design with a plot size of 5x4 m^2, with five replications. The row-to-row distance was 60 cm and the plant-to-plant dis-tance was 45 cm and distance between each replicate was 30 cm. Lufenuron 5.4 EC 30 g a.i./ha and 60 g a.i./ha along with the untreated control were manually sprayed on cabbage at an interval of 15 days on reproductive stage using a knapsack sprayer. Spraying was done during morning hours in such a way as to give uniform coverage on foliage and to avoid drift. Inderon® 1ml/lit was mixed with spray fluid as sticker.

For insecticide residue analysis, cabbage head

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sample was collected during the first harvest from the treatments after third round of spraying. The interval between the last spray and the first harvest was 10 days. Soil samples were also collected at the time of first harvest. Control samples were collected from untreated plots.

Samples of cabbage head and soil were collected from the last day of spray at different time intervals *viz.* 0 (1 hr after application), 1, 2, 5, 7, 10 and 15 days after application. One kilogram of cabbage heads and 2 kg of soil samples were collected from each treated and untreated plots. Field samples were transported to the laboratory immediately after harvest. Each cabbage head was chopped finally and divided into four quarters; which were subjected to sample preparations. These portions of each laboratory sample were separately packed in a plastic bag, labeled and stored at -24°C until analysis.

Twenty grams of cabbage sample was placed in a 250 mL homogenizer cup to which 100 mL of methanol was added. The mixture was macerated at 7000 rpm for 5 min in a high-speed homogenizer. The extract was filtered through flter paper topped with 1 cm of Celite 545 in a porcelain Buchner funnel. The fitrate was then quantitatively transferred to a 250 mL round bottomed flask and was partially evaporated under a rotary evaporator until 20 mL of the fltrate remained. The concentrate was transferred to another 500 mL separating funnel, followed by sequential addition of 30 mL of saturated sodium chloride solution, 100 mL of water and 50mL of n-hexane : diethyl ether (9:1, v:v). The organic phase was dehydrated over 40 g of anhydrous sodium sulfate after vigorous shaking for 3 min. The extraction was repeated twice using the same volume of the organic solvent. The organic phase was combined and evaporated to dryness in a vacuum rotary evaporator at 40°C. The dry residue was dissolved in 10 mL (5 mL x 2 times) of n-hexane.

A chromatographic column (18 mm i.d., 65 cm height) was slurry packed with 5 g of silica gel in n-hexane and topped with 3 g of anhy-drous sodium sulfate. The column was pre-washed with 50mL of n-hexane. The sample extract was loaded and followed by 50 mL of dichloromethane:n-hexane (20:80, v:v). The column was further loaded with 100 mL of dichloromethane:n-hexane:acetonitrile (49:50:1, v:v:v) and the elute was collected. The elute was concentrated to dryness in a rotary vacuum evaporator at 40°C. The residue was dissolved again in 2 mL of methanol prior to HPLC. Lufenuron residues were determined by high performance liquid chromatography (HPLC, a Cyperlab LC100) equipped with UV detector (245 nm). The column used was C18 with ambient temperature at 40°C. The mobile phase was methanol: water (80 : 20) at a flow rate of 0.8 mL / min. A 20 µL aliquot of each sample was injected each time for residue analysis. The representative retention time of lufenuron was 3.41 minutes. A stock standard solution of lufenuron (0.1 mg/mL) was prepared by dissolving the standard in methanol. Working solutions were prepared by serial dilution of the stock solution. All solutions were stored in a refrigerator at 4°C until use.

The half-life of lufenuron was estimated by assuming that the recorded residues were a sum of the residues after each of the two applications and that both were assumed to follow ûrst-order kinetics. Chemical order of reaction was determined based on the rate law (rate = k (conc A)^{m}where k = rate constant; A=chemical concentration; m=molarity). The power (^{m}) to where, the concentration of A raised in the rate expression describes the order of the reaction.

Power (m)	Order of Reaction	Condition
0	Zero	Rate is **independent** of the concentration of reactant. Doubling concentration has **no** effect on rate.
1	First	Rate is **directly proportional** to the concentration of the reactant. Doubling the concentration increases the rate by a factor 2
2	Second	Rate is to the **square** of the concentration of the reactant. Doubling the concentration increases the rate by a factor 4

The half-life was calculated using *Pesticide Residue Half Life Calculator* ©software developed by Department of Soil Science, Tamil Nadu Agricultural University, Coimbatore based on Regupathy and Dhamu (2001).

The final quantification was worked out using the following formula with the parameters from chromatogram

$$\text{Residues (ppm)} = \frac{A_s W_{std} V_s}{A_{std} W_s A_{sj}}$$

where,

A_s - Peak area of the sample

A_{std} - Peak area of the standard

W_{std} - Weight of the standard in ng

W_s - Weight of the sample in g

V_s - Volume of the sample (final extract in ml)

A_{sj} - Aliquot of the sample injected in ml

Results and Discussion

The per cent recovery values of lufenuron 5.4 EC from soil and cabbage samples were 86 and 95

Table 1. Dissipation of lufenuron 5.4 EC on soil and cabbage heads in temperate region

Days after spraying	Lufenuron (µg/g)*			
	Soil		Cabbage	
	30 g a.i./ha	60 g a.i./ha	30 g a.i./ha	60 g a.i./ha
0	BDL	BDL	0.1958	0.3267
1	BDL	BDL	0.1521	0.2626
3	BDL	BDL	0.0730	0.1040
5	BDL	BDL	0.0196	0.0359
7	BDL	BDL	BDL	BDL
Limit of Detection (LOD) = 0.01 µg/g				
Limit of quantification (LOQ) = 0.04 µg/g				

* (Mean of three replications)

BDL – Below Detectable Limit

per cent, respectively. Garrido *et.al.* (2000) reported that the lowest detectable concentrations of the lufenuron were between 0.3 and 6.0 ng/L and recoveries ranged from 95 to 104 per cent for ground water samples spiked at 10 ng/L. The recoveries of lufenuron on grapes (*Vitis vinifera)* were observed from 91.97 to 95.25 per cent at fortification levels of 0.1, 0.5 and 1.0 mg/kg. and the half-life was 2.79 days (Ehab *et al.,* 2013; Markoglou *et. al.,* 2007).

Lufenuron residues declined consistently with time in soil as well as in cabbage (Table 1). At the lower application rate (30 g a.i./ha) residues persisted in cabbage up to 3 days whereas, at the higher rate (60 g a.i./ha) residue was detected up to 5 days. However, in the case of soil, irrespective of application rate, the lufenuron residues were below detectable limit even after 1 hour of application.

On cabbage, the initial deposits of lufenuron 5.4 EC @ 30 and 60 g a.i/ha were 0.196 and 0.327 µg/g , respectively which dissipated to below detectable levels on five and seven days after spraying respectively (Table.1) The results of the present study was in accordance with the findings of Ehab Hassan *et al.* (2013), who reported that the average initial deposit in grapes was 1.85 mg kg^{-1}at single application rate. The decline behavior of the persistent residues was completed following seven transformations and the best fit was selected among them. Based on the coefficient of determination, the best fit observed was the first order reaction for both the doses with significance of 1 per cent level at 30 g a.i. håand 5 per cent level at 60 g a.i. ha^{-1}(Table 2). The intercept (a), slope of

Table 2. Dissipation pattern parameters and correlation coefficients for lufenuron 5.4 EC on cabbage heads

Dosage	Function	Initial deposition (a)	Degradation reaction rate constant (b or *k*)	$T_{½}$	r	r^2	Modified r^2	Significance
30 g a.i. / ha	First order	3.1492	-0.4659	1.4877	-0.985	0.9702	0.95	*
	1.5^{th}order	0.1697	0.0976	0.7202	0.9522	0.9067	0.25	*
	2^{nd}order	-0.0149	0.0905	-0.1646	0.9145	0.8363	-196.47	NS
	RF first order	3.6046	-1.147	0.3651	-0.9333	0.871	0.71	NS
	RF 1.5 first order	0.0823	0.2342	0.0603	0.8793	0.7732	-1.63	NS
	RF 2^{nd}order	-0.09	0.2127	0.179	0.8272	0.6843	-21.02	NS
	Inverse P L	-0.2904	0.489	4.1267	0.8227	0.6768	-0.16	NS
60 g a.i. / ha	First order	3.6381	-0.4591	1.5097	-0.9951	0.9902	0.97	* *
	1.5th order	0.1377	0.0722	0.79	0.9748	0.9502	0.56	*
	2nd order	-0.0027	0.0499	-0.0541	0.9421	0.8876	-293.79	NS
	RF first order	4.103	-1.1425	0.368	-0.9529	0.908	0.74	*
	RF 1.5 first order	0.0701	0.1755	0.0685	0.9113	0.8305	-0.91	NS
	RF 2nd order	-0.0459	0.1186	0.1498	0.8625	0.7439	-41.27	NS
	Inverse P L	-0.288	0.4912	4.1006	0.8471	0.7176	-0.11	NS

RF- Root function; Inverse PL - Inverse Power Law; * Significant at 1% level; ** Significant at 5% level; $T_{½}$– Half life of pesticide

regression lines (b) and half life ($t_{0.5}$) with modified $r^2$are presented in Table 2. Likas and Tsiropoulos (2011) reported that dissipation of three insect growth regulator (IGR) insecticides (flufenoxuron, lufenuron and tebufenozide) in grapes ranged from 0.011 to 0.018 mg/kg/day. In tomato Farag *et. al.* (2012) reported that the average initial deposit of lufenuron was 1.299 mg/kg at first order reaction and residue dissipated to below LOQ of 0.03 mg/kg upto 21 days. Ehab *et. al.,* (2013) reported that residues were analyzed by HPLC and it dissipated in grape fruits following first order kinetics. The average initial deposit of in grape fruits was 1.85mg/kg at single application rate. The reported limit of quantification (LOQ) was 0.01mg/kg. Accordingly, dissipation experiments on grapes showed that the half-life ($T_{½}$) of lufenuron was 2.79 days.

The half life was 1.49 and 1.51 days at 30 g a.i/ha and 60 g a.i/ha, respectively. Trinidad *et al.,* (2004) reported that determining optimal relationships between benzoyl phenyl urea residues and time fitted well in first-order for diflubenzuron, triflumuron, hexaflumuron and flufenoxuron in zucchinis and Root Function (RF) first-order models for the five insecticides in peppers and for lufenuron in zucchinis (summer squash).

The limit of detection (LOD) and limit of quantification (LOQ) were estimated as 0.01 and 0.04 µg/g, respectively. More than 85 per cent of the residues dissipated on the third day after spraying. The half life was 1.49 days for 30 g a.i. $ha^{1}$and 1.51 days for 60 g a.i. ha^{-1}. Since, lufenuron residues were below detectable levels on 7^{th} day after

application at 30 g a.i. $ha^{-1}$and 60 g a.i. ha^{-1}, 7 days as waiting period was suggested.

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