Residue dynamics and household decontamination techniques for some non-approved pesticides on cabbage head and cropped soil

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Abstract: - The persistence study of some non-approved pesticides *viz.*, fenvalerate, imidacloprid and profenofos on cabbage heads and cabbage cropped soil was conducted using gas chromatography-mass spectrometry after application of recommended single and double doses. QuEChERS technique was employed for sample preparation methodology and validated, as per SANTE 2019 guidelines. Residue analysis in cabbage cropped soil was also undertaken at the harvesting stage. Samples were extracted with acetonitrile and cleaned up with anhydrous magnesium sulphate and primary secondary amine (PSA). Average recoveries were in the accepted range of 80-120%. Half-life and waiting period were calculated. The efficiency of different household decontaminants in mitigating the residues of from the cabbage heads were evaluated from consumption point of view. Among the different decontaminants unwrapping of the outer leaves from the cabbage head proven to be most efficient treatment as it removes 63-70 per cent of residues.

Key-Words: - Cabbage head, Cropped soil, Residues, Maximum residual limit, Decontamination
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1 Introduction

Fruits and vegetables make up about 22% of food production globally (Grunwald, 2021). Besides the diverse vegetable flora, their sufficiently rich content of nutrients, phytonutrients and dietary fibres make vegetables an appealing dietary constituent for good health (FAO, 2020). To fulfil the dual objective of sustainable food supply and protective nutritional effect, intake of 200–600g of vegetables per day is recommended (Loken *et al.,* 2020). Cabbage (*Brassica oleracea var. capitata L.*) constitutes an indispensable part in the human diet because of the consumer's priority, nutritional aspects and easy market availability year-round at accessible price. With respect to economic importance, it holds the second position after tomato in Asia (Eifediyi and Remison, 2010). India occupies the second rank globally in the production of cabbage after China. In Himachal Pradesh cabbage is grown both as main season as well as an off-season summer crop (Anonymous, 2018).

The climate change has culminated in the growth of pest pressure (Newton *et al.,* 2011) and pesticide abuse in an effort to sustain the horticultural yields which leads to plates loaded of pesticides. Human exposure to pesticides can occur through multiple routes, but the contaminated food poses highest risk. Extreme use of pesticides in brassicas is mainly attributed to the pesticide resistance history of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Furlong *et al.,* 2013). Many studies reported that the main pesticide residues were organophosphates, pyrethroids and organochlorine (Machekano *et al*., 2019).

The Central Insecticide Board and Registration Committee (CIBRC) have approved approximately 18 pesticides to manage the pests infesting cabbage like chlorpyrifos 20.0 per cent EC, indoxacarb 15.80 per cent SC, cypermethrin 10 per cent EC etc. against diamond back moth and insecticides like acetamiprid 20 per cent SP and cyantranilliprole 10.26 per cent OD for the control of aphids and tobacco caterpillar respectively (CIBRC, 2022). But the residues status by pesticide monitoring studies speaks a whole different story. The application of non-recommended pesticides against specific crop pest is still carried out by farmers. Pesticide use in sister cole crops is the perfect example to quote as pesticides like imidacloprid and profenofos are recommended by CIBRC to be used against the cauliflower pests but not against cabbage insect pests. In the current scenario where doubling the indian farmer's income is the goal, when the major holdings are marginal, use of particular crop wise recommended insecticide is not expected to be followed at bulk level.

Thus, the present study was undertaken to monitor the insecticide residues of non-approved pesticides of three different groups i.e. fenvalerate (synthetic pyrethroid), imidacloprid (neonicotinoid) and profenofos (organophosphate) in cabbage and to evaluate some economical and simple household decontamination methods from the consumer safety point of view. Safer the food, better the lives. With every consumed bite, one is exposed to illness from contamination. Hence, the concern of food safety for developing countries where pesticide consumption is prominent and a major part of the population lives below the poverty line needs to be look after (WHO, 2015).

2 Materials and Method

2.1 *Reagents and Chemicals*

The technical grade analytical standard of insecticide formulation were obtained and reanalysed with acetonitrile extract with respect to the purity of the active ingredient. All the necessitous chemicals like sodium chloride (NaCl), analytical grade anhydrous sodium sulfate (Na_2SO_4) and $MgSO_4$ activated anhydrous (AR grade) were procured from Merck Specialties Pvt. Ltd., Worli, Mumbai. Primary Secondary Amine (PSA) were obtained from Agilent Technology, USA. All fundamental solvents like ethyl acetone, acetonitrile, water and n-hexane (HPLC grade) were obtained from the standard manufacturer and redistilled in all-glass apparatus before use. The suitability of the solvents and other chemicals were ensured by running reagent blanks before actual analysis.

2.2 Preparation of standard stock solutions

A standard stock solutions of 400 ppm of each tested insecticide were prepared from Dr. Ehrenstorfer (Augsburg, Germany) certified reference material (CRMs) of insecticide standards procured from supplier. Working solutions of 40 ppm, 10 ppm and 1 ppm were prepared by serial dilutions from each stock solution, respectively for producing an calibrating curve and stored at 4^0 C.

2.3 Experimental design and insecticides application

To carry out the scheduled studies on the cabbage fruit (early maturing var. Golden acre), supervised field trial was laid out in a Randomized Block Design (RBD) with eight treatments replicated thrice with individual plot size of 15 x 8 ft^2 following all good agricultural practices as per standard package of practices recommended by the university. For the persistence studies total of two foliar spray of the aimed three insecticides i.e. fenvalerate (20% EC), imidacloprid (17.8% SL) and profenofos (50% EC) were given. The first spray was given after head initiation and second spray at 10 days after first spray. For the decontamination studies the application of the of the premix formulation of the test insecticides were sprayed using knapsack sprayer appropriately at head maturation stage. Each insecticide was applied at two concentrations i.e. single and double doses. The control plots were treated with water only

2.4 Instrumentation

For quantification of fenvalerate residues, a gas chromatograph (Agilent 7890B) equipped with electron capture detector (ECD) was used. A fused silica capillary DB-5 column DB-5 column of 30m long, 0.25mm ID and 0.25µm film thickness was used to resolve target compounds using nitrogen as carrier gas at constant flow rate of 1 ml min^{-1}. The detector and injection port were operated at 300º C and 250º C. The oven temperature was programmed as follows: Initial temperature 80º C for 3 minutes, raised to 150º C at the rate of 30º C min^{-1} with a hold time of 2 min, further raised to 205º C at the rate of 3º C min^{-1} and finally ramped to 260º C (22 min) at the rate of 10º C min^{-1}. For the determination of profenofos residues a gas chromatograph (Agilent 7890B) equipped with flame photometric detector was used with other dimensions remaining constant as GC-ECD. For FPD hydrogen and zero air were used at constant flow.

High performance liquid chromatography (Shimadzu LC- 20 AT) equipped with photo diode array (PDA) detector (λ_{max} 260 nm) was carried out using reverse phase Merck LiChrosorb® RP C18 (5µm) column (2.1 mm X 30 cm) and acetonitrile: water (60:40, v/v) as mobile phase with pump flow at the rate of 0.60 mL min^{-1} to detect and quantify the imidacloprid residues.

Residues were quantified by comparison of peak height/peak area of standards with that of unknown or spiked samples run under identical conditions and the conformation was done by mass spectrometer and retention times and Limit of Quantification (LOQ) are enlisted in Table 1.

2.5 Method validation

The method was validated as per SANTE 2019 guidelines to evaluate its accuracy, precision and suitability. The parameters included linearity and recovery. The recovery was determined by spiking the untreated cabbage heads and soil samples at 0.05, 0.10, 0.25, 0.50 and 1.00 ppm levels with different test insecticides. Fortified samples were processed as per the procedure described for analysis of sample and linearity was tested.

2.6 Sample preparation

For the dissipation studies about 1 kg heads of marketable size were randomly collected from each plot, packed in polyethylene bags and brought to the laboratory and processed further. To carry out the decontamination studies the head samples were collected after 48 hours (2 days) of the spray and dried 20-30 minutes on filter paper at room temperature.

2.6.1 Cabbage heads

Head samples were chopped into small pieces, homogenized in high volume homogenizer and prepared following QuEChERS method for the residue analysis. For the extraction purpose, sub sample of 15 g of cabbage head was weighed into a 50 mL centrifuge tube and 30 ml acetonitrile was added to it and homogenized at 15,000 rpm for 3 minutes. Anhydrous sodium

chloride (3 g) was added to homogenized sample for phase separation and then centrifuged at 3000 rpm for 3 minutes. An aliquot of 18 ml was transferred to another 50 ml centrifuge tube containing 9 g anhydrous sodium sulphate and shaken well. The acetonitrile extract subjected further to cleanup by dispersive solid phase extraction (DSPE). 11ml fraction from 18 ml aliquot was transferred over anhydrous magnesium sulphate (1150 mg) and PSA (400 mg) in a 15 ml centrifuge tube, shaken well and then centrifuged at 3000 rpm for 5 minutes. The 6 ml aliquot was taken to turbo glass tube and evaporated to dryness at 45° C in turbo evaporator in the presence of zero air. The residues were dissolved in 3 ml n-hexane and injected 1 µl into gas chromatograph for estimation of fenvalerate and profenofos whereas imidacloprid residues were dissolved in 3 ml of acetonitrile: water mixture (60:40), filtered through PTFE filter (0.45µm pore size) and injected 20 µL into HPLC.

2.6.2 Cabbage cropped soil

Soil samples were analysed by another QuEChERS technique, modified for analysis of soil (Asensio-Ramos *et al*., 2010). Extraction was carried out by adding 20 ml acetonitrile in 10 g sieved ground dry soil sample. Clean up was performed by 4 g of anhydrous magnesium sulphate, 1 g of sodium chloride, 1.50 g of magnesium sulphate and 0.25 g of PSA. After repeated serial centrifugation, 4 ml aliquot of the supernatant was taken in a turbo tube and evaporated to dryness in presence of zero air at 45° C. The dried residues of fenvalerate and profenofos cropped soil were dissolved in 2 ml of n-hexane for injection (1µl) in to GC-ECD and residues of imidacloprid cropped soil were dissolved in acetonitrile: water mixture (60:40) for injection (20µl) into HPLC.

2.7 Decontamination of pesticide residues in head

Economical and easily available materials like common salt (NaCl), sodium bicarbonate (Baking soda/NaHCO3), acetic acid (Vinegar/CH3COOH), Veggie Clean (from Marico) etc. were integrated for decontamination studies. In treatment one (T1) each replicated cabbage head samples were washed under running tap water for 1 min. In the second treatment (T_2) 1kg head samples were soaked in 2-4 litre of lukewarm water (45-50°C) for 10 minutes with 15 rpm. In third treatment (T_3) 1% NaCl aqueous solution was used for soaking the 1 kg heads in 2-4 litre of for 10 minutes with 15 rpm. In the next succeeding treatments 1 kg heads were soaked in 2-4 litre of 5% $NaHCO_3$ (T_4), 2% acetic acid (T_5), 0.01% $KMnO_4$ (T_6) aqueous solution for 10 minutes with 15 rpm. In the next succeeding treatments popular marketed products like Veggie Clean from Marico (T_6), Nim wash from ITC (T_7) and Arka Herbiwash, Product of IIHR Banglore (T_8) were exploited. 1 kg heads were soaked in 2-4 litre of aqueous solutions of each above mentioned products for 10 minutes with 15 rpm. In the last treatment (T_{10}) outer leaves were unwrapped from the cabbage heads.

3 Results

3.1 Method validation

To check the efficiency of extraction and clean-up procedures, recovery studies were carried out in the present experiment. The recovery studies of fenvalerate, imidacloprid and profenofos were evaluated by spiking the cabbage heads and cropped soil at five fortification levels viz. 0.05, 0.10, 0.25, 0.50 and 1.00 mg kg^{-1}, respectively and processed further by following the above mentioned methodology. The average recovery values from fortified samples were in the accepted range i.e. 80–120 per cent as per SANTE guidelines (SANTE, 2019). The limit of quantification (LOQ) for all the test insecticides was found to be 0.05mg kg^{-1} (Fig 1, 2 and 3).

3.2 Dissipation of pesticides in cabbage head and cropped soil

The average initial deposits of fenvalerate were 1.097 mg kg^{-1} and 1.922 mg kg^{-1} due to the application of fenvalerate at the rate of 60 g a.i. ha^{-1} and 120 g a.i. ha^{-1} (Fig, 4) when applied as Fenvalerate 20 EC. The initial deposit of fenvalerate on cabbage heads at recommended dose were 1.7 times lower than that obtained from double the recommended dose. The initial deposits of fenvalerate (1.097 mg kg^{-1}) dissipated to 0.588, 0.250 and 0.094 after 1, 3 and 5 days of spraying at the recommended dose (60 g a.i. ha^{-1}) and at double the

recommended dose (120 g a.i. ha^{-1}), the initial deposits of 1.922 mg kg^{-1} dissipated to 1.047,0.361, 0.180 and 0.080 mg kg^{-1} in 1, 3, 5 and 7 days respectively. The dissipation of the initial deposit were found to be 46.42, 77.19, 91.40 and 100 per cent at 1, 3, 5 and 7 days interval, respectively when applied at recommended dose.

At double the recommended dose, the registered dissipation pattern was 45.55, 81.20, 90.65, 95.86 and 100 at 1, 3, 5, 7 and 10 days intervals, respectively. Deposits from single dosage samples reached below determination limit (BDL) after 7 days; whereas at double dose, initial deposit dissipated after 7 days to 0.080 mg kg^{-1} and the residues were found below LOQ (< 0.05 mg kg^{-1}) on 10^{th} day after last spray application.

The average initial deposits of imidacloprid at single dose (25 g a.i. ha^{-1}) and double dose (50 g a.i. ha^{-1}) were found to be 0.279 mg kg^{-1} and 0.714 mg kg^{-1} when applied as Leopard 17.8 SL. Residue deposits at recommended the double dose is 2.5 times higher than the single dose. At recommended dose, the initial deposits of imidacloprid (0.279 mg kg^{-1}) dissipated to 0.260 and 0.107 mg kg^{-1} with per cent dissipation values of 6.83 and 61.49 at 1 and 3 days interval, respectively whereas at the higher dose the initial deposits of 0.714 mg kg^{-1} dissipated to 0.515, 0.232 and 0.102 mg kg^{-1} with per cent dissipation values of 27.82, 67.55 and 85.67 per cent at 1, 3 and 5 days interval. Persistence data showed (Fig. 5) that the imidacloprid residues on cabbage heads reduced to BDL within 5 days at single dose whereas at double dose residues reached below BDL at 10 days on cabbage fruits.

The average initial deposits of profenofos at single dose (500 g a.i. ha^{-1}) and double dose (1000 g a.i. ha^{-1}) were found to be 2.536 mg kg^{-1} and 4.278 mg kg^{-1} when applied as Curacron 50 EC (Table 2). The initial residues of profenofos at single dose (2.536 mg kg^{-1}) dissipated to 1.100, 0.423, 0.135 and 0.055 mg kg^{-1} after 1, 3, 5 and 7 days from last foliar application, respectively. At double dose, the corresponding values dissipated to 1.826, 0.870, 0.273, 0.398 and 0.059 mg kg^{-1} from an initial deposit of 4.278 kg^{-1} after 1, 3, 5, 7 and 10 days from last scheduled spray, respectively. The residues at single dose were observed to be below limit of quantification (< 0.05 mg kg^{-1}) on 10 th day after last foliar application; while residues dissipated to 0.059 mg kg^{-1} at double dose on 10^{th} day and then reached below limit of quantification (< 0.05 mg kg^{-1}) at 15^{th} day after last foliar application of the test insecticide. Persistence data showed that the profenofos residues on cabbage heads dissipated by 56-97.84 per cent at single dose and 57.32 - 98.63 per cent at double dose (Fig. 6).

The residues of fenvalerate, imidacloprid and profenofos in cabbage cropped soil were found to be below determination level (0.05 mg kg^{-1}) after harvest of cabbage heads from the treatments having application of test insecticides at recommended standard and double doses. The suggested safe waiting period for fenvalerate, imidacloprid and profenofos were 8.15, 6.79 and 12.38 days, respectively (Table 2).

3.3 Decontamination of pesticides in cabbage heads

The results of decontamination studies done against the different treatments viz. washing with Running tap water (T_1), Luke warm water (T_2), 1% NaCl (T_3), 5 % $NaHCO_3$ (T_4), 2 % Acetic acid (T_5), 0.01% $KMnO_4$ (T_6), veggie clean (T_7), Nimwash (T_8), Arka herbiwash (T_9) and Removing the outer leaves (T_{10}) revealed that treatment T_{10} i.e. removal of outer leaves from cabbage heads (63-70 per cent) was the most effective treatment among all the other treatments for all three insecticides followed by washing with saline water. The saline water (1%), $NaHCO_3$ (5%), Acetic acid (2%) and $KMnO_4$ (0.01 %) washing provided upto 68.01, 62.73, 61.55 and 57.21 per cent relief, respectively from insecticide residues. The tap water washing and luke warm water washing of cabbage heads turned out to be least effective (Table 3).

4 Conclusion

"Owing to pesticides" important role in agricultural development, there is a heavy dependence on pesticide applications to meet the huge demand for food production by an

increasing population. This causes environmental stress and has detrimental health effects on humans worldwide. Human exposure to pesticides can occur through multiple routes, but the contaminated food poses highest risk. Cumulative effects of low-level exposures through pesticide residues in food have created concern and scientific and political debate for more than a century.It is the need of the hour that every consumer rich or poor should have knowledge to mitigate residues own their own at household level and the concept of waiting periods should be well popularized and adopted.

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Table 1: Retention time and Limit of Quantification (LOQ) of insecticides

Name of Insecticide	Retention Time (minutes)	Limit of Quantification ($mg\ kg^{-1}$)
Fenvalerate	42.040,42.981	0.05
Imidacloprid	6.02	0.05
Profenofos	28.902	0.05

Table 2: Statistical constants of test insecticides in cabbage

Pesticide	Dosage (g a.i. /ha)	Statistical Constants			
		Regression equation (y = a + bx)	Correlation coefficient (r)	RL_{50} (Days)	Waiting period (Days)
Fenvalerate	60	y = –0.0145 – 0.2084x	-0.9884	1.45	6.44
	120	y = –0.2259 – 0.1945x	-0.9952	1.55	8.15
Imidacloprid	25	y = –0.5088– 0.1458 x	-0.9646	2.06	5.11
	50	y = –0.1320 – 0.1702 x	-0.9994	1.77	6.79
Profenofos	500	y = –0.3549 - 0.2465x	-0.9961	1.22	6.91
	1000	y = –0.4731 – 0.1560 x	-0.9195	1.92	12.38

Table3: Effect of decontamination processes on reduction of insecticide residues in cabbage heads

Treatments	Fenvalerate		Imidacloprid		Profenofos	
	Mean Relief (%) ± SD		Mean Relief (%) ± SD		Mean Relief (%) ± SD	
	60 g a.i. ha^{-1}	**120 g a.i. ha^{-1}**	**25 g a.i. ha^{-1}**	**50 g a.i. ha^{-1}**	**500 g a.i. ha^{-1}**	**1000 g a.i. ha^{-1}**
Running tap water washing	19.94 ± 7.06	21.98 ± 6.03	21.45 ± 6.02	20.05 ± 9.79	22.73 ± 3.60	23.25 ± 2.85
Lukewarm water washing	23.80 ± 6.65	24.74 ± 1.35	27.72 ± 6.57	30.05 ± 7.61	30.80 ± 4.51	25.67 ± 1.94
Saline water washing	58.05 ± 6.01	61.17 ± 3.68	64.44 ± 2.17	68.01 ± 2.97	63.83 ± 3.21	64.05 ± 1.96
$NaHCO_3$ solution dipping	53.98 ± 3.77	59.52 ± 3.15	61.10 ± 3.73	62.73 ± 2.17	57.83 ± 1.71	60.68 ± 0.76
Acetic acid solution dipping	47.54 ± 6.20	52.44 ± 4.38	54.49 ± 4.50	57.08 ± 4.04	55.37 ± 1.21	61.55 ± 1.15
$KMnO_4$ solution dipping	33.52 ± 3.21	45.09 ± 2.98	53.87 ± 5.98	57.21 ± 1.20	40.23 ± 4.57	47.88 ± 2.32
Veggie clean solution dipping	35.71 ± 17.11	47.41 ± 2.75	47.39 ± 4.02	49.40 ± 1.80	50.28 ± 3.25	49.26 ± 1.34
Nimwash solution dipping	32.53 ± 6.95	49.45 ± 6.74	45.86 ± 4.15	48.78 ± 7.18	49.69 ± 6.11	50.38 ± 1.38
Arka herbiwash solution dipping	35.86 ± 5.47	49.47 ± 5.18	47.13 ± 3.84	48.88 ± 4.35	50.39 ± 4.14	50.16 ± 1.41
Outer leaves removed	63.55 ± 5.57	66.86 ± 3.85	68.38 ± 3.83	70.95 ± 1.06	65.56 ± 1.51	65.61 ± 0.84

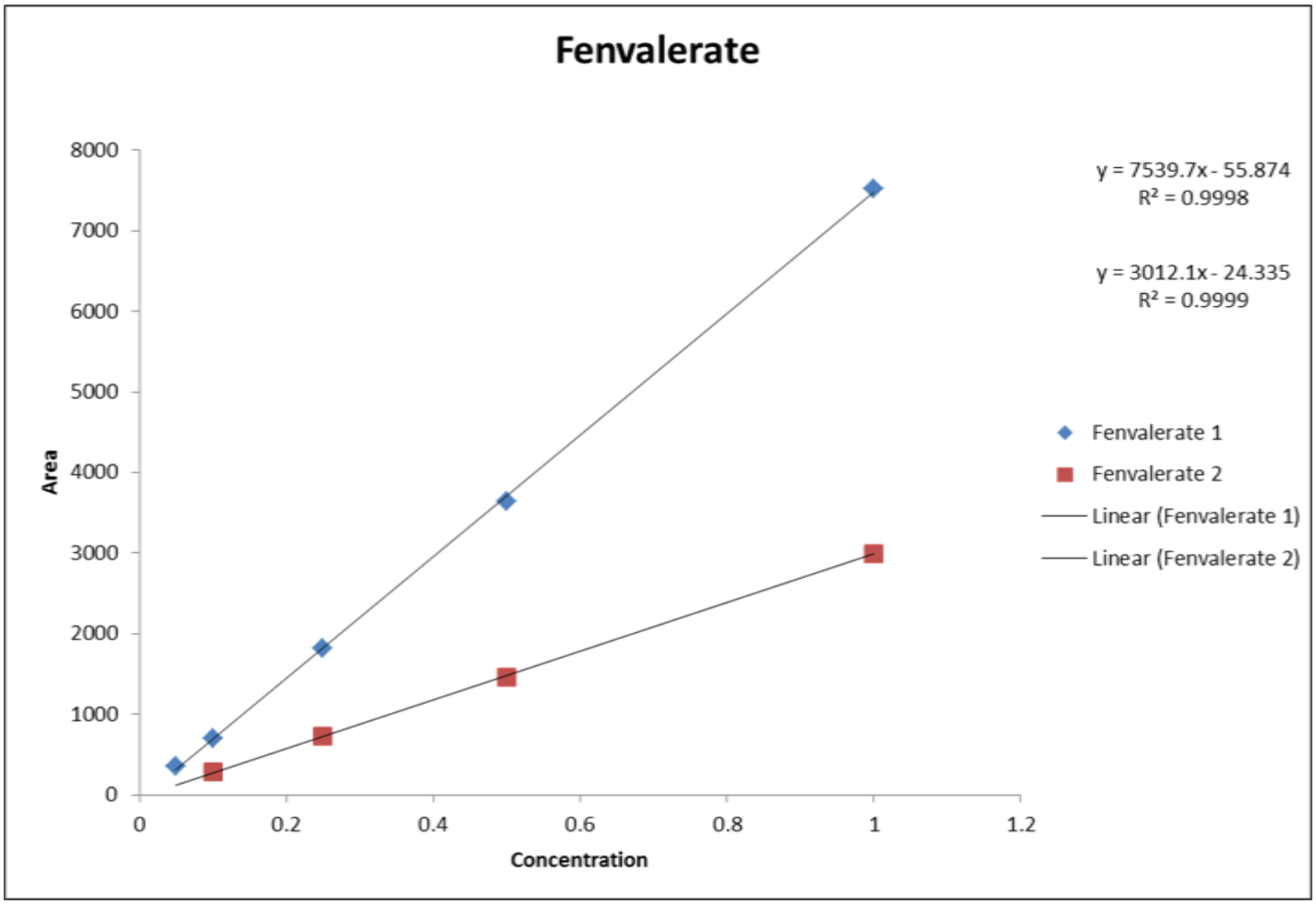

Fig. 1 Calibration curve between concentration of fenvalerate Versus Gas chromatograph peak area depicting linearity of response

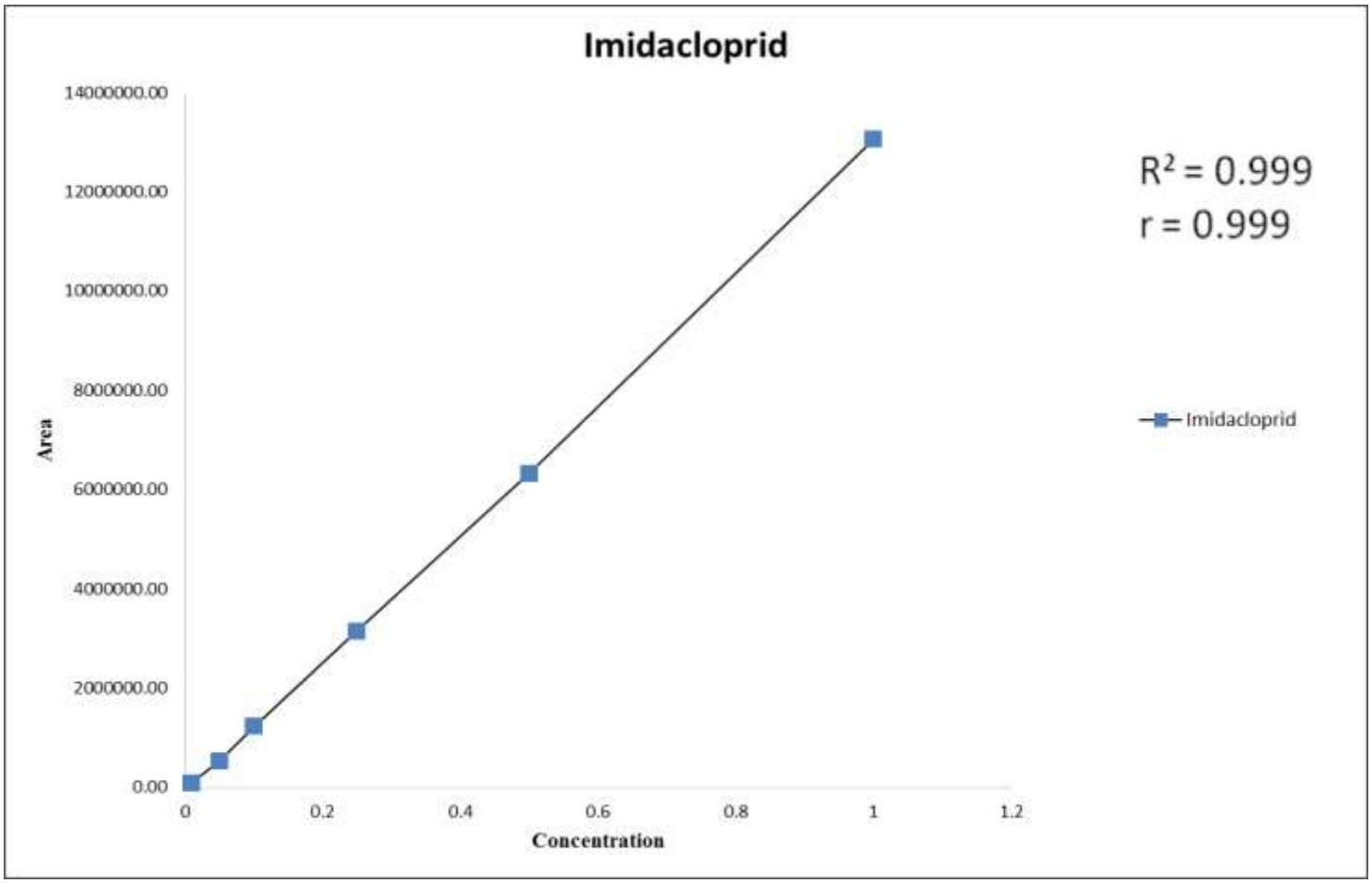

Fig. 2 Calibration curve between concentration of imidacloprid Versus Gas chromatograph peak area depicting linearity of response

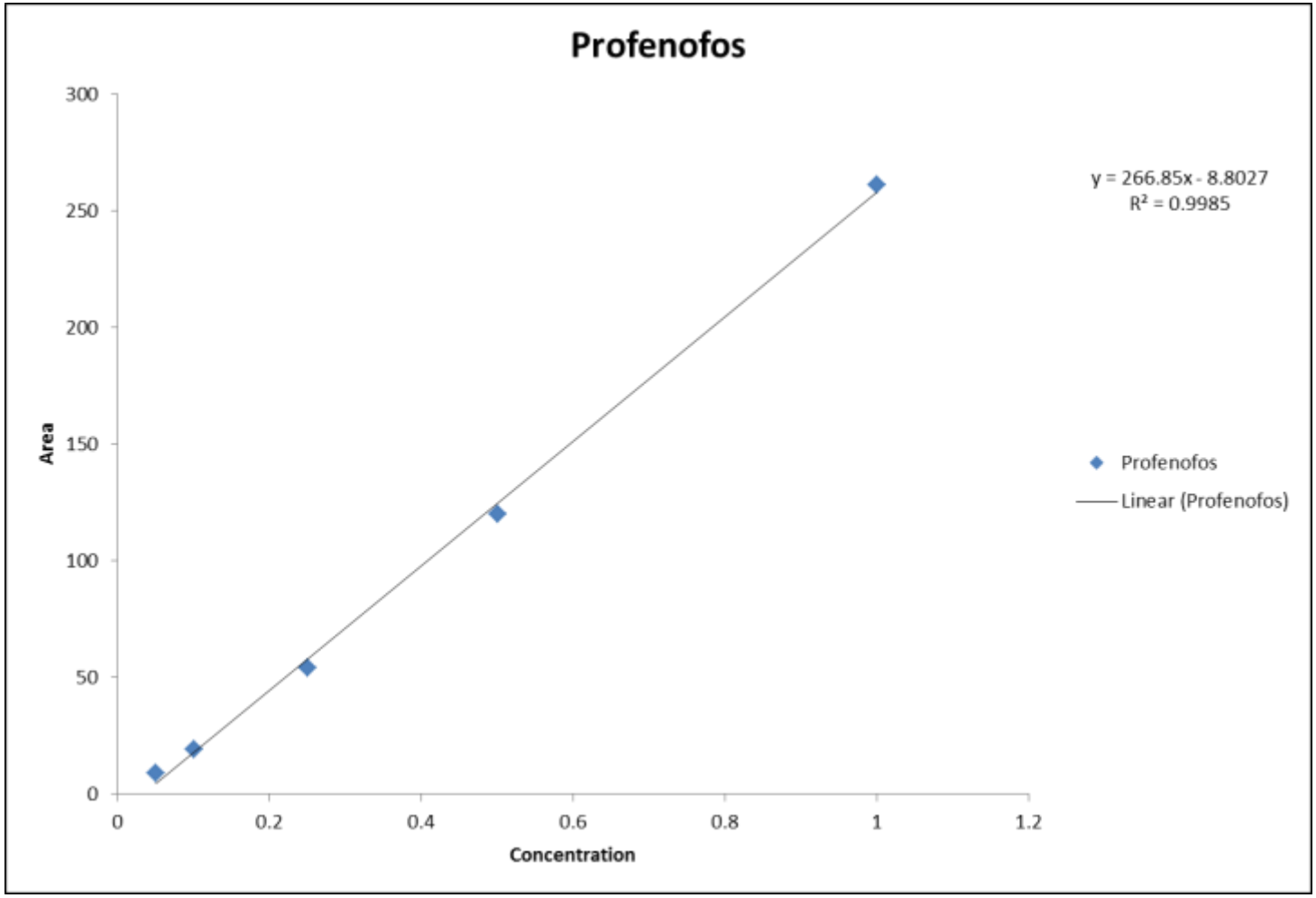

Fig. 3 Calibration curve between concentration of profenofos Versus Gas chromatograph peak area depicting linearity of response

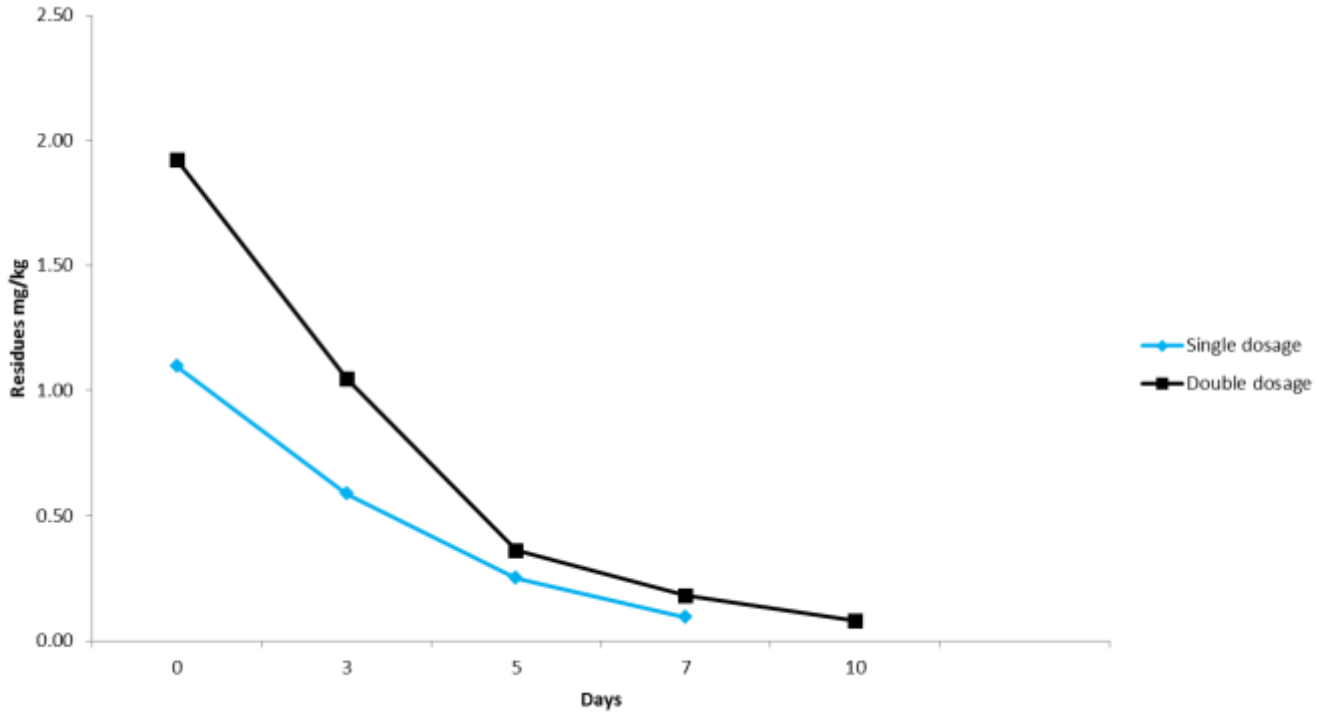

Fig. 4 Persistence of fenvalerate (@ 60 and 120 g a.i. ha^{-1}) after application(Fenval 20 EC) on cabbage heads

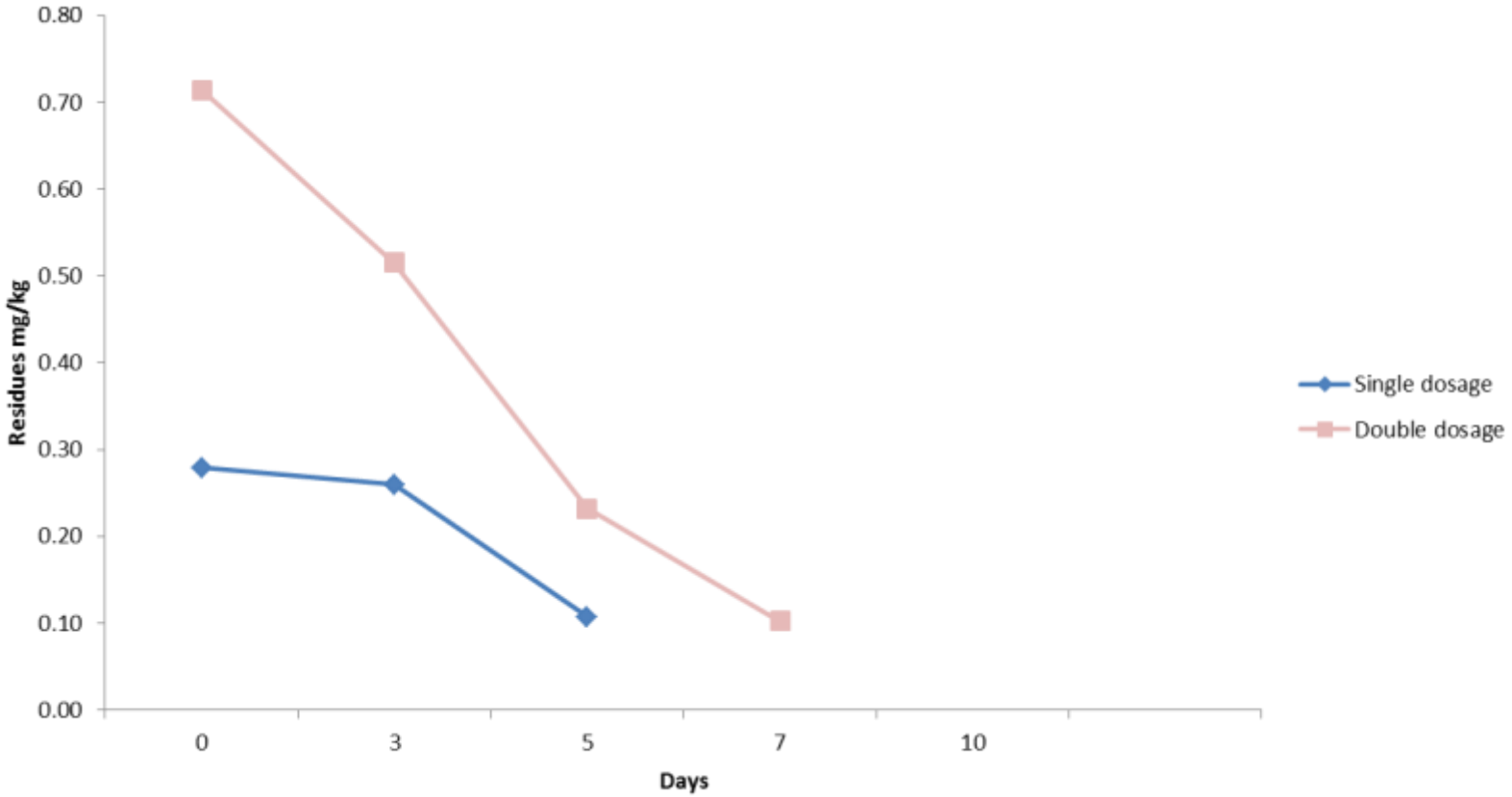

Fig. 5 Persistence of imidacloprid (@ 25 and 50 g a.i. ha^{-1}) after application (Leopard 17.8 SL) on cabbage heads

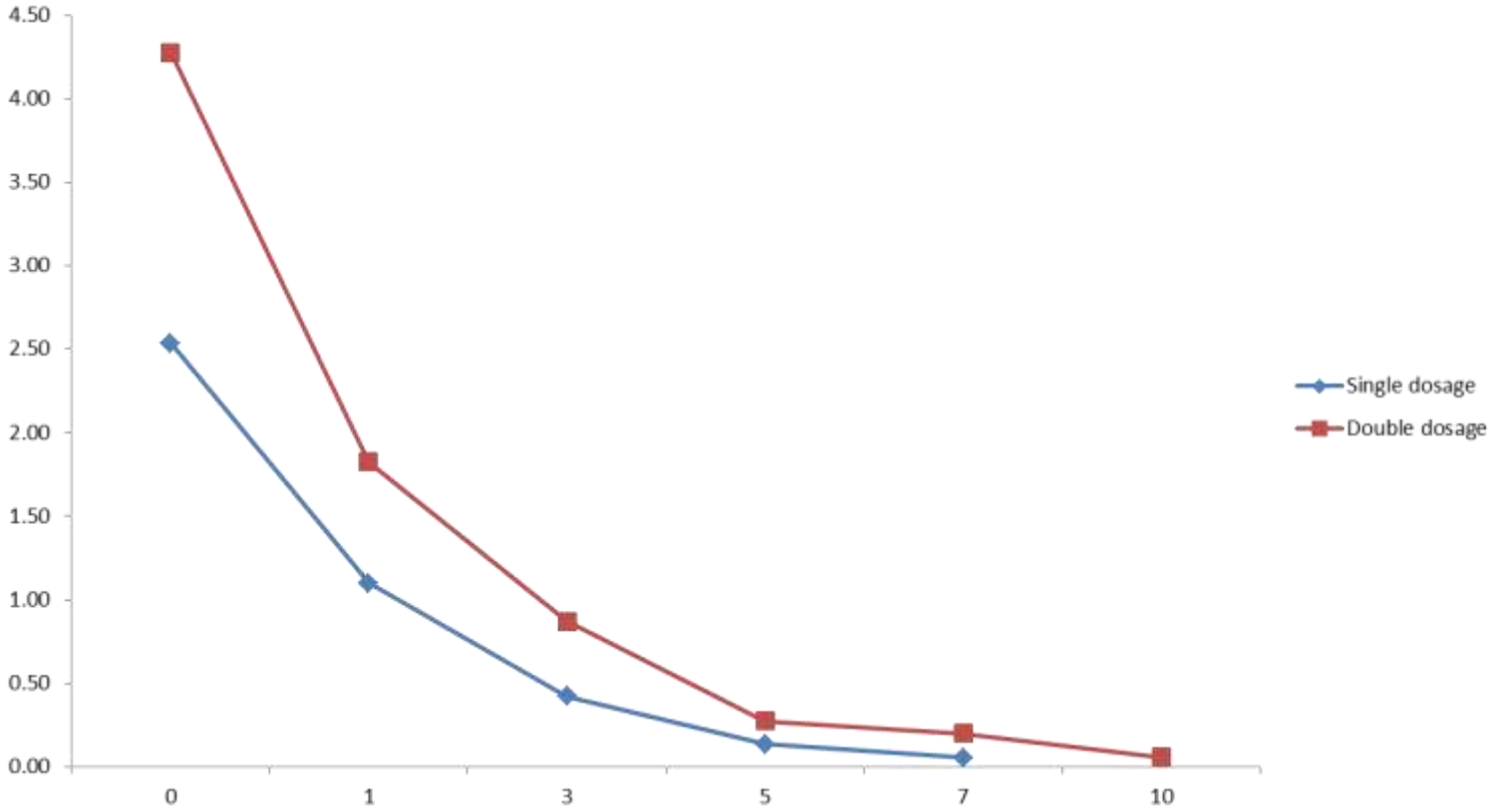

Fig. 6 Persistence of profenofos (@500 and 1000 g a.i. ha^{-1}) after application (Curacron 50 EC) on cabbage heads