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Persistence and Effect of Processing on Reduction of Chlorpyriphos in Chilli

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Abstract

Persistence behavior of chlorpyriphos in chilli was studied following application of formulation of 20 EC @ 160 (single dose) and 320 (double dose) g a.i.ha^{-1} at fruiting stage. Samples of green chilli and soil under crop were drawn at different time intervals and quantified by gas liquid chromatography equipped with electron capture detector. The initial deposits of residue on the chilli fruit were 0.397 and 1.021 mg kg^{-1} at single and double dose, respectively. Residues of chlorpyriphos dissipated to more than 75% after 10 days at both the dosages. The half-life period of chlorpyriphos in chilli was recorded to be 6.02 days at single dose and 5.67 days at double dose. Washing process was found effective in reducing the residues by 67.75 and 71.60 percent at, respective doses.

Keywords: Chlorpyriphos; Chilli; Residues; Persistence; Half-life; Washing; Soil

Introduction

Pesticides help the farmers to increase their income by improving the quality of crop, saving crop losses, increase crop productivity and reduce cost of production. In recent years, the role and contribution of pesticides have increased much more, especially in the country like India because of fast growth of population; the demand for food supply will continue to grow steadily. Due to regular use of pesticides, the residues in food commodities seldom exceed the maximum residue limits (MRLs) set by the food authorities. As a result, consumption of pesticide treated food commodities become risky. So it becomes necessary that, pesticide should be effective against pest along with its toxicologically acceptable residues in food commodity [1]. For this reason, proper assessing of pesticide residues is very important for reducing health hazard to consumers. Chilli (*Capsicum annum*) is an essential pillar of the cuisines of India. It is attacked by various insect pests resulting in yield losses. The crop is attacked by various insect pests resulting 51 species of insects and 2 species of mites. Thrips, mites and pod borer are serious among its pests [2].

Chlorpyriphos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothionate] is an organo phosphorus broad spectrum insecticidal active ingredient registered for application to more than 40 different food commodities. It is a stable compound in neutral and acidic conditions [3]. It kills insects by disrupting their nervous system and is effective against both sucking and chewing insects and has been widely used to control pests of various vegetables [4]. It is non-systemic, fairly persistent, and highly soluble in organic solvents like acetone, xylene, and methylene chloride [5]. Cholinesterase inhibition is the mode of action of chlorpyriphos and is the cause of potential toxicity in human [6,7].

There is currently an increasing concern and awareness about the hazards of pesticides to consumers. Even with the adoption of integrated pest management, farmers believe in the control of pests using pesticides because of their quick effect. Therefore, the present study was designed to determine the residual persistence and effect of processing on reduction of chlorpyriphos residues in chilli.

Materials and Methods

Chemicals and reagents

Chlorpyriphos formulation (20 EC) used for field application was procured from local market. Solvents and reagents like dichloromethane, acetone, sodium chloride, and anhydrous sodium sulfate all were procured from Merck (Darmstadt, Germany). Before use all the common solvents were redistilled in glass apparatus. By running reagent blanks the suitability of all the solvents was ensured before actual analysis.

Field experiment

The crop was raised at the Research Farm of Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar following recommended agronomic practices. Plot size was 25 m^2 with spacing 60 × 30 cm (rows × plants) in triplicate with randomized block design (RBD). Before spraying, chilli fruits (*Variety*: HPH-2024) in all plots/replicates were tagged and sprayed with chlorpyriphos 20 EC using knap sap sprayer at single dose(160 g a.i ha^{-1}) and double dose (320 g a.i ha^{-1}) along with a control plot where no insecticide was applied. The volume of water used at the time of spray was @ 500 Lha^{-1}.

The soil under crop was of light texture with low content of organic matter; other relevant properties of the soil were EC 2 dSm^{-1}; K 10.08, P_2O_5 15 kg ha^{-1} with pH 7.6 and organic carbon 0.67 percent.

Sampling

The composite sample about 250 g of tagged chilli fruits were collected randomly separated from the control and treated plots of each treatment at 0 (1 hr after spray), 1, 3, 5, 7, 10, 15 and 30 days after the application of insecticide, packed in paper bags, and brought to the laboratory for processing. In the laboratory the samples were divided in two lots, one was processed as such and other was kept to study the effect of washing on reduction of residues. After thorough mixing and quartering of each part, 25 g representative sample of chilli was used for processing and analysis.

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Soil samples (500 g) under crop were also collected separately from 5-10 different sites with the help of a steel auger from the depth of 0-15 cm. Soil collected from different sites was pooled and sieved to remove extraneous matter, including stones/pebbles. After thorough mixing, a sub sample of about 250 g was taken from pooled sample of each treatment and transported to the laboratory. The samples were processed and analyzed at the Pesticide Residue Laboratory, Department of Entomology, CCS HAU, Hisar.

Extraction

Residues of chlorpyriphos from chilli were extracted by adopting the method of Bhardwaj et al. [7]. Representative 25 g sample of chilli was chopped into small pieces and macerated in mixer grinder and shaken with acetone (100 ml) on mechanical shaker for 1 hr and kept overnight in an Erlenmeyer flask. Extract was filtered through 2–3 cm layer of anhydrous sodium sulphate and the filter was washed with acetone (2×10 ml). The filtrate was subjected to liquid-liquid partitioning in 1-L separator funnel and diluted with 4–5 times brine solution (10% sodium chloride solution), and the contents partitioned first with dichloromethane (75,75 ml) and then with hexane (75,75 ml). Combined both the fractions and treated with 300 mg activated charcoal powder for about 2-3 hrs at room temperature. When the solution became clear, it was filtered through Whatman filter paper no.1. The clear extract was concentrated using a rotary vacuum evaporator. The extract was finally made up to 2 ml and added to the liquid – solid chromatography column.

Clean-up

Clean-up of samples was done by using column chromatography. Glass columns (60 cm × 22 mm i.d) were packed compactly with silica gel sand-witched in between two layers of anhydrous sodium sulphate. Prepared column were pre-washed using 40 ml hexane. The column was loaded with the concentrated extract and eluted with a solution of hexane: acetone (9:1 v/v). Combined the organic phases and concentrated to about 5 ml on a rotary vacuum evaporator. Finally, the extract was concentrated to dryness on gas manifold evaporator and final volume was made to 2 ml in n-hexane and analyzed by GC.

Washing

The whole fruits of chilli were washed under running tap water for 1 min by gentle rubbing with hands and the water was discarded. These washed samples were kept on blotting paper just to remove the excess of water following method of Walter et al. [8]. Then the samples were extracted, cleaned up in a similar manner as the raw samples were processed and analyzed.

Soil

Ground, sieved and dry representative (15 g) sample was mixed with 1-2 drops of ammonia solution and left for half an hour. Mixed the soil thoroughly with 0.3 g activated charcoal, 0.3 g Florisil and 10 g of anhydrous sodium sulphate and packed compactly in a glass column (60 cm × 22 mm i.d) in between two layers of anhydrous sodium sulphate as per method of Kumari et al. [9]. Residues were eluted with 125 ml solution of hexane: acetone (9:1 v/v) at flow rate of mL min^{-1}. The elute was concentrated on rotary vacuum evaporator and made the final volume to 2 ml in n-hexane for GC analysis.

Estimation by gas liquid chromatography

The cleaned extracts analyzed on gas chromatograph (Shimadzu-2010) equipped with ^{63}Ni electron capture detector (ECD) and HP-1 capillary column provide good results. Operating conditions were as per details: Temperature (°C): Oven: 150 (5 min^{-1}) →8 min^{-1}→190 (2 min) →15 min^{-1}→280 (10 min), Injection port: 280°C and detector: 300°C. Flow rate of nitrogen (carrier gas) was maintained at 60 ml min^{-1} and through column 2 ml min^{-1} with split ratio 1:10. Under these operating conditions, the retention time of chlorpyriphos was found to be 13.991 min. The residues of chlorpyriphos in samples were identified and quantified by comparing retention time and area of sample chromatograms with that of standards run under identical conditions.

Chilli and soil samples were spiked with chlorpyriphos at two concentration levels (0.010 and 0.025 mg kg^{-1}) processed and analyzed as per the methodology described above to check the validity of the method. Percent recoveries in chilli were 90.50 and 92.10 while in soil were 92.70 and 93.95 at two fortification levels, respectively. As the percent recovery obtained were more than 90%, therefore, the results have been presented as such without applying any correction factor. Limit of determination/quantification (LODe /LOQ) was 0.010 mg kg^{-1}.

Results and Discussion

The results of chlorpyriphos residues after application @ 160 and 320 g a.i.ha^{-1} detected in chilli samples collected from the Research Farm of CCS HAU, Hisar are presented in Table 1. The results indicated that at raw stage the highest chlorpyriphos residues were found in chilli. The initial deposits of chlorpyriphos at single and double dose were observed to be 0.397 and 1.021 mg kg^{-1}, respectively. In the start, residues dissipated slowly i.e. 0.385, 0.278 and 0.180 in case of single dose while 0.997, 0.695 and 0.485 in case of double dose on 1, 3 and 5th day after application. The residues reached below detectable levels (0.010 mg kg^{-1}) on 15th and 30th days of treatment in single dose and double dose, respectively, showing per cent dissipation of 97.48 and 98.14 per cent. Residue data were subjected to statistical analysis for computation of regression equations, half-life ($t_{1/2}$) values and percent degradation. The residues dissipated with half-life period of 6.02 days at single dose and 5.67 days at double dose. Residues of chlorpyriphos did not follow the first order kinetics. But the residues followed the pseudo first order kinetics (R^2) with 0.900 and 0.856 for single and double dose, respectively. Subhash et al. [10] studied the persistence behavior of chlorpyriphos, cypermethrin and monocrotophos in okra. The residues of all the three insecticides reached below detection limit (BDL) showing complete dissipation with in 15, 17 and 19 days respectively, when it was applied 100, 200 and 300 g a.i. h^{-1}. Jyot et al. [2] reported that the average initial deposits of chlorpyriphos on chilli were 0.59 and 2.02 mg kg^{-1} following the application of chlorpyriphos at 500 and 1,000 ga.i.ha^{-1}. These residues reached below the determination limit of 0.01 mgkg^{-1} after the 10th and 15th with half-life period of 4.43 and 2.01 days at single and double doses, respectively. Samriti et al. [11] observed that when chlorpyriphos applied on okra @ 200 and 400 g a.i ha^{-1}, residues on 7th and15th day of application reached below detection limit (BDL) of 0.010 mg kg^{-1} in single and double dose, respectively and reported half-life period of 3.15 days at single dose and 3.46 days at double dose following first order kinetics. Similar type of observation with chlorpyriphos in chilli was reported by Waghulde et al. [12]. Present results seem to be in conformation with earlier reports.

Effect of processing

Chilli fruits were subjected to processing like washing in order to investigate the reduction of residues up to 5th day of sampling. The results pertaining to the effect of washing on the removal of chlorpyriphos residues applied at 160 and 320 g a.i. ha^{-1} on chilli fruits

are presented in Table 2. It has been found that washing was found effective in reducing the residues on 0 (1 h) days after application. The average initial deposits of chlorpyriphos was 0.397 and 1.021 mg kg^{-1} at single and double dose on chilli fruits were reduced to 0.128 and 0.290 mg kg^{-1} as a result of simple washing with tap water showing the loss of 67.75 and 71.60 percent at, respective doses. In case of third day samples, the residues were reduced to 0.180 and 0.425 mg kg^{-1} thereby, accounted 35.25 and 38.84 percent residues loss at single and double doses, respectively. Sunayana et al. [13] studied the effect of washing alone with tap water to check the percent reduction in residues of chilli, when fipronil was applied on chilli crop @ 50 and 100 g a.i.ha^{-1}. As a result of simple washing percent reduction was observed 42.05 and 45.42 at single and double dose respectively. Rani et al. [14] reported that processing was very effective in reducing the residues of chlorpyriphos in tomato fruits when applied @ 400 and 800 g a.i. ha^{-1}. Samriti et al. [11] reported that due to washing 13–35% reduction of chlorpyriphos was observed in okra when applied @ 200 and 400 g a.i. ha^{-1}.

Chlorpyriphos residues in soil samples

In soil, initial deposit of chlorpyriphos on 0 (1 h after treatment) days at single dose was 0.439 mg kg^{-1} and 0.903 mg kg^{-1} at double dose (Table 3). Under study period of 30 days, residues reached to the levels of 0.037 and 0.088 mg kg^{-1} at single and double dose, respectively. The dissipation after 30 days was observed to be 91.57 per cent for single dose and 90.25 per cent for double dose.

Jyot et al. [2] reported that residues of chlorpyriphos and cypermethrin were found to be <0.01 mg kg^{-1} for both these insecticides at the single (500 g a.i ha^{-1}) and double dosages (1000 g a.i ha^{-1}) collected 15 days after the last spray. Persistence of chlorpyriphos in soil under tomato crop was studied by Rani et al. [14] with active application of chlorpyriphos at 400 and 800 g a.i. ha^{-1}. In soil samples, residues of chlorpyriphos reached below detectable level of 0.010 mg kg^{-1} after 5 and 10 days after spray at single and double dose, respectively. Samriti and Kumari [3] reported that the residues of chlorpyriphos in soil under okra crop dissipated below determination level of 0.005 mg kg^{-1} on 5 days at single dose (200 g a.i ha^{-1}) and 7 (400 g a.i ha^{-1}) days at double dose reported.

Conclusion

From the above outcome, it is obvious that the application of pesticides in agriculture is necessary for better crop production against the possible health hazards arises due to the presence of pesticide residues in food. The half-life values for chlorpyriphos following three applications at the single and double dose on chilli fruits were observed to be 6.02 and 5.67 days, respectively. The residues of chlorpyriphos studied in chilli at two doses, reached to below detectable value of 0.01 mg kg^{-1} on 15th and 30th in single and double dose, respectively. Meticulous processing with simple household practices like washing, dislodged the residues by 68 -72 per cent and thereby ensures the safety of the chilli fruits to the consumers.

Days after treatment	Residue (mg kg^{-1})			
	Single Dose (160 g a.i. ha^{-1})		Double Dose (320 g a.i. ha^{-1})	
	Average	% Dissipation	Average	% Dissipation
0(1 h)	0.397 ± 0.101	-	1.021 ± 0.226	-
1	0.385 ± 0.017	3.02	0.997 ± 0.098	2.35
3	0.278 ± 0.033	29.97	0.695 ± 0.111	31.92
5	0.180 ± 0.016	54.65	0.485 ± 0.015	52.49
7	0.134 ± 0.010	66.24	0.360 ± 0.085	64.74
10	0.085 ± 0.012	78.58	0.258 ± 0.032	74.73
15	0.010 ± 0.007	97.48	0.019 ± 0.007	98.14
30	BDL	-	BDL	-
$t_{1/2}$ = 6.02 days			$t_{1/2}$ = 5.67 days	

BDL: 0.01 mg kg^{-1}

Table 1: Persistence and dissipation of chlorpyriphos residues in chilli.

Days after treatment	Residue (mg kg^{-1})					
	Single Dose (160 g a.i. ha^{-1})			Double Dose (320 g a.i. ha^{-1})		
	Initial residues ± SD	Washing ± SD	% Reduction	Initial residues ± SD	Washing ± SD	% Reduction
0(1 h)	0.397 ± 0.101	0.128 ± 0.110	67.75	1.021 ± 0.226	0.290 ± 0.031	71.60
1	0.385 ± 0.017	0.157 ± 0.012	59.22	0.997 ± 0.098	0.354 ± 0.101	64.49
3	0.278 ± 0.033	0.180 ± 0.016	35.25	0.695 ± 0.111	0.425 ± 0.111	38.84
5	0.180 ± 0.016	BDL	-	0.485 ± 0.015	0.405 ± 0.075	16.49

Table 2: Effect of processing on chlorpyriphos residues (mg kg^{-1})* in chilli.

Days after treatment	Residue (mg kg^{-1})			
	Single Dose (160 g a.i. ha^{-1})		Double Dose (320 g a.i. ha^{-1})	
	Average	% Dissipation	Average	% Dissipation
0(1 h)	0.439 ± 0.011	-	0.903 ± 0.012	-
3	0.304 ± 0.013	30.75	0.635 ± 0.006	29.67
7	0.149 ± 0.008	66.05	0.315 ± 0.023	65.11
15	0.078 ± 0.006	82.23	0.165 ± 0.010	81.72
30	0.037 ± 0.006	91.57	0.088 ± 0.007	90.25

Table 3: Persistence and dissipation of chlorpyriphos residues in soil.

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