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RESEARCH ARTICLE

Dissipation Pattern of chlorpyriphos in/on Green Chillies

Vinothkumar. B*, Muralitharan. V and Shanmugapriya.R
*Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore - 641 003.

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ABSTRACT

Supervised field trials were conducted to study the dissipation of chlorpyriphos at Devarayapuram village, Coimbatore District, Tamil Nadu, India on chillies TNAU hybrid CO 1, during December 2016 - March 2017. The chillies plots were sprayed with chlorpyriphos 20 EC @ 200 g a.i. ha^{-1} and 400 g a.i. ha^{-1} twice at 15 days interval. Samples were collected and processed immediately. The residues were extracted by following the modified QuEChERS method. The residues of chlorpyriphos were estimated using Gas Chromatograph (GC) (Shimadzu, GC-2010) equipped with autosampler and Flame Photometric Detector. Percent recoveries of chlorpyriphos in green chillies were found to be consistent and they were 98.76, 88.40 and 94.98 per cent for 0.05, 0.25 and 0.5 µg g^{-1}, respectively. Good linearity was achieved with a correlation coefficient of 0.999. The Limit of Detection and Limit of Quantitation was found to be 0.05 µg g^{-1} and 0.015 µg g^{-1}, respectively. The mean initial deposits of chlorpyriphos were observed to be 3.47 and 5.12 µg g^{-1} on green chillies at 200 and 400 g a.i. ha^{-1}, respectively. These residues were dissipated to 97.34 and 95.99 percent at the recommended and double the recommended dosages, respectively, after 7 days of the last application of the pesticide. The dissipation pattern of chlorpyriphos in green chillies was computed following seven transformations and the best fit observed was first-order kinetics for both the doses. The half-life values of chlorpyriphos on green chillies were found to be 1.41 and 1.65 days and calculated safe waiting periods were 6.17 and 8.23 days, respectively for recommended and double the recommended dose.

Keywords: *Green chillies, Chlorpyriphos, Dissipation, Residues, Waiting period.*

INTRODUCTION

Chillies (*Capsicum annum* L.), is an important vegetable and condiment crop grown throughout the world and it has large commercial, dietary and therapeutic values (Reddy *et al.*, 2017). In India, chillies is cultivated in an area of 774.9 lakh ha with an annual production of 1492.1 lakh tonnes (Horticultural Statistics, 2015).Famous chillies growing states in India are Andhra Pradesh, Telangana, Karnataka, Maharashtra and Tamilnadu which constitute nearly 75 per cent of the total area under chillies. Chillies crop is known to be attacked by 51 species of insects and 2 species of mites belonging to 27 families under 9 orders along with snail and 2 species of millipedes both in nursery and main field (Reddy and Puttuswamy, 1983 and 1984). Chillies suffer major quantitative and qualitative loss in production due to chilli thrips, *Scirtothrips dorsalis* Hood and yellow mite, *Polyphagotarsonemus latus* (Banks) and fruit borers *Helicoverpa armigera* (Hubner) (Raghu, 2014). These pests not only cause reduction in yield, but also act as vectors for several viral diseases and cause complete failure of the crop. A number of insecticides are being frequently used, to combat these pests. However, some of these insecticides leave residues on pods and these residues may persist up to harvest. The presence of insecticides residues in the harvested chillies is posing a problem at the time of export and in recent times importing countries have rejected few consignments. Pesticide use has increased rapidly over the last two decades at the rate of 12 oercent per year (Thacker *et al*., 2005). Many farm gate chillies samples showed presence of insecticide residues (Singh *et al*., 1999).

As per the insecticides Act of 1968 (www.cibrc.nic.in), 37 insecticide formulations are registered and recommended for use to encounter the various pests of chillies. However the usage of pesticides in farm level varies with pest status, recommendation by state agricultural departments, judious uuse of non recommended insecticides without following preharvest intervals are common phenomenon at farmer's level. Since chillies is an vital component

*Corresponding author's e-mail: vinothkumar@tnau.ac.in

in the food, assessment of of toxic substances and contaminants in the chillies are verymuch essential. As the older molecules like organophosphorus compounds are cheap and easily available in the retail pesticide market, farmers spray these insecticides more frequently and judiciously on vegetable crops without following any PHI. The extensive and irrational use of insecticides resulted in the presence of residues of insecticides on chillies is likely to be associated with severe ill effects on human health. Hence, it is imperative to study the persistence of insecticides on edible crops to ensure human safety. It is important to ensure that the levels of harvest time residues of insecticides on food stuff do not pose any hazard to consumers and are admissible in domestic as well as international trade. In this context, the present study was carried out to investigate the residual behavior and risk assessment of chlorpyriphos on green chillies fruits at different time intervals.

MATERIAL AND METHODS

The supervised field trial was conducted to study the dissipation of chlorpyriphos on chillies TNAU hybrid CO 1 at Devarayapuram village, Coimbatore District, Tamil Nadu, India, during December 2016 - March 2017. The crop was maintained well by adopting standard agronomic practices as per the recommendations of Tamil Nadu Agricultural University, Coimbatore. The experiment was laid out in a randomized block design in a plot size of 50 m^2 and replicated thrice, including untreated control. The chillies plots were sprayed with chlorpyriphos 20 EC @ 200 and 400 g a.i. ha^{-1} using hand operated knapsack sprayer twice at fruit inintation stage and 15 days after the first spray. It is ensured that the test chemical should not been used arlier in the field which is taken for our study. One kilogram of fruit sample from each replication was collected at 0 (one hour after spraying), 1, 3, 5, 7, 10, 15, 21 and 30 days after spraying from chlorpyriphos treated plots as well as an untreated control. The samples should be transported immediately after collection and processed by chopping in to small pieces. Sub samples weighing 500 g was taken and homogenized with a high-speed mixer grinder. Homogenized samples were stored in the wide mouth glass bottles at -20 °C until further use.

The reference standard of chlorpyriphos (99.7 % purity) was purchased from M/S Sigma Aldrich, Bangalore, India. Hexane (C_6H_{14}), sodium chloride (NaCl) and anhydrous magnesium sulfate ($MgSO_4$) were purchased from M/s. Merck India Ltd., Mumbai, India. Sodium chloride and magnesium sulfate were heated at 650ºC for 4 h for activation and kept in a desiccator until use. Graphitized Carbon Black (GCB) and Primary Secondary Amine (PSA) (Bondesil 40 µm) were purchased from M/s. Agilent Technologies, USA. Primary stock solutions of chlorpyriphos(400 µg ml^{-1}) standard were prepared with C_6H_{14} (v/v) in a volumetric flask. An intermediate stock solutions of 100 µg ml^{-1} and 10 µg ml^{-1} were prepared from primary stock solution and working standards was prepared from intermediate stocks. The stock solutions were stored in the deep freezer at -20 °C and working standards were stored at 4ºC until further use.

The residues of chlorpyriphos were estimated using Gas Chromatograph (GC) (Shimadzu, GC-2010) equipped with auto sampler and Flame Photometric Detector (FPD). Chromatographic separation was achieved with capillary column (DB-5, 30 m x 0.25 mm x 0.25 µm) with injection port temperature of 280ºC and column temperature programme of 150ºC, hold for 2 min; increased @ 4ºC min^{-1} into 200ºC, hold for 7 min.; increased @ 2ºC min^{-1} to 230ºC, hold for 0 min; increased 3.5 ºC min^{-1} to 280ºC, hold for 10 min. Nitrogen was used as carrier gas with a flow rate of 1.0 ml min^{-1}. Hydrogen and zero air were used as detector gases in the flow rate of 80 and 120 ml min^{-1}, respectively. An injection volume of 1 µL was used and the total run time was 60.799 min. Residues of chlorpyriphos were quantified by the comparison of peak height/ peak area of standards with that of unknown or spiked samples run under identical conditions of operation.

The residues were extracted by following the modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Anastassiades *et al.* 2003). A 50 ml centrifuge tube was taken to hold 10 g of the homogenized representative sample with 20 ml acetonitrile. Vegourous shaking was done for about one minute. Four gram of magnesium sulphate (Mg SO_4) and 1 gram of sodium chloride (NaCl) was added in the centrifuge tube and shaken well by vortex mixture. The tube was centrifuged at 6000 rpm for 10 minutes. Four gram of anhydrous sodium sulphate (Na_2SO_4) was taken in the test tube containing 9 ml of supernatant transferred from the centrifuge tube.100 mg PSA, 600 mg anhydrous $MgSO_4$ and 10 mg GCB were taken in 15 ml centrifuge tube and 6 ml of supernatant from the testube was added and vortexed for 1 minute and then centrifuged for 10 minutes at 3000 rpm. The upper extract (4 ml) was transferred into a turbovap tube and concentrated to dryness under a gentle stream of nitrogen in a turbovap LV at 40 °C. Hexane (1ml) was added to test tube, shaken well reconstituted to 1 ml and transferred into a 1.5 ml glass auto sampler vial for analysis.

The validation of analytical method was performed for parameters *viz.,* specificity, linearity, Limit of Detection (LOD), Limit of Quantitation (LOQ), recovery, repeatability and ruggedness along

with measurement uncertainty (SANTE 2017). The specificity of the chlorpyriphos was assessed by injecting standard solution six times at one concentration (0.05 µg ml^{-1}). The linearity study was performed by injecting five different concentrations of the standard of chlorpyriphos between 0.05 and0.8 µg ml^{-1} with three replications. Validity of the test method was ensured with recovery of test chemical spiked at three different concentrations *viz.,* 0.05, 0.25 and 0.5 µg g^{-1} in 10 g of homogenized untreated chillies fruit samples and replicated thrice along with untreated control. , The LOD and LOQ were calculated using a linear regression model. Recovery of test chemical @ 0.05µg g^{-1} level was done with six replications to estimate the reproducibility, repeatability and ruggedness The control samples were analyzed and the result indicated that the blank did not contribute any interference with the target compound. The measurement uncertainty was calculated based on the procedure given by Ellison and Williams (2012) and Magnusson and Örnemark (2014).

The chlorpyriphos degradation pattern was analyzed by applying seven transformation functions, as suggested by Hoskins (1961) and Timme *et al.* (1986). The half-life was calculated based on Regupathy and Dhamu (2001) and the best-fit degradation model was determined. The safe waiting period was worked out as per the formula is given by Handa *et al.* (1999) using Codex / FSSAI Maximum Residual Limit (MRL)

$$Safewaitingperiod(T_{toi}) = \frac{\log K_2 - \log(MRL/tolerance)}{|\log K_1|}$$

Where, K_1 is the slope of the regression line (b), always negative sign used as a positive number and K_2 is the apparent initial deposit obtained by extrapolating the line back to zero time.

RESULTS AND DISCUSSION

Green chillies were spiked with chlorpyriphos at three concentration levels (0.05, 0.25 and 0.5 µg g^{-1}) and analyzed as per the methodology described above to estimate the trueness (recovery) of the method. Percent recoveries of chlorpyriphos in green chillies were found to be consistent and they were 98.76, 88.40 and 94.98 per cent for 0.05, 0.25 and 0.5 µg g^{-1}, respectively. Quantification was accomplished using a standard curve (Fig .1), prepared by diluting the stock solution.Good linearity was achieved with a correlation coefficient of 0.999 (Fig. 2). The precision of the method was determined by repeatability studies of the method and expressed as relative standard deviation (RSD) values. The RSD for repeatability ranged from 2.13 to

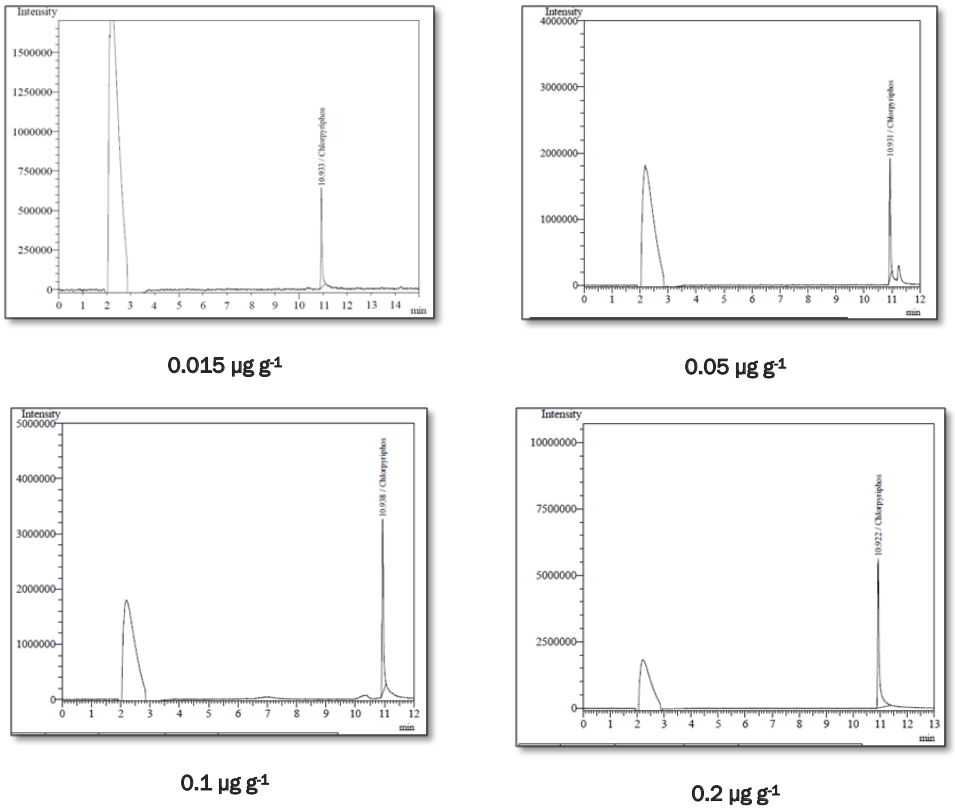

Figure 1. Standard chromatograms of chlorpyriphos in Gas chromatograph

3.11 percent.The percent recoveries of chlorpyriphos in green chillies were more than 85 percent. Therefore, the results have been presented as such without applying any correction factor. In general, the residues of chlorpyriphos were determined by a comparison of the peak areas of the reference standards with those of the unknown or spiked samples run under identical working conditions of

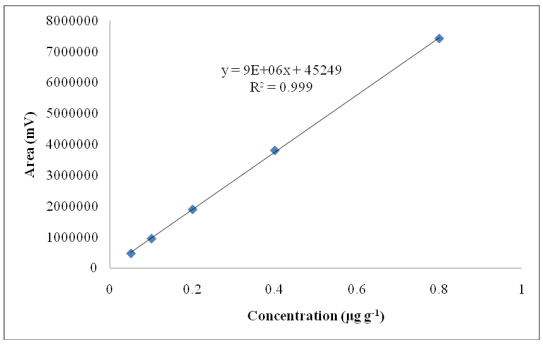

Figure 2.Calibration curve of chlorpyriphos in gas chromatograph

the instruments employed. For chlorpyriphos, the limit of quantification was found to be 0.05 µg g^{-1} and the limit of detection found to be 0.015 µg g^{-1}.

The overall results of the analysis of green chillies fruits following the second application of chlorpyriphos 20 EC at 200 and 400 g a.i. ha^{-1} are presented in Table 2. The mean initial deposits of chlorpyriphos were observed to be 3.47 and 5.12 µg g^{-1} on green chillies fruits following the second application at 200 and 400 g a.i. ha^{-1}, respectively.

Table 1. Per cent recovery of chlorpyriphos in green chillies

Fortification (g g^{-1})	Mean % recovery	RSD of Recoveries (%)
0.05	98.76	3.11
0.25	88.40	2.13
0.5	94.98	2.80

These deposits dissipated to 0.80 and 1.07 µg g^{-1} after three days at single and double dosages, respectively, there by showing corresponding losses of about 76.84 and 79.05 percent. Around 90 percent of these residues got dissipated in five days at both these dosages. These initial residues were dissipated to 97.34 and 95.99 percent at the recommended and double the recommended dosages, respectively, after seven days ofthe last application of the insecticides (Table 2). The dissipation pattern of chlorpyriphos in green chillies was computed following seven transformations and the best fit observed was first-order kinetics for both the doses (200 and 400 ga.i ha^{-1}) (Table 3). The statistical parameters like intercept (a), slope of regression lines (b) and half-life are presented in Table 3. The half-life values of chlorpyriphos on green chillies were found to be 1.41 and 1.65 days at recommended and double the recommended dose, respectively. Since Codex MRL is not available for chlorpyriphos in chillies,the Food Safety Standards Authority of India (FSSAI) MRL value (0.2 µg g^{-1} for chillies) was referred and calculated safe waiting periods were 6.17 and 8.23 days, respectively for recommended and double the recommended dose.

Table 2. Persistence and dissipation of chlorpyriphosresidues in/on chillies

DAT	Chlorpyriphos residue in g g^{-1}									
	Chlorpyriphos 20 EC @ 200 g a.i. ha^{-1}					**Chlorpyriphos 20 EC @ 400 g a.i. ha^{-1}**				
	R1	R2	R3	Mean	Dissipation (%)	R1	R2	R3	Mean	Dissipation (%)
0 (1 hr)	3.315	3.458	3.651	3.47	-	5.261	5.114	4.975	5.12	-
1	1.672	1.856	1.625	1.72	50.57	2.586	2.921	2.197	2.57	49.81
3	0.715	0.923	0.776	0.80	76.84	1.014	1.203	0.998	1.07	79.05
5	0.384	0.296	0.356	0.35	90.05	0.483	0.512	0.586	0.53	89.70
7	0.092	0.103	0.082	0.09	97.34	0.221	0.192	0.202	0.21	95.99
10	BDL	BDL	BDL	BDL	100.00	0.058	0.075	0.062	0.07	98.73
15	BDL	BDL	BDL	BDL	100.00	BDL	BDL	BDL	BDL	100.00

DAT: (Days after Treatment) BDL: Below Detectable level (0.05 µg g^{-1}), ND: Not Detected

The results of the present study is in accordance with the findings of Kaushik Banerjee and Banerjee (2002), who found that dissipation levels of chlorpyriphos on grapes at 1 and 15 days after treatment were 20.49 and 98.94 per cent for 500 g a.i. ha^{-1} of chlorpyriphos and 18.78 and 98.87per cent for 1000 g a.i. ha^{-1} of chlorpyriphos, respectively. The safe waiting periods were 6.14 and 8.10 days following foliar spray at lower (500 g a.i. ha^{-1})and higher (1000 g a.i. ha^{-1}) concentration, respectively, for chlorpyriphos on grapes. Raina and Raina (2008) reported the dissipation of chlorpyriphos following its application at 500 and 1,000 g a.i.ha^{-1} for two consecutive years, 2004 and 2005 and the average initial deposits varied from 0.56 to 0.86 and 1.29 to1.43 µg g^{-1}, respectively. No residue

was detected15 days after the last application of the pesticide at both dosages. Samriti *et al.* (2011) reported that the average initial deposits of chlorpyriphos on okra were 0.067 and 0.129 µg g^{-1} following the application of chlorpyriphos at 200 and 400 ga.i.ha^{-1}.These residues declined below the determination limit of 0.01 µg g^{-1} after the10thand 15thday of application in single and double doses,

Table 3. Correlation coefficient and half life for chlorpyriphosin/on chilliesby different methods of transformations of residues data

Function	Chlorpyriphos 20 EC @200 g a.i.ha^{-1}						Chlorpyriphos 20 EC @400 g a.i.ha^{-1}					
	a	b	r	R^2	Mod R^2	T Half	a	b	r	R^2	Mod R^2	T Half
First order	1.18	-0.49	-0.994**	0.987	0.99	1.41	1.45	-0.42	-0.997**	0.994	0.96	1.65
1.5th order	0.31	0.37	0.942*	0.888	-5.5	0.34	0.17	0.32	0.968**	.937	-40.29	0.22
2nd order	-1.16	1.37	0.867^{NS}	0.751	-3.05	-0.85	-1.82	1.28	0.893*	0.797	-1.74	-1.42
RF First order	1.67	-1.33	-0.955*	0.913	0.74	0.27	2.14	-1.38	-0.977**	0.955	0.7	0.25
RF 1.5th order	0.04	0.93	0.851^{NS}	0.725	-381.3	0.007	-0.21	0.97	0.877*	0.769	-267.13	-0.04
RF 2nd order	-1.93	3.34	0.749^{NS}	0.561	-1.45	0.34	-2.97	3.70	0.766^{NS}	0.586	-0.71	0.64
Inverse power law	-0.35	0.42	0.805^{NS}	0.647	0.5	5.13	-0.52	0.51	0.818*	0.669	0.59	3.94

a - Intercept; b – Slope; r - regression coefficient; RF- root function; * significant at 5 % level; ** significant at 1 % level

respectively. Spraying of readymix formulation Action-505EC (50 %+cypermethrin 5 %) at 400 and 800 ga.i.ha^{-1}, on tomato showed average initial deposits of 0.117and 0.253 µg g^{-1} chlorpyriphos. These residues reduced to 0.010 and0.038 µg g^{-1} on the seventh day, accounting for the lossof more than 82–91 percent. The residues persisted beyond 10 and 15 days at the single and double doses, respectively (Gupta *et al.* 2011).Gagan Jyot *et al.,* (2013) reported that, Half life period of chlorpyriphos in chillies when sprayed with Nurelle-D 505 (chlorpyriphos 50 %+cypermethrin 5 %) at 1 and 2 Lha^{-1} were found to be 4.43 and 2.01 days, respectively and residues of chlorpyriphosdissipated to more than 80 percent after 10 days atboth the dosages.Half life period of chlorpyriphos 20 EC @ 300 g a.i. ha^{-1} was 2.37 days in tomato and 2.83 days in green peas (Sushil Ahlawat *et al.,* 2017).

CONCLUSION

To conclude, present study indicated that the chlorpyriphos 20 EC sprayed on green chillies @ 200 and 400 g a.i ha^{-1} dissipated to Below Detectable Level (BDL) (< 0.05 µg g^{-1}) on 7 and 10 days after treatment, respectively with calculated half-life of 1.41 and 1.65 days, respectively and safe harvesting period of 6.17 and 8.23 days, respectively.

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