



## Dissipation and persistence pattern of chlorantraniliprole residues in tomato under open field conditions and its estimation through UHPLC-PDA

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**ABSTRACT:** Field experiment was carried out to study the dissipation and persistence of chlorantraniliprole in tomato (cv. Kashi Vishesh) sprayed @ 30 and 60 g a.i. ha<sup>-1</sup>. The initial residues ranged from 0.54 to 0.74 and 0.92 to 1.20 mg kg<sup>-1</sup>. Residues further declined below Limit of Quantification (LOQ) of 0.05 mg kg<sup>-1</sup> after 5<sup>th</sup> day, respectively. The T<sub>1/2</sub> of chlorantraniliprole on tomato after the 2<sup>nd</sup> spray was calculated to be 1.02 and 1.32 days whereas after the 3<sup>rd</sup> spray it was observed to be 0.90 days at both the dosages. The safe waiting period for tomato was suggested to be 1 day after application, if followed the GAP.

**Keywords:** Chlorantraniliprole, residues, tomato, waiting period, dissipation, UHPLC

### INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is a solanaceous vegetable crop having a good source of nutrients. It is the world's largest consumed vegetable, widely grown throughout the world and ranked first among the canned vegetables (Choudhary, 1996). The fruits of tomato can be consumed both in raw form as well as in cooked form. It is grown under tropical and subtropical and temperate regions. Biotic and abiotic stresses are two major factors responsible for the reduction of quality and quantity production and productivity. In India, *Helicoverpa armigera* is the most serious pest and is responsible for huge economic losses by reducing the quantity, quality thereby market value (Singh *et al.*, 2011; Reddy and Zeharm, 2004). In India around 5 to 55 per cent losses reported due to fruit borer in tomato growing regions. Under favourable conditions, damage caused by the pest may go up to 88 per cent (Selvanaryanan and Narayanasamy, 2006). Chemical control serves as an important tool of pest management that is employed largely against the management of above pests in tomato. The injudicious use of the chemicals leads to the problem of residue, resistance as well as the outbreak of secondary pests. This resulted in development of newer molecules such as chlorantraniliprole with a unique mode of action for the safety of human beings as well as other beneficial organisms on different plants, including tomatoes.

Chlorantraniliprole is a new insecticide with systemic action of anthranilic diamide group (Cordova *et al.*, 2006). The population of fruit borer larvae was minimum in plot with chlorantraniliprole 18.5 SC treated with mean larval population of 1.14 larvae/plant (Wasu *et al.*, 2020). The reduction per cent of fruit borer larval population was highest at 3<sup>rd</sup> day of after application with per cent

reduction of 98.04. When rynaxypyr imposed @ 40 g a.i. ha<sup>-1</sup> showed a high level of insecticidal activity against lepidopteran insect pests and less toxic to mammals attributed by its higher selectivity to insects over mammalian ryanodine receptors (Ghosal *et al.*, 2012). Besides its insecticidal activity against lepidopteran pest, it is also equally effective in controlling the population of whitefly, leaf miners, beetle, and termite species (Babu *et al.*, 2019). Chlorantraniliprole 18.5 per cent SC is used against fruit borers of chillies and tomato, bollworms in cotton, tobacco caterpillar, okra fruit borer and diamond back moth in cabbage (Anon., 2020).

The rational recommendation for an insecticide must achieve effective control of target pest without leaving residues on the produce. Pesticide use on any crop leads to its residues which further dissipate with time. The dissipation and persistence of pesticide residues depend upon the physico-chemical properties of the pesticide used, dose of pesticide used and nature of the crop on which sprayed and weather parameters of that locality. Most of the pesticides applied may affect non target organism may be due to inefficient application system which further increases the problems of pesticide residues in the environment. To safe guard the problems arising out of pesticide use, it is always advisable for judicious use of pesticide. The amount of residues present in different commodities for different pesticide, supervised field trails are to be conducted. The dissipation and persistence nature of pesticides on different crops can be obtained by conducting supervised field trials. Data from supervised trials give reliable estimates of the residue levels that are likely to persist in food commodities at the time of harvest. To ensure consumer safety, the

**Table 1. Per cent recovery of chlorantraniliprole from spiked samples of tomato**

Spiked Level (mg kg <sup>-1</sup> )	Replicates		Percent Mean Recovery ± SD	RSDr
	Amount recovered	Percent recovery		
0.05	0.042	84.00	88.00 ± 5.292	6.013
	0.043	86.00		
	0.047	94.00		
0.25	0.240	96.00	89.60 ± 5.769	6.438
	0.212	84.00		
	0.220	88.00		
0.5	0.459	91.80	90.60 ± 4.937	5.449
	0.473	94.60		
	0.425	85.00		

RSDr = (Relative Standard Deviation (Repeatability), SD = (Standard Deviation)

persistence pattern of pesticides on edible parts of crops must be known. The current field experiment was carried out with an objective to study the dissipation and persistence of chlorantraniliprole in tomato at different time intervals and also to suggest waiting periods for chlorantraniliprole on tomato.

## MATERIALS AND METHODS

**Insecticide:** Analytical standard of chlorantraniliprole (purity 97.28 per cent) was obtained from Dr. Erhenstrofer, India, chlorantraniliprole 14.5 SC formulations were used for field application. Standard stock solutions of chlorantraniliprole (1mg mL<sup>-1</sup>) were pre arranged with respect to HPLC grade acetonitrile, the standard solutions were further diluted to have different concentrations and injected into the instrument to see the linearity by plotting a calibration curve. The storage temperature for all these standard solutions were kept around -4°C prior to use.

**Raising of the crop:** A field experiment with three treatments and three replications was carried out at Agricultural Research Farm, Tirhut College of Agriculture (TCA), Dholi, Muzaffarpur, Bihar, India. Tomato (cv. Kashi Vishesh) was raised in a randomized block design according following the recommended agronomic practices for this region. The seedlings were transplanted in the first week of November at proper spacing (30cm x 45cm) in 25 m<sup>2</sup> plots.

**Application of insecticide:** The chlorantraniliprole 18.5 SC was applied on tomato crops at doses of 30 and 60 g a.i. ha<sup>-1</sup>, respectively by using High Pressure Lithium Battery Operate and Knapsack Sprayer of 15 L capacity. The 1<sup>st</sup> spraying was done at 50 per cent flowering/ fruit initiation stage & subsequently 2 sprays were done at 10

days interval. Amount of volume used while spraying was 500 litres ha<sup>-1</sup>.

**Sampling:** About half kg samples of marketable size tomato fruits were randomly taken from each plot at “0 (2 hours), 1, 3, 5, 7 and 10 days after the second and 0 (2 hours), 1, 3, 5, 7, 10 and 15 days” after third application of the of insecticide. The collected samples were brought to the pesticide residue laboratory, Department of Entomology for further analysis. Samples were chopped and 15 g of macerated samples were kept in the refrigerator at 4 °C. The extraction and cleaned up was done on next day of sampling.

**Residue analysis of tomato samples:** “Quick, Easy, Effective, Rugged and Safe (QuEChERS)” technique with slight changes is used for processing of tomato samples for residue analysis (Anastassiades *et al.*, 2003). A grinded tomato sample (15g) was transferred to a 50 mL polypropylene centrifuge tube later kept overnight in refrigeration. Samples were taken from the refrigerator and 30 mL of ACN (HPLC grade) was added to each tube. To each centrifuge tube, for phase separation, (10 ± 0.1 g) NaCl was added and agitated for 10 minutes at 50 rpm on a rotospin (Tarson®). Sample was centrifuged for 3 min at 2,500 rpm. Moisture, if any, was removed from aliquot of acetonitrile by sodium sulphate anhydrous (10 ± 0.1 g) followed by clean-up through “dispersive solid phase extraction (DSPE)”. For this, a polypropylene tube constituting “0.15 ± 0.01 g PSA sorbent and anhydrous MgSO<sub>4</sub> (0.90 ± 0.01 g) was prepared for an aliquot of 6 mL which was thoroughly mixed by vortex spinix (Tarson®). Once again centrifuged for 3 min at 2,500 rpm and finally a 3 mL aliquot was taken for estimation of residues of chlorantraniliprole for residue analysis.

**Table 2. Residues of chlorantraniliprole in tomato**

Days after spraying	2 <sup>nd</sup> spray				3 <sup>rd</sup> spray			
	Dose @ 30 g a.i. ha <sup>-1</sup>		Dose @ 60 g a.i. ha <sup>-1</sup>		Dose @ 30 g a.i. ha <sup>-1</sup>		Dose @ 600 g a.i. ha <sup>-1</sup>	
	Mean of Residue (mg kg <sup>-1</sup> ) ± SD	Per cent dissipation	Mean of Residue (mg kg <sup>-1</sup> ) ± SD	Per cent dissipation	Mean of Residue (mg kg <sup>-1</sup> ) ± SD	Per cent dissipation	Mean of Residue (mg kg <sup>-1</sup> ) ± SD	Per cent dissipation
Before application	<LOQ	-	<LOQ	-	< LOQ	-	< LOQ	-
0 (2hrs after spray)	0.60 ± 0.076	-	0.97 ± 0.047	-	0.70 ± 0.068	-	1.10 ± 0.083	-
1	0.18 ± 0.031	70.00	0.33 ± 0.053	65.97	0.21 ± 0.036	70.00	0.35 ± 0.055	68.18
3	0.07 ± 0.015	88.33	0.11 ± 0.015	88.86	0.06 ± 0.015	91.43	0.10 ± 0.015	90.09
5	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
7	<LOQ	-	< LOQ	-	<LOQ	-	<LOQ	-
10	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
15	-	-	-	-	<LOQ	-	<LOQ	-

LOQ = Limit of Quantification (0.05 mg kg<sup>-1</sup>)

**Table 3. Dissipation parameters of chlorantraniliprole residue in tomato**

Dissipation parameters	Doses after 2 <sup>nd</sup> spray		Doses after 3 <sup>rd</sup> spray	
	30 g a.i. ha <sup>-1</sup>	60 g a.i. ha <sup>-1</sup>	30 g a.i. ha <sup>-1</sup>	60 g a.i. ha <sup>-1</sup>
K <sub>1</sub> (b)	-0.295	-0.227	-0.336	-0.336
K <sub>2</sub> (a)	1.687	1.889	1.770	1.976
T <sub>1/2</sub>	1.02	1.32	0.90	0.90
T <sub>tol</sub>	0.31	0.49	0.4	0.58
R <sup>2</sup>	0.933	0.882	0.964	0.973
Y	-0.295x + 1.687	-0.227x + 1.889	-0.336x+1.7703	-0.336x+1.9767
K <sub>1</sub> =	“Slope of the regression line”			
K <sub>2</sub> =	Initial deposit obtained as in the regression equation”			
T <sub>1/2</sub> =	“Residual half-life (in days)”			
T <sub>Tol</sub> =	“Time (in days) required for the pesticide residue to reach below the maximum residue limit of 0.6 mg kg <sup>-1</sup> ”			
R <sup>2</sup> =	“Coefficient of determination”			

**Estimation:** The estimation of chlorantraniliprole was done through UHPLC (High Pressure Liquid Chromatography) developed with “Photodiode Array Detector (PDA)”. The peak of chlorantraniliprole was detected at a  $\lambda_{max}$  of 260 nm when Mobile phase {ACN: HPLC water (70:30)} was given with a flow rate of 0.3 ml min<sup>-1</sup>, injected at a volume of 20 µl using C<sub>18</sub> column at a temperature of 40 °C.

**Determination of residues:** The residues of chlorantraniliprole in tomato were matched with the retention time of respective standards, whereas, estimated by peak area. Retention time for chlorantraniliprole was observed to be 4.327 min., correspondingly when injected under above mentioned conditions.

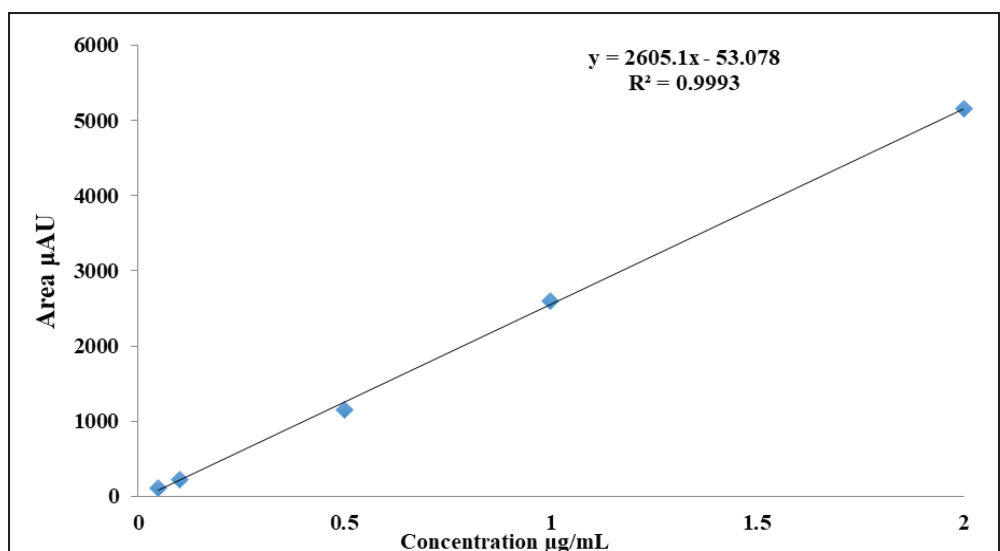


Fig. 1 Linearity curve of chlorantraniliprole standards

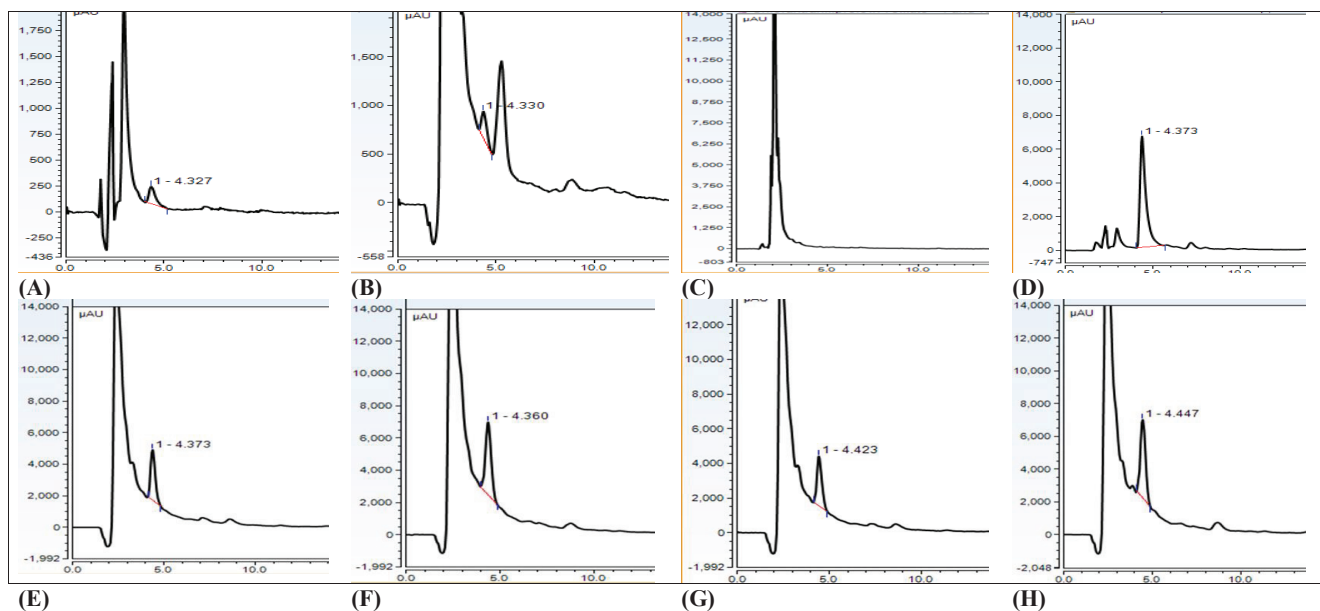


Fig 2. HPLC chromatograms for tomato samples of (A)Chlorantraniliprole standard @  $0.05 \mu\text{g mL}^{-1}$ ; (B)Tomato spiked with chlorantraniliprole @  $0.05 \mu\text{g mL}^{-1}$ ; (C)Tomato control; (D)Chlorantraniliprole standard  $1 \mu\text{g mL}^{-1}$ ; (E)Chlorantraniliprole @  $30 \text{ g a.i.ha}^{-1}$  after 2<sup>nd</sup> spray; (F)Chlorantraniliprole @  $60 \text{ g a.i.ha}^{-1}$  after 2<sup>nd</sup> spray; (G)Chlorantraniliprole @  $30 \text{ g a.i.ha}^{-1}$  after 3<sup>rd</sup> spray; (H)Chlorantraniliprole @  $60 \text{ g a.i.ha}^{-1}$  after 3<sup>rd</sup> spray

## RESULTS AND DISCUSSION

**Limit of detectability of chlorantraniliprole residues in tomato:** The full-scale deflection was obtained with 5 ng of the standard of chlorantraniliprole, respectively. Samples of tomato were processed and terminal volume was composed to 3 mL. The sample load 20  $\mu\text{L}$  for chlorantraniliprole was injected to observe the maximum load of samples can be analysed without any

interference peak in the area relating to the compound estimated. The LOQ (Limit of quantification) and LOD (Limit of detection) for chlorantraniliprole was found to be  $0.05 \text{ mg kg}^{-1}$  and  $0.017 \text{ mg kg}^{-1}$ , respectively.

**Recovery of chlorantraniliprole in tomato:** Mean recoveries of chlorantraniliprole in tomato samples spiked with 0.05, 0.25 and  $0.5 \text{ mg kg}^{-1}$  levels range about 84.00 to 96.00 per cent and found to be were greater than 80 per cent (Table 1 and Fig. 2). The quantitative determination of chlorantraniliprole tomato was validated as stated by

bio analytical method recommendations described in the SANCO guidelines (Anon., 2021).

The calibration curves in relation to chlorantraniliprole generate a linear relationship with different conc. of 0.05, 0.1, 0.5, 1 and 2  $\mu\text{g ml}^{-1}$  (Fig. 1). The linearity curve was prepared taking different concentrations of standards starting with the concentration equal to LOQ. The curve shows good linearity with  $R^2$  value of more than 0.99 implies that the detector used and standard prepared were fit for further analysis of chlorantraniliprole residues.

Determination of Repeatability ( $RSD_r$ ) by spiking chlorantraniliprole through developed analysis method at different concentrations to different substrates. The repeatability ( $RSD_r$ ) for chlorantraniliprole in tomato at 0.05, 0.25 and 0.5  $\text{mg kg}^{-1}$  level correspond to 6.01, 6.43 and 5.44 per cent, respectively (Table 1).

**Estimation of chlorantraniliprole residues in tomato:** The quantitative estimates of chlorantraniliprole (30 and 60 g a.i.  $\text{ha}^{-1}$ ) residues in tomato, at different time interval, after 2<sup>nd</sup> and 3<sup>rd</sup> spray were presented in Table 3. Similarly, the representative chromatograms for 0 day samples with reference to second and third sprays for recommended and double doses was presented in Fig. 2.

At recommended dose, the mean initial deposit of chlorantraniliprole after 2<sup>nd</sup> spraying was found to be 0.60  $\text{mg kg}^{-1}$ . The residue of chlorantraniliprole in tomato fruits was found to be dissipated to a mean level of 0.18  $\text{mg kg}^{-1}$  after one day of 2<sup>nd</sup> spray which represented a dissipation of about 70.00 per cent residues. The mean level of residue after three days of spraying was found to be 0.07  $\text{mg kg}^{-1}$  and represented a dissipation of about 83.00 per cent residues. After 5<sup>th</sup> days of spraying, the residues in tomato fruits were found below the limit of quantification (0.05  $\text{mg kg}^{-1}$ ) (LOQ) (Table 2, Fig. 2).

At double dose, the mean initial deposit of chlorantraniliprole after 2<sup>nd</sup> spraying was found to be 0.97  $\text{mg kg}^{-1}$ . The residue of chlorantraniliprole in tomato fruits was found to be dissipated to a mean level of 0.33  $\text{mg kg}^{-1}$  after one day of 2<sup>nd</sup> spray which represented a dissipation of about 65.97 per cent residues. The mean level of residue after three days of spraying was found to be 0.11  $\text{mg kg}^{-1}$  and represented a dissipation of about 88.86 per cent residues. After 5<sup>th</sup> days of spraying, the residues in tomato fruits were found below the limit of quantification (0.05  $\text{mg kg}^{-1}$ ) (LOQ) (Table 2 and Fig. 2).

After 3<sup>rd</sup> spraying, the mean initial deposits of chlorantraniliprole @ 30 g and 60 g a.i./ha was found to be 0.70  $\text{mg kg}^{-1}$  and 1.10  $\text{mg kg}^{-1}$ , respectively. At recommended

dose, dissipation of about 70.00 and 91.62 per cent was observed after one and three days of spraying, respectively. Similarly, in double doses, dissipation of about 68.18 and 91.00 per cent was observed after one and three days of spraying. The residues reached below the limit of quantification after five days of spraying in both the recommended as well as in double doses (Table 2 and Fig. 2).

According to the findings of investigation, higher the doses of application led to higher amount of initial deposits. The overall results of the experiment revealed that, the mean initial deposits of chlorantraniliprole @ 30 g a.i.  $\text{ha}^{-1}$  and 60 g a.i.  $\text{ha}^{-1}$  varied from 0.54 to 0.74  $\text{mg kg}^{-1}$  and 0.92 to 1.20  $\text{mg kg}^{-1}$ , respectively. The residue of chlorantraniliprole both at a recommended and double dose dissipated to below LOQ after five days of spraying.

Similar results also reported by Paramasivam (2020) who studied the dissipation of chlorantraniliprole on tomato at Tamilnadu. Kabadad and Gali (2018) studied the dissipation pattern of four sprays of chlorantraniliprole at 0.20  $\text{ml L}^{-1}$  (single dose) and 0.40  $\text{ml/L}$  (double dose) on cabbage. Chlorantraniliprole residues reached BDL by 3<sup>rd</sup> day, showing 100 per cent dissipation of residue at respective dose. Reddy *et al.* (2017) who observed that the mean deposits of 0.56  $\text{mg kg}^{-1}$  chlorantraniliprole in chilli following application @ 30 g a.i.  $\text{ha}^{-1}$ . Vijayshree *et al.* (2012) also found that mean deposits of 0.55  $\text{mg kg}^{-1}$  following application of chlorantraniliprole 18.5 SC on cowpea. But Singhla *et al.* (2020) observed the initial deposits of 0.21  $\text{mg kg}^{-1}$  and 0.46  $\text{mg kg}^{-1}$  in okra fruits when fruits were sprayed with chlorantraniliprole @ 25 and 50 g a.i.  $\text{ha}^{-1}$ , respectively, which are less than the residues reported in this study may be because of lower dose applied. The amount of initial deposit of any pesticide residues mainly depends upon the amount of dose applied on that crop. Similarly, Kar *et al.* (2013) reported deposits of 0.18 and 0.29  $\text{mg kg}^{-1}$  of residues of chlorantraniliprole on cauliflower following application @ 9.25 and 18.50 g a.i.  $\text{ha}^{-1}$ . Not only the dose but also the type of crop also influences the initial deposits. The results of present study particularly the deposit of chlorantraniliprole on tomato are different from the findings by Sonia (2019) who reported higher initial deposits of 1.17  $\text{mg kg}^{-1}$  and 2.36  $\text{mg kg}^{-1}$  at 30 and 60 g a.i.  $\text{ha}^{-1}$  in tomato, respectively.

**Waiting period for chlorantraniliprole on tomato:** The half-life values are described simply and broadly as the time required to dissipate initial residues to half (Gunther and Blinn 1955). Time taken for residue to reach below MRL (T<sub>tol</sub>) and T<sub>1/2</sub> in days were computed by using the formula given by Hoskins (1961).



The Maximum Residue Limit (MRL) of chlorantraniliprole on tomato was approved at 0.6 mg kg<sup>-1</sup> (FSSAI). For chlorantraniliprole insecticide, a linear relationship was determined by plotted log concentration of residue against the time. It confirms, that the declination of the chlorantraniliprole residues showed first order kinetic reaction. The T<sub>1/2</sub> of chlorantraniliprole on tomato were calculated to be 1.02 days and 1.32 days when applied at the rate of 30 and 60 g a.i. ha<sup>-1</sup> after 2<sup>nd</sup> spray, whereas after 3<sup>rd</sup> spray it was observed to be 0.90 days at both the dosages (Table 3). The mean initial deposits of chlorantraniliprole on tomato was observed to be below the MRL after 1 day of spraying at both the dosages after 2<sup>nd</sup> and 3<sup>rd</sup> spraying. Thus, the present study suggested that, a waiting period of one day for chlorantraniliprole at single and double dose is required, provided that followed good agricultural practices were followed.

These findings were in accordance with the (Sonia, 2019) who found that half live of chlorantraniliprole was 1.57 days and 1.98 days at recommended and double dosages on tomato, respectively. Similar results were also recorded by (Paramasivam, 2020) who analysed the tomato sample as well as soil sample treated with chlorantraniliprole and found that the half-life of chlorantraniliprole was 1.26 and 1.77 days in fruits and soil respectively. Similar result was also reported by (Kar *et al.*, 2013), in cauliflower, who concluded a half-life value (T<sub>1/2</sub>) at recommended dosages (9.25 g a.i. ha<sup>-1</sup>) of chlorantraniliprole was found to be 1.36 days. Vijayasree *et al.* (2013) reported a waiting period of 0.62 days when we go for the spraying of chlorantraniliprole 18.5 % SC and the half-life was found to be 1.31 days on cowpeas. Shams EL Din *et al.* (2015) found that the residues of chlorantraniliprole were higher in winter and summer seasons of 2012, the lowest residues were in 2013 for winter seasons and in 2014 for the summer seasons. The least half-life and PHI were 2.441 and 6 days in the summer season of 2014, whereas it was the longest in the winter season of 2012 with 2.988 and 7.400 days, respectively.

## CONCLUSION

The residues of chlorantraniliprole in tomato dissipated and reached below LOQ after 5 days at recommended and double dose. After 2<sup>nd</sup> spray, the half-life values of chlorantraniliprole on tomato were calculated to be 1.02 and 1.32 day at 30 and 60 g a.i. ha<sup>-1</sup> whereas after 3<sup>rd</sup> spray it was observed to be 0.90 days @ 30 and 60 g a.i. ha<sup>-1</sup>. The present study suggested a waiting period of 1 day for chlorantraniliprole following application at recommended dose on tomato if followed good agricultural practices.

## ACKNOWLEDGEMENT

Authors thank the Head, Department of Entomology, Rajendra Prasad Central Agricultural University for providing facilities and encouragement.

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*MS Received: 16 May 2022*

*MS Accepted: 29 May 2022*