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Bioefficacy, phytotoxicity and insecticide residue dynamics of chlorantraniliprole in brinjal (*Solanum melongena*) under field condition

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ABSTRACT

Brinjal shoot and fruit borer (BSFB) (*Leucinodes orbonalis* (Gennadius)) causes significant economic harm to brinjal (*Solanum melongena* L.) production. The present study was carried out at research farm of ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh to standardize the optimum dose of chlorantraniliprole 18.5% SC (CAP) to manage BSFB under field conditions and to quantify the residue of CAP in brinjal for food safety. Three CAP doses (20, 40, and 80 g a.i./ha) along with an old generic insecticide (cypermethrin 25% EC @50 g a.i./ha) as a check and an untreated control were examined during 2017–19. CAP applications of 40 and 80 g a.i./ha were similarly efficient against BSFB and resulted in a significantly higher yield of brinjal fruit. The population of borers was reduced significantly in the treatment, CAP @40 g a.i./ha as compared to the untreated control. Phytotoxic effects of chlorantraniliprole 18.5% SC were not observed. Natural enemies of the brinjal ecosystem, viz. predatory pentatomid bug [*Eocanthecona furcellata* (Wolff)], ladybird beetle [*Coccinella septempunctata* (Linn.)], and spider were unaffected by CAP treatment. When CAP was applied @40 and 80 g a.i./ha, chlorantraniliprole degraded from brinjal fruits with half-lives of 4.85 and 7 days, and pre-harvest intervals of 7 and 10 days were prescribed, respectively. Even at greater application doses, residues in a person's meals were found to be lower than the maximum permissible intake (24.96 mg/person/day) in day-zero samples. Chlorantraniliprole @40 g a.i./ha can be an effective alternative to the conventional insecticides against BSFB in brinjal, ensuring quality, sustainability, and safety.

Keywords: Bioefficacy, Chlorantraniliprole, Dose optimization, Phytotoxicity, Residue dissipation

Eggplant, also known as brinjal (*Solanum melongena* L.), is a popular solanaceous vegetable crop in India. Brinjal fruits are high in important minerals and phytochemicals, and they provide a variety of health benefits. India accounts for about 72.9% of global brinjal production. The brinjal crop is infested by several insect pests throughout its growth period and brinjal shoot and fruit borer (BSFB) [*Leucinodes orbonalis* (Gennadius)], (Lepidoptera: Crambidae), which causes 8–37% shoot damage and 17–93% fruit damage, is more serious, making brinjal cultivation unprofitable.

Chemical pesticides are mostly used in its management by the farmers. However, pesticide resistance, reappearance of major pests, secondary pest outbreaks, residues in food, contamination of groundwater, and human health dangers have all resulted from their intensive, and indiscriminate usage (Halder *et al*. 2017). Recently, certain new pesticide compounds have been commercially available that are environment friendly, have a novel mode of action, are less persistent in the environment and have reduced toxicity to non-target organisms (Kodandaram *et al*. 2010). Anthranilic diamide is one such new family of pesticide chemistry, with chlorantraniliprole (CAP) as the primary component. For the management of insect pests in a few selected crops in India, two CAP formulations (chlorantraniliprole 0.4% G and chlorantraniliprole 18.5% SC) are recommended (CIB&RC (DPPQS) 2018). However, its bio-efficacy and dose as a suitable option for conventional insecticides for managing borer pest(s) must be investigated, as well as its toxicity to natural enemies and phytotoxicity on plant parts. Furthermore, it is essential to comprehend the insecticide's dissipation pattern in the crop, as this will aid in determining the CAP's persistence and efficiency. Furthermore, the CAP risk assessment will suggest human safety from the perspective of consumers.

Therefore current study was carried out to examine the efficacy, toxicity, and residue dynamics of CAP in the brinjal ecosystem since knowledge on bioefficacy and residue features of CAP, specifically in brinjal is limited in field conditions. The findings of this study will help pest protection expert to manage BSFB on brinjal crops with a correct dose, spray schedule, and residue dynamics

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of chlorantraniliprole 18.5% SC, as well as understand its human safety.

MATERIALS AND METHODS

Chemicals, reagents and apparatus: Sigma Aldrich, India, provided the certified reference standard of CAP. HPLC grade, analytical grade chemicals and solvents, as well as primary secondary amine (PSA), were purchased from Merck, India. BR Biochem Life Sciences Private Ltd, India, provided polytetra fluoroethylene membrane filters (13 mm diameter with a pore size of 0.2 m).

Field experiments: Field experiments were conducted in a randomized block design (RBD) with 5 treatments and 3 replications at the research farm of ICAR-Indian Institute of Vegetable, Varanasi, Uttar Pradesh, for two consecutive cropping periods 2017–18 and 2018–19 (October to March). In this study, Brinjal cv. Kashi Taru was used and cultivated in 5 m × 5 m raised beds with 60 cm × 45 cm spacing. The crop was grown using standard agronomic procedures. The chemical treatments for the control of the brinjal borer pest included 3 dosages of chlorantraniliprole 18.5% SC @20, 40, and 80 g a.i./ha, respectively, i.e. half of the recommended dosage (T_1), recommended dosage (T_2), and double the recommended dosage (T_3), and a standard check treatment of cypermethrin 25% EC @50 g a.i./ha (T_4). During the trial, an untreated control (T_5) was also kept. The first spray was applied when the borer infestation reached the economic threshold level, followed by 2 further sprays spaced 15 days apart.

Bioefficacy of Chlorantraniliprole 18.5% SC against shoot and fruit borer in brinjal: The damage percentage of *L. orbonalis* populations on shoots and fruits was calculated. Infested/damaged shoots were measured in percentages from 5 plants chosen at random per plot 1 day before, 3, 7, and 10 days after each spraying. Fruit damage was calculated and presented in percentages from harvested fruits 15 days after each spray. For each treatment, the percentage reduction over control (PROC) of the BSFB over the untreated control was calculated as:

$$\text{PROC} = \frac{(\text{Count in control} - \text{Count in treatment})}{\text{Count in control}} \times 100$$

Phytotoxicity of chlorantraniliprole 18.5% SC on brinjal: The phytotoxic effects of chlorantraniliprole 18.5% SC on brinjal leaves, shoots, and fruits were measured using the technique described by Kodandaram *et al*. (2017) at the recommended (40 g a.i./ha) and double the recommended (80 g a.i./ha) concentrations.

Safety of chlorantraniliprole 18.5% SC to natural enemies: Five plants in each treatment were counted for natural enemies such as the pentatomid bug [*Eocanthecona furcellata* (Wolff)], lady bird beetle [*Coccinella septempunctata* (Linn.)], and spiders. One day before and 10 days after each spray, the observation was made. Natural enemy population reductions have been calculated. The treatments were categorized according to the IOBC toxicity classes based on the findings (Boller *et al*. 2005).

Fruit yield and economics: Each treatment's mature brinjal fruits were picked on a regular basis. Fruits that were infested and those were not separated, weighed, and expressed in kg/ha. Similarly, the following method was used to perform the incremental cost-benefit analysis for individual treatments:

$$\text{Incremental Cost-benefit ratio (ICBR)} = \frac{\text{Net return (₹/ha)}}{\text{Cost of treatment (₹/ha)}} \times 100$$

Residue dynamics

Sample collection: On 0 (2 hours after foliar application of CAP), 1, 3, 5, 7, 10, and 15 days after spraying (DAS), brinjal fruit samples (about 500 g) were obtained from the three CAP treatments as well as the control treatments. Samples for residue analyses were taken only after 3 foliar applications of CAP.

Preparation of reference standard solutions: To make the stock solution, 10 mg of chlorantraniliprole standard was dissolved in 10 ml of acetonitrile solvent. Acetonitrile was used to make the calibration standard solutions (10, 100, 250, 500, 750, and 1000 g/litre). For matrix matched standard (MMS) preparation, the control brinjal matrix was used.

Chlorantraniliprole extraction: The macerated brinjal sample (15 g) was combined thoroughly with acetonitrile (20 ml) in a vortex. The macerated samples were then treated with 1 g sodium chloride and 4 g anhydrous sodium sulphate (Na_2SO_4). The contents were homogenized for 10 min before being centrifuged at 5000 RPM for 10 min. In a rotary evaporator, the supernatant solvent was evaporated. To dissolve the concentrated extract, 2 ml methanol was added to the content. PSA (0.02 g) and Na_2SO_4 (0.2 g) were used to clean up the extract. The contents were well combined in a vortex mixer before being centrifuged at 10000 RPM for 5 min. The supernatant solvent was sieved and introduced into HPLC using PTFE filter paper.

Instrumentation and method validation: HPLC-DAD was used to calculate chlorantraniliprole using the method reported by Sahu *et al.* (2019). In the HPLC-DAD system, the filtered extract (20 micro litre) was injected. The C18 column (25 cm × 4.6 mm × 5 m) was purchased from Merck, India. The mobile phase was HPLC grade acetonitrile and water (90:10 V/V) at a flow rate of 0.4 ml per minute. At 8.30 min, the chlorantraniliprole peak appeared. The λ_{max} of CAP was 230 nm.

Safety evaluation: The residue values were converted to theoretical maximum daily intake (TMDI) and compared to the maximum allowable intake to estimate CAP dietary exposure (MPI). Dietary exposures were calculated by multiplying brinjal residue levels (mg/kg) by the per capita mean consumption of 14.26 g brinjal per day (NSS report no. 541 66/1.0/3, 2012). According to European Commission data, the acceptable daily intake (ADI) of CAP is 1.56 mg/kg body weight/day. MPI values for children and adults were obtained by multiplying the child's (16 kg) and adult's (60 kg) body weights by the ADI value (Hingmire *et al*. 2015).

Statistical analysis: The data on the infestation of *L. orbonalis* on eggplant shoots and fruits was run by SQRT. PROC GLM was used to construct the ANOVA (SAS version 9.3; SAS Institute 2011). When the ANOVA was significant, Turkey's significance test results at the 0.05 probability level were used to evaluate and compare the means as they were changed by the treatments. For the measurement of the residual half-life (T1/2), the CAP residue data in brinjal fruits were log transformed and plotted against time to fit the first order kinetics as described by Hoskin (1961).

RESULTS AND DISCUSSION

Bioefficacy of chlorantraniliprole 18.5% SC against shoot and fruit borer of brinjal: Table 1 shows the infestations of BSFB before and after three rounds of treatment for two consecutive years. Before spraying, shoot damage ranged from 31.59–35.26% in different treatments throughout the first season. Chlorantraniliprole 18.5% SC @20, 40, and 80 g a.i./ha considerably reduced the *L. orbonalis* infestation (10.56, 7.40 and 8.26% shoot damage, respectively). Shoot damage was 26.49% in the untreated control group (Table 1). The two doses of CAP @40 and 80 g a.i./ha were statistically equal in efficacy and retained considerable superiority over CAP @20 g a.i./ha and cypermethrin 25% EC @50 g a.i./ha. In both years, the trend was similar in all 3 rounds of application. CAP @80 g a.i./ha showed the greatest reduction in shoot damage during 2018–19 (72.60 PROC). CAP dosages of 40 and 80 g a.i./ha produced statistically similar results.

In different treatments, the average fruit damage before the first spraying ranged from 27.65–31.25%. During the first year, the highest dose of CAP, 80 g a.i./ha, showed the maximum reduction in fruit damage (69.85%) compared to the untreated control. Treatment at the indicated dose of 40 g a.i./ha was equally effective (68.63%) and statistically at par for the first year, followed by treatment @20 g a.i./ha (63.09%) (Table 1). The average fruit damage caused by BSFB after 3 rounds of spray in plots treated with CAP @80, 40, and 20 g a.i./ha was 10.34, 11.25, and 12.37%, respectively, in the second year. For all 3 CAP doses, the per cent decreases in fruit damage over untreated control were 73.99, 71.71, and 68.89, respectively (Table 1). The check insecticide, cypermethrin 25% EC, was found to be far less effective against BSFB damage than all 3 CAP dosages tested, with reductions of only 21.18, 38.21, 48.57, and 16.79, 39.15, 50.38 percent over two years, respectively.

During the two successive years, the plots sprayed with chlorantraniliprole (@80 g a.i./ha) had the highest brinjal fruit yield (464.2 and 449.3 q/ha) and were statistically comparable to the dose of 40 g a.i./ha (459.7 and 442.8 q/ha). CAP @40 g a.i./ha treatment, the maximum ICBR (1:4.30) was realized (Table 2). Two dosages of CAP, 80 and 40 g a.i./ha, produced statistically equal marketable fruit yields.

All the 3 chlorantraniliprole dosages tested were effective in reducing the incidence of *L. orbonalis* on brinjal shoots and fruits. At 40 and 80 g a.i./ha, the bioefficacy of CAP was statistically comparable to that of other treatments and the untreated control. Rajavel (2011) reported that the highest dose of CAP, 60 g a.i./ha, was the best treatment for brinjal, with the lowest amount of shoot damage (4.99%) compared to the untreated control. Although the brinjal fruit yield in CAP @80 g a.i./ha was the highest, it was statistically comparable to the dose of 40 g a.i./ha during both the years. At 40 g a.i./ha, CAP had the highest ICBR (1:4.30) and could be recommended for managing the BSFB.

Chlorantraniliprole 18.5% SC phytotoxicity on brinjal: Even after foliar sprayings of CAP at double the recommended dose (80 g a.i./ha), no phytotoxic effects (chlorosis, wilting, necrosis, etc.) were observed on brinjal leaves, shoots, flowers, or fruits.

Effect of chlorantraniliprole 18.5% SC to natural enemies (NEs): During the brinjal growing season, common predators such as the predatory pentatomid insect, lady bird beetle, and spiders were commonly observed. In all of the treatments, CAP had no discernible effect on the predatory fauna (Fig 1). However, the untreated control had much greater pooled mean numbers of these predatory fauna in both years. According to the IOBC categories for NEs all 3 doses of CAP examined were classed as "N" (harmless or slightly harmful). Halder *et al*. (2020) reported

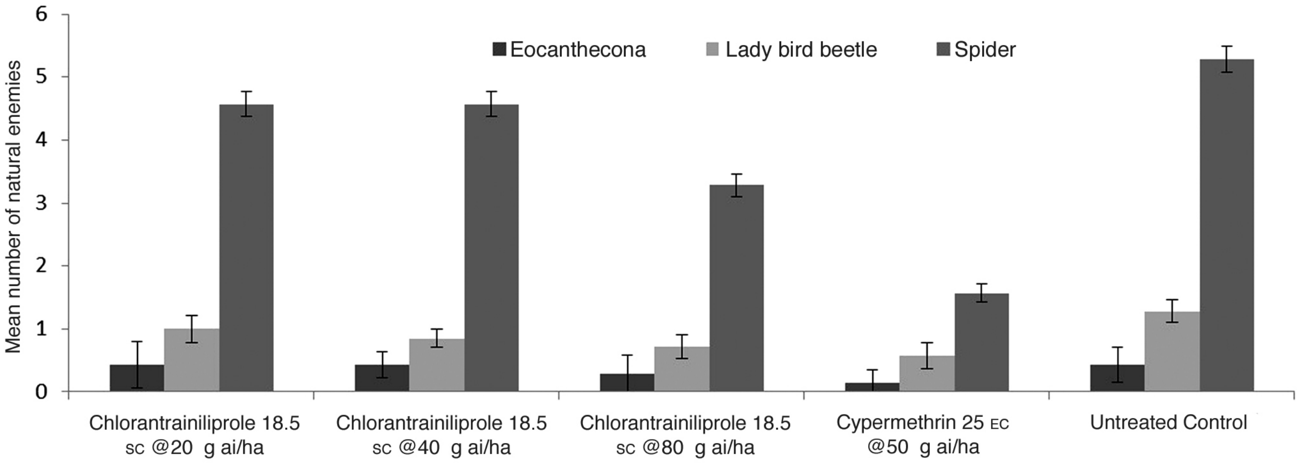

Fig 1 Effect of chlorantraniliprole 18.5% SC on natural enemies in brinjal ecosystem.

Table 1 Effect of chlorantraniliprole 18.5% SC on brinjal shoot and fruit damage (%)

Treatment	Brinjal shoot damage (%)													
	First Year (2017–18)							Second Year (2018–19)						
	PTC	1st spray		2nd spray		3rd spray		PTC*	1st spray		2nd spray		3rd spray	
		Number	PROC	Number	PROC	Number	PROC		Number	PROC	Number	PROC	Number	PROC
T_1	33.95	16.49 (24.34^{b})	49.89	13.26 (21.77^{ab})	52.10	10.56 (19.43^{ab})	60.14	26.45	13.69 (22.12^{b})	49.17	10.29 (19.18^{a})	58.29	10.11 (19.01^{b})	27.09
T_2	35.26	12.64 (21.25^{a})	61.59	10.67 (19.53^{a})	61.45	7.40 (16.32^{a})	72.06	28.95	10.56 (19.43^{a})	60.79	9.11 (18.06^{a})	63.07	7.98 (16.93^{a})	66.13
T_3	32.67	11.28 (20.07^{a})	65.73	10.08 (18.98^{a})	63.58	8.26 (17.22^{a})	69.04	27.26	9.47 (18.41^{a})	64.84	8.64 (17.59^{a})	64.98	7.23 (16.14^{a})	69.31
T_4	31.59	23.49 (29.33^{c})	28.62	18.64 (25.94^{c})	32.66	15.67 (23.71^{c})	40.85	28.85	21.06 (27.67^{c})	21.80	17.26 (24.93^{b})	30.04	14.85 (23.07^{c})	36.97
T_5	34.25	32.91 (35.32^{d})	--	27.68 (32.06^{d})	--	26.49 (31.30^{d})	--	27.06	26.93 (31.58^{d})	--	24.67 (30.11^{c})	--	23.56 (29.31^{d})	--
CD (P= 0.05)	--	4.57 (2.11)	--	4.11 (2.52)	--	4.78 (2.97)	--	--	4.15 (2.59)	--	4.15 (2.60)	--	4.24 (2.67)	--
	Brinjal fruit damage (%)													
T_1	27.65	19.95 (26.89^{b})	36.18	14.56 (22.84^{ab})	49.14	11.26 (20.06^{a})	63.09	35.29	19.67 (26.69^{ab})	38.97	15.45 (23.54^{ab})	56.39	12.37 (21.02^{a})	68.89
T_2	30.49	16.48 (24.34^{a})	47.28	12.14 (20.83^{a})	57.60	9.57 (18.50^{a})	68.63	33.46	17.95 (25.44^{a})	44.31	14.18 (22.53^{a})	59.98	11.25 (20.05^{a})	71.71
T_3	29.94	15.13 (23.29^{a})	51.60	12.87 (21.45^{a})	55.05	9.20 (18.14^{a})	69.85	30.26	16.05 (24.01^{a})	48.81	12.95 (21.52^{a})	63.45	10.34 (19.22^{a})	73.99
T_4	31.25	24.64 (30.09^{c})	21.18	17.69 (25.25^{c})	38.21	15.69 (23.73^{b})	48.57	34.47	26.82 (31.51^{c})	16.79	21.56 (28.01^{c})	39.15	19.73 (26.73^{b})	50.38
T_5	29.36	31.26 (34.30^{d})	--	28.63 (32.67^{d})	--	30.51 (33.84^{c})	--	31.41	32.23 (34.90^{d})	--	35.43 (36.83^{d})	--	39.76 (39.39^{c})	--
CD (P= 0.05)	--	3.19 (1.92)	--	3.66 (2.18)	--	5.17 (3.56)	--	--	3.21 (2.30)	--	4.68 (2.75)	--		

*PTC, Pre-treatment Count. Figures in parentheses are X+0.5 square root transformed values. In column, means followed by common letters are not significantly different at (P≤ 0.05). Treatment details are given in Materials and Methods.

Table 2 Economics of different treatments in brinjal

Treatment	Average yield (q/ha)	Increase in yield over control	Per cent increase in yield over control	Cost (₹) of cultivation (A)	Cost (₹) of plant protection inputs (3 sprays) (B)	Total cost (₹) (A+B)	Total return/ income (₹)	ICBR
T_1	422.9	73.5	21.04	202960/-	3400	204360	845800	1:4.14
T_2	451.3	101.9	29.16	202960/-	6800	209760	902600	1:4.30
T_3	456.8	107.4	30.74	202960/-	13600	216560	913600	1:4.22
T_4	389.8	40.4	11.56	202960/-	600	203560	779600	1:3.83
T_5	349.4			202960/-	--	202960	698800	1:3.44

Average cost of brinjal @ ₹2000/Quintal; Cost of cultivation includes all the expenditures to raise the brinjal crop except plant protection measures for brinjal shoot & fruit borer; Spray volume, 500 litre of water.

Treatment details are given in Materials and Methods.

that *Eocanthecona furcellata*, is a potential polyphagous pentatomid predator of various lepidopteran pests of vegetables in India. In the rice ecosystem, sub-lethal dosages of CAP were safer to the generalist predator *Cyrtorhinus lividipennis* (Zhang *et al*. 2015).

Dissipation and risk assessment of chlorantraniliprole 18.5% SC in brinjal: With a regression co-efficient of 0.992, the six-point solvent-matched calibration curve was fitted linearly. The standardized method's LOD and LOQ were 0.003 mg/kg (S/N ratio 3:1) and 0.006 mg/kg (S/N ratio 10:1). The matrix effect was less than 12%. At 3 distinct concentrations (50 µg/kg, 100 µg/kg, and 250 µg/kg), the recoveries of CAP from brinjal were ranged from 76–87%. At LOQ, the relative standard deviation (RSD) was 13.50%. According to the SANTE requirements (70–110% recovery percentage and 20% RSD value), the procedure is satisfactory (SANTE/11813/2017) (SANTE 2017). The dissipation of CAP from brinjal at 3 doses (T_1, CAP @20 g/ha; T_2, CAP @40 g/ha; and T_3, CAP @80 g/ha) is depicted in Table 3. The CAP residues in brinjal were 0.126 mg/kg, 0.149 mg/kg, and 0.174 mg/kg, respectively, for T_1, T_2, and T_3 treatments. At 10 days after sampling, CAP residues were below detectable limit (BDL) in both T_1 and T_2 treatments, however at 15 days after sampling, CAP residue was detected 0.006 mg/kg of brinjal in T_3 treatment. With higher than 0.96 regression co-efficient values, the dissipation rate followed first order kinetics (Table 3). The half-lives of CAP from 3 treatments were found to be 4.95, 4.85, and 7.00, respectively.

Vijayasree *et al*. (2015) reported that initial CAP residues on brinjal fruits @30 and 60 g a.i./ha were 0.72 and 1.48 mg/kg, respectively. The chemical nature of the pesticides, rate of application, and biochemical properties of the plant matrix could all play a role in the difference in CAP dissipation and half-life dissipation values. Environmental factors like temperature, wind, relative humidity, and sunshine hours may have contributed to the loss of CAP in the current findings. Pesticide dissipation was also linked to pesticide dilution in plant matrix as a result of crop growth. Furthermore, the CAP residue acquired in this investigation

Table 3 Persistence and dietary exposure of chlorantraniliprole in/on brinjal under different treatments

Time (Days)	Treatment (g a.i./ha)					
	T_1		T_2		T_3	
	Residue (mg/kg)	Dietary exposure (mg/person/day)	Residue (mg/kg)	Dietary exposure (mg/person/day)	Residue (mg/kg)	Dietary exposure (mg/person/day)
0	0.127±0.010	0.0018	0.149±0.011	0.0021	0.175±0.004	0.0025
1	0.106±0.009	0.0015	0.127±0.002	0.0018	0.152±0.010	0.0022
3	0.061±0.007	0.0009	0.082±0.007	0.0012	0.134±0.006	0.0019
5	0.027±0.008	0.0004	0.042±0.009	0.0006	0.088±0.006	0.0013
7	0.009±0.005	0.0001	0.012±0.004	0.0002	0.049±0.008	0.0007
10	0.007±0.003	0.0001	0.007±0.004	0.0001	0.022±0.004	0.0003
15	BDL*	--	BDL	--	0.006±0.001	0.0001
21	BDL	--	BDL	--	BDL	--
Kinetics and half-life of chlorantraniliprole in/on brinjal under different treatments						
Regression equation	y = -0.139x - 0.869		y = -0.143x - 0.756		y = -0.099x - 0.658	
Regression co-efficient	0.960		0.964		0.978	
Half-life (days)	4.95		4.85		7.00	

*BDL, Below the detection limit.

was sufficient to keep the BSFB under control for 10–15 days. For the management of BSFB, a second application of CAP or another pesticide can be recommended.

The CAP ADI was 1.56 mg/kg of body weight/day. The MPIs of a child and an adult were found to be 24.96 mg/person/day and 93.6 mg/person/day, respectively. The dietary exposures of CAP on each sampling day were lower than the MPI value for all 3 dosages, based on an average daily consumption of 14.26 g of brinjal. The MRL is proposed at 0.03 mg/kg in the Government of India's Gazette Notification (F. No.01-SP (PAR)-Notification-Pesticides/Stds-FSSAI/2017). Based on the MRL value, we could propose the PHI of 7 and 10 days when CAP applied @40 and 80 g a.i./ha, respectively. Since the CAP did not show any acute toxicity to mammals as well as natural enemies hence it can be considered as a low risk insecticide.

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