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Peristence behavior of Carbosulfan in Brinjal (*Solanum melongena L.*) Forage

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Abstract

Plant foliage are widely utilized as cattle feed, especially vegetables due to their rich nutritive and fiber content. Brinjal, the wide cultivated vegetable crop for its fruits, which is a major vegetarian food dish, leaves lot of plant material to be destroyed, which is utilized as livestock feed in some parts of the world. Brinjal intake a huge pesticide load to withstand the enormous insects, diseases and nematodes that damage the crop, which leaves the residues to accumulate in the plant parts including the foliage, which can be detrimental to the cattle that feed on them. In this regard, the current research was conducted to investigate the persistence behaviour and dissipation dynamics of a carbamate chemical, carbosulfan, which is capable to manage the major brinjal insect pests such as shoot and fruit borer. The results revealed that the residues reached below the detectable level (BDL) at 14 day after three rounds of spraying of carbosulfan at the recommended dose of 250g a.i.ha^{-1}, which was 2.071 and 5.09µg g^{-1} at the higher doses of 500 and 1000g a.i.ha^{-1} respectively. However, those residues reached below the detectable level on 21st day after spraying at all levels of application of the chemical. The residues were below the maximum permissible residue limit (MRL) of 0.1µg g^{-1} at the recommended dose of 250 g ai ha^{-1} making it a suitable insecticide for managing brinjal pests without any risk as cattle feed.

Keywords: Carbosulfan, residues, brinjal leaves, eggplant, safe harvest, *Solanum melongena*

Introduction

Vegetables, form an indispensable component of the human diet, have attained the third level in accumulating pesticides residues next to cotton and paddy.[1] Brinjal (*Solanum melonogena* L.) is cultivated for its tender fleshy fruit for preparation of several cuisines globally. Brinjal is highly productive and considered apoorman's crop.[2] It is cultivated worldwide in an area of more than 1,60,000ha with a production of over 50million Mt.[3] Brinjal hosts ahuge number of pests from planting till harvest.[4] Insecticides belonging to diverse classes such as organochlorines, organophosphates, carbamates and synthetic insecticides are the chiefsource of pest management in brinjal, owing to their effective and swift action. Carbosulfan(2,3-diydro-2, 2-dimetyl-7-benzo-furnanyl (di-n-butyl amino-thio methyl carbamate) is a novel carbamate chemical effective against effective against brinjal pests.[5] Carbosulfan (Figure 1) is carbofuran derivative, interrupting cholinesterase activity, extensively utilized to manage borers and sucking pests in the agricultural and horticultural ecosystems as well as soil-dwelling insects and nematodes.[6,7] Brinjal, widely known as 'Poormans' vegetable is a prime vegetable fondly consumed by diverse categories of people globally; therefore, receives huge pesticide load to check the pests. The insecticide carbosulfan is a persistent pesticide and all parts of brinjal plants receive the chemical. The risk of carbosulfan in edible brinjal fruits iscorrespondingly present in leaves alsowhich serves as fodder for cattles, specially goats.[8] Therefore, it is inevitable to assess the risk of carbosulfan in brinjal foliage to avoid hazards to cattle. In this regard, an exhaustive investigation on the persistence and dissipation behavior of carbosulfan and its risk assessment in brinjal foliage was conducted to determine the safe waiting period to feed the foliage for cattle.

Materials and Methods

Field Experiment: A replicated and randomized field experiment was conducted to evaluate the harvest time residues of carbosulfan at Urumandampalayam village, Vellakinaru, Coimbatore, on the brinjal variety CO^{-2}, with ten treatments replicated four times and the plot size was 20m^2. The carbamate chemical carbosulfan (Marshal 25 EC) was sprayed in three rounds @ 250, 500 and 100 g a.i.ha^{-1} in a fortnightly interval. The initial spraying was given 2 weeks after transplantation of seedling with a pneumatic knapsack sprayerwith a fluid rate of 500lha^{-1} and subsequent sprays at 14 days interval.

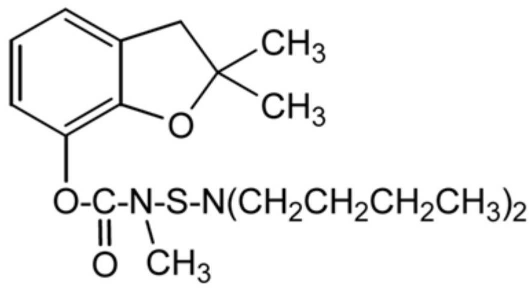

Figure 1 Structural formula of carbosulfan.

Sampling*:* The samples of brinjal foliage werehandpicked from the field and composed collectively from various treatments at 0 (onehour after spraying,), 3, 7,14 and 21 days after spraying. The samples were placed iceboxes and brought to pesticide residue analysis lab immediately to avoid residue deterioration. The foliage was cut into tiny pieces, mixed and a subsample of 50grams was taken for residue analysis and analyzed instantly.

Extraction, cleanup and estimation: A representative sample of 50 gram of leaves was extracted wit 100 ml of 9: 1 methanol: pH buffer (4.925 g of K_2PO_4 + 0. 465 g of K_2PO_4) in a Waring Laboratory blender, filtered wit Buchner Funnel and condensed in a rotary vacuum flame evaporator (BuchiRoto vapor R-14$^{®}$).[9] The condensed extract was extracted thrice with dichloromethane and 250 ml of saturated sodium chloride in a separating funnel and further condensed to dryness in flame evaporator. In Gas chromatography, the glass columns of 50 X 1.5cm I.D was used for column cleanup, where the tip was plugged with cotton wool and filled with anhydrous sodium sulphate for 2cm level, followed by 8 cm of Florisil®: charcoal mixture (8 g of deactivated Florisil®+ 1.5 g of acid washed charcoal) and overlaid with 2 cm layer of anhydrous sodium sulphate. The Floredil® was activated at 135°C for six hours in a hot air oven and cooled in a desiccator and then deactivated with 4 per cent freshwater. The condensed dichloromethane extract was loaded onto the column and eluted with a mixture of 9:1 hexane and ethyl acetate, which was condensed again to dryness for further analysis procedures.

Derivatisation: The condensed extract was dissolved in 1 ml of reactant solution (1.0g of 1-fluoro 2,4-dinitrobenzene in 100ml of acetone) and transferred quantitatively to stoppered 25X190mm test tubes. The phosphate buffer (Phosphate buffer of pH 11- 25.0g of Na_2HPO_4 was dissolved in 2480ml of distilled water and 20ml of 1M NaOH solution) was added to this mixture, mixed well and kept in water bath and the temperature was maintained at 50°C for 30 minutes. The extract was then extracted in a separating funnel with exactly two 25ml portions of n-hexane and the organic layer was collected and the hexane extract was condensed and retained for final determination.

Operation Parameters: The carbosulfan residues were dissolved in 10 ml of hexane for final injection (2 µl) in Chemito model 2685 Gas Chromatography (GC) equipped with ^{63}Ni electron capture detector (ECD) and fitted with a glass column (3 % OV 17) of 1.8 m X 2.0 mm I.D, with the operating conditions as injector port (255°C), detector (280°C), oven temperature (235°C), column temperature (300°C), carrier gas (nitrogen) 20 ml min $^{-1}$,. The carbosulfan residues were detected at retention time of 8.0th minute under the operating conditions of Gas Chromatography.

Recovery studies: The analytical method of carbosulfan was standardized with the known purity standards in Gas Chromatography. Recovery studies of carbosulfan was done by fortifying ten grams of leaves with 0.1, 0.5, 1.0 and 1.5µg g^{-1} of carbosulfan technical grade (92.5 % purity) solution. The residues were extracted and estimated as per the method mentioned above.

Determinability: Gas chromatography has a minimum detectable level of 0.1ng for carbosulfan. The lowesquantitative limits (LOQ) of GC for brinjal leaves considering 10 g weight was 0.001µg with a final volume of the extract being 2ml and injection volume of 2µl, below which the residual level is considered as below detectable level (BDL)

Data Analysis: The insecticide degradation pattern was analyzed by applying seven transformation functions [10, 11]. The half-life was calculated using Pesticide Residue Half-life Calculator software developed by Department of Soil Science, Tamil Nadu Agricultural University, Coimbatore[12] nd the best fit degradation model was determined. The safe waiting period was worked out as per the formula given below as.[13,14]

$$\text{Safe waiting period (TMRL)} = \frac{\log K_2 - \log (\text{MRL/tolerance})}{\log K_1}$$

Results

The residual analysis methodology was validated with standardsofcarbosulfan with known purity using Gas Chromatography. Recovery studies were carried out to test the validity of the current methodology and the results were furnished in Table 1. The linearity of the calibration curve was established in the range of 0.1 to 1.5µg g^{-1}(4 levels) with a correlation coefficient of (R2) 0.994 in brinjal leaves (Figure 2). The standard chromatograms of carbosulfan at different levels of spiking were illustrated in Figure 3. The recovery percentage of the brinjal leaves ranged from 78.36 to 84.87 per cent, with the average recovery of 81.36, 83.01, 83.02 and 79.96 percent at the fortification level of 0.1, 0.5, 1 and 1.5 µg g^{-1}. The recovery of carbosulfan on brinjal leaves was more than 80 percent indicating the reliability of the analytical method followed.The minimum sensitivity or detectable level of Gas chromatography was 0.1ng for carbosulfan. The lower quantitative limits of GC for brinjal leaves considering 10g weight were 0.01µg with the final volume of the extract being 2ml and injection volume of 2µl. The residual values below 0.01µg is considered as below detectable Level (BDL).

Table 1 Recovery percentage of carbosulfan in brinjal leaves

Spiking level (µg g^{-1})	Recovery per cent (%)				
	R1	R2	R3	Mean ± SD	RSD
0.1	79.32	84.36	80.41	81.36±2.65	3.26
0.5	82.14	83.21	83.69	83.01±0.79	0.96
1.0	83.23	84.87	80.96	83.02±1.96	2.37
1.5	78.36	79.14	82.37	79.96±2.13	2.66

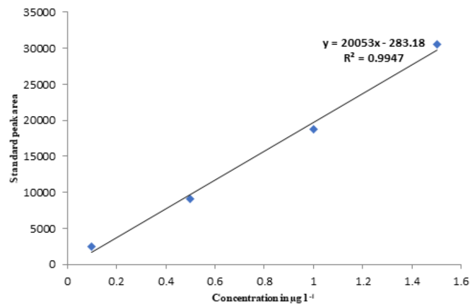

Figure 2 Linearity calibration curve of carbosulfan in brinjal leaves.

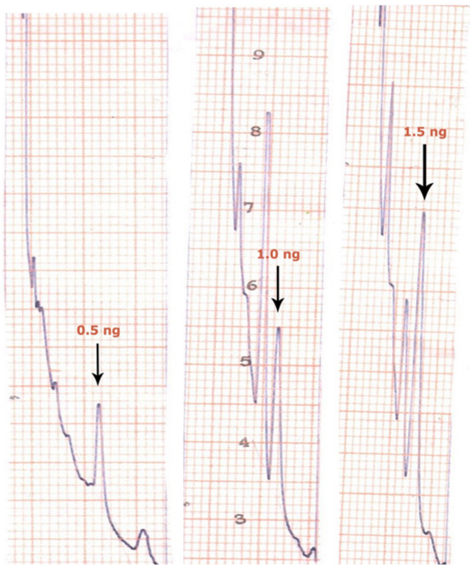

Figure 3 Standard chromatograms of carbosulfan.

The residues of carbosulfan in brinjal leaves at 250 g a.i. ha^{-1} were 30.92, 20.0, 13.0 and 6.0µg g-1at 0, 1,3, and 7 days after spraying and reached below detectable level at 14th day after spraying. The initial residue of 46.34µg g^{-1}declined to 26.58, 15.85, 8.05, 2.071 and 0.06.34 µg g^{-1}at 1,3,7,14 and 21 days after spraying of carbosulfan at double the dose of 500 g.a.i.ha^{-1}. Similarly, the initial residues of 99.07µg g^{-1}declined to 55.2, 31.02, 19.4, 5.09 and 0.09µg g^{-1}at 1, 3, 7, 14 and 21 days after spraying of four times the dose of carbosulfan at1000 g.a.i.ha^{-1}. The residues reached BDL after 14 days of application at the normal dose of carbosulfan, whereas the double and four times the dose of carbosulfan which reached BDL at 30 days of application (Figure 4; Table 2).

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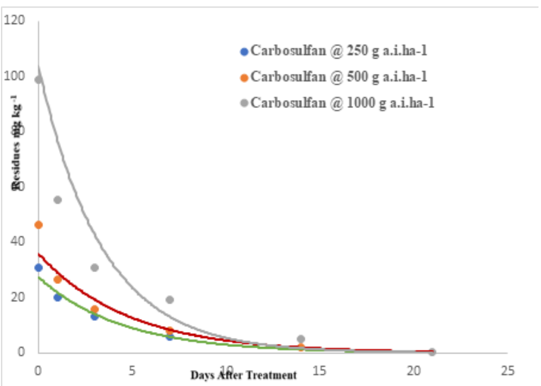

Figure 4 Dissipation of carbosulfan residues in brinjal

Table 2 Degradation and persistence of carbosulfan in/on brinjal fruits

DAT	Carbosulfan @ 250 g a.i.ha^{-1}		Carbosulfan @ 500 g a.i.ha^{-1}		Carbosulfan @ 1000 g a.i.ha^{-1}	
	Mean ± SD	%D	Mean ± SD	%D	Mean ± SD	%D
0	30.92±3.87	-	46.34±5.80	-	99.07± 12.40	-
1	20±3.19	34.9	26.58±4.24	42.64	55.2±8.80	45.87
3	13±1.34	55.7	15.85±1.64	65.79	31.02± 3.20	68.05
7	6.01±0.87	80.56	8.05±1.17	82.60	19.4±2.82	80.40
14	BDL	96.7	2.071±0.16	95.50	5.09±0.40	95.49
21	BDL	100	46.34±5.80	100.0	0.09±0.01	99.07

DAT - Days after treatment; R - Residues (µg g^{-1}); % D - Percentage degradation; ND – Not Detected, BDL – Below Detectable Level

leaves.To study the degradation and persistence of carbosulfan in brinjal leaves the samples were collected at varying intervals and the level of carbosulfan persisting in the brinjal leaves were determined. The data on the residues of carbosulfan and the corresponding elapsed time were subjected to simple regression analysis and the time required for the applied carbosulfan to get degraded to its 50 per cent of the applied dose (T0.5) was computed. The data was also fit into different mathematical models to depict the degradation curves.[9,10] Linearisation of the data was attempted by transforming the time (X) and/or the residues left over in the brinjal leaves (Y). Based on the regression coefficients (R^2), best-fit model was chosen as that model possessing the highest R^2 value. From the regression analysis, the intercept (a), slope (b) and the half-life (T0.5) were derived.

The data were further used to compute intercept (a), slope (b) and half-life ($T_{0.5}$) and their lower and upper limit values for 95 per cent confidence limit (Table 3). It was seen that the intercept, an index of initial deposit increased with the increasing dose of carbosulfan applied. The rate of degradation as represented by the slope of the function was also increasing with the increase in dosage of carbosulfan (Figure 5). The half-life derived for different doses ranged from 2.94 to 3.36 days. The best fit model was chosen based on the R^2 value arrived for different models. For all the doses of carbosulfan applied on brinjal leaves, the degradation pattern followed first order function (Table 4).

Discussion

The recovery studies revealed a satisfactory recovery of carbosulfan residues (Table 1) with mean per cent recoveries of 81.36, 83.01, 83.02 and 79.96percent and the Relative Standard Deviation (RSD) of 3.26, 0.96, 2.37and 2.66when samples were spiked at 0.1, 0.5, 1.0 and 1.5µg g^{-1} respectively, which is in accordance with earlier reports that the mean recovery value of carbosulfan was 91.00 and 97.30 per cent in potatoplant.[15] A carbosulfan recovery of 66 and 98 percent was exhibited when spiked between 0.03 and 0.25µg g^{-1}on orange homogenates.[16]

Table 3 Dissipation pattern ofcarbosulfan in brinjal fruits with statistical parameters

Treatments	a	LL	UL	b	LL	UL	T(0.5)	LL	UL	Prediction equation
Carbosulfan 25 EC @ 250 g a.i.ha^{-1}	7.955	8.154	7.757	-0.235	-0.207	-0.263	2.941	3.289	2.594	Y=7.955-0.235X
Carbosulfan 25 EC @ 500 g a.i.ha^{-1}	8.186	8.578	7.794	-0.208	-0.153	-0.263	3.324	4.199	2.440	Y=8.186-0.208X
Carbosulfan 25 EC @ 1000 g a.i.ha^{-1}	8.944	9.310	8.577	-20.6	-0.172	-0.240	3.362	3.916	2.807	Y=8.944-0.206X

a - Intercept; b - Slope;$T_{(0.5)}$ - Half-life ; UCL-Upper Confidence Limit; LCL-Lower Confidence Limit;T Half- Half life

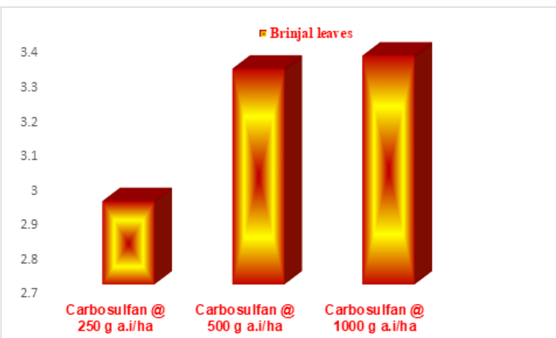

Figure 5 Half life period of carbosulfan in brinjal leaves

Immediately, an hour after spraying, carbosulfan deposits were 30.92µg g^{-1}at the recommended dose @ 250 g ai. ha^{-1}, which dissipated to 20.00 and13.00µg g^{-1}in 1st and 3rd day registering 34.9 and 55.7 percent dissipation, 6.01in 7th with 80.56 percent dissipation. The residues reached below detectable limit at 14th DAT, but 96.7 per cent dissipation was detected on 14th day and reached 100 per cent dissipation on 21st day (Table 2).

The primary deposit of 46.24µg g^{-1} at 1 hour after spraying dissipated to 46.34, 26.58, 15.85, 8.05 and 2.071 µg g^{-1}with a dissipation percentage of 42.64, 65.79, 82.60 and 95.50 at 1,3,7 and 14th DAT and reached below detectable level on 21st day at double the recommended dose of 500 g a.i.ha$^{-1.}$ The largest dose of carbosulfan*ie.* 1000 g a.i.ha^{-1} recorded an initial deposit of 99.07µg g^{-1}, which dissipated to 55.2,31.02, 19.4, 5.09 and 0.009 mg kg^{-1} with a dissipation per centage of 45.87, 68.05, 80.40, 95.49 and 99.07 per cent and reached below detectable limit on 28th DAT recording 100 per cent dissipation (Figure 4).

The carbosulfan residues extracted ib brinjal leaves immediately after application was 30.92, 46.34 and 99.07g g^{-1} respectively for 250, 500 and 1000 g a.i.ha$^{-1.}$ It was quite expected that higher levels of application resulted in higher amounts of initial deposits on brinjal leaves. The quantity of carbosulfan after the expiry of 14 days was

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BDL, 2.071 and 5.09 µg g^{-1} for the three doses of carbosulfan. The current findingswere in line with previous findings that carbosulfan residues were detected till 7 DAS and they were above MRL till 3 DAS with earlier reports that carbosulfan residues in brinjal plant was detected till 7 DAS.[17]

Table 4 Correlation coefficient for carbosulfan in brinjal leaves by different methods of transformation of residue datawith statistical parameters

Function	Carbosulfan 25 EC @ 250 g a.i.ha^{-1}		Carbosulfan 25 EC @ 500 g a.i.ha^{-1}		Carbosulfan 25 EC @ 1000 g a.i.ha^{-1}	
	r	r^2	r	r^2	r	r^2
First order	**0.998**	**0.978**	**0.980**	**0.934**	**0.986**	**0.903**
1.5th order	0.947	-0.613	0.970	0.871	0.897	-37.14
2nd order	0.873	-70.08	0.920	-9.68	0.763	-1.48
RF First order	0.917	0.617	0.970	0.910	0.937	0.679
RF 1.5th order	0.775	-46.12	0.854	-16.08	0.730	-8.93
RF 2nd order	0.662	-1.76	0.895	-2.32	0.567	-7.055
Inverse PL	0.839	0.742	0.731	0.820	0.832	0.860

RF- Root Function; Inverse PL- Inverse Power Law

Similar findings were previously conveyed that the residues of carbosulfan of carbosulfan persisted in brinjal fruits only between 3-5 days of dosages of 187.5, 375.5 g a.i.ha^{-1}[18] and that carbosulfanresidues deteriorated beyond 3 DAT for the dosage of 1.5 mlÂ·L^{-1} rate and 10 DAT for 3.0 mlÂ·L^{-1} rate.[19] Reports beforehand revealed that traces of carbosulfan residues (0.01µg g^{-1}) was detected in brinjal leaves after 20 days of application.[20] Such findings were also conveyed previously that the residues of betacyfluthrin in brinjal fruits declined progressively and become non-detectable after seven days.[21] The residues of carbosulfan, a chief metabolite of carbosulfanranged from 58-60ppb and 50-60ppb at first and third harvest respectively when applied as split application at 1.0kg a.i.ha^{-1} and 2.0kg a.i.ha^{-1}on brinjal crop.[22] The carbosulfan residues persevered in chickpea plant parts (roots and shoots) over 90 days but were in trace in seeds.[23]

Usually, brinjal leaves as cattle feed will be harvested after the fewharvests of brinjal fruits and there will not be produce anymore. The first harvest of brinjal leaves after spraying was on 14th day, were below the maximum permissible limit at the recommended dose of 250g.a.i.ha^{-1}and considered a very meager amount of 2.07 and 5.09µg g^{-1} of residues was detected. Thus, brinjal foliage harvested after spraying carbosulfan were found to be safe with no risks at the recommended dose, which is in line with previous research findings of brinjal plant parts sprayed with normal, doble and four times the dose of carbosulfan, displayed a below detectable level of residues at first and third harvest.[24] The samples from the third harvest did not possibilityreveal the presence of carbosulfan at the minimum detectable limit of 0.002mgkg$^{-1.}$ The results indicated that carbosulfan residues detected in brinjal leaves both at first and third harvest werewell below the prescribed limit for the different doses applied. Though high level of reisdues persisted in the foliage at the firt harvest, the residues dissipated quickly and reached t below detectable level at third harvest in higher doses of carbosulfan (500 and 100 g a.i.ha^{-1}).

The dissipation scenario of carbosulfan was plotted in diverse models and the best fit was chosen on highest R values. The modified regression equation (R^2) was calculated for arriving the best fit of the dissipation of carbosulfan in brinjal foliage. The dissipation rate of carbosulfan in brinjal leaves followed first order function and seemed to fit first order kinetics.[25] The carbosulfandegration kinetics in brinjal leaves revealed that the intercept *ie*. Initial deposit (a) and the slope *ie. The degardatiin rate* (b) increased with the increased dose of carbosulfan treatment (Tables 3&4). The half-life of carbosulfan increased with the increase in levels of carbosulfan. The half-life of carbosulfan in brinjal foliage ranged from 2.94 to 3.36 days. The safe waiting period for harvest being 12.6, 14.19 and 16.89 days for normal, double and four times the doses of carbosulfan (Figure 5). Hence it may be recommended to harvest the brinjal foliage for feeding livestock after allowing a waiting period of 15 days which is in conformation with the safe waiting period of 12-30 days according to the previous research investigations.[26] These results are also in accordance with earlier workers[27] reporting the safe waiting period for harvest of carbosulfan at 250, 500 and 1000 g a.i.ha^{-1}were 12.36, 14.19 and 16.89 respectively. Such an observation was earlier reported that the safe waiting period for harvest of brinjal fruits when deltamethrin was applied @ 10-15 g a.i ha^{-1}was 5 days and the initial deposits dissipated within 5 days of application.[28] This was also in accordance with the previous findings that the half-life of fluvalinate in brinjal was 7 days.[29]

Conclusion

The current investigation of carbosulfan residues in brinjal foliage, provided anadequate knowledge for safe waiting period for harvesting foliage for cattle feed under agroclimatic conditions. A satisfactory outcome thatcarbosulfan residues detected in brinjal foliage at normal, double and four times the dosage were well below the prescribed limit was professed and hence the carbamate chemical, carbosulfan is well recommended for managing th brinjal pests without any negative impact on the environment.

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Ethical Statement

All institutional and national guidelines for the care and use of plant material and chemicals were followed.

Disclosure statement

No potential conflict of interest was reported by the authors.

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