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PERSISTENCE OF BETA-CYFLUTHRIN AND IMIDACLOPRID RESIDUES IN CASHEW

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ABSTRACT

The QuEChERS analytical method in combination with GC and LC-MS/MS was validated to quantify the insecticide residues in immature kernel, mature kernel, cashew apple and in the field soil. The method performance was satisfactory with the LOQ, recovery and RSD values. Residue persistence of betacyfluthrin and imidacloprid on cashew was carried out after foliar spray of the combination formulation, betacyfluthrin 90 g ℓ^{-1} + imidacloprid 210 g ℓ^{-1} OD (Solomon) at recommended (2.5 ml 10 ℓ^{-1}) and double the recommended (5 ml 10 ℓ^{-1}) doses. The residues of betacyfluthrin and imidacloprid were at below LOQ of 0.05 mg kg^{-1} in all the samples at recommended and double the recommended dose. Soil sample collected at the time of harvest did not show betacyfluthrin and imidacloprid residues at their detection limit of 0.05 mg kg^{-1}. The analytical method standardized for the extraction of betacyfluthrin and imidacloprid residues in cashew matrices offers advantages such as simplicity and also, less time consuming.

Key words: Cashew, combination insecticide, Solomon, QuEChERS, method validation, dissipation, harvest time residues, immature kernels, mature kernels, cashew apple, soil

Cashew (*Anacardium occidentale* L.) is one of the most important plantation crop. Though cashew nuts/ kernels are the main consumption part, cashew apples rich in iron, vitamins and minerals are processed into jam, jelly, juice and soft drinks. Cashew is reported to be infested by >180 insect pests. The most serious are tea mosquito bug (*Helopeltis antonii* Signoret), the stem and root borer (*Plocaederus ferrugineus* L., *P. obesus, Batocera rufomaculata* De Geer) and thrips (*Scirtothrips dorsalis* Hood.). These attack cashew both spatially and temporally resulting in 11-55% loss in yield (Saroj, 2015; Zote et al., 2018). Timely management measures are essential prevent their damage. Insecticides such as lambdacyhalothrin, profenophos, acetamiprid, triazophos, imidacloprid, endosulfan, monocrotophos, cypermethrin and carbaryl were widely used by farmers against cashew pests (Ramesh Kumar et al., 2015; Saroj, 2015).

Combination formulations of insecticides with different modes of action and broad spectrum activity are considered more effective (Casida et al., 2013). Betacyfluthrin (cyano (4-fluoro-3-phenoxyphenyl) methyl 3-(2, 2-dichloroethenyl)-2, 2-dimethylcyclopropane carboxylate), a synthetic pyrethroid in the combination having contact activity is recommended for lepidopteran pests. Imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine), a neonicotinoid with systemic action is widely recommended against sucking pests. The combination product of beta cyfluthrin (9%) + imidacloprid (21%) was introduced in India by Bayer Crop Science Ltd. as Solomon 300 OD and recommended against sucking pests and borers in chilli (Giraddi et al., 2017) and brinjal (Bhargava et al., 2003). Betacyfluthrin 90% + imidacloprid 210% OD (Solomon 300 OD) 1.5 ml for 10 l was found effective in management of tea mosquito bug and thrips in cashew (Zote et al., 2018). Application of insecticides lead to pesticide residues in food commodities which in turn results in health hazards. Information is available on persistence and dissipation behavior of residues of betacyfluthrin and imidacloprid on cashew and hence the present study.

MATERIALS AND METHODS

The material of betacyfluthrin (93% purity), imidacloprid (98.6% purity) its commercial formulation Solomon 300 OD were obtained from Bayer Crop Science Limited, India. Analytical-grade sodium chloride (≥ 99% purity), anhydrous magnesium sulphate (≥ 99.5% purity), trisodium citrate dihydrate and disodium hydrogen citrate sesquihydrate (Sigma Aldrich) were obtained from Merck, Mumbai, India. The magnesium sulphate was activated at 600°C for a minimum of 4 hr in a muffle furnace before use and stored in an airtight desiccator. Primary secondary amine (PSA, 40 µm), florisil and graphitized carbon black (GCB) were obtained from Agilent Technologies, Palo Alto, CA, USA. The standard stock solutions (400 µg ml^{-1}) were prepared by weighing 10± 0.1 mg of certified reference material (CRM) using calibrated analytical balance. The standards of betacyfluthrin and imidacloprid were weighed into a calibrated Class A volumetric flask (25mL) and dissolved with HPLC grade hexane and acetonitrile respectively for making 25 ml volume. The secondary stock solution (40 µg ml^{-1}) was prepared from the stock solution in 25 ml volume by transferring 2.5 ml. The prepared solutions were stored at -20ºC until use. All reagents and solvents used were of HPLC grade. The calibration curves were established by injecting standard solutions of both beta cyfluthrin and imidacloprid prepared in hexane and acetonitrile respectively in the range of 0.01-1.0 µg ml^{-1} (0.01, 0.05, 0.1, 0.5 and 1.0 µg ml^{-1}). Standard concentration at five levels (0.01, 0.05, 0.1, 0.5 and 1.0 mg l^{-1}) were prepared by adding the betacyfluthrin and imidacloprid with blank extracts of corresponding cashew matrices and soil separately. The untreated immature kernels, mature kernels, cashew apple and soil were fortified with betacyfluthrin and imidacloprid residues at 0.05, 0.25 and 0.50 mg kg^{-1} and extracted as per the procedure explained below with six replications each. Recovery percentage was calculated using the Microsoft Excel 2007 software.

The study on persistence of betacyfluthrin g l^{-1} 90 + imidacloprid 210 g l^{-1} OD (Solomon 300 OD) in cashew was carried out in Elayamuthur, Udumalpet (10.476 N, 77.293 E) during February-May, 2019. The field experiment was conducted in a randomized block design (RBD) with three treatments and replicated thrice. The cashew orchard (variety VRI-3) was maintained as per the recommended agronomic practices in 60 m^2 per plot with spacing of 3 x 2 m. Foliar application of commercial formulation of betacyfluthrin 90 g l^{-1} + Imidacloprid 210 g l^{-1}) (Solomon OD) at recommended and double the recommended doses and water spray constituted the untreated control. First foliar application was initiated at fruit setting stage and second at 15 days interval using a high volume knapsack sprayer using 500 l ha^{-1} of water as spray fluid. Samples of immature kernels were collected at 0 (within 2 hr of last application), 3, 7, 14, 21 and 35 days after second spraying. Mature kernel, cashew apple and soil samples were collected at the time of harvest. The field soil samples were collected from the depth of 0-15 cm in the experimental field at random in each replication.

Samples of immature and mature kernel (500 g/ replication) cashew apple (1 kg/ replication) were collected and homogenized in a high volume homogenizer (Robot Coupe, Germany) for 2 min. Immature and mature kernels were processed after the removal of outer shell to represent the edible part. Soil samples (1 kg/ replication) were shade dried by spreading on a polythene sheet on a hard surface and powdered using a wooden mallet, sieved through 2 mm sieve and stored in polythene bag for further analysis. The extraction and cleanup procedure for betacyfluthrin in cashew matrices and soil was done based on the method developed by Silva et al. (2014) with few modifications in sample weight taken for extraction and sorbents used in cleanup step. For extraction a representative sample of 2 g (immature and mature kernel, cashew apple and soil) was weighed into 50 ml polypropylene centrifuge tube, 10 ml of acetonitrile was added and mixed using a vortex mixer for one min. After that, 4.0 g of anhydrous magnesium sulphate ($MgSO_4$), 1.0 g of sodium chloride (NaCl), 1.0 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate salts were added and mixed well in a vortex mixer for 1 min. for proper mixing followed by centrifugation (centrifuge ROTA 4 V/FM) at 6,000 rpm for 10 minutes. To 4.0 ml of supernatant, each 100 mg primary secondary amine (PSA), florisil and 600 mg of anhydrous $MgSO_4$ were added for removing the co extracts. After centrifugation for 10 min at 3,000 rpm, two ml of supernatant evaporated to near dryness and the residue was dissolved in one ml hexane and filtered into a 2.0 ml vial using 0.22 µm nylon filter for GC-ECD (Shimadzu, GC-2010) analysis.

For extraction and cleanup for imidacloprid residues in cashew matrices and soil QuEChERS (Quick, Easy, Cheap, Effective, Robust and Safe) method was followed with slight modifications (Anastassiades et al., 2003). Homogenized samples of 5.0 g immature kernel, 5.0 g of mature kernel and 10.0g of cashew apple were weighed into a 50 ml polypropylene centrifuge tube and to this 10.0 ml of acetonitrile was added and vortexed for 1 min. To each tube 4.0 g of anhydrous $MgSO_4$, 1.0 g of sodium chloride salts were added, vortexed for one minute and centrifuged at 6,000 rpm for 10 min. From the upper supernatant, 6.0 ml extract was transferred into 15 ml small centrifuge prefilled with 100 mg PSA, 25 mg of graphitized carbon black (GCB) and 600 mg of anhydrous $MgSO_4$. The tubes were shaken well and centrifuged at 3,000 rpm for 10 min. Finally, one ml of supernatant was directly filtered in to 2.0 ml vial using 0.2 µm nylon membrane filter for LC-MS/MS analysis.

For soil the same procedure was followed with the addition of 10 ml of distilled water before extraction and 10 mg GCB in the cleanup step and analysed in LC-MS/MS. Analysis of the betacyfluthrin residues was carried out in a gas chromatograph (Shimadzu GC 2010, Japan) equipped with electron capture detector (ECD). The extracts were injected and separation was performed in GC equipped with DB-5 (30 m x 0.25 mm x 0.25 µm) column. An injection volume of 1 µl was made with the injector temperature set at 280ºC in split (1:5) mode and pressure at 74.2 kPa. The flow of carrier gas (He) was set at 0.80ml min^{-1} with a linear velocity of 38.1 cm sec^{-1}. The column temperature was set as 150ºC, hold for 2 min; increased @ 4ºC min^{-1} to 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 11 min. The injection volume was 1µl. The injection port and detector temperature were set at 280 and 300 ºC.

Liquid chromatograph coupled to a triple quadrupole mass spectrometer (LC-MS/MS, Waters Alliance LC and Acquity TQD) with an electrospray ionization (ESI) interface in positive mode was used for imidacloprid residue analysis using Xterra analytical column C18, 5 µm (4.8 x 250 mm) (Waters, Milford, MA, USA). The column temperature was set at 30ºC. The mobile phases were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) (70:30 ratio), run at a flow rate of 0.5 mL min^{-}1. The injection volume was 20 µL and imidacloprid residues were eluted in an isocratic mode within 15 min. Optimum MS/MS, parameters set included voltage at 3.5 kV; ion source temperature at 150ºC; desolvation temperature at 500ºC; flow rates of cone gas and desolvation gas at 50 and 1100 l h^{-}1. To identify the parent and daughter ions, working standard solutions of imidacloprid at 0.5 µg ml^{-1} in acetonitrile containing 0.1% (v/v) of formic acid were directly infused into the mass spectrometer and chromatograms were recorded in the full scan mode.

RESULTS AND DISCUSSION

The method has been validated in terms of linearity, accuracy and precision in spiked samples. The results of linearity studies showed good linearity with coefficients of determination (R^2) > 0.99 for both beta-cyfluthrin and imidacloprid for matrix-matched standard calibration curves of cashew matrices with five points calibration ranging from 0.01 to 1.0 mg l^{-1}. Method accuracy was estimated by recovery experiment at three concentration levels and the results are summarized in Table 1. Currently the QuEChERS method is the most popular analytical method in the field of pesticide residue analysis in food commodities. Hence we tried QuEChERS method with few modifications for recovery studies. In the present method the samples of untreated cashew immature kernels, mature kernels, cashew apple and soil were fortified with betacyfluthrin and imidacloprid at 0.05, 0.25 and 0.50 mg kg^{-1} and extracted with six replications in each concentration. The mean per cent recoveries were in the acceptable range of 70 to 120% and < 20 % RSD for all the matrices studied (Fig. 1). Thus, the performance of the method adopted in this study was considered satisfactory for both β-cyfluthrin and imidacloprid (SANTE, 2019).

Since cashew is a complex matrix containing high levels of phenolics, sugar and organic acids, there is every possibility of matrix interference during instrument analysis. In order to improve the recovery rate, different buffering salts and sorbents were employed in extraction and cleanup step. In the present method, sample weight of 2 g was taken for all the matrices except for cashew apple (10 g). Florisil and GCB were tried in the d-SPE cleanup. Florisil was used as a sorbent and GCB to remove pigments along with PSA sorbent (readily adsorbs organic acids in the matrix) in order to accomplish better clean- up and to improve the recovery rate. Buffering salts such as trisodium citrate dihydrate and disodium hydrogen citrate sesquihydrate were added to maintain the pH during the extraction and to achieve good recovery of pesticides.

As far as cashew is concerned, a very few published literatures are available regarding the methods for pesticide residue analysis. Earlier, Silva et al. (2014) developed a multiresidue method for simultaneous determination of 20 multiclass pesticides using liquid chromatography coupled to mass spectrometry using electrospray ionization source (LC-ESI-MS/MS). In this method, 10 g of homogenized sample was extracted with 10 ml acetonitrile and PSA was the cleanup sorbent. Recoveries obtained were of 78-110% with precisions of 3.3-5.5%, at all three levels 5, 50, 100 ng g^{-1} of spiked imidacloprid. Dorea and Lancas (1999) reported a multiresidue extraction method based on matrix solid-phase dispersion (MSPD) and capillary gas chromatography-electron capture detection (GC-ECD) for quantification of diazinon, malathion, parathion, methidathion and pyrazophos and one synthetic pyrethroid permethrin in Brazilian cashew nuts and obtained an acceptable average recovery of 101.4% at 0.1 to 0.6 ppm levels in cashew nut.

The standardized method was successfully applied for studying residue and dissipation pattern of beta-cyfluthrin and imidacloprid residues after application of combination insecticide Solomon (Betacyfluthrin 90 g l^{-1} + imidacloprid 210 g l^{-1} OD). In treated plots also, beta-cyfluthrin and imidacloprid residues were at below LOQ (0.05 mg kg^{-1}) in the immature kernels collected in all sampling intervals *viz*., 0 (within 2 hr after last spray), 3, 7, 14, 21 and 35 days after second spraying. Mature kernel, cashew apple and soil collected at the time of harvest (68 days after last spray) also showed no residues at detectable level in the recommended and double the recommended doses, respectively. Hence, consumption of cashew apple and nuts does not pose risk to consumers.

Field experiments on residue and persistence pattern of quinalphos (Geetha and Regupathy, 1999), monocrotophos, endosulfan and lamdacyhalothrin (Suresh Kumar et al., 2004) in cashew nuts revealed that the residues below detectable limits irrespective of the doses tested. The initial deposits (1 hr after treatment) of monocrotophos, endosulfan and lamda-cyhalothrin was 36.16, 67.79, 31.39 mg kg^{-1}, respectively. After shelling, the concentrations of residues were reduced to 2.32, 2.63 and 0.90 mg kg^{-1}, respectively. The insecticide residues reached below detectable limit (BDL) in the nuts after roasting and removal of testa layer. Similarly monitoring studies revealed that cashew nut samples collected from market were free from pesticide residues and found safe for consumption. This was likely due to presence of two protective coverings in the form of the outer shell and inside testa layer. Furthermore, the residues reached below detectable levels after shelling, peeling and roasting processes (Ramesh Kumar et al., 2004). Residue and persistence studies were conducted earlier using a combination formulation of betacyfluthrin and imidacloprid in crops such as okra, chilli, brinjal, mango, chickpea, tea etc. Difference in residue persistence and dissipation pattern was observed among fresh as well as processed commodities. Study on persistence of betacyfluthrin and imidacloprid in mango indicated that the residues dissipated with the half-lives of 2.4 and 2.6 days and 3.06 and 4.16 days, respectively. Mature mango fruits at harvest were free from residues of both insecticides. A safe preharvest interval of 8 days is recommended for consumption of mango fruits (Mohapatra et al*.,* 2011). In okra, half-lives were 0.91 and 0.68 days and 0.85 and 0.96 days for betacyfluthrin and imidacloprid at single and double the doses, respectively (Sahoo et al., 2012). The dissipation of betacyfluthrin 8.91% + imidacloprid 19.81% OD in chilli was studied and found 4.20 and 3.80 days as the half lives of betacyfluthrin and imidacloprid. Betacyfluthrin reached LOQ on 15th day after spraying while, imidacloprid reached LOQ on 7 days after spraying (Anju Viswanathan and Ambily Paul, 2019).

In the present study, an analytical method was developed and validated using GC-ECD for betacyfluthrin and LC-MS/MS for imidacloprid residues in cashew. The proposed method is simple, less time-consuming and has high cleanup ability and exhibited better recovery rate of target pesticides.

Table 1. Recovery of betacyfluthrin and imidacloprid (cashew matrices and soil) using GC and LC-MS/MS

Selected analytes	Fortification (mg kg^{-1})	Immature kernel		Mature kernel		Cashew apple		Soil	
Beta-cyfluthrin		*Mean Recovery± SD (%)	RSD (%)	*Mean Recovery ± SD (%)	RSD (%)	*Mean Recovery± SD (%)	RSD (%)	*Mean Recovery± SD (%)	RSD (%)
	0.05	88.26± 6.59	7.46	103.99± 2.51	2.42	96.16± 4.75	4.94	111.88 ± 5.48	4.89
	0.25	105.09± 1.89	1.80	84.43± 2.82	3.34	100.10± 6.72	6.71	104.97± 1.33	1.27
	0.50	102.24± 4.14	4.05	82.36± 3.83	4.65	111.19± 3.06	2.75	100.23± 0.44	0.43
Imidacloprid	0.05	96.70± 7.95	8.22	109.72± 4.02	3.67	98.95± 3.04	3.07	102.12± 4.38	4.29
	0.25	101.69± 4.97	4.89	99.50± 2.20	2.21	110.29± 6.17	5.59	99.19± 5.71	5.76
	0.50	103.73± 2.29	2.20	108.20± 6.14	5.67	91.72± 2.40	2.62	96.87± 5.11	5.27

*Mean of 6 replicate analyses; SD- Standard Deviation; RSD- Relative Standard Deviation

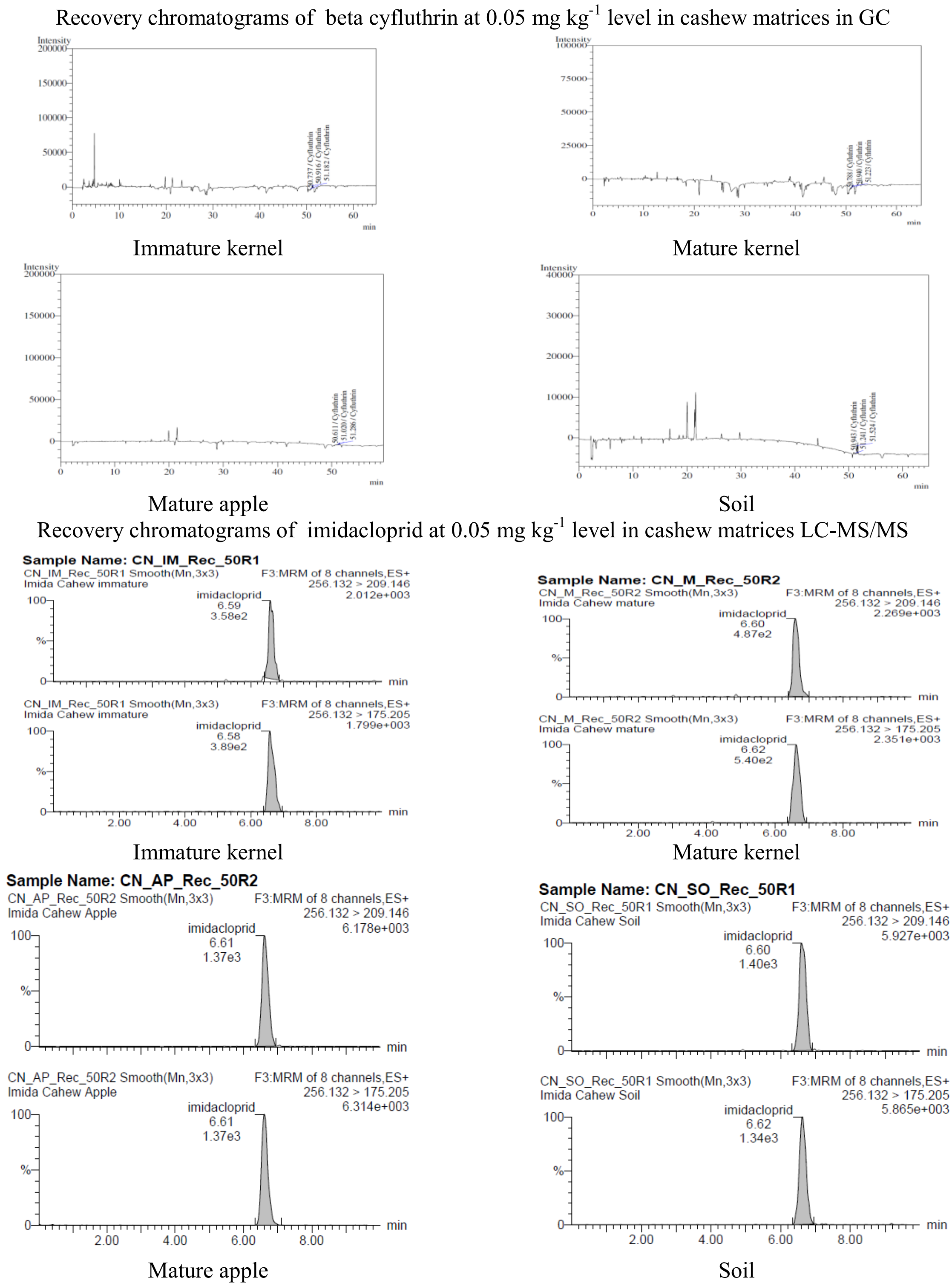

Fig. 1. Chromatograms of recovery of betacyfluthrin and imidacloprid (cashew matrices analysed in GC and LC-MS/MS)

Hence, the validated method was successfully used for studying persistence and dissipation of betacyfluthrin and imidacloprid in cashew crop. The residues of betacyfluthrin and imidacloprid were at below limit of quantification (0.05 mg kg^{-1}) on the day of spray itself in the immature kernels collected after 2 hr of application, mature kernels, cashew apple and soil collected at the time of harvest. Hence, application of Solomon (betacyfluthrin 90 g l^{-1} + imidacloprid 210 g l^{-1} OD) for cashew pest management can be concluded as safe from consumption point of view. The proposed method can be used for monitoring of betacyfluthrin and imidacloprid residues from market cashew nut samples.

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AUTHOR CONTRIBUTION STATEMENT

KB conceived and designed research. KB, VM, AS and PT conducted field experiment. CS and PK conducted lab experiment, NT and PK analyzed data. CS and KB wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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