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NOTE

Evaluation of Imidacloprid Residues in Okra Fruits by LC-MS/MS

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Residues of imidacloprid applied as foliar spray at every ten day's interval to control leaf hopper (*Amrasca bigutulla bigutulla*, Ishida) on okra fruits were determined by LC-MS/MS at three successive pickings following each application at flowering-fruiting stage. Residues were the highest at the first picking after spray and lowest at the third picking and were in the range of 0.10 to 0.16 ug g^{-1}.The residues in fruits at all the pickings following all applications remained below the maximum residue limit of 0.5 µg g^{-1} for imidacloprid on okra. The farmer's practice of spraying imidacloprid in okra every 10 days at the recommended dose of 0.3 mL L^{-1} was therefore found to be safe if the produce was harvested at least two days after the previous application of the insecticide. Leaf hopper population was effectively controlled by application of imidacloprid.

Key words: Imidacloprid, pesticide residue, LC-MS/MS, leaf hopper, okra

Okra, (*Abelmoschus esculentus* L.Moench), is a popular and important commercially cultivated vegetable grown in all parts of india. It is cultivated in more than 0.5 million hectares with an annual production of 6.35 million tonnes. In the states of Karnataka and West Bengal it is grown throughout the year. India alone produces more than 70 per cent of total okra produced in the world.

The productivity of okra is significantly reduced by leaf hopper, *Amrasca bigutulla bigutulla* Ishida. The nymphs and adults of this pest suck the plant sap mainly from the lower surface of leaves and cause symptoms of yellowing and leave curling upwards. This is known as hopper burn which results in complete desiccation of the leaves and stunted plant growth. Leafhoppers do not have effective natural enemies. Also, it has been reported by several workers that leafhopper population in okra or cotton has a significant positive correlation with the maximum temperature and a significant negative correlation with the rainfall[2,3]. The chemical control is a popular method for control of this pest especially in summer months. Foliar spray of imidacloprid has been reported to be extremely effective in controlling this pest[4]. Yield loss in unprotected crop can be as high as 40 to 60 per cent and therefore farmers in South India often spray imidacloprid once in 10 days to control this pest in okra during summer.Such regular applications of imidacloprid in okra during summer may prove to be hazardous to human health as it may lead to harmful residues on the crop and therefore, the present study was conducted to estimate its residues following its periodic applications. In plants imidacloprid metabolizes into several metabolites but, unchanged parent compound has been found to be the major component[5] following its application as a foliar spray. So in the present study only imidacloprid residues were monitored.

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MATERIALS AND METHODS

Chemicals

Imidacloprid certified reference material (99.9% purity) and methanol (LC-MS grade), ammonium formate and formic acid were purchased from M/s Sigma -Aldrich, India.Confidor 200SC of imidacloprid was purchased from Bayer Crop Science, India Ltd., Mumbai. Magnesium sulphate heptahydrate was purchased from M/s Avantor Performance Materials India Limited (brand Rankem), which was heated in a muffle furnace at 600 °C for 5 hours after which it was ground in a mixer-grinder to a fine powder of anhydrous magnesium sulphate. Anhydrous sodium acetate (AR grade) was procured from M/s Thomas Baker Pvt. Ltd., India.

LC-MS/MS

LC-MS/MS analysis was performed with an Agilent (California, USA) 1290 HPLC hyphenated to Agilent 6460 C triple quadrupole mass spectrometer with ESI probe in

the positive mode. The analytical column used was a Zorbax (CA, USA) make Eclipse plus C-18, 100 mm × 3 mm i.d., 1.8 µm particle size. The column temperature was 30°C, injection volume 1 µL and flow rate 0.5 mL min^{-1}. The mobile phase consisted of 5mM ammonium formate and 0.01% formic acid in water (A) or methanol (B). The gradient programme used was as follows: Time 1.0 min = 15% B, 6.0min = 50% B, 12.0 min = 95% B, 17.5 min = 95% B, 18.0 min = 15% B.The column oven temperature was 40 °C. Under the above conditions imidacloprid eluted out at a retention time of 4.5 min. The MS conditions were, nebulizer-30psi, sheath gas flow 11 L min^{-1}, capillary voltage +3000V, sheath gas temperature 375 °C.

Experimental

A field experiment was conducted in a plot measuring 25 × 25m.Okra (variety- Arka Anamika) was grown in sub-plots measuring 3.5 × 3.0 m with a spacing of 20 cm x 1m during summer of 2014. Spraying of imidacloprid on the crop was initiated at 15 days after sowing, when the plants were very small, and repeated at every 10 days interval till 65 days after sowing in 15 experimental sub-plots.Thus, a total of 6 sprays were given at an application rate of 0.3 mL L^{-1} (1000 L spray solution per hactare) using a gator sprayer.Five control sub-plots were maintained without spraying imidacloprid but spraying with only water. The first two sprays of imidacloprid were given at vegetative stage of the crop i.e. at 15 and 25 days after sowing. The third spray was given at 35 days after sowing and residues of imidacloprid analysed in okra fruits starting with first picking at 37 days after sowing (2 days after last spray or 2 DALS), followed by analysis of subsequent pickings at 6 and 9 DALS. Thus, in all, four applications of imidacloprid were made on the crop at its fruiting stage (at 35, 45, 55 and 65 days after sowing) and a total of 12 pickings of okra fruits were made, three pickings at 2nd , 6th and 9th day after each application at fruiting stage. Jassid nymphs were counted in control as well as treated plants in order to find the effectiveness of imidacloprid treatment. Three middle leaves were sampled per plant and in all 5 plants per plot were sampled. Thus, jassid population in a total of 15 leaves was counted from each plot.

Residue estimation

Okra fruits were picked at random from each sub-plot and a total of 1 kg fruits was sampled at each picking/sampling and analysed immediately. Sample preparation was carried out as per QuEChERS technique[6] with minor modifications. The okra fruits were mixed, quartered, cut into small pieces and homogenized using a Waring blender and 15g of the homogenized sample was taken in a 100 mL centrifuge tube to which 15 mL of 1% acetic acid in acetonitrile was added. To this 5g anhydrous magnesium sulphate and 1.5g sodium acetate were added and the contents mixed thoroughly by using a vortex mixer for 2 min. The mixture was centrifuged at 4000 rpm for 10 min. Four mL of supernatant from the centrifuge tube was taken in another centrifuge tube to which 50mg primary secondary amine (PSA) and 150mg anhydrous magnesium sulphate had been added, the mixture was again vortexed for 1 min. and then centrifuged, the supernatant from this was analysed directly using LC-MS/MS equipment.

At first the LC-MS/MS fragmentor voltage for imidacloprid certified reference standard was optimized so as to produce the greatest signal for the precursor ion. The protonated molecule (m/e = 256) was used as the precursor ion. At the optimal fragmentor signal, imidacloprid standard was injected at a concentration of 10 µg mL^{-1} to determine collision energies for both the quantifying and qualifying ions. The collision energies were optimized for each of the ions and the voltages that gave the best sensitivity were selected. The MRM(multi reaction monitoring) transitions thus selected for imidacloprid are shown in Table 1.Samples showing ion ratios within the range of ±20 per cent were accepted as that of imidacloprid residues. Recovery experiments were carried out on okra fruits by fortifying the same with three replicates at six concentrations of imidacloprid viz. 0.005, 0.010, 0.015, 0.020, 0.050 and 0.1 µg g^{-1}. This was compared with okra samples for matrix matched calibration.

RESULTS AND DISCUSSION

Leaf hopper population

The leaf hopper population on okra leaves (Table 2) showed a reduction in number when imidacloprid was applied. However, due to early showers, the temperature was lower than usual during the period of study and therefore the number was not high even in untreated plants.

Table 1. Optimization of multi reaction monitoring (MRM) parameters for analysis of imidacloprid

Precursor ion	Product ion	Fragmention voltage	Collision energy
256	209	100	10
256	175	100	13

Table 2. Imidacloprid residues at various pickings of okra and jassid count

Spray no.	Mean§ Imidacloprid residues in (µg g^{-1} ± SD) at			Av. No. of leaf hoppers on leaves $plot^{-1}$*	
	Picking			Untreated leaves	Treated leaves
1				16.0	8.0
2				16.7	6.3
	1 (2 DALS)	2 (6 DALS)	3(9 DALS)		
3	0.112 ± 0.012	0.013 ± 0.102	0.008 ± 0.004	15.3	3.0
4	0.158 ± 0.011	0.058 ± 0.033	0.006 ± 0.002	17.3	3.3
5	0.163 ± 0.002	0.035 ± 0.003	0.017 ± 0.002	8.7	3.3
6	0.106 ± 0.004	0.023 ± 0.001	0.011± 0.001	10.5	3.0

MRL (UK/EC) for imidacloprid in okra = 0.5 µg g^{-1}
DALS: Days after last spray; § Average of three replicates collected across all treated sub-plots; *Nymphs + adults recorded on 75 plants (3 leaves $plant^{-1}$)

Imidacloprid residues

The limit of quantification of imidacloprid in okra, using triple quadrupole mass spectrometry was found to be 0.005 µg g^{-1}. This was determined by analysis of matrix matched recovery standards. No residue of imidacloprid was detected in any of the fruits sampled from the untreated control sub-plots. The mean residue of imidacloprid (average of three replications) at different pickings of okra grown in treated sub-plots is presented in Table 2. The residues were the highest in fruits picked two days after the last spray. At these pickings, the imidacloprid residues in okra ranged from 0.10 to 0.16 µg g^{-1} which was less than the maximum residue limit (MRL, UK/EC) of 0.5 µg $g^{-1}$for imidacloprid on okra. The fruits sampled at the next picking at 6 days after last spray, had much lesser residues 0.01 to 0.06 µg g^{-1} and those at the third picking after each application contained only about 0.01 µg g^{-1} of imidacloprid. Thus, the farmer's practice of spraying imidacloprid on okra every 10 days seems to be safe from the point of residues. However, harmful residues are likely to remain on the crop if the dose of application is exceeded since often the dose at which application is made is higher than the recommended dose of 0.3 mL L^{-1}. Also, leaf hopper population on okra crop seemed to be well controlled by the application of imidacloprid at 10 days interval (Table 2). Earlier workers[7] have also reported that imidacloprid residues dissipated quickly on okra with a half life of approximately 2 days. However, imidacloprid residues have been found in market samples of okra and other vegetables in India[8,9] and therefore it is prudent to evaluate the safety of common farmer's practices involving this insecticide in vegetables, as has been carried out in the present study.

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