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Residues of Thiamethoxam and Acetamaprid, Two Neonicotinoid Insecticides, in/on Okra Fruits (*Abelmoschus esculentus* L)

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Synthetic pesticides are important component of modern agriculture. About 13-14% of total pesticide used in India, are consumed in vegetable crops only. The produce is harvested at short intervals and consumed fresh in many cases. The survey of market samples show high level of pesticides (Agnihotri,1999) in vegetables. Therefore target specific and extremely low dose pesticides are being introduced specially for vegetables. Okra (*Abelmoschus esculentus* L) is an annual vegetable crop, which is attacked by a number of insect pests like shoot/fruit borer, jassids and aphids resulting in the reduction of yield to an extent of 69% and quality of fruits (Dewan et al. 1967). To protect the crop from such pests various pesticides from different class are applied (Rawat and Sahu, 1973; Patel et al. 2001). Neonicotinoids (Yamamoto, 1996) represents a novel and distinct chemical class of insecticides with remarkable chemical and biological properties with low application rates (Yamamoto and Casida, 1999). The effectiveness of imidacloprid, a chloroneonicotinoid insecticide, has been reported on okra, brinjal and chilli fruits against various insect pests (Mote et al. 1994; Jarande and Dethe, 1994). Acetamaprid ((E)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine; 1, Figure 1) has been introduced by Nippon Soda with common structural features of first generation neonicotinoids (Maienfisch et al., 1999). Thiamethoxam [(EZ)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene (nitro) amine; 2, Figure 1] is a novel neonicotinoid belonging to sub class of thia nicotinyl compounds and it represents the first example of second generation neonicotinoids with a unique structure and outstanding insecticidal activity (Maienfisch et al., 1999a) introduced by Novartis. Both are systemic insecticides for soil and foliar applications and control a variety of pests such as aphids, whiteflies, thrips, beetles, leaf hopper, bugs and borers in fruiting, corm, tuberous vegetables, cotton and fruits (Roberts and Hutson, 1999b). GC method has been used for the determination of acetamaprid and its degradation product in soil by Tokieda et al, 1999c. HPLC method has also been developed for the detection of thiamethoxam from water, soil and vegetables (Singh et al, 2004; Karmakar et al, 2005). In this study we report the dissipation and residues of these two neonicotinoids viz., thiamethoxam and acetamaprid by HPLC in okra fruits following foliar applications at different stages of crop growth.

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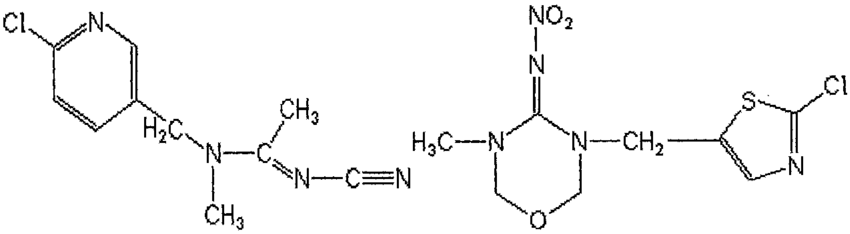

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Figure 1. Chemical structures of acetamaprid (1) and thiamethoxam (2)

MATERIALS AND METHODS

Technical grade thiamethoxam was obtained from M/s Syngenta India Ltd. Acetamaprid was extracted from formulation and purified by column chromatography over silica gel. Finally it was crystallized from benzene before use. All the solvents were analytical grade and glass distilled. Anhydrous sodium sulphate (AR grade) was used as a drying agent for different samples. HPLC grade acetonitrile and water were used for HPLC analysis. Formulation Actara^R (25% WG) and Pride^R (20% SP) for thiamethoxam and acetamaprid respectively were purchased from market.

Field experiment was conducted at IARI, New Delhi, during August to November, 2003. Okra (variety Pusa kranti) was grown in plots of 5 x 8m using RBD replicated 4 times with 50 cm spacing between the rows and 30 cm between plants. Two sets of experiments were conducted. In the first experiment foliar application of thiamethoxam and acetamaprid at recommended doses of 140g and 75g a.i. ha^{-1} respectively was given in separate replicated plots at the time of flowering. Okra fruits were harvested and analysed for the insecticide residues from these plots. In the second experiment for the waiting period studies two sprays of each pesticide were given in separate replicated plots. First spray was given at the time of flowering and a second spray of both the insecticides (thiamethoxam and acetamaprid) at recommended doses was given at the time of fruiting (in the month of November) in separate respective plots. From this experiment fruit samples were collected at 0, 1, 3, 5 and 7 days for respective insecticide analysis.

The okra sample (50g) was blended in a blender with acetone (100 ml) and contents were filtered through Buchner funnel using suction. The extraction was done twice more with the same solvent (50 ml each time) and filtered in the same way. The combined filtrate was then concentrated by evaporating the solvent on a rotary vacuum evaporator at 35-40 °C to 2-3ml. The extract concentrate was diluted with 150ml of 10% NaCl (aq.) solutions and partitioned with hexane (25ml), which was discarded. The aqueous phase was extracted thrice with dichloromethane (50+50+30 ml). The organic phase was dried over anhydrous sodium sulphate, evaporated at rotary evaporator and finally exchanged with acetonitrile prior to HPLC analysis. Recovery experiments for both the insecticides were conducted with fortified okra samples at the level of 0.1 and 0.5μg g^{-1}.

A reverse phase high performance liquid chromatographic technique was used for quantitative analysis. A Hewlett Packard HPLC instrument (series 1100) equipped with degasser, quarternary pump, photo diode-array detector connected with rheodyne injection system (20μl loop) and a computer (model Vectra) was used for analysis. The stationary phase consisted of Lichrospher on RP-18 packed stainless steel column (250mm×4mm i.d). Chromatogram was recorded in a Windows' NT based HP chemstation programme. Acetonitrile : water at 1ml min^{-1} flow rate was used as mobile phase using a gradient elution system described in table 1. Wavelengths 254 and 270 nm were selected for absorption maxima of thiamethoxam and acetamaprid respectively. Under these conditions of analysis standard solutions of thiamethoxam and acetamaprid and the okra sample extracts were injected in HPLC.

Table 1. HPLC gradient elution system for the analysis of thiamethoxam and acetamaprid

S.No.	Time (min.)	Flow rate (ml min^{-1})	Water (%)	Acetonitrile (%)
1.	0.0	1.00	85	15
2	11.0	1.00	60	40
3	13.0	1.00	10	90
4.	15.0	1.00	85	15

RESULTS AND DISCUSSION

Purified acetamaprid gave a single spot on TLC and a sharp melting point, 99°C. Under the described condition of HPLC thiamethoxam and acetamaprid resolved as sharp peaks at 6.45 and 8.90 min. respectively. Instrument detection limit of 0.02μg ml^{-1} was observed for both the insecticides with a sensitivity of 4 ng. Clean up with hexane partitioning removed chlorophyll and no matrix interference was observed during HPLC analysis of thiamethoxam and acetamaprid in okra samples (Figure 2 and 3). Average recovery of thiamethoxam and acetamaprid form okra fruits ranged between 84-89%. Okra fruits from first experiment, where application was given at the time of flowering, showed no detectable residues of both the insecticides. Okra samples from the second experiment, where insecticide application was given at flowering as well as fruiting time, were analysed at 0, 1, 3, 5 and 7days after second application. Initial deposits of 0.475 and 0.335μg g^{-1} was observed for thiamethoxam and acetamaprid respectively following two application @ 140 and 75g a.i ha^{-1} for each pesticide. Residues dissipated rapidly and more than 50% dissipation was observed on 3rd day after application (Table 2). 95.2 and 80% dissipation was

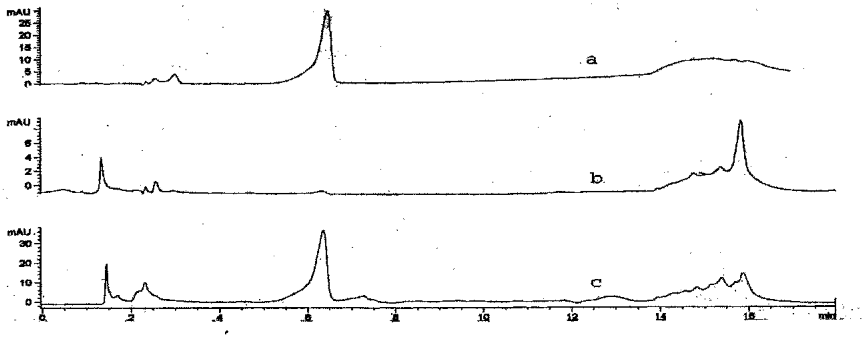

Figure 2. HPLC chromatogram showing recovery of thiamethoxam: Standard thiamethoxam (a); Control okra fruits (b); Fortified okra fruits (c)

recorded on 5th day for thiamethoxam and acetamaprid respectively. Residues were below detectable limits on 7th day after application. The data analysis by logarithmic plots showed that residue of thiamethoxam and acetamaprid in okra fruits dissipated with half-lives of 1.3 and 2.3 days respectively. Dissipation of both the insecticides followed first order rate kinetics (Figure 4). The application of these insecticides at the time of flowering effectively controlled the attack of whitefly and thus no viral infection was observed in the crop. But this application alone was not sufficient to control the borers at the time of fruiting. Two sprays (1st at flowering and 2nd at fruiting) of thiamethoxam in other experiment were able to control the pests at the time of fruiting also. Similar results were found in

Table 2. Persistence of thiamethoxam and acetamaprid residues in okra fruits

Analysis time (*Application time*)	Insecticide amount remaining ($\mu g\ g^{-1}$)	
	Thiamethoxam (@ 140g a.i. ha^{-1})	Acetamaprid (@ 75g a.i. ha^{-1})
Harvest (*At flowering*)	BDL	BDL
Days after 2nd Application (*At flowering & fruiting*)		
0	0.475±0.07(0)	0.335±0.03(0)
1	0.306±0.02(35.5)	0.190±0.05(43.3)
3	0.20±0.04(57.9)	0.125±0.03(62.7)
5	0.023±0.01(95.2)	0.05±0.02(80.0)
7	BDL	BDL
Half life	1.3 days	2.3 days

* Average of three replicates; Figure in parenthesis indicates % dissipation

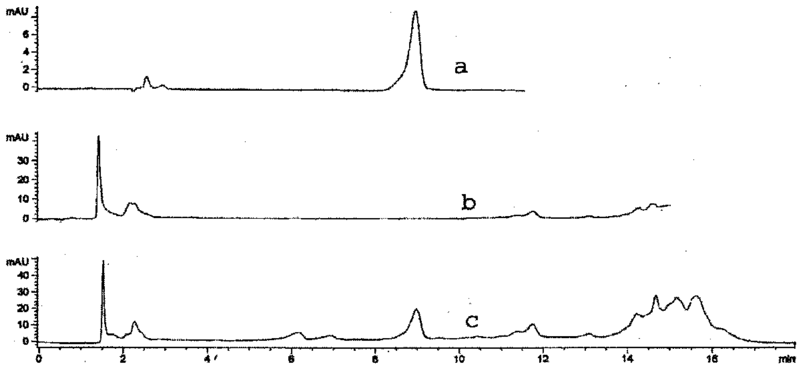

Figure 3. HPLC chromatogram showing recovery of acetamaprid: Standard acetamaprid (a); Control okra fruits (b); Fortified okra fruits (c)

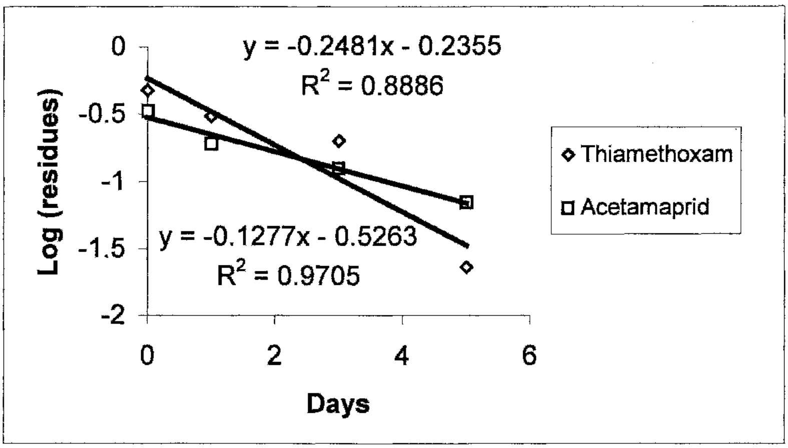

Figure 4. Dissipation of thiamethoxam and acetamaprid residues on okra fruits

case of acetamaprid. The plots with two sprays of acetamaprid were free from borer attack. The results were also supportive from the yield, which was approximately 5% higher than the first experiment.

The half life of acetamaprid in citrus has been reported as 12.7 days by Li et al., 2000. In this crop generally four applications per season are given at a higher application rate. The half life of thiamethoxam as reported on other crops is also higher. But in okra under Indian tropical conditions it was found quite short for both the insecticides (1.3 and 2.3 days for thiamethoxam and acetamaprid respectively), which may be due to the rapid vegetative growth of the okra fruits.

MRL of these pesticides on okra has not been fixed. On 7th day after application the residues were below or less than limit of detection (LOD 0.02ppm). The studies can be useful for prescribing the safe waiting period for okra.

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