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Sarswati Negi
Department of Entomology, YSP University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

ID Sharma
Department of Entomology, YSP University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Tanuja Banshtu
Department of Entomology, YSP University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

RS Chandel
Department of Entomology, YSP University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Correspondence
Sarswati Negi
Department of Entomology, YSP University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Persistence of acephate and methamidophos in/on okra (*Abelmoschus esculentus* L.)

Sarswati Negi, ID Sharma, Tanuja Banshtu and RS Chandel

Abstract
The persistence study on okra fruits recorded initial deposits due to single dose each of acephate and methamidophos as 1.858 and 0.048 mg kg^{-1}, respectively. However, at double dose the deposits were 3.337 and 0.068 mg kg^{-1} for respective insecticide. The initial deposits of 0.291 and 0.483 mg kg^{-1} at single dose and 0.443 and 0.056 mg kg^{-1} at double dose were observed for acephate and methamidophos, respectively, in okra cropped soil. Acephate residues persisted in okra fruits upto 7 and 10 days at single and double dose, respectively. The safe waiting periods on okra were suggested as 8.2 days for acephate, respectively. The washing of okra fruits by tap water, saline water and lukewarm water were found effective in removing the residues upto 41.87, 50.00 and 58.13 percent, respectively. Open pan and microwave cooking provided upto 56.20 and 63.50 percent relief, respectively from insecticide contamination.

Keywords: Vegetables, okra, pesticides, GCMS

Introduction
Okra, *Abelmoschus esculentus* (L.) Monech, is an important vegetable crop in India. It is infested by several insect pests among which shoot and fruit borer is the most destructive [1]. The average fruit infestations have been estimated to be 35-76 percent [2]. In order to control the damage caused by insect pests, substantial amounts of insecticides are used by the farmers, even during fruiting stage. Indiscriminate use of insecticides, particularly at fruiting stage and non adoption of safe waiting period leads to accumulation of residues in consumable produce. Organophosphorus insecticides like acephate though not recommended are being used for the control of insect pests in vegetable crops [3]. In plants and soil, acephate is converted into toxic compound methamidophos which also have insecticidal activity. Residues of methamidophos in vegetables can harm consumers. Recently residues of above insecticides have been reported in different vegetables including okra. Keeping this in mind, the investigations were undertaken to study the persistence of acephate in okra [4].

Material and Methods
The supervised field trials were conducted at the experimental farm and the residue analysis was done in the Pesticide Residue Laboratory (PRL) of the department. The okra crop was raised in randomized block design in Twenty four plots each of size 3×2 m^2 were prepared. The plants were planted at planting distance of 45 cm ×15 cm, as per standard package of practices [5].

Extraction and Clean up
Okra fruits (1kg) were collected from each replication on 0 (2hr after application), 1, 3, 5, 7, 10,15 and 20 days after second application of insecticides and samples were brought to the Pesticide Residue Laboratory (PRL) and processed immediately. The estimation of residues of insecticides was carried out by QuEChERS method [6]. The entire Laboratory sample (1kg) was cut and homogenized in grinder properly. Weighed 15g homogenized sample in a 50 mL centrifuge tube and tube was kept in deep freezer for 10 min. To this, added 30 ml acetonitrile and 9g anhydrous sodium sulphate, shaken for 5 minutes at 50 rpm in Rotospin mixer and then centrifuged at 3300 rpm for 3 minutes. Transferred 11 mL supernatant to the 15 mL tube containing 400 mg PSA. The contant was vortexes for 30 sec and then centrifuged at 3000 rpm for 3 minutes. The 6 mL supernatant was transferred to 30 mL glass turbo tube and evaporated in turbo evaporator to dryness at 45 ^{0}C in the presence of nitrogen current.

The residues were redissolved in 3 mL n-hexane and performed GC-MS analysis using operation parameters as mention in table1.

Recovery

The analytical method for estimation of acephate and methomodophos residues in okra fruits has been validated by conducting recovery studies using control samples. A 15 g control sample of blended okra fruits was taken in 50 ml centrifuge tubes in three replicates and each were spiked with acephate and methomodophos separately at required fortification levels i.e. 0.05, 0.10, 0.25, 0.50 and 1.00 mg/kg by adding an appropriate volume of working standard. This mixture was then shaken, in order to attain a proper homogeneity of insecticides in the sample. The extraction and cleanup procedure was followed as described earlier. Percent recovery was calculated by using following formula.

$$\text{Percent recovery} = \frac{\text{Quantity of pesticide recovered}}{\text{Quantity of pesticide added}} \times 100$$

Results and Discussion

The average recoveries of mixture of pesticides from different substrates fortified @ 0.05, 0.10, 0.25, 0.50 and 1.00 mg kg-1 for okra are shown in Table 1 and Table 2 respectively. For the amount fortified ranging from 0.05 to 1.00 mg/kg in, acephate showed % recovery values ranging between 94.00 and 120.00. These present findings are in agreement with those of [7] where the recovery of acephate obtained from olives ranged between 83.00-110.00 percent with fortification levels ranged between 0.01- 2.00 mg kg^{-1}. [8] recovered 83.00-117.00 and 73.00-116.00 percent acephate from tomato fruits and soil, respectively. The recovery of acephate from mango orchard soil was in the range of 89.75–91.44 percent [9, 10]. reported recovery of acephate ranged between 82.00-92.33 percent from brinjal cropped soil, at 0.05-1.00 mg kg^{-1} fortification levels. methamidophos showed % recovery values ranging between 78.00 and 109.67.

The initial deposits of acephate and methamidophos at double dose on okra fruits were found to be 1.7 and 1.4 times higher than single dose. These present findings are in agreement with those of [11] who reported 1.6 fold increases in deposits due to higher dose over lower one on mango. Our results are also in a close similarity to [12, 13] who reported an increase of about 2 fold in deposits for double dose over single dose on cotton crop [10]. Reported 1.9 and 1.6 times increase in deposits of acephate on capsicum and brinjal fruits due to higher dose over lower dose.

The residue data indicated that initially there was a rapid loss of insecticide residues followed by slower rate of dissipation in test insecticides from fruits. According to persistence data acephate exhibited 50.48 to 96.71 percent dissipation at single dose, respectively. Whereas, at double dose, the dissipation observed for the respective insecticide was 55.29 to 97.57 percent, respectively. The dissipation behaviour of the evaluated insecticides showed that in okra fruits residues of these reached below detectable limit with in 7 and 10 days at single and double dose, respectively. The results showed a close similarity with those of [14, 15], who reported 94.85 and 99.00 percent dissipation of acephate within 10 days and its initial deposits on capsicum and pakchoi recoded to half in 1.8 and 1.36 days, respectively. The dynamic degradation results of acephate showed that immediately (2 hours) after its application on okra at the respective doses, methamidophos residues found were 0.048 and 0.068 mg kg^{-1}. These residues increased further to 0.085 and 0.088 mg kg^{-1} on 3rd and 5th day of sampling at the respective doses. The increase in concentration of methamidophos residues due to degradation of acephate when the insecticide was sprayed on vegetables has been reported by various workers [8, 15, 16].

The half life values of acephate at single and double doses were 1.8 and 1.9 days, respectively, which indicating faster dissipation of the molecule irrespective of doses. Our finding are supported by [15, 12], according to them the half life of acephate was 1.4 days in pakchoi and 1.9-2.0 days in cotton crops. Similar results were obtained by [17], who calculated half-life value of acephate in grapes as 3.01 days.

The waiting period of acephate has been calculated as 8.2 and 11.3 days for single and double dose, respectively. A pre-harvest interval of 7 days for acephate has been established in vagetables [18, 17]. also suggested 5.13 days waiting period for acephate on treated grape berries.

Acephate residues reached below limit of determination after 5 days of application. Methamidophos residues persisted below the limit of determination at single dose and 3 day at double dose, respectively. The present studies are in conformity with those of [10, 14] who observed similar trend of dissipation of acephate in brinjal and capsicum cropped soils.

The half life of acephate deposits in okra cropped soil at single dose was found 1.7 days while it was 2.2 days at double dose [10, 14]. Observed similar trend of dissipation of acephate in brinjal and capsicum cropped soils.

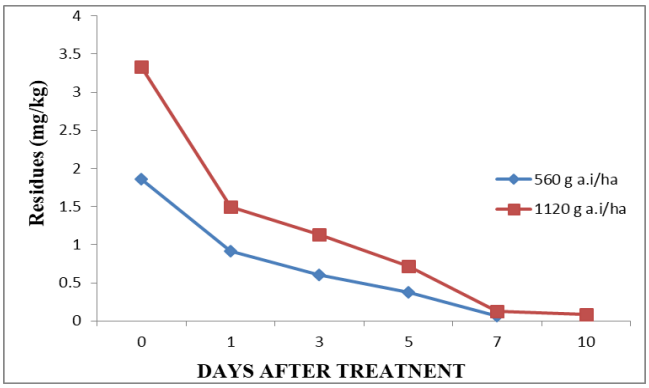

Fig 1: Dissipation of acephate from okra fruits

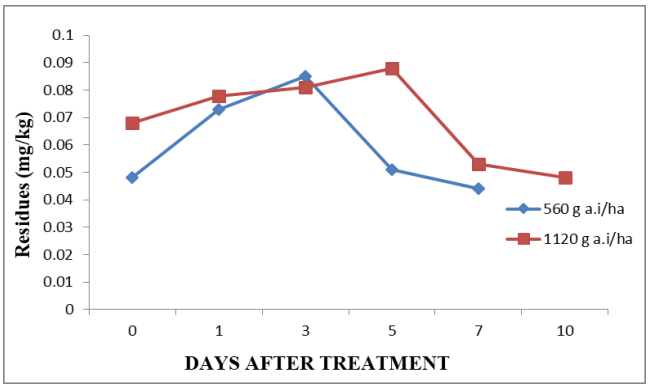

Fig 2: Residues of methamidophos after application of acephate from okra fruits

Table 1: GC-MS Parameters

Column type	DB-5 (30mt, 0.25mm ID, 0.25µm
Column temperature	
Injection temperature	250 ^{0}C
Interface temperature	280 ^{0}C
Ion source temperature	250 ^{0}C
Injection volume	1µl

Table 2: Recovery of acephate and methamidophos from fortified okra fruits

Amount added (mg kg^{-1})	acephate		Methamidophos	
	Average of three replications ± SD (mg kg^{-1})	Recovery (%)	Average of three replications ± SD (mg kg^{-1})	Recovery (%)
0.05	0.060 ± 0.001	120.00	0.039 ± 0.001	78.00
0.10	0.101 ± 0.003	101.00	0.110 ± 0.000	109.67
0.25	0.235 ± 0.003	94.00	0.258 ± 0.011	103.20
0.50	0.498 ± 0.069	99.60	0.489 ± 0.076	97.73
1.00	0.999 ± 0.000	99.90	1.002 ± 0.014	100.23

Table 3: Residues of acephate and methamidophos on okra

Interval (Days)	Residues of acephate and methamidophos on okra ± SD (mg kg^{-1})			
	Treatment at 560 g a.i. ha^{-1}		Treatment at 1120 g a.i. ha^{-1}	
	Acephate	Methamidophos	Acephate	Methamidophos
0	1.858 ± 0.026	0.048 ± 0.000	3.337 ± 0.073	0.068 ± 0.002
1	0.920 ± 0.071	0.073 ± 0.002	1.492 ± 0.015	0.078 ± 0.002
3	0.603 ± 0.064	0.085 ± 0.002	1.138 ± 0.000	0.081 ± 0.001
5	0.373 ± 0.010	0.051 ± 0.001	0.722 ± 0.091	0.088 ± 0.003
7	0.061 ± 0.002	0.044 ± 0.001	0.130 ± 0.008	0.053 ± 0.001
10	BDL	BDL	0.081 ± 0.007	0.048 ± 0.002
15			BDL	BDL

Conclusion

The analytical method was suitable for analysis of acephate and methamidophos in okra. The recoveries of both compounds were within the satisfactory range. Acephate residues from the standard dose treatment of 560 g a.i. ha-1 persisted on okra for 7 and 10 days, respectively. Methamidophos, the metabolite of acephate, was detected up to 7 and 10 days. Though methamidophos residue level was lower compared to acephate, it was detected in okra as long as acephate was detected. Acephate is rapidly translocated from soil to plant and translocation occurs in the direction of roots to foliage, not from foliage to root But the chances of acephate entering into successive crops was ruled out as the field soil was free from residues of acephate and methamidophos after 30 days.

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