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Persistence Behavior of Combination Mix Crop Protection Agents in/on Eggplant Fruits

Irani Mukherjee · Aman Kumar · Ashok Kumar

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Abstract Persistence behavior of two combination mix formulations of insecticides viz. profenofos + cypermethrin (Roket 44EC) and chlorpyrifos + cypermethrin (Nacraj-505) were studied in eggplant fruits following spray application at recommended and double dose. Half-life values were calculated from first order dissipation kinetics, in case of Roket 44EC, residues of cypermethrin on fruits dissipated with half-life of 2.15–2.31 days, whereas residues of profenofos dissipated with the half-life of 0.91–1.86 days. Profenofos residues persisted beyond 7 days in soil, whereas residues of cypermethrin were below detection limit on 0-day itself. Dissipation of chlorpyrifos residues in the combination mix with cypermethrin (Nacraj-505) followed first order kinetics with the half-life values of 3.27–3.10 days. In soil, chlorpyrifos residues were below detectable limit even on 0-day at recommended dose of application. More than 90% dissipation was recorded on 7 day, although residues persisted beyond 15 days at double the recommended dose. Half life of dissipation for cypermethrin calculated from first order dissipation kinetics varied from 2.19 to 3.27 days. In soil, no residues of cypermethrin were detected even on 0-day at recommended dose.

Keywords Residues · Dissipation · Combination mix · Crop protection agents · Profenofos · Chlorpyrifos · Cypermethrin · Eggplant · Waiting period

I. Mukherjee (✉) · Aman Kumar
Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110012, India
e-mail: mukrj_irani@yahoo.com

Ashok Kumar
Division of Agronomy, Indian Agricultural Research Institute, New Delhi 110012, India

Egg plant (*Solanum melongena* L.) is an important and indigenous vegetable crop of India. It contributes 9% of the total vegetable production of the country. It is due to improvement in production technology, protection measures and the genetic improvement which has shown significant advancement in yield, quality, diseases and insect-pest resistance. The yield of long, round, oblong and small round varieties have reached at 50, 65, 60 and 40 t/ha, respectively, whereas, F1 hybrids raised it to 62.5, 79, 75 and 50 t/ha in these respective groups. Egg plants grown on nearly 550,000 ha in India, making the country the second largest producer after China with a 26% world production share. Major egg plant producing states include: West Bengal (30% production share), Orissa (20%), and Gujarat and Bihar (around 10% each). Egg plant is prone to attack from insect pests and diseases, the most serious and destructive of which is the fruit and shoot borer (FSB) *Leucinodes orbonalis*. To protect the crop from severe damage chemical pesticides like endosulfan, fenvalalerate, fluvalinate (Mukherjee and Gopal 1992; Gopal and Mukherjee 1993). Other insecticides have also been found effective under field conditions (Rai et al. 2010). Some bio pesticides have also been reported to be effective in the control of pests of eggplant (Tripathi et al. 2003). Injudicious use of pesticides not only poses health hazard to the farm workers, they leave harmful pesticide residues on the crop and soil. Numbers of monitoring studies carried out worldwide have reported the residues of pesticides in fruits and vegetables (Arora 2008; Azizi and Hadian 2008; Mukherjee 2003). Development of pest resistance to existing pesticides is a major problem faced by farmers. A new crop protection agent, flubendiamde was found to be effective against shoot borer of brinjal (Tohnishi et al. 2005). To combat this problem use of mixture of crop protection agents (pesticide mixtures) is a promising

option. In India, a number of ready mix formulations containing mixture of an organophosphate and a synthetic pyrethroid are registered for use on various crops (Regupathy et al. 2004). There are as many as 17 ready mix formulations registered in India. The ready mix formulations can be classified in two broad categories based on the risk of developing resistance, low risk and high risk insecticide mixtures. Each insecticide mixture should not have cross resistance to populations of pests. The mixtures should have significant synergism, and most importantly should be able to delay the resistance. These insecticides, separately or in combination mixture, have been found effective in controlling insect pests of vegetables (Gupta et al. 2007). However, very scarce information is available in literature on the dissipation behavior of individual component present in ready mix formulations in/on egg plant crop. Therefore, present investigation was undertaken to investigate the dissipation kinetics and residue behavior of individual components in the ready mix formulations of cypermethrin + profenofos and cypermethrin + chlorpyrifos in/on egg plant fruit and soil, following spray application.

Materials and Methods

All the solvents used were glass distilled at their boiling point. Adsorbents like silica gel (60–120 mesh) and florisil, sodium sulfate and sodium chloride were washed with distilled acetone and air-dried and activated at 350°C for 4 h in muffle furnace. All glass apparatus was washed with chromic acid, distilled water and rinsed with acetone before use. Analysis was carried out on gas chromatograph instrument Model Varian CP 3800 (GLC) equipped with electron capture detector (ECD) and CP-Sil 5 CB (25 m × 0.25 mm × 0.25 μm) column. Commercial ready mix formulation Nacraj-505 (M/S Konark India Ltd, Hyderabad, India) and Roket 44EC (M/S PI Industries Ltd, Gujarat, India) were purchased from local market. Analytical grade chlorpyrifos (purity 100%), and cypermethrin (purity 94%) were procured from AccStandards, whereas profenofos (purity 94.3%) was purchased from Sigma-Aldrich. Chemical structures of the active ingredients present in these formulations are given in Fig. 1. Stock solution of the individual pesticide (~1,000 mg/mL) was prepared by accurately weighing 10 mg of pesticide and dissolving in 10 mL of acetone. Stock solutions of chlorpyrifos and cypermethrin (1 mL each) were mixed in 10 mL capacity volumetric flask and filled with hexane up to the mark to get 100 mg/mL standard mixture. This mixture was serially diluted with hexane to get working standard of 1 mg/mL concentration for quantification. Similarly stock solutions of profenofos and cypermethrin were mixed and diluted to get standard mixture of concentration 1 mg/mL.

Experiment was conducted at the research fields of Division of Agronomy, Indian Agricultural Research Institute, New Delhi. Eggplant crop (variety Pusa Shymala) was raised in the 5 × 5 m^2 plots, following recommended agricultural practices. Five set of treatments, each replicated thrice with randomized block design (RBD) was laid. Crop was sprayed at 50% flowering followed by 2nd spray at 50% fruit formation stage with ready mix formulation of chlorpyrifos and cypermethrin (Nacraj-505, @ 800 and 1,600 mL/ha) and profenofos and cypermethrin (Roket 44EC @ 1 and 2 L/ha). Control plots were not sprayed with any insecticide. Eggplant fruit samples (200 g) were collected periodically after 2nd application from each treatment. Samples collected on 0 (2 h after spray), 1, 3, 5, 7, 10 and 15 days were processed on the same day. Soil samples (~1 kg, 0–15 cm soil depth) from each treatment were collected on 0, 3, 7 and 15 days after spray. Samples were dried under shade dried, crushed in pestle and mortar and sieved through 2 mm sieve. Soil (20 g), in duplicate, from each treatment was weighed and processed. Eggplant fruits were cut into small pieces and a representative 50 g sample (in duplicate) was taken after quartering. The samples were extracted in a Waring blender with 50 mL distilled acetone. The extract was filtered Whatman No. 1 filter paper using Buchner funnel. The solid residues were transferred into the jar and extracted two more times with acetone (2 × 30 mL). The acetone extracts were combined and concentrated using rotary evaporator. The concentrated extract was quantitatively transferred to separatory funnel, diluted with 150 mL 2% aqueous sodium chloride solution and partitioned thrice with dichloromethane (50 mL each). The dichloromethane phases were combined and evaporated completely for column clean up. Soil samples were mixed with 200 mg of 1:1 mixture of charcoal and florisil. This dry slurry was packed in the glass column in between two layers of sodium sulfate. The column was eluted with 100 mL of hexane: acetone mixture (8:2). The extract was concentrated under reduced pressure. No further clean up is required for soil samples. The eggplant fruit extract was cleaned by adsorption column chromatography using silica gel as adsorbents. Glass column (30 × 1.5 cm i.d.) was packed with silica gel (10 g) using hexane, in between two layers of anhydrous sodium sulfate (2 g). The column was washed with 50 mL hexane. The dichloromethane extract was concentrated to dryness, residues dissolved in 1–2 mL hexane and quantitatively transferred on to the column using small portions of hexane. The column was eluted with 100 mL mixture of hexane and acetone (8:2). The extract was again concentrated to near dryness under reduced pressure. Residues of chlorpyrifos, profenofos and cypermethrin were analysed by GLC fitted with electron

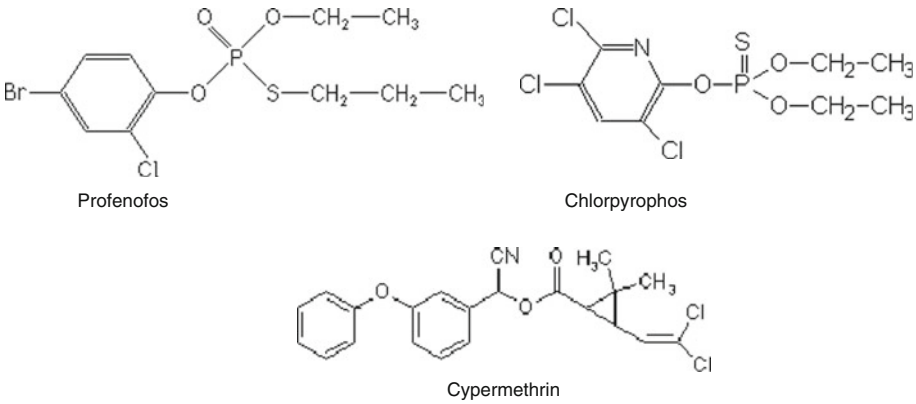

Fig. 1 Chemical structures of active ingredients present in ready mix formulations Roket 44EC (profenofos + cypermethrin) and Nacraj-505 (chlorpyrifos + cypermethrin)

capture detector. The operating conditions for simultaneous determination of chlorpyrifos and cypermethrin (active ingredient of combination mix Nacraj-505) were: temperatures: detector 300°C, injector 280°C and column programmed as 200°C for 3 min, increased @ 20°C/min to 270 and hold for 3 min. Total run time was 9.50 min with the retention time of chlorpyrifos at 3.13 min and cypermethrin at 7.64 min (Fig. 2). The GLC operating conditions for simultaneous determination of profenofos and cypermethrin (active ingredient of combination mix Roket 44EC) were standardized as: temperatures: detector 300°C, injector 280°C and column programmed as 170°C for 1 min, increased @ 10°C/min to 230 and hold for 3 min, increased @ 30°C/min to 270 and hold for 5 min. Under the set conditions, total run time was 15.33 min with the retention time of profenofos at 5.80 min and cypermethrin at 10.91 min (Fig. 2). Instrument detection limit for chlorpyrifos and profenofos was 0.001 mg/mL whereas for cypermethrin it was 0.005 mg/mL. The cleaned extracts of eggplant and soil were concentrated to dryness and re-constituted in 10 mL of hexane. The residue data was subjected to regression analysis and half-life values were calculated based on first order dissipation kinetics. Recovery studies were carried out by fortifying the untreated control samples of eggplant and soil with the pesticides mixture. Average recoveries of chlorpyrifos and cypermethrin from fortified eggplant fruits and soil at 0.5 mg/g level ranged from 89% to 97% and 84% to 93%, respectively, whereas for profenofos and cypermethrin combination mixture average recoveries were in the range of 82%–927% and 85%–89%, respectively from eggplant fruits and soil. Standard chromatograms of pesticide mixtures chlorpyrifos + cypermethrin and profenofos + cypermethrin, are presented in Fig. 2.

Results and Discussion

The residue data for Roket 44EC (profenofos + cypermethrin) and Nacraj-505 (chlorpyrifos + cypermethrin) on eggplant fruits are presented in Table 1. Regression equations and half-life values are presented in Table 2. Figure 3 shows the percent dissipation of different pesticides on egg plant fruits. Residue pattern in soil is presented in Table 3.

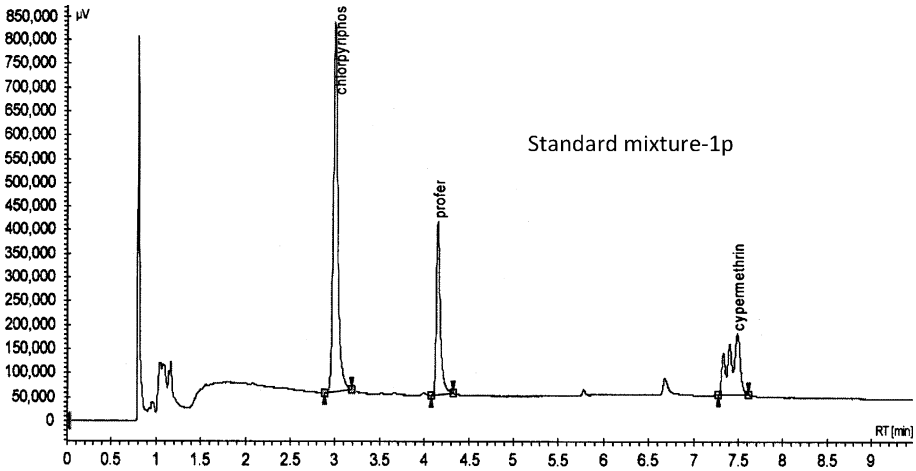

Fig. 2 Standard chromatograms of pesticide mixtures profenofos, chlorpyrifos and cypermethrin

Table 1 Residues of cypermethrin, profenofos and chlorpyrifos in/on eggplant fruits

Insecticide	Formulation	Treatment (mL/ha)	Average residues (mg kg^{-1})						
			0	1	3	5	7	10	15
Cypermethrin	Roket 44EC	1	0.52 ± 0.03	0.28 ± 0.01	0.18 ± 0.01	0.12 ± 0.02	0.057 ± 0.03	0.016 ± 0.02	ND
		2	1.13 ± 0.03	0.87 ± 0.02	0.48 ± 0.03	0.32 ± 0.04	0.21 ± 0.05	0.09 ± 0.06	0.04 ± 0.05
Profenofos		1	0.575 ± 0.02	0.250 ± 0.04	0.041 ± 0.03	0.014 ± 0.02	ND	ND	ND
		2	1.854 ± 0.03	0.554 ± 0.04	0.159 ± 0.05	0.05 ± 0.04	0.024 ± 0.04	ND	ND
Cypermethrin	Nacraj-505EC	800	0.051 ± 0.04	0.032 ± 0.05	0.015 ± 0.05	0.011 ± 0.04	0.005 ± 0.03	ND	ND
		1,600	0.064 ± 0.04	0.056 ± 0.04	0.042 ± 0.05	0.024 ± 0.02	0.015 ± 0.03	ND	ND
Chlorpyrifos		800	0.053 ± 0.05	0.044 ± 0.04	0.023 ± 0.03	0.015 ± 0.04	0.012 ± 0.04	0.005 ± 0.03	ND
		1,600	0.234 ± 0.03	0.168 ± 0.02	0.095 ± 0.02	0.065 ± 0.03	0.042 ± 0.03	0.024	0.007

Following application of ready mix formulation of profenofos and cypermethrin (Roket 44EC) at recommended and double the recommended dosages on egg plant crop, the initial deposits (2 h after application) of profenofos on egg plant fruits were 0.575 and 1.854 mg kg^{-1} (Table 1). The residues dissipated with time and by day 7, residues were below detectable limit at the recommended rate of application, while 0.024 mg kg^{-1} residues were detected at double the recommended dose, accounting to the loss of 98%–100% (Fig. 3). Dissipation of profenofos from eggplant fruits followed first order kinetics and registered half-life values of 0.91 and 1.86 days at low and high rate of application, respectively (Table 2). Soil samples analysis showed the initial deposits of 0.048 and 0.057 μg g^{-1} at low and high dose. Residues dissipated with time and no residues were detected in samples collected on day 7 and 15 at recommended and double dose, respectively (Table 3).The average initial deposits of cypermethrin ready mix formulation of profenofos and cypermethrin (Roket 44EC) were 0.52 and 1.13 mg kg^{-1}, respectively at recommended and double the recommended dosage (Table 1). Residues on 7-day samples were 0.057 and 0.21 mg kg^{-1}, amounting to the loss of 84%–92% (Fig. 3). At low dose, no residues were detected in 15th day sample, however at high dose residues of cypermethrin persisted beyond 15 days. Cypermethrin residues dissipated with the half-life of 2.19–3.27 days, following first order kinetics (Table 2). The residues of cypermethrin were below detectable limit in soil samples even on 0-day at both the spray concentrations (Table 3).

Application of ready mix formulation Nacraj-505, initial deposits of chlorpyrifos were 0.053 and 0.234 mg kg^{-1} on egg plant fruits at recommended and double the recommended rate of application (Table 1). Residues dissipated to 0.005 and 0.024 mg kg^{-1} on day 10 amounting to the loss of more than 99% (Fig. 4). Residues persisted beyond 10 and 15 days at low and high rate of application. Dissipation of chlorpyrifos residues from egg plant fruits followed first order kinetics with the half-life values of 3.27 and 3.106 days at low and high rate of application (Table 2). In soil, chlorpyrifos residues were below detectable limit even on 0-day at recommended dose of application (Table 3). However, at double the recommended dose average initial deposits were 0.015 mg kg^{-1}, which dissipated by day 3 to 66.6% and there after was non-detectable by 7 day (Table 3), Samples of eggplant and soil collected 2 h after pesticide application showed average initial deposit of cypermethrin as 0.051 and 0.064 mg kg^{-1} at the recommended and double the recommended dose. More than 90% dissipation was recorded on day-7 although residues persisted beyond 15 days at double the recommended dose. At lower dose residues persisted beyond 7 days. Half life of dissipation for cypermethrin calculated from first order

Table 2 Regression equation and half-life values of different insecticides on egg plant fruits

S. no.	Ready mix formulation	Formulation dose	Insecticides	Regression equation Y	R^2	$T_{1/2}$ (days)
1.	Roket 44EC	1 L/ha	Profenofos	$-0.0.328x + 0.281$	0.99	0.91
			Cypermethrin	$-0.139x + 2.11$	0.98	2.15
		2 L/ha	Profenofos	$-0.1615x + 2.254$	0.98	1.86
			Cypermethrin	$-0.137x + 1.695$	0.99	2.31
2.	Nacraj-505	800 mL/ha	Chlorpyrifos	$0.0923x + 0.699$	0.98	3.27
			Cypermethrin	$-0.1371x + 1.695$	0.99	2.19
		1,600 mL/ha	Chlorpyrifos	$-0.0979x + 1.319$	0.99	3.10
			Cypermethrin	$-0.0926x + 2.115$	0.97	3.27

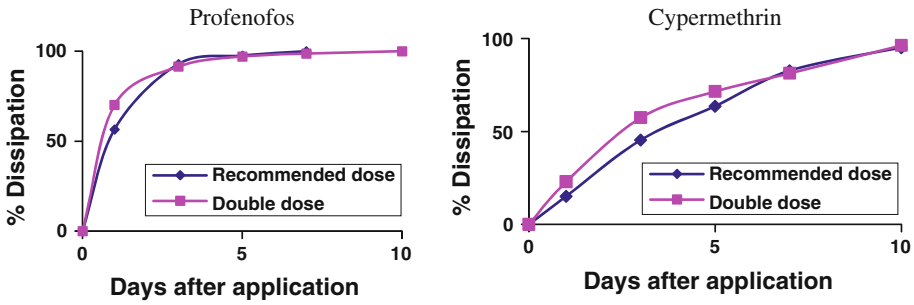

Fig. 3 Percent dissipation of profenofos and cypermethrin in ready mix formulation (Rocket 44)

Table 3 Residues of profenofos +cypermethrin and chlorpyrifos + cypermethrin in field soil

Formulation	Dose	Insecticide	Residues			
			Days after spray			
			0	3	7	15
Roket 44EC	1 L/ha	Profenofos	0.048	0.015 (68.7)[a]	ND (100)	–
		Cypermethrin	ND			
	2 L/ha	Profenofos	0.062	0.031 (50.0)	0.014 (77.4)	ND (100)
		Cypermethrin	ND	–	–	–
Nacraj-505	800 mL/ha	Chlorpyrifos	ND	–	–	–
		Cypermethrin	ND	–	–	–
	1,600 mL/ha	Chlorpyrifos	0.015	0.005 (66.6)	ND (100)	–
		Cypermethrin	0.036	0.015 (58.3)	ND (100)	–

[a] Figure in parenthesis show percent dissipation

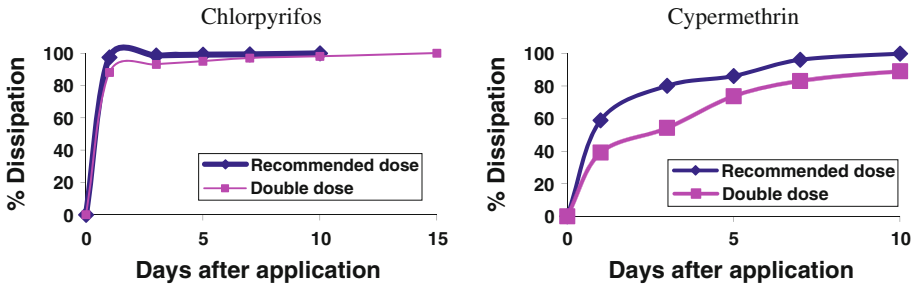

Fig. 4 Percent dissipation of chlorpyrifos and cypermethrin in ready mix formulation (Nacraj-505)

dissipation kinetics varied from 2.19 to 3.27 days. In soil, no residues of cypermethrin were detected even on 0-day at recommended dose. At double dose, initial deposits in soil were 0.036 mg kg^{-1}. On day-3, residues decreased to 0.015 mg kg^{-1} level, recording a dissipation of 58.3% and became non-detectable by 7 day.

At the recommended dose of application, the residues of cypermethrin and chlorpyrifos are below the Codex MRL value of 0.5 mg kg^{-1} even on 0-day for both the pesticides. Based on the results waiting period of 1-day is suggested for the combination mix of cypermethrin and chlorpyrifos on egg plant from consumer's safety point of view. The residues of profenofos on brinjal were below the Codex MRL of 0.05 mg kg^{-1} as documented by day-3; hence a safe waiting period of 3 days is suggested for the combination mix profeofos and cypermethrin.

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