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Residue Behavior of Combination Mix Formulations in/on Bittergourd and Their Efficacy Against Melon Fruitfly

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Abstract Persistence behavior of three combination mix formulations of insecticides viz. chlorpyriphos + cypermethrin (Action-505), profenofos + cypermethrin (Roket 44EC) and triazophos + deltamethrin (Anaconda) and their bioefficacy against melon fruitfly were studied in bittergourd fruits following spray application. Half-life values of the constituent insecticides calculated from first order dissipation kinetics were ~2–3 days. Based on acceptable daily intake values, safe waiting period of 3-days is suggested for all the three combination mix formulations at recommended dose of application. Anaconda (1 L/ha) was found to be most effective against Melon fruitfly as it gave 11.72 % (number/number) and 10.93 % (weight/weight) damage as compared to control 41.13 % and 41.16 %, respectively. Anaconda at lower and higher dose (1 and 2 L/ha) was not significantly different. Rocket (2 L/ha) and Action 505 (2 L/ha) were also found effective.

Keywords Residues · Bittergourd · Combination mix formulations · Bioefficacy · Melon fruitfly · Profenofos · Chlorpyrifos · Triazophos · Cypermethrin · Deltamethrin

Bittergourd (*Momordica charantia*) is one of the most popular vegetables grown in India. It is commonly known as bitter melon or *karela*. The fruits of bittergourd are rich in folate and vitamin C and are used in a variety of culinary preparations. The medicinal value of the gourd in the treatment of infectious diseases and diabetes is attracting the attention of scientists worldwide. The melon fruitfly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) is a serious pest of bittergourd and distributed widely in temperate, tropical and sub-tropical regions of the world (Palada and Chang 2003). This fruitfly is difficult to control because its maggots feed inside the fruits, protected from direct contact by insecticides. Synthetic pyrethroids like lambda-cyhalothrin, deltamethrin, fenvalerate and organophospates like malathion, dichlorvos, acephate and triazophos have been used effectively in controlling melon fruitfly in cucumber and bittergourd (Waseem et al. 2009; Oke 2008; Jha 2008; Bhatnagar and Yadava 1992; Ranganath et al. 1997; Reddy 1997). In a laboratory bio-assay study Dong et al. (2002) have reported that abamectin has the greatest potential for controlling the *B. cucurbitae*. Patnaik et al. (2004) reported that among different treatments acephate at 0.15 % significantly reduced the populations of *B. cucurbitae* and *Amrasca biguttula biguttula* and recorded the highest fruit yield (62.7 q/ha) and benefit: cost ratio (11.6:1). Considering that different group of insecticides have been found effective, it was felt that combination mixtures containing insecticides of different mode of action may prove more effective against fruitfly. Keeping in mind that the fruits of bittergourd are consumed afresh, a field trial was conducted for evaluation of three combination mix formulations for melon fruitfly management and their safety to consumers.

Materials and Methods

Commercial ready mix formulations viz. Action-505 EC (M/S Tropical Agrosystem India (P) Ltd, Chennai, India)

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containing 50 % chlorpyriphos and 5 % cypermethrin, Roket 44 EC (M/S PI Industries Ltd, Gujarat, India) containing 40 % profenofos and 4 % cypermethrin and Anaconda Plus (M/S Crop Chemicals India Ltd, Kot Kapura, India) containing 35 % triazophos and 1 % deltamethrin were purchased from local market. Chemical structures of the active ingredients present in these formulations are given in Fig. 1. Analytical grade chlorpyriphos (purity 100 %), deltamethrin (purity 98.5 %), and cypermethrin (purity 94 %) were procured from AccuStandards, whereas profenofos (purity 94.3 %) and triazophos (purity 70 %) were purchased from Sigma-Aldrich. Stock solutions of the individual pesticides ($\sim$1,000 μg mL^{-1}) were prepared by accurately weighing 10 mg of pesticide and dissolving in 10 mL of acetone. Stock solutions of chlorpyriphos, profenofos, deltamethrin and cypermethrin (1 mL each) were mixed in 10 mL capacity volumetric flask and volume was made up with *n*-hexane to get 100 μg mL^{-1} standard mixture. This mixture was further serially diluted with *n*-hexane to get the working standards of lower concentrations for quantification of pesticide residues in samples. Stock solution of triazophos was also diluted serially to get solutions of lower concentrations. All the solvents used in the study were glass distilled at their boiling point. Silica gel was activated at 110°C for 4 h. Sodium sulfate and sodium chloride were washed with distilled acetone and air-dried before heating them at 250°C for 4 h in the oven. All the glass wares were soaked in chromic acid solution and washed thoroughly with water. These were rinsed with acetone and air-dried before use.

Field experiment was conducted in a randomized block design with three replicates in the experimental plots of Indian Agricultural Research Institute. Bittergourd var. *Chaman* (F_1 *Hybrid*) was sown with a plant spacing of 50 cm

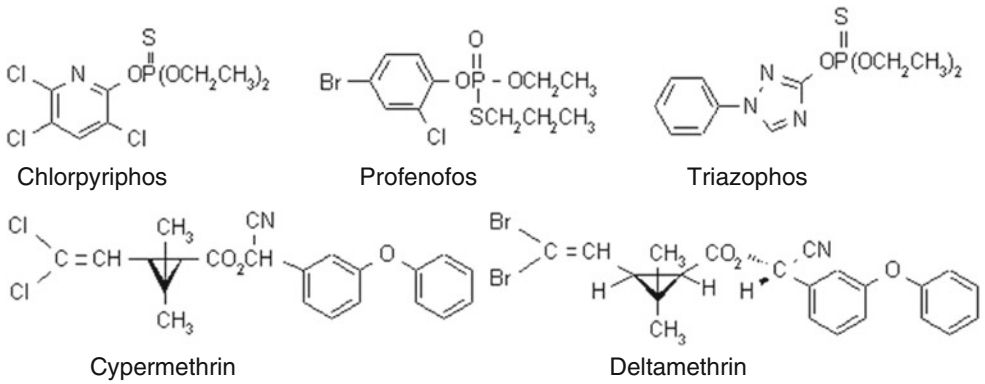

Fig. 1 Chemical structures of active ingredients present in ready mix formulations Action-505 (chlorpyriphos + cypermethrin), Roket 44EC (profenofos + cypermethrin) and Anaconda Plus (triazophos + deltamethrin)

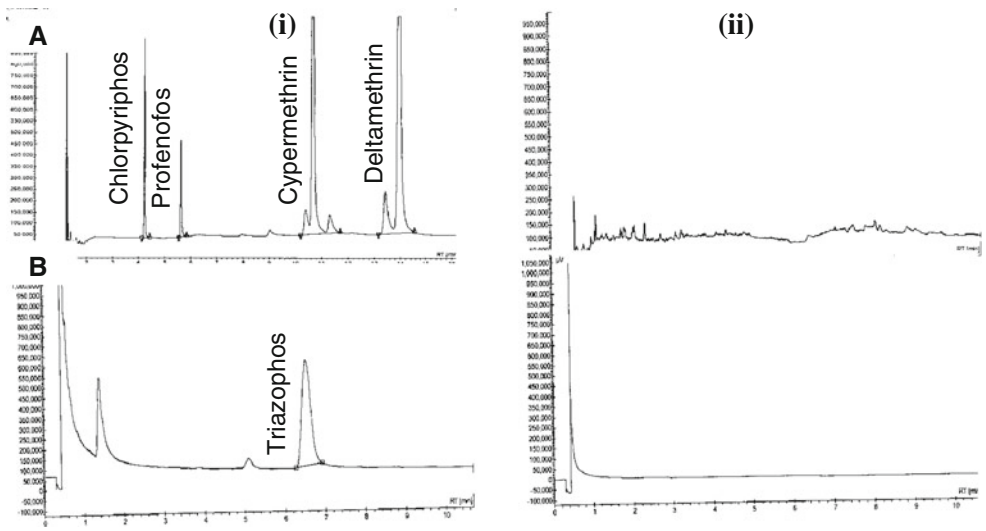

Fig. 2 **a** GLC-ECD chromatogram of (*i*) standard mixture of chlorpyriphos, profenofos, cypermethrin and deltamethrin (*ii*) Unspiked control **b** GLC-TSD chromatogram of (*i*) triazophos (*ii*) Unspiked control

and the distance between two ridges 200 cm. Each replicate had ten healthy plants. Six treatments viz., Action 505 (cypermethrin 5 % and chlorpyriphos 50 %) @ 800 and 1,600 mL/ha, Rocket (cypermethrin 4 % and profenofos 40 %) @ 1 and 2 L/ha, Anaconda (deltamethrin 1 % + triazophos 35 %) @ 1 and 2 L/ha with three replication were planned. The low and high spray doses corresponded to 400 and 800 g a.i./ha for profenofos and chlorpyriphos, 350 and 700 g a.i./ha for triazophos, 40 and 80 g a.i./ha for cypermethrin and 10 and 20 g a.i./ha for deltamethrin. Control plots with only water spray were maintained for comparison. Two foliar sprays using 500 L/ha water were given at fortnightly interval starting from flowering stage. All the treatments were evaluated against melon fruitfly, *Bactrocera cucurbitae* in bittergourd. Samples of fruits were collected on different days after 2nd foliar spray for residue analysis and for bioefficacy studies.

For bioefficacy evaluation fruits were collected at 5 days interval. The number and weight of healthy and damaged fruits were recorded and percent damage was calculated. Data was statistically analyzed after converting percent values into angular transformation.

For residue analysis fruit samples were collected on 0, 1, 3, 5, 7, 10 and 15 days. Fruits were picked up randomly from the replicated plot and pooled together for each treatment. The fruits were cut into small pieces and 50 g representative subsample, in triplicate, was drawn by quartering technique. The sample was transferred to blender jar with 50 mL acetone and macerated for 3 min. The contents were filtered through Buchner funnel using Whatman No. 1 filter paper. The solid residues were transferred back to jar and extraction was repeated two more times using 40 mL acetone each time. The extracts were pooled together and concentrated in a rotary evaporator at 50°C. The concentrated extract was transferred to separatory funnel, diluted with 250 mL 10 % aqueous NaCl solution and partitioned thrice with dichloromethane (3 × 30 mL). The organic phases were pooled and passed through anhydrous sodium sulfate for removing traces of moisture. The extract was cleaned by adsorption column chromatography using silica gel as adsorbent and 10 % acetone: hexane as eluting solvent. The cleaned extract was concentrated in rotary evaporator. Finally the residues were dissolved in 5 mL hexane and analysed by gas liquid chromatography (GLC). A Varian CP 3800 GLC equipped with electron capture detector (ECD) and CP-Sil 5 CB (25 m × 0.25 mm × 0.25 μm) column was used for analysis of chlorpyriphos, profenofos, cypermethrin and deltamethrin. The operating temperatures were detector 300°C, injector 280°C and oven programmed as 200°C for 1 min, increased @ 10°C/min to 230 and hold for 1 min, again increased @ 30°C/min to 270 and hold for 10 min. IOLAR I grade nitrogen was used as carrier gas at a flow rate of 2 mL/min. Under these conditions, total run time was 16.33 min with the retention

Table 1 Residues of insecticides in bittergourd fruits

Formulation	Constituent insecticide	Rate of application (g a.i./ha)	Residues (μg g^{-1})						
			0 day	1 day	3 days	5 days	7 days	10 days	15 days
Action 505	Cypermethrin	40	0.13 (0.0)[a]	0.079 (39.2)	0.043 (66.9)	0.032 (75.4)	0.020 (84.6)	ND (100)	
		80	0.207 (0.0)	0.135 (34.8)	0.105 (49.3)	0.055 (73.4)	0.038 (81.6)	ND (100)	
	Chlorpyriphos	400	0.372 (0.0)	0.209 (43.8)	0.140 (62.4)	0.074 (80.1)	0.038 (89.8)	0.014 (96.2)	0.009 (97.6)
		800	0.472 (0.0)	0.246 (47.9)	0.169 (64.2)	0.091 (80.7)	0.046 (90.3)	0.032 (93.2)	0.015 (96.8)
Rocket	Cypermethrin	40	0.114 (0.0)	0.098 (14.0)	0.072 (36.8)	0.034 (70.2)	0.015 (86.8)	0.003 (97.4)	ND (100)
		80	0.225 (0.0)	0.213 (5.3)	0.111 (50.7)	0.060 (73.3)	0.023 (89.8)	0.009 (96.0)	ND (100)
	Profenofos	400	0.26 (0.0)	0.159 (38.8)	0.097 (62.7)	0.052 (80.0)	0.027 (89.6)	0.010 (96.2)	0.006 (97.7)
		800	0.336 (0.0)	0.146 (56.5)	0.101 (69.9)	0.054 (83.9)	0.028 (91.7)	0.019 (94.3)	0.009 (97.3)
Anaconda	Deltamethrin	10	0.037 (0.0)	0.028 (24.3)	0.010 (73.0)	0.004 (89.2)	ND (100)		
		20	0.059 (0.0)	0.037 (37.3)	0.014 (76.3)	0.008 (86.4)	ND (100)		
	Triazophos	350	0.209 (0.0)	0.120 (42.6)	0.048 (77.0)	0.023 (89.0)	0.018 (91.4)	ND (100)	
		700	0.478 (0.0)	0.393 (17.8)	0.252 (47.3)	0.135 (71.8)	0.084 (82.4)	0.056 (88.3)	ND (100)

[a] Figure in parenthesis show percent dissipation

Table 2 Regression equation for first order dissipation of insecticides on bittergourd

Formulation	Constituent insecticide	Dose (g ai/ha)	Regression equation Y = a − bX*	Correlation coefficient	Half-life (days)
Action 505	Cypermethrin	40	Y = −0.9570 − 0.110X	0.967	2.8
		80	Y = −0.7140 − 0.102X	0.980	2.9
	Chlorpyriphos	400	Y = −0.5500 − 0.110X	0.958	2.7
		800	Y = −0.4870 − 0.097X	0.954	3.1
Rocket	Cypermethrin	40	Y = −0.8060 − 0.156X	0.955	1.9
		80	Y = −0.5610 − 0.146X	0.987	2.1
	Profenofos	400	Y = −0.6900 − 0.113X	0.964	2.7
		800	Y = −0.6860 − 0.099X	0.942	3.0
Anaconda	Deltamethrin	10	Y = −1.3980 − 0.198X	0.994	1.9
		20	Y = −1.2560 − 0.176X	0.984	2.1
	Triazophos	350	Y = −0.7610 − 0.155X	0.957	2.3
		700	Y = −0.3280 − 0.098X	0.984	3.2

*Y = Log C_t (Log Residues); a = Log C_0 (Log apparent initial concentration); b = dissipation constant; X = t (time)

Table 3 Maximum permissible intake and theoretical maximum daily intake values for insecticides on bittergourd

Formulation	Dose (L formulation/ha)	Constituent insecticide	Rate of application (g ai/ha)	ADI (mg/kg/day)	MPI (mg/person/day)	TMDI (mg/person/day)						
						0 day	1 day	3 days	5 days	7 days	10 days	15 days
Action 505	0.8	Cypermethrin	50	0.05	2.5	0.033	0.020	0.011	0.008	0.005		
		Chlorpyriphos	500	0.01	0.5	0.093	0.052	0.035	0.019	0.010	0.004	0.002
	1.6	Cypermethrin	100	0.05	2.5	0.052	0.034	0.026	0.014	0.010	0.000	0.000
		Chlorpyriphos	1,000	0.01	0.5	0.118	0.062	0.042	0.023	0.012	0.008	0.004
Rocket	1.0	Cypermethrin	40	0.05	2.5	0.029	0.025	0.018	0.009	0.004	0.001	
		Profenofos	400	0.01	0.5	0.065	0.040	0.024	0.013	0.007	0.003	0.002
	2.0	Cypermethrin	80	0.05	2.5	0.056	0.053	0.028	0.015	0.006	0.002	0.000
		Profenofos	800	0.01	0.5	0.084	0.037	0.025	0.014	0.007	0.005	0.002
Anaconda	1.0	Deltamethrin	10	0.01	0.5	0.009	0.007	0.003	0.001			
		Triazophos	350	0.001	0.05	0.052	0.030	0.012	0.006	0.005		
	2.0	Deltamethrin	20	0.01	0.5	0.015	0.009	0.004	0.002			
		Triazophos	700	0.001	0.05	0.120	0.098	0.063	0.034	0.021	0.014	

MPI = ADI × average body weight (50 kg); TMDI = Residues × average daily consumption of commodity (0.250 kg); residues safe when TMDI < MPI

time of 4.22, 5.61, 10.61 and 13.88 min for chlorpyriphos, profenofos, cypermethrin and deltamethrin, respectively (Fig. 2a(i)). Residues of triazophos were quantified separately in GLC equipped with thermoionic specific detector (TSD) and CP-Sil 5 CB (15 m × 0.53 mm) column. The operating temperature conditions were: detector 300°C, injector 280°C and column programmed as 200°C for 1 min, increased @ 3°C/min to 220, again increased @ 30°C/min to 250 and hold for 2 min. Carrier gas used was Nitrogen (IOLAR I grade) with the flow rate of 10 mL/min. The flame gases were hydrogen and air with the flow rate of 4.2 and 175 mL/min, respectively. Total run time was 10.67 min with the retention time of triazophos at 6.53 min (Fig. 2b(i)). Concentration versus detector response curve was plotted by injecting different concentrations of the standard mixture in the GLC. The response was found to be linear in the range of 0.005–1.0 μg mL^{-1} for chlorpyriphos, profenofos, cypermethrin and deltamethrin and 0.001–1.0 μg mL^{-1} for triazophos.

Instrument detection limit was 0.01 ng for chlorpyriphos, profenofos, cypermethrin and deltamethrin and 0.002 ng for triazophos. Recovery studies were carried out by fortifying the untreated control samples of bittergourd fruits with the pesticides mixture at 0.5 μg g^{-1} fortification level. The average recoveries from bittergourd fruits varied from 89 % to 96 % for chlorpyriphos, 88 %–92 % for profenofos, 86 %–90 % for cypermethrin, 89 %–94 % for deltamethrin and 90 %–93 % for triazophos. The method of analysis was found to be satisfactory as the recoveries of all the five pesticides were above 80 %. Unspiked control sample of bittergourd did not show any interfering peaks in the GLC-ECD (Fig. 2a(ii)) and GLC-TSD (Fig. 2b(ii)) chromatograms.

The residue data was subjected to first order kinetics (log $C_t = \log C_0 - Kt/2.303$), where C_t is concentration after a lapse of time (t); C_0 is apparent initial concentration, and K is the dissipation constant. The value of K was calculated by the formula, K = slope × 2.303, and the half-life was calculated from the value of K by the formula, $T_{1/2} = 0.693/K$.

Results and Discussion

The residue data for Action-505 (chlorpyriphos + cypermethrin), Roket 44 EC (profenofos + cypermethrin) and Anaconda plus (triazophos +deltamethrin) on bittergourd fruits are presented in Table 1. Regression equations for first order dissipation kinetics and half-life values are presented in Table 2.

Application of Action 505 on bittergourd crop resulted in initial deposits of 0.130 and 0.207 μg g^{-1} of cypermethrin and 0.372 and 0.472 μg g^{-1} of chlorpyriphos when applied at the dosages of 0.8 and 1.6 L/ha. Residues of cypermethrin persisted up to 7 days and chlorpyriphos up to 15 days. Similarly, application of Rocket at 1 and 2 L/ha resulted in initial deposits of 0.114 and 0.225 μg g^{-1} of cypermethrin and 0.260 and 0.336 μg g^{-1} of profenofos. Residues of cypermethrin persisted up to 10 days and chlorpyriphos up to 15 days. Combination premix Anaconda application resulted in initial deposits of 0.037 and 0.059 μg g^{-1} of deltamethrin and 0.209 and 0.478 μg g^{-1} of triazophos when applied at 1 and 2 L/ha. Residues of deltamethrin persisted up to 5 days and triazophos up to 7–10 days. The residues of all the insecticides dissipated with time and on 7th day 81 %–100 % dissipation was recorded (Table 1). The dissipation of residues followed first kinetics and the calculated half-life values varied from 1.9 to 3.2 days (Table 2).

The maximum residue level (MRL) for the insecticides pertaining to this study are not available for bittergourd. Since MRLs are not available, waiting periods were calculated based on dietary intake and acceptable daily intake (ADI) values (Table 3). The calculated dietary intake (TMDI) values for the constituent insecticides of Action 505 and Rocket i.e cypermethrin, chlorpyriphos and profenofos even on the day of spraying and also at double dose were less than maximum permissible level (MPI) meaning that the bittergourd fruits were safe for consumption even just after spray. However, for additional safety, a waiting period of 3 days is suggested. In case of Anaconda, residues of deltamethrin were safe on 0 day after spray, however, residues of triazophos became safe only 1 day after spray at recommended dose and 5 days in case of double dose. Therefore, a waiting period of 3 days is suggested for Anaconda on bittergourd at recommended doses. At higher spray dose, safe waiting period of 5-days must be observed.

Table 4 Impact of various insecticides on damage due to fruitfly, *Bactrocera cucurbitae* in bittergourd

Treatments	Dosage (L/ha)	Damage (%)	
		Number basis	Weight basis
Action 505	0.8	17.2 (24.51)*[c#]	16.1 (23.67)[c]
	1.6	12.5 (20.70)[b]	13.2 (21.31)[bc]
Rocket	1.0	12.4 (20.59)[b]	15.7 (23.35)[c]
	2.0	11.6 (19.79)[a]	11.6 (19.91)[b]
Anaconda	1.0	11.7 (19.99)[a]	10.9 (19.29)[ab]
	2.0	9.4 (17.78)[a]	8.6 (17.00)[a]
Control	–	41.1 (39.90)[e]	41.2 (39.91)[f]
SEM±		0.95	0.85
CD (0.05)		2.82	2.53
CD (0.01)		3.86	3.46

* Figures in parenthesis are angular transformed values

[#] Means followed by same alphabet do not differ significantly by DMRT ($P = 0.05$)

The infestation by *B. cucurbitae* in various treatments is summarized in Table 1. Infestation on number basis in various treatments varied from 9.4 % to 20.4 %, while it was 41.0 % in control. On weight basis, damage ranged from 8.6 % to 21.9 %, while it was 41.2 % in untreated check. Anaconda (1 L/ha) was found to be most effective as it gave least damage 11.7 % on number basis and 10.9 % on weight basis as compared to control 41.1 % and 41.2 %, respectively. Lower and higher doses (1 and 2 L/ha) of Anaconda were not significantly different. Rocket (2 L/ha) and Action 505 (2 L/ha) were also found effective (Table 4). The superior efficacy of the Anaconda might be due to the presence of deltamethrin and triazophos in it. Separately triazophos and deltamethrin have been used successfully for the management of fruitfly (Reddy, 1997; Oke 2008). Sood and Sharma (2004) have also reported deltamethrin (37.5 g a.i./ha) to be most effective in the field against *B. cucurbitae* on summer squash. Next best treatment was found to be Rocket containing profenofos and cypermethrin. Sood and Sharma (2004) also reported cypermethrin (75 g a.i./ha) as next best treatment after deltamethrin.

Even though, Anaconda premix formulation gave maximum efficacy against fruitfly, the waiting period was observed to be 3–5 days. The Action 505 and Rocket premix formulation were little less effective than Anaconda but from safety point of view were found to be better and therefore could be used for the control of fruit-fly in bittergourd.

References

Bhatnagar KN, Yadava SRS (1992) An insecticidal trial for reducing the damage of some cucurbitaceous fruits due to *Dacus cucubitae* Coq. Indian J Entomol 54:66–69

Dong YJ, Chang LL, Chen CC (2002) Laboratory bioassay of ten insecticides against the melon fruitfly (*Bactrocera cucurbitae*). J Agric Res China 51:66–72

Jha S (2008) Bio-ecology and management of fruitfly and Epilachna beetle in bittergourd. SAARC J Agric 6(1):1–15. http://www.saarcagri.net/documents/download_SJA/sja_v_6_i_1/BIO-ECOLOGY.pdf. Accessed 08 June 2012

Oke OA (2008) Effectiveness of two insecticides to control melon fruitfly (*Bactrocera Cucurbitae* Coq.) in cucumber (*Cucumis sativus* L). crop at Anse Boileau Seychelles. Eur J Sci Res 22(1): 84–86

Palada MC, Chang LC (2003) Suggested cultural practices for bittergourd. International cooperators' guide, AVRDC publications # 03-547, pp 1–5. http://www.scribd.com/doc/81022537/Bitter-Gourd. Accessed on 08 June 2012

Patnaik HP, Sarangi PK, Mohapatra P (2004) Studies on the incidence of fruit flies and jassids on summer bittergourd and their control. Orissa J Hort 32:87–90

Ranganath HR, Suryanarayana MA, Veenakumari K (1997) Management of melon fly (*Bactrocera* (Zeugodacus) *cucurbitae* Coquillett) in cucurbits in South Andaman. Insect Environ 3: 32–33

Reddy AV (1997) Evaluation of certain new insecticides against cucurbit fruitfly (*Dacus cucurbitae* Coq.) on bittergourd. Ann Agric Res 18:252–254

Sood N, Sharma DC (2004) Bioefficacy and persistent toxicity of different insecticides and neem derivatives against cucurbits fruitfly, *Bactrocera cucurbitae* Coq. on summer squash. Pestic Res J 16(2):22–25

Waseem MA, Nagangoud A, Patil BV, Prabhuraj A, Hussain A (2009) Efficacy of some insecticides against melon fly, *Bactrocera cucurbitae* coquillett on cucumber. Karnataka J Agric Sci 22(3):701–702