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Comparative efficacy, non-target toxicity and economics of seven novel pre-mixed formulations against *Maruca testulalis* G. and *Aphis craccivora* K. infesting cowpea

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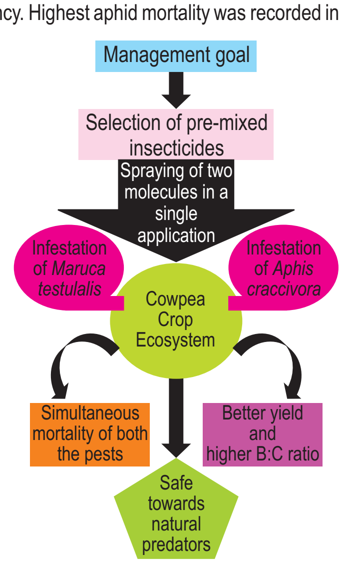
Abstract

Aim: Cowpea is one of the most important legume vegetables commercially cultivated crop in India and is severely prone to attack by both chewing and sucking insect pests. As the most effective tool of IPM, proper selection of ready-mix insecticides is required for eco-friendly and simultaneous management of these insect pests to reduce the cost of protection. The main objective of the study was to evaluate the relative field-effectiveness *vis.-a-vis.* non-target toxicity and economics of seven new generation pre-mixed formulations against spotted pod borer (*Maruca testulalis* G.) and bean aphid (*Aphis craccivora* K.) infesting cowpea.

Methodology: Two rounds of insecticides spraying were done at 40-50% flowering stage, followed by next at 15 days interval. The number of target pests was counted 24 hrs before first spray followed by 3, 7, 10 and 14 days after first and second spray, respectively. Data of pod infestation was recorded 10 days after each spray and at harvest. Percent reduction or increase (+) of *Chrysoperla sp.* and *Micraspis discolor* was also worked out at 15 days after each spray. Mean yield of green pods were recorded and converted into quintals per hectare.

Results: Chlorantraniliprole+thiamethoxam showed maximum impact (60.68%) than emamectin benzoate + fipronil (60.66%) and flubendiamide + thiacloprid (60.35%) considering the mean percent reduction of pod damage caused by pod borer, while later proved most superior among all the test combinations in percent reduction of *M. testulalis* larval population with higher persistency. Highest aphid mortality was recorded in pyriproxyfen + fenpropathrin (80.71% and 80.90%) and fipronil + imidacloprid (79.24% and 79.54%) treated plots after first and second spray, respectively, during both the years. Chlorantraniliprole + thiamethoxam and flubendiamide + thiacloprid proved least toxic to the prevailing predatory fauna *Micraspis discolor* (Fabricius) and *Chrysoperla* sp., with less than 10% mortality after 15 days of each insecticide imposition. Highest green pod and seed yield was also recorded from flubendiamide+thiacloprid (13.78 and 1.91 q ha^{-1}) treated plots and highest cost: benefit ratio (1: 6.8) was obtained from both the combinations of flubendiamide + thiacloprid and emamectin benzoate+fipronil.

Interpretation: The outcome of this study experiment will encourage the farming community in selecting new generation pre-mixed insecticides like flubendiamide 24%+thiacloprid 24% SC or chlorantraniliprole 10%+thiamethoxam 20% SC or emamectin benzoate 5%+fipronil 15% WDG, those might sustain toxicity against spotted pod borer and bean aphid for a longer period with least possibility of resistance development.



Introduction

Cowpea is a nutritionally important legume crop grown in semi arid and sub-humid tropics of Asia for both vegetables and pulses (Kumar and Kumar, 2015). High protein content of 23%, carbohydrate 67%, fat 1.3%, fibre 1.8% and moister 8-9% are responsible to enrich the nutritional value of this crop (Jefferson, 2009). Majority of people in many developing countries are involved with cowpea cultivation and production (Singh and Singh, 2015), but the productivity is still low than its potential mainly due to the attack of different insects.

Spotted pod borer, *Maruca testulalis* is the most formidable and potential pest of cowpea, causing significant damage to the crops by infesting reproductive parts and tender pods (Kanhere *et al.*, 2012). Scrapping followed by feeding on the seeds inside the pod by this pest, results in direct damage and a considerable yield loss up to 60% in cowpea (Pandey *et al.*, 1991). Moreover, the typical cryptic feeding habit of *M. testulalis* larvae protects themselves from natural enemies and other environmental factors (Singh and Singh, 2015). On the other hand, bean aphid, *Aphis craccivora* (Koch) is one of the most important sap feeders which is solely responsible to cause 20-40% yield loss in cowpea (Reddy *et al.*, 2014) and a major threat to cowpea growers throughout the country (Gauns *et al.*, 2014). Entire seedling to pod bearing stage is most vulnerable to aphid attack when both nymphs and adults suck cell sap and cause stunting, crinkling and curling of leaves, delayed flowering, shriveling of pods and ultimately reduction in yield. Pesticide mixture may be in the form of tank-mix or pre-mix formulation that entails exposing individuals in a pest population to each of the active ingredient simultaneously (Hoy, 1998). The mixed pesticide formulations decrease labour cost by reducing the rounds of applications (Blackshaw *et al.,* 1995), simultaneous by increase the mortality of different groups of arthropod pests having separate and distinct feeding habits (Warnock and Cloyd, 2005) and delaying resistance development against a particular pesticide by various pests (Bielza *et al.*, 2009). Resistance problem in aphids and borers with higher mortality of natural enemies due to indiscriminate use of non-selective synthetic molecules, opened the new modern era of using mixtures of new molecules having novel mode of action for sustainable management of these pests. Hence, in order to manage these pests in cowpea, various control strategies like physical, biological, regulatory and chemical are followed and experimented by many researchers (Sharah and Ali, 2008); however, management practices for poor and marginal farming communities (Singh and Singh, 2015). Thus, the pre-mix insecticides having different mode of action may serve as a ready-reckoner to the farmers, which can be applied on the hypotheses that they would complement the action of each other in simultaneous management of two target insect pests having different feeding habits. Keeping these views in backdrop, present study was undertaken to investigate the field bio-effectiveness *vis.-a-vis*. non-target toxicity and economics of some new generation pre-mixed formulations against spotted pod borer, *M. testulalis* and aphid, *A. craccivora* in cowpea.

Materials and Methods

Field bio-effectiveness was conducted on cowpea during June to September (*kharif* season) of 2014 and 2015 with a promising cultivar "*Kashi Kanchan* (VRCP-4)" at Horticulture Instructional Farm of Bidhan Chandra Krishi Viswavidyalaya, located at Mondouri, Nadia, West Bengal, India. The climate of the area is sub-humid tropical with average annual rainfall 1607.2 mm. Sowing was done in the third week of June in 5 × 4 m^2 plot size and 40 cm (row to row) × 20 cm (plant to plant) spacing followed by recommended horticultural practices during both the years (Ahlawat and Shivakumar, 2012). A battery operated sprayer (V-Dyut Delux, Code: VBD09/TPHB4/SS/ WOB, ASPEE Sprayers and Farm Mechanised Equipment, Mumbai, 440 064, India) fitted with hollow cone nozzle of 16 l capacity was used for foliar spray of insecticides at the recommended level of @ 600 l spray volume with neutral pH per hectare.

Experiments were set in a completely randomized block design with eight treatments *viz*. flubendiamide 24% + thiacloprid 24% SC @ 175 ml ha^{-1} (Tatagar *et al*., 2014), fipronil 40% + imidacloprid 40% WG @ 150 g ha^{-1} (Patil *et al*., 2009), indoxacarb 14.5% + acetamiprid 7.7% SC @ 400 ml ha^{-1} (Kamble *et al*., 2014), cartap hydrochloride 50% + buprofezin 10% SC @ 600 ml ha^{-1} (Sarkar and Roy, 2015), pyriproxyfen 5% + fenpropathrin 15% EC @ 600 ml ha^{-1}, chlorantraniliprole 10% + thiamethoxam 20% SC @ 180 ml ha^{-1} , emamectin benzoate 5% + fipronil 15% WDG @ 750 g ha^{-1} (Sarkar *et al*., 2015) and one untreated control and each treatment was replicated thrice (Selvaraj *et al*., 2015). Treatments were imposed coinciding ETL of the target pests, *M. testulalis* and *A. craccivora* (at 40-50% flowering stage of cowpea) followed by next at 15 days interval. The number of aphid nymphs and adults (counted visually with a 10x magnifying lens on a white paper sheet after removing from 15 cm shoot tip with the help of a camel hair brush) and pod borer larvae (counted visually from flowers, buds and pods separately and the average values were worked out) were recorded early in the morning (between 5.45 to 7.00 am) from ten randomly selected and tagged plants in each replication 24 hrs before first spray, followed by 3, 7, 10 and 14 days after first and second spray, respectively. The total number of pods and pods damaged by *M. testulalis* were recorded 10 days after each spray and at harvest. From these data, percent pod damage was calculated. Percent reduction or increase in each of the pest population was converted into per cent mortality by the formula of Henderson and Tilton (1955).

The percent reduction or increase (+) of important predators *Chrysoperla sp*. and *Micraspis discolor* prevailing in the crop agro ecosystem was also worked out based on their number at 15 cm shoot tip on ten randomly selected and tagged plants (Kumar and Kumar, 2015) at 15 days after each insecticidal spray. Mean yield of green pods were recorded from each of the treated plots and converted into q ha^{-1}. The cost benefit ratios were worked out according to Sarao and Kaur (2014) for evaluating the cost effectiveness of the treatments (Singh and Singh, 2015). Observed data on natural enemies' reduction were presented by bar graphs with standard errors using MS Excel sheet. Field-efficacy data were subjected to statistical analysis with one way ANOVA using SPSS (version 18.0: Inc., Chicago, IL, USA) software. Mean values were separated by Duncan's Multiple Range Test (Gomez and Gomez, 1984) at $p < 0.05$ for interpretation of the results.

Results and Discussion

Percent mortality of *M. testulalis* and *A. craccivora* against different treatment schedules during 2014 and 2015 is shown in Table 1, which revealed that all the pre-mixed formulations were significantly superior over untreated control in the reduction of larval population and nymphal and adult stages, respectively. After first round of spray application, emamectin benzoate + fipronil was found to be most effective combination (74.13%), followed by chlorantraniliprole + thiamethoxam (73.40%) and flubendiamide + thiacloprid (72.02%) respectively, in overall mean percent reduction of *M. testulalis* larval population over untreated control ($F_{7,14}$ = 10.25, P = 0.0012). Kaushik *et al*. (2016) reported that two rounds of spray at 50% flowering stage, followed by 25 days after first spray can be recommended to the farmers to manage *M. vitrata* in cowpea. Similarly, it is also clear that during the first season, lowest percent pod damage by *M. testulalis* was recorded in case of emamectin benzoate + fipronil which was statistically at par with chlorantra-niliprole + thiamethoxam and pyriproxyfen + fenpropathrin 10 days after first spray ($F_{7,14}$ = 14.34, P = 0.001), whereas at 10th day after second spray, percent pod damage varied between 9.59-12.18% in different treatments found statistically at par with each other (Table 2). Patel *et al*. (2012) acknowledged that emamectin benzoate was highly effective over indoxacarb and spinosad in managing *M. testulalis* in cowpea. Effectiveness of emamectin benzoate against lepidopteran borer in vegetables has also been confirmed by Anil and Sharma (2010). At the same time, highest mean percent reduction of all the stages of aphid population was registered by pyriproxyfen + fenpropathrin (80.71%), followed by fipronil + imidacloprid (79.24%); whereas cartap hydrochloride + buprofezin, flubendiamide + thiacloprid and chlorantraniliprole + thiamethoxam was statistically at par in the mitigation of aphid after first spray ($F_{7,14}$ = 9.68, P = 0.001). Neonicotinoid insecticides proved much promising against cowpea aphid under field, as well as laboratory condition (Abd-Ella, 2014) which corroborates the

Table 1 : Relative efficacy of different pre-mix formulations against spotted pod borer *Maruca testulalis* and bean aphid *Aphis craccivora* infesting cowpea during kharif season of 2014 and 2015

Treatments	Dose (g or ml ha^{-1})	PTC (per 10 plants)	Mean % reduction/increase (+) of larval population of *M. testulalis* after 1st spray (pooled data of 2014 and 2015)	Mean % reduction/increase (+) of larval population of *M. testulalis* after 2nd spray(pooled data of 2014 and 2015)	Mean % reduction/increase (+) of nymphs and adults of *A. craccivora* after 1st spray(pooled data of 2014 and 2015)	Mean % reduction/increase (+) of nymphs and adults of *A. craccivora* after 2nd spray(pooled data of 2014 and 2015)
Flubendiamide 24%+ Thiacloprid 24% SC	175	11.50	72.02abc	79.99a	73.55c	77.30bc
Fipronil 40%+ Imidacloprid 40% WG	150	11.75	64.24e	66.78cd	79.24ab	79.54ab
Indoxacarb 14.5%+ Acetamiprid 7.7% SC	400	12.25	70.57bc	69.25b	71.81d	70.02cde
Cartap hydrochloride 50%+ Buprofezin 10% SC	600	13.00	68.38cd	68.67bc	74.66c	74.37c
Pyriproxyfen 5%+ Fenpropathrin 15% EC	600	11.25	68.34d	68.76bc	80.71a	80.90ab
Chlorantraniliprole 10%+ Thiamethoxam 20% SC	180	12.75	73.40ab	77.91ab	72.74cd	72.43cd
Emamectin benzoate 5%+ Fipronil 15% WDG	750	12.00	74.13a	75.65ab	70.29d	71.96cd
Untreated control	-	12.50	+ 39.17	+ 44.21	+ 21.83	+ 29.19
LSD (0.05)	NS	3.17	2.96	2.61	2.27	
SEM±	NS	1.34	1.40	1.03	0.89	

PTC = Pre Treatment Count; Mean values followed by different letters are significantly different (otherwise statistically at par) at p<0.05 by Duncan's Multiple Range Test

Table 2 : Relative effectiveness of different pre-mix formulations against percent pod damage of cowpea by spotted pod borer *Maruca testulalis* during kharif season of 2014 and 2015

Treatments	Dose (g or ml ha^{-1})	Percent pod damage by *M. testulalis* during 2014			Mean % pod damage during season I	Percent pod damage by *M. testulalis* during 2015			Mean % pod damage during season II	Overall mean % pod damage (pooled)	Overall mean % reduction of pod damage over control
		10 days after1st spray	10 days after2nd spray	At harvest		10 days after1st spray	10 days after2nd spray	At harvest			
Flubendiamide 24%+ Thiacloprid 24% SC	175	13.79bcde	10.83b	8.51bc	11.04	12.24de	8.71b	6.52c	9.16	10.10	60.35
Fipronil 40%+ Imidacloprid 40% WG	150	15.34b	12.18b	9.58b	12.37	14.38bcd	11.91b	9.97b	12.09	12.23	51.98
Indoxacarb 14.5%+ Acetamiprid 7.7% SC	400	14.29bc	11.92b	9.59b	11.93	14.71bc	11.88b	10.01b	12.20	12.07	52.61
Cartap hydrochloride 50%+ Buprofezin 10% SC	600	13.99bcd	11.49b	9.57b	11.68	15.23b	12.10b	9.95b	12.43	12.06	52.65
Pyriproxyfen 5%+ Fenpropathrin 15% EC	600	12.46cde	10.29b	9.22bc	10.66	12.76cde	10.31b	7.80bc	10.29	10.48	58.85
Chlorantraniliprole 10%+ Thiamethoxam 20% SC	180	12.13de	9.59b	8.12bc	9.95	11.85e	10.26b	7.82bc	9.98	9.97	60.68
Emamectin benzoate 5%+ Fipronil 15% WDG	750	11.86e	10.05b	7.74c	9.88	12.99bcde	10.22b	7.23c	10.15	10.02	60.66
Untreated control	-	27.87a	27.13a	21.77a	25.59	29.03a	25.20a	21.83a	25.35	25.47	-
LSD (0.05)	3.38	6.16	5.14	7.25	4.64	10.19	3.26	7.09	NS	NS	
SEM±	0.90	1.45	1.48	2.19	1.48	2.57	0.87	2.50	NS	NS	

Mean values followed by different letters are significantly different (otherwise statistically at par) at $p<0.05$ by Duncan's Multiple Range Test

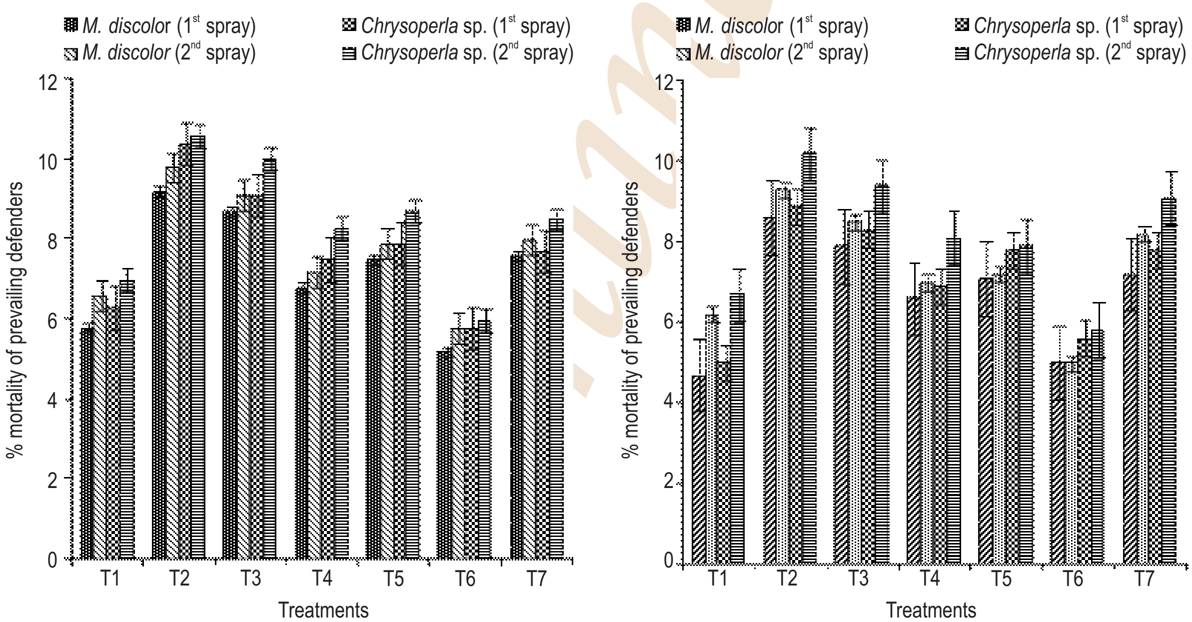


Fig. 1 : Percent mortality of prevailing natural enemies of cowpea crop-ecosystem after first and second spray of different pre-mix insecticides against spotted pod borer and aphid during kharif season of 2014 (A) and 2015 (B). (Vertical column followed by vertical bars are standard error of the mean)

Table 3: Yield of cowpea and economics of different pre-mix insecticides against spotted pod borer *Maruca testulalis* (Geyer) and bean aphid *Aphis craccivora* (Koch) during kharif season of 2014 and 2015

Treatments	Dose(g or ml ha^{-1})	Mean yield of cowpea during 2014 and 2015 (q ha^{-1})		Price of chemicals (Rs ha^{-1})	Labour cost (Rs ha^{-1})	Total cost of treatment (Rs ha^{-1})	Gross return (Rs ha^{-1})	Net return (Rs ha^{-1})	Net profit over control (Rs ha^{-1})	C:B ratio
		Green pod	Seed							
Flubendiamide 24%+ Thiacloprid 24% SC	175	13.78	1.91	2200.00	1176.00	3526.00	37463.4	33937.4	24059.8	1: 6.8
Fipronil 40%+Imidacloprid 40% WG	150	11.59	1.49	1760.00	1176.00	3086.00	30905.7	27819.7	17942.1	1: 5.8
Indoxacarb 14.5%+ Acetamiprid 7.7% SC	400	10.16	1.10	1825.00	1176.00	3151.00	26023.5	22872.5	12994.9	1: 4.1
Cartap hydrochloride 50%+ Buprofezin 10% SC	600	9.57	0.98	1650.00	1176.00	2976.00	24221.3	21245.3	11367.7	1: 3.8
Pyriproxyfen 5%+ Fenpropathrin 15% EC	600	10.32	1.18	1240.00	1176.00	2566.00	26758.3	24192.3	14314.7	1: 5.6
Chlorantraniliprole 10%+ Thiamethoxam 20% SC	180	12.62	1.72	1980.00	1176.00	3306.00	34158.2	30852.2	20974.6	1: 6.3
Emamectin benzoate 5%+ Fipronil 15% WDG	750	13.25	1.84	2020.00	1176.00	3346.00	36040.4	32694.4	22816.8	1: 6.8
Untreated control	-	4.15	0.56	0.00	1176.00	1326.00	11203.6	9877.6	-	-
LSD (0.05)	1.59	0.83	-	-	-	-	-	-	-	
SEM±	0.32	0.20	-	-	-	-	-	-	-	

Labour charges (skilled): Rs. 196.00 per day as per govt. of W.B. Labour Commission Circular, 2013; Sprayer charges Rs. 50.00 per day.
Price of cowpea pod Rs. 2000.00 per quintal and cowpea seed Rs. 5185.00 per quintal (http://agmarket.nic.in, All India level price range (Rs $quintal^{-1}$) accessed on 11.02.2016)

findings of the present study. Swarnalata *et al*. (2015) proclaimed that imidacloprid 0.005% was significantly effective treatment over thiamethoxam, fipronil and cartap hydrochloride against cowpea aphid.

Almost similar trend of effectiveness of different pre-mixed formulations against spotted pod borer larvae and bean aphid nymphs and adults was encountered after second round of spray (Table 1), where flubendiamide + thiacloprid (79.99%) and pyriproxyfen + fenpropathrin (80.90%) provided highest mean percent reduction of the target pests. Lowest mean percent pod damage was also recorded in flubendiamide + thiacloprid treated plots followed by chlorantraniliprole + thiamethoxam, emamectin benzoate + fipronil and pyriproxyfen + fenpropathrin ($F_{7,14}$ = 20.27, P = 0.0001) during 2015 (Table 2). These findings carborate the previous findings of Anusha *et al.* (2014) who reported that flubendiamide provided highest protection against pod borers of cowpea and was superior over emamectin benzoate and profenofos. Similarly, Dey *et al*. (2012) also conceded the efficacy of flubendiamide at 36 and 48 g a.i. ha^{-1} in enhancing yield against the attack of *M. testulalis* in pigeonpea. Moreover, chlorantraniliprole + thiamethoxam and emamectin benzoate + fipronil was statistically at par in the mean percent reduction of larval population of *M. testulalis* ($F_{7,14}$ = 12.32, P = 0.0001), whereas fipronil + imidacloprid followed by flubendiamide + thiacloprid also proved to be significantly more effective than other combinations in the mean percent reduction of all the stages of *A. craccivora* ($F_{7,14}$ = 17.48, P = 0.0002). Superiority of pre-mixed formulation of flubendiamide + thiacloprid over emamectin benzoate and indoxacarb against *M. testulalis* has been reported by Shivaraju *et al.* (2011). Findings of the present study are in conformity with the findings of Swami *et al.* (2010) who substantiated that flubendiamide registered significantly less incidence of *M. testulalis* in black gram. Mahalakshmi *et al*. (2016) also reported that integration of bio-pesticides with chemical compounds is an effective tool in the management of spotted pod borer on pulse crops. Richardson and Kutz (2007) acknowledged that juvenile hormone analogue pyriproxyfen, was highly effective in managing soybean aphid. Reddy *et al*. (2014) also conceded the efficacy of different insecticides in controlling the incidence of cowpea aphid.

The non-target toxicity of different treatments were recorded on prevailing natural enemies in cowpea eco-system has been depicted on Fig. 1. During first season, fipronil + imidacloprid showed highest percent reduction of *M. discolor* after 15^{th} day of first (SEm ± 0.25) and second (SEm ± 1.08) imposition. Chlorantraniliprole + thiamethoxam followed by flubendiamide + thiacloprid showed mild effect on all the test combinations in the reduction of *M. discolor* after first and second spray. All the test combinations exhibited less than 11% mortality of *Chrysoperla* sp. during 2014. Similar trends were also observed during the second study period 2015. Pyriproxyfen + fenpropathrin and emamectin benzoate + fipronil proved quite similar in the safety evaluation against both the predatory fauna and found statistically at par. But, fipronil + imidacloprid exhibited 8.6% and 8.9% reduction of *M. discolor* after 15 days of first (SEm ± 1.82) and second (SEm ± 0.90) spray, whereas 9.3% and 10.2% reduction of *Chrysoperla* sp. after 15 days of first (SEm ± 0.39) and second (SEm ± 1.35) spray respectively. Pre-evaluated results of higher toxicity of fipronil against *Chrysoperla* (Medina *et al.*, 2004) and imidacloprid against aphid feeding coccinellid predators (Andreev and Atanasova, 2005) fully support the present findings. Moreover, eco-toxicological evaluation of clorantraniliprole against *Chrysoperla* (Hussain *et al.*, 2012), thiamethoxam against coccinellid predators (Dhaka *et al.*, 2009) and flubendiamide against *Chrysoperla* and Coccinellids (Dey *et al.*, 2012) are in parity with the present findings, where all these chemicals had mild effect on these aphid feeding predatory fauna.

Mean green pod and seed yield of cowpea was higher in plots treated with different insecticidal combinations than untreated control (Table 3). The highest green pod ($F_{7,14}$ = 17.72, P = 0.0003), as well as seed yield ($F_{7,14}$ = 13.91, P = 0.0001) was obtained in the treatment of flubendiamide + thiacloprid followed by emamectin benzoate + fipronil and chlorantraniliprole + thiamethoxam with 13.78, 1.91; 13.25, 1.84 and 12.62, 1.72 q ha^{-1} respectively. Highest benefit: cost ratio was obtained from flubendiamide + thiacloprid and emamectin benzoate + fipronil, which was superior over chlorantraniliprole + thiamethoxam, fipronil + imidacloprid and pyriproxyfen + fenpropathrin. Cartap hydrochloride + buprofezin showed lowest economic yield with least benefit: cost ratio among all the pre-mixed formulations.

Based on the findings of present investigation, it can be concluded that new generation pre-mixed molecules like flubendiamide 24% + thiacloprid 24% SC @ 175 ml ha^{-1} or chlorantraniliprole 10% + thiamethoxam 20% SC @ 180 ml ha^{-1} or emamectin benzoate 5% + fipronil 15% WDG @ 750 g ha^{-1} can be an effective combination for sustainable management of spotted pod borer and bean aphid infesting cowpea from entomological, eco-toxicological and economic point of view, which will enhance the choice of farming community in selecting chemicals from different groups in near future.

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