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Effects of Nitrogenous Fertilization and some Insecticides on the Natural Enemies of Transgenic Bt Cotton Pests

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Abstract. Field assessment of integrated management of BT cotton pests was carried out in two consecutive seasons; 2016/2017 and 2017/2018 in the experimental research farm of the Kassala University, Sudan. Four insecticides (imidacloprid, abamectin, profenofos, and lufenuron) from different chemical groups and with a different mode of action and three levels (43.81 kgha^{-1}, 87.62 kgha^{-1}, and 131.43 kgha^{-1}) of soil-applied nitrogen were tested in the IPM package. Insecticides were applied at the recommended doses. A total of 7 weekly counts of prevailing natural enemies [*Chrysoperla carnea* (Stephens) and *Aenasius bambawalei* Hayat] were carried out for 4 weeks before spraying and 3 weeks post spraying starting the 3rd week from spraying. Results indicated that nitrogen level exerted no significant effects on the populations of prevailing natural enemies; *C. carnea* and *A. bambawalei.* Based on average post spray counts and percentage reduction over the control, all insecticide treatments significantly reduced the population of the natural enemies. Imidacloprid and profenofos exerted the highest reduction of *C. carnea* population followed by abamectin and lufenuron (56.03, 48.25, 07.78 and 06.61% respectively) in the first season, while profenofos, imidacloprid, lufenuron, and abamectin induced the highest reductions (55.94, 45.05, 17.57 and 17.33% respectively) in the second season. On the other hand, reductions in *A. bambawalei* population (abundant in season 1 only) followed the order; imidacloprid, lufenuron, abmectin, and profenofos (03.95, 02.63, 202.63, and 01.32%, respectively). Interactions between nitrogen fertilization and insecticide were not significant as measured by the population of prevailing natural enemies.

Keywords: Bt Cotton; Natural Enemies; Nitrogen; Insecticides Sudan.

Cotton (*Gossypium hirsutum* L) is considered the leading fiber crop, grown in more than 80 countries (Giri *et al.* 2016). More than 1,300 species of insects were reported as pests of cotton worldwide; among them are bollworms and sap-sucking pests (Matthews & Tunstall 1994). Bt toxins produced by transgenic cotton can effectively control lepidopterous species but fail to control sap-sucking pests (Hofs *et al.* 2004; Sharma & Pampapthy 2006). While there is a decrease in the status of bollworms; the sap feeders, *viz*. aphids, jassids, mirids, and mealybugs are currently emerged as serious pests in Bt cotton (Vennila 2008). The fact that modified insect-resistant Bt cotton received far fewer doses of insecticides may consequently favour the increase in the population of sucking insect pests (Men *et al.* 2005).

Further higher densities of Bt cotton responds to higher doses of nitrogen by producing significantly higher yields (Bharathi *et al.* 2012) but they provide congenial substratum for the growth and development of sucking pests throughout the growing period (Anusha *et al.* 2017). Further such higher nitrogen doses were also reported to reduce the plant resistance and consequently increase the occurrence of serious insect herbivores such as leafhoppers, whiteflies, and thrips (Ahmed *et al.* 2007) and subsequently increase the crop damage (Ge *et al.* 2003).

The combination of nitrogen fertilization with insecticides treatments were found to decrease the population of sucking pests and increase the yield of Bt cotton (Fadlelmawla *et al.* 2020), but they could destroy the beneficial natural enemies and non-target arthropods (Naranjo 2001; Fernandes *et al.* 2010) in most cropping systems and subsequently, cause pest resurgence or outbreak of secondary pests. The indiscriminate use of insecticides may promote resistance to the resurgence of sap-sucking pests (Rohini *et al.* 2012) and urged the search for safe alternatives to synthetic chemicals (Munoza *et al.* 2013).

Natural enemies of insects play an important role in regulating agricultural pests under normal conditions. Therefore, utilization of natural enemies in biological control (through importation, augmentation, or conservation) comprises an important component of many integrated pest management programs. Among effective natural enemies used in IPM programs is *Chrysoperla carnea* (Stephens) (Chrysopidae, Neuroptera) and *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae). *C. carnea* is a predator on many soft-bodied insects (Principi & Canard 1984; Chakraborty & Korat 2010). Scopes (1969) reported that the *C. carnea* possesses a wide range of hosts such as whiteflies, thrips, mealy bugs, aphids, besides the eggs of different arthropods. It has significant potential for commercialization and uses against a variety of crop pests in combination with other strategies of insect pest management (Chakraborty & Korat 2010).

In laboratory experiments, Mota *et al.* (2012) found that the biology and development of *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) larvae fed on *Aphis gossypii* Glover (Hemiptera: Aphididae) reared on leaves of Bt cotton were not affected by Cry1Ac toxin, possibly because these aphids

do not tend to accumulate the Bt-toxin.

Aenasius bambawalei has been identified and reported as a solitary endoparasitoid on *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). *A. bambawalei* was found to parasitize cotton mealy bug in Pakistan (Mahmood 2008) and in India (Tanwar *et al.* 2008). It is the only dominant and aggressive parasitoid reported so far, responsible for the decline of mealy bug after its initial establishment (Rishi *et al.* 2009). Adult females parasitize the third instar nymphs of *P. solenopsis* (Ashfaq *et al.* 2010) and kill the host before maturity. The parasitization resulted in reddish-brown mealy bug mummies easily distinguishable from the healthy colony (Prasad *et al.* 2011). The parasitization efficiency of the parasitoid from field-collected mealy bugs was 57.2 % whereas, under laboratory conditions, it was 60.6 % (Rishi *et al.* 2009) and up to 80% under favorable conditions (Vennila 2010).

The high economic losses of Bt cotton caused by sap-sucking pests and the expected negative environmental impacts of the chemical control initiated our interest to develop an a simple IPM package for the management of these pests. The specific objectives of this study were to evaluate the negative impact of the designed IPM package on the natural abundance of the prevailing natural enemies (*C. carnea* and *A. bambawalei*). The designed package included three levels of nitrogenous fertilization and four insecticides (imidacloprid, abamectin, profenofos, and lufenuron) from different chemical groups. The efficacy of the IPM package on the abundance of sap-sucking insects, cottonseed yield, and net profit was covered in another study (Fadlelmawla *et al.* 2020).

MATERIAL AND METHODS

Location, treatments, and experimental design. The current study was carried out for two consecutive seasons; 2016/2017 and 2017/2018 in the experimental research farm (15.3261, 35.5895) of the Faculty of Agriculture and Natural Resources of Kassala University, Sudan. Split-plot design was adopted with three doses of nitrogen (as urea) as the main factor with five insecticide treatments (including the control) as sub-factors (sub-plots). Each treatment was replicated three times. The plot area was 4x5 m^2 each divided into six ridges. Treatments were randomly distributed at the two levels.

Sowing and cultural practices. The source of cotton seeds (Sini 1 cultivar) was Halfa Algadidah Agric. Corporation. Seeds were sown on August 5th in both seasons (2016/2017 and 2017/2018). Cultural practices followed the recommendation of Halfa Algadidah Agric. Corporation except for the nitrogen fertilization, which was done at three levels (as the main factor), before the 3rd irrigation (one month from sowing). Insecticides were sprayed on November 5th (three months after sowing).

Nitrogen fertilization (factor I). Urea (46% N_2) was evenly distributed in all plots and immediately followed by irrigation. Three levels [N1(43.81), N2(87.62), and N3(131.43) kg/ha] of urea were used.

Insecticide's treatments (factor II). Four types of insecticides from different chemical groups (imidacloprid, abamectin, profenofos, and lufenuron) were sprayed by pneumatic knapsack sprayer, at spray volume of 100 L per 0.42 ha. The source of the applied insecticides is the local agents in Sudan. Dosage and application rates were shown in Table1.

Counts of natural enemies. All counts of natural enemies were recorded weekly for seven weeks. Counts were done at 6 am and continued for few minutes after sunrise. Counts were taken from plants in the inner four ridges. Four pre-spray counts (from the 11th of October to the 1st of November) and three post-spray counts (from the 8th to the 22nd of November) were carried out in the two seasons.

Eggs of *C. Carnea* per 100 leaves were counted in each plot. The mealy bug (*A. bambawalei*) infestation was estimated by the number of mealy bug mummies per 10 randomly selected plants in each plot.

Data, transformation, and analysis. The counts of natural enemies (*C. carnea* and *A. bambawalei*) were transformed to $(X+0.5)^{1/2}$ before statistical analysis. Transformed data were analyzed by the Analysis of variance (ANOVA) and means were separated by Tukey's honestly significant difference test.

RESULTS AND DISCUSSION

Effect of nitrogenous fertilization on the natural enemies. The aim of the study is to assess the impact of the designed IPM package on the natural enemies (C. *carnea* and *A. bambawalei*) abundant in the experimental plots during the two seasons. The abundance of the C. *carnea* was assessed by the number of eggs while that of *A. bambawalei* was assessed by the number of infested mealy bug mummies. *C. carnea* eggs were found throughout the various weeks in both seasons while the parasitoid *A. bambawalei* was found in season one only. The absence of the latter in season two might be explained by the unavailability of the host (mealy bug) in the experimental plots although was found parasitizing mealy bug in adjacent fallow land weeds (specially *Abutilon*) but never spread to the cotton in that season due to the lack of suitable transport mechanisms (animals, humans etc.) during that season. The occurrence of the two natural enemies, *C. carnea* eggs and *A. bambawalei*, was not significantly affected by the applied nitrogen fertilizer and there is no direct association between the level of the nitrogen and the population of the two natural enemies (p=0.05; Table 2; Figure 1). However, there is a slight dose-related increase in the counts of *C. carnea* eggs observable during the 2nd and 3rd week while the increase in the counts of infested mealy bug mummies was noticeable during the 3rd and 4th week (Table 2; Figure1). This may be elucidated by the fact that natural enemies do not feed directly on plants, however; they might indirectly be influenced by nitrogen fertilization through insect host (Couture *et al.* 2010; Garratt *et al.* 2010). Nitrogen is one of the most frequently used fertilizers in agricultural production systems. Its application potentially exerts variety of bottom-up effects, which can significantly alter tritrophic interactions through different mechanisms (Chen *et al.* 2010).

Table 1. The insecticides used in the study.

Insecticide		Group	Dose (g ai/ha)	Application rate (L/ha)
Common name	Trade name			
Lufenuron	Match 5%EC	Chitin synthesis inhibitor (IGR)	47.50	0.950
Profenofos	Selecron 720EC	Organophosphate	720.00	1.000
Abamectin	Vertemic 18EC	Avermectin (Microbial)	09.72	0.540
Imidacloprid	Confidor 200EC	Neonicotinoid	26.00	0.130

Table 2. Effects of the nitrogenous fertilization on the natural abundance of *Chrysoperla carnea* eggs

Seasons	Treatment	Average of pre-spray counts				Average of post-spray counts		
		Week 1	week 2	week 3	week 4	week 5	week 6	week 7
I	N1	1.47 a	2.53 a	3.60 a	4.33 a	2.67 a	2.07 a	1.66 a
	N2	1.07 a	2.80 a	3.80 a	3.60 a	2.60 a	2.00 a	1.73 a
	N3	1.33 a	2.87 a	3.87 a	3.53 a	2.60 a	2.00 a	1.80 a
	Standard error ±	0.32	0.42	0.41	0.33	0.38	0.27	0.33
	LSD	ns	ns	ns	ns	ns	ns	ns
	GM	1.29	2.73	3.76	3.82	2.62	2.02	1.73
	RSD%	9.77	9.37	4.57	13.32	6.00	8.67	8.03
	Treatment	**Average of pre-spray counts**				**Average of post-spray counts**		
		week 1	**week 2**	**week 3**	**week 4**	**week 5**	**week 6**	**week 7**
II	N1	1.64 a	2.60 a	3.60 a	3.73 a	3.53 a	3.20 a	2.88 a
	N2	1.23 a	2.80 a	3.67 a	2.60 a	3.27 a	2.60 a	2.53 a
	N3	1.48 a	2.87 a	3.75 a	2.75 a	3.00 a	3.00 a	2.87 a
	Standard error ±	0.23	0.41	0.41	0.52	0.17	0.41	0.50
	LSD	ns	ns	ns	ns	ns	ns	ns
	General mean	1.45	2.76	3.67	2.94	3.27	2.93	2.76
	RSD%	6.11	5.80	6.33	6.80	10.93	7.60	10.35

N1, N2 and N3 = levels of nitrogen doses. LSD = least significant difference. RSD = relative standard deviation. ns = not significant.

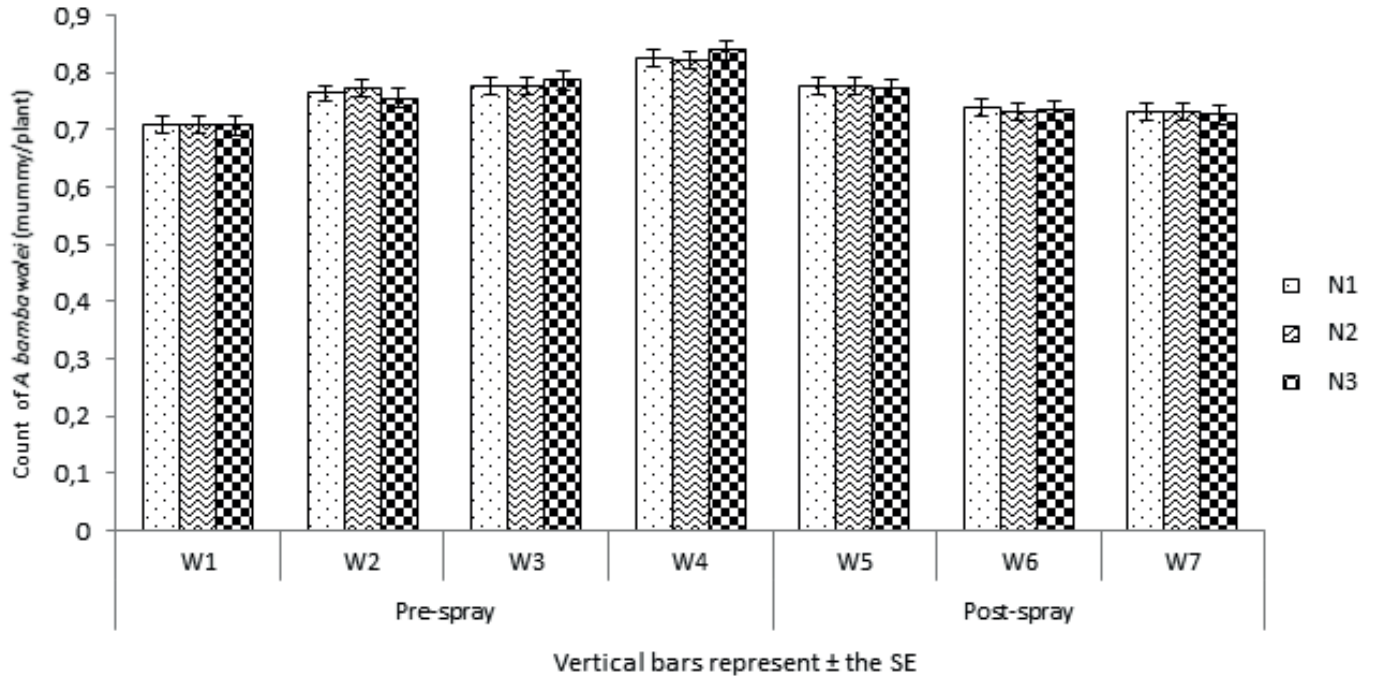

Figure 1. Pre and post-spray count of *A. bambawalei* (mummy/plant) pre and post-spray in season 1.

Asiimwe *et al.* (2016) stated that plant quality can influence the impact of natural enemies. Improving the nutritional value of host-plant enhanced the development and fertility of some herbivorous insects (Awmack & Leather 2002), which enhances also the performance and population density of some predators and parasitoids (Hunter 2003). Generally, plant quality influences the population dynamics of the insect through changes in their fertility, existence, distribution, competition, and natural enemy induced death (Walker *et al.* 2008; Zhu *et al.* 2020). So, alterations in the quality of host plant may indirectly affect natural enemies through the effects on their prey (Giles *et al.* 2002; Harvey *et al.* 2003). Zhu *et al.* (2020) reported that the nitrogen application to rice plants prolonged the development of *Anagrus flaveolus*, increased wing size, decreased female fecundity, and decreased the parasitism by reducing the searching efficiency of *A. flaveolus*. Nitrogen has profound effects on natural enemy efficiency and increases the dependence on insecticides (Zhu *et al.* 2020). The differences between authors reporting significant indirect effects of nitrogen fertilization on natural enemies might be explained by differences in the strain of the pest or natural enemies studied.

Effects of the insecticides on the abundance of the natural enemies. Results of pre-spray counts (Table3) indicated no significant differences in the abundance of *C. carnea* eggs in various experiment plots. Counts of the eggs were relatively high in season II compared to season I. Imidacloprid and profenofos exerted the highest significant reduction in the counts of *C. carnea* eggs (Table 3) throughout the three post spray counts. There is no significant difference between these insecticides in the two seasons however both are significantly different from other treatments. These results agree with the findings of many authors; Gandhi *et al.*

Table 3. Impact of the applied insecticides on the average of natural abundance of *Chrysoperla carnea* eggs.

Seasons	Treatment	Pre-spray means	Average of post-spray counts			Average of post-spray counts	Reduction (%)
			Days				
			3	10	17		
I	Lufenuron	4.11 a	2.67 a	2.00 a	2.52ab	2.40a	06.61
	Profonofos	4.22 a	1.00 b	1.44 b	1.56b	1.33b	48.25
	Abamectin	3.33 a	2.67 a	2.00 a	2.44 ab	2.37a	07.78
	Imidacloprid	4.44 a	1.00 b	0.99 c	1.40b	1.13b	56.03
	Control	3.00 a	2.78 a	2.22 a	2.70a	2.57 a	-
	Standard error ±	0.41	0.25	0.17	0.23	0.18	-
	LSD	ns	0.74 ***	0.51 ***	0.66 ***	0.50***	-
	General mean	3.82	2.02	1.73	1.73	2.13	-
	RSD%	13.32	6.00	8.67	8.03	14.63	-
	Treatment	Pre-spray means	Average of post-spray counts			Average of post-spray counts	Reduction (%)
			Days				
			3	10	17		
II	Lufenuron	3.91 a	3.11 b	3.56 a	3.33 a	3.33 b	17.57
	Profonofos	3.22 a	2.11 c	1.36 c	1.86 b	1.78 c	55.94
	Abamectin	3.84 a	3.44 b	3.22 a	3.37 a	3.34 ab	17.33
	Imidacloprid	4.22 a	2.44 bc	1.89 b	2.33 b	2.22 c	45.05
	Control	2.67a	3.56 a	3.78 a	4.04 a	4.04 a	-
	Standard error ±	0.281	0.358	0.290	0.310	0.2	-
	LSD	ns	0.781*	0.823**	0.780**	0.67***	-
	General mean	2.94	2.93	2.67	2.99	2.99	-
	RSD%	6.80	22.36	27.84	11.38	11.94	-

LSD = least significant difference. RSD = relative standard deviation. ns = not significant. Significant at* (0.05), **(0.01), ***(0.001)

(2005) and Brunner *et al.* (2001) who found that imidacloprid is toxic to *the green lacewing predator, C. carnea* under laboratory conditions, while Boueno & Reitas (2004) found abamectin is innocuous and lufenurom is toxic to the eggs and larvae of *Chrysoperla externa* under laboratory conditions. Abamectin, lufenuron were not significantly differ from the untreated set in most post-spray counts and not significantly different from each other. This result agrees with Moura *et al.* (2012) who found abamectin slightly harmful to *C. externa* 1st instar larvae and in contrast harmless to the 2nd instar larvae under laboratory conditions. Based on the accumulated post spray mean of counts and the percentage reduction, the insecticide hazards to *C. carnea* eggs can be ranked as follows; imidacloprid, profenofos, abamectin, and lufenuron, in season 1 and profenofos, imidacloprid, lufenuron, and abamectin in season 2. Similar to effect of the designed IPM package on Bt cotton sucking pests (Fadlelmawla *et al.* 2020) there is no significant interactions between nitrogen and insecticide treatments measured by the count of *C. carnea* eggs, at p=0.05.

Aenasius bambawalei was abundant in season I only (Table 4) and this may be explained by the absence of its main host in Bt Cotton during that season. There are no significant differences in the parasitoid counts (measured by the number of infested mealy bug mummies) between the various experimental units in both pre and post-spray counts except imidacloprid treatments during the second post-spray counts (after 10 days) (Table 4).

Table 4. Impact of the applied insecticides on the natural abundance of *Aenasius bambawalei*

Treatment	Pre-spray means	Average of pre-spray counts			Accumulated post-spray mean	Reduction (%)
		Days				
		3	10	17		
Lufenuron	0.84 a	0.77 a	0.73 ab	0.73 a	0.74 bc	02.63
Profonofos	0.84 a	0.76 a	0.74 a	0.72 a	0.74 bc	02.63
Abamectin	0.84 a	0.78 a	0.74 a	0.72 a	0.75 ab	01.32
Imidacloprid	0.86 a	0.76 a	0.71b	0.72 a	0.73 c	03.95
Control	0.78a	0.79 a	0.75 a	0.74 a	0.76 a	-
Standard error ±	0.014	0.013	0.009	0.009	0.005	-
LSD	ns	ns	0.026 *	ns	0.016*	-
General mean	0.83	0.77	0.73	0.73	0.74	-
RSD%	6.33	5.00	7.17	4.43	1.15	-

LSD = least significant difference. RSD = relative standard deviation. ns = not significant. *= significant at 0.05.

Being a completely endo-parasitoid (i.e., all developmental stages were found inside the body of the host and therefore protected from direct contact with applied insecticides) may explain the slight percentage reduction in the population of this parasitoid induced by the applied insecticides. The significant reduction in the number of infested mummies observed after 10 days in imidacloprid treated plots may be explained by the toxicity of this insecticide to *A. bambawalei* as reported by Bhatt & Jalpa (2018) under laboratory conditions. Based on the accumulated post spray count and the percentage reduction, the hazards of the insecticides to *A. bambawalei* can be ranked as follows imidacloprid, lufenuron, profenofos, and abamectin. Similar to effect of the designed IPM package on Bt cotton sucking pests (Fadlelmawla *et al.* 2020) there is no significant interactions between nitrogen and insecticide treatments measured by the count of *A. bambawalei mumies*, at p=0.05.

Nitrogen level exerted no significant effects on the populations of prevailing natural enemies; *C. carnea* and *A. bambawalei.* All insecticide treatments significantly reduced the population of natural enemies. Imidacloprid and profenofos exerted the highest reduction of *C. carnea* population followed by abamectin and lufenuron in the first season, while profenofos, imidacloprid, lufenuron, and abamectin induced the highest reductions in the second season. On the other hand reductions in *A. bambawalei* population (abundant in season 1 only) followed the order; imidacloprid, lufenuron, abmectin, and profenofos. Interactions between nitrogenous fertilization and insecticide treatments were not significant as measured by the population of prevailing natural enemies.

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SUPPORT MATERIAL

1- Table S1. The nitrogen dosages
2- Table S2. Effects of nitrogen fertilization on the abundance of *Aenasius bambawalei* in season 1
3- Table S3. Counts of *Chrysoperla carnea* (eggs number) affected by nitrogen*insecticides
4- Table S4. Counts of *Aenasius bambawalei* (mealybug mummies) affected by nitrogen*insecticides
5- Table S5. *C. carnea* analysis of variance summary containing the mean square (MS) for the 7-week counts in the two seasons
6- Table S6. *A. bambawalei* analysis of variance summary containing the mean square (MS) for the 7-week counts in season1

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SUPPLEMENTARY MATERIALS

Table S1. The nitrogen dosages.

Dose level	Urea (46%)/feddan	Urea (46%)/ha	Nitrogen (kg/ha)
N_1	40.00	95.24	43.81
N_2	80.00	190.48	87.62
N_3	120.00	285.72	131.43

Table S2. Effects of nitrogen fertilization on the abundance of *Aenasius bambawalei* in season 1.

Treatment	Pre-spray				Post-spray		
	W1	W2	W3	W4	W5	W6	W7
N1	0.707 a	0.763 a	0.775 a	0.825 a	0.775 a	0.738 a	0.730 a
N2	0.707 a	0.772 a	0.777 a	0.822 a	0.776 a	0.730 a	0.730 a
N3	0.707 a	0.755 a	0.786 a	0.839 a	0.772 a	0.734 a	0.726 a
SE±	0.000	0.013	0.012	0.014	0.010	0.004	0.008
LSD	ns	ns	ns	ns	ns	ns	ns
GM	0.707	0.763	0.779	0.828	0.774	0.733	0.729
CV%	0.00	6.80	5.95	6.33	5.00	7.17	4.43

W1, W2, ...W7 = Weeks of counts. N1, N2 and N3 = levels of nitrogen doses. SE = standard error. LSD = least significant difference. GM = general mean. CV = coefficient of variation. ns = not significant. *, **, and *** = significant

Table S3. Counts of *Chrysoperla carnea* (eggs number) affected by nitrogen*insecticides.

Season 1												
Insecticide	Week 5				Week 6				Week 7			
	N1	N2	N3	Mean	N1	N2	N3	Mean	N1	N2	N3	Mean
L	2.67	2.67	2.67	2.67	2.00	2.00	2.00	2.00	2.89	2.67	2.00	2.52
P	1.00	1.00	1.00	1.00	1.33	1.33	1.67	1.44	2.22	1.00	1.44	1.56
A	2.67	2.67	2.67	2.67	2.00	2.00	2.00	2.00	2.67	2.67	2.00	2.44
I	1.00	1.00	1.00	1.00	0.96	1.00	1.00	0.99	2.22	1.00	0.99	1.40
C	3.00	2.67	2.67	2.78	2.00	2.33	2.33	2.22	3.11	2.78	2.22	2.70
Mean	2.07a	2.00a	2.00a	2.02	1.66a	1.73a	1.80a	1.73	2.62a	2.02a	1.73a	1.73
Season 2												
Insecticide	Week 5				Week 6				Week 7			
	N1	N2	N3	Mean	N1	N2	N3	Mean	N1	N2	N3	Mean
L	3.33	2.67	3.33	3.11	3.33	3.67	3.67	3.56	3.33	3.11	3.56	3.33
P	2.67	1.67	2.00	2.11	1.73	0.67	1.67	1.36	2.11	2.11	1.36	1.86
A	3.33	3.33	3.67	3.44	3.33	3.00	3.33	3.22	3.44	3.44	3.22	3.37
I	2.67	2.00	2.67	2.44	1.67	1.67	2.33	1.89	2.67	2.44	1.89	2.33
C	4.00	3.33	3.33	3.56	4.33	3.67	3.33	3.78	4.78	3.56	3.78	4.04
Mean	3.20a	2.60a	3.00a	2.93	2.88a	2.53a	2.87a	2.76	3.27a	2.93a	2.76a	2.99

Table S4. Counts of *Aenasius bambawalei* (mealybug mummies) affected by nitrogen*insecticides.

Insecticide	Week 5				Week 6				Week 7			
	N1	N2	N3	Mean	N1	N2	N3	Mean	N1	N2	N3	Mean
L	0.79	0.75	0.77	0.77a	0.73	0.75	0.71	0.73ab	0.71	0.75	0.73	0.73a
P	0.77	0.77	0.75	0.76a	0.75	0.73	0.75	0.74a	0.73	0.73	0.71	0.72a
A	0.77	0.77	0.82	0.79a	0.75	0.71	0.75	0.74a	0.73	0.73	0.71	0.72a
I	0.75	0.79	0.75	0.76a	0.709	0.71	0.71	0.71b	0.75	0.71	0.71	0.72a
C	0.79	0.79	0.77	0.79a	0.749	0.75	0.749	0.75a	0.73	0.73	0.77	0.74a
Mean	0.78a	0.78a	0.77a	0.77	0.74a	0.73a	0.73a	0.73	0.73a	0.73a	0.73a	0.73

No significant differences in all insecticides*nitrogen interaction means at 5% probability.

Table S5. *Chrysoperla carnea* analysis of variance summary containing the mean square (MS) for the 7 week counts in the two seasons.

Source	Df	Season 1							Season 3						
		W1	W2	W3	W4	W5	W6	W7	W1	W2	W3	W4	W5	W6	W7
Replications	2	0.29	2.07	1.76	2.29	2.47	0.62	0.69	0.33	1.76	1.76	1.99	3.25	1.95	3.69
Nitrogen	2	0.6ns	0.47ns	0.29ns	2.96ns	6.20ns	2.69ns	1.09ns	0.65ns	0.29ns	0.08ns	1.20ns	4.53ns	5.40ns	5.15ns
Error 1	4	1.56	2.63	2.56	1.62	2.17	1.06	1.59	0.78	2.56	2.53	3.99	0.52	2.45	3.77
Insecticides	4	0.5	1.03	1.24	3.48	6.19***	7.56***	12.2***	0.19	1.19	0.97	2.81ns	9.86***	4.94***	8.76***
Nitrogen x Insecticides	8	0.3	1.80	1.84	1.43	0.59ns	0.61ns	1.17ns	0.35	1.87	1.70	0.68ns	0.42ns	0.45ns	0.70ns
Error 2	24	0.52	1.28	1.26	1.51	0.57	0.27	0.46	0.40	1.26	1.15	0.71	1.15	0.76	0.87
Total	**44**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CV%	**-**	**9.77**	**9.37**	**4.57**	**13.32**	**6.00**	**8.67**	**8.03**	**6.11**	**5.80**	**6.33**	**6.80**	**10.93**	**7.60**	**10.35**

*=significant at 5% probability. **=significant at 1% probability. ***=significant at 0.01% probability. ns=not significant.

Table S6. *Aenasius bambawalei* analysis of variance summary containing the mean square (MS) for the 7 week counts in season1.

Source	Df	Season 1						
		W1	W2	W3	W4	W5	W6	W7
Replications	2	0.00	0.003	0.003	0.005	0.000	0.003	0.000
Nitrogen	2	0.00 ns	0.001 ns	0.001 ns	0.001 ns	0.000 ns	0.000 ns	0.000 ns
Error 1	4	0.00	0.003	0.002	0.003	0.002	0.000	0.001
Insecticides	4	0.00 ns	0.001 ns	0.003 ns	0.004 ns	0.001 ns	0.002 *	0.000 ns
Nitrogen x Insecticides	8	0.00 ns	0.001 ns	0.001 ns	0.001 ns	0.002 ns	0.000 ns	0.001 ns
Error 2	24	0.00	0.001	0.002	0.002	0.002	0.000	0.000
Total	44	-	-	-	-	-	-	-
CV%	-	0.00	6.80	5.95	6.33	5.00	7.17	4.43

*=significant at 5% probability. **=significant at 1% probability. ***=significant at 0.01% probability. ns=not significant.

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