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Original article

Stink bug *Agonoscelis* spp. (Heteroptera: Pentatomidae) – An emerging threat for seed production in alfalfa crop (*Medicago sativa* L.) and their successful management

Haider Karar [a,*], Muhammad Amjad Bashir [b], Abdul Khaliq [c], Muhammad Jaffar Ali [d], Reem Atalla Alajmi [e], Dina M. Metwally [e,f]

[a] Mango Research Institute, Multan, Pakistan
[b] Department of Plant Protection, Faculty of Agricultural Sciences, Ghazi University Dera Ghazi Khan, Pakistan
[c] Cotton Research Institute, Khanpur, India
[d] Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan
[e] Zoology Department College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[f] Department of Parasitology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt

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ABSTRACT

Fodder crops play an important role in sustainable agriculture as they provide feed for animals, which is ultimately converted to human food. Alfalfa is one of the most important fodder crops having high nutritive value for livestock. However, seed production of alfalfa crop is seriously affected by several factors and the highest reduction in seed yield is caused by stink bug infestation. The current study evaluated different insecticides to control stink bugs during 2016–17. The efficacy of ten insecticides, i.e., acephate, dimethoate, malathion, chlorpyriphos, bifenthrin, lambdacyhalothrin, deltamethrin, acetamiprid, imidacloprid and carbosulfan was tested on *Agonoscelis* spp. (Heteroptera Pentatomidae). The mortality of stink bug was recorded at one, three, five, seven, ten and fifteen days after insecticide application. Similarly, the population of pollinators was recorded before and one, three and five days after the application of insecticides. It was observed that acetamiprid (81.14%) and acephate (80.65%) caused the highest mortality of stink bug and proved most effective. Insecticides application decreased the pollinators' population one day after spray; however, it was rehabilitated three days after insecticide application. Insecticide application increased seed yield from 28.05 kg/acre (during last four year without chemical control) to 116 kg/acre in 2016–17 (with chemical control). It is concluded that acetamiprid and acephate can be successfully used in integrated management program of increasing alfalfa seed production.

1. Introduction

Fodder crops are regarded as strong pillars of sustainable agriculture as they provide feed for livestock, which is converted to human food, i.e., milk and meat. Legumes among fodder crops play a vital role in grassland ecosystems globally (Singh et al., 2010). Alfalfa is important legume crop having high nutritive value for livestock as it contains 16–25% crude protein and 20–30% fiber (Babu et al., 2014). Alfalfa is considered as a rich source of minerals because 100 g of alfalfa contain 79 mg potassium (K), 70 mg phosphorus (P), 32 mg calcium (Ca), 27 mg magnesium (Mg), 6 mg sodium (Na), and trace amounts of iron (Fe), zinc (Zn) and manganese (Mn). Fodder and seed yield of alfalfa is low in Pakistan as compared to other developing countries. Several factors are responsible for yield reduction, including poor field management, unjustifiable last cutting, changing climatic conditions and poor management of insets, pests and diseases. Numerous insect pests attack alfalfa crop, which significantly reduce yield and quality of produce (Godfrey et al., 2013). Stink bug is considered as one of the most destructive pest of alfalfa at reproductive stage among sucking pests. Stink bug is a major pet of economically important

* Corresponding author.
E-mail addresses: haider853@gmail.com (H. Karar), khaliq2387@gmail.com (A. Khaliq).

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crops worldwide, and responsible for transmission of various diseases. The population of stink bug is increased when plants reach to maturity or pod formation. It sucks sap from the developing pods and negatively affect seed formation. It is highly polyphagous (Hoebeke and Carter, 2003) and may cause tremendous economic damage to crops globally.

Stink bug is a mobile insect and can travel from nearby agricultural and wild hosts to farms. This movement is linked to crop phenology and availability of suitable food sources (Jones and Sullivan, 1981). It is not only notorious pest of alfalfa pods, but has >60 host plants (Bernon et al., 2004). According to an estimate, global annual crop losses due to stink bugs alone are higher than other insects. For example, in cotton losses are $31 million (Williams, 2009) and in soybean $60 million (McPherson and McPherson, 2000). Similarly, in grain and legume crops stink bug feeding declines the quality of the produce or fruiting bodies can be lost (Hall and Teetes, 1982; Espino and Way, 2008). It is not only a pest of legume crops, but also considered as severe pests of maize (Negron and Riley, 1987; Ni et al., 2010), and can cause 100% crop losses. According to Wheeler (2001), sucking by alfalfa bug, *A. lineolatus* can decrease ~50% seed yield, while Sekulić et al. (2005) reported that alfalfa bug reduced the seed yields by 20–90%.

Pollinators also play an important role in seed production. So there is a need to develop effective integrated pest management strategies to reduce major crop losses. Stink bug is a recently noticed pest in Pakistan; therefore, cultural and biological strategies are unavailable. This has necessitated immediate, insecticide-based management program for the control of stink bug (Leskey et al., 2012b, Basit et al., 2021), while other long-term strategies can be developed for maximum seed production in alfalfa. Insecticides are considered the only immediate effective option available for minimizing economic losses.

Alfalfa is a cross pollinated crop and its optimum seed production depends upon the activity of pollinators. Among pollinators, bees play an important role in fertilization of flowers. It is necessary to screen different groups of insecticides with different modes of action to evaluate the chemicals which ensure minimum damage to pollinators for maximum seed production of alfalfa. Although fodder crops are attacked by various pests, these are seldom treated with pesticides as these are directly fed to the livestock. However, fodder crops are kept in the field for flowering and seed formation and not fed to livestock. Chemical treatment of fodder crops for seed production purpose not only enhances seed yield but also safe because as it is not used as feed for livestock.

The purpose of this study was to test the efficacy of different insecticides against stink bug. Nonetheless, testing the damage caused by insecticides to natural pollinators such as honey bees was the second objective of the study.

2. Materials and methods

The experiments were conducted at Fodder Research Institute, Sargodha, Pakistan during 2016–17. Details of different insecticides used in the study is given in Table 1. One alfalfa variety was used as experimental material and last cutting was left in the field to obtain seeds. The tested insecticide were used in water solution and applied by manual knapsack sprayer at the rate of 100 L solution acre^{-1}. The experiment was arranged in randomized complete block design (RCBD) with three replications. The data relating to stink bug density was recorded before spray, then 1, 3, 5, 7, 10 and 15 days after insecticide application by using ten net sweeps per plot. All insecticides were sprayed at 6.00 PM to save pollinators' population. The data regarding activity of pollinators were recorded at 10.00 AM before and then one, three and five days after spray. Before each insecticide application, sprayer was thoroughly cleaned with clean water to avoid insecticide mixture.

2.1. Mortality rate

Percent mortality of stink bug was calculated by using following formula:

$$\text{Percent mortality} = \frac{(P1 - P2)}{P1} \times 100$$

Population before spray = P1
Population after spray = P2

2.2. Pollinators

The population of pollinator was recorded from alfalfa seed crop at five different places. The handmade square meter was kept in the field and the population of all visiting insects was counted. Average pollinators populations were calculated per square meter.

2.3. Yield data

The crop was harvested in during June 2017 and kept on concrete floor for drying. After three days the crops was threshed and seed yield was recorded. The yield data of last four years was collected from farm manager, Fodder Research Institute, Sargodha and comparative graphs were prepaed.

2.4. Statistical analysis

All treatments were compared with control to assess the performance of insecticides. The data were subjected to statistical analy-

Table 1
Information of insecticides with different mode of action used against stink bug.

S/ No	Insecticides		Formulation	Group	WHO hazard classification	IRAC group	Dose (ml g^{1-} Acre^{1-})	Mode of Action
	Trade Name	Common Name						
1	Codedor	Acephate	75SP	Organophosphates	ii	1B	400	Acetylcholinesterase (AChE) inhibitors
2	Danadim	Dimethoate	40EC	Organophosphates	ii	1B	400	Acetylcholinesterase (AChE) inhibitors
3	Route	Malathion	57EC	Organophosphates	iii	1B	500	Acetylcholinesterase (AChE) inhibitors
4	Cordelia	Chlorpyriphos	40EC	Organophosphates	ii	1B	500	Acetylcholinesterase (AChE) inhibitors
5	Welthrin	Bifenthrin	10EC	Pyrethroids	ii	3A	300	Sodium channel modulators
6	Karate	Lambdacyhalothrin	2.5EC	Pyrethroids	ii	3A	300	Sodium channel modulators
7	Deltashine	Deltamethrin	2.5EC	Pyrethroids	ii	3A	400	Sodium channel modulators
8	Starlet	Acetamiprid	20SP	Neonicotinoids	ii	4A	125	Nicotinic acetylcholine receptor (nAChR) competitive modulators
9	Imidacloprid	Imidacloprid	20SL	Neonicotinoids	ii	4A	250	Nicotinic acetylcholine receptor (nAChR) competitive modulators
10	Advantage	Carbosulfan	20EC	Carbamates	ii	1A	500	Acetylcholinesterase (AChE) inhibitors

sis using Statistix version- 9 (www.statistix.com/free trial.html) and means were compared by Tukey's HSD.

3. Results

3.1. Mortality rate one day after spray

The effects of various insecticides on mortality of stink bug population are presented in Table 2. There was a significant difference ($P < 0.01$) among treatments. Carbosulfan and bifenthrin caused 95.65% and 84.62% mortality and were statistically similar with deltamethrin, imidacloprid, dimethoate, chlorpyrifos, and acetamiprid, while malathion caused 52.63% morality.

3.2. Mortality rate three days after spray

The mortality rate of stink bug three days after insecticide application is given in Table 2. Acephate and acetamiprid caused 100% mortality rate three days after application and were statically similar to imidacloprid and carbosulfan, which caused 80.0% and 75.46% mortality, respectively. Lambdacyhalothrin and bifenthrin caused 29.55% and 23.21% mortality, respectively.

3.3. Mortality rate five days after spray

Significant differences ($P < 0.01$) were observed among different insecticides five days after spray. Acephate caused the highest mortality (100%), followed by acetamiprid (82.76%) and carbosulfan (52.61%) five days after application, while lambdacyhalothrin and bifenthrin did not cause any mortality.

3.4. Mortality rate seven days after spray

Significant variation ($P < 0.01$) was noted among different insecticides for mortality seven days after application. Acephate (84.62%) and acetamiprid (81.82%) caused the highest mortality than other insecticides seven days after spray. However, lambdacyhalothrin, deltamethrin, dimethoate, malathion and bifenthrin resulted in the lowest mortality (Table 2).

3.5. Mortality rate ten days after spray

The effect of different insecticides on mortality rate of stink bug population ten days after spray is presented in Table 2. The highest mortality was recorded with acetamiprid (81.82%) and acephate (68.56%), while lambdacyhalothrin, chlorpyrifos, deltamethrin, dimethoate, malathion and bifenthrin caused no mortality ten days after spray.

3.6. Mortality rate fifteen days after spray

Data regarding mortality revealed significant variations ($P < 0.01$) among tested insecticides fifteen days after spray. The highest mortality was observed with acetamiprid (67.73%) and acephate (61.54%) fifteen days after spray. However, lambdacyhalothrin, chlorpyrifos, deltamethrin, dimethoate, malathion and bifenthrin caused no mortality.

3.7. Cumulative mortality of stink bug

Data regarding stink bug mortality in alfalfa seed crop on cumulative basis are shown Fig. 1. The results revealed that mortality in acephate and acetamiprid sprayed plots was 80.65% and 81.14%, respectively, while the mortality of stink bug in other insecticides sprays plots ranged from 16.59% to 44.35%.

Table 2
Comparison of percent mortality of stink bug after spray on alfalfa crop during 2016–17.

S. No	Insecticides		Pop. of stink bug B. spray	Dose (ml g $^{1-}$ 100liter^{1-} solution)	Mortality rate of Stink Bug after spray						Avg mortality
	Trade Name	Common Name			One day Mean ± SE	Three days Mean ± SE	Five days Mean ± SE	Seven days Mean ± SE	Ten Days Mean ± SE	Fifteen days Mean ± SE	
1	Codedor 75SP	acephate	5.61	400	69.23 ± 1.04c	100.00 ± 0.00a	100.00 ± 0.00a	84.61 ± 1.93a	68.56 ± 1.36b	61.54 ± 1.87a	80.65
2	Danadin 40EC	dimethoate	4.67	400	78.57 ± 1.28bc	50.00 ± 0.78c	28.57 ± 0.95d	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00b	26.19
3	Route 57EC	malathion	6.21	500	52.63 ± 0.82d	47.37 ± 0.96 cd	31.58 ± 0.71d	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00b	21.93
4	Cordelia 40EC	chlorpyrifos	7.67	500	73.91 ± 1.12bc	42.61 ± 0.88d	29.87 ± 0.76d	15.22 ± 1.15c	0.00 ± 0.00d	0.00 ± 0.00b	26.93
5	Welthrin 10EC	bifenthrin	4.33	300	84.62 ± 2.76ab	23.21 ± 1.19e	0.00 ± 0.00e	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00b	17.97
6	Karate 2.5EC	lambdacyhalothrin	5.19	300	70.00 ± 1.40c	29.55 ± 0.98e	0.00 ± 0.00 e	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00b	16.59
7	Deltashine 2.5EC	deltamethrin	4.17	400	80.00 ± 0.92bc	44.12 ± 0.82 cd	30.00 ± 1.40d	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00b	25.68
8	Starlet 20SP	acetamiprid	5.67	125	72.73 ± 2.21bc	100.00 ± 0.00a	82.76 ± 1.14b	81.82 ± 1.39a	81.82 ± 1.46a	67.73 ± 1.81a	81.14
9	Imidacloprid 20SL	imidacloprid	4.96	250	80.00 ± 0.83bc	80.00 ± 0.94b	30.00 ± 1.12d	11.29 ± 0.38c	0.00 ± 0.00d	0.00 ± 0.00b	33.54
10	Advantage 20EC	carbosulfan	7.67	500	95.65 ± 0.49a	75.46 ± 0.73b	52.61 ± 0.81c	33.91 ± 1.36b	7.22 ± 0.27c	1.26 ± 0.10b	44.35
11	Control		5.26	–	0.00 ± 0.00e	2.01 ± 0.04f	0.00 ± 0.02e	0.00 ± 0.00 d	0.00 ± 0.00d	0.00 ± 0.00b	2.01
	Tukey's HSD 5				12.78	7.06	7.48	8.37	5.64	7.27	
	F-Value				101.57	518.80	491.97	397.47	753.44	336.48	

Different letter within column indicate significant differences ($p < 0.5$) among treatments ($n = 3$).

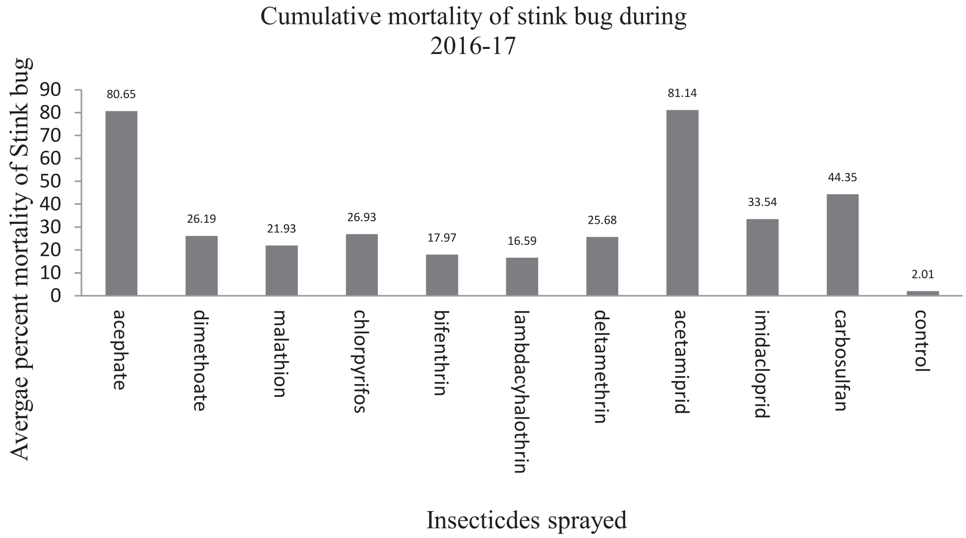

Fig. 1. Cumulative percent mortality of stink bug in alfalfa seed crop.

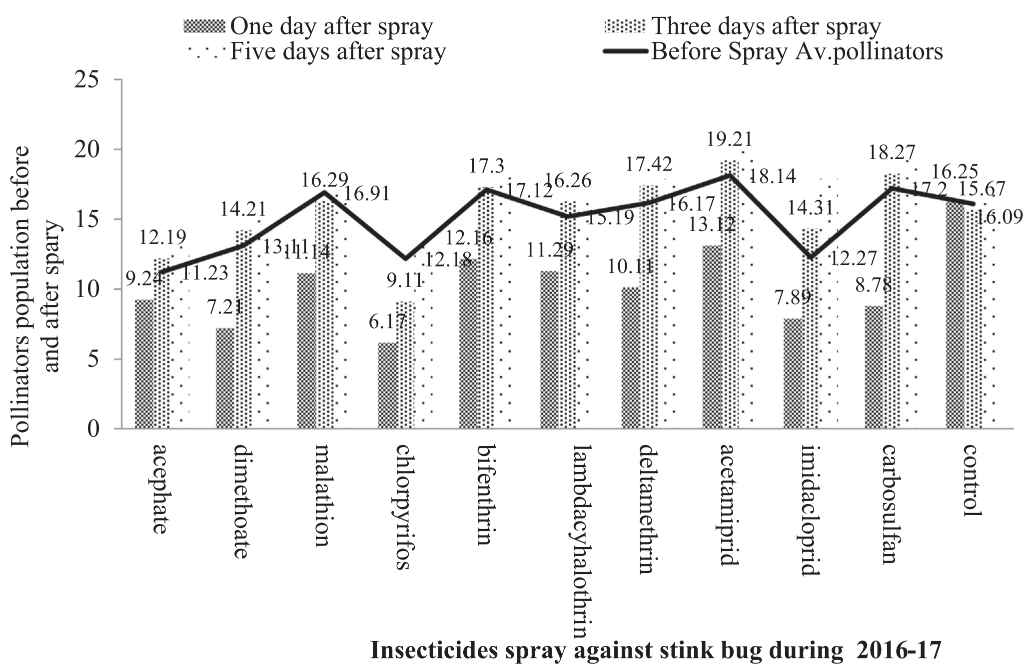

Fig. 2. Effect of different pesticides on population of pollinators before and after spray.

3.8. Pollinator population before and after spray

The effects of different insecticides on pollinators' populations are shown in Fig. 2. All insecticides significantly reduced the populations of pollinators one day after spray. The highest and the lowest reduction were recorded with chlorpyrifos and acetamiprid, respectively one day after spray. However, negligible effect of insecticides on pollinators' population was observed three days after spray (Fig. 2).

3.9. Seed yield during last five years

Seed yield of alfalfa crop and population of stinkbug during last five years are shown in Fig. 3. Inverse relationship was noted between seed yield and stinkbug population during various years. Seed yield of alfalfa crop in 2012–13, 2013–14, 2014–15, 2015–16 and 2016–17 was 42.0, 10.5, 22.5, 37.2 and 116.0 ag acre^{-1} respectively, while stinkbug population was 4.19, 5.93, 6.09, 4.74 and 0.02 individuals per net sweep, respectively.

4. Discussion

Production of alfalfa seed depends upon several factors, including last cutting date, weather conditions, availability of pollinators, and infestation of insect pests and diseases. Insect pests are considered the utmost important factors, which need special attention. Different management practices are being opted globally, including cultural, biological and chemical to overcome insect pests of various crops. Among these control measures chemical control is considered to be the most effective and efficient strategy, which saves the crop from pest outbreak. In our experiment, four different groups of insecticides with different modes of action were

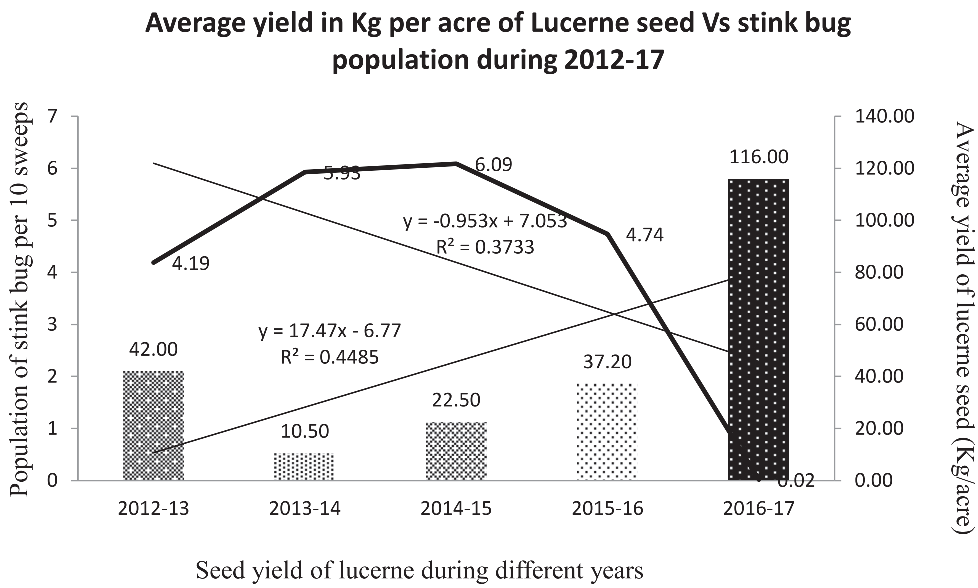

Fig. 3. Comparison of alfalfa seed production in 2016–17 with last four years yield at FRI-Sargodha.

tested against stink bug under field conditions. Our results suggested that all insecticides were statistically different and had significant impact on stink bug mortality when compared to control plot. On numerical basis, highest mortality was observed with acetamiprid and acephate, which proved as the most effective insecticide (Table 2). The results are in agreement with Karar and Khaliq (2019) who concluded that acetamiprid and acephate proved as best insecticides against seed pods feeding stink bug and increased yield. Furthermore, it was noted that carbosulfan showed knock down effects and remained effective for short period against stink bug. Similar results have been reported by Wallingford (2012) and Lee et al. (2013) stating that organophosphates, neonicotinoids, pyrethroids and carbamates insecticides are effective and efficient strategy to manage stink bug. Similarly, described that acetamiprid caused greatest mortality of stink bug, i.e., 93–100%. Furthermore, our results are also supported by who partially confirmed that pyrethroids, organophosphate, acephate, carbamates, methomyl and oxamyl, neonicotinoids dinotefuran, imidacloprid, thiamethaxim, clothianidin, and acetamiprid were effective in controling stink bug. In our study it was observed that pyrethroids like bifenthrin, lambdacyhalothrin and deltamethrin caused the highest mortality of stink bug one day after spray but after three days the mortality was reduced. These results are in line with Leskey *et al.* (2012) who reported that pyrethroids have knockdown effects but many bugs recover within 7 days. Similarly in our results carbamate like carbosulfan has also knock down effect and remained effective for period of five days.

Seed yield of crop and stink bug population had inverse relationship, when stink bug population increased the seed yield of alfalfa was dramatically decreased (Fig. 3). When stink bug population was controlled by insecticides, there was a significant increase in seed yield. This is first study in Pakistan that identified that stink bug population dramatically reduced seed yield of alfalfa crop and its control by insecticides increased seed yield. Alfalfa is cross pollinated and for seed production it is important to enhance the activity of pollinators. It had been observed that insecticides spray had negligible effect on the population of pollinators because population of pollinators rehabilitated three days after spray

Comparison of alfaalfa seed production in 2016–17 with last four years data clearly depicted that maximum seed yield of alfalfa was obtained during 2016–17 as compared to last four years yield. The data shows that there is much gap between previous years and current year yield. The reasons could be that there is lack of knowledge regarding detrimental effect of stink bug on seed yield of crop and importance of pollinators activity to enhanced seed yield.

5. Conclusions and recommendations

Increased activities of pollinators and management of stink bug play a key role in increasing alfalfa seeds production. The current study indicated that acetamiprid and acephate are the most effective insecticides for the management of stink bug. Carbosulfan was also effective to some extent for short period of time, with knock down effect. These insecticides have diverse mode of action and can be included in the IPM module which will be very helpful in planning future program of pest management It is further recommended that spray should be done after 6.00 pm to save and increased activity of pollinators in seed production.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author's contributions

HK developed idea and layout of the study. HK wrote up the manuscript. NM reviewed and rephrased the manuscript. MJA statistically analysis, AK collected data and MH,NH help in preparation of manuscript.

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