DOI: 10.53992/njns.v10i3.275 NUST Journal of Natural Sciences, Vol. 10, Issue 3, 2025

Research Article

Impact of synthetic and botanical insecticides on jassid *(Amrasca biguttula)* and their influence on cotton production

Zafar Hussain Shah[1*], Hakim Ali Sahito[1], Abdul Manan Shaikh[1], Zulfiqar Ali Sahito[2], Fahmeeda Imdad Sahito[3], Pir Arshad Ahmed Jan[4]

[1]Department of Zoology, Shah Abdul Latif University, Khairpur, Sindh, Pakistan.
[2]Department of Environmental Science, Zhejiang University, Hangzhou, China.
[3]Department of Teacher Education, Shah Abdul Latif University, Khairpur, Sindh, Pakistan.
[4]Government Boys Degree College, Tando Muhammad Khan, Sindh, Pakistan.

***Corresponding author's email:** lakyarializafar@yahoo.com

Abstract

The research was conducted on the comparison of some synthetic and botanical insecticides against jassid on the cotton crop in the district Khairpur, Sindh. The four experiments were performed: Experiment-1, on five treatments: T_1= Acephate, T_2= Nitenpyram, T_3= Boiled Neem Leaves extract, T_4= Neem Oil extract, and T_5= Control (unsprayed), which were replicated and applied four times at the ETL of jassid. Among the tested insecticides, Nitenpyram was proven highly effective and reduced the jassid population density % (81.65±5.21), followed by Neem Oil (65.75±8.44), Acephate (64.44±6.71), and Boiled Neem Leaves (55.20±17.11), while in the control plot, the mean population was (1.75±0.27). Experiment-2 was on the impact of natural enemies against jassid in the control plot, the overall results of the 6 fortnightly intervals showed the mean population of jassid per leaf and their natural enemies per plant as; (3.13, 1.19); (3.33, 2.02), (4.13, 2.07), (3.06, 1.38), (1.93, 0.73) and (1.56, 0.39), respectively. Whereas Experiments 3 and 4 were performed on green bolls' weight and cotton production, respectively. The Neem Oil was found best with an average weight of the green boll (13.86 g) and production of 230 kgs, followed by Boiled Neem Leaves (12.73 g) with 212 kgs, Nitenpyram (11.38 g) with 178 kgs, Acephate (11.28 g) with 168 kgs, while in the control plot (10.51 g) with 150 kgs. So, both synthetic and botanical insecticides proved to be better in controlling cotton jassid, while botanical insecticides were found to be better in increasing green bolls' weight and cotton production.

Keywords: *G. hirsutum*, *A. biguttula*, Insecticides, Boll Weight, Cotton Production.

Article History: **Received:** 7 Apr 2024, **Revised:** 11 Nov 2024 , **Accepted**: 25 Oct 2025, **Published:** 18 Nov 2025.
Creative Commons License: NUST Journal of Natural Sciences (NJNS) is licensed under Creative Commons Attribution 4.0 International License.

Introduction

Cotton, *Gossypium hirsutum* (L.) family; Malvaceae, recognized with "white gold" of the sub-continent, is an economically important product of our country currently cultivated in 80 countries on 31.8 million hectares around the world, producing more than 24.9 million tons of cotton fiber annually [1 - 3]. It is utilized in different

structures like oil, lint, and nourishment, whereas; contributed to about 80% of the country's oil production [4]. Among cotton-growing countries of the world, China produced 25%, the USA, 19%, India, 14% and Pakistan, 9%. In the same way, major consumers are China, 35%; India 15%, and Pakistan 10% [5]. In Pakistan, seed cotton production increased by 7.97% in the year 2017-18, with (11.582 million bales, as compared to 2016-17 with (10.727 million bales). Sindh province performed better in the increment % of cotton production as compared to other provinces during 2017-18, which was 12.31% (4.253 million bales) compared to the previous year (3.787 million bales) [6].

There are different variables as restrictive elements for quality cotton production. These elements incorporate delay due to extreme rainfall or high temperatures at the blooming stage, and the sudden outburst of pests up to 35-45% loss in cotton yield [5, 7]. Roughly; 1326 types of insects have been recorded infesting cotton throughout the world and 150 have been reported in Pakistan, Among these, the sucking insect pest, jassid, *A. biguttula* is mostly found in the plant species of family Solanaceous and Malvaceous in Southeast Asian countries, Thailand and India [8], with 25 species on cotton throughout the world, that took a status of agricultural pest in Indo-Pak [9]. The development and growth of young plants are reduced due to severe “Hopper burn” which causes the shedding of buds and bolls of the cotton plant, and ultimately yield is reduced [10]. Habitually, jassid is a cotton pest of the early phase, but its population increased throughout the period of cotton growing. For the management of soft-bodied sucking insect pests, numerous endeavors have been conducted by introducing parasites and predators as biological control; the majority of these are beetles (Coccinellidae), Lacewings (Chrysopidae), and Spiders (Araneae) [11]. *C. septempunctata* is known as a potential predator, feeding on delicate-bodied insect pests, and the *O. insidiosus* (Say) (Hemiptera: Anthocoridae) is a general predator of sucking insect pests [12].

The conventional strategy for managing cotton insect pests is chemical control; however, just utilized as the last alternative [13], sensibly following suitable spray techniques and at the time of Economic Threshold Level (ETL) for reducing insecticides’ burdens. In Pakistan, aggregate, 80% of insecticide utilization is applied to cotton, and in the world, it is estimated that about 50% of the recent yield of cotton is credited to agrochemicals utilized as part of crop protection and production. Numerous pesticides have been utilized for controlling cotton insect pests [14], such as neonicotinoids, pyrethroids, organophosphates, and IGRs [15 - 19] on the sucking complex of the cotton crop. For controlling jassid’s attack under ETL (1 to 1.5/leaf), Acephate 75 SP and Dimethoate 40 EC turned out to be the best up to the 7th day of the spray [20]; likewise, Mospilan and Confidor were ideal [21]. Certainly, agrochemicals have a noteworthy part in enhancing production in food production; in any case, fear of the negative effects of these chemicals has emerged on human well-being and the environment. The agricultural producers apply around US$40 billion worth of pesticides every year [22]. The haphazard utilization of hazardous chemical pesticides also provides enormous residual effects on the soil, water, and plants, as well as the predators and other natural enemies. Synthetic insecticides bring about a reduction of different useful microbes, flora, and fauna; furthermore, many serious diseases are caused by these in humans. By observing the present scenario, there is a direct need to develop an eco-friendly and efficient pest control approach for piercing-sucking insects of cotton [14].

Botanical sprays are a promising option to replace chemical insecticides for controlling insect pests as these are secure,

low-cost, locally produced, environmentally supportive, and easily available compounds, derived from different sources [23]. Among these, the Neem tree (*Azadirachta indica*) was more effective in controlling many key crop pests, and it is still used in different countries of the world for storing grains. Many naturally active compounds, like meliantriol, salannin, deacetyl azadirachtinol, vepol, and sulfur, are present in the neem plant; however, the chief component is azadirachtin [24 - 25]. Neem parts show different properties like repellent, antifeedant, growth-repressing, and toxic against insect pests [26]. The most extreme reduction was observed by neem seed extract (64.69%), followed by neem oil (57.74%), tobacco (52.91%), and asafoetida (46.52%) [27 - 28]. The major objectives of this research study are to compare the efficacy of various synthetic and botanical insecticides against the sucking pest, cotton jassid, and the impact of natural enemies against jassid in the control plot was kept under observations with the effect of insecticides on green bolls' weight, and on the cotton production to find out more effectual insecticide for controlling jassid under field conditions at district Khairpur, Sindh.

Materials and Methods

To assess the impact of various synthetic and botanical insecticides against cotton jassid at pre- and post-treatments under field conditions:

The field experiments were carried out at Lakyari Agricultural Farm (Location = 27º44'63" N, 68º45'18" E), Saidi Lower near Kamaldero, taluka Gambat, district Khairpur Mir's, Sindh, during the Kharif season, 2017-18, to investigate the efficacy of different synthetic and botanical insecticides against cotton jassid. The seeds of cotton variety (FH-114) were sown on ridges, from north to south in a Randomized Complete Block Design (RCBD) with a treatment size of 2178 sq. feet each. There were five treatments: T_1 = Acephate 75 SP (Safate), T_2 = Nitenpyram 10 SL (Amrasca), T_3 = Boiled-Neem leaves (BNL) extract, T_4 = Neem oil, and T_5 = Control (unsprayed) plot, as shown in Table 1 and replicated 4 times Table 2.

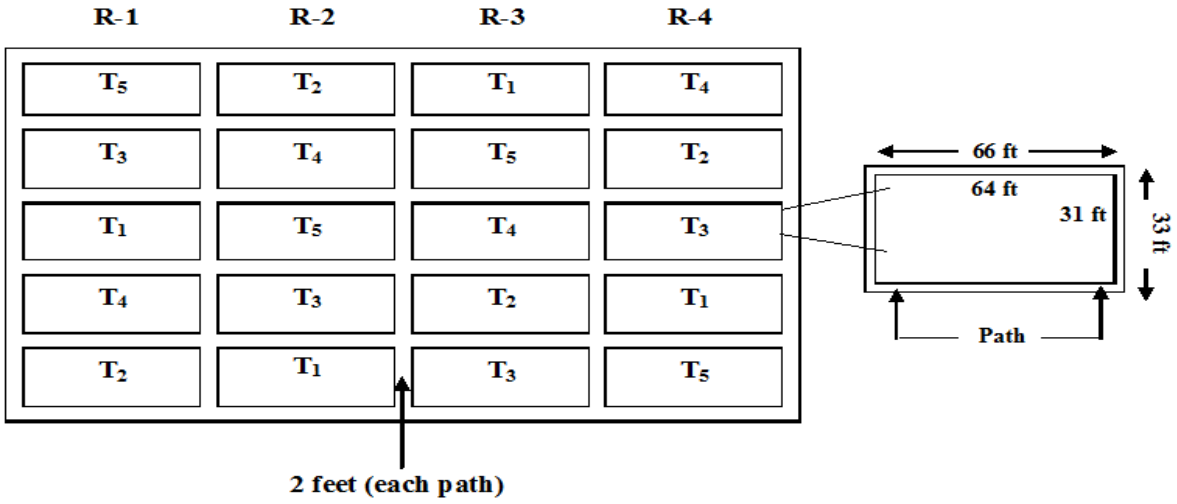
A space of 2 feet was maintained between treatments and replications for separating plots from one another, whereas a space of 18" was kept between furrow to furrow/ridge to ridge and (9" to 12") between plant to plant. Thinning, weeding, and other agronomical practices were manually done. Before sowing the cotton crop, the pre-weedicide, Stomp 455 CS (Pendimethalin), was applied to control the weeds. The full kit of the sprayer person was also maintained, like the face with the

Table 1: Synthetic and botanical insecticides used against jassid under field conditions of the cotton crop during, 2017-18.

S/No	Insecticides (Active ingredient and trade name)	Group or compound	Dose / Acre*1	Company / Distributor	Dose / Tank*2
1	Acephate 75 SP (Safate)	Organophosphate	250 g	R. B. Avari Enterprises (Pvt.) Ltd.	50 g
2	Nitenpyram 10 SL (Amrasca)	Neonicotinoid	200 ml	Agri Farm Services (Pvt.) Ltd.	40 ml
3	Boiled Neem Leaves (BNL)	Azadirachta indica	10 kg	Local hand made	2 kg
4	Neem Oil	Azadirachta indica	500 ml	Local hand made	100 ml
5	Control plot	Without the use of insecticide			

*1 Acre = 0.40 hectare, *2 Tank = 20 liters liquid container for spray

Table 2: The layout of research plot treatments (n=5) of synthetic and botanical insecticides under cotton field conditions during 2017-18.



mask, hands with gloves, whole body with clothes, and long boots/shoes as precautionary measures, spraying with the direction of the wind.

The spraying of the synthetic and botanical insecticides was done against the jassid after their economic threshold level (ETL) at one and a half ($1^1/_2$) months after the germination of the crop, as the plant bore 5-6 leaves to attain the initial pests. For checking the efficacy of insecticides, the cotton crop was sprayed with the help of a knapsack hand sprayer early in the morning to avoid a flare-up of sucking insect pests from the field conditions. For this purpose, the four sprays were done to check their proper efficacy, and finally, the comparison was done between treatments and control plots, located in the same research trial agricultural field. All the insecticides were used at their recommended doses under field conditions.

Boiled neem leaves (BNL) extract

For this research purpose, only 2 kg leaves of Neem, *A. indica,* were collected from locally grown trees and boiled in 2 liters of water. When water remained near 1 liter, it was poured into another flask and filtered with the help of a muslin cloth, and then the extract was obtained, which was sprayed on the cotton cultivated crop. The recommended botanical insecticides for the cotton crop were sprayed at 5 liters/acre as described by [29].

Neem oil extract

Usually, the neem oil was available in the local market of district Khairpur or at the Pansare store, but in this research trial, 100% pure hand-made neem oil was utilized for controlling and observing the pests. The five percent (5%) solution of neem oil was used to check the effectiveness against the jassid. For this purpose, the recommended dose (5 ml of neem oil in 1 liter of water) of approximately 100 liters of the solution was used for one acre. Due to the insolubility of neem oil in water, it was emulsified by adding 100 g of surf Excel to enhance the effectiveness of the solution [27, 30].

Sampling methods

The collection of data was done at pre-treatment (before the spray) and post-treatment (after the spray), i.e., before 24 hours and after 24 hours (h), 48 h, 72 h, 7th day, and 12th day of each spray. The population density of the jassid was counted randomly on 3 leaves (1 from the top, 1 from the middle, and 1 from the

bottom) of the plant. Overall, 20 plants were observed from a single treatment. While the reduction % of the insecticides was calculated by using Henderson and Tilton's (1955) formula [31]:

$$\text{Corrected } \% = \left(1 - \frac{\text{n in Co before Treatment} \times \text{n in T after Treatment}}{\text{n in Co after Treatment} \times \text{n in T before Treatment}}\right) \times 100$$

Where,

n = Insect population, T = Treated, and
Co = control

Statistical analysis

After the collection of data, it was subjected to analysis of variance (ANOVA), and the mean values were compared with the LSD test by using the Analytical Statistics Package 8.1 software (USA).

To observe the occurrence of jassid and natural enemies in the control plot of the cotton crop

The population of sucking pest jassid and natural enemies (predators and parasites) was recorded on the 15th day on a fortnightly interval basis after the emergence of the insects on plant leaves up to the harvesting of the cotton crop, in the control plot under field conditions. The population fluctuation of the jassid was observed per leaf, whereas the population of natural enemies (predators and parasites) was checked on the whole plant body.

To observe the impact of different synthetic and botanical insecticides on the weight of green (un-opened) bolls of cotton

After the appearance of cotton bolls in above mentioned five treatments (Table 1), on every 15th day, the 10 green (unopened) bolls were selected randomly from each treatment of synthetic and botanical sides, including the control plot. The green bolls were picked from the bottom, middle, and top, representing all boll sizes on the plant. After collection, the composite sample on an accurate scale was calibrated in grams. Dividing the weight (in grams) by the number of bolls picked gave an approximate average green boll weight per plot as described by [32]. For this purpose, the Electronic Digital Scale Model KD-160 made in Germany was kept at the Entomological Laboratory, Department of Zoology. The calculation was made for several samples taken to represent the field, from the green bolls' appearance to the harvest of the cotton crop. Finally, the comparison between treatments was observed by weighing green bolls with the effect of synthetic and botanical insecticides. Further, the information on the weight of green bolls was displayed through a different examination of the mean values.

To determine the socio-economic impact (benefit-cost ratio) of different synthetic and botanical insecticides against jassid on the cotton crop

After the opening of the matured bolls, the cotton was picked from those and kept separately for each treatment (Table 1), then weighed out to calculate the income of the different treated plots separately for synthetic and botanical insecticides, including the control plot. The calculation was made on the expenditures of all treated plots separately, and finally, the socio-economic impact (benefit-cost ratio) of different synthetic and botanical insecticides, including the control plot, was assessed to assess the net benefits of the applied synthetic and botanical insecticides against the jassid. The total expenditures incurred from cultivation to harvesting of the crop, including the costs of seed purchase, field layout preparation, sowing, thinning, weed management, irrigation, fertilizing, and harvesting. As there were no changes in the incurred costs, it remained similar for all of the treatments except for the cost of insecticides, sprayer person, and

cotton picking. Gross income was calculated for each treatment by multiplying the weight of the seed cotton by the existing price in the local market. Whereas the net income was obtained by subtracting the total expenses from gross income, and the benefit-cost ratio (BCR) was formulated by dividing net income by total expenditures [33].

Results

The research study was conducted to evaluate the efficacy of different synthetic and botanical insecticides against the sucking insect pest, jassid (*A. biguttula biguttula*) of the cotton crop under field conditions of the district Khairpur, Sindh, Pakistan because no proper study on this menacing pest was conducted since, in this tropical region. So, this research was conducted for the proper management of cotton jassid to encourage the farmers as they did not approach the suitable production of cotton. Therefore, the results of the current research are given as below:

Efficacy of various synthetic and botanical insecticides against jassid on the cotton crop at pre- and post-treatments under field conditions

It was observed that the jassid is vulnerable to the cotton crop, found underside the leaves from starting to the harvesting of the crop and damaging it by sucking the sap of the cells. That's why it was necessary to control this sucking pest with various synthetic and botanical insecticides at their recommended doses based on time intervals. For this purpose, four sprays were done.

First spray

The results of the 1st spray determined that among the different insecticides, the mean of the jassid population was (3.06) in pre-treatment of data collection when sprayed with Nitenpyram insecticide, the post-treatment data showed a reduction of (72.36%) after 24 hours (h) of spray. Therefore, after 48 h was found (85.77%), after 72 h (76.11%), on the 7th day (91.48%) and 12th day (32.52%) with the overall maximum reduction % of (71.65±10.35), followed by Acephate (60.32±4.95), Neem oil (43.27±18.11) and Boiled neem leaves (40.04±22.43), whereas; in control plot (2.32±0.34) mean of jassid population was found as shown in (Table 3). Therefore, the analysis of variance (ANOVA) showed a significant difference among all the insecticides (DF= 4, 20; F= 6.06; P= 0.001) utilized for controlling the cotton jassid at (P<0.05).

Table 3: Mean and reduction % of cotton jassid at pre- and post-treatment of different synthetic and botanical insecticides after first spray.

Insecticides	Pre-treatment	Post-treatments					Mean±SE and Reduction%±SE
		24 h	48 h	72 h	7th day	12th day	
Acephate	3.46	1	0.93	0.53	0.63	0.58	0.73±0.10
	Reduction %	59.26	64.53	72.01	63.5	42.31	60.32± 4.95 [a]
Nitenpyram	3.06	0.6	0.33	0.4	0.13	0.6	0.41±0.09
	Reduction %	72.36	85.77	76.11	91.48	32.52	71.65±10.35 [a]
BNL	2.4	1.66	0.53	0.36	0.2	0.9	0.73±0.26
	Reduction %	2.51	70.86	72.59	83.29	-29.06	40.04± 22.43[a]
Neem Oil	3.26	0.66	0.36	1	1.06	1.13	0.84±0.15
	Reduction %	71.46	85.43	43.94	34.81	-19.3	43.27±18.11[a]
Control plot	4.13	2.93	3.13	2.26	2.06	1.2	2.32±0.34 [b]

(Each value is a mean of 4 replications, means in column followed by different letters are significantly different at P≤0.05).

Nitenpyram and Acephate provided better outcomes up to the 7th day, while Neem oil reduced the jassid population up to the 3rd day.

Second spray

The findings of the 2nd spray demonstrated that the mean of the jassid population was (2.74) in the pre-treatment of data collection when Nitenpyram insecticide was applied. The post-treatment data illustrated the reduction of (82.25%) was recorded after 24 h of spray and after 48 h was found (94.78%), at 72 h (50.05%), on 7th day (84.94%), on 12th day (80.71%) with overall highest reduction % of (78.55±7.53), followed by Neem oil (74.44±5.24), Acephate (73.14±6.13), and Boiled neem leaves (57.12±16.12), while in control plot (1.69±0.25) mean of jassid population was recorded as shown in (Table 4). The ANOVA demonstrated the significant difference among all the insecticides (DF= 4, 20; F= 13.96; P= 0.001) utilized for controlling the sucking pest population at (P<0.05). Nitenpyram, Neem oil, and Acephate provided better outcomes up to the 12th day against cotton jassid.

Third spray

The results of the 3rd spray described that among the tested insecticides, the meaning of the jassid population was (2.06) at pre-treatment of Nitenpyram insecticide. The post-treatment data illustrated the reduction of (91.52%) after 24 h, (95.97%) at 48 h, (91.40%) at 72 h, (92.40%) on the 7th day, and (49.86%) on the 12th day with an overall maximum reduction % of (84.23±8.63), followed by Neem oil (74.14±7.86), Boiled neem leaves (65.01±16.24) and Acephate (50.82±12.92), whereas; in control plot (1.57±0.30) mean of jassid population was calculated as shown in (Table 5). Therefore, the ANOVA demonstrated a significant difference among all the insecticides (DF= 4, 20; F= 16.01; P= 0.001) utilized for controlling the cotton jassid population at (P<0.05). Nitenpyram and Neem oil provided better outcomes up to the 12th day, while Boiled Neem leaves and Acephate up to the 7th day after the third spray.

Table 4: Mean and reduction % of cotton jassid at pre- and post-treatment of different synthetic and botanical insecticides after second spray.

Insecticides	Pre-treatment	Post-treatments					Mean±SE and Reduction%±SE
		24 h	48 h	72 h	7th day	12th day	
Acephate	3.64	0.96	0.66	0.06	0.4	0.46	0.51±0.15
	Reduction %	61.14	74.09	96.24	67.62	66.6	73.14± 6.13 [a]
Nitenpyram	2.74	0.33	0.1	0.6	0.14	0.2	0.27±0.09
	Reduction %	82.25	94.78	50.05	84.94	80.71	78.55± 7.53 [a]
BNL	2.48	1.33	0.66	0.1	0.06	0.76	0.58±0.23
	Reduction %	20.98	61.97	90.8	92.87	19.01	57.13±16.12[a]
Neem Oil	3.6	0.8	0.13	0.46	0.33	0.46	0.44±0.11
	Reduction %	67.26	94.84	70.86	72.99	66.23	74.44± 5.24 [a]
Control plot	3.33	2.26	2.33	1.46	1.13	1.26	1.69±0.25 [b]

(Each value is a mean of 4 replications, means in column followed by different letters are significantly different at P≤0.05).

Table 5: Mean and reduction % of cotton jassid at pre- and post-treatment of different synthetic and botanical insecticides after third spray.

Insecticides	Pre-treatment	Post-treatments					Mean±SE and Reduction%±SE
		24 h	48 h	72 h	7th day	12th day	
Acephate	2.06	0.76	0.46	0.2	0.3	0.65	0.47±0.10
	Reduction %	50.44	69.07	71.33	62.01	1.24	50.82±12.92 b
Nitenpyram	2.06	0.13	0.06	0.06	0.06	0.33	0.13±0.05
	Reduction %	91.52	95.97	91.4	92.4	49.86	84.23±8.63 a
BNL	2.46	1.06	0.13	0.13	0.06	0.69	0.41±0.20
	Reduction %	42.12	92.68	84.4	93.64	12.21	65.01±16.24 ab
Neem Oil	2.63	0.53	0.26	0.46	0.06	0.26	0.31±0.08
	Reduction %	72.93	86.31	48.35	94.05	69.06	74.14±7.86 ab
Control plot	3.13	2.33	2.26	1.06	1.2	1	1.57±0.30 c

(Each value is a mean of 4 replications; means in column followed by different letters are significantly different at P≤0.05).

Fourth spray

The results of the 4th spray determined that the mean of the jassid population was (2.00) at pre-treatment when Nitenpyram insecticide was applied. The post-treatment data illustrated the reduction of (95.41%) after 24 h of spray. Therefore, after 48 h was found (97.70%), at 72 h (100.00%), on the 7th day (100.00%) and 12th day (100.00%) with the overall maximum reduction % of (98.62±0.92), followed by Neem oil (74.40±12.27), Acephate (70.01±10.05) and Boiled neem leaves (57.90±17.30), whereas; in control plot (1.43±0.23) mean of jassid population was calculated as shown in (Table 6). Therefore, the ANOVA demonstrated the significant difference among all the insecticides (DF= 4, 20; F= 20.06; P= 0.001) utilized for controlling the pest population at (P<0.05). Among these, Nitenpyram provided the best outcomes up to the 12th day, while Neem oil and Acephate up to the 7th day after spray.

Table 6: Mean and reduction % of cotton jassid at pre- and post-treatment of different synthetic and botanical insecticides after fourth spray.

Insecticides	Pre-treatment	Post-treatments					Mean ± SE and Reduction%±SE
		24 h	48 h	72 h	7th day	12th day	
Acephate	2.06	0.53	0.17	0.06	0.19	0.43	0.28±0.09
	Reduction %	60.64	87.37	91.59	74.34	36.13	70.01±10.05 b
Nitenpyram	2	0.06	0.03	0	0	0	0.02±0.01
	Reduction %	95.41	97.7	100	100	100	98.62±0.92 a
BNL	2	1	0.06	0.06	0.23	0.58	0.39±0.18
	Reduction %	23.5	95.41	91.34	68.01	11.26	57.90±17.30 b
Neem Oil	2.36	0.4	0.1	0.13	0.06	0.56	0.25±0.10
	Reduction %	74.07	93.52	84.1	92.93	27.39	74.40±12.27 b
Control plot	3.06	2	2	1.06	1.1	1	1.43±0.23 c

(Each value is a mean of 4 replications; means in column followed by different letters are significantly different at P≤0.05).

Overall sprays

The summary of the overall four sprays indicated that the maximum reduction % of (81.65±5.21) was obtained by Nitenpyram, followed by Neem oil (65.75±8.44), Acephate (64.44±6.71), and Boiled neem leaves (55.20±17.11), as compared to the control plot (1.75±0.27) with an overall mean population of jassid as shown in Figure 1. Therefore, the ANOVA demonstrated the least significant difference (LSD) among all the insecticides (DF= 4, 20; F= 17.45; P= 0.001) utilized for controlling the sucking insect pest, jassid population at (P<0.05). Among all the tested insecticides, Nitenpyram was found to be best up to the 12th day, while Neem oil and Acephate up to the 7th day. These insecticides reduced the jassid population in overall sprays and gave the best management of jassid under field conditions of the cotton crop.

Occurrence and regression analysis of jassid and natural enemies in the control plot of the cotton crop

The IPM strategy for controlling cotton jassid by natural enemies (biological control) is of great importance. For this, in the control (un-sprayed) plot, based on fortnightly (the interval of 15 days), the population of sucking pest, jassid and natural enemies (predators, and parasites) i.e., Pirate bug (*Orius insidiosus*), Ladybird beetle / Coccinellids (*Coccinella septempunctata)*, Green Lacewing (*Chrysoperla carnea*), Miridbug (*Rhabdomiris striatellus*), and Spiders were observed on randomly selected 20 plants from July to September. The overall results of the 6 fortnightly intervals showed the mean population of jassid per leaf and their natural enemies per plant such as (3.13, 1.19); (3.33, 2.02), (4.13, 2.07), (3.06, 1.38), (1.93, 0.73) and (1.56, 0.39), respectively. The maximum population of jassid and natural enemies was observed in the third fortnightly in August as shown in Figure 2. The regression analysis showed a positive correlation (88%) between the population of Jassid and natural enemies because the population fluctuation of natural enemies increases with the increase in the population of Jassid as shown in Figure 3.

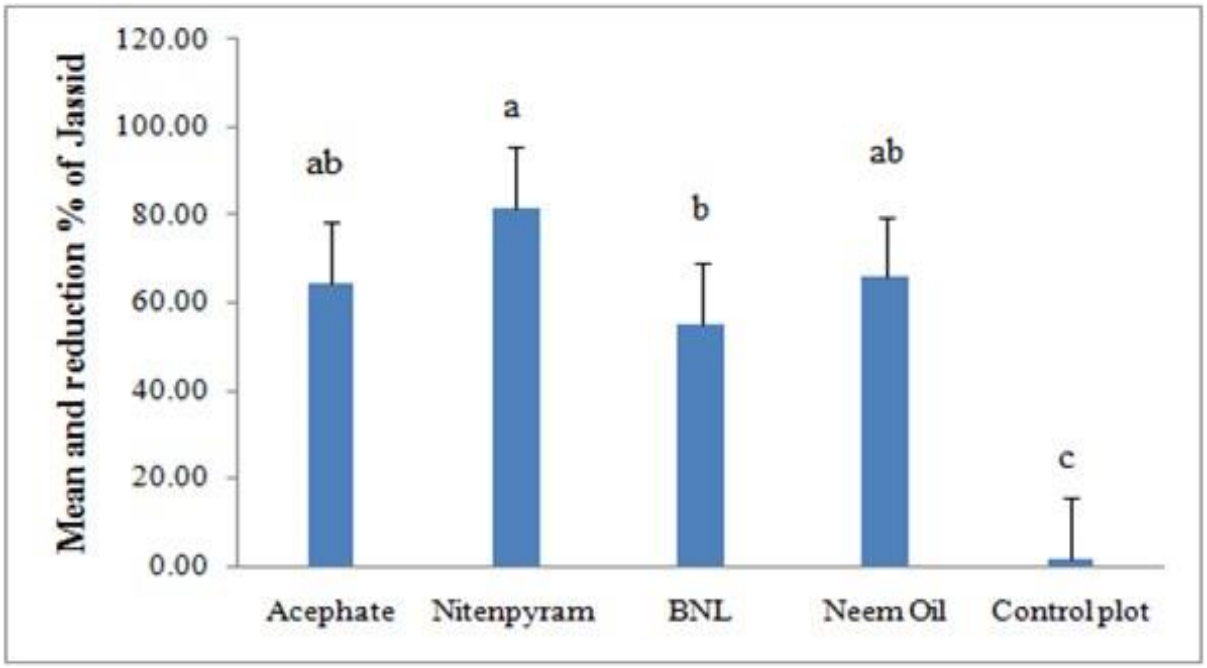


Figure 1: Overall mean and reduction % of cotton jassid at post-treatments of different synthetic and botanical insecticides under field conditions.

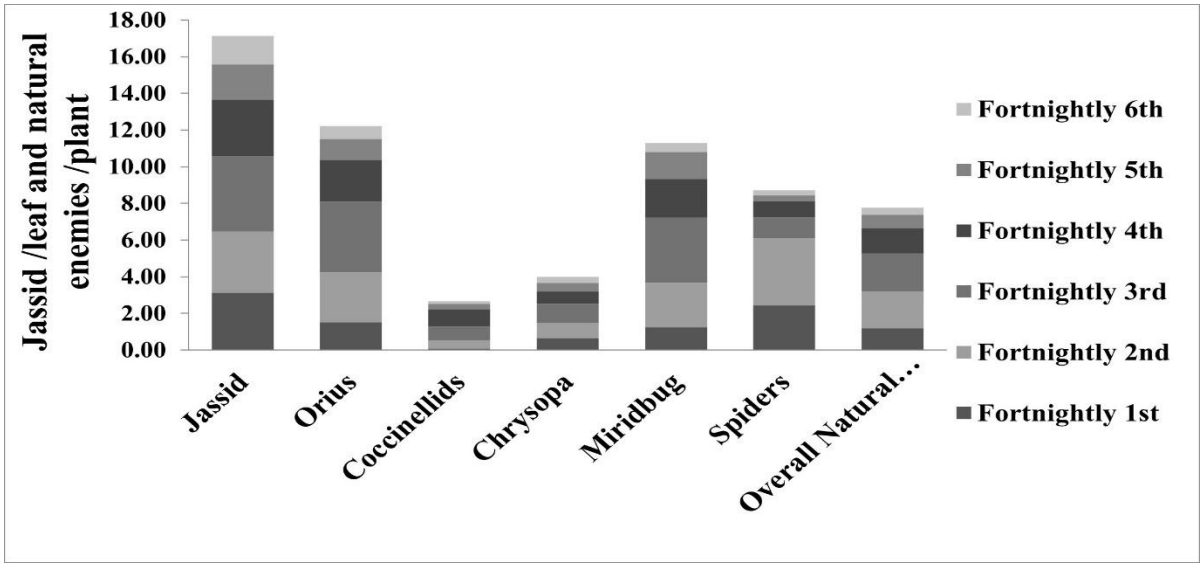


Figure 2: The population of jassid and natural enemies in control plot of the cotton crop.

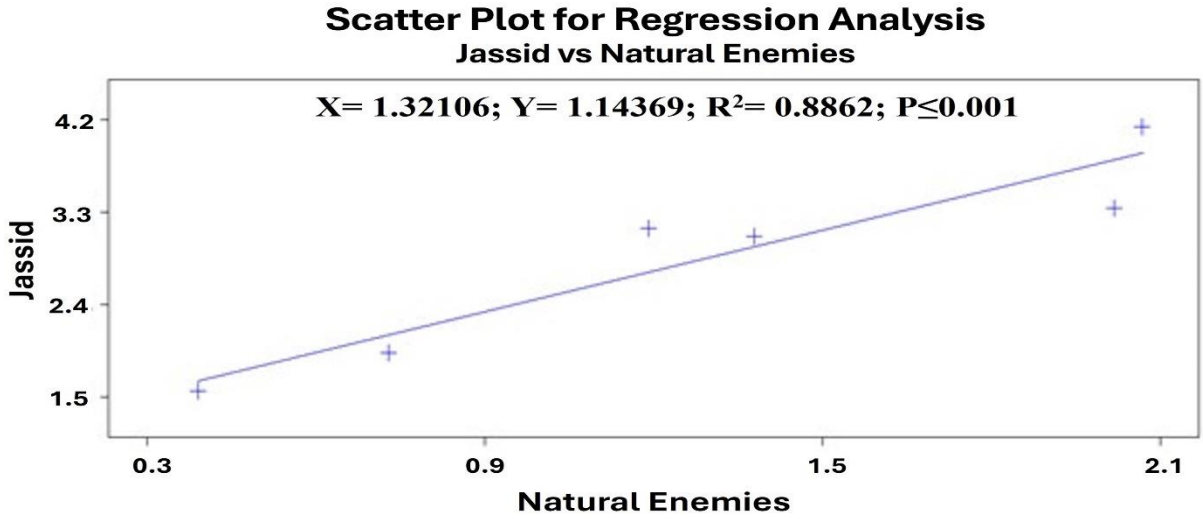


Figure 3: Regression analysis of jassid and natural enemies in the control plot of the cotton crop under field conditions.

Impact of different synthetic and botanical insecticides on the weight of green (un-opened) bolls of cotton

For checking the impact of different synthetic and botanical insecticides on the weight of green bolls of cotton under field conditions, the ten bolls were selected randomly after their appearance from each plot of synthetic and botanical side including the control plot on every 15th day from July to October. The results of their average weight were recorded in grams for each treatment, separately. The overall maximum mean of green boll weight (13.86 g) was observed in the Neem oil used plot as shown in (Table 7), followed by Boiled neem leaves (12.73 g), Nitenpyram (11.38 g), and Acephate (11.28 g) while the minimum weight was found in control plot (10.51 g). Therefore, the ANOVA indicated a significant difference (DF= 4, 35; F= 26.50; P= 0.001) among all the treatments. The botanical insecticides used plots gave overall more heavy green bolls as compared to synthetic insecticides. The maximum weight of the green boll of cotton was measured at 21 grams in the neem oil used plot. Hence botanical insecticides had a good impact on the weight of green bolls of the cotton crop.

Table 7: Weight in grams of green bolls of cotton at fortnightly interval basis.

Insecticides	Fortnightly								Mean ± S. E
	1st	2nd	3rd	4th	5th	6th	7th	8th	
Acephate	2.4	7.2	11.2	15.2	17.8	14.8	12.8	8.8	**11.28**±1.76[c]
Nitenpyram	2.6	6.2	10.4	14	18	14.8	13	12	**11.38**±1.74[c]
BNL	3	7.4	12	16	20	16	14	13.4	**12.73**±1.89[b]
Neem Oil	3.4	8.4	13.2	16.5	21	18.6	16.4	13.4	**13.86**±2.01[a]
Control plot	1.8	5.8	10	14	17	14.1	11.8	9.6	**10.51**±1.73[d]
Mean	**2.64**	**7**	**11.36**	**15.15**	**18.76**	**15.66**	**13.6**	**11.44**	**11.95**±1.83

(Each value is a mean of 4 replications, means in column followed by different letters are significantly different at P≤0.05).

The socio-economic impact (benefit-cost ratio) of different synthetic and botanical insecticides against the jassid on the cotton crop

For checking the socio-economic impact (benefit-cost ratio) of different synthetic and botanical insecticides against the sucking insect pest, jassid on the cotton crop under field conditions, the expenditures, and income of all the treatments were recorded separately as given in (Table 8) and (Table 9). The findings determined that the Neem oil was found to be more effective in terms of cotton yield, with the overall production of 230 kgs of seed cotton, followed by Boiled neem leaves 212 kgs, Nitenpyram 178 kgs, Acephate 168 kgs, and Control plot 150 kgs. While the total overall production of the five treatments was 938 kgs. The maximum BCR (benefit-cost ratio) was observed in botanical insecticides (2.74, and 2.44) as compared to synthetic insecticides (1.96, and 2.17), and the control plot (2.10). The botanical insecticides gave more production, especially the Neem oil was found to be the best in terms of cotton production.

Table 8: Overall cotton production in various synthetic and botanical insecticides' used plots of the cotton crop under field conditions.

Name of Insecticide	Treatment/plot Size in feet	Cotton Production in Kgs	Income (Kgs x 95)	Expenses	Net Profit	BCR
Acephate	2178 feet for each treatment and replicated 4 times	168	15960	5390	10570	1.96
Nitenpyram		178	16910	5340	11570	2.17
BNL		212	20140	5380	14760	2.74
Neem oil		230	21850	6360	15490	2.44
Control Plot		150	14250	4600	9650	2.1
Grand Total	**43560**	**938**	**89110**	**27070**	**62040**	**2.29**

Table 9: Overall cotton expenses in various synthetic and botanical insecticides' used plots of the cotton crop under field conditions.

Expenses\ Insecticides	**Acephate**	**Nitenpyram**	**BNL**	**Neem Oil**	**Control plot**
Plough	900	900	900	900	900
Seed	130	130	130	130	130
Pre-weedicide	220	220	220	220	220
Weeding	600	600	600	600	600
Fertilizers	1250	1250	1250	1250	1250
Insecticides	450	300	-	800	-
Sprayer Person	160	160	160	160	-
Picking	1680	1780	2120	2300	1500
Total	**5390**	**5340**	**5380**	**6360**	**4600**

Sprayer Person = 40 / Tank, Picking = 10 / Kg. (All values are in Pakistani currency Rupees).

Discussion

The efficacy of different synthetic and botanical insecticides was checked against the sucking insect pest, jassid (*Amrasca biguttula biguttula*) of the cotton crop under field conditions because this type of research was conducted first time in the region, district Khairpur. For this purpose, four experiments were conducted such as the efficacy of synthetic and botanical insecticides, the comparison of pests and natural enemies under the control plot, the impact of insecticides on green bolls' weight, and lastly the cotton production (benefit-cost ratio). The experiment first was carried out to check the efficacy of synthetic and botanical insecticides against cotton jassid from seedling up to the harvest. It appeared on the cotton plant leaves in the early stages and crossed the economic threshold level (ETL) after one and a half months of the germination of the cotton seeds. The spraying of the different synthetic and botanical insecticides was done at that time to control this notorious and nuisance-sucking insect pest, which reduced the pest population but after the 7th day increased in the following weeks up to the (4.13 / leaf) in August. The population of jassid fluctuated, decreased, and transformed till the harvesting of the crop. The results are supported by [7], who observed 6.56 jassids per leaf in August.

In this present study, Nitenpyram was found to be more effective than the other tested insecticides against sucking pests, jassid, up to the 12th day in all four sprays, followed by Neem oil, Acephate, and Boiled Neem leaves. From these, Nitenpyram and Acephate insecticides were also used by [15, 17, 18, 34]. Neem oil also gave better results against jassid up to the 7th day in all four sprays. All the tested insecticides gave better results within 48 and 72 hours after spray in reducing the jassid population as compared to the 7th and 12th day after spray. Among the synthetic insecticides, Nitenpyram gave better results than Acephate against cotton jassid under field conditions. Our results are similar to [35 - 38], who also found Nitenpyram better against cotton jassid. Among botanical insecticides, Neem oil was found to be better in suppressing cotton jassid as compared to boiled neem leaves. Our results are supported by [39 - 41], who found Neem oil more effective in controlling cotton jassid. Further supported by [42], who also found neem oil more effective against cotton jassid.

The second experiment was carried out for biological control, which is of great importance in reducing the various harmful pests. In this current research, the population of sucking insect pest jassid and natural enemies (predators and parasites)

was recorded in the control plot under field conditions. The observed natural enemies were Orius, Coccinellids, Chrysopa, Miridbug, and Spiders. The maximum mean population of predators was observed in August. Our results are similar to [43], which documented the maximum population of predators as 1.73±0.37 and the lowest as 0.19±0.08 per plant. These predators, *Coccinella spp., Chrysoperla sp.*, *Geocoris* sp., *Orius sp.,* and Spiders were actively feeding on sucking insect pests. *Chrysoperla* was found as the most frequent predator species [44 - 45]. The maximum population of *C. septempunctata* (1.42 per leaf on 10th August), Dictyna sp (0.56. per leaf on 19th September) and *S.* invicta (1.22 per leaf on 20th June) was reported [7]. They further said that pests and natural enemies completely disappeared after mid-October.

In the third experiment, the impact of different synthetic and botanical insecticides was checked out on the weight of green (un-opened) bolls of cotton, which was never done before on green bolls of cotton, and the findings showed that the botanical insecticides were better in increasing the weight of green bolls of cotton as compared to synthetic insecticides under field conditions. The maximum weight of the single green boll was recorded (21 g) in the neem oil used plot on the 5th fortnightly in August. The overall mean of the weight of green boll throughout the season was also observed in the neem oil used plot (13.86 g), followed by BNL, as compared to synthetic insecticides. Our results are similar to [46], who also used botanical pesticides and observed an increment in the weight of dry bolls and yield of seed cotton of sprayed varieties with recommended doses of *Datura alba* and nimbokil in comparison to the control plot. Our results are further supported by [47], who used Gibberellic acid (GA_3) treatments in 2011 and found increased weight in sprayed berry vines as compared to unsprayed vines. The maximum berry weight was observed (2.83 g) at the dose of 25 ppm on other plants.

The fourth experiment was performed at the time of the harvesting of the crop. In which the production of different plots treated with synthetic and botanical insecticides was compared. The expenditures and income of all the synthetic and botanical treatments, including the control, were recorded separately. The botanical insecticides were found to be better than synthetic insecticides, i.e., Neem oil was recorded as a more effective insecticide in terms of cotton yield, with an overall production of 230 kgs of cotton as compared to the other 4 treatments. Our results are in line with investigated the extracts of eight indigenous plants against sucking insect pests on okra and found maximum pod yield (3178.7 kg/ha) in neem-treated plots [48]. Further, [49] observed the maximum yield in plots treated with 1.5% and 2% neem oil as compared to that in the control. [50] found a maximum insecticidal Cost-Benefit Ratio (1: 16.54) by imidacloprid, followed by acetamiprid (11.06), acephate (10.38), thiamethoxam (7.05), and diafenthiuron (6.13). Further maximum cost-benefit ratio was recorded (18.67) at one jassid leaf^{-1} in field conditions of cotton.

Conclusion

It was concluded that the sucking insect pest, jassid (*A. biguttula biguttula*), was notably present on the cotton crop's green leaves from its appearance until harvest. Among all the tested insecticides, Nitenpyram and Neem oil proved to be the most effective in controlling this insect pest. Both synthetic and botanical insecticides demonstrated effective control of cotton jassid. Additionally, in the control plot, the observed natural enemies, such as predators and parasites, were found to be effective against cotton jassid. Botanical insecticides were also found to enhance the weight of green bolls and, consequently,

increase cotton production. This study concludes that neem derivatives serve as a promising alternative to synthetic insecticides for controlling the sucking insect pest, cotton jassid (*A. biguttula biguttula*).

Future prospects

The present study suggests that more research work should be carried out on the techniques for long-lasting storage of botanical insecticides and residual effects on insects.

Conflict of interest

All authors have declared that there is no conflict of interest regarding the publication of this article.

References

1. Sahito, H.A., Lanjar, A.G., Nahiyoon, A.A., Khajjak, A.S., Memon, S.A. & Mal, B. Seasonal occurrence of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) and its natural enemies on different varieties of cotton crop. Pakistan Journal of Entomology. 2011; 26(1): 17-24.
2. Ramesh, Krishnamurthy, K.N., Shilpa M., Maruthi, Sharanabasappa & Channabasava. ARIMA models for forecasting production of cotton crop in selected districts of Karnataka. The Pharma Innovation Journal. 2022; SP-11(3): 535-546.
3. Khamidov, M. & Khamraev, K. Water-saving irrigation technologies for cotton in the conditions of global climate change and lack of water resources. In IOP Conference Series: Materials Science and Engineering. 2020; 883(1): 012077.
4. Ozyigit, I.I., Kahraman, M.V. & Ercan, O. Relation between explant age, total phenols, and regeneration response in tissue cultured cotton (*Gossypium hirsutum* L.). African Journal of Biotechnology. 2007; 6(1): 003-008.
5. Kannan, M., Uthamasamy, S. & Mohan, S. Impact of insecticides on sucking pests and natural enemy complex of transgenic cotton. Current Science. 2004; 86(5): 726-729.
6. GoP, (Government of Pakistan). The Karachi Cotton Association: Executive Committees' Report 2017-18. Karachi: The Cotton Exchange, I.I. Chundrigar Road. 2018.
7. Ashfaq, S., Khan, I.A., Saeed, M., Saljoqi, A.U.R., Manzoor, F., Sohail, K., Habib, K. & Sadozai, A. Population dynamics of insect pests of cotton and their natural enemies. Sarhad Journal of Agriculture. 2011; 27(2): 251-253.
8. Pradeepa, S. & Regupathy, A. Generating baseline data for insecticide resistance monitoring in cotton leafhopper, *Amrasca devastans* (Distant). Resistance Pest Management Newsletter. 2002; 11(2): 4-6.
9. Ali, M., Ashfaq, M., Akram, W., Sahi, S.T., & Ali, A. The physio-morphic characters of the brinjal (*Solanum melongena* L.) plant and their relationship with the jassid (*Amrasca biguttula* (Ishida)) population fluctuation. Pakistan Journal of Agricultural Sciences. 2012; 49(1): 67-71.
10. Sahu, B.K. & Samal, I. Sucking pest complex of cotton and their management: A review. The Pharma Innovation Journal. 2020; 9(5): 29-32.
11. Gerling, D., Alomar, Ò. & Arnò, J. Biological control of *Bemisia tabaci* using predators and parasitoids. Crop Protection. 2001; 20(9): 779-799.
12. Kumar, R., Tian, J.C., Naranjo, S.E. & Shelton, A.M. Effects of Bt Cotton on *Thrips tabaci* (Thysanoptera: Thripidae) and Its Predator, *Orius insidiosus* (Hemiptera: Anthocoridae). Journal of Economic Entomology. 2014; 107(3): 927-932.
13. Amjad, M., Bashir, M.H. & Afzal, M. Comparative resistance of some cotton cultivars against sucking insect pests.

Pakistan Journal of Life and Social Sciences. 2009; 7(2): 144-147.
14. Ashfaq, M., ul Ane, M.N., Zia, K., Nasreen, A. & Hasan, M. The correlation of abiotic factors and physico-morphic characteristics of (*Bacillus thuringiensis*) Bt transgenic cotton with whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) and jassid, *Amrasca devastans* (Homoptera: Jassidae) populations. African Journal of Agricultural Research. 2010; 5(22): 3102-3107.
15. Sahito, H.A., Shah, Z.H., Ruk, M., Shah, M.Z. & Mangrio, W.M. Toxicant efficacy of some insecticides against whitefly, *Bemesia tabaci* under cotton field conditions at Khairpur-Sindh. Academic Journal of Entomology. 2015; 8(4): 193-200.
16. Abbas, G., Ahmed, A., Amer, M., Abbas, Z. & Rehman, M. Comparative efficacy of pesticides against sucking insects pest of cotton (*Gossypium hirsutum* L.) Crop under arid conditions. Science International. 2016; 28(3): 2679-2682.
17. Shah, Z.H., Sahito, H.A., Shar, G.A., Kousar, T., Mangrio, W.M. & Kanhar, K.A. Toxicity of different insecticides against mealybug, *Phenacoccus solenopsis* (Tinsley) under cotton field conditions. Pakistan Journal of Entomology. 2016; 31(1): 39-50.
18. Sahito, H.A., Shah, Z.H., Kousar, T., Mangrio, W.M., Mallah, N.A., Jatoi, F.A. & Kubar, W.A. Comparative efficacy of novel pesticides against jassid, *Amrasca biguttula biguttula* (Ishida) on cotton crop under field conditions at Khairpur, Sindh–Pakistan. Singapore Journal of Scientific Research. 2017; 1-8.
19. Sahito, H.A., Kousar, T., Mangrio, W.M., Mal, B., Mastoi, A.H., Jamali, Y.A., Jatoi, F.A. & Abro, A.H. Comparative Crop Production and Protection Costs of Cotton Crop under Different Pest Management Strategies of Cotton Mealybug, *Phenacoccus Solenopsis* (Tinsley). Annals of Romanian Society for Cell Biology. 2022; 26(1): 763-775.
20. Sanghi, A.H., Aslam, M., Khalid, L. & Javed, S. To investigate the comparative efficacy of insecticides against cotton jassid *Amrasca devastans* (Distant) (Cicadellidae: Homoptera) under field conditions of Rahim Yar Khan. Pakistan Journal of Entomology. 2013; 28(1): 1-6.
21. Aslam, M., Razaq, M., Shah, S.A. & Ahmad, F. Comparative efficacy of different insecticides against sucking pests of cotton. Journal of Research Science. 2004; 15(1): 53-58.
22. Popp, J., Pető, K. & Nagy, J. Pesticide productivity and food security. A review. Agronomy for sustainable development. 2013; 33: 243-255.
23. Lengai, G.M.W. & Muthomi, J.W. Biopesticides and their role in sustainable agricultural production. Journal of Biosciences and Medicines. 2018; 6(6): 7-41.
24. Akhtar, M. Nematicidal potential of the neem tree *Azadirachta indica* (A. Juss). Integrated Pest Management Reviews. (2000); 5(1): 57-66.
25. Muhammad, A. & Kashere, M.A. NEEM, *Azadirachta indica* L. (A. Juss): An Eco-Friendly Botanical Insecticide for Managing Farmersâ€™ Insects Pest Problems-A Review. FUDMA Journal of Sciences. 2020; 4(4): 484-491.
26. Diaz, G.E., Collado, J.L., Jimenez, J.A.V., Acosta, F.O., Colina, G.O. & Diaz, E.C. Azadirachtin concentration, insecticide efficacy and phytotoxicity of four neem *Azadirachta indica* A. Juss. extracts. Agrociencia (Montecillo). 2010; 44(7): 821-833.
27. Fiaz, M., Hameed, A., ul Hasan, M. & Wakil, W. Efficacy of plant extracts on some cotton (*Gossypium hirsutum*) pests: *Amrasca biguttula biguttula* Ishida and *Thrips tabaci* Lindeman. Pakistan Journal of Zoology. 2012; 44(1): 277-283.

28. Noonari, A.M., Abro, G.H., Khuhro, R.D. & Buriro, A.S., Efficacy of bio-pesticides for management of sucking insect pests of cotton, *Gossipium hirsutum* (L.). Journal of Basic and Applied Sciences. 2016; 12: 306-313.
29. Ali, S.S., Ahmed, S.S., Rizwana, H., Bhatti, F., Khoso, A., Mengal, M.I., Jatoi, J.H., Bugti, A., Rind, M.A. & Shahwani, S.A. Efficacy of different bio-pesticides against major sucking pests on brinjal under field conditions. Journal of Basic and Applied Sciences. 2017; 13: 133-138.
30. Patham, I.K. Protocols for using botanical extracts. Society for Elimination of Rural Poverty. Community Managed Sustainable Agriculture Supported by Farmers, Women's Groups, and NGOs. 2011; 1-18.
31. Henderson, C.F. & Tilton, E.W. Tests with acaricides against the brown wheat mite. Journal of Economic Entomology. 1955; 48(2): 157-161.
32. Boman, R. Estimating cotton yield using boll counting. *Cotton*. Okstate. Edu; OSU Southwest Research and Extension Center: Altus, OK, USA. 2012; pp. 1-4.
33. Biswas, G.C. Incidence, damage potential and management of jassids in groundnut field. Bangladesh Journal of Agricultural Research. 2015; 40(3): 507-512.
34. Shah, Z.H., Sahito, H.A., Kousar, T., Rind, M.M., Jatoi, F.A. & Mangrio, W.M. Integrated Pest Management of Cotton Thrips, *Thrips tabaci* (Lindeman, 1889) through Selected Pesticides under Vitro Conditions. International Journal of Research Studies in Zoology. 2017; 3(4): 76-83.
35. Karar, H., Babar, T.K., Shahazad, M.F., Saleem, M., Ali, A. & Akram, M. Performance of novel vs. traditional insecticides for the control of *Amrasca biguttula biguttula* (Hemiptera, Cicadellidae) on cotton. Pakistan Journal of Agricultural Sciences. (2013); 50(2): 223-228.
36. Baker, M.A., Makhdum, A.H., Nasir, M., Imran, A., Ahmad, A. & Tufail, F. Comparative efficacy of synthetic and botanical insecticides against sucking insect pest and their natural enemies on cotton crop. Journal of Mountain Area Research. 2016; 1(1): 1-4.
37. Nawaz, S., Razaq, M., Sarwar, Z.M., Sajjad, M., Ubaid, S.A., Zulfiqar M.A. & Haider, U. Determination of economic threshold level (ETL) of jassid, *Amrasca biguttula biguttula* (Cicadellidae: Homoptera). Pakistan Journal of Agriculture Research. 2019; 32: 28-32.
38. Hafeez, F., Aslam, A., Iftikhar, A., Naeem, A., Akhtar, M.F. & Saleem, M.J. Field evaluation and economic assessment of different insecticides against cotton jassid, *Amrasca devastans* Dist. Journal of Innovative Sciences. 2020; 6(1): 24-29.
39. Khattak, M.K., Mamoon-ur-Rashid, Hussain, S.A.S. & Islam, T. Comparative effect of neem (*Azadirachta indica* A. Juss) oil, neem seed water extract and Baythroid TM against whitefly, jassids and thrips on cotton. Pakistan Entomologist. 2006; 28: 31-37.
40. Khan, M.H., Ahmad, N., Rashdi, S.M.M., Rauf, I., Ismail, M. & Tofique, M. Management of sucking complex in Bt cotton through the application of different plant products. Pakistan Journal of Life Sciences. 2013; 1(1): 42-48.
41. Rajput, L.B., Gilal, A.A., Bukero, A. & Wahocho, N.A. Impact of Different Oils as Biopesticides against Sucking Insect Pests in Cotton. Journal of Basic and Applied Sciences. 2017; 13: 34-40.
42. Asif, M.U., Muhammad, R., Akbar, W., Sohail, M., Tariq, J.A. & Ismail, M. Comparative efficacy of Neem derivatives and imidacloprid against some cotton pests. Journal of Entomology and Zoology Studies. 2018; 6: 113-117.

43. Sahito, H.A., Abro, G.H., Mahmood R. & Malik, A.Q. Survey of mealybug, Phenacoccus solenopsis (Tinsley) and effect of bio-ecological factors on its population in different ecological zones of Sindh. Pakistan Journal of Agriculture Agricultural Engineering and Veterinary Sciences. 2011b; 27: 51-65.
44. Syed, A.N., Ashfaq, M. & Khan, S. Comparison of development and predation of *Chrysoperla carnea* (Neuroptera: Chrysopidae) on different densities of two hosts (*Bemisia tabaci*, and *Amrasca devastans*). Pakistan Entomologist. 2005; 27(1): 41-44.
45. Colmenarez, Y., Gibbs, I.H., Ciomperlik, M. & Vasquez, C. Biological control agents of cotton pests in Barbados. Entomotropica. 2016; 31(18): 146-154.
46. Khan, M.A., Rashid, A. & Chohan, R.A. Biological control of bacterial blight of cotton using some plant extracts. Pakistan Journal of Agricultural Sciences. 2000; 37: 3-4.
47. Shah, S.U.S., Khan, A., Khan, M.A., Farooq, K., Riaz Chattha, M., Javed, M.A., Gurmani, Z.A., Hussain, A. & Iftikhar, M. Effects of gibberellic acid on growth, yield and quality of grape cv. *Perlet*, International Journal of Biology and Biotechnology. 2015; 12(3): 499-503.
48. Iqbal, J., Ali, H., Hassan, M.W. & Jamil, M. Evaluation of indigenous plant extracts against sucking insect pests of okra crop. Pakistan Entomologist. 2015; 37(1): 39-44.
49. Rashid, M.M., Khattak, M.K. & Abdullah, K. Evaluation of botanical and synthetic insecticides for the management of cotton pest insects. Pakistan Journal of Zoology. 2012; 44: 1317-1324.
50. Bharpoda, T.M., Patel, N.B., Thumar, R.K., Bhatt, N.A., Ghetiya, L.V., Patel, H.C. & Borad, P.K. Evaluation of insecticides against sucking insect pests infesting Bt cotton BG-II. The Bioscan. 2014; 9(3): 977-980.