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RESEARCH ARTICLE

MOLECULAR CHARACTERIZATION OF COTTON JASSID AND THEIR RESPONSE AGAINST RELATIVELY NEWER PESTICIDES IN TWO COTTON VARIETIES OF BANGLADESH

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ABSTRACT

Cotton, known as the "monarch of fibers" for meeting global textile demands, faces a decline in production due to natural threats like the cotton jassid insect. In Bangladesh, where this insect has caused production losses of 20% to 50%, government support for upland cotton cultivation is challenged. Two experiments at BSMRAU's entomological field in 2021-2022 aimed to address this issue. The first confirmed the presence of the jassid through molecular analysis, while the second studied how different cotton varieties responded to biopesticides. Three different treatment combinations using different dosages of biorational insecticides on CB-12 and CB-14 cotton varieties were implied to see the interactions for revealing the biopesticidal effects on species cotton cultivars. The study identified the cotton jassid insect by analyzing its mitochondrial DNA, targeting cytochrome oxidase subunit-1 gene. The resulting nucleotide sequences were assigned accession numbers ranging from OR362770 to OR362772. A phylogenetic analysis further confirmed the insect's identity as the cotton jassid, with strong support indicated by a 94% bootstrap value. This study delves into the impact of various treatments and different cotton plant varieties on chlorophyll and anthocyanin levels in parts of plants infested by the jassid. Principal component analysis (PCA) demonstrated the significant role of two environmentally-friendly insecticides in controlling the cotton jassid across two distinct cotton varieties. Notably, there were significant differences observed in chlorophyll and anthocyanin levels among the treatments and cotton varieties. Variety CB-12 consistently exhibited higher levels of chlorophyll and anthocyanin, suggesting it may possess a greater resilience to pests. These findings underscore the importance of selecting appropriate cotton varieties and employing effective treatment strategies to manage jassid infestations and enhance crop productivity. This research provides valuable insights for the promotion of sustainable cotton cultivation for supporting textile industry in Bangladesh.

KEYWORDS

Anthocyanin, biopesticide, Cotton cultivars, chlorophyll, PCA, phylogeny

1. INTRODUCTION

Cotton is considered to be the “king of fibre”, and commercially grown in more than 50 countries of the world. In the 2021 calendar year, Bangladesh imported 8.5 million bales of cotton spending more than $3 billion. This year in 2023, Bangladesh, followed by China, has become the second largest importer of cotton. US-based international cotton trade analyst Cotton Connect has conducted several studies on cotton production potentialities in Bangladesh. The studies suggest that Bangladesh can possibly increase cotton production from 150,000 to 1 billion bales minimum.

Cotton productivity and quality are influenced by factors such as climate, crop variety, pest infestation, harvest frequency, and ginning processes (Azad et al., 2011). To support the textile industry's demand for cotton fiber in Bangladesh, the government has been providing subsidies for upland cotton cultivation (*Gossypium hirsutum* L.) since 1977. However, cotton farmers have shown reluctance to engage in cotton farming due to issues related to insect pests. These pests encompass a range of organisms, including insects, mites, nematodes, and others, which can diminish the quality and yield of cotton crops. They pose a significant threat to cotton production, leading to both reduced crop yields and quality. Among these biotic stresses, the cotton jassid (*Amrasca biguttula*) stands out as a major pest that inflicts significant damage on cotton crops (Jaber and Ownley, 2018).

Cotton jassid is a polyphagous pest that feeds on various plant species, including cotton, okra, and hibiscus, and has a wide geographical distribution. The pest causes damage by piercing and sucking the sap from the plants, which leads to stunted growth, leaf curling, and ultimately reduced yield. Cotton jassid infestation has been reported in many cotton-growing countries, including India, Pakistan, China, Egypt, and the United States (Lopez and Sword, 2015). The pest is known to cause yield losses ranging from 20% to 50%, depending on the severity of the infestation and the cotton cultivar. In addition, cotton jassid infestation can also increase the susceptibility of cotton plants to other diseases, such as cotton leaf curl virus (CLCuV). Management of cotton jassid infestation is challenging due

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to the pest's ability to rapidly develop resistance to insecticides (Farooq et al., 2011).

Genetic analysis serves as a valuable tool for understanding how genes are inherited and determining the number of genes involved in a particular mechanism. To decipher the genetics of traits, researchers commonly employ segregating populations. In modern species classification and identification, the mitochondrial cytochrome C oxidase subunit I (COX1) gene region is frequently used as a genetic marker (Zhao et al., 2014). DNA-based methods are generally considered faster and more precise than alternative techniques and are applicable to all cell types.

Controlling cotton pests involves a range of strategies, including cultural practices such as crop rotation, the cultivation of resistant cultivars, and effective irrigation management. Chemical and biological control methods are also employed. Synthetic pesticides, used in chemical control, are the predominant means of managing cotton pests but have raised concerns due to their potential environmental and human health impacts (Roy, 2016). Overreliance on chemical pesticides has led to the development of pest resistance in jassid populations and adverse effects on both the environment and human health. Biological control, which involves the use of natural enemies like parasitoids and predators, has been explored as an alternative to chemical pesticides. However, its effectiveness can be influenced by factors such as the availability of natural enemies and environmental condition (Allegrucci et al., 2017). Neonicotinoids are considered a promising option due to their lower toxicity to mammals, reduced issues with pest resurgence, environmental friendliness, selectivity in pest management, and minimal harm to natural enemies.

Microbial control involves the use of various microorganisms such as bacteria, fungi, viruses, and nematodes to control pest populations. These microorganisms have specific modes of action and are highly target-specific, making them an effective and safe alternative to chemical insecticides. Mietkiewski observed spectacular mortality of *Galleria mellonella* larvae occurred due to *B. bassiana*, applied to soils (Mietkiewski et al., 1997). Several studies have reported the use of entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* for the control of jassids. Among these fungi, *Beauveria bassiana* has garnered significant attention for its effectiveness against various insect pests, including jassids. These fungi work by infiltrating the insect's outer covering, germinating inside their bodies, ultimately leading to their demise[9]. Furthermore, they have the potential to hinder jassids' reproductive capabilities, further reducing their population. Research by Mantzoukas revealed that applying *B. bassiana* during the early stages of a jassid infestation is more efficient than later applications (Mantzoukas et al., 2015). Importantly, these fungi pose minimal harm to non-target organisms, are biodegradable, highly specific to insects, and are considered safe for both the environment and human health. In laboratory and greenhouse settings, Imidacloprid and other neonicotinoids have been found to enhance the efficacy of *B. bassiana* against white grub (*Popillia japonica*) larvae, although this synergy may not extend to field conditions (Morales- Rodriguez and Peck, 2009).

In Bangladesh, there have been limited studies on the use of *B. bassiana* for cotton jassid control. Its potential as a microbial control agent against cotton jassid in Bangladesh is promising *B. bassiana* has demonstrated effectiveness in managing jassids across various agricultural scenarios, showcasing its versatility and adaptability. Utilizing *B. bassiana* as a biological control agent offers several advantages over traditional chemical insecticides. One study even reported a significant reduction in jassid populations and an increase in cotton yields when employing *B. bassiana* (Rondot and Reineke, 2018). Nevertheless, there remains a need for further research to optimize the application of *B. bassiana* within the context of cotton farming in Bangladesh and to develop more sustainable and environmentally friendly pest management practices for the country's cotton industry.

In this study, our primary objectives were threefold. Firstly, we aimed to determine the identity of the cotton jassid through a comprehensive analysis of mitochondrial DNA. Secondly, we sought to investigate and quantify the effectiveness of two distinct biopesticides in mitigating the prevalence of jassid infestations in cotton crops. Lastly, we endeavored to uncover the varying responses of different cotton varieties to jassid infestations when treated with these biopesticides. These objectives collectively contribute to a deeper understanding of cotton jassid management strategies and provide valuable insights for sustainable cotton cultivation practices.

2. Materials and Methods

The study comprised two distinct experiments conducted in the Department of Entomology at Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. The initial experiment was dedicated to the molecular confirmation of the Jassid species present in the experimental field. This involved the meticulous collection of Jassid specimens using standardized sampling techniques, followed by DNA extraction, PCR amplification of a specific mitochondrial DNA region (COI gene), sequencing of amplified DNA fragments, and subsequent sequence analysis by comparing them to reference sequences in the NCBI GenBank database.

In contrast, the second experiment was designed to investigate how two different cotton varieties, CB-12 and CB-14, responded to the application of biopesticides. The experimental setup included controlled plots for each cotton variety, the application of selected biopesticides (Imidacloprid and *B. bassiana*) at recommended dosages and timings, regular monitoring of Jassid populations, and the quantification of chlorophyll and anthocyanin content in the cotton plants. Statistical data analysis was employed to evaluate the effectiveness of the biopesticides and assess varietal responses. The study was conducted over ten months, from July 2021 to April 2022, in a subtropical climate with specific soil characteristics. The experimental field underwent meticulous land preparation, including soil conditioning and leveling, followed by the application of recommended manure and fertilizer doses.

The experimental design involved a randomized complete block design (RCBD) with three replications, subdividing the field into 18 plots. The three treatments, including a control, were applied to the two cotton varieties to assess their efficacy in managing Jassid infestations. The research encompassed essential agricultural practices such as seed collection, treatment, intercultural operations, irrigation, and weeding to ensure healthy crop growth. Harvesting was conducted in five pickings, with cotton bolls harvested at their peak ripeness. Overall, the comprehensive methodology employed in this study provides a robust foundation for reliable and replicable research findings in the field of cotton Jassid management and varietal responses to biopesticides.

2.1 Monitoring and Collection of Cotton Jassid

All plants in the plots were closely observed every day for the purpose of studying the incidence of Jassid. The jassids were collected from experimental site. The occurrence of pests was observed through visual search in all plots and their number per plot was noted. For recording Jassid incidence, underside of leaves was considered and number of Jassid per selected leaves. At this stage, the observation was made once a week. The collected specimens were preserved in 99.9% Ethanol prior to DNA extraction.

2.2 Molecular Identification Of Jassid Species Collected From Experimental Site.

The molecular analyses were conducted in the Advanced Entomology Laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. The molecular identification was done by the following method:

2.3 Extraction of DNA

Genomic DNA was extracted from recently collected jassid specimens by *using QAGEN DNeasy Blood and Tissue kit,* following the manufacturer's instructions. Samples were vortexed with adding 180 µl Buffer ATL and 20 µl proteinase K. Sample was incubated at 55° C for 48 hours. DNA extraction was completed by adding two wash buffer AW1 and AW2 with Buffer AE and lysis buffer AL incorporated with elusion buffer AE, as per manufacturer instruction. All centrifugation steps were completed at room temperature. The colony mates of the specimens used for DNA analysis were preserved in the Advanced Entomology Laboratory, BSMRAU after DNA extraction

2.4 PCR (Polymerase Chain Reaction)

Amplification of DNA was done by polymerase chain reaction (PCR) *TaKaRa Ex Taq* PCR kit, according to the manufacturer's instructions. The kit contains 10x *Ex Taq* Buffer (20 mM Mg^{2+} plus) and dNTP mixture (2.5 mM each). The storage buffer contains 20 m MTris-HCl (pH 8.0), 100

mMKCl, 0.1 mM EDTA, 1mM DTT, 0.5 % Tween 20, 0.5 % Nonidet P-40 and 50 % glycerol. dNTP mixtures contain TAPS, KCl, $MgCl_2$, DTT, dATP, dGTP, dCTP with activated salmon sperm DNA. Reaction mixtures for PCR for consisted of *TaKaRa Ex Taq* (0.25 µl), 10x Ex Taq Buffer (5 µl), dNTP mixture (4 µl), a pair of oligonucleotide primers (0.2-1.0 µM). For conducting the PCR, PCR machine (GTQ-Cycler 96) was used from the Department of Horticulture of BSMRAU. The detailed primer configuration and PCR status are presented in Table 1 and Figure 1 respectively. (Zhao et al., 2014).

Table 1: Primer Name and Corresponding Position

Region	Name	Direction	Sequence (5'-3') [a]	Position	Annealing Temperatu-re(°C)
Mitochondrion Cytochrome oxidase Subunit -1	CO1 1-3[b]	Forward	ATAATTTTTTTTATAGTTATACC	1981-2002	54°C
	CO1 2-4[b]	Reverse	TCCTAAAAAATGTTGAGGAAA	3063-3083	

[a] (1) Crozier *et al*., 1994, [b] Used for both PCR and sequence

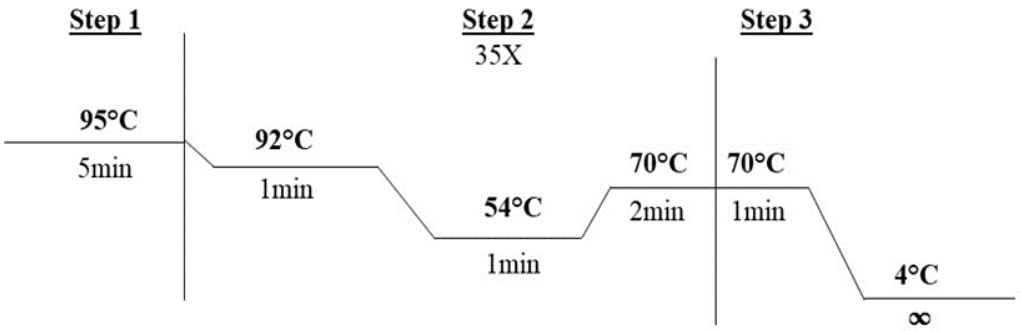

Figure 1: Operational Status of PCR for COI Gene

2.5 Sequencing And Submission To NCBI Genbank, USA For Accession

The PCR products were sequenced by using the facilities from GENEWIZ, (Azanta life science from China). Upon completing all the steps as gel electrophoresis, Exostar, cycle sequencing and ethanol precipitation, the nucleotide sequence data have been received. The obtained sequences were then submitted to the National Center for Biotechnology Information (NCBI), USA for receiving the accession number. Upon receiving the accession numbers from NCBI of all the bee samples, the nucleotide sequence data have been processed for further analysis.

2.6 Measurement Of Biochemical Content Of Healthy And Infested Cotton Leaf

In our study, the measurement of biochemical content in both healthy and infested cotton leaves played a crucial role in assessing the impact of pest infestations on plant health. We focused on several key biochemical parameters to make comparisons between the two conditions.

Firstly, we determined the chlorophyll content in the leaves. This involved taking fresh leaf samples weighing 200 mg and placing them in small vials containing 5 ml of 80% acetone, which were then covered with aluminum foil and kept in the dark for 24 hours. The supernatant was adjusted to a final volume of 10 ml, and the absorbance of the extract was measured at 470, 645, and 663 nm using a UV-visible spectrophotometer. We used 80% acetone as a blank for reference. The chlorophyll content, including both chlorophyll-a and chlorophyll-b, as well as carotenoid content, was calculated using specific equations and expressed as milligrams per gram of fresh weight (mg/g FW) based on the methodology outlined by Lichtenthaler in 1987.

Additionally, we assessed the anthocyanin content in the leaves using a spectrophotometric approach, following the pH differential method as described by Lee et al. in 2005. This method involved using two buffer systems, potassium chloride buffer (pH 1.0, 0.025 M) and sodium acetate buffer (pH 4.5, 0.4 M). Leaf samples weighing 0.2 grams were ground with 1 ml of extraction buffer (a mixture of methanol, water, and concentrated HCl solution), followed by centrifugation at 14,000 rpm. The supernatant was then mixed with the respective buffers, and absorbance measurements were taken at 510 nm and 700 nm wavelengths using a UV-visible spectrophotometer. Anthocyanin content was quantified using a specific formula.

To analyze our data, we employed rigorous statistical methods. Molecular nucleotide sequences were analyzed using MEGA 11 software. The recorded biochemical data were systematically compiled and organized for statistical analysis, and all statistical procedures were conducted using computer software programs, particularly the statistical R package. This comprehensive approach ensured the reliability and scientific rigor of our research findings, allowing for a detailed understanding of the biochemical responses of cotton leaves to pest infestations and the molecular identification of the involved species.

3. Results

In this chapter, the findings of the present study are presented through two distinct sections. The initial portion of the experiment was dedicated to verifying the Jassid species on a molecular level, while the latter part concentrated on assessing how two different cotton varieties responded to biopesticide treatments.

3.1 Molecular identification of *Amrasca biguttula biguttula*

The identification of Cotton jassid species was done by mitochondrial DNA analysis of *cytochrome oxidase subunit-1* gene. The nucleotide sequences were submitted to NCBI GenBank, USA and received the accession number.

Table 2: Sampled Cotton jassid with assigned nucleotide accession number from NCBI GenBank

SI No.	Name	GenBank Accession No
01	Amrasca biguttula voucher	OR362770
02	Amrasca biguttula voucher	OR362771
03	Amrasca biguttula voucher	OR362772

3.2 Phylogenetic Study Of *Amrasca Biguttula Voucher*

The phylogenetic analysis of the Jassid sample from BSMRAU was conducted using MEGA 11 software, as detailed by Tamura (Tamura et al., 2004). The evolutionary history was inferred through the application of the Neighbor-Joining method, as originally described by Saitou and Nei (Saitou and Nei, 1987). To assess the robustness of the phylogenetic tree, a bootstrap test with 500 replicates was performed, and the percentage of replicate trees in which the associated taxa clustered together is indicated adjacent to the branches, following Felsenstein's work (Felsenstein, 1985). Evolutionary distances were calculated using the Maximum Composite Likelihood method, based on Tamura's methodology (Tamura et al., 2004). Ambiguous positions in each sequence pair were eliminated, using the pairwise deletion option, resulting in a final dataset comprising a total of 487 positions.

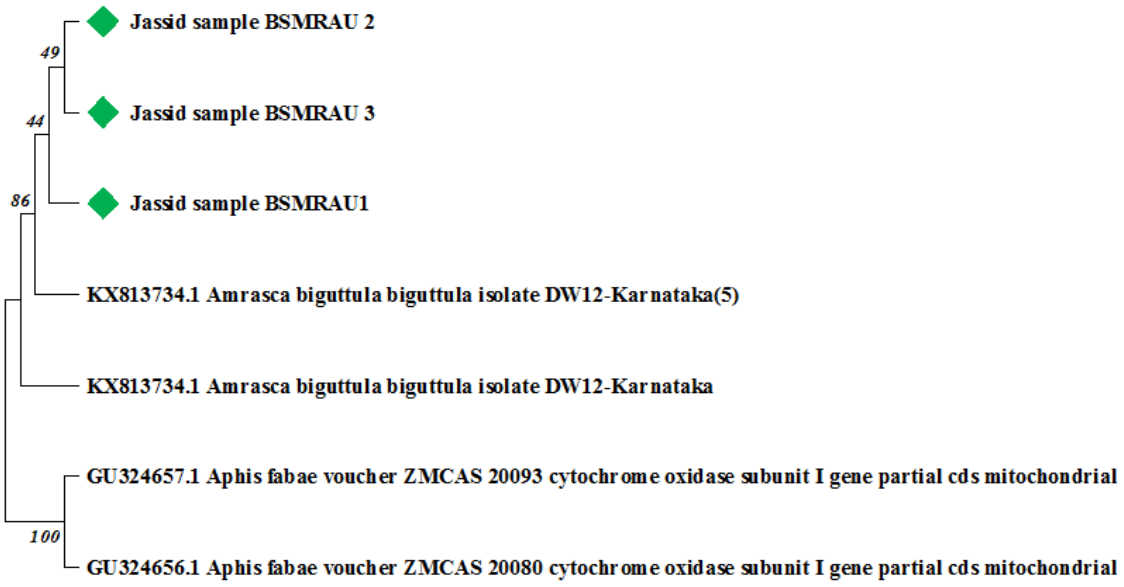

Figure 2: Molecular phylogenetic analysis of *Amrasca biguttula* by Neighbor-Joining method. (The taxon with green triangles denotes sample from Bsmrau, Bangladesh and others are the reference sequence from Genbank)

The phylogenetic tree indicates that the Jassid sample BSMRAU 2 and Jassid sample BSMRAU 3 are closely related to each other with bootstrap value 49. But they are distantly related to Jassid sample BSMRAU 1 and are in different clades with bootstrap value 44. All the jassid of BSMRAU are closely related to KX813734*.1 Amrasca biguttula biguttula* isolate DW12-Karnataka (5) with the bootstrap value 86.

3.3 Biochemical Analysis Of Infected And Healthy Cotton Leaves

Analyzing the biochemical composition of cotton leaves offers valuable insights into the physiological and metabolic condition of these crucial plant components. This comprehensive assessment commonly encompasses the measurement of chlorophyll content, a critical parameter for evaluating photosynthetic activity, along with the quantification of anthocyanin levels, which serves as an indicator of the plant's response to stress. The biochemical analysis of cotton leaves provides essential data that can be used to fine-tune agricultural techniques, ultimately leading to improvements in both crop yield and quality.

3.4 Correlation Matrix Among Cotton Leaf Sample Variables

Correlation among the variables was examined through correlation matrices, where shades of blue to white indicated positive correlations, and transitions from white to red indicated negative correlations among the leaf sample variables (as illustrated in Figure 3). In the graph, it's evident that the chlorophyll content of fresh cotton leaves displayed a robust negative correlation with jassid infestation in the top, lower, and middle leaves. Moreover, the chlorophyll content of infected cotton leaves exhibited a negative correlation with jassid infestation in the top and lower leaves, although no significant correlation was observed with jassid infestation in the middle leaves. Interestingly, a contrasting trend emerged where jassid infestation in the top leaves and jassid infestation in the lower and middle leaves showed a positive correlation.

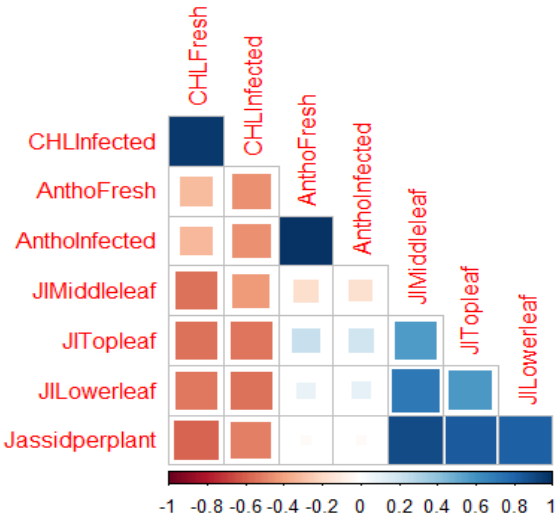

Figure 3: Correlation matrix among the variables of cotton leaf samples.

There was a notable positive correlation between jassid infestation and the number of jassids per plant in the lower and middle leaves. However, there was no significant correlation identified between jassid infestation in the top, middle, or lower leaves and the number of jassids per plant when considering anthocyanin content in both fresh and infected cotton leaves. Furthermore, no observable correlation was detected between chlorophyll content in fresh and infected leaves and the anthocyanin content in fresh and infected leaves, as shown in Figure 3.

3.5 Exploring Principal Component Analysis (PCA) For Understanding Cotton Jassid Infestation Variables

Principal component analysis (PCA) was done among the healthy and infested cotton leaf sample variables of two cotton variety and it was found that the first two components could explain more than 82% of the variation presented in Figure 4. Where dimension 1 contributed 54% and dimension 2 contributed 28.4%.

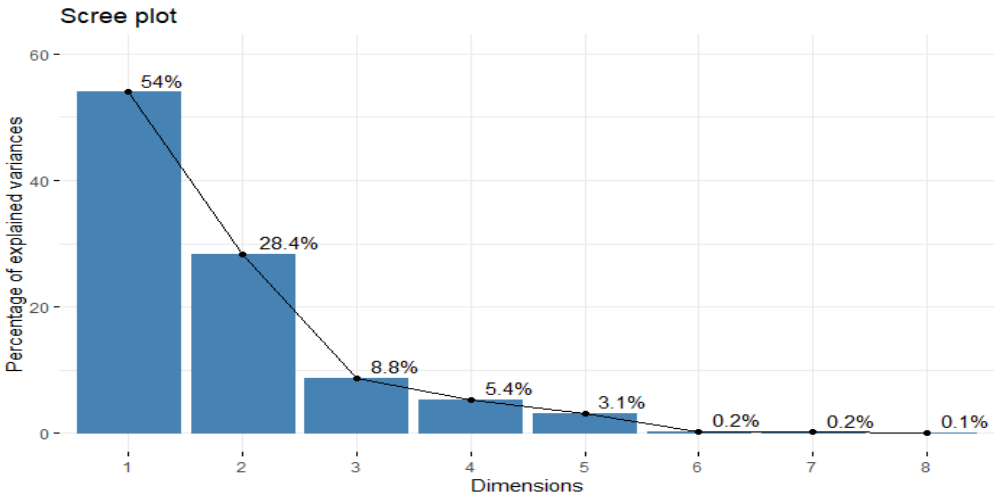

Figure 4: Principal Component analysis (PCA) among the healthy and infested leaf sample variables of two cotton variety.

The results of a Principal Component Analysis (PCA) involving various variables related to anthocyanin and chlorophyll content, as well as jassid infestation in leaves. The PCA results are described here under the following subheadings:

1. **Positive Relationship with Dimension 1 and 2 for Anthocyanin Content in Infected and Fresh Leaves:** This suggests that anthocyanin content in both infected and fresh leaves contribute positively to both principal components (dimensions 1 and 2) and might be important factors in explaining the variability.

2. **Negative Relationship with Dimension 1 and 2 for Chlorophyll Content in Infected and Fresh Leaves:** Conversely, chlorophyll content in infected and fresh leaves is negatively correlated with both dimension 1 and 2. This indicated that lower chlorophyll levels are associated with higher values of both principal components.

3. **Positive Correlation with Dimension 1 and Negative Correlation with Dimension 2 for Jassid Infestation Metrics:** The number of jassids per plant and jassid infestation in different parts of the leaves (top, middle, and lower) are positively correlated with dimension 1 but negatively correlated with dimension 2. This implies that dimension 1 might represent a factor associated with increased jassid infestation.

4. **Strong Positive Correlation among Jassid Infestation Parameters, Particularly Anthocyanin:** Among the various parameters related to jassid infestation, anthocyanin content shows a strong positive correlation with other variables. This suggested that anthocyanin content is highly interrelated with other jassid-related factors in your dataset.

5. **Strong Negative Correlation for Chlorophyll Content in Infected and Fresh Leaves:** Chlorophyll content in both infected and fresh leaves showed a strong negative correlation in both dimensions. This indicated that lower chlorophyll content is associated with higher values of both dimension 1 and dimension 2.

6. **Possible Variability in Correlations due to Jassid Infestation Rate:** The correlations among these variables may differ based on the infestation rate of jassids. This suggested that the relationships between these variables might change or become more pronounced under different levels of jassid infestation.

The PCA analysis resulted Anthocyanin content and jassid infestation seem to be important factors, while chlorophyll content has a negative influence on the two principal dimensions. The specific correlations and their strengths can provide valuable insights into the underlying relationships within the varieties, which can be useful for further analysis or experimentation.

Figure 5 displays the variance contributions in a PCA. In this analysis, anthocyanin levels in both infected and fresh leaves exhibit positive associations with both the first and second dimensions, whereas chlorophyll content in infected and fresh leaves demonstrates negative correlations with both dimensions. Furthermore, the number of jassids per plant and jassid infestation in different leaf sections (top, middle, and lower) are positively linked to the first dimension but negatively tied to the second dimension. Among the eight parameters related to jassid infestation, anthocyanin displays a strong positive correlation with the other variables. Conversely, chlorophyll content in infected and fresh leaves exhibits a strong negative correlation across both dimensions. It's important to note that these correlations among the variables might vary depending on the infestation rate of jassids.

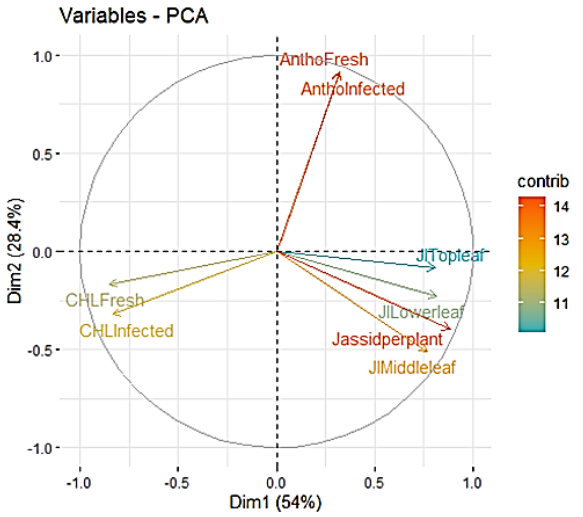

Figure 5: Principal component analysis representing different leaves variable for two varieties of cotton.

The contribution of different variables of cotton samples from Bangladesh in PC loadings plot analysis is presented with 2 dimensions in Figure 6.

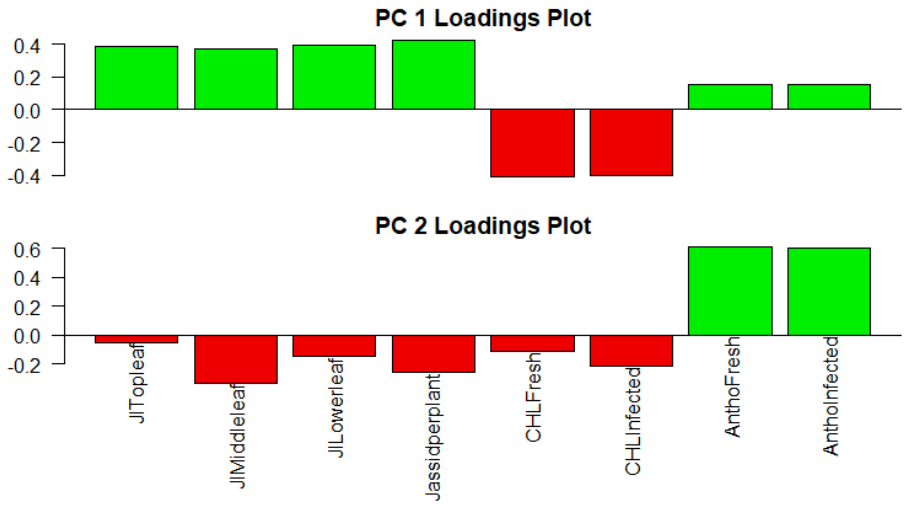

Figure 6: Principal component Loading plot representing contribution of different variables of cotton leaf samples

Anthocyanin content of both fresh and infected leaf was found to have strong positive correlation in dimension 1 and dimension 2. On the other hand, Chlorophyll content of both fresh and infected leaf had strong negative correlation with both dimensions. Whereas the number of jassid per plant, jassid infestation in top, middle and lower leaves had positive correlation with dimension 1, but negatively correlated with dimension 2.

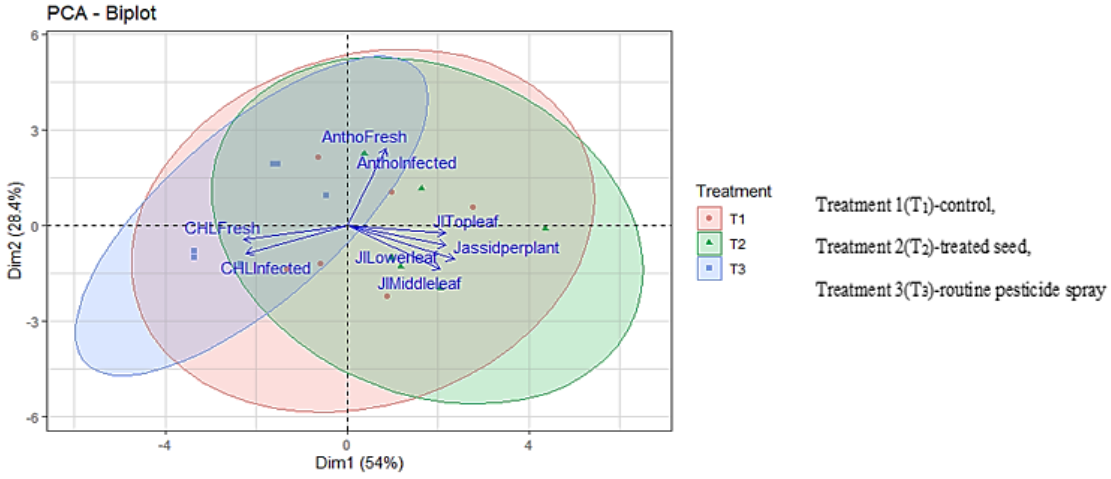

Figure 7: Biplot generated through principal component analysis corresponding with different treatments

Figure 7 illustrated how various factors related to cotton leaves are involved in a PCA under different treatment conditions. In this specific study of cotton leaf samples, we observed the following patterns:

- Under the T1-control treatment, which occupied the largest portion of both dimensions, there was a positive correlation between anthocyanin content in fresh leaves and anthocyanin content in infected leaves in both dimension 1 and dimension 2.
- In the case of the T2-treated seed treatment, which also covered both dimensions, all the parameters displayed positive correlations with both dimension 1 and 2, except for the chlorophyll content of the leaves.
- With the T3-routine pesticide spray treatment, which also extended across both dimensions, anthocyanin content in fresh and infected leaves exhibited positive correlations in both dimensions, while chlorophyll content in infected and fresh leaves showed negative correlations in both dimensions.

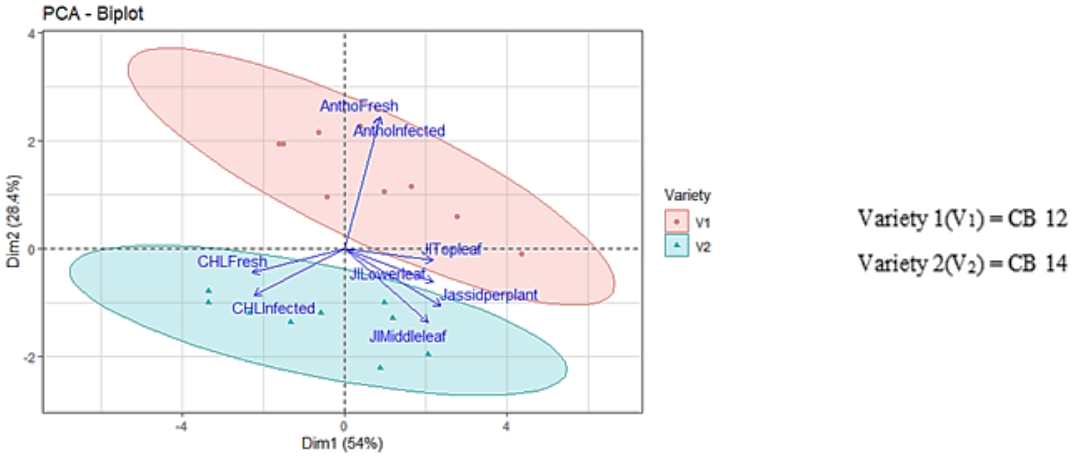

Figure 8: Biplot generated through principal component analysis corresponding with different varieties of cotton.

The engrossment of cotton leaf variables in PCA with its different varieties of cotton is presented in Figure 8. In this study of cotton leaf samples:

- Variety-1 (V1), represented by CB 12, occupies the largest portion of both dimensions in the PCA plot. Here, there is a positive correlation between anthocyanin content in fresh leaves and anthocyanin content in infected leaves in both dimension 1 and dimension 2.
- Variety-2 (V2), represented by CB 14, exhibits a negative correlation in dimension 2. Additionally, chlorophyll content shows a negativecorrelation in both dimension 1 and 2, while the other variables display positive correlations on dimension 1.
- The data provided in Table-3 reveals significant differences among the treatments when it comes to jassid infestation in cotton plants. In the context of agricultural practices, it is crucial to assess how different treatments impact a plant's response to environmental stressors, particularly pests. Among these pests, jassids play a substantial role in affecting plant growth and overall health. This section explores the influence of treatments, specifically T1, T2, and T3, on essential plant parameters in areas exposed to jassid infestations.

Table 3: Effects of different treatments on different parameters of cotton leaf

Treatments	Top leaf	Middle leaf	Lower leaf	Jassid/ plant	CHL Fresh	CHL Infected	Anthocyanin Fresh	Anthocyanin Infected
T1	11.61a	10.28ab	3.28a	8.53a	39.33b	7.41b	24.44a	98.47a
T2	11.16a	13.78a	4.78a	9.84a	20.61c	3.28c	24.43a	98.45a
T3	4.48a	4.43b	1.42a	3.85a	59.95a	10.70a	24.43a	98.46a

Data expressed as mean ± SE. Means within a row followed by same letter(s) are not significantly different according to TukeyHSD mean comparison test- at < 0.01*, * T1: Control Treatment; T2: Seed treatment with *Beauvaria bassiana*; T3: Routine foliar spray

Data were collected to quantify chlorophyll content (CHL) and anthocyanin content in both fresh and infected plant parts. Additionally, observations were recorded regarding the presence of jassids on the plants. The plant segments analyzed included the top leaf, middle leaf, and lower leaf. Treatment T2 displayed the highest presence of jassids, with a recorded value of 9.84a, while Treatment T3 exhibited the lowest presence at 3.85a. Notably, Treatment T2 generally showed higher chlorophyll content, particularly in the middle and lower leaf segments, compared to T1 and T3. In terms of anthocyanin content, minor variations were observed between the treatments in both fresh and infected states, with all treatments displaying similar values. Treatment T1 had an anthocyanin content of 24.44a in the fresh state and 98.47a in the infected state, Treatment T2 exhibited 24.43a and 98.45a, and Treatment T3 showed 24.43a and 98.46a in the respective fresh and infected states. These findings underscore the influence of different treatments on chlorophyll content and jassid presence across various plant segments, highlighting Treatment T2 as distinctive for its higher chlorophyll levels and increased jassid presence.

These findings underscore the significant impact of different treatments on chlorophyll content and the presence of jassids across various plant segments. Treatment T2, in particular, demonstrated higher chlorophyll content and a greater presence of jassids compared to the other treatments. However, anthocyanin content exhibited minimal variation between the treatments. In summary, this section illuminates the influence of various treatments on chlorophyll and jassid presence in different plant segments. Notably, treatment T2 emerged as a distinctive treatment, displaying higher chlorophyll content and a greater presence of jassids. These results hold implications for agricultural strategies and pest management approaches.

Table 4: Effects of Different Varieties On Different Parameters Cotton Leaf

Variety	Top leaf	Middle leaf	Lower leaf	Jassid/ plant	CHL Fresh	CHL Infected	Anthocyanin Fresh	Anthocyanin Infected
V1	10.32a	8.47a	3.36a	7.33a	34.44b	5.51b	36.98a	145.54a
V2	7.85a	10.52a	2.95a	7.48a	45.48a	8.75a	11.89b	51.38b

Data expressed as mean ± SE. Means within a row followed by same letter(s) are not significantly different according to TukeyHSD mean comparison test- at < 0.01*, *V1: Cotton Board 12; V2: Cotton Board 14

Based on the data presented in Table 4, there were no significant differences observed between the two different varieties when it came to the number of jassid per plant and jassid infestation in the top, middle, and lower leaves. A fundamental aspect of modern agriculture involves discerning how different plant varieties respond to environmental stressors, particularly pest infestations. This study aims to explore the impact of two distinct varieties, denoted as V1 and V2, on several critical parameters within jassid-infested plant parts. Data collection involved the measurement of chlorophyll content (CHL) and anthocyanin content in both fresh and infected plant parts for varieties V1 and V2. Additionally, the presence of jassids on the plants was recorded. Analysis of the data revealed notable differences in chlorophyll content between the two varieties across all plant segments. Variety V1 consistently exhibited higher CHL content compared to V2. Similarly, V1 displayed higher anthocyanin content in both fresh and infected states. Conversely, the presence of jassids did not significantly differ between the two varieties. The findings underscore the substantial impact of varietal selection on chlorophyll and anthocyanin content in jassid-affected plant parts. Variety V1 demonstrated a heightened capacity for chlorophyll and anthocyanin production, suggesting its potential advantage in dealing with pest pressure. These results hold significant implications for crop management and varietal selection strategies. In conclusion, this research elucidates the pronounced influence of plant variety on chlorophyll and anthocyanin production in the presence of jassid infestations. Variety V1 exhibited superior performance in these parameters, implying its potential suitability for pest-prone agricultural environments. This study contributes valuable insights to the ongoing discourse on varietal selection and pest management, facilitating informed decisions for enhancing crop productivity and sustainability.

The data presented in Table-5, which focuses on the interaction between treatment and varietal factors, highlights notable disparities in the chlorophyll and anthocyanin content of both infected and fresh leaves across various treatment and variety combinations. In the realm of contemporary agriculture, a comprehensive comprehension of the ramifications of various treatments and varietal interactions on plant health stands as a paramount necessity for the optimization of crop yield and quality. Pests, exemplified by jassids, manifest the potential to exert substantial influence over plant growth and overall vitality. Consequently, the principal objective of this investigation is to scrutinize the repercussions of diverse treatments denoted as T1, T2, and T3, as well as varieties represented by V1 and V2, on distinct segments of the plant when subjected to the presence of jassids.

Table 5: Effect of different treatments and varietal interaction in different jassid affected part

Interaction	Top leaf	Middle leaf	Lower leaf	Jassid/ plant	CHL Fresh	CHL Infected	Anthocyanin Fresh	Anthocyanin Infected
V1:T1	14.33a	6.56a	4.23a	8.31a	35.83d	5.46d	36.97a	145.57a
V1:T2	11.10a	13.90a	4.23a	9.65a	15.83f	2.80f	36.97a	145.50a
V1:T3	5.53a	4.96a	1.63a	4.02a	51.66b	8.26c	36.99a	145.55a
V2:T1	8.90a	14.00a	2.33a	8.74a	42.83c	9.36b	11.91a	51.36b
V2:T2	11.23a	13.66a	5.33a	10.02a	25.40e	3.76e	11.90b	51.39b
V2:T3	3.43a	3.90a	1.21a	3.69a	68.23a	13.13a	11.88b	51.38b

Data expressed as mean ± SE. Means within a row followed by same letter(s) are not significantly different according to TukeyHSD mean comparison test- at < 0.01*, * T1: Control Treatment; T2: Seed treatment with *Beauvaria bassiana*; T3: Routine foliar spray; V1: Cotton Board 12; V2: Cotton Board 14

Data was systematically gathered from plant specimens that underwent a spectrum of treatments and belonged to different varieties. The components of the plant subjected to analysis encompassed the top leaf, middle leaf, and lower leaf. Notably, meticulous attention was directed towards recording the presence of jassids on the plant, alongside the collection of measurements pertaining to chlorophyll content (CHL) and anthocyanin content within both uninfected, fresh plant parts and those afflicted by jassid infestation. The findings presented herein disclose a spectrum of effects precipitated by distinct treatments and varietal interactions on chlorophyll and anthocyanin content, thus elucidating variances across diverse plant segments. Employing a statistically sound framework, these variances have been systematically unveiled through the utilization of letter-based annotations, designating the statistical significance observed within and between treatments and varieties. For instance, within the "Jassid/plant" column, a uniform grouping denoted by the letter 'a' signifies the absence of significant differences concerning jassid presence between the various treatments and varieties.

The ramifications unearthed herein offer profound insights into the plant's response to jassid infestation, as well as the efficacy of distinct treatments and varietal choices in ameliorating these impacts. The profound understanding derived from these interactions possesses the potential to wield significant influence over agricultural practices, directly contributing to the enhancement of crop resilience and the augmentation of overall yield, particularly in the face of pest pressures. In summation, this empirical inquiry unequivocally underscores the pivotal role that treatment and varietal selection play in molding the plant's response to jassid infestation, with a pronounced emphasis on the modulation of chlorophyll and anthocyanin content within various plant segments. These empirically derived insights offer substantial contributions to the ongoing discourse surrounding pest management strategies, thereby furnishing valuable guidance to both agricultural practitioners and researchers, enabling them to make informed decisions aimed at elevating crop productivity and ensuring long-term sustainability.

4. Discussions

In our study, we utilized mitochondrial DNA to trace parental inheritance in jassid species, a method recognized for its dependability in this context. While attempts have been made to employ nuclear DNA, mitochondrial DNA's maternal trait inheritance makes it the preferred choice. Our molecular analysis based on the COI gene within mitochondrial DNA resulted in a comprehensive phylogenetic tree, which strongly supported the jassid species under investigation, consistent with previous findings (Kranthi et al., 2002).

Studying a plant's canopy throughout its growth cycle provided valuable insights into its response to environmental factors. We observed specific areas of the canopy exhibiting reddish-purple or red colors, indicative of potential anthocyanin accumulation. Our research, in line with Table-5, revealed that variety-1 (CB-12) demonstrated a greater capacity for anthocyanin production compared to variety-2 (CB-14).

We also explored the relationship between leaf reddening, induced by various environmental stresses, and anthocyanin synthesis, a pigment responsible for this phenomenon. Our findings supported the notion that biotic factors, such as jassids, can induce leaf reddening and downward cupping effects (Praharaj , 2010). However, it's important to note that reddened foliage is less photostable due to reduced light utilization and increased risk of photodamage (Close and Beadle, 2003). This suggests that anthocyanin might act as a protective screen, reducing photoinhibition and facilitating efficient resorption of nutrients (Hoch et al., 2001).

While foliar anthocyanin has been associated with plant resistance to herbivory, our findings suggest that anthocyanin accumulation alone may not be solely responsible for induced resistance (Costa- Arbulu et al., 2001; Coley and Kursor, 1996). The observed reddening was attributed to increased anthocyanin and decreased chlorophyll levels, possibly related to rootlet death impacting water and nutrient uptake. Furthermore, reductions in chlorophyll content, such as the significant decreases in chlorophyll a, chlorophyll b, and total chlorophyll observed in jassid-infested cotton plants, indicated a negative impact on photosynthetic capabilities (Reddall et al., 2007). This aligns with previous studies demonstrating a decrease in photosynthetic pigments due to insect damage (Hung et al., 2013).

Our findings, consistent with Golawska and Huang emphasize higher concentrations of photosynthetic pigments in non-infested leaves compared to jassid-infested plants(Golawska et al., 2010; Huang et al., 2014). This reduction in pigments could potentially lead to slower plant growth and reduced yield (Almeselmani et al., 2006). While *B. bassiana* has shown promise as an endophyte in enhancing plant traits in previous studies our focus was on its impact on jassid infestations, not on the specific growth-promoting mechanisms (Jaber and Ownley, 2018). The presence of *B. bassiana* decreased jassid infestation rates, although we acknowledge that higher concentrations were not tested due to funding limitations. Imidacloprid, a neonicotinoid insecticide, effectively controlled jassid populations in cotton fields, with the foliar application yielding particularly positive results. This aligns with prior research supporting the efficacy of imidacloprid in pest control (Almeselmani et al., 2006; Elbert et al., 1998).

In conclusion, our study provides essential groundwork for utilizing *B. bassiana* as a biopesticide in integrated pest management strategies for cotton jassid control. Future research should focus on developing suitable formulations and delivery methods for larger-scale assessments under more authentic field conditions.

5. Conclusions

This study had a dual focus, aiming to uncover the molecular characteristics of cotton jassids and assess the response of two cotton varieties to biopesticides. The key findings of our research can be succinctly summarized as follows:

Firstly, we successfully determined the genetic identity of cotton jassids by analyzing the COI gene in mitochondrial DNA. Our molecular analysis unequivocally confirmed the presence of the *Amrasca biguttula biguttula* species of cotton jassids, aligning with nucleotide sequence data available in the NCBI GenBank under accession numbers OR362770, OR362771, and OR362772.

Secondly, our investigation into the impact of two biopesticides on jassid populations yielded noteworthy results. Imidacloprid emerged as the more effective choice compared to *B. bassiana* in reducing jassid infestations within the cotton field. Imidacloprid-treated plots displayed fewer instances of jassid infestations and exhibited higher levels of chlorophyll content. It is important to note, however, that anthocyanin content was observed to be lower in Imidacloprid-treated plots when contrasted with those treated with *B. bassiana.*

Lastly, our varietal response analysis shed light on significant differences between the two cotton varieties, CB-12 (variety-1) and CB-14 (variety-2). Notably, variety-1 experienced a significant reduction in chlorophyll content compared to variety-2, while the accumulation of anthocyanin content displayed an inverse trend between the two varieties. These variations were attributed to jassid infestation. Importantly, despite receiving identical management practices and fertilization, the distinct responses of the two cotton varieties underscore the critical importance of selecting the appropriate variety to effectively address pest-related challenges. Overall, these findings contribute valuable insights to the field of cotton pest management and varietal selection for sustainable cotton cultivation practices.

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