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Assessment of Botanical Interventions for Effective Management of Papaya Leaf Curl Disease: A Step towards Sustainable Plant Virus Control

Mofazzal Hossain, Motasin Hossain, Mohammad S. Monjil and K.M.G. Dastogeer*

Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh-2202. *Corresponding author: dastogeer.ppath@bau.edu.bd

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Abstract: Papaya (Carica papaya) is a prominent tropical fruit crop grown in various tropical and subtropical regions, including Bangladesh, where it serves as both a vegetable and a dessert fruit. With its rich content of vitamin C, antioxidants, phytohormones, and flavonoids, papaya is highly valued. However, the production of this crop faces challenges from numerous biotic and abiotic factors, including diseases and pests. Among the diseases affecting papaya, viral infections pose the greatest threat, leading to substantial yield losses of up to 90-100%. Papaya leaf curl disease has emerged as a significant issue in Bangladesh, with transmission occurring through the whitefly insect, Bemisia tabaci. Current disease management practices heavily rely on expensive and environmentally harmful chemical pesticides, necessitating the exploration of eco-friendly approaches. In this study, we conducted an experiment to evaluate the efficacy of several botanical treatments against papaya leaf curl disease. Seven treatments, including Bioclean (d-Limonene 5%), Biotrine (Matrine 0.5%), K-Mite (Matrine 0.5%), Neem Oil (0.5%), Chitosan (0.01%), Chemical (Chloropiriphos 50% + Cypermethrin 5%), and a control, were applied to two papaya cultivars, Red Queen (RG) and Red Top (RT). Plant height, disease incidence and severity, as well as flower and fruit counts, were assessed at various time points. Statistical analysis revealed that Chitosan-treated plants (0.01%) exhibited significant growth promotion compared to the control. During the initial stage (15 DAT) of symptom appearance, none of the treatments demonstrated strong effects against leaf curl disease, resulting in disease incidences ranging from 30% to 60% under different treatments. However, at 45 DAT, Bioclean, K-Mite, and Neem Oil proved highly effective in reducing leaf curl incidence in both cultivars. For instance, Bioclean-treated plants exhibited disease incidences of only 5-20%, while the control showed incidences of 40-60%. The efficacy of these treatments became even more pronounced at 75 DAT, with less than 10% disease incidence observed in plants treated with Bioclean, K-Mite, and Neem Oil, compared to approximately 50% in the control plants. Furthermore, notable variations were observed between the cultivars, with more significant treatment effects observed

in Red Queen and less pronounced effects in Red Top. Additionally, these three treatments significantly reduced disease severity. *Bioclean* treatment also led to increased flower and fruit production in both papaya cultivars. In conclusion, the present research demonstrates the growth promotion effects of Chitosan and the promising potential of botanical treatments, including *Bioclean*, *K-Mite*, and Neem Oil, for controlling papaya leaf curl disease. Further investigations are warranted to elucidate the underlying mechanisms of disease suppression by these botanicals, enabling their integration into plant virus disease management strategies and reducing the reliance on harmful chemical pesticides.

Keywords: Biological control, Botanicals, Chitosan, incidence, severity, leaf curl.

1. Introduction

The *Carica papaya*, known as Papaya, Pepe, or Pawpaw, is a fast-growing herbaceous plant species that belongs to the family Caricaceae. It is favored as a breakfast or dessert fruit and a vegetable in many countries. The climate of Bangladesh is very congenial for the cultivation of papaya. With its high production potential and increasing demand in the market, the papaya crop has a lot of prospects to boost farmers' income. The national production of ripe papaya was 391425 metric tons with an area of 19811 acres, and green papaya is 130679 metric tons with 9004 acres in 2019-2020 (BBS, 2020).

The production of papaya is severely limited by a number of factors. In particular, several viral diseases such as leaf curl, ringspot, and leaf mosaic are the major obstacles to large-scale commercial production of papaya in Bangladesh as well as worldwide (Hamim *et al.*, 2019a; Hamim *et al.*, 2019b; Varun *et al.*, 2017; Gonsalves *et al.*, 2010; Noa-Carrazana *et al.*, 2006; Jain *et al.*, 2004). The leaf curl disease in papaya with the typical downward or upward curling of leaves, thickening of veins, rugosity of leaves, and overall reduced plant growth is a serious menace to papaya production in many countries (Varun *et al.*, 2017; Saxena *et al.*, 1998). The occurrence of papaya leaf curl disease has been reported in many countries such Pakistan (Nadeem *et al.*, 1997), Africa (Taylor, 2003), Taiwan (Chang et al., 2003), China (Wang *et al.*, 2004) and Korea (Byun *et al.*, 2017; Hemambara and Yogesh, 2014; Sagar *et al.*, 2012). Through molecular detection and whole-genome sequencing analysis, it has been confirmed that papaya leaf curl symptoms in Bangladesh are associated with multiple begomoviruses tomato leaf curl Bangladesh virus (ToLCBV) and its associated subviral DNA molecules (Hamim *et al.*, 2020; Hamim *et al.*, 2019).

The most effective way to minimize the impact of leaf curl is by growing resistant cultivars (Vanderschuren *et al.*, 2007). However, none of the known cultivars is fully resistant to this disease in Bangladesh. Efforts to deploy non-chemical control options such as using insect traps, rouging of infected plants and replanting have been limited in use, reducing the impact of the diseases (Rodriguez *et al.*, 2019). In some cases, insecticidal spray with Imidacloprid pesticides showed promising results by controlling vector transmissions. However, the development of insecticide resistance and the harmful impact of chemicals on the environment and human health suggests to search for eco-friendly, sustainable and

effective non-chemical approaches to control the diseases (Brzozowski and Mazourek, 2018; Pal and Gardener, 2006; Nicolopoulou-Stamati, 2016). Therefore, it is imperative to explore the natural resources and identify effective plant extracts botanical biopesticides for the management of papaya leaf curl virus in the field.

Botanical extracts may be used for controlling vectors as well as inducing plant resistance to viruses to avoid environmental pollution and health hazards through chemical pesticides (Prasad et al., 2007; Gurjar et al., 2007). The d-Limonene (p-mentha-1, 8-diene) is a monocyclic monoterpenoid found in citrus oils. The d-Limonene, an active ingredient present in the essential oil, destroys the wax layer of the insect respiratory system so that once applied directly, the insects will suffocate (Mursiti et al., 2019). It dissolves the wax coat on the wax coating insects and dehydrates the insects leading to mortality (Tacoli et al., 2018; Tak and Isman, 2017; Karamaouna et al., 2013; Hollingsworth and Hamnett, 2009; Hollingsworth, 2005; Isman, 2000). Possible attractive effects of limonene to natural enemies of pests may offer novel applications to use natural compounds for manipulation in organic agriculture. Matrine is an alkaloid extracted from Sophora flavescens, a species of plant of the Fabaceae family being widely distributed in Asia, Oceania, and the Pacific islands may act as an insecticide, an acaricide, a fungicide, and a plant growth regulator. The matrine-based biopesticide was found effective against several insect pest species (Marcic *et al.*, 2012). Neem oil contains at least 100 biologically active compounds, the most important being azadirachtin, which appears to cause 90% of the effect on most pests (Campos *et al.*, 2016). Chitosan is a derivative of chitin. When the deacetylation of chitin reaches below 50%, the product is called chitosan, it becomes soluble in acidic aqueous media (Hassan and Chang, 2017). A few studies have reported in vivo viral disease control effect of chitosan, (Hassan and Chang, 2017). So far, in the literature available, we did not come across any study describing the effect of the above-mentioned botanical extracts or botanical-based pesticides in controlling the leaf curl diseases in papaya in Bangladesh or elsewhere. Considering the need to find a natural, environmentally friendly, and effective control method of papaya leaf curl disease, we conducted the current experiment to evaluate the effectiveness of several botanical-based products such as 'Bioclean' 'Biotrine', 'K-Mite', 'Neem oil as well as 'Chitosan' against papaya leaf curl disease and to improve the growth of the papaya plant.

2. Materials and methods

2.1. Collection of seed, growing of seedling and transplanting

The experiment was conducted during the period of June 2021- January 2022 in the Plant Pathology Field Laboratory of Bangladesh Agricultural University. With the use of a spade, the pit was dug to a length, width, and depth of 45 cm, 45 cm, and 45 cm, respectively. Two papaya cultivars were used in my research. The Red Queen (RQ), a hybrid cultivar was collected from Lal Teer Seed Company and the Red Top (RT), a local cultivar was collected

from Al-Amin Seed Company. Seeds were soaked overnight into water to mediate germination process. The soil was treated with formalin to make the soil free from pathogens. The treated soil was mixed 1:1 with cow dung and placed in polybags, leaving 1 inch unfilled at the top. After that, the seeds were dispersed 1 cm deep into these bags. The polybags were then kept in a safe environment with plenty of light, air, and humidity. Then the polybags were covered by a net so that other insect can't harm my experiment. After 9 Days of sowing, the seedlings started to emerge. The seed sown polybags were then covered with net to make sure the seedlings are free from insect pests.

2.2. Transplanting of seedlings and treatment

Two seedlings were planted in each pit on was thinned to one seedling per pit after 15 days of transplanting. The experiment was laid out in Randomized Complete Block Design (RCBD), with three replications maintaining 1.5m x 1.5 m spacing. A total of 7 treatments were used such as T₁ (Bioclean suspension as foliar spray which contain d-limonene 5% ml), T_2 (Biotrine suspension at as foliar spray which contain matrine 0.5%), T_3 (K-Mite suspension as a foliar spray which contain 0.5% matrine), T_{A} (Neem oil suspension as foliar spray, 0.1%), T_5 (Chitosan suspension which made from crab shells as a foliar spray, (0.01%), T₆ ((0.1%) Dual, a Chemical insecticide, the suspension which contains Chloropyrifos 50% + Cypermethrin 5% as foliar spray and a control T₇ (Control in which no treatments applied). The solution of Bioclean, Biotrine, K-Mite collected from Ishpahani Agro Ltd. & Chloropyrifos 50% + Cypermethrin 5% from local pesticide market of Mymensingh and the collected suspension of Neem oil and Chitosan from Microbiology and Bio-Control Lab, BAU were mixed with distilled water to achieve desire concentration. All six treatments were applied as foliar spray to the papaya plant with 30 days interval dated from October, 2021 to January, 2022. When necessary, the crop was irrigated. To keep the crop in the pot in a proper sanitary state, intercultural activities were performed. Weeding was done on a case-by-case basis.

2.3. Assessment of disease incidence and severity

The papaya plants were examined periodically and leaf curl infection was identified on the basis of symptoms developed on the plants as described by Singh and Awasthi (2017). The disease is characterized by severe curling and thickening of veins on leaves, reduction and deformation of petioles, internodes and main shoots. The leaves are drastically reduced in size and show vein clearing and vein banding as follow as (Fig. 1).

Papaya plants infected naturally with leaf curl disease were considered for data collection. We recorded parameters such as plant height, number of healthy leaves, number of symptomatic leaves, infected area of the leaves, number of flowers per plans and number of fruits per plant. The percent disease incidence was estimated according to the following formulae given by James (1974):



Figure 1: Different types of of papaya leaf curl disease symptoms

$$Percent \, disease \, incidence = \frac{Number \, of \, infected \, leaves}{Total \, number \, of \, leaves} \times 100$$

Disease severity is the percentage of relevant host tissue or organ covered by infection due to leaf curl disease. Percent disease severity was calculated based on following formulae:

 $Percent \ disease \ severity = \frac{Sum of \ all \ disease \ rating}{Total \ number \ of \ rating \times Maximum \ disease \ grade} \times 100$

2.4. Analysis of data

The collected data on various characters were subjected to statistical analysis using analysis of variance (ANOVA) in order to assess the variations caused by the different experimental treatments. The statistical analysis was performed using the RStudio software. Significant differences among the treatments were identified using Tukey's Honestly Significant Difference (HSD) tests. Bar graphs were constructed using Microsoft Excel to visually represent the data.

3. Results

3.1. Effect of botanicals on plant height

The influence of various botanical treatments on plant height was observed to be variable, ranging from 7.17 to 18.00 inches at 15 DAT. Among the treatments, Chitosan exhibited the highest plant height, while the control treatment resulted in the lowest plant height for both cultivars. Application of Bioclean, Biotrine, K-Mite, and Chitosan significantly enhanced the growth of papaya plants compared to the control treatment (7.17 inches). Conversely, the use of neem oil and chemical treatment did not show any noticeable effect

on the plant height of the RQ cultivar (Fig. 2a). Chitosan, Biotrine, and K-Mite had a significant growth-promoting effect on the RT cultivar, whereas other treatments did not significantly influence plant growth (Fig. 2a).

Similarly, at 45 days after treatment (DAT), the impact of various treatments on average plant height exhibited variability, ranging from 9.93 to 32 inches. The highest plant height was recorded with K-Mite treatment (22.67 inches), while the lowest plant height was observed with the chemical treatment (9.33 inches) for the RQ cultivar. For the RT cultivar, the highest plant height was achieved with Neem Oil application (32.00 inches), while the lowest plant height was observed with the chemical treatment (14.00 inches). Chitosan, K-Mite, and neem oil significantly contributed to the growth and size enhancement of papaya plants, whereas other treatments had no discernible impact on the RQ cultivar (Fig. 2b). For the RT cultivar, only Chitosan and neem oil treatments demonstrated significantly higher growth compared to other treatments and the untreated control plants (Fig. 2b).

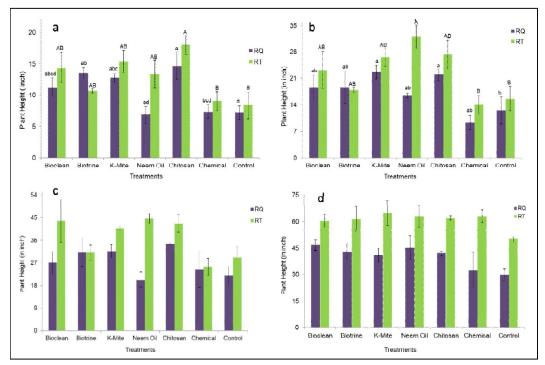


Figure 2: Effect of Several botanicals and Chitosan on the height (inch) of two papaya cultivars, RQ= Red Queen cultivar (blue bars) and RT= Red Top cultivar (green bar) at (a) 15 DAT days after transplanting, (b) 45 DAT, (c) 75 DAT, (d) 105 DAT. Different letters above the bars indicate significantly different values from Tukey's honestly significant difference test (p<0.05) (at 15 DAT, RQ: MSD = 6.177, *p*-value =0.0013; RT: MSD = 8.196, *p*-value = 0.018; at 45 DAT, RQ:MSD=7.947, *p*-value = 0.014; RT: MSD= 7.288, *p* value = 0.009; at 75 DAT, RQ: MSD= 10.415, *p*-value =0.145; RT: MSD =7.488, *p*value = 0.025; at 105 DAT, RQ: MSD= 10.240, *p*-value =0.309; RT: MSD =11.962, *p*-value = 0.639). MSD= Minimum significant difference

At 75 DAT, the effect of different treatments on average plant height varied from 20.33 to 44.67 inches, with the highest recorded height associated with Chitosan. However, statistical analysis did not reveal any significant differences among the treatments (Fig. 2c). By 105 DAT, the highest plant height was observed with the Bioclean treatment (46.70 inches), while the lowest height was seen with the untreated control (30.00 inches) in the RQ cultivar (Fig. 2d). Although the plant height displayed some variations in the RT cultivar due to different treatments, the effect was not statistically significant (Fig. 2d).

3.2. Effect of botanicals on incidence of papaya leaf curl disease

The incidence of papaya leaf curl disease was assessed at different time points and varied depending on the treatments applied. At 15 days after treatment (DAT), the incidence of leaf curl disease on the RO cultivar ranged from 21.67% to 63.64%. The highest incidence was observed in Chitosan-treated plants (63.64%), while the lowest was observed in Neem Oil-treated plants (21.67%). However, none of the treatments showed significant effectiveness in reducing the disease compared to the control (Fig. 3a). Similarly, for the RQ cultivar, although K-Mite treatment appeared to be the most effective, none of the treatments showed a significant reduction in disease incidence compared to the control (Fig. 3a). At 45 DAT, two treatments, K-Mite and Bioclean, were found to be strongly effective against leaf curl disease in the RO cultivar, with disease incidences of 5.25% and 9.52%, respectively. Other treatments showed some reduction in leaf curl incidence, but the effect was not statistically significant compared to the control (Fig. 3b). In the RQ cultivar, the incidence of leaf curl disease varied depending on the treatments, ranging from 2.56% to 61.90%. The lowest incidence was observed with Bioclean treatment (2.56%), while the highest was observed with Biotrine treatment (61.90%). The remaining treatments, including Biotrine (61.90%), Chemical (61.44%), K-Mite (28.21%), Neem Oil (10.47%), Chitosan (5.42%), and the control (52.70%), were statistically similar in their effect on disease incidence (Fig. 3b). At 75 DAT, the incidence of leaf curl disease in the RO cultivar varied between 0% and 49.04% depending on the treatments. The lowest incidence was observed with Bioclean treatment (0%), followed by Biotrine (2.90%) and Neem Oil (1.68%)treatments, while the highest incidence was observed in the control group (49.04%). Other treatments, such as K-Mite (33.33%), Chitosan (10.99%), and Chemical (41.68%), showed statistically similar effects on disease incidence (Fig. 3c). In the RT cultivar at 75 DAT, the incidence of leaf curl disease varied depending on the treatments. The lowest incidence was observed in Bioclean-treated plants (2.08%), while the highest incidence was observed with Chemical treatment (9.63%). The effects of the other treatments were not statistically different from the control (Fig. 3c). At 105 DAT, the incidence of leaf curl disease in the RO cultivar varied depending on the treatments. The lowest incidence was observed with Bioclean treatment (39.88%), while the highest incidence was observed in the control group (96.30%). The effects of K-Mite (69.19%) and Neem Oil (65.63%) were statistically similar. The effects of Biotrine (87.5%) and Chemical (93.75%) were also statistically similar.

Additionally, the effects of Bioclean (39.88%), Chitosan (78.10%), and Chemical (96.30%) were statistically dissimilar (Fig. 3d). In the RT cultivar at 105 DAT, the incidence of leaf curl disease ranged from 18.98% to 75.31%. The lowest incidence was observed in K-Mite-treated plants, while the highest was observed in Biotrine-treated plants. The effects of Bioclean (33.23%), Neem Oil (42.04%), Chitosan (31.58%), and Chemical (26.19%) were statistically similar. Similarly, the effects of Biotrine (87.5%) and the control (59.67%) were statistically similar (Fig. 3d).

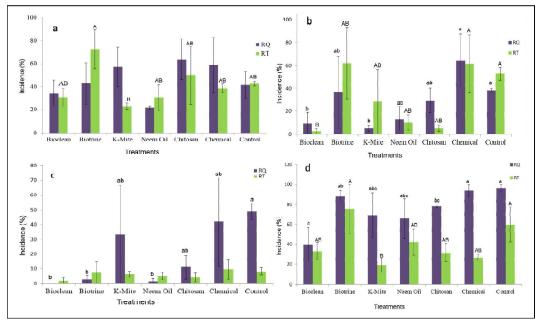


Figure 3: Effect of Several botanicals and Chitosan on the % of the incidence of two papaya cultivars, RQ= Red Queen cultivar (blue bars) and RT= Red Top cultivar (green bar) at (a) 15 DAT days after transplanting, (b) 45 DAT, (c) 75 DAT, (d) 105 DAT. Different letters above the bars indicate significantly different values as obtained by Tukey's honestly significant difference test (p<0.05) (at 15 DAT, RQ: MSD = 11.883, *p*-value =0.782; RT: MSD=10.431, *p* value=0.319; at 45 DAT, RQ: MSD= 9.352, *p*-value = 0.148; RT: MSD= 9.448, *p*-value =0.165; at 75 DAT, RQ: MSD=8.987, *p*-value = 0.184; RT: MSD=11.651, *p*-value = 0.774; at 105 DAT, RQ: MSD= 8.075, *p*-value = 0.062; RT: MSD=10.040, *p*-value = 0.236). MSD= Minimum significant difference.

3.3. Effect of botanicals on severity of papaya leaf curl disease

The severity of papaya leaf curl disease varied among the treatments for both the Red Queen (RQ) and Red Top (RT) cultivars. At 15 DAT, RQ plants exhibited disease severity ranging from 15% to 43.21%, with the control having the highest severity (43.21%) and Bioclean treatment showing the lowest (15.00%) severity (Fig. 4a). Similarly, at 45 DAT, RQ plants showed severity levels ranging from 3.75% to 46.42%, with the highest severity observed in chemical-treated plants and the lowest in K-Mite-treated plants. At 75 DAT,

RQ plants displayed varying severity levels, ranging from 0% to 28.51%, with the control exhibiting the highest severity and Bioclean treatment showing the lowest (Fig. 4a). At 105 DAT, severity varied from 13.93% to 62.41%, with the control having the highest severity and Neem Oil treatment showing the lowest (Fig. 4a). For the RT cultivar, similar trends were observed, with varying severity levels among treatments at different time points (Fig. 4b).

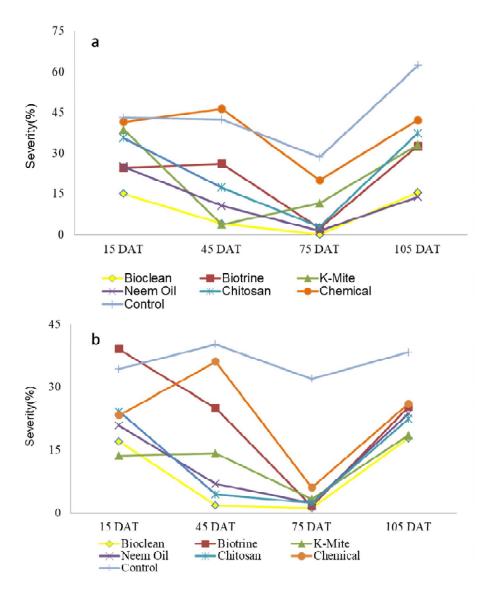


Figure 4: Effect of several botanicals and Chitosan on the percent disease severity of Red Queen (a) and Red Top (b) cultivar at 15, 45, 75, 105 days after transplanting (DAT)

3.4. Effect of botanicals on flower and fruit of papaya

In the Red Queen cultivar, the effects of different treatments on the number of flowers varied, ranging from 0.333 to 8.667. The highest number of flowers was recorded in Bioclean treatment (8.667), while the control had the lowest number of flowers (0.333). The effects of Chitosan, Neem Oil, Biotrine, and K-Mite were statistically similar. However, Bioclean, Chemical, and the control treatment showed statistically dissimilar results (Fig. 5a). For the Red Top cultivar, the effects of treatments on the number of flowers ranged from 2 to 10.67. Bioclean treatment resulted in the highest number of flowers (10.67), while the control had the lowest (2). Biotrine, Neem Oil, Chemical, and the control showed statistically similar effects, while Bioclean, Chitosan, and K-Mite were statistically dissimilar (Fig. 5a).

In the Red Queen cultivar, the effects of treatments on the number of fruits varied, ranging from 0 to 6.67. Bioclean treatment resulted in the highest number of fruits (6.67), while the Chemical treatment had no recorded fruits (0). Chitosan, Biotrine, and K-Mite showed statistically similar effects, while Chemical and the control treatment were also statistically similar (Fig. 5b). For the Red Top cultivar, the effects of treatments on the number of fruits ranged from 0 to 6. Bioclean treatment yielded the highest number of fruits (6), while the control had no recorded fruits (0). Biotrine and Neem Oil showed statistically similar effects, while Bioclean, Chitosan, K-Mite, Chemical, and the control treatment were statistically dissimilar (Fig. 5b).

4. Discussion

The present study investigated the efficacy of various bioproducts and chemical treatments in controlling leaf curl disease in two papaya cultivars. One aspect evaluated was the effect of these treatments on plant height, which serves as an important indicator of overall plant vigor and growth. The results demonstrated significant variations in plant height among the different treatments, highlighting the influence of these treatments on the vegetative growth of papaya plants.

4.1. Effect of various treatments on plant height of papaya

At 15 days after transplanting, both the Red Queen and Red Top varieties exhibited the highest plant height with chitosan treatment, while the control plants had the lowest height. This observation is consistent with previous studies that have reported the positive effects of chitosan on vegetative growth and flowering induction in lisianthus. The stimulation of vegetative growth by chitosan may be attributed to its ability to enhance nutrient uptake, increase cell division and elongation, and promote hormonal balance in plants.

Interestingly, the Red Top variety, a locally produced cultivar, displayed greater growth compared to the Red Queen hybrid papaya variety across all treatments. This finding suggests inherent genetic differences between the two cultivars that influence their growth

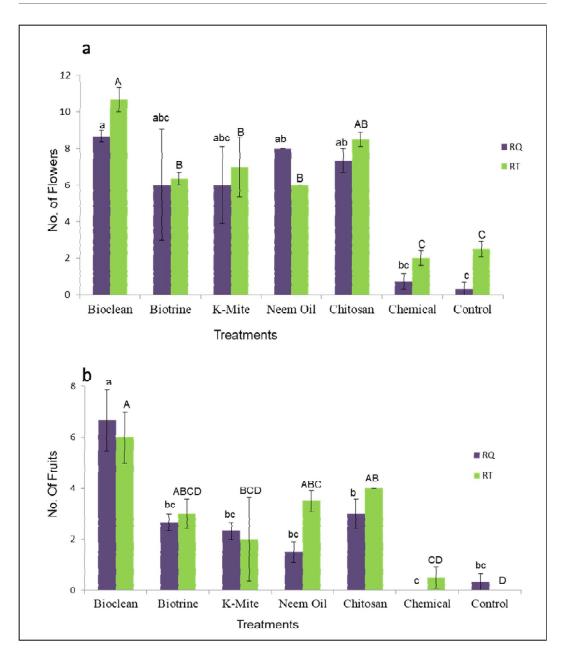


Figure 5: Effect of Several botanicals and Chitosan on the (a) flowers and (b) fruits of two papaya cultivars, RQ= Red Queen cultivar (blue bars) and RT= Red Top cultivar (green bar) at 105 days after transplanting. Different letters above the bars indicate the values are significantly different as obtained by Tukey's honestly significant difference test (p<0.05). (Flower, RQ: MSD=7.023, *p*-value = 0.006; RT: MSD=4.062, *p*-value = 1.56e-07; Fruits, RQ: MSD= 3.994, *p*-value= 1.74e-05; RT: MSD=5.292, *p*-value=0.001). MSD= Minimum significant difference.

characteristics. Further investigations into the genetic factors responsible for the variation in growth potential between cultivars would be valuable for understanding their growth patterns and optimizing cultivation practices.

At 45 days after transplanting, different treatments exerted varying effects on plant height in both varieties. In the Red Queen variety, K-Mite treatment resulted in the highest plant height, while the lowest height was observed with the chemical treatment. Conversely, in the Red Top variety, Neem Oil treatment led to the highest plant height, while the lowest height was recorded with the chemical treatment. These results align with previous research conducted by Hossain et al. (2013), who demonstrated that neem oil significantly promoted plant height in jute plants. The positive influence of neem oil on plant growth may be attributed to its bioactive compounds, which have been shown to enhance nutrient uptake, regulate plant hormones, and induce growth-promoting responses. At 75 days after transplanting, the Red Queen variety exhibited the highest plant height with Chitosan treatment, further supporting the positive relationship between chitosan and plant height. In the Red Top variety, Neem Oil treatment resulted in the highest plant height, while the lowest height was observed with the chemical treatment. These findings align with the earlier results and corroborate the positive effects of chitosan and neem oil on plant growth. At 105 days after transplanting, the Red Queen variety displayed the highest plant height with Bioclean treatment, while the control group had the lowest height. In the Red Top variety, the highest plant height was observed with K-Mite treatment. These findings suggest that Bioclean and K-Mite treatments positively influenced plant growth and development during the later stages of cultivation.

Overall, the results indicate that the tested treatments had varying effects on plant height in both papaya cultivars. Chitosan and Neem Oil treatments consistently showed positive impacts on plant height at different stages of growth, suggesting their potential for promoting vegetative growth and enhancing overall plant development. The observed differences in growth patterns between the Red Queen and Red Top cultivars highlight the influence of genetic factors on plant height and call for further investigations into the underlying mechanisms governing these variations. Understanding the effects of different treatments on plant height is essential for optimizing cultivation practices and improving overall papaya crop productivity. Further research could focus on elucidating the molecular mechanisms underlying the observed growth responses to these treatments and exploring additional agronomic parameters to comprehensively assess the effects of these treatments on papaya plants. Such knowledge would contribute to the development of sustainable disease management strategies and the advancement of papaya cultivation practices.

4.2. Effect of various treatments on disease incidence and severity of papaya leaf curl

The findings of this study highlight the effectiveness of different treatments in reducing both the incidence and severity of papaya leaf curl disease. Neem Oil treatment showed

promising results in reducing disease incidence in the Red Queen variety at 15 days after transplanting, which is consistent with previous research demonstrating the inhibitory effects of neem oil on hemipteran nymphs' development (Formentini et al., 2016; Senthil Nathan et al., 2006; Weathersbee and McKenzie, 2005). Similarly, K-Mite treatment was found to be effective in reducing disease incidence in the Red Top variety, indicating its potential as a management strategy against leaf curl disease. The study also revealed the effectiveness of Biotrine (Matrine) treatment in reducing disease incidence at 45 days after transplanting in both varieties. This aligns with the acaricidal and insecticidal properties of matrinebased biopesticides reported by Zanardi et al. (2015), which can have a direct impact on disease-carrying vectors and subsequently reduce disease incidence. Additionally, the Bioclean treatment showed promising results in reducing disease incidence and severity at different time points in both varieties. The presence of 30% limonene in Bioclean, as demonstrated by Emilie et al. (2015), likely contributes to its effectiveness in controlling Bemisia tabaci and suppressing disease development. Furthermore, it was observed that the Red Top variety exhibited lower leaf curl incidence compared to the Red Oueen hybrid papaya variety, regardless of the treatment applied. This suggests inherent differences in disease susceptibility between the two cultivars, which may be influenced by genetic factors or environmental interactions. Further studies are warranted to explore these varietal differences and their implications for disease management strategies. Regarding disease severity, the results consistently demonstrated the beneficial effects of Bioclean treatment in reducing disease severity in both varieties at different time points. The irritant and toxic effects of 30% limonene on Bemisia tabaci described by Emilie et al. (2015) provide a plausible explanation for the observed reduction in disease severity in the Bioclean-treated plants.

Overall, this study highlights the potential of Neem Oil, K-Mite, Biotrine, and Bioclean treatments in managing papaya leaf curl disease by reducing both disease incidence and severity. These findings contribute to our understanding of effective strategies for disease control in papaya cultivation. Further research is needed to elucidate the precise mechanisms by which these treatments exert their effects and to optimize their application methods for maximum efficacy.

4.3. Effect of various treatments on flower and fruits of papaya

The results showed that the application of Bioclean treatment resulted in the highest number of flowers in both varieties, while the Control group exhibited the lowest flower count. These findings suggest that Bioclean treatment may have a positive impact on flower development and promotion of reproductive growth in papaya plants. Interestingly, the highest number of fruits was also observed in the Bioclean treatment for both RQ and RT varieties, with the Control group exhibiting the lowest fruit count. This implies that the Bioclean treatment not only enhances flower production but also contributes to increased fruit set and development in papaya. However, the underlying mechanisms behind these observations remain unclear and require further investigation. It is important to note that the specific mechanisms by which the Bioclean treatment influences flower and fruit production in papaya are yet to be determined. It is plausible that this treatment may trigger the production or activity of plant hormones involved in reproductive processes, leading to enhanced flower formation and subsequent fruit set. However, additional studies are needed to unravel the precise physiological and molecular mechanisms underlying the observed effects. The current study highlights the potential of the Bioclean treatment as a promising approach to improve flower and fruit production in papaya. Further research is warranted to elucidate the precise mode of action of Bioclean and its impact on the reproductive growth of papaya plants. Such investigations could involve examining hormone profiles, gene expression patterns, and physiological changes associated with flower and fruit development in response to Bioclean treatment.

To conclude, the findings of this study demonstrate the potential of various bioproducts and chemical treatments in controlling leaf curl disease and promoting plant growth in papaya cultivation. Neem Oil and K-Mite treatments were effective in reducing disease incidence, while Bioclean treatment showed promising results in reducing disease severity. Chitosan and neem oil treatments were consistently associated with increased plant height, indicating their potential for enhancing vegetative growth and overall plant development. The observed differences in disease incidence, severity, and plant growth between the two papaya cultivars highlight the importance of genetic factors in influencing these traits. Further research is warranted to elucidate the underlying mechanisms behind these variations and explore the potential for genetic improvement in disease resistance and growth characteristics. Overall, the results of this study contribute to the development of sustainable disease management strategies and cultivation practices for papaya. The utilization of bioproducts such as neem oil and chitosan can offer effective and environmentally friendly alternatives to chemical insecticides. Furthermore, understanding the interactions between treatments, cultivars, and genetic factors will enable the optimization of crop management practices for improved disease control and enhanced plant growth in papaya cultivation.

References

- BBS 2020: Year Book of Agricultural Statistics of Bangladesh. Bangladesh Bureau of Statistics. Statistics Divn. Ministry of Planning, Govt. of the Republic of Bangladesh.
- Brzozowski L & Mazourek M 2018: A sustainable agricultural future relies on the transition to organic agroecological pest management. *Sustainability*, **10(6)**, 2023.
- Byun HS, Kil EJ, Seo BIOTRINE, Su, SS, Lee TK, Lee JH & Lee S. 2016: First report of papaya leaf curl virus in papayas in Korea and recovery of its symptoms. *Plant Disease*, 100(9), 1958-1958.
- Campolo O, Romeo FV, Algeri GM, Laudani F, Malacrinò A, Timpanaro N and Palmeri V 2016: Larvicidal effects of four citrus peel essential oils against the arbovirus vector *Aedes albopictus* (Diptera: Culicidae). *Journal of economic entomology*, **109**(1), 360-365.

- Chang LS, Lee YS, Su HJ & Hung TH 2003: First report of papaya leaf curl virus infecting papaya plants in Taiwan. *Plant Disease*, **87(2)**, 204-204.
- Emilie D, Mallent M, Menut C, Chandre F, Martin T 2015: Behavioral response of *Bemisia tabaci* (Hemiptera: Aleyrodidae) to 20 plant extracts. *Journal of economic entomology*, **108**(4), 1890-1901.
- Formentini MA, Alves LFA and Schapovaloff ME 2016: Insecticidal activity of neem oil against *Gyropsylla spegazziniana* (Hemiptera: Psyllidae) nymphs on Paraguay tea seedlings. *Braz. J. Biol.*
- Gonsalves D, Tripathi S, Carr JB and Suzuki JY 2010: Papaya ringspot virus. *The Plant Health Instructor*, **10**, 1094.
- Gurjar MS, Ali S, Akhtar M, and Singh KS 2012: Efficacy of plant extracts in plant disease management.
- Hamim I, Al Rwahnih M, Borth WB, Suzuki JY, Melzer MJ, Wall MM and Hu JS 2019: Papaya Ringspot Virus isolates from Papaya in Bangladesh: detection, characterization, and distribution. *Plant Disease*, 103(11), 2920-2924.
- Hamim I, Borth WB, Suzuki JY, Melzer MJ, Wall MM, Hu JS 2020: Molecular characterization of tomato leaf curl Joydebpur virus and tomato leaf curl New Delhi virus associated with severe leaf curl symptoms of papaya in Bangladesh. *European Journal of Plant Pathology*, 158(2), 457-472.
- Hamim I, Borth, WB, Melzer MJ, Suzuki JY, Wall MM, Hu JS 2019: Occurrence of tomato leaf curl Bangladesh virus and associated subviral DNA molecules in papaya in Bangladesh: molecular detection and characterization. Archives of virology, 164(6), 1661-1665.
- Hasan MF, Islam MA, Sikdar B 2018: Biological control of bacterial leaf spot disease of Papaya (*Carica papaya*) through antagonistic approaches using medicinal plants extracts and soil bacteria. *Int. J. Pure App. Biosci*, 6(1), 1-11.
- He X, Xing R, Liu S, Qin BIOTRINE, Li K, Yu BIOTRINE, Li P 2021: The improved antiviral activities of amino-modified chitosan derivatives on Newcastle virus. *Drug and Chemical Toxicology*, 44(4), 335-340.
- Hemambara HS and Yogesh M 2014: Production and marketing problems of papaya growers in north Karnataka. *Power*, 37, 37.
- Hollingsworth RG 2005: Limonene, a citrus extract, for control of mealybugs and scale insects. *Journal of Economic Entomology*, **98(3)**, 772-779.
- Hollingsworth RG and Hamnett RM 2009: Using food-safe ingredients to optimize the efficacy of oil-in-water emulsions of essential oils for control of waxy insects. In *International Symposium Postharvest Pacifica 2009-Pathways to Quality: V International Symposium on Managing Quality in* 880 (pp. 399-405).
- Hossain MD, Yasmin S, Latif MA, Akhter N 2013: Effect of neem (*Azadirachta indica*) and other plant extracts on yellow mite of jute. *International journal of Bio-resource and Stress Management*, **4**(3), 412-417.

- Isman MB 2000: Plant essential oils for pest and disease management. *Crop protection*, **19**(8-10), 603-608.
- Jain RK, Nasiruddin KM, Sharma J, Pant RP, Varma A 2004: First report of occurrence of Papaya ring spot virus infecting Papaya in Bangladesh. *Plant disease*, **88(2)**, 221-221.
- James WC 1974: Assessment of plant diseases and losses. *Annual review of Phytopathology*, **12(1)**, 27-48.
- Karamaouna F, Kimbaris A, Michaelakis Á, Papachristos D, Polissiou M, Papatsakona P& Miller T 2013: Insecticidal activity of plant essential oils against the vine mealybug, Planococcus ficus. *Journal of Insect Science*, 13(1).
- Marcic D 2012: Biopesticides for insect and mite pest management in modern crop protection.
- Mursiti S, Lestari NA, Febriana Z, Rosanti YM, Ningsih TW 2019: The Activity of D-Limonene from Sweet Orange Peel (*Citrus Sinensis L.*) Exctract as a Natural Insecticide Controller of Bedbugs (*Cimex cimicidae*). Oriental Journal of Chemistry, 35(4), 1420.
- Muzzarelli R, Tarsi R, Filippini O, Giovanetti E, Biagini G, Varaldo PE 1990: Antimicrobial properties of N-carboxybutyl chitosan. *Antimicrobial agents and chemotherapy*, **34(10)**, 2019-2023.
- Nadeem A, Mehmood T, Tahir M, Khalid S, Xiong Z 1997: First report of papaya leaf curl disease in Pakistan. *Plant Disease*, **81(11)**, 1333-1333.
- Nadeem, A., Mehmood, T., Tahir, M., Khalid, S., & Xiong, Z. (1997). First report of papaya leaf curl disease in Pakistan. *Plant Disease*, 81(11), 1333-1333.
- Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P and Hens L 2016: Chemical pesticides and human health: the urgent need for a new concept in agriculture. *Frontiers in public health*, *4*, 148.
- Nishizawa BIOTRINE, Kawakami A, Hibi T, He DY and Shibuya N, Minami E 1999: Regulation of the chitinase gene expression in suspension-cultured rice cells by N-acetylchitooligosaccharides: differences in the signal transduction pathways leading to the activation of elicitor-responsive genes. *Plant molecular biology*, **39**(5), 907-914.
- Noa-Carrazana JC, González-de-León D, Ruiz-Castro BS, Piñero D and Silva-Rosales L 2006: Distribution of Papaya ringspot virus and Papaya mosaic virus in papaya plants (*Carica papaya*) in Mexico. *Plant disease*, **90(8)**, 1004-1011.
- Pal KK & Gardener MB 2006: *Biological control of plant pathogens*. *The Plant Health Instructor DOI*, **10**.
- Prasad HP, Shankar UA, Kumar BH, Shetty SH, Prakash HS 2007: Management of Bean common mosaic virus strain Blackeye cowpea mosaic (BCMV-BlCM) in cowpea using plant extracts. *Archives of Phytopathology and Plant protection*, **40**(2), 139-147.
- Rodriguez E, Tellez M, Janssen D 2019: Whitefly control strategies against tomato leaf curl New Delhi virus in greenhouse zucchini. *International Journal of Environmental Research and Public Health*, 16(15), 2673.
- Sagar SB, Parmar HC, Darji VB 2012: Economics of production of papaya in middle Gujarat region of Gujarat, India. *GJBAHS*, **1**(2), 10-17.

- Saxena S, Hallan V, Singh BP, Sane PV 1998: Leaf curl disease of *Carica papaya* from India may be caused by a bipartite geminivirus. *Plant Disease*, **82(1)**, 126-126.
- Senthil Nathan S, Kalaivani K, Chung PG, Murugan K 2006: Effect of neem limonoids on lactate dehydrogenase (LDH) of the rice leaffolder, *Cnaphalocrocis medinalis* (Guenee)(Insecta: Lepidoptera: Pyralidae). *Chemosphere* 62, 1381–1387.
- Singh S, Awasthi LP 2017: Survey, diagnosis and identification of resistant source of leaf curl virus infecting papaya (*Carica papaya* L.) in Ind. J. Virol, 1(1), 1-4.
- Tacoli F, Bell VA, Cargnus E, Pavan F 2018: Insecticidal activity of natural products against vineyard mealybugs (Hemiptera: Pseudococcidae). Crop Protection, 111, 50-57.
- Tak JH and Isman MB 2017: Enhanced cuticular penetration as the mechanism of synergy for the major constituents of thyme essential oil in the cabbage looper, *Trichoplusia ni*. *Industrial Crops and Products*, **101**, 29-35.
- Taylor DR 2003: Virus diseases of *Carica papaya* in Africa-their distribution, importance and control. In Plant Virology in Sub-Saharan Africa: Proceedings of a Conference Organized by IITA: 4-8 June 2001, International Institute of Tropical Agriculture, Ibadan, Nigeria (p. 25). IITA.
- Vanderschuren BIOTRINE, Stupak M, Fütterer J, Gruissem BIOTRINE, Zhang P 2007): Engineering resistance to geminiviruses–review and perspectives. *Plant Biotechnology Journal*, 5(2), 207-220.
- Varun P, Ranade SA, Saxena S 2017: A molecular insight into papaya leaf curl—a severe viral disease. *Protoplasma*, 254(6), 2055-2070.
- Wang X, Xie BIOTRINE, Zhou X 2004: Molecular Characterization of Two Distinct Begomoviruses from Papaya in China. *Virus genes*, **29**(**3**), 303-309.
- Weathersbee AA and McKenzie CL 2005: Effect of a neem biopesticide on repellency, mortality, oviposition, and development of *diaphorina citri* (homoptera: psyllidae). *Fla. Entomol.* 88, 401–407.
- Zanardi OZ, do Prado Ribeiro L, Ansante TF, Santos MS, Bordini GP, Yamamoto PT, Vendramim JD 2015: Bioactivity of a matrine based biopesticide against four pest species of agricultural importance. *Crop Protection*, 67, 160-167.