Research Article

Comparative Efficacy of Different Plant Extracts to Manage the Cotton Leaf Curl Virus Disease and its Vector (*Bemisia tabaci* L.)

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Abstract | Cotton is the king of fibers and it is an important cash crop of tropical and subtropical regions. The present research was carried out to evaluate the comparative efficacy of different against the cotton leaf curl virus vector (*Bemisia tabaci*) and indirectly the disease incidence. Leaves extracts of four plant extracts i.e. *Calotropis gigantea, Zingiber officinale, Allium cepa* and *Azadirachta indica* were evaluated at 3 % concentration against eggs hatchability and adult emergence of the whitefly in lab condition. Two consecutive sprays were applied to assess the relative impact of different plant extracts against adult whitefly population and the disease incidence of cotton leaf curl virus disease in filed conditions. *Azadirachta indica* was found most effective to inhibit the egg hatchability and adult emergence as compared to other plant extracts applied in lab conditions. In field conditions, *Azadirachta indica* was found most effective to minimize whitefly population and to lower the disease incidence. From the present study, we may conclude that the leaf extract of *Azadirachta indica* at 3% concentration may be used to minimize the whitefly population and to lower the disease incidence of cotton leaf curl virus disease under filed conditions.

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Introduction

Cotton (*Gossipium hirsutum*) belongs to genus "Gossypium" and family "Malvaceae" is an important fibrous cash crop. It is called the king of fibers commonly known as "White Gold". Cotton is a crop of tropical and sub-tropical regions; it is cultivated in more than 80 countries in the world with the production of 123.6 million bales (Dohlman et al., 2019).

Pakistan is the 5th largest cotton producing country in the world after India, China, United States and Brazil (Statista, 2018). In 2018, cotton was cultivated on 2373 thousand hectares (12.1 less than the previous year) with the production of 9.861 million bales which is 17.1 % lower than the last year (Pakistan, 2019). The decrease in cultivation area and the production is due to variety of different reasons i.e. market problems, heavy losses caused by the pink boll worm and the increased attack of sucking insects especially of whitefly, hence, the losses by CLCuV may be assumed to increase.

Cotton leaf curl virus disease is caused by cotton leaf curl virus (Sattar et al., 2013) and is vectored by

whitefly (*Bemisia tabaci*) (Rajagopalan et al., 2012). The disease has dreadful history in Pakistan when the estimated losses of 4.98 million bales of cotton worth US $ 7.4 billion were recorded (Zafar et al., 1997). In cotton fields of Pakistan, the disease averagely reduces plant height (40.6%), boll weight (33.8%) and number of bolls per plant (72.5%), fiber length (3.44%), fiber strength (10%) and elongation percentage up to (10%) (Ahmed, 1999; Mahmood et al., 1996). Generally, the disease lowers 30% biological yield of cotton crop in Pakistani fields (Ashraf et al., 2013; Hassan et al., 2016). Cotton leaf curl virus disease appears characteristically on the leaves. Initially, tertiary veins of the leaf are thickening due to photosynthates blockage. With the passage of time, the secondary leaf veins are affected and leaf curling starts which results into reduced leaf surface area for photosynthesis and obstacle in photosynthates passage. The infected plants seem to be dwarf as compared to the healthy ones. In severe conditions, a small leaf-like growth (enation) appears below the leaf (Singh, 2009).

Use of plant extracts are novel in plant disease management but has not generated much interest by scientists. These plant extracts are unique in their action not only inhibiting pathogen growth but also having tonic effect on plant health. They are cheap, eco-friendly and can be used at very low doses. These are easy to use and have broad spectrum potential.

The present research was carried out to evaluate the comparative efficacy of different plant extract i.e. *Calotropis procera* L., *Zingiber officinales* L., *Azadirachta indica* L. and *Allium cepa* L. against whitefly (vector for leaf curl virus of cotton) and indirectly the cotton leaf curl virus disease incidence.

Materials and Methods

Preparation of the aqueous plant extracts

Leaves of *Calotropis gigantea, Zingiber officinale, Allium cepa* and *Azadirachta indica* were taken, thoroughly washed with tap water and dried in the sun. Giving brittle appearance, leaves were grinded by electric grinder. Twenty five gram powder of each extract was taken dissolves in 100 ml of acetone solvent. Extracts were mixed thoroughly by electric stirrer. The aliquot was poured in to plastic tubes and centrifuged at 6000 rpm for 5 minutes. After centrifugation, extract was taken out by the help of pippet and solution was passed through Whatman No.1 filter paper. The resultant was considered to be standard and 3% dilutions were made.

In vitro evaluation of plant extracts against eggs hatchability and adult emergence of the whitefly

To evaluate effect of plant extracts on eggs hatchability, the yellowish colored whitefly eggs present on the lower portion of the leaves of cotton plant were identified and the infested leaves were collected and transferred the Petri plates. Petri plates were arranged in Completely Randomized Design with three repeats. Plant extracts were sprayed separately on and the plates were incubated at 28°C. Data was recorded before and after the application. Number of nymphs were calculated after five days of the application.

The check the relative efficacy of plant extracts on adult emergence, the counted nymphs were placed on the cotton leaf already present in petri plate. Petri plates were arranged in Completely Randomized Design with three repeats. Plant extracts were sprayed separately on and the plates were incubated at 28°C. In control, only sterile water was sprayed. Data was recorded before and after the application. Number of adults were calculated after five days of the application.

Effect of plant extracts on the adult population of whitefly

For evaluation of plant extracts of adult whiteflies, seeds of FH-941 cotton variety were sown in Seed of FH-cotton varieties were sown in experimental area of Plant Virology, Plant Pathology Research Institute, Faisalabad, Pakistan using "dibbler" on beds at 6 cm depth by keeping plant to plant and row to row distance 25 and 75 cm, respectively. Experiment was conducted in Randomized Complete Block Design (RCBD) with three repeats. All agronomic practices were done. Five plant extracts, i.e. *Calotropis gigantea, Zingiber officinale, Allium cepa* and *Azadirachta indica* were evaluated at 3% concentration against whitefly population. In negative control, nothing was applied. Two consecutive sprays were applied at 15 days interval. Whitefly population was recorded at 10:00 am after 7 days of each application.

Disease incidence data of cotton leaf curl virus disease was recorded after ten days of each application by using the following formula.

$$\text{Disease Incidence \%} = \frac{\text{Total No. of infected plants}}{\text{Total no. of plants}} \times 100$$

Statistical analysis

Recorded data was analyzed through Analysis of Variance (ANOVA) and treatments means were compared by Fisher's Least Significant Difference (LSD) test. Data was processed statistically through SAS (9.3) software (Inc., 2011-2012) and was represented by Microsoft Excel (2019).

Results and Discussion

Effect of plant extracts against eggs hatchability and adult emergence of the whitefly in lab conditions

Significant effect of different plant extracts at 3 % concentration on egg hatching and adult emergence of whitefly was recorded after five days of application. Maximum egg hatching percentage was recorded in control treatment (70 %) where only sterile water was sprayed. *Azadirachta indica* was found most effective to inhibit the egg hatchability as compared to other plant extracts applied. No significant difference between *Allium cepa* and *Zingiber officinale* extracts in eggs hatchability of whitefly. *Calotropis gigantea* was found least effective to inhibit the egg hatching, however, it was effective as compared to control. Similar results trend was seen on adult emergence from the nymph.

Maximum adult emergence percentage was recorded in control treatment (66 %) where only sterile water was sprayed. *Azadirachta indica* was found most effective to inhibit the adult emergence as compared to other plant extracts applied. No significant difference between *Calotropis gigantea* and *Zingiber officinale* extracts in adult emergence of whitefly (Table 1).

Table 1: *Effect of plant extract against egg hatchability and adult emergence of whitefly.*

Treatments	Egg hatchability(%)	Adult emergence(%)
Control	70 A	66 A
Calotropis gigantea	68 B	64 B
Zingiber officinale	65 C	63 B
Allium cepa	65 C	61 C
Azadirachta indica	63 D	58 D
LSD	0.81	0.8

Values sharing similar letters do not differ significantly.

Whitefly and aphids are important pests and plant virus vectors. Neem (*Azadirachta indica*) has shown immense potential as a pest and vector control agent because of its insecticidal, antifeedent, antiecdysternoidal and antiviral properties (Koul et al., 1990). Coudriet et al. (1985) reported the significant reduction in egg hatchability on cotton leaves after the application of botanical insecticides.

Effect of plant extracts on the adult population of whitefly in field conditions

Significant impact of plant extract was seen against the adult population of whitefly in filed conditions. The two foliar applications of plant extracts reduced the whitefly populations siginificantly. After the 1st application maximum, whitefly was recorded in control treatment where nothing was applied. *Azadirachta indica* was found most effective to minimize the whitefly population as compared to the other plant extracts. *Allium cepa* was found least effective as compared to the *Azadirachta indica* but was more effective with respect to the *Calotropis gigantea* and *Zingiber officinale*. *Calotropis gigantea* and *Zingiber officinale* was found least effective plant extracts to reduce the whitefly population. Significant decrease in whitefly population was observed after 2nd application of plant extract comparing to the 1st foliar spray. No difference of population was observed in control treatment in 1st and 2nd application. No significant difference in efficacy between *Calotropis gigantea* and *Zingiber officinale* was observed and was found less effective as compared to the *Allium cepa* and *Azadirachta indica*. The extract of *Azadirachta indica* at 3% concentration. was found most effective to minimize the whitefly population (Table 2).

Table 2: *Effect of plant extract on whitefly population in field conditions.*

Treatments	1st Application	2nd Application
Control	7 A	7 A
Calotropis gigantea	6 B	4 C
Zingiber officinale	6 B	4 C
Allium cepa	4 C	3 D
Azadirachta indica	2 D	1 D
LSD (Treatments X Applications)	0.53	

Values sharing similar letters do not differ significantly.

Mamoon-ur-Rashid et al. (2012) conducted field trials to compare the effect of neem (Azadirachta indica A. Juss) oil at 1%, 1.5% and 2% and neem seed water extract at 1%, 2% and 3% concentration with that of synthetic insecticide (Polytrin C® 440 EC) against *Bemisia tabaci, Amrasca devastans, Thrips*

tabaci, Earias insulana, Pectinophora gossypiella and *Helicoverpa armigera*. Neem oil at 2% and neem seed water extract at 3% significantly reduced the whitefly, jassids and thrips infestation up to 12 days after spray as compared to that in the control. Mohan and Katiyar (2000) assessed the impact of insecticides for controlling bollworms and sucking pests such as jassid and whitefly. Neem treated plots recorded relatively less whitefly population, whereas, continuous use of cypermethrin induced 10-fold resurgence.

Efficacy of plant extracts against cotton leaf curl virus disease incidence

Plant extracts significantly reduced the disease incidence of cotton leaf curl virus disease. *Azadirachta indica* was found most effective to lower the disease incidence as compared to other plant extracts. *Allium cepa* was found less effective as compared *Azadirachta indica*, however, was found more effective as compared to the *Calotropis gigantea* and *Zingiber officinale*. *Calotropis gigantea* was found least effective to minimize the disease incidence but was effective when compared with the control treatment. Significant decrease in disease incidence was noticed after the 2nd spray as compared to the 1st one. Similar pattern of efficacy of plant extracts was recorded as was seen after the 1st application. *Azadirachta indica* was found most effective plant extract as compared to the other treatments (Figure 1).

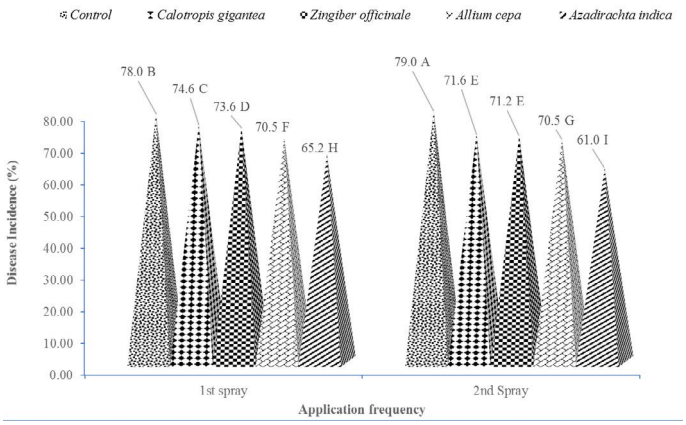

Figure 1: *Comparative efficacy of different plant extracts at 3 % concentration against disease incidence of CLCuV between two different application times; LSD (No. of applications X Treatments) = 0.51.*

The cotton leaf curl virus disease is only biologically transmitted through whitefly. Whitefly transmits the virus in a persistent manner from infected to the healthy plant. So, if we have to manage the disease, we must have to deal with its vector. The continuous use of insecticides has developed the resistance in the whitefly, it may cause the serious threat to disease progression in future. The use of safe bio-products may be an alternate option to coup the dilemma. In present study, the leaf extracts of *Azadirachta indica* lowered the whitefly population, hence may be a good option to manage the cotton leaf curl virus disease. The leaves of *Azadirachta indica* actively contains the chemical "Azadirechtine" which may be responsible for controlling the whitefly population. It means that it has also insecticidal/repellent properties. Hypothesis can be built that it may induce herbivore induced resistance in the plant which obstructs the whitefly feed; research is needed to explore this phenomenon.

Saeed et al. (2018) used *Datura stramonium* (plant extract) and Aviara (homoeopathic product) at 10 and 15 % concentrations against the whitefly population. Datura stramonium was found more effective as compared to Aviara (Homoeopathic)

Ali et al. (2010) reported that plant extracts from the *Azadirachta indica*, *Caleotropics procera*, *Eucalyptus globuls* L., *Allium sativum* L, *Datura stramonium* L, *Aloe barbadensis* and salicylic acid 0.02% were assessed against *Bemisia tabaci* and cotton leaf curl virus disease under field conditions. *A. indica* extract and Salicylic acid were found to be most effective against *B. tabaci* and CLCuV disease.

Moazzam et al. (2000) applied three concentrations of neem oil (0.1, 0.5 and 1%), fresh neem leaves and seed (10 g each) extracts, neem flavonoids (glycosides and aglycone, both at 3% concentration in water or ethanol), or nimbokil (0.5% in water at pH 6.5) on both sides of leaf surface. Among the treatments, only foliar application of neem leaf extract and 1% neem oil completely inhibited disease transmission (100%). Neem seed extract resulted in 80% reduction of CLCuV transmission.

Conclusions and Recommendations

The present study was aimed to assess the comparative potential of different plant extracts against the whitefly population and disease incidence of cotton leaf curl virus disease. From the present study, we found that the leaf extract of *Azadirachta indica* at 3% concentration may be used to minimize the whitefly population and to lower the disease incidence of cotton leaf curl virus disease in field conditions.

Author's Contribution

Waseem Abbas: Conceived the idea.
Shakeel-ur-Rehman: Result and discussion.
Abdul Rashid: Technical Input.
Muhammad Kamran: Introduction and Methodology.
Muhammad Atiq: Conclusion and references.
Muhammad Ehetisham ul Haq: Overall Management of the article.

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