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Management of *Cotton Leaf Curl Virus* Disease and its Vector through *In Vivo* Evaluation of Organic Nutritional Amendments, Organic Oils and Insecticides

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Abstract

Cotton (*Gossypium hirsutum* L.) is the most important cash crop having a great agro-economic significance throughout the world. *Cotton leaf curl virus* (CLCuV) is the most devastating pathogen of cotton, which is responsible for causing huge economic yield losses. The present study was focused on the application of organic nutritional amendments (farm yard manure, spent compost, decomposed leaves compost and kitchen waste compost) in the soil for the enhancement of plant vigor as well as for increasing the ability of plants to survive against the viral attack. The applications of organic oils (canola, sunflower and cotton seed) were done at 25% concentration (25% oil and 75% vinegar) on young cotton plants in order to create hurdle against insect vector infestation (*Bemisia tabaci* Genn.) and insecticides (Imidiacloprid (Imidacloprid 25% WP), Megamos (Acetamparid 20% SC and Bifenthrin (Bifenthrin 10% EC)) were sprayed at their standard doses in the field for maximum mortality of whitefly (*Bemisia tabaci* Genn.) with 7 days interval. The trial was conducted at the research area of Department of Plant Pathology, University of Agriculture, Faisalabad under randomized complete block design (RCBD) with three replications. The recorded data was subjected for analysis of variance (ANOVA) and the means were compared using least significance difference (LSD) test.

Keywords: CLCuV; Organic nutritional amendments; Organic oils; Insecticides

Introduction

Cotton (*Gossypium hirsutum* L.) is an important fiber crop belongs to family Malvaceae. Due to its immense economic importance it is also called "white gold". Cotton has multipurpose uses and it provides basic products, which are hulls, lint, linters, meal, and oil [1]. Cotton is grown around sixty countries of the world. Worldwide production of cotton is 119.8 million bales. In Pakistan, cotton is grown on an area of 2.806 million hectares with production of 13.983 million bales [2]. There are about 75 destructive diseases of cotton [3]. Among the biotic factors, *cotton leaf curl* disease (CLCuD) is the most destructive disease of cotton, which is caused by *Cotton leaf curl virus* (CLCuV). CLCuV is a single stranded DNA virus belongs to genus Begomovirus and family Geminiviridae [4]. Origin of this disease was in Nigeria in 1912 [5]. In Pakistan, this disease was first reported from Tiba Sultan Pur near district Multan in 1967 on variety S-12 [6] and since after its origin it became a serious threat to successful crop yield because of being unnoticed. In 1992, CLCuV appeared in epidemic form causing a very huge loss of 0.543 thousand bales [7]. From its appearance in epidemic form to up till now this disease is the main point of consideration up till now [8]. Primary host, alternate host and vector population are the most important factors on which the disease occurrence is based [9]. Symptomological studies of CLCuV on cotton plant reveals that this disease causes upward and downward curling of leaves accomplished by vein thickening [10]. Regarding severe attack of disease, a cup shaped laminar growth of leaf occurs on the lower side of leaf known as enation [11]. Infection of CLCuV at seedling stage is very much destructive causing enormous yield losses [12]. CLCuD affects the crop yield as well as characters involved in fiber quality like fiber bundle strength, fiber fitness, ginning out turn percentage, fiber uniformity index and maturity ratio of staple length [13]. CLCuV is transmitted by an insect vector known as whitefly (*Bemisia tabaci*) [14]. Whitefly (*B. tabaci*) is a member of order Hemiptera and family Aleyrodidae. Virus is transmitted in a persistent and circulative fashion [15]. The present experiment was being focused on the eco-friendly management of *Cotton leaf curl virus* (CLCuV) and its vector.

Materials and Methods

Development of disease screening nursery

A disease-screening nursery was established in research area of Department of Plant Pathology at University of Agriculture, Faisalabad. For this purpose, the soil was properly pulverized and small plots were prepared for sowing. The experiment was conducted under RCBD where delinted cotton seeds were sown at R × R distance of 75 cm and P × P distance of 30 cm.

Collection of organic waste materials

Organic waste material i.e. farm yard manure (FYM) was collected from the cow shed in University of Agriculture, Faisalabad. Fallen leaves of different trees were collected from different trees while compost was collected from Mushroom Lab. Institute of Horticultural Sciences, University of Agriculture, Faisalabad and Kitchen waste material including peels of fruits and vegetables was collected from the waste bins. These waste materials were properly decomposed by placing them under a polyethene sheet in sunlight. After decomposition these waste materials were used as nutritional amendments.

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Incorporation of waste materials in soil

Before sowing of cotton, soil was properly pulverized and mixed with the decomposed organic waste materials in three replications. Deep ploughing was done for its complete incorporation in the soil followed by irrigation. The impact of waste materials was checked on root length, shoot length and number of bolls per plant of the test variety.

Preparation and application of organic oils

Oils of canola (*Brassica napus*), cotton seed (*Gossypium* spp.) and sunflower (*Heliathus annus*) were collected and their 25% concentrations were prepared i.e. 25 mL oil and 75 mL of vinegar and sprayed on cotton seedlings with one week interval at different crop stages.

Collection and application of insecticides

Insecticides viz. Bifenthrin (bifenthrin 10% EC), Imidacloprid (Imidacloprid 20% EC) and Megamos (Acetamaprid 20% SC) were collected and tested to control whitefly vector by preparing their concentrations at their standard doses.

Data recording

Data regarding disease incidence was recorded on the basis of symptom appearance using the following disease rating scale (Table 1) developed by Akhtar et al. [16] and assessment of disease was done as:

$$\text{Disease Incidence (\%)} = \frac{\text{No. of Infected Plants}}{\text{Total no. of Plants}} \times 100$$

Then percentage disease decrease over control was calculated as:

$$\text{\% Disease Decrease over Control} = \frac{\text{Disease Incidence in Control-Disease Incidence in Treatment}}{\text{Disease Incidence in Control}} \times 100$$

Whitefly infestation

Data regarding whitefly infestation was collected by random selection of leaves from upper, middle and lower portion of each plant and whiteflies were counted by placing a convex mirror below the lower side of leaf then taking mean of all portions of a plant.

Statistical analysis

The data was subjected to statistical analysis and comparison of means was done with the help of least significant difference (LSD) test at 5% level of significance.

Results

Effect of organic nutritional amendments against CLCuV and whitefly under field conditions

Organic based nutritional amendments used were farm yard manure, spent compost, leaf compost and Kitchen waste along with their combinations i.e. farm yard manure + Kitchen waste, farm yard manure + spent compost, farm yard manure + leaf compost, spent compost + leaf compost, spent compost + Kitchen waste and Kitchen waste + leaf compost. These amendments after proper decomposition were incorporated in the soil and then the sowing of cotton was done and disease incidence was found the lowest in the treatment having all organic amendments in a mixture form as it helps in proper growth and functioning of plants resulting in active metabolism of the plant leading to reduced disease incidence with maximum control among all other treatments as compared to control while leaf compost showed minimum control among all as compared to control. Mixture of all amendments showed significant response in reducing the disease incidence and showed significant impact among all treatments. Kitchen waste + leaf compost and leaf compost showed less significant behavior (Figure 1). In case of whitefly infestation, mixture of all amendments showed significant impact in decreasing whitefly infestation while leaf compost had a least significant impact in reducing whitefly infestation (Table 2).

Evaluation of organic oils and insecticides against CLCuV and whitefly under field conditions

The oils of sunflower (*Heliathus annus* L.), canola (*Brassica napus* L.) and cotton seed (*Gossypium* spp.) were collected and their formulations were prepared at 25% i.e. 25 mL of oil in 75 mL of vinegar to make the solution up to 100 mL. Similarly, insecticidal formulations of three insecticides i.e. Imidacloprid (Imidacloprid 25% WP), Megamos (Acetamaprid 20% SC) and Bifenthrin (Bifenthrin 10% EC) were prepared at their recommended doses and both organic oils and insecticides were sprayed at 1 week interval. The time interval between organic oil sprays and insecticidal sprays was 2 hours. The results revealed that after 3rd spray of canola oil and bifenthrin (Bifenthrin 10% EC) maximum disease reduction occurred while sunflower oil and megamos (Acetamaprid 20% SC) showed minimum disease reduction after 3rd spray as compared to control (Figure 2). Considering whitefly infestation, after 3rd spray significant reduction was observed by the application of canola oil and bifenthrin (Bifenthrin 10% EC) while minimum reduction was observed by the application of sunflower oil and megamos (Table 3).

Discussion

For the purpose of management of a disease, organic soil amendments were applied before sowing and the results showed that using all amendments in proper proportion have a significant impact on growth and related plant parameters. Application of all organic nutritional amendments in the soil before sowing leads to good soil nutritional status as well as it provides all the necessary nutrient

Symptoms	Disease index (%)	Disease rating	Response
Complete absence of symptoms	0	0	Immune
Thickening of few small scattered veins or only presence of leaf enations on one or few leaves of a plant observed after careful observations	0.1-10	1	Highly Resistant
Thickening of small group of veins, no leaf curling, no reduction in leaf size and boll setting.	10.1-20	2	Resistant
Thickening of all veins, minor leaf curling & deformity of internode with minor reduction in leaf size but no reduction in boll setting	20.1-30	3	Moderately Resistant
Severe vein thickening, moderate leaf curling followed by minor deformity of internodes and minor reduction in leaf size and boll setting.	30.1-40	4	Moderately Susceptible
Severe vein thickening, moderate leaf curling & deformity of internodes with moderate reduction in leaf size and boll setting followed by moderate stunting.	40.1-50	5	Susceptible
Severe vein thickening, leaf curling, reduction in leaf size, deformed internodes and stunting of the plant with no or few boll setting	>50	6	Highly Susceptible

Table 1: Disease rating scale for the assessment of CLCuV disease [16].

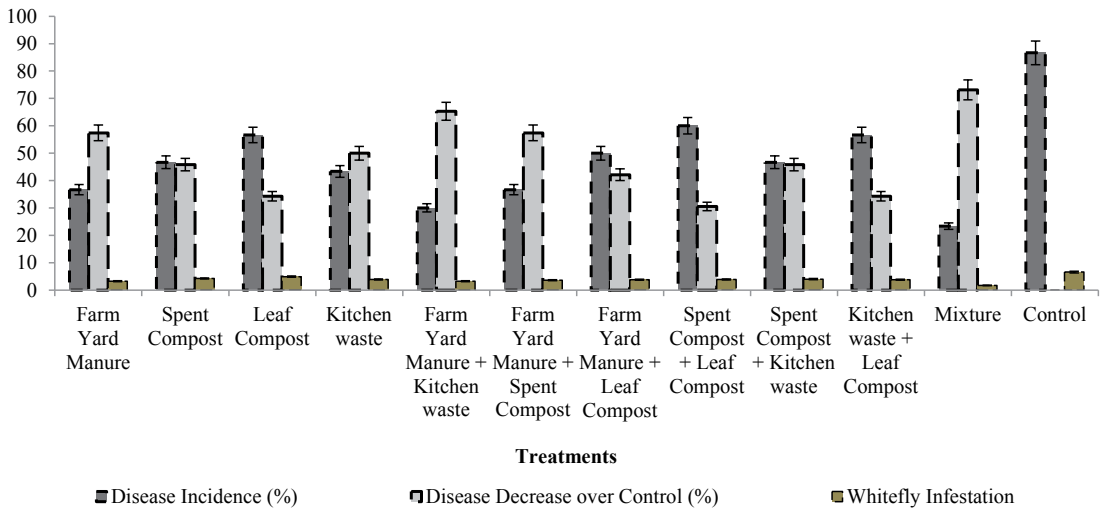

Figure 1: Effect of organic nutritional amendments against CLCuV incidence and whitefly infestation.

Treatments	Disease Incidence (%)	Disease Decrease over Control (%)	Whitefly Infestation
Farm Yard Manure	36.67ef	57.41	3.22d
Spent Compost	46.67cde	45.83	4.22bc
Leaf Compost	56.67bc	34.26	4.89b
Kitchen waste	43.33de	50	3.89cd
Farm Yard Manure + Kitchen waste	30fg	65.28	3.22cd
Farm Yard Manure + Spent Compost	36.67ef	57.41	3.56cd
Farm Yard Manure + Leaf Compost	50bcd	42.13	3.78cd
Spent Compost + Leaf Compost	60b	30.56	3.89cd
Spent Compost + Kitchen waste	46.67cde	45.83	4bcd
Kitchen waste + Leaf Compost	56.67bc	34.26	3.78cd
Mixture	23.33g	73.15	1.67e
Control	86.67a	0.00	6.56a
LSD	10.930		1.1053

Table 2: Evaluation of organic nutritional amendments against CLCuV incidence and whitefly infestation under field conditions.

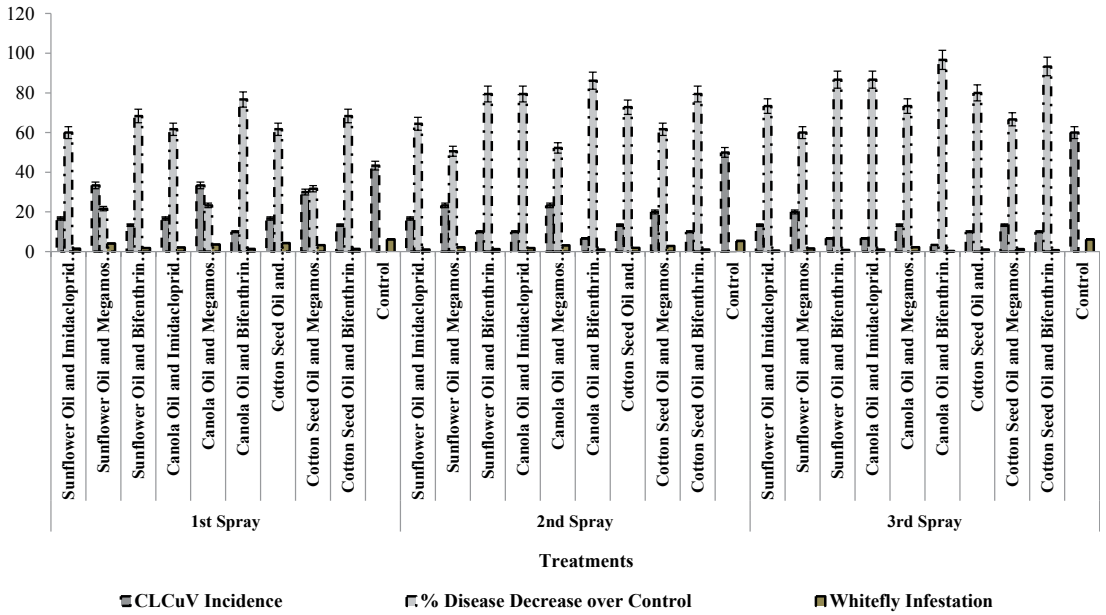

Figure 2: Effect of organic oils and insecticides against CLCuV incidence and whitefly infestation.

Treatments	1st Spray			2nd Spray			3rd Spray		
	CLCuV Incidence (%)	Disease Decrease over Control (%)	Whitefly Infestation	CLCuV Incidence (%)	Disease Decrease over Control (%)	Whitefly Infestation	CLCuV Incidence (%)	Disease Decrease over Control (%)	Whitefly Infestation
Sunflower Oil and Imidacloprid (Imidacloprid 25% WP)	16.67c	60.00	1.46d	16.67bcd	64.44	1.0f	13.33bc	73.33	0.5d
Sunflower Oil and Megamos (Acetamaprid 20% SC)	33.33ab	21.67	4.1abc	23.33b	50.56	2.2cd	20b	60.00	1.6bc
Sunflower Oil and Bifenthrin (Bifenthrin 10% EC)	13.33c	68.33	1.8cd	10cd	79.44	1.3ef	6.67c	86.67	0.8cd
Canola Oil and Imidacloprid (Imidacloprid 25% WP)	16.67c	61.67	2.1bcd	10cd	79.44	1.8de	6.67c	86.67	1.0cd
Canola Oil and Megamos (Acetamaprid 20% SC)	33.33ab	23.33	3.6bcd	23.33b	52.22	3.1b	13.33bc	73.33	2.2b
Canola Oil and Bifenthrin (Bifenthrin 10% EC)	10.00c	76.67	1.4d	6.67d	86.11	1.0f	3.33c	96.67	0.3d
Cotton Seed Oil and Imidacloprid (Imidacloprid 25% WP)	16.67c	61.67	4.3ab	13.33bcd	72.78	1.9de	10bc	80.00	1.0cd
Cotton Seed Oil and Megamos (Acetamaprid 20% SC)	30b	31.67	3.3bcd	20bc	61.67	2.8bc	13.33bc	66.67	1.3bcd
Cotton Seed Oil and Bifenthrin (Bifenthrin 10% EC)	13.33c	68.33	1.4d	10.00cd	79.44	1.0f	10.00bc	93.33	0.7cd
Control	43.33a	0.00	6.2a	50a	0.00	5.4a	60.00a	0.00	6.2a
LSD	10.748		2.5497	10.748		0.6968	11.388		0.9393

Table 3: Effect of organic oils and insecticides against CLCuV incidence and whitefly infestation.

elements, which are important for plant growth, and increases the plant vigor. By improving the nutritional status of cotton plant, the disease incidence can be minimized up to a considerable extent with applying heavy doses of chemicals, which leads to toxic level of chemicals in the end product. Furthermore, it improves soil physico-chemical properties, which increases the organic matter content in soil necessary for appreciable growth and development of plant. Rider et al. also explained the process of compositing that it is mainly depends upon the potential of the microbes provided by the organic matter. Many of the environmental factors effect the composting process like temperature, heating, aeration etc. Temperature is important factor, which control the activity of the microbes. Heating is responsible for the production of thermophilic organisms, which have more capacity and ability to degrade the compounds and to kill the pathogens like fungi, nematodes and viruses. Boulter et al. [17] also described that Aerobic conditions must be essential during composting to prevent the odour but if there is too much moisture content then anaerobic conditions should be maintained. The ratio of carbon and nitrogen is around 25 and it actually gives the rate of decomposition of the matter, therefore only solid, carbon-based matter should be decomposed. Gill and Meelu [18] observed that nearly 12 tons/ha farm yard manure can be a better substitute for 40 Kg of N for rice. Moreover, residual effect of FYM gave 30 Kg of nitrogen and 14.2 Kg of phosphorus to succeeding crop. For the management of vector and virus, organic oils were prepared at 25% concentration and then insecticides were used after 2 hours on plants the results depicted that canola oil and bifenthrin (bifenthrin 10% EC) had a significant impact on whitefly infestation and disease incidence. The results depicted have a close resemblance with Butler et al. [19] conducted an experiment on using cotton seed oil and detergent on cotton plant against the insect vector and reported 62% to 75% decrease through cotton seed oil and 56% decrease using detergent. Butler and Henneberry [20] observed that applying cottonseed oil and soyabean oil at the rate of 1% to 2% in water causes significant reduction in white fly population on cotton, squashes, water melon and cucumber. Khan et al. [21] concluded that application of nimbokil at the 3% concentration was found more effective in limiting the egg hatchability, adult emergence, adult whitefly population and cotton leaf curl disease severity. At 2% and 3% concentration of furnace oil was also very effective in control of white fly population. However, 0.5% and 1% concentration of nimbokil and 1% and 2% concentration of furnace oil were statistically found more effective in reducing whitefly infestation and CLCuV disease severity. Amjad et al. [22] used seven insecticides named Megamos 20SL, Actara 25WG, Polo 500SC, Sitara 25WP, Nighaban 20EC, Thiodan 35EC and Confidor 200SL were applied at recommended doses in the field and evaluated to check their effectiveness in Dera Ghazi Khan. Application of these insecticides was done at economic threshold level (ETL) of whitefly and after seven days of treatment a substantial mortality rate was observed. The most effective insecticides were Megamos and Confidor while Actara was found least effective.

Conclusion

Mixture of all organic nutritional amendments was found to be most effective pre-sowing soil amendment for the management of CLCuD and whitefly and spray of canola oil and bifenthrin (bifenthrin 10% EC) was most effective against CLCuV incidence and whitefly infestation.

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