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Field Evaluation of Selected Bio Pesticides for the Control of Leaf Curl Virus Disease in Capsicum (*Capsicum annuum* L.)

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ABSTRACT

Leaf curl is a major biotic stress for cultivation of capsicum (*Capsicum annuum* L.). Mainly it is caused by chilli leaf curl virus which is transmitted by white fly (*Bemisia tabaci*). The indiscriminate use of synthetic pesticides to control chilli leaf curl virus in capsicum leads to severe health hazards and environmental pollution. Bio pesticides are safe, environmental friendly alternative for synthetic pesticides. A study was conducted to evaluate the effectiveness of three bio pesticides for the control of chilli leaf curl virus in capsicum. A field experiment was designed in Randomized Complete Block Design. Neem seed based bio pesticide (T_2), *Metarhizium anisopliae* based bio pesticide (T_3) and *Verticillium lecanii* based bio pesticide (T_4) were applied once in four weeks after transplanting. The control treatment (T_1) remained without applying any pesticides. Data on disease severity index, disease incidence, pest species, pest amount, growth parameters and yield parameters were recorded. *Metarhizium anisopliae* based bio pesticide (T_3) showed significantly lowest disease severity index and disease incidence values and highest values for yield parameters than other three treatments. Therefore, this study concludes that *Metarhizium anisopliae* based bio pesticide (T_3) is efficient in controlling chilli leaf curl virus in capsicum.

KEYWORDS: Bio pesticide, *Capsicum annuum*, Disease severity index

INTRODUCTION

Capsicum (*Capsicum annuum* L.) is a popular vegetable in Solanaceae family. The centre of origin of capsicum is in Central and South America. The edible portion of capsicum plant is the pod which is an optimum food for inclusion in weight loss diets, containing low amounts of calories, fat, and sodium. They are also a good source of dietary fiber, vitamin A, C and potassium. China is a major capsicum producing country in the world (Anon, 2016a).

In Sri Lanka capsicum is considered as one of the important vegetable crops due to high demand, export potential and nutritional value. Capsicum is widely cultivated in Badulla, Nuwara Eliya and Puttalam districts. Annual capsicum production in Sri Lanka is 10,381 mt/year and extent of production is 2,812 ha (Anon, 2013).

Thrips (*Seirtothrips dorsalis* H.), mites (*Hemitarsonormus latus),* aphids, white flies (*Bemisia tabaci*) and pod borers are the major pests of capsicum. Collar rot, seedling rot, anthracnose, bacterial wilt, leaf spot, narrow leaf disorder and leaf curl are the major diseases of capsicum (Anon, 2016b).

Leaf curl is a major biotic stress for capsicum cultivation in Sri Lanka. It can occur at any stage from nursery to harvesting in life cycle of capsicum plant. Leaf curl is caused by chilli leaf curl virus (CLCV), mites and thrips. Among them leaf curl due to virus infection is more prevalent in South India, Sri Lanka and other tropical countries (Abeygunawardena, 1969).

Chilli leaf curl virus is transmitted by white fly and curl induced by mites and thrips feeding injury (Johnpulle, 1939). Leaves are the plant part which shows initial symptoms of leaf curl disease. Leaves are reduced in size, strongly crumpled and curled upward. The diseased plants give bushy appearance and bearing few flowers and fruits.

Infected capsicum plants should be removed from the field as soon as identifying the symptoms by bagging and discarding to prevent the spread of white flies on them that may carry the virus. Removal of weeds, avoid growing other Solanaceae crops near to capsicum and chemical control are the other methods used to control leaf curl disease (Anon, 2016c).

Most of the farmers are commonly applying synthetic pesticides to plants as a chemical control method in order to suppress the sucking pest population and reduce the spread of leaf curl virus disease in capsicum due to its fast action on pest control and easiness in usage.

However, the indiscriminate use of chemical pesticides have resulted in several risks and ill effects to human as well as to the environment such as social health hazards, ecological imbalance both in flora and fauna, and environmental pollution through pesticide residues in the soil, water, fodder and food crop beside destruction of the natural enemies of pests (Purohit and Gehlot, 2006). Due to that there is a moving trend of reducing synthetic

pesticide usage and finding new sources to control economically harmful pests.

Bio pesticides are certain types of pesticides derived from natural materials such as animals, plants, microorganisms and certain minerals. They can be considered as a viable alternative for synthetic pesticides due to which are known for their qualities of bio rationality, bio deterioration and safe to environment (Purohit and Gehlot, 2006).

Neem (*Azadirachta indica)* is an evergreen plant in family Meliaceae. Different parts of the neem tree are used as a bio pesticide due to its effect on more than 200 insect species and some nematodes, fungi, bacteria and viruses. It contains several active chemicals, which work in different ways. As a result of this, pests are unlikely to become resistant to neem. The most well-known chemical in neem is azadirachtin. Neem is easy to prepare and use, and is environmentally safe and not harmful to human and animals (Purohit and Gehlot, 2006).

Verticillium lecanii and *Metarhizium anisopliae* are two entomopathogenic fungi which are used as bio pesticides due to their ecofriendly and sustainable nature. The first use of *Metarhizium anisopliae* as a microbial agent against insects was in 1879. It has been reported to infect approximately 200 species of insects and other arthropods. Also *Metarhizium anisopliae* is not infectious or toxic to mammals. The activity of *Verticillium lecanii* depends on the strain of the fungus. Fungal strains with large spores infect whiteflies (Anon, 2014).

Therefore, this study was conducted to identify a suitable bio pesticide for effective control of chilli leaf curl virus in capsicum.

MATERIALS AND METHODS

Experimental Site

The study was carried out at the farm field of Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura situated in the low country intermediate zone (IL_{1a}), at an elevation of 30 m from mean sea level. The study was carried out from January to May, 2016. During the period of study, the average day temperature was 32.7 °C, average night temperature was 23.2 °C and relative humidity in the field was 80% respectively.

Field Layout

Land was ploughed to 15-20 cm depth and 16 raised beds (2×2 m) were prepared with 16 planting holes in each bed. Between row and within row spacing was 40 cm and 40 cm. Drains with 40 cm width were prepared between beds to improve drainage. Four treatments were arranged in a Randomized Complete Block Design (RCBD) with four replicates.

Crop Establishment and Maintenance

Capsicum seedlings of the variety CA-8 were raised in a cell plug tray nursery and transplanted in the field at the height of 15-20 cm with 3-4 true leaves. As a basal dressing, organic and inorganic fertilizer was applied to each planting hole according to the recommendation of the Department of Agriculture, Sri Lanka. Irrigation, weeding and other cultural practices were also done according to Department of Agriculture recommendations (Anon, 2016b).

Type of Bio Pesticides Tested

Three bio pesticides (Table 1) were applied once by spraying on to the plant canopy. Application of treatments commenced four weeks after transplanting.

Commercial preparations of *Metarhizium anisopliae* (spores 1.15% w/w) based bio pesticide (T_3) and *Verticillium lecanii* (spores 1.15% w/w) based bio pesticide (T_4) were applied according to the concentration given in the product label.

Fifty grams of dried neem seeds were crushed into small pieces and steeped 24 h in a cloth bag suspended in 1000 ml of water. After 24 h solution was filtered and it was used as neem based bio pesticide (T_2).

Table 1. Bio pesticide treatments used in the experiment

Treatment	Bio pesticide	Application dosage g/4m²
T_1	No pesticide (control)	-
T_2	Neem (Azadirachtin)	12.5 g/250 ml water
T_3	TERMARON (*Metarhizium anisopliae*)	1 g/128 ml water
T_4	*Verticillium lecanii*	1 g/128 ml water

T_1- Control, T_2- Neem, T_3-Metarhizium anisopliae, T_4- Verticillium lecanii

Data Collection

Disease Incidence (DI)

$$= \frac{\textit{Number of infected plants in plot}}{\textit{Number of plants in plot}} \times 100$$

Disease Severity Index (DSI)

$$= \frac{\textit{Total number of infected leaves}}{\textit{Total number of leaves}} \times 100$$

To determine the DSI of affected plants a score scale was prepared. The scores were given according to the extent of leaf curl symptom development in affected leaves of individual plant infected by CLCV in each plot (Table 2).

Table 2. Disease severity index score scale

Score	Symptom	Disease severity (%)
1	Partially curled	0-25
2	Fully curled	0-25
3	Partially curled	26-50
4	Fully curled	26-50
5	Partially curled	51-75
6	Fully curled	51-75
7	Partially curled	76-100
8	Fully curled	76-100

Growth and Reproductive Parameters

Plant height was recorded from the base of the plant to the highest point of the plant at the age of 75 days and 100 days. Numbers of branches and number of flowers were recorded at the age of 75 days and 100 days. At the age of 100 days number of pods per plant was recorded in a sample of ten randomly selected plants from each treatment in each block.

Fresh weight and dry weight of roots, stems and leaves were recorded in a sample of five randomly selected plants at the age of 120 days from each treatment in each block. Dry weight of roots, stems and leaves were measured by drying plant parts in an oven at 105 °C for 24 h.

Number of Pest Species Available in a Plot

Number of pest species available in a plot was manually counted.

Number of Pests Available on a Plant

Number of individual pests available on a plant was counted manually.

Number of pest species available in a plot and number of pests available on a plant were observed from 7.00 a.m. to 9.00 a.m., 24 h before and 72 h after treatment application in each treatment in each block.

Yield Parameters

Total number of harvestable pods was counted and weighed to obtain overall yield as fresh matter in each treatment in each block. Length of the pod without petiole was recorded and circumference of the pod was measured at the widest point of the pod. Fresh weights and dry weights of pods were recorded in a sample of three randomly selected pods from each treatment in each block. Dry weight of the pods was measured in an oven at 105 °C for 24 h.

Statistical Analysis

Obtained data from the study was analyzed using Statistical Analytical System (SAS) software version 9.2.

RESULTS AND DISCUSSION

Disease Severity Index (DSI) and Disease Incidence (DI)

Significant differences were observed in DSI and DI values among the treatments (Table 3). Control recorded the highest DSI and DI values, while *Metarhizium anisopliae* based bio pesticide recorded the lowest DSI and DI values.

With the increase of DSI value, number of totally infected leaves per plant was also increased. Leaves of infected plants were small and curled. Consequently the photosynthetic area of the plant is reduced causing a negative effect to the final yield.

Increased DI value indicates the higher number of totally infected plants. As a control method leaf curl infected plants should be removed from the field in order to reduce the spread of virus to other healthy plants. As a result of removing plants from the field yield reduction has taken place.

According to the results, *Metarhizium anisopliae* based bio pesticide could maintain lower disease severity in plants and low number of infected plant.

Table 3. Effect of treatments on disease severity index (DSI) and disease incidence (DI)

Treatment	DSI	DI
T_1	16.05^a	0.20^a
T_2	11.92^a	0.18^a
T_3	7.55^b	0.13^b
T_4	13.25^a	0.19^a
cv	21.46	14.13
R-square	0.80	0.83

Means followed by the same letter within a column are not significantly different at 0.05 levels. T_1- Control, T_2- Neem, T_3-Metarhizium anisopliae, T_4- Verticillium lecanii

Growth and Reproductive Parameters

The height of the capsicum plants was significantly different among the treatments at both stages of the growth measurements (Table 4). Neem based bio pesticide and *Verticillium lecanii* recorded the higher values, while control and *Metarhizium anisopliae* recorded lower value.

Significant differences were observed among the number of branches in the treatments at both stages of the growth measurements (Table 4).

The number of flowers was significantly different among the treatments at the age of 75

days. However, it was not significantly different among the treatments at the age of 100 days (Table 4).

Number of pods per plant was significantly different among the treatments (Table 4).

The total fresh weight was significantly different among the treatments while total dry weight was not significantly different among the treatments (Figure 1). Control recorded the lowest dry weight and *Verticillium lecanii* based bio pesticide recorded the highest value.

All three treatments recorded higher vegetative and reproductive growth than the control treatment (T_1). After infection of a plant by CLCV virus it multiplied within the infected plant cells that suppress the normal functions of the plant. As a result plant growth and development retards and it get stunted, appear as a deformed bush that produce few small leaves and few flowers and fruits. Further, fruits get deformed due to virus infection. Thus control of spreading virus by vectors and other means are important to obtain a healthy, well grown plant and higher yield.

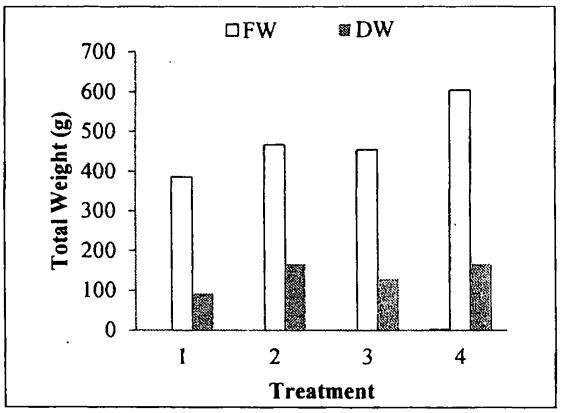

Figure 1. Effect of treatments on the fresh weight and dry weight of capsicum plant. *1- Control, 2- Neem, 3- Metarhizium anisopliae, 4- Verticillium lecanii, DW- Dry weight, FW- Fresh weight*

Number of Pest Species Available in the Plot

No significant differences were observed among the treatment (Table 5) which ranged from 11.37 to 6.88. Control recorded the highest number while *Metarhizium anisopliae* based bio pesticide recorded the lowest number.

Number of Pests Available on a Plant

No significant differences were observed among the treatments (Table 5) which ranged from 9.12 to 7.75. Control recorded the highest number while *Metarhizium anisopliae* based bio pesticide recorded the lowest number.

When number of pests available on the plant and number of pest species available in the plot increase, it will increase the spread of CLCV in the fields as the pests are the vectors who transmit CLCV between plants. Hence, control of pests is important in order to suppress the leaf curl disease in capsicum.

Table 5. Effect of treatments on the pest population available on the plant

Treatment	Pest amount	Pest species
T_1	9.12^a	11.37^a
T_2	8.88^a	7.62^a
T_3	7.75^a	6.88^a
T_4	8.25^a	8.12^a
Chi-square	0.2077	2.2183
Pr>Chi-square	0.9763	0.5284

Means followed by the same letter within a column are not significantly different at 0.05 level. T_1- Control, T_2- Neem, T_3- Metarhizium anisopliae, T_4- Verticillium lecanii

Yield Parameters

Total mass of harvestable pods, circumference, fresh weight and dry weight of pods were significantly different among the treatments (Table 6). *Metarhizium anisopliae* based bio pesticide recorded the highest values and control recorded the lowest values.

Number of harvestable pods and length of harvested pods were not significantly different among the treatments (Table 7).

Table 6. Effect of treatments on the yield parameters of capsicum

Treatments	Mass (g)	FW (g)	DW (g)	Circumference (cm)
T_1	23.9^b	14.6^c	1.5^b	6.2^b
T_2	55.4^b	20.0^{ba}	2.0^a	6.6^{ab}
T_3	114.8^a	22.1^a	2.4^a	7.0^a
T_4	55.5^b	17.6^{bc}	1.9^{ab}	6.3^b

Means followed by the same letter within a column are not significantly different at 0.05 levels.
Note: FW-Fresh weight, DW-Dry weight. T_1- Control, T_2- Neem, T_3-Metarhizium anisopliae, T_4- Verticillium lecanii

Table 4. Effect of treatment on the growth and reproduction of capsicum plant

Treatment	Growth parameters						
	At age of 75 days			At age of 100 days			
	Height(cm)	Branches	Flowers	Height(cm)	Branches	Flowers	Pods
T_1	25.47^b	5.52	6.27	35.09^b	6.65	6.94	4.31
T_2	29.42^a	7.92	9.22	39.26^a	8.88	6.78	5.54
T_3	25.96^b	5.85	6.38	35.47^b	6.46	6.47	3.51
T_4	28.60^a	9.62	9.77	38.18^a	10.05	6.14	4.20

Means followed by the same letter within a column are not significantly different at 0.05 level. T_1- Control, T_2- Neem, T_3-Metarhizium anisopliae, T_4- Verticillium lecanii

Table 7. Effect of treatments on the yield parameters of capsicum

Treatment	Number	Length(cm)
T_1	5.25	9.5[a]
T_2	8.12	10.2[a]
T_3	11.75	10.0[a]
T_4	8.87	9.3[a]

Means followed by the same letter within a column are not significantly different at 0.05 levels; T_1- Control, T_2- Neem, T_3- Metarhizium anisopliae, T_4- Verticillium lecanii

Even though the growth parameters in T_3 were significantly lower at 75 days and 100 days (Table 4), T_3 was not significantly different in yield parameters with other treatments. However, it produced higher number of harvestable pods with higher pod length (Table 7).

In contrast, T_2 and T_4 were having significantly higher growth parameters at 75 days and 100 days and significantly lower yield parameters (Table 6).

Due to higher vegetative growth, plant root, stem and leaf development enhanced and total biomass increased (Figure 1). Consequent to the infection by CLCV normal functions of the plants have changed. In addition, external appearance of the plant changed as the leaves become smaller and curled. As a result of this the photosynthetic area of the plant has reduced resulting low dry matter accumulation in the pod. Because of that final yield of the plant has reduced.

CONCLUSIONS

The results revealed that all three bio pesticides have better performance than the control treatment (T_1) in minimizing disease incidence as well as improving growth and yield performances. Out of them *Metarhizium anisopliae* based bio pesticide (T_3) has given the best results for disease severity index, disease incidence, yield and pod characters. Due to that it controlled disease occurrence and produced higher yield. Therefore, *Metarhizium anisopliae* based bio pesticide could be recommended for farmers to control leaf curl disease in capsicum. Further, this study should be repeated under different soil and climatic conditions with use of different varieties for the confirmation of results.

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REFERENCES

Abeygunawardena, D.V.W. (1969). Diseases of cultivated plants. The Colombo Apothecaries Co. Ltd, 1969.

Anon. (2013). Sri Lanka Export Development Board. Available from http;//www.edb.gov/industry capability report (Accessed 14 December 2015).

Anon. (2014). The Entomopathogenic Fungus. Available from http;//www.entomology.wisc.edu (Accessed 14 May 2016).

Anon. (2016a). Banana peppers. Available from http;//www.wikipedia.org/banana pepper (Accessed 12 May 2016).

Anon. (2016b). Department of Agriculture, Sri Lanka. Available from http;//www.agridept.gov.lk/crop recommendations (Accessed 7 May 2016).

Anon. (2016c). Banana pepper. Available from http;//www.gardeningknowhow.com/banana pepper (Accessed 14 May 2016).

Johnpulle, A.L. (1939). Chilli leaf curl experiments. *Tropical Agricultural research*, **62**, 28- 30.

Purohit, S.S. and Gehlot, D. (2006). Trends in Organic Farming in India, Agrobios (India), Agro house, Behinl Nasrani cinema, Chopasani road, Jodhpur, India. 242-244.