APPLIED BIO-SYSTEMS TECHNOLOGY

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Evaluation of the Efficacy of Bio Rational Pesticides for Eco Friendly Management of Leaf Curl Complex in Chili (*Capsicum annum*)

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Abstract

Background: Leaf curl complex can be identified as a major disease found in most of the Solanaceous vegetable crops, including green chili. Thrips (*Scirtothrips dorsalis* H.) and white flies (*Bemisia tabaci*) are the major vectors responsible for the spread of Leaf curl complex. Farmers tend to use synthetic chemicals as the main control method of this disease. Continuous use of synthetic insecticides results in serious health and environmental hazards. Therefore, application of bio rational pesticides is one of the best alternatives for it. This experiment was conducted to evaluate the efficiency of six bio rational pesticides for the control of leaf curl complex in chili.

Methods: A combination of natural plant extract (T_2), Neem (Azadiractin) based product (T_3), *Beauveria bassiana* based product (T_4), *Metarhizium anisopliae* based product (T_5), *Saccharopolyspora spinosa* based product (Spinosad) (T_6) and a combination of *Beauveria bassiana* and *Metarhizium anisopliae* based product (T_7) were applied to chili plants, starting from three weeks after transplanting, while control (T_1) remained without any pesticide application. The Disease Incidence (DI) and Disease Severity Index (DSI) were calculated for each treatment.

Results: Mean DSI and DI values reported significant differences among the treatments ($P<0.05$). The control treatment recorded the highest DSI value (44.3±2%), while the treatment 6 (Spinosad) recorded the lowest DSI value (31.1±4%). Similarly, the control plot recorded the highest DI (73.6±1%) value, while T_6 (Spinosad) plot recorded the lowest DI value (58.8±3%). Spinosad bio rational pesticide (T_6) showed a significantly lower disease severity and disease incidence.

Conclusions: Based on the overall findings, Spinosad bio rational pesticide (T_6) was identified as the bio rational with a high potential to control leaf curl complex in chili. Further studies are suggested to optimize the effect and minimize the hazard to pollinators.

Keywords: Bio Rational, Green Chili, Leaf Curl Complex, *Saccharopolyspora spinosa*

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INTRODUCTION

Chili (*Capsicum annum*), which is considered as a popular cash crop in Sri Lanka belongs to the family *Solanaceae.* Chili has several species in the genus Capsicum that are widely consumed throughout the world as a condiment or a vegetable. Central and South America is considered as the center of origin of chili. The pod is consumed as the edible portion of chili plant, which helps to prevent cancers, heart problems, treat infections, and combat mood swings. Further, chili is also a good source of dietary fiber, Vitamins B6 and C, Sodium and Potassium. India is the major country that produces dried chilis and green chilis in the world. In Sri Lanka, chili is considered as one of the important condiments due to high demand, export potential and nutritional value. Chili is widely cultivated in Anuradhapura, Monaragala, Ampara, Puttalam, Kurunegala, Hambantota districts and Mahaweli System H [1].

The crop is infested by more than 21 insects and non- insect pest. Leaf curl virus is one of the major limiting factors in the cultivation of the crop [2]. Thrips (*Scirtothrips dorsalis* H.), mites (*Hemitarsonormus latus*), aphids, white flies (*Bemisia tabaci*), nematodes and pod borers are the major pests of chili, while fusarium wilt, collar rot, bacterial spot, seedling rot, anthracnose, bacterial wilt, narrow leaf disorder and leaf curl are the major diseases associated with chili cultivation. Leaf curl complex is a major biotic stress for chili cultivation in Sri Lanka caused by begomoviruses [1]. This disease is identified as a complex because it is a combination of thrips attack, mites attack and leaf curl virus. Thrips and mite attacks and involvement of viruses transmitted by whitefly are the major reasons for chili leaf curl disease complex [3]. Initial symptoms of leaf curl disease appear from the leaves of the plant. After infection of leaf curl complex reduction of leaf area, upward curling in the leaves, crinkling appearance and vein banding can be observed. The diseased plants show bushy appearance, stunting in plant, bunchy leaves, shortening of petioles and internodes and finally leads to bearing few flowers and fruits [4].

Since, Leaf Curl Complex (LCC) is not a seed borne disease, the responsible virus can remain in the cultivated lands through alternative host crops such as tobacco and other solanaceous crops like tomato, capsicum and weeds. In nurseries, chili plants are frequently prone to infection of LCC during the seedling and vegetative stages [5]. Once the chili plants are affected by this disease, those 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. Other methods that can be used to control LCC are removal of weeds, avoiding growing of other host crops near to chili, cultivation of resistant or moderately resistant varieties, Integrated Pest Management (IPM) approach and chemical control [1].

Therefore, farmers are facing the necessity to protect this high value crop from diseases, leading to application of large amounts of pesticides, that has caused the development of pest resistance, phytotoxicity on fruits, human health hazards, destruction of beneficial microbes and environmental pollution. Therefore, the recent trend is going on the improvement of non-chemical or ecofriendly methods for the management of this disease, which has provided impacts to more extensive exploration of natural resources and to identify effective plant extracts or botanical bio rational pesticides for the management of leaf curl virus by reducing vector population in the field [6].

Bio rational pesticides can be defined as certain types of pesticides that are derived from natural materials including animals, plants, microbes and certain minerals. They can be considered as a viable alternative to synthetic pesticides, which are known for their qualities of bio rationality, bio deterioration and low impacts on the environment [1]. Neem (*Azadirachta Indica*) is an evergreen plant that belongs to the family Meliaceae. Different parts of the neem tree, like seed kernel, leaves, roots and stem parts are used to extract bio rationals. The effect of neem can be used to control more than 200 insect species and some nematodes, fungi, bacteria and viruses. Azadirachtin is the widely used active ingredient extracted from neem. Neem based products are popular and available because of their easy preparation, environmentally safe nature and not being harmful to humans and animals [1].

Beauveria bassiana and *Metarhizium anisopliae* are two entomopathogenic fungi, which are used as bio rational pesticides because of their ecofriendly behaviour. *Metarhizium anisopliae* is used to control of pests like termites, thrips etc., while *Beauveria bassiana* is used to control pests like aphids, thrips and whitefly [2]. Both *Beauveria bassiana* and *Metarhizium anisopliae* spores when in contact with the outer surface of the insect, start germination and begin to grow from outside of the skeleton to inside causing the insect to die [7]. Spinosad was the first commercialized active ingredient in the unique class of insect control products, the spinosyns that have a natural origin and are produced from the metabolites of the naturally occurring soil bacterium *Saccharopolyspora spinosa.* Spinosyns are highly active on insect pests including the orders Lepidoptera, Diptera, Hymenoptera, Thysanoptera and some Coleoptera [8].

However, limited studies have attempted to evaluate the effectiveness of using biorationals in controlling pests in chili cultivation in Sri Lanka. Identifying such bio rational could significantly elevate the sustainability of the chili cultivation, while ensuring minimum negative impacts on the environment. Therefore, this study was conducted to identify a suitable biorational pesticide for effective control of LCC in chili.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the farm field of the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP), which is located in the Low Country Intermediate Zone (IL_{1a}), at an elevation of 30 m from the mean sea level. The experiment was carried out from November to April 2022. During the period of the experiment, the average day temperature and Relative Humidity levels were 33.1^{0}C and 80%, respectively.

Field Layout

Land was deep ploughed up to 15-20 cm in depth. A total of 21 raised beds were prepared at the size of 2.5 × 2.5 m with each bed having 12 planting holes. Between row and within row spacing were 90 cm and 60 cm, respectively. In addition, 50 cm width drains were prepared to ensure the drainage between beds. Seven treatments were arranged in a Randomized Complete Block Design (RCBD) with three replicates.

Crop Establishment and Maintenance

Chili seedlings of the variety MI 2 were transplanted in the field after three weeks of seed sowing. Compost was incorporated as a basal dressing to each planting hole and top dressing was applied one month after field establishment, according to the recommendation of the Department of Agriculture (DOA), Sri Lanka. Irrigation, weeding and other cultural practices were practiced according to the recommendation of DOA [9].

Types of Bio Rational Pesticides Tested

Six bio rational pesticides (Table 1) were applied by spraying onto the plant canopy. Treatment application started after three weeks of transplanting. Preparations of bio rational pesticides, Natural plant extracts [1 L/1 ac] (T_2), neem preparation [3200ml/ 1 ha] (T_3), *Beauveria bassiana* [spores 1.15% w/w] (T_4), *Metarhizium anisopliae* [spores 1.15% w/w] (T_5), Spinosad [200-240 ml/1 ac] (T_6) and *Beauveria bassiana* + *Metarhizium anisopliae* [spores 1.15% w/w] (T_7), were applied according to the concentrations given in the product label.

Table 1: Bio Rational Pesticides Tested in Experiment

Trt	Bio Rational Pesticide	Application Dosage (g/ml/6.25m²)
T_1	No pesticide (Control)	-
T_2	Natural plant extract SARUSARA	1.5ml/250ml
T_3	Neem (Azadirachtin) LAKGRO NEEM	75ml/150ml
T_4	*Beauveria bassiana*	1.56g/312ml
T_5	*Metarhizium anisopliae*	1.56g/312ml
T_6	Spinosad SUCCESS	0.25ml/250ml
T_7	*Beauveria bassiana* + *Metarhizium anisopliae*	1.56g/312ml

Note: *Trt; Treatment*

Data Collection

As the growth and reproductive parameters, the plant height of the chili plants was recorded by measuring from the base of the plant to the highest point of the plant at the age of 60 days and 75 days, separately in a sample of ten randomly selected plants from each treatment in each block. In addition, the number of branches and number of flowers were counted. At the age of 75 days, number of pods per plant was recorded in a sample of ten randomly selected plants from each treatment in each block. Each plot was visited by a trained field assistant from 7.00 a.m. to 9.00 a.m., 24 hours before and 72 hours after bio rational application and the number of pest species available in each plot and the average number of pests available on plants were recorded. Based on the recorded data, the Disease Incidence (DI) and Disease Severity Index (DSI) were calculated using the equations 1 and 2.

An eight-point based scoring scale was prepared based on the development extent of leaf curl symptoms in affected leaves to determine the DSI level of affected plants, as shown in Table 2 [10], the scores were given in the Table 2.

Table 2: Disease Severity Index Score Scale

Score	Symptoms	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

Statistical Analysis

The obtained data of the study was analyzed using Statistical Analytical System (SAS) software (version 9.4). Mean separation was done by Analysis of Variance (One-Way ANOVA), followed by the Tukey's pair-wise comparison at a significance level of 5%.

RESULTS AND DISCUSSION

Disease Severity Index (DSI) and Disease Incidence

Significant differences were observed in mean DSI values among the treatments (Table 3). The control treatment recorded the highest DSI value (44.3±2%), while the plot maintained under T_6 treatment (Spinosad) recorded the lowest DSI value (31.1±4%). When the total number of infected leaves per plant increased, the DSI values also increased. Due to the infection by LCC, plant leaves become small and curled, which results in the decrease of photosynthetic area of the plant causing a reduction of the yield. Therefore, treatments should be done in the nursery period as well as on the growth period [10].

$$Disease\ Incidence\ (DI) = \frac{Number\ of\ infected\ plants\ in\ a\ plot}{Number\ of\ plants\ in\ a\ plot} \times 100 \quad (1)$$

$$Disease\ Sevirity\ Index\ (DSI) = \frac{Total\ number\ of\ infected\ leaves}{Total\ number\ of\ leaves} \times 100 \quad (2)$$

Significant differences were observed in the mean DI values among the treatments (Table 3). Similar to DSI, the control plot recorded the highest DI (73.6±1%) value, while T_6 (Spinosad) plot recorded the lowest DI value (58.8±3%). Increased DI value indicated a higher number of infected plants, thereby suggesting a higher population of pests. Spinosad demonstrated its effect on the spread of LCC by controlling the disease vectors of LCC. According to the results, Spinosad bio rational pesticide could be effectively used to maintain a lower disease severity and low number of infected plants.

Table 3: Effect of Treatments on Disease Severity Index (DSI) and Disease Incidence (DI)

Treatment	DSI ± SE	DI± SE
T_1	$44.3±2^{a}$	$73.6±1^{a}$
T_2	$38.9±2^{ab}$	$66.7±2^{ab}$
T_3	$39.6±2^{ab}$	$67.8±1^{a}$
T_4	$37.2±1^{ab}$	$67.8±2^{a}$
T_5	$40.5±2^{ab}$	$67.8±1^{a}$
T_6	$31.1±4^{b}$	$58.8±3^{b}$
T_7	$38.8±2^{ab}$	$65.7±1^{ab}$

Note: *Values are mean ± standard error. Different superscript letters within a column indicate significantly different means at a 0.05 significance level, resulted by One-Way ANOVA, followed by the Tukey's pair-wise comparison. T_1: Control; T_2: Sarusara; T_3: Neem; T_4: Beauveria bassiana; T_5: Metarhizium anisopliae; T_6: Spinosad; T_7: Beauveria bassiana + Metarhizium anisopliae*

Growth and Reproductive Parameters

There were no significant differences observed in height, number of branches, number of flowers and number of pods among the treatments (Table 4). Chilis are generally self-pollinating but cross pollination is also common [11]. The control treatment recorded the highest number of pods. The control plot was not treated with any bio rational pesticides and that could have resulted the enhanced pollination by pollinators like bees, ants etc. Despite the lowest DSI and DI values, the plot treated with Spinosad (T_6) recorded the lowest number of pods. This can be attributed to the effect of Spinosad on pollinators like honeybees and other flower habituating insects of order Thysanoptera [9].

Sarusara (T_2) and *Beauveria bassiana*+ *Metarhizium anisopliae* (T_7) recorded the second highest number of pods, though it had lesser number of flowers. This observation could be due to the safer effect of those preparations for the pollinators and the possible content of plant growth hormones in the products based on plant extracts [12]. In this experiment, all treatment applications were done in the morning to avoid the negative effect of prevailed higher temperature on the efficiency of bio rational, due to quick evaporation of the applied bio preparations. Concurrently, morning is the time period at which insects including pests and pollinators like honeybees are active [13]. Therefore, bio rational pesticides like Spinosad that are with a high toxicity against pollinators should be applied at a time of the day when the pollinators are less active and the relevant pests are active.

Number of Pests Available on a Plant

Pest amount denoted significant differences among the treatments, ranging from 0 to 4.0 (Table 5). All other treatments recorded no pests, except the control treatment that recorded the highest number of pests (4.0). When the number of pests and pest species increase, the possibility of spreading LCC can also increase, since vectors are the mechanism of the spread of LCC in chili. Hence, vector control is an essential practice in order to control the LCC in chili. This proves the effect of bio rational treatments on controlling pest incidence. Also, based on the LCC incidence recorded in all treatments, there was a lower quantity of food source for the pests in the affected leaves. Therefore, with time the

Table 4: Effect of Treatment on the Growth and Reproduction of Chili

Treatment	Growth Parameters						
	At the Age of 60 Days			At the Age of 75 Days			
	Height	Branches	Flowers	Height	Branches	Flowers	Pods
T_1	24.3±2[a]	20.7±1[a]	15.0±2[a]	33.3±3[a]	30.0±4[a]	17.0±4[a]	6.6±3[a]
T_2	24.2±1[a]	16.7±4[a]	14.7±3[a]	31.5±1[a]	22.7±4[a]	15.0±2[a]	4.0±3[a]
T_3	24.9±1[a]	17.7±1[a]	13.0±1[a]	33.5±2[a]	29.0±2[a]	19.0±3[a]	3.7±1[a]
T_4	24.3±1[a]	17.3±2[a]	16.0±4[a]	32.4±1[a]	26.0±2[a]	14.3±1[a]	33±0[a]
T_5	21.8±1[a]	11.0±1[a]	11.3±1[a]	29.4±1[a]	20.7±3[a]	16.0±1[a]	1.7±2[a]
T_6	24..9±1[a]	16.0±2[a]	15.3±1[a]	34.0±1[a]	27.7±2[a]	21.3±4[a]	2.0±1[a]
T_7	25.0±1[a]	14.6±2[a]	16.3±2[a]	33.4±1[a]	28.3±1[a]	18.0±1[a]	4.0±2[a]

Note: *Values are mean ± standard error. Different superscript letters within a column indicate significantly different means at a 0.05 significance level, resulted by One-Way ANOVA, followed by the Tukey's pair-wise comparison. T_1: Control; T_2: Sarusara; T_3: Neem; T_4: Beauveria bassiana; T_5: Metarhizium anisopliae; T_6: Spinosad; T_7: Beauveria bassiana + Metarhizium anisopliae.*

number of pests has reduced, since thrips and whitefly much prefer to feed on younger leaves [14]. Furthermore, there may be repellant effects by the applied pesticides, especially by the odour of the pesticides. Therefore, as a result, pest population has become reduced.

Table 5: Effect of Treatments on Pest Population Available on Plants

Treatment	Average Number of Pests	Number of Pest Species
T_1	4	2
T_2	Not Detected	Not Detected
T_3	Not Detected	Not Detected
T_4	Not Detected	Not Detected
T_5	Not Detected	Not Detected
T_6	Not Detected	Not Detected
T_7	Not Detected	Not Detected

CONCLUSIONS

The findings of the current study suggests that all six bio rational pesticides have a significant effect on the disease severity and disease incidence of the Leaf Curl Complex in chili, with compared to the control treatment (T_1). The best result for the control of LCC in chili was observed from the treatment applied with Spinosad (T_6). Hence, this treatment can provide the best yield performance as well, since a reduced number of affected leaves in plants represents lower DSI and DI values. The increased photosynthesis can result in an increased yield as well. Therefore, Spinosad bio rational pesticide could be suggested as the best option to control LCC in chili.

However, this experiment should be again conducted under different climatic conditions and protected environments, while starting the application of the treatment at the nursery period. Plants should be continued until the economic lifespan of the crop as well. Furthermore, attention should be given to studying the optimum time and other methods of bio rational pesticide application to avoid negative effects on the pollinators and to improve efficiency of applications.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

AUTHORS' CONTRIBUTIONS

AR: Conducted the field experiments and wrote the manuscript. BR: Conceptualized and designed the research, supervised the field experiments and reviewed the manuscript. IK: Performed data analysis. SS: Assisted field experiments. All authors read and approved the manuscript.

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