EVALUATION OF DIFFERENT BIO-AGENTS AND BOTANICALS AGAINST LEAF CURL DISEASE OF CAPSICUM (*Capsicum annuum*)

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EVALUATION OF DIFFERENT BIO-AGENTS AND BOTANICALS AGAINST LEAF CURL DISEASE OF CAPSICUM (*Capsicum annuum*)

BY

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CERTIFICATE

*This is to certify that thesis entitled, "*THE BIOLOGICAL ELICITOR POTENTIAL OF ENDOSPHERIC BIO-AGENTS AND BOTANICALS AGAINST PEPPER LEAF CURL VIRUS (PELVC)*" submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* MASTER OF SCIENCE IN PLANT PATHOLOGY, *embodies the result of a piece of bona fide research work carried out by,* MST. ATKIYA RAHMAN MITHI *Registration No.* 19-10252 *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated:
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Dedicated
to
My Beloved Parents and Supervisor

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The author

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ABSTRACT

A filed experiment was conducted in central farm of Sher-e-Bangla Agricultural University and lab experiment was conducted for multiplication of selected bio-agent in lab of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka during the period from July, 2020 to June, 2021. The study was carried out to evaluate the suppressive effects of selected endospheric bio-agents viz. *Trichoderma harzianum*, *Trichoderma viride*, *Metarizium anisopliae*, *Verticilium lecanii, Bauveria bassiana* and botanicals namely Neem, Nishinda, Bhat and Lantana leaf extracts for management of *Pepper leaf curl virus* (*PeLCV*). In response to different selected treatments, percent disease incidence and disease severity on the basis of visible symptoms, analysis of chlorophyll contain in diseases and healthy leaves, yield and yield attributes were recorded. An encouraging performances was observed in reducing the disease intensity in terms of plant infection and appearance the visible symptoms in leaves in comparison to manage at 25, 55, 85 days after transplanting (DAT) which was after 7 days of treatments application. Among the selected endospheric bio-agents, the lowest disease incidence and severity was found in T_7 (*Verticilium lecanii*) treatment followed by T_9 (*Bauveria*) and the highest in T_6 (*T. viride*) treatment. Among the selected botanicals, the lowest disease incidence and severity was found in T_1 (Neem leaf extract) and the highest was found in T_4 (Lantana leaf extract). The chlorophyll contain was tested based on visible symptoms in the field conditions. From the results, it was found that among the treatments, the highest chlorophyll contain was recorded in T_4 treatment (Lantana, 2.23×10^{-03} and 5.52×10^{-03}) and the lowest chlorophyll contain in T_1 treatment (Neem, 0.51×10^{-03} and 1.65×10^{-03}) in case of both types of samples. The effect of different treatments on yield and yield contributing characters also studied and significance variation was observed. From the findings of the present study, it may be concluded that bio-agent, *Verticilium lecanii* or *Bauveria* and botanical, Neem or Bhat leaf extracts can be used as ecofriendly approach for management of *Pepper leaf curl virus* (*PeCLV*). However, further investigation is needed to justify the present findings.

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INTRODUCTION

The pepper (*Capsicum annuum* L.) belonging to family Solanaceae, is used worldwide either as a vegetable or a condiment (Doymaz and Pala, 2002). Pepper (Capsicum) is a tropical and an important agricultural crop and one of the popular vegetables, not only because of its economic value, but also for the combination of color, taste and nutritional values of its fruit (Barham *et al*., 2010; Kouassi and Koffi-Nevry, 2012). The genus Capsicum is part of the large solanaceae family, which, among the more than 90 genera and 2500 species of flowering plants, includes commercially important vegetables such as tomato, potato, chilli and eggplant. This genus is native to tropical and subtropical (Davenport, 2004). Vegetable crops play essential role in the diet, health and livelihood of rural population in the world and Bangladesh. Owing to their flavour, colour, taste and their high content in vitamins, vegetables are important raw materials to pharmaceutical and agro-industries, making them important cash crops to trader's and smallholder farmers in developing country (Moriones *et al*., 2017). Attempts of domestication of vegetables for food and economic purposes has been envisaged centuries ago to feed the ever-growing world's population especially in developing countries where food security burden has ever been an urgent task. In recent years indeed, in spite of significant progress registered in crop improvement technologies, lot of efforts are still needed to establish a stable balance between the needs and supplies.

Factually, the global vegetable's yield trend indicates a steadily drop, which has been ascribed to a set of constraints topped by diseases of which viral attacks are of great significance worldwide (Tolin and Fayad, 2016). Among plant viral infections, germiniviruses, inciting *Pepper Leaf curling virus* (*PeLCV*), and transmitted by whitefly (*Bemisia tabaci*) has been cited among economically impactful plant viral diseases (Gangwar and Gangwar, 2018). Considering the virulence, the broad host range, the dispersion, such diseases

deserve particular attention as it may become epidemic with huge consequences on local and global food security. However, neither local researcher nor the government has considered this issue thought a proper diagnosis of the disease, its host range in the local environment, and the implementation of sustainable control measures would be of paramount importance. A range of chemically diverse synthetic insecticides such as organophosphate, organochlorine and so others, targeting whiteflies as principal vector are currently utilized in endemic areas (Oliveira *et al*., 2013). Nonetheless, the inherent carcinogenic, mutagenic and teratogenic effects, resistant whiteflies genesis (Naveen *et al*., 2017; Shadmany *et al*., 2015), related to synthetic insecticides have motivated the search of eco-friendly approaches (Faoro and Gozzo, 2015). Likewise, efforts are being granted at harnessing endophytes that share many functions and are complementarity with their host to impart fitness in crops under aversive conditions (Eke *et al*., 2016, Niu *et al*., 2018). The so-called endophytes, standing for plant inhabiting microbes are thought to sense physiological changes in stress afflicted plants, and adjust plant gene expression accordingly (De Palma *et al*., 2019). Such fine monitoring of host gene expression has further been demonstrated by (Mayo *et al*., 2016) showing a repression of circumstantial useless genes in favour of defence-related counterparts in common bean (*P. vulgaris L*) primed with *Trichoderma velutinum* under *Rhizoctonia solani* assault. Many other research attempts have depicted the capability of *Trichoderma* species to systemically induce resistance in several hosts against various phytophagous (Eke *et al*., 2016) bacteria (Salas-Marina *et al*., 2015), nematodes (Al-Hazmi and Tariq Javeed, 2016), fungi (Das *et al*., 2019) and viruses (El-Sharkawy *et al*., 2014). Meanwhile, the diversity of earth's ecosystems, and the coevolution concept, reiterates the importance of investigating T*richoderma* from geographically diverse areas as their metabolomics and subsequent efficacy may vary accordingly. On the other hand, consortium rather than single biological control agents (BCA) are currently strongly advocated since it is likely more

effective and transposable in open field trial (Eke *et al*., 2016; Wehner *et al*., 2010). In the present study, screened out the endophytic bio-agents and botanicals for the growth and suppression of *PeLCV* in Pepper plants under field conditions.

Different biological control agents (BCA's) can be used for the control of plant diseases. These include fungi, bacteria and actinomycetes. Biological control of plant pathogens is an eye catching alternative to decrease heavy dependence of modern agriculture on costly chemical fungicides, which not only cause environmental pollution but also lead to the development of resistant strains (Harman *et.al*., 2004). A recent list of mechanisms are *viz*., mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, solubilisation and sequestration of inorganic nutrients, induced resistance and inactivation of the pathogens enzymes (Lewis and Lumsden, 2001). Apart from bio-control ability, the BCA's possess other traits such as rhizosphere competence, tolerance of fungicides, saprophytic competitive ability, ability to tolerate high and low temperatures, adaptability to different edaphic conditions, good searching ability, host specificity, high reproduction rate, short life cycle, adaptability, well adapted to different stages of life cycle of target host, able to maintain itself after reducing host population (Okigbo and Ikediugwu, 2000) have showed that *Trichoderma viride* displaced the naturally occurring mycoflora on the surface of the turmeric. To develop an effective disease management programme, the compatibility of potential bio agents with fungicides is essential. Combinations of fungicides and compatible bio agents in an IDM strategy protects the seeds and seedlings from soil borne and seed borne inoculum (Dubey and Patel, 2012). Combining antagonists with synthetic chemicals eliminates the chance of resistance development and reduces the pesticides application. Species of *Trichoderma* have growth promoting capabilities that may or may not be integral to biological control (Dubey *et al*., 2007). *Trichoderma harzianum* isolated from rhizome rot suppressive soils reduced the disease and

increased plant growth and yield (Ram *et al*., 1999). It has been reported that many *Trichoderma* species has an innate and/or induced resistance to many fungicides but the level of resistance varies with the fungicide (Omar, 2006). The combined use of BCA's and chemical pesticides has attracted much attention in order to obtain synergistic or additive effects in the control of diseases (Locke *et al*., 1985). The virulence and the difficulty to control viral diseases is certainly related to their physio-pathology. Compared to phytophagous pathogens, viruses weaken the host innate immune system and subtilize the cell machinery to establish itself in plant tissues (Li *et al*., 2014). As a matter of fact, the plant functioning, productivity and survival are seriously compromised. Disease suppression in resistant cultivars. Also, among the inducible compounds,

Peppers leaf curl virus (PeLCV), transmitted by whitefly is a cosmopolitan plant viral disease threatening important crops. Chemical elicitors have been used to curb the trend regardless of their setbacks on environment and mankind. Biological control of plant pathogens is an eye catching alternative to decrease heavy dependence of modern agriculture on costly chemical fungicides, which not only cause environmental pollution but also lead to the development of resistant strains. For example some of the reported suppressive effects bio-agent likewise, *Trichoderma* spp. and their consortia against *PeLCV*. The capability of bioagents to release salicylic acid under *in vitro* conditions appear to have prominent role in orchestrating PeLCV suppression. The aim of this proposed study is to evaluate the compatible Bio-agents and selected Botanical extracts against *Pepper* (*Capsicum annuum* L.) *leaf curl virus* (*PeLCV*). In this proposed study, the species of selected compatible bio-agents viz. *Trichoderma viride Verticillium lecanii*, *Metarhizium anisopliae* and *Beauveria bassian* and selected botanical extracts viz. Neem leaves extract, Bhat leave extract, Lantana leave extract and Nishinda leave extract were used to study its compatibility under field conditions.

Objectives:

The research work was carried out to achieve the following specific objectives-

- To evaluate the suppressive effects of selected endospheric bio-agents and botanicals against Pepper leaf curl disease
- To determine the effect of selected bio-agents and botanicals on yield and yield attributes

Review of Literature

Capsicum annuum L. is an economically interesting crop in horticulture, and several protocols have been reported to induce microspore embryogenesis and plant regeneration in different varieties (De *Vaulx et al*., 1981; Mityk´o *et al*., 1999; Mityko *et al.*, 1995; Dolcet-Sanjuan *et al*., 1997; B´ar´any *et al*., 2001).

Pepper is a warm climate crop and requires 25-27°C for optimum seed germination and emergence (Hartmann *et al*., 1988).

These compounds can cause a variety of adverse health effects, including carcinogenic and immunosuppressive activities (WHO and IARC 1993; Pfohl-Leszkowicz and Manderville, 2007).

The use of Bell pepper as a food ingredient, it is also used in traditional medicine especially treating symptoms such as stomach ache, diarrhoea, dysentery. Most gastroenteritis may be a result of contaminated food (Tchiegang *et al*., 1999; Boxman *et al*., 2007)

Peppers include many species and hundreds of varieties and types. They are consumed as fresh unripe fruits, ripened red or other colors and dried forms. The different species, varieties and consumption forms vary in their nutritional and anti-oxidant contents (Howard *et al*., 2000).

Peppers contain wide array of phytochemicals such as vitamins, phenolics and flavonoids that are important anti-oxidants which may reduce degenerative diseases (Howard *et al*., 2000; Abdul Salam, 2015).

Pepper is one of the most important vegetable crops contributing to significant foreign exchange earnings in Sub-Saharan Africa (Osei Bonsu *et al*., 2003).

Foodborne gastroenteritis affects several million people yearly throughout the world. It is one of the main causes of mortality in infants, children, and the elderly (Ogunshe *et al*., 2006).

In the Mediterranean region, pepper is established using seedling transplants for open field production in early spring or into protected cultivation in late summer (Basak *et al*., 2006).

A general fertilizer recommendation for one growing season for Bell pepper is 1000 *lbs* of 18:20:15 (NP_2O_5-K_2O) per acre, or approximately 2.3 *lbs*. of the same nutrient ratio for every 100 sq ft. (Hartz *et al*., 2008).

Peppers are rich in many minerals, vitamins and amino acids essential for human health and growth (Pawar *et al*., 2011)

Bell peppers can be transplanted as a seedling or direct-seeded in to the ground (Trinklein, 2012).

They grow best in temperatures of 75-85°F (24-30°C) (Hartz *et al*., 2008), but can tolerate Guam's hotter temperatures, and can be cultivated all year round on Guam. In tropical regions, bell peppers grow as perennial plants (can grow for more than one season) plants, but because plants are tender and are easily killed by frost, bell peppers are most often cultivated as an annual (one growing season) in temperate regions (Trinklein, 2012).

Around 30 different species are present in the genus Capsicum, out of which 5 species are domesticated which are *Capsicum annuum* L., *Capsicum frutescens* L., *Capsicum chinense* Jacq., *Capsicum baccatum* L., *Capsicum pubescens* Ruiz and Pav. (Sokona *et al*., 2013).

They also are very high in potassium, magnesium, iron and rich in calcium and phosphorus (Pawar *et al*., 2011; Abdul Salam, 2015).

It has high amount of vitamin C and other vitamins such as vitamin A, E and B-complex group of vitamins such as a thiamin (vitamin B_1), riboflavin (vitamin B_2), niacin (vitamin B_3) and pyridoxine (vitamin B_6). Some

minerals such as a potassium, iron, manganese, magnesium and many different flavonoids like lutein, zea-xanthin, β carotene, α-carotene, cryptoxanthin are also present in chili pepper. The amount of nutritional constituents present in 100 g chilli of different form such as raw green, raw red, and cayenne or spices are represented in Table (Chakrabarty *et al*., 2017).

Peppers are rich in vitamin C, vitamin A, vitamin E most B vitamins and in particular vitamin B_5 (Howard *et al*., 2000; Ganguly *et al*., 2017).

Capsicum spp. contains different vitamins, minerals and amino acid which are very beneficial for our nourishment and better health (Saleh *et al*., 2018).

Most mycotoxins are chemically and thermally stable and cannot be destroyed during most food processing operations. Mycotoxins have become an important issue in relation to the food safety requirements for international marketing of agri-food commodities for human and animal consumption (Costa *et al*., 2019).

Co-occurrence of mycotoxins in Capsicum (Santos *et al*., 2011) pepper products can be contaminated with aflatoxins, ochratoxin A, fumonisins, zearalenone, trichothecenes, and patulin. Among those, aflatoxins and ochratoxin A are considered the most abundant and commonly found toxins in Capsicum peppers (Costa *et al*., 2019).

There are approximately 400 diverse varieties of Capsicum grown in the world (Banya *et al*., 2020).

Diseases of capsicum

The pathogen enters the plant through root wounds or penetrate the plant at sites of secondary root emergence and spreads to the plant through the vascular system. *R. solanacearum* remains in the deeper layers of soil, grows endophytically, moves with water and also have an association with the weeds (Wang and Lin, 2005).

Bacterial wilt is one of the most important diseases which is widely distributed among the pepper growing areas of the world (Hayward, 1991; Denny *et al*., 2006).

Pod rotting and Frogeye leaf spot also known as Cercospora leaf spot (*Cercospora capsici*) was also an important disease with varying severity levels from three to five in the region of Ethiopia (Mohammed *et al*., 2009).

The outbreak of Powdery mildew was also reported in southern Ethiopia (Bekele *et al*., 2012).

The disease is most commonly seen in the coastal regions and foothills of India. The pathogen *Ralstonia solanacearum* Smith (Yabuuchi *et al*., 1995) earlier known as *Pseudomonas solanacearum* Smith or *Burkholderia solanacearum* Smith causing bacterial wilt disease of *Capsicum* spp., is known to infect several agricultural crops. It is difficult to assess the loss in the yield due to bacterial wilt as it varies from crop to crop, cultivar, climate, soil type, the presence of root-knot nematodes and strain pathogenicity. Bacterial wilt is ranked as one of the most important plant diseases in the entire world as it causes cent per cent yield losses in solanaceous vegetables (Jyothi *et al*., 2012).

There are several diseases like bacterial wilt, anthracnose, wet rot, Phytophthora rot, leaf curl and powdery mildew which infect pepper crop worldwide (Dhaliwal, 2015).

Capsicum is infected by a number of diseases, among them wilt complex has gained major importance in the State of Jammu and Kashmir from the last 15-18 years and the losses caused by disease are up to the extent of 30-40 per cent (Jaber and Araj, 2018).

Major fungal diseases of capcicum are damping off (*Pythium aphanidermatum* and *Phytophthora* spp.), leaf spots (*Cercospora capsici* and *Alternaria solani*), anthracnose and ripe rot (*Colletotrichum capsici*) and

fruit rot and leaf blight (*Phytophthora* spp.), powdery mil dew *Erysiphe cichoracearum* and *Leveillula taurica*, Early blight (*Alternaria solani*), wilt (*Fusarium oxysporum*), frog eye rot (*Phaeoramularia capsicicola*), leaf spot (*Septoria lycopersici*), fruit spot (*Phoma destructiva*), stem rot (*Macrophomina phaseoli*), dry rot (*Scterotium rolfsii*) and fruit rot (*Phomopsis* spp.), respectively. The post-harvest rots are caused by *Aspergillus terreus, A. candidus*, *A. niger*, *Fusarium moniliforme, F. sporotrichioides, Paecilomyces variotii* and *Penicillium corylophilum* (Bose *et al*., 2002; Gupta and Paul, 2002; Gupta and Thind, 2018).

A significant amount of Hot pepper damage and yield losses associated with various disease complexes like Phytophthora blight, Fusarium wilt disease and Leaf spot around Harato area and villages which have a potential pathogenicity to devastate hot pepper in a short period of time. Hence an attempt was made to evaluate different hot pepper varieties against these complex fungal pathogens to identify the best performed varieties. Therefore, the objective of this paper was laid with response of different hot pepper varieties against major fungal diseases under open field and greenhouse conditions. (Muvea *et al*., 2018).

The present situation indicates that the pathogens (Fungal, bacterial and viral) are devastating the crop at various growth stages from nursery to harvesting and significant yield loss is observed which limits the production of hot pepper by small holder farmers. Hot pepper is attacked by complex wilting disease caused by fungi such as blight (*Phytophthora capsici*), *Rhizoctonia* and *Fusarium* spp. Not only in Western Ethiopia, but also in the country as a whole (Gobie, 2019).

Taxonomy

The chromosome number of *C. annuum* is 2n = 24 and *C. annuum* var. *glabriusculum* is tetraploid (2n = 48) from Florida (Emboden, 1961).

Previously this taxon was named *C. annuum* var. *aviculare* (Dierb) (D'arcy and Eshbaugh, 1973).

Capsicum chinense is characterized as a small stout shrub up to 1.5m tall, glabrous to puberulent with two flowers or more at a node, the flowers are pendant and have a prominent constriction between the base of the calyx and the pedicel, especially when in fruit, the flower lacks calyx teeth, the corolla is dull white rarely greenish white, anthers are blue to violate rarely yellow, the style and stigma are rarely exerted more than 1mm and the fruit of many different colors, contain seeds that are cream to yellow (D'arcy and Eshbaugh, 1974).

Capsicum frutescens is a species of the lowlands, it is a small shrub or tree like shrub up to 2m tall, it can be herbaceous to woody, typically two or more flowers are present per node, flowers lack a prominent constriction between the base of the calyx and the pedicel, calyx teeth are absent, the corolla is greenish white, anthers are blue to violate rarely yellow, the style and stigma are rarely exerted 1.5mm or more beyond the anthers and the immature fruit is green without dark pigmentation while mature fruit is red or very rarely orange, erect and deciduous , the seeds are cream to yellow (D'arcy and Eshbaugh, 1974).

Capsicum annuum is a small shrub 2m tall with white to bluish-white flowers, most often node, calyx teeth are lacking or short, rarely exceeding 0.5mm and there is no prominent constriction between the base of the calyx and the pedicel (D'arcy and Eshbaugh, 1974).

The proposed wild ancestor of this species is *C. annuum* var. *glabriusculum* (Dunal) Heiser and Pickersgill (1975).

The solanaceae is a complex, cosmopolitan, family comprised of at least 98 genera and as many as 2716 species including capsicum, an economically important plant (Hunziker, 2001, Olmsted and Bohs, 2006), Many genera are poorly understood, generic boundaries within the family remain poorly

defined and the source of taxonomic confusion. Within several genera, e.g. Solanum, little is known about relationship of species. The genus Capsicum was originally characterized as having rotate corollas. This description was modified when heiser and smith (1958) described *C. Cardenasii* as having campanulate corollas. Later description of *C. scolnikianum* (Hunziker, 1960) and *C. friburgense* (Barboza and Bianchetti, 2005) confirmed that the campanulate corolla is found among other capsicum species.

Eshbaugh (1984) provides maps of the hypothetical distribution of the domesticated pepper species at the time of European discovery as extrapolated from Heiser (1976), Eshbaugh (1975) and McLeod *et al*., (1982).

Eshbaugh *et al*. (1983) suggested that *C. annuum C. fruitescens* and *C. chinense* form a closely linked group that evolved in the lowland tropics of Latin America and the Caribbean with *C. annuum* eventually dominating Mexico, *C. fruitescens* the Caribbean and *C. chinense* Amazonas. Columbus and subsequent explorers of Mesoamerica were responsible for introducing *C. annuum* chilli peppers to Europe while Portuguese explorers introduced *C. chinense* to Eastern Europe, Africa and Asia (Eshbaugh, 1983; Andrews, 1993).

In 1988 Pickersgill wrote that the status of *C. annuum C. fruitescens* and *C. chinense* could be legitimately questioned. Some taxonomists continue to treat these three domesticated taxa as distinct species while corresponding wild forms intergrade to such an extent that it is often impractical, if not impossible, to give them distinct taxonomic names (Eshbaugh *et al*., 1983; Eshbaugh 1993).

Bird dispersal of capcicum seeds has recently been documented (Levey *et al*., 2006).

Capsicum is a genus comprised of as many as 36 species with at least five taxa from Southern Brazil not yet treated taxonomically (Pozzobon *et al*.,

2006). Several new species of capsicum have recently been describe from Bolivia (Nee *et al*., 2006).

Biological Control Agent (BCA)

A prerequisite for the commercial use of BCA's is that inoculum retains high cell viability and can easily be transported and applied (Kloepper and Schroth, 1981).Furthermore, proper timing and placement of applications can greatly reduce the amount of BCA required (Fravel, 2005).

Several bacteria and fungi have been reported as BCA's against *V. Dahlia* (Tjamos *et al*., 2004; Malandraki *et al*., 2008; Zheng *et al*., 2011; Veloso and Dıaz, 2012).

These BCA's are capable of colonizing the root system and protecting plants against *V. dahliaeby* employing several mechanisms of action, such as antibiosis, competition for nutrients or space on roots, and triggering of induced systemic resistance (ISR) (Tjamos *et al.*, 2005; Malandraki *et al*., 2008; Veloso and Dıaz, 2012; Li *et al*., 2013).

Beauvericin forms Na^+ and K^+ complexes leading to increased permeability of natural and artificial membranes (Ovchinnikov *et al*., 1971).

Beauvericin shows antibiotic activity against several bacteria like *Bacillus subtilis*, *Escherichia coli*, *Mycobacterium phlei*, *Sarcinea lutea*, *Staphylococcus aureus* and *Streptococcus faecalis* (Ovchinnikov *et al*., 1971).

Another toxin secreted by B. bassiana is the cyclo-octadepsipeptid e called bassianolide (Suzuki *et al*., 1977).

Bassianolide, like beauvericin, is an ionophore antibiotic but diVers in its reaction to different cations (Suzuki *et al*., 1977; Kanaoka *et al*., 1978).

Beauveria species toxicity for animals and plants, even their insecticidal activity, remains generally unknown. Where tested, they give negative tests, with the exception of Beauveriolide I (Mochizuki *et al*., 1993). They are also

produce beauveriolides and beauverolides which are peptides structurally related to beauvericin and bassianolid e (Namatame *et al*., 1999).

Beauvericin has been isolated from the entomopathogeni c fungi *Beauveria spp.* and *Paecilomyces spp.* and the plant-pathogeni c fungi *Fusarium spp.* and *Polyporus fumosoroseus* (Grove and Pople, 1980; Gupta *et al*., 1991; Plattner and Nelson, 1994).

Furthermore, beauvericin has moderate insecticidal properties (Suzuki *et al*., 1977; Kanaoka *et al*., 1978; Champlin and Grula, 1979; Zizka & Weiser, 1993; Gupta *et al*., 1995).

The entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales), is largely used as an alternative to chemical pesticides for the biocontrol of insect pests and is the active ingredient of several commercial products used worldwide for sustainable pest management (De Faria and Wraight, 2007; Saranraj and Jayaprakash, 2017).

In the last decade, the capability of *B. bassiana* to endophytically colonize a wide range of host plants has been proven as well as its capacity to induce plant resistance against insect pests and pathogens (Ownley *et al*., 2004; Quesada-Moraga *et al*., 2006; Ownley *et al*., 2008; Vega, 2008; Quesada-Moraga *et al*., 2009; Vega *et al*., 2009; Quesada-Moraga *et al*., 2014; Vidal and Jaber, 2015; Wei *et al*., 2020).

Metarhizium anisopliae, on the other hand, is not as common in nature as *B. bassiana*. However, the fungus has also been reported as a plant tissue colonizer, plant growth enhancer, or as a naturally occurring endophyte (Hu and St. Leger, 2002; Akello and Sikora, 2012; Liao *et al*., 2014; Behie *et al*., 2015; Greenfield *et al*., 2016).

Artificial inoculation of plants with entomopathogenic fungi and their establishment as endophytes has been successfully demonstrated in several arable crops such as sweet pepper (Jaber and Araj, 2018).

Trichoderma is one of the most studied BCA's in plants, being part of several commercial formulations. This may be explained by its antimicrobial activity arising from mechanisms such as antibiosis, mycoparasitism or competition (Zeilinger *et al*., 2016); ability to induce systemic resistance and plant defence (Hermosa *et al*., 2012); positive effects on seed germination and plant growth (Zhang *et al*., 2016); and versatility and cosmopolitan distribution (Hermosa *et al*., 2012).

However, although the beneficial role of *Trichoderma* on plant growth and productivity is universally accepted, some negative effects have also been reported (Menzies, 1993; Marın-Guirao *et al*., 2016).

The successful use of *Trichoderma* species against *F. circinatum* has been documented in in vitro trials (Iturritxa *et al*., 2011; Moraga-Suazo *et al*., 2011; Martınez-Alvarez *et al*., 2012) and in planta (Lopez- Lopez *et al*., 2016).

However, strains of *T. harzianum* and *T. viride*, which proved to be effective in vitro, failed to control the disease in *P. radiata* at the seed and seedling stages (Martınez- Alvarez *et al*., 2012, 2016).

Botanicals

Neem oil is one of the most promising substances in the current approach to pest control. Neem oil is a product of the Indian neem tree *Azadirachta indica* (A.) Juss. It possesses a variety of insecticidal properties, such as repellency (Bina *et al*., 2017), antifeedancy, toxicity, and growth disruption, against numerous pest species (Saxena, 1989).

Several members of the mahogany family (Meliaceae), to which neem and chinaberry (*Melia azedarach*) belong, are economically important timber species, and the wood and bark of some of these contain appreciable concentrations of limonoids that have antifeedant and/or growth inhibitory properties against pest insects (Assabgui *et al*., 1997; McLaughli *et al*., 1997).

The biochemical effect (growth inhibition, feeding deterrence, oviposition inhibition) of neem oil against more than 30 Lepidopteran pests (Senthil-Nathan, 2013) has been well documented. The main active ingredient is azadirachtin, a tetranortriterpenoid, which was isolated from the seeds of *Azadirachta indica* by Butterworth and Morgan (Butterworth and Morgan, 1968) and is known to disrupt insects' metamorphosis (Tomlin, 1997). Neem oil is a contact insecticide, but even systemic activity has been documented (Osman and Port, 1990).

Vitex negundo stem bark yields leucoanthrocyanidins (chopra *et al*., 1956; Hussain *et al.,* 1992).

Basu and Singh (1944), Gupta and Behari (1973) and Joshi *et al*. (1974), reported isolation of n-Tritriacontane, n-hentriacontanol, nhentricontane, n-pentatricontane, n-nonacosane, β sitosterol, phydroxybenzoic acid and 5-oxyisophthalic acid; 3, 4- dihydroxybenzoic acid was also isolated from the seeds of *Vitex negundo*.

GS, (1980) isolated three new flavones glycosides which were identified as 3,6,7,3',4'-Pentamethoxy-5-Oglucopyranosyl- rhamnoside, vitexin cafeate, 4'-O-methyl myricetin- 3-O-[4''-O-β-D-galactosyl]-β-D-galactopyranoside from *Vitex negundo*.

Li and Guan (1987), Chandra and Babber (1987), Banerji *et al*. (1988), Kosankar *et al*. (2000) reported from the leaves and twig of *Vitex negundo*, a stilbene derivative, characterised as 4,4'-dimethoxy-trans-stilbene, along with five flavones, 5,6,7,8,3'4'5- heptamethoxy, 5-hydroxy-6,7,8,3'4'-pentamethoxy (5-Odesmethylnobiletin), 5-hydroxy-6,7,8,3',4',5-hexamethoxy (gardenin A), 5-hydroxy-6,7,8,4'-tetramethoxy (gardenin B) and 5-hydroxy-7,3',4',5'-tetramethoxyflavone(corymbosin).

Bhargava (1989) and Telang *et al*. (1999) isolated two flavones 5,7,3'-trihydroxyflavone, 6,8,4'-trimethoxyflavone from *Vitex negundo*.

Kuo-Chung *et al.* (1989), Singh *et al*. (2004) and reported isolation of terpinen-4-ol, α-terpineol, sabenine, globulol, spathulenol, β-farnesene, farnesol, bis (1,1dimethyl) methylphenol, α-pinene, β- pinene, linalool, terpinyl acetate, caryophyllene epoxide, caryophyllenol along with viridiflorol from *Vitex negundo*.

Vitex negundo Leaves contain an alkaloid nishidine, flavonoids like flavones, luteolin-7-glucoside, casticin, iridoid glycoside, an essential oil and other constituents like vitamin-C, carotene, benzoic acid, β-sitosterol and C-glycoside (Hussain *et al*., 1992).

Vitex negundo seeds contain hydrocarbons, β-sitosterol, benzoic acid and phthalic acid (Hussain *et al*., 1992), anti-inflammatory diterpene, flavonoids, artemisin, tri terprnoids (Chawla *et al*., 1992).

Chawla *et al*. (1992) reported a flavonoid artemetin from *Vitex negundo*.

Chawla *et al*. (1992) and Hebbalkar *et al*. (1992) reported triterpenoids 3β-acetoxyolean-12-en-27-oic acid, 2α,3α- dihydroxyoleana-5,12-dien-28-oic acid, 2β,3α-diacetoxyoleana-5,12- dien-28-oic acid and 2α,3β-diacetoxy-18-hydroxyoleana-5,12-dien- 28-oic acid from *Vitex negundo*.

Chawla *et al*. (1992) and Ono *et al*. (2004) isolated a new phenyl dihydro naphthalene-type lignan, vitedoin A, a new phenyl naphthalene-type lignan, vitedoamine A and a new trinorlabdane-type diterpene, vitedoin B from the seeds of *Vitex negundo* along with five known lignin derivatives. Their chemical structures were determined mainly on the basis of NMR and MS data.

The studies of Dariyat and Lagurin (1994), revealed a four iridoids in the pharmacologically-active fraction of the leaves of *Vitex negundo* L. which were identified as 2'-p-hydroxybenzoyl mussaenosidic acid, agnuside & lagundinin. The data obtained for 2'-phydroxybenzoyl mussaenosidic acid modifies a previous assignment while lagundinin is a newly identified iridoid. Three of the iridoids contain glucosyl and p-hydroxybenzoic acid

moieties. In addition to the four iridoids which were reported, two other iridoids were known to occur in the leaves of *Vitex negundo*, aucubin and nishindaside.

Ragasa *et al*. (1999) from the chloroform extract of Vitex negundo leaves performed the structure elucidation of vitexilactone and casticin. This is first report on the isolation of vitexilactone from *Vitex negundo*, its structure elucidation by NMR. Casticin was earlier reported as a constituent of *V. negundo*.

Krishna *et al*. (2002) isolated β-amyrin, epifriedelinol and oleanolic acid from the heartwood of *Vitex negundo*.

Singh *et al*. (2003) isolated the twelve pure compounds, namely viridiflorol, squalene, 5-hydroxy-3,6,7,3',4'-pentamethoxy flavone, 5-hydroxy-3,7,3',4'-tetramethoxy flavones, 5,3-dihydroxy- 7,8,4- trimethoxy flavanone, p-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, luteolin 7-glucoside, isoorientin, agnuside and 2'-phydroxybenzoyl mussaenosidic acid and characterized by spectral data (UV, IR, NMR, & MS) from the different leaf extracts. Squalene is reported for the first time from the *V. negundo* leaves. This is the first report of the isolation of squalene from the leaves.

Diaz *et al.* (2003), revealed the known flavones vitexicarpin, methylated, acetylated and six new acylated derivatives, identified as 3'-Benzoyloxy-5-hydroxy-3,6,7,4'-tetramethoxyflavone, 5,3'- Dibenzoyloxy-3,6,7,4' tetramethoxyflavone, 5,3'-Dipropanoyloxy- 3,6,7,4'-tetramethoxyflavone, 5,3'-Dibutanoyloxy-3,6,7,4'-tetramethoxyflavone, 5,3'-Dipent-4-enoyloxy 3,6,7,4'-tetramethoxyflavone, 5,3'-Dihexanoyloxy-3,6,7,4' tetramethoxyflavone from the chloroform extract of the leaves of *Vitex negundo*.

Chandramu *et al*. (2003) proved the presence of two pentacyclic triterpenoids, betulinic acid (3β-hydroxylup-20-(29)-en-28-oic acid) and

ursolic acid (2β-hydroxyurs-12-en-28-oic acid) from Vitex negundo leaves along with three other compounds; an aliphatic alcohol n-hentriacontanol, p-hydroxybenzoic acid.

Malik and Khan (2004) and Dayal (2004) from the root of *Vitex negundo* isolated Vitexoside a new flavonoid glycoside and agnuside, R-dalbergiphenol.

Singh *et al*. (2010) proved the presence of volatile oil which contains ten volatile components like α-copaene, β-caryophyllene, β-elemene, camphene, α-thujene, α-pinene, sebinene, linalool, stearic acid and behenic acid in *Vitex negundo*.

Sun (1989), Pan *et al*. (1989), Mallvarapu (1994), Singh (1999) and Rameshwar and Virendra (2000), reported isolation of α-elemene, δ-elemene, β-elemene, β-eudesmol, camphor, camphene, careen, 1,8- cineol, 1-oceten-3-ol, γ-terpinine, α-phellendrene, β-phellendrene, α- guaiene, abieta-7,13-diene, neral, geranial, bornyl acetate, nerolidol, β-bisabolol, cedrol from *Vitex negundo*.

Malik *et al*. (2006) from the methanolic extracts of the Bhat roots isolated eight lignans, identified as negundin A, negundin B, 6- hydroxyl-4-(4-hydroxy-3-methoxy)-3-hydroxymethyl-7-methoxy-3, 4-dihydro-2-naphthaledehyde, vitrofolal E, (+)-lyoniresinol, (+)- lyoniresinol-3α-O-β-D-glucose, (+)-(-)-pinoresinol and (+)- diasyringaresinol.

Methanolic extract of Bhat (*Clerodendrum infortunatum* Linn.) were studied against carbon tetrachloride induced hepatotoxicity in rats. This study was evaluated by assaying the serum biochemical parameters like glutamate pyruvate transaminase (ALT), glutamate oxaloacetate transaminase (AST), alkaline phosphatase (ALP), bilirubin and total protein. Malondialdehyde (MDA) level, as well as reduced glutathione (GSH) content and catalase activity (CAT) was determined. The substantially elevated serum enzymatic levels of AST (SGOT), ALT (SGPT), ALP and total bilirubin were restored

towards normalization significantly by the extract. The hepatoprotective activity of the methanolic extract was further supported by histological studies as the normal rats did not show any abnormal state in their liver architecture. The results of this study revealed that methanol extract of Bhat (*Cleodendrum infortunatum*) has moderate hepatoprotective activity. The hepatoprotective activity of the methanolic extract of Clerodendrum infortunatum may due to the presence of flavonoids, terpenoids and saponins in the extract. (Sannigrahi *et al*., 2009).

The plant was also found to contain triterpenes, steroids and flavonoids (Sannigrahi *et al*., 2009).

Saponin (SN) isolated from Bhat (*Cleodendrum infortunatum*) leaves exhibited protection from writhing induced by 1.2% v/v acetic acid in Adult Swiss albino mice. SN1 was administered ip at doses of 30, 50, 75 and 100 mg/kg and standard drug used were aspirin, paracetamol and morphine sulphate. In hot plate method, SN not only produced analgesia in mice but also potentiated the analgesic action of pentazocine and aspirin (Pal *et al*., 2009).

Saponin (SN) was isolated from Bhat (*Cleodendrum infortunatum*) leaves also showed anticonvulsant activity. The anticonvulsant activity was tested by leptazol induced seizures. SN was administered ip in different doses (20-100) mg/kg body weight. It was observed that SN decreased the duration of seizures and gave protection in a dose dependent manner against leptazol induced convulsions. The results suggested that the saponin (SN) has significant anticonvulsant effect (Pal *et al*., 2009).

Bhat (*Clerodendrum infortunatum* Linn.) leaves on preliminary chemical analysis were found to contain saponin, clerodin (a bitter diterpene) 4, 6 and some enzymes. Leaves also contain a fixed oil which consists of Glycerides of Lenoleic, oleic, stearic and lignoceric acid. Luperol and β-sitosterol from roots. Clerosterol identified as 5, 25-sigmastadien_3β-ol, clerodolone as lup_20(30)-en-3β-diol-12-one and clerodone as 3β-hydroxy-lupan-12-one

and a steroidal glycoside from roots (Jorn.Ind.chem.society.1967,44,549). Previous phytochemical investigation of the plant revealed the presence of alkyl sterols and 2,-(3, 4-dehydroxyphenyl) ethanol 1-O-α-2rhamnopyranosyl-(1→3)-β-D-(4-O-caffeoyl) glycopyranoside (acteoside) in this plant (Modi *et al*., 2010).

Ethanolic extracts of Bhat (*Clerodendron infortunatum*) showed significant activity against four clinical strains of bacteria Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, and E.coli. The standard drug used was tetracycline (100 mcg/ml).Three strains of fungi namely Aspergillus niger, Aspergillus flavus and Candida albicans. In both the cases, the ethanolic extracts at dose concentration of 500 mcg/ml showed significant antimicrobial activity (Modi *et al*., 2010).

Alcohol and aqueous extracts from the leaves of Bhat (*Cleodendrum infortunatum*) were investigated for their anthelmintic activity against pheretima posthuma and five concentrations (5, 10, 15, 20 and 25 mg/ml) of each extracts were studied in activity, which involved the determination of time of paralysis and time of death of the worm. Both the extracts exhibited significant anthelmintic activity at highest concentration of 100 mg/ml (Modi *et al*., 2010).

Ethanolic extract of leaves of Bhat (*Cleodendrum infortunatum*) were examined for their antioxidant effects at various concentrations(0.020-0.10)mg/ml in the DPPH radical scavenging assay, (20-100) mcg/ml in FRAP assay (Ferric Reducing Antioxidant Power) in comparison to Vitamin C in same conc. Respectively. The ethanolic extracts were also examined for the Hydrogen peroxide radical scavenging assay. The results of the study revealed that the plant extract has significant antioxidant activity (Modi *et al*., 2010).

The leaves of *Lantana camara* Linn. are used as a bechic, antitumoral, antibacterial and antihypertensive agent (Taoubis *et al*., 1997).

Several tri-terpenoids, napthaquinones, flavonoids, alkaloids and glycosides isolated from this plant are known to exert diverse biological activities including cytotoxic and anticancer properties (Ghisalberti, 2000).

Etiology of virus

The viruses belonging to the family Geminiviridae are among the major limitations that cause huge losses to vegetable crop production. Geminiviruses are controversially one of the most baleful plant viruses in the entire world and cause a vast threat to global food security. They are ample in tropical and subtropical environments, where insects that spread these viruses are plentiful. The aetiology of chilli leaf curl disease found in tropical and subtropical areas had proved to be elusive till 1977 when circular single-stranded DNA (ssDNA) containing geminiviruses were reported (Harrison *et al*., 1977).

The genomes of begomoviruses commonly contain one or two circular, single-stranded DNA components of 2.5–3 kb in size, known as DNA A and DNA B (Mayo, 1999; Hanley-Bowdoin *et al*., 1999).

ICTV raised geminivirus group to the status of family Geminiviridae in its XI International Congress of Virology held in 1999 in Sydney. The standards for demarcation of species of the members of family Geminiviridae were revised by ICTV and on the basis of genome organization, distinguished insect vector and host range instead of three, there are four genera distinguished (Mastrevirus, Curtovirus, Begomovirus and Topocuvirus) (Fauquet and Stanley, 2003).

The two components of bipartite begomoviruses share a highly conserved common region (CR), approximately 200 nucleotides (nt) in length which consists of reiterative sequence-specific replicase (Rep) binding motifs called iterons and a nonanucleotide stem-loop structure (TAATATTAC),

required for replication of the viral genome (Moffat 1999; Fauquet and Stanley 2003).

On the basis of the geminate morphology of the virus particles and circular ssDNA as their genomic component, geminiviruses were perceived as a separate group by International Committee on Taxonomy of Viruses (ICTV) in 1978 (Rishi 2004).

Begomoviruses are described by twin icosahedral particles, approximately 18 9 30 nm in size (Stanley *et al*., 2005).

Most of the monopartite begomoviruses are consociated with a satellite molecule of approximately 1.4 kb, known as the betasatellite, which is necessary for symptom appearance but depends fully upon the helper virus for its replication, encapsidation and cell-to-cell movement (Mansoor *et al*., 2003; Briddon and Stanley 2006).

For the first time, it was substantiated that chilli leaf curl disease (ChiLCD) was caused by a complex consisting of the monopartite, ChiLCV and a betasatellite, Tomato leaf curl Bangladesh betasatellite (ToLCBDB) (Chattopadhyay *et al*., 2008).

Both bipartite and monopartite begomovirus with alpha and beta satellites have been reported to be associated with ChiLCVD (Hussain *et al.,* 2004; *George et al*., 2014).

The genus begomovirus (Geminiviridae) with 288 species is the largest genus of all viral taxonomy currently recognized by the ICTV (Brown *et al*., 2015).

Recently on the basis of genome organization, nucleotide sequence similarities and biological properties, the geminiviruses are now classified into nine genera—Becurtovirus, Begomovirus, Capulavirus, Eragrovirus, Grablovirus, Mastrevirus, Curtovirus, Topocuvirus and Turncurtovirus (Zerbini *et al*., 2017).

Vector and host range

The non-viral aetiology of leaf curl disease complex was experimentally proved, and it was concluded that it was the result of damage caused by thrips (upward curl) and mites (downward curl) (Amin, 1979).

On the basis of host range (*C. annuum*, *C. frutescens*, *C. microcarpum*, *Solanum lycopersicum* and *Nicotiana tabacum*) and transmission, it was reported that the ChiLCVD has been caused by the agent of tobacco leaf curl (Muniyappa and Veeresh, 1984).

Whitefly *B. tabaci* was first described in Greece in 1889 as a pest of tobacco known as tobacco whitefly (*Aleyrodes tabaci*) (Gennadius, 1889).

In 1897, whitefly was known as sweet potato whitefly (*B. inconspicua*) as it was first reported on sweet potato in the New World in the USA (Brown *et al*., 1995).

Further extension of its geographical range has occurred to include temperate climate areas; the species is now globally assorted and found on all continents except Antarctica (*Martin et al*., 2000).

Association between plant viruses and their insect vectors is very much complicated. Some plant viruses are carried in the insect's feeding apparatus and can be acquired and inoculated within seconds or minutes (non-persistent transmission). Other viruses circulate in the body and can be transmitted only after the incubation period of hours to days (persistent transmission). ChiLCVD causing by begomoviruses is transmitted by whitefly *B. tabaci* in a persistent manner. Whiteflies are small piercing and sucking insects of the family Aleyrodidae, order Hemiptera, which have been consociated with agriculture and with the transmission of plant viruses for many years (Czosnek *et al*., 2002).

It is a polyphagous pest and is noted on more than 600 plant species spreading more than 60 plant viruses (Rishi, 2004).

Whitefly was able to transmit ChiLCV from field samples to 50–100% of chilli test plants, which produced typical disease symptoms (Senanayake *et al*., 2007).

In an epidemic of chilli leaf curl disease at Jodhpur (Rajasthan), it was concluded that several isolates were effectually transmitted by whitefly, all of which produced severe leaf curl symptoms in chilli (Senanayake *et al*., 2012).

A single whitefly was able to transmit the virus, and eight or more whiteflies per plant resulted in 100% transmission. The minimum acquisition access period (AAP) and inoculation access period (IAP) were 180 and 60 min, respectively. The virus was only able to infect five species, viz, *C. annuum*, *Carica papaya*, *S. lycopersicum*, *N. tabacum* and *N. benthamiana*, out of the 25 species tested from various families, viz, Asteraceae, Caricaceae, Cucurbitaceae, Euphorbiaceae, Leguminosae, Malvaceae and Solanaceae (Senanayake *et al*., 2012).

ChiLCV was also reported to infect species like *Petunia hybrida*, *Amaranthus sp.*, *Mentha spicata* and *Mirabilis jalapa* (Al-Shihi *et al.*, 2014; *George et al*., 2014; Saeed *et al.*, 2014; Nehra and Gaur 2015; Jaidi *et al*., 2017).

Infection of ChiLCV and Tomato leaf curl betasatellite on watermelon was reported first time by Shahid *et al*., (2017) at Oman.

Materials and Methods

An experiment was conducted to evaluate the efficacy of biological elicitor potential of endospheric bio -agents and botanicals against *Pepper leaf curl virus* (*PeLCV*). The selected treatments were evaluated through a field experiment in natural conditions. Materials used and Methodology followed in this study are included in this chapter.

3.1. Experimental site

The field experiment was conducted in central farm of Sher-e-Bangla Agricultural University and lab experiment was conducted in Bio-agents laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 (Appendix: II).

3.2. Experimental duration

The experiment was carried out during the period July, 2020 to June, 2021.

3.3. Climate

The experimental site is situated in a subtropical environment that has three distinct seasons: summer (March to April), monsoon (May to October), and winter season (November to February). The investigation was carried in the winter or popularly known as the Rabi season (November 05, 2020 – March 20, 2021), and the major meteorological data (air temperature, relative humidity, and rainfall) of this period were collected from Bangladesh Meteorological Department (Climate & weather division), Dhaka, which is presented in the Appendix III.

3.4. Soil

The experiment done in central farm of Sher-e-Bangla Agricultural University which belongs to the Madhupur Tract (AEZ-28). The soil used in the experiment had a silty loam texture, low organic matter content, and dark olive-grey color. The analytical data of the soil sample collected from the experimental area was determined in the Soil Resource 20 Development Institute (SRDI), Soil Testing Laboratory, Khamarbari, Dhaka and were presented in Appendix V.

3.5. Planting Material

Seeds of BU capsicum -1 were used as the test crop for this experiment. It was collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. Healthy uniform capsicum seeds were selected, soaked in water overnight, and sown on Nov 05, 2020.

3.6. Design and treatments of the experiment

The experiments were laid out in randomized complete block design (RCBD). In total ten (10) treatments with three replications were codified to achieve the stated specific objectives. Treatments codified for this study are given below-

T_0 – Control

T_1 – Neem leaves extract

T_2 – Nishinda leaves extract

T_3 – Bhat leaves extract

T_4 – Lantana leaves extract

T_5 – *Trichoderma harzianum*

T_6 – *Trichoderma viride*

T_7 – *Verticillium lecanii*

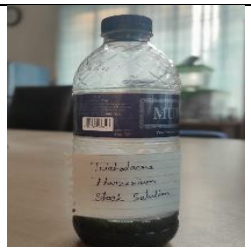
T_8 – *Metarhizium anisopliae*

T_9 – *Beauveria bassiana*

3.7. Experimental Work

3.7.1. Collection of Bio-agents

The selected endospheric bio-agents was provided by ACI Agro Chemical Limited. The details of bio-agents used in the study are given below-

Number	Name of Bio-agents	Types of Bio-agent	Picture
01.	*Trichoderma harzianum*	Bio-pesticide	
02.	*Trichoderma viride*	Bio-pesticide	
03.	*Verticillium licanii*	Entomophagous fungus	
04.	*Metarhizium anisopliae*	Entomophagous fungus	
05.	*Beauveria bassiana*	Entomophagous fungus	

3.7.2. Multiplication of bio-control agents

The selected bio-agents were cultured and multiplied in artificial media PSA and appearance of mycelium and spores were studied through microscopy.

3.7.3. Method of bio-agents preperation

3.7.3.1. *Verticillium lecanii*

The pour plate technique was adopted for the inoculation of *Verticillium lecani* 1.50 % LF (Bio-Catch) into the solid medium. One gram of liquid formulation of *Verticillium lecani* 1.50 % LF (Bio-Catch) was thoroughly mixed in a flask containing 99 ml of sterile distilled water labelled flask 1:1:10,000 dilution (10^{-4}) and serially diluted upto 10^{-6}. Four replicates were maintained for each dilution factor. Pipetted out 1ml of supernatant solution and transferred into petri dish. The melted agar medium was poured into Petridis containing the suspension of *Verticillium lecani* 1.50 % LF (Bio-Catch). The inoculation was carried out aseptically by using laminar air flow chamber. Allowed the medium to solidity for few minutes. Then labelled each petri dish with inoculation date.

3.7.3.2. *Metarhizium anisopliae*

The pour plate technique was adopted for the inoculation of *Metarhizium anisopliae* 1.15 % WP (Bio Magic) into the solid medium. One gram of powdered formulation of *Metarhizium anisopliae* (Bio Magic) was thoroughly mixed in a flask containing 99 ml of sterile distilled water labelled flask 1:1:10,000 dilution (10^{-4}) and serially diluted up to 10-8. Four replicates were maintained for each dilution factor. Pipetted out 1ml of supernatant solution and transferred into petri dish. The melted agar medium was poured into petri dish containing the suspension of *Metarhizium anisopliae* (Bio Magic). The inoculation was carried out aseptically by using laminar air flow chamber. Allowed the medium to solidity for few minutes. Then labelled the each petri dish with inoculation date.

3.7.3.3. *Beauveria bassiana*

The pour plate technique was adopted for the inoculation of *Beauveria bassiana* 1.50 % LF (Bio-Power) into the solid medium. One gram of liquid formulation of *Beauveria bassiana* 1.50 % LF (Bio-Power) was thoroughly mixed in a flask containing 99 ml of sterile distilled water labelled flask 1:1:10,000 dilution (10^{-4}) and serially diluted up to 10^{-8}. Four replicates were maintained for each dilution factor. Pipetted out 1ml of supernatant solution and transferred into petri dish. The melted agar medium was poured into petri dish containing the suspension of *Beauveria bassiana* 1.50 % LF (Bio-Power). The inoculation was carried out aseptically by using laminar air flow chamber. Allowed the medium to solidity for few minutes. Then labelled each petri dish with inoculation date.

3.7.3.4. *Trichoderma viride* and *Trichoderma harzianum*

For the treatment purpose *Trichoderma viride* solution was collected from the available sources (ACI Company) and diluted with distilled water. The diluted solution was applied in Petridis in cup method.

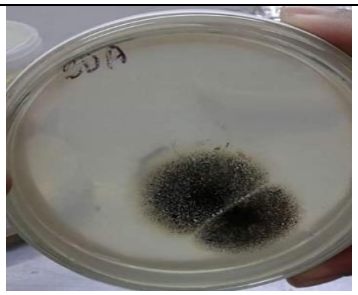
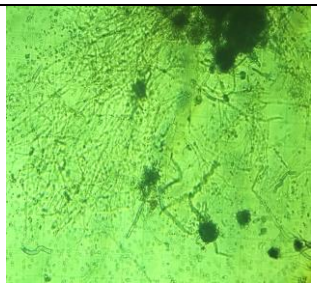
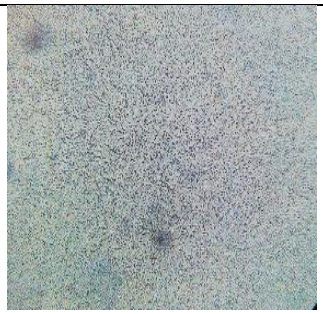
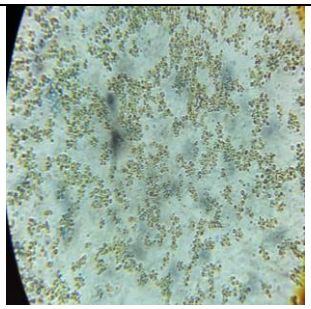
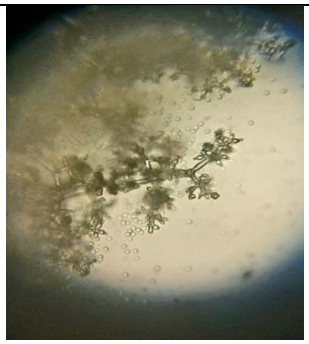
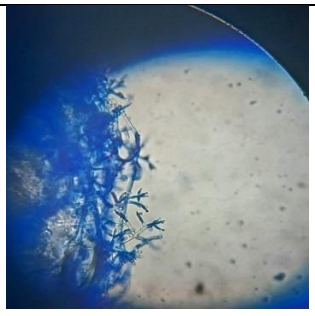
Bio-agents	Pure culture of Bio-agents	Morphological structures
Verticillium lecanii		
Materhizium anisopliae		
Beauveria bassiana		
Trichoderma viride		
Trichoderma harzianum		

Plate no 1. Collection of Bio-agents

3.7.4. Collection Botanicals

The selected botanicals were collected from the Sher-e-Bangla Agricultural University Campus. The details of botanicals used in the study are given below-

No.	Selected Botanical	Botanical name	Picture of Leaves	Picture of Extracts
1.	Neem leaves extract	*Azadirachta indica*		
	Bhat leaves extract	*Clerodendrum infortunatum*		
3.	Nishinda leaves extract	*Vitex negundo*		
	Lantana leaves extract	*Lantana camara*		

Plate no 2. Collection of Botanicals

3.7.5. Preparation botanicals extract

For extraction of juice, required amount of respective leaves of each botanicals was taken and washed in tap water. The leaves were blended in an electric blender adding equal amount of sterile water for 1:1 solution. The blend was filtered through sterile cheese cloth. The supernatant was diluted in equal amount of sterile water for 1:2 solution.

3.7.6. Field experiment

3.3.6.1. Planting materials

In this research work, seeds of capsicum (Mesi) were used as planting materials. The seeds were collected from Siddik Bazar Seed Market, Dhaka.

3.7.6.2. Seedling Preparation

For the seedlings preparation, seeds were soaked overnight in distil water. Seedlings were grown in a seed bed of the experimental field of SAU. The soil of seed bed was mixed with Furadan 5G and covered the whole soil with polythene sheet to sterilize the soil. Then it was mixed with desired amount of fertilizers and cowdung. Finally, the seeds were sown in individual row and proper care was taken for better germination and seedling development.

a) Sowing of Seed

b) Seedlings of capsicum

Plate no 3. Seedling Preparation

3.7.7. Land Preparation and Transplanting of Seedling

3.7.7.1. Land Preperation

The selected land for the experiment was first opened on 16 November, 2020 by power tiller then mixed with cowdung and expose to the sun for a week. After one week the land was ploughed and cross-ploughed several times with a power tiller and laddering was done to obtain good tilth. Weeds and stubble's were removed and the large clods were broken into smaller pieces to obtain a desirable tilth of soil for sowing of seeds.

3.7.7.2. Layout Preparation

The experiment was carried out in a Randomized Complete Block Design (RCBD) with three replications and each treatment contains 3 plots. The total number of unit plots was 30. After removal of the weeds, stubbles and dead roots, the land was leveled and the experimental plot was separated to make the unit plots and were prepared as 15cm raised beds.

Figure 1. Preperation of Layout

3.7.7.3. Transplantation

Solid and uniform measured 30 days old seedlings were removed independently from the seed beds. The seed beds were watered before evacuating the seedlings to limit the root injury. The seedlings were relocated in the pits of the exploratory plots toward the evening of 11 November, 2018 keeping up a dividing of 40 cm and 60 cm between the lines and plants, individually. Light water system was given following relocating by utilizing a watering stick. So as to hole filling and to check the outskirt impact, some additional seedlings were likewise relocated around the fringe zone of the test field.

3.7.8. Intercultural Operations

When transplantation is done in the plots it was always kept under careful observation and various intercultural operations; gap filling, netting, irrigation, weeding, cleaning and drainage preparation and application of bio-agents and botanicals were accomplished as and when necessary for better growth and development of the capsicum plants.

3.7.8.1. Gap filling

Gap filling was done after one week of transplantation. The seedlings were taken from the same source and a minor gap filling was done where it was necessary.

3.7.8.2. Netting

Net was used because capsicum is destroyed by bird. Birds eaten capsicum.

3.7.8.3. Weeding

Weeding and mulching were accomplished as and whenever necessary to keep the crop free from weeds, for better soil aeration and to break the soil crust. It also helps in conservation of soil moisture. Four weeding were done manually at 15, 30, 45 and 55 DAS to keep the plots free from weeds.

3.7.8.4. Experimental plot cleaning and Draineg Preparation:

Experimental plot cleaning and Draineg Preparation was done when necessary.

3.7.8.5. Irrigation and drainage

Irrigations were given throughout the growing season as and when necessary. Stagnant water effectively drained out at the time of excess water.

3.7.8.6. Fertilizer application

Cowdung MoP, TSP, Boron, zink and one third of Urea were applied in the final preparation of the field. Krishibid joibo sar was applied in the rhizosphere of the plant at 10^{th} days after transplanting. Remain one third Urea applied in the flowering stage. And last one third Urea applied at the fruiting stage.

3.7.8.7. Application of bio-agents and botanicals

Selected bio-agents and extracted botanicals were added directly to the selected pots/plants rhisoaphere as a treatment for checking the biological elicitor potentiality of capsicum plant.

a) Prepared bio-agents and botanicals

b) Application of bio-agents and botanicals in individual plants

Plate 4. Bio-agents and botanicals application in the field

3.7.9. Parameters assayed

Data were collected in respect of the plant growth characters. The following parameters were set up for data recording before and after harvesting.

1. Disease incidence (%)
2. Disease severity (%)
3. Chlorophyll Content
4. Fruits per Plant
5. Individual Fruit Weight
6. Fruit weight per plant
7. Fruit diameter

3.7.9.1. Disease incidence (%)

Disease incidence was measured in percentage on the basis of infected plant at 25, 55 and 85 days after transplanting.

Firstly I count how many plant are infected per plot. Per plot have 9 plant then following the formula and get disease incidence in percent.

$$\text{Disease incidence (\%)} = \frac{\text{Infected plant per plot}}{\text{Total plant per plot}} \times 100$$

3.7.9.2. Disease severity (%)

Disease severity was measured in percentage on the basis of infected leaf at 25, 55 and 85 days after transplanting.

Firstly I count infected leaf per plant and then count all leaf of that plant. Then following the formula and get disease severity in percent.

$$\text{Disease severity (\%)} = \frac{\text{Infected leaf per plant}}{\text{Total leaf per plant}} \times 100$$

3.7.9.3. Chlorophyll Content

For measurement the chlorophyll content samples were collected from the experimental field. In total six samples, three diseased and three healthy samples were collected randomly from each of the plot. The following protocol was followed for chlorophyll determination:

1. The selected leaf samples are collected and kept in separate polythene bag.
2. After collection, the leaf samples are immediately taken to the crop physiological laboratory for subsequent analysis.
3. Around 20 mg leaf sample was weighed and poured into glass vial containing 20 ml 80% acetone solution.
4. The glass vials are kept into dark condition for 48 hours.
5. After 48 hours chlorophyll was determined by using double beam spectrophotometer at 663 and 645 nm wave length and chlorophyll was determined by using the following formula

$$\text{Chlorophyll (a+b)}^{g-1}\text{ leaf tissue} = \frac{[20.2\ (D645) + 8.02\ (D663)] \times v}{1000 \times w}$$

Where,

D = optical density regarding of the chlorophyll extract at wave length of 663 and 645 nm

v = Final volume (ml) of the 80% acetone with chlorophyll extract

w = Weight of fresh sample in g

Data was presented in tabular and graphically.

3.7.9.4. Fruits per Plant

Harvesting the fruit 4 or 5 days interval. Every harvesting day I was collected the data and entry the data book. The sum of the all harvested fruits per plant from 1st harvesting day to last harvesting day was the fruits per plant.

Figure 2. Fruits bearing healthy Plant

3.7.9.5. Individual Fruit Weight

Every individual fruit was measured with weight machine and get individual fruit weight.

3.7.9.6. Fruit weight per plant

Measured the individual fruit weight and collected them in the data book. When harvested the fruit last day then got all fruit weight per plant. Then following the formula and get Fruit weight per plant

$$\text{Fruit weight per plant} = \frac{\text{Sum weight of all fruits}}{\text{Number of Fruits}}$$

3.7.9.7. Fruit diameter (cm)

At first harvested the fruit then measured the diameter of this fruit by measuring tape in horizontally.

3.7.10. Data analysis

The collected numeric values from the overall experiments will be normalized, and subjected to the analysis of variance (ANOVA) using the Statistix-10 statistical software. The differences between generated mean values will measured through the Duncan's paired based comparison test. The differences in means will then confirmed when the mean errors will be under the threshold of 5%, which will the significance.

RESULTS

The present study was conducted to evaluate the selected endospheric bio-agents and botanicals against the *Pepper leaf curl virus* (*PeLCV*). The management of *PeLCV* in response to different selected treatments, percent disease incidence and disease severity on the basis of visible virus and virus like symptoms, analysis of Chlorophyll contain, yield and yield attributes were recorded. The results have been presented in tables, figures and graphs under headings and sub-headings.

4.1. Effect of selected treatments on disease incidence (%) of *Pepper leaf curl virus* (*PeLCV*) at different days after transplanting (DAT)

The effect of different treatments on disease incidence (%) of *Pepper leaf curl virus* (*PeLCV*) was observed based on visible symptoms. Disease incidence was recorded three times at 25, 55, 85 days after transplanting (DAT) which was after 7 days of treatments application.

At 25 DAT, the highest disease incidence (67.00%) was recorded in T_2 (Nishinda) which statistically similar with T_9 (*Beauveria bassiana*, 63.33%) and identical with T_4 (Lantana), and T_0 (Control), which was 59.33% and 59.33% respectively. The lowest disease incidence (40.33%) was recorded in T_8 (*Metarhizium anisopliae*) which was statistically identical with T_1 (Neem, 44.00%). In remaining treatments, the disease incidence was found moderate at 25 DAT which was in range of 52.00% - 55.67%.

At 55 DAT, the highest disease incidence (81.67%) was recorded in T_0 (Control) which statistically similar with T_2 (Nishinda) and T_4 (Lantana) which was 78.00% and 78.00% respectively. The lowest disease incidence (59.33%) was recorded in T_1 (Neem) which statistically similar with T_7 (*Verticillium lecanii*, 59.33%). In other treatments moderate disease incidence was found that was in range 67.00% -74.33 %.

At 85 DAT, the highest disease incidence (92.67%) was recorded in T_0 (Control) which statistically similar with T_4 (Lantana), disease incidence was 92.67%. The lowest disease incidence (63.00%) was recorded in T_7

(*Verticillium lecanii*) which statistically identical with T_1 (Neem), T_9 (*Beauveria bassiana*), T_2 (Nishinda) T_3 (Bhat), T_5 (*Trichoderma harzianum*), T_8 (*Metarhizium anisopliae*), T_6 (*T. viride*) which was 74.33%, 74.33%, 81.67%, 81.67%, 81.67%, 85.33%, 89.00% respectively. Results are presented in table 1.

Table 1. Effect of slected endospheric bio-agents and botanicals on disease incidence (%) on the basis of visible symptoms at 25, 55, 85 DAT

Treatment	Disease Incidence 25 DAT	Disease Incidence 55 DAT	Disease Incidence 85 DAT
Control (T_0)	59.33 ab	81.67 a	92.67 a
Neem (T_1)	44.00 bc	59.33 b	74.33 ab
Nishinda (T_2)	67.00 a	78.00 a	81.67 ab
Bhat (T_3)	55.67 abc	74.33 ab	81.67 ab
Lantana (T_4)	59.33 ab	78.00 a	92.67 a
T. harzianum (T_5)	52.00 abc	70.67 ab	81.67 ab
T. viride (T_6)	55.67 abc	70.67 ab	89.00 ab
Verticillium lecanii (T_7)	55.67 abc	59.33 b	63.00 b
Metarhizium (T_8)	40.33 c	70.67 ab	85.33 ab
Beauveria (T_9)	63.33 a	67.00 ab	74.33 ab
CV (%)	17.31	14.11	16.60

4.2. Effect of selected treatments on disease severity (%) of *Pepper leaf curl virus* (*PeLCV*) at different days after transplanting (DAT)

The effect of different treatments on disease severity (%) of *Pepper leaf curl virus* (*PeCLV*) was observed based on visible symptoms. Disease severity was recorded three times at 25, 55, 85 days after transplanting (DAT) which was in the same date of disease incidence recorded.

At 25 DAT, the highest disease severity (55.57%) was recorded in T_5 (*Trichoderma harzianum*) which was statistically identical with T_6 (*Trichoderma viride*) and T_8 (*Metarhizium anisopliae*) which was 50.87% and 51.78% respectively. The lowest disease severity (28.22%) was recorded in T_7 (*Verticillium lecanii*) which was statistically identical with T_3 (Bhat) and T_9 (*Beauveria bassiana*) which was 36.33% and 37.11%

respectively. In remaining treatments, the disease severity was found moderate at 25 DAT in range of 41.97% - 43.89%.

At 55 DAT, the highest disease severity (58.13%) was recorded in T_5 (*Trichoderma harzianum*) which was statistically similar with T_6 (*Trichoderma viride*) and T_0 (control) which was 58.13% and 53.89% respectively and statistically identical with T_8 (*Metarhizium anisopliae*, 53.11%). The lowest disease severity (33.13%) was recorded in T_7 (*Verticillium lecanii*) which is statistically identical with T_3 (Bhat, 37.00%). In other treatments moderate disease severity was found that was in range of 42.33% - 49.13%.

At 85 DAT, the highest disease severity (71.77%) was recorded in T_0 (Control) which was statistically identical with T_5 (*Trichoderma harzianum*, 63.22%). The lowest disease severity (38.23%) was recorded in T_3 (Bhat) which statistically identical with T_7 (*Verticillium lecanii*), T_4 (Lantana), T_2 (Nishinda), T_1 (Neem), T_9 (*Beauveria bassiana*), which was 42.23%, 48.43%, 49.89%, 52.33%, 55.67% respectively. In remaining treatments, T_6 (*Trichoderma viride*, 58.89%) and T_8 (*Metarhizium anisopliae*, 58.13 %) disease severity was found moderate which was statistically similar. Results are presented in table 2.

Table 2. Effect of selected endospheric bio-agents and botanicals on disease severity (%) on the basis of visible symptoms at 25, 55, 85 DAT

Treatment	Disease Severity 25 DAT	Disease Severity 55 DAT	Disease Severity 85 DAT
Control (T_0)	41.97 abc	53.89 a	71.77 a
Neem (T_1)	40.77 abc	49.13 abc	52.33 bcd
Nishinda (T_2)	43.89 abc	48.00 abc	49.89 bcd
Bhat (T_3)	36.33 bc	37.00 bc	38.23 d
Lantana (T_4)	39.57 abc	44.87 abc	48.43 bcd
T. harzianum (T_5)	55.57 a	58.13 a	63.22 ab
T. viride (T_6)	50.87 ab	58.13 a	58.89 abc
Verticillium lecanii (T_7)	28.22 c	33.13 c	42.23 cd
Metarhizium (T_8)	51.78 ab	53.11 ab	58.13 abc
Beauveria (T_9)	37.11 bc	42.33 abc	55.67 abcd
CV (%)	22.67	19.82	21.01

4.3. Chlorophyll Content

The effect of different treatments on chlorophyll content was tested based on visible symptoms in the field conditions. For measuring the chlorophyll content, fresh and diseased leaves were collected as a tested samples.

In fresh leaf, the highest chlorophyll content (2.23×10^{-03}) was recorded in T_4 treatment (Lantana). The lowest chlorophyll content (0.507×10^{-03}) was recorded in T_1 treatment (Neem). In remaining treatments, the chlorophyll content was found moderate in range of 0.507×10^{-03}- 1.80×10^{-03}. It was observed that chlorophyll content was recorded in T_5 treatment (*Trichoderma harzianum*, 0.780×10^{-03}) statistically not similar with T_3 treatment (Bhat, 0.820×10^{-03}) but both was statistically identical with T_6 (*Trichoderma viride*) which was 0.800×10^{-03}.

In diseased leaf, the highest chlorophyll content (0.552×10^{-03}) was recorded in T_4 (Lantana). The lowest chlorophyll content (0.165×10^{-03}) was recorded

in T_1 (Neem). In remaining treatments, the chlorophyll content was found moderate in range of 1.65 X 10^{-03} - 4.37 X 10^{-03}.

From the result it was revealed that the highest chlorophyll content was recorded in T_4 treatment (Lantana) and the lowest chlorophyll content in T_1 treatment (Neem) in case of both types of samples (Table 3).

Table 3. Feature of the Chlorophyll Content in fresh and disease sample

Treatment	**Fresh Leaf**	**Diseased Leaf**
Control (T_0)	1.65 X 10^{-03} c	2.22 X 10^{-03} h
Neem (T_1)	0.51 X 10^{-03} i	1.65 X 10^{-03} j
Nishinda (T_2)	0.85 X 10^{-03} f	3.18 X 10^{-03} d
Bhat (T_3)	0.82 X 10^{-03} g	2.98 X 10^{-03} e
Lantana (T_4)	2.23 X 10^{-03} a	5.52 X 10^{-03} a
T. harzianum (T_5)	0.78 X 10^{-03} h	2.55 X 10^{-03} g
T. viride (T_6)	0.80 X 10^{-03} gh	3.60 X 10^{-03} c
Verticillium lecanii (T_7)	1.03 X 10^{-03} e	2.62 X 10^{-03} f
Metarhizium (T_8)	1.32 X 10^{-03} d	1.82 X 10^{-03} i
Beauveria (T_9)	1.80 X 10^{-03} b	4.37 X 10^{-03} b
CV (%)	1.38	0.58

4.4. Fruits per Plant and Individual Fruit Weight

The highest fruit per plant (8.1633) was recorded in T_7 (*Verticillium lecanii*) which was statistically similar with T_3 (Bhat) which was 7.4967. The lowest fruit per plant (4.0533) was recorded in T_8 (*Metarhizium anisopliae*). The highest fruit per plant and the lowest fruit per plant both are statistically identical with T_4 (Lantana), T_6 (*Trichoderma viride*), T_2 (Nishinda), T_9 (*Beauveria bassiana*), T_0 (Control), T_1 (Neem) T_5 (*Trichoderma harzianum*) which was 7.0000, 6.4467, 6.3900, 5.9967, 5.8867, 5.8333 and 5.3867 respectively. The range of harvesting fruit per plant was 4.0533 - 8.1633.

The highest Individual Fruit Weight (100.07gm) was recorded in T_4 (Lantana) which was statistically similar with T_0 (Control), T_6 (*Trichoderma viride*), T_8 (*Metarhizium anisopliae*), T_5 (*Trichoderma harzianum*) which was 99.45gm, 97.78gm, 97.36gm and 95.83gm respectively. The lowest

Individual Fruit Weight (69.38) was recorded in T_2 (Nishinda) which statistically identical with T_7 (*Verticillium lecanii*, 77.79 gm). Results are presented in table 4.

Table 4. Effect of treatments on yield and yield contributing characters

Treatment	**Fruits/Plant**	**Individual Fruit Weight (gm)**
Control (T_0)	5.89 ab	89.83 abc
Neem (T_1)	5.83 ab	77.79 cd
Nishinda (T_2)	6.39 ab	95.83 a
Bhat (T_3)	7.50 a	99.45 a
Lantana (T_4)	7.00 ab	97.78 a
T. harzianum (T_5)	5.39 ab	81.29 bcd
T. viride (T_6)	6.45 ab	97.36 a
Varticilium lecanii (T_7)	8.16 a	100.07 a
Metarizium (T_8)	4.05 b	69.38 d
Bauveria (T_9)	5.99 ab	92.03 ab
CV (%)	29.84	8.14

4.5. Relationship between fruit per plant and percent disease incidence at 85 DAT

From the relationship study between fruit per plant with percent disease incidence at 85 DAT, it was revealed that among the botanical treatments, number of fruit per plant increased with decreased of percent disease incidence. And also the bio-agents treatments, number of fruit per plant increased with decreased of percent disease incidence (Figure 3).

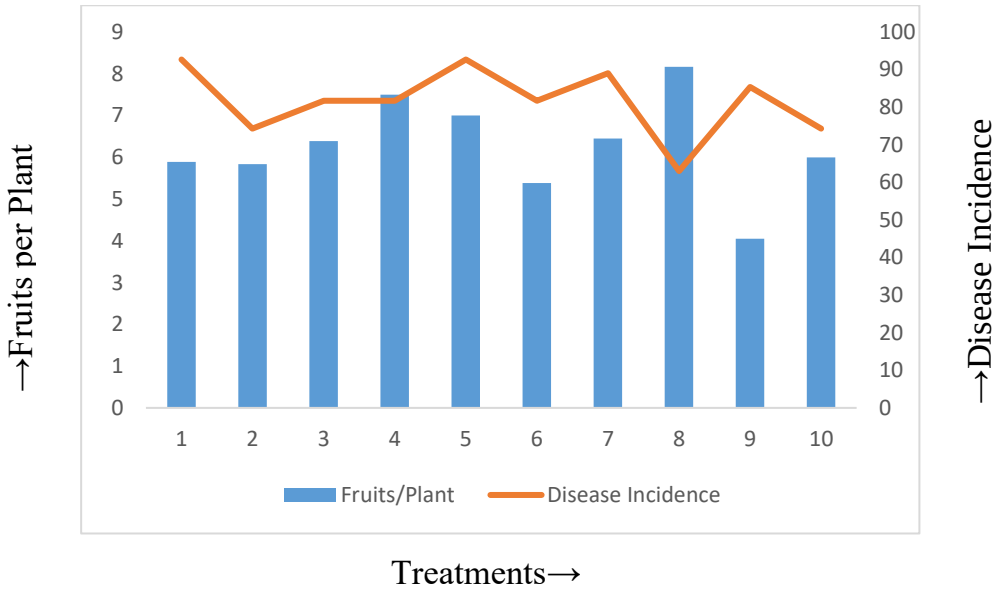


Figure 3. Relationship between fruit per plant and percent disease incidence

[Here, 1– Control (T_0) , 2– Neem leaves extract (T_1), 3– Nishinda leaves extract (T_2), 4– Bhat leaves extract (T_3), 5– Lantana leaves extract (T_4), 6– *Trichoderma harzianum* (T_5), 7– *Trichoderma viride* (T_6), 8– *Verticillium lecanii* (T_7), 9– *Metarhizium anisopliae* (T_8), 10– *Beauveria bassiana* (T_9)]

4.6. Relationship between fruit per plant and percent disease severity at 85 DAT

From the relationship study between fruit per plant with percent disease severity at 85 DAT, it was also revealed that in case of botanical treatments, number of fruit per plant increased with decreased of percent disease severity (%). And also the bio-agents treatments, number of fruit per plant increased with decreased of percent disease severity (Figure 4).

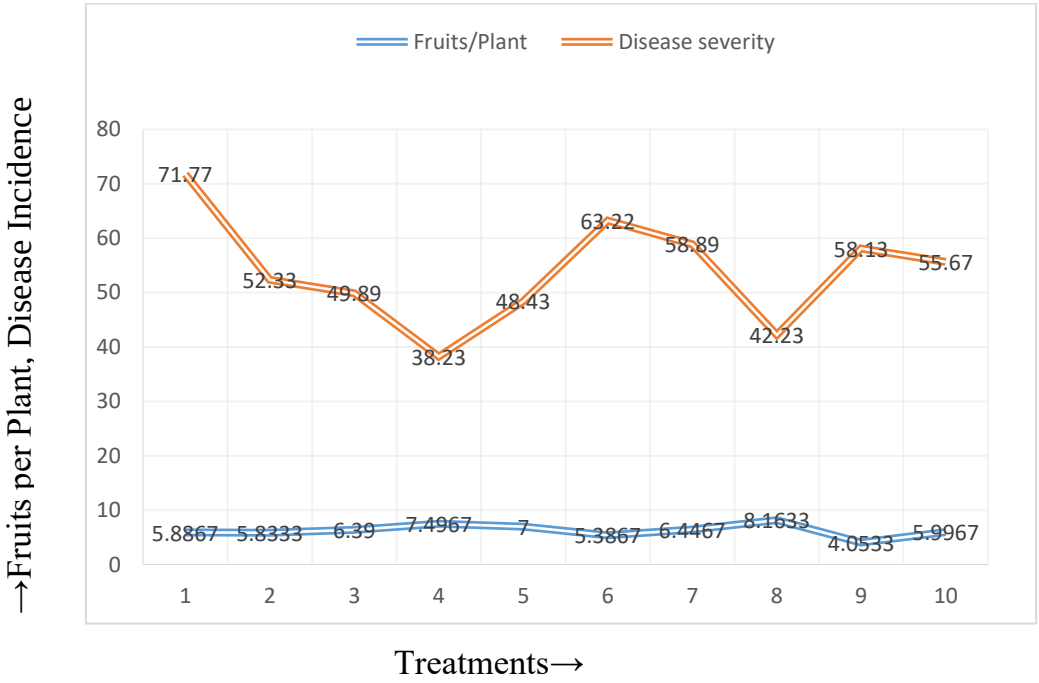


Figure 4. Relationship between percent disease severity and fruit per plant

[Here, 1– Control (T_0) , 2– Neem leaves extract (T_1), 3– Nishinda leaves extract (T_2), 4– Bhat leaves extract (T_3), 5– Lantana leaves extract (T_4), 6– *Trichoderma harzianum* (T_5), 7– *Trichoderma viride* (T_6), 8– *Verticillium lecanii* (T_7), 9– *Metarhizium anisopliae* (T_8), 10– *Beauveria bassiana* (T_9)]

4.7. Relationship between individual fruit weight (gm) and percent disease incidence at 85 DAT

From the relationship study between individual fruit weight (gm) with percent disease incidence at 85 DAT, it was revealed that in case of both types of treatments, botanicals and bio-agents, individual fruit weight (gm) increased with decreased of percent disease incidence (Figure 5).

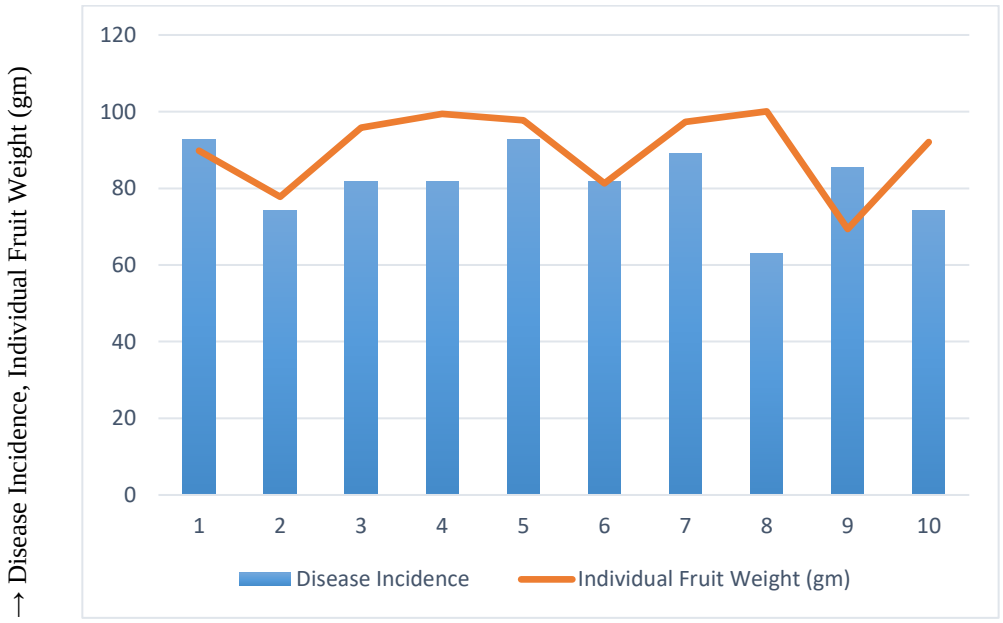


Figure 5. Relationship between percent disease Incidence and individual fruit weight (gm)

[Here, 1– Control (T_0) , 2– Neem leaves extract (T_1), 3– Nishinda leaves extract (T_2), 4– Bhat leaves extract (T_3), 5– Lantana leaves extract (T_4), 6– *Trichoderma harzianum* (T_5), 7– *Trichoderma viride* (T_6), 8– *Verticillium lecanii* (T_7), 9– *Metarhizium anisopliae* (T_8), 10– *Beauveria bassiana* (T_9)]

4.8. Relationship between individual fruit weight (gm) and percent disease severity at 85 DAT

From the relationship study between individual fruit weight (gm) and percent disease severity at 85 DAT, it was revealed that among the botanical treatments, individual fruit weight (gm) increased with decreased of percent disease severity (%). And also the bio-agents treatments, individual fruit weight (gm) increased with decreased of percent disease severity (Figure 6).

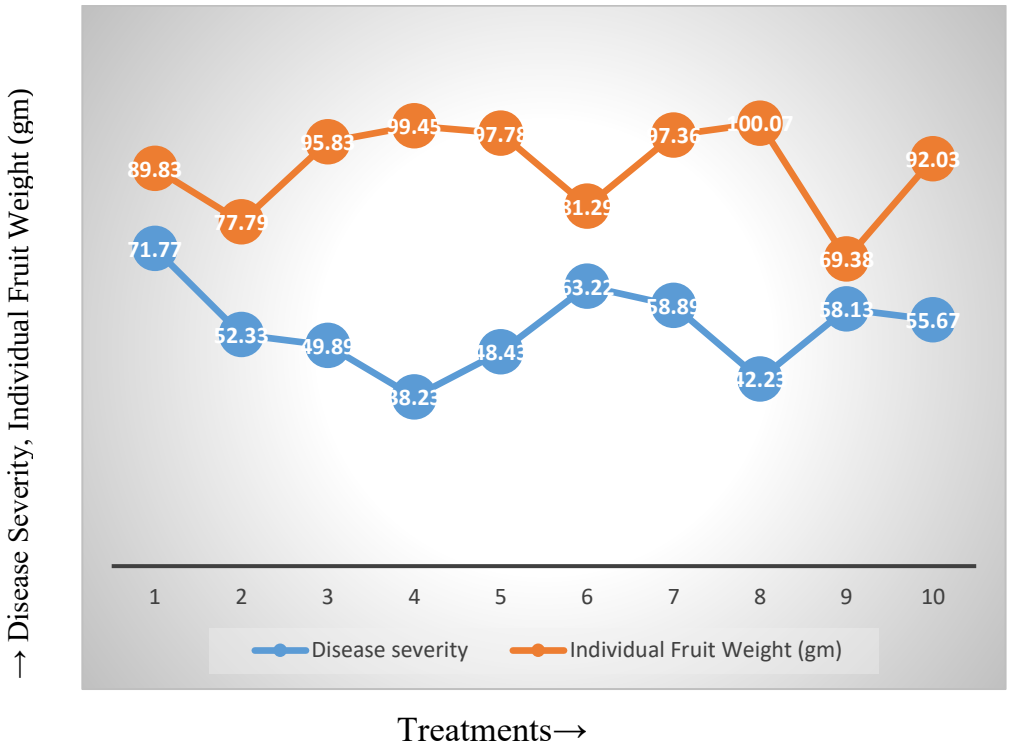


Figure 6. Relationship between disease severity and individual fruit weight (gm)

Here, 1– Control (T_0) , 2– Neem leaves extract (T_1), 3– Nishinda leaves extract (T_2), 4– Bhat leaves extract (T_3), 5– Lantana leaves extract (T_4), 6– *Trichoderma harzianum* (T_5), 7– *Trichoderma viride* (T_6), 8– *Verticillium lecanii* (T_7), 9– *Metarhizium anisopliae* (T_8), 10– *Beauveria bassiana* (T_9)

DISCUSSION

Capsicum is one of the high value vegetables and has great demand in the world market. When it comes to the history of capsicums, they were originated in the Americas, but are now spread worldwide. It is also known as bell pepper, sweet pepper and green pepper. Usually, its look like a bell, hence it's being called as bell pepper in many countries. Capsicum is like fleshier and the shape, intensity of pungency, color is different from chilli. All the cultivars are very mild in pungency and even some of them are non-pungent. It is used as salad, stews, stir fries, baked and stuffed dishes, salsa, pizzas and cheeses and pickles. It is also being used for producing paprika which is used for coloring foods, flavoring and in sauces. It can be grown in controlled and protected environments such as poly-house, shade-house, net-house and greenhouse. It grown in protected area has more demand due to produce good quality fruits. The crop management practices of capsicum are almost similar to chilli. One can expect more yield from polyhouse, greenhouse or shade net when compared to open field cultivation. Capsicum can also be grown indoors/back yards, in pots and containers as well. It is rich in vitamins such as A, C, minerals and antioxidants. Growing colored capsicum is increasing day by day due to their attractive color and to use specially in salads. (Kumar *et al*., 2006).

There are many constants in capsicum cultivation either control or protective conditions and also in field condition. In Bangladesh, some biotic factors are the major thread in capsicum cultivation like pathogenic attack. The range of pathogens afflicting in peppers is very high and includes fungi, viruses, bacteria, nematodes and insect pests. Fungal diseases are Anthracnose, Cercospora (frogeye) leaf spot, Charcoal rot, Choanephora blight (wet rot), Damping-off and root rot, Downy mildew, Fusarium stem rot, Fusarium wilt, Gray mold, Phytophthora blight, Powdery mildew, Southern blight, Verticillium wilt. Viral diseases are Pepper leaf curl, Chili

leaf curl, Alfalfa mosaic, Andean potato mottle, Beet curly top, Chilli veinal mottle, Cucumber mosaic, Pepper golden mosaic complex (previously Texas Pepper, Serrano Golden Mosaic, and Pepper Mild Tigre Viruses), Pepper huasteco, Pepper mild mottle, Pepper mottle. Bacterial diseases are Bacterial spot, Bacterial wilt, Bacterial canker and Syringae seedling blight. Root knot nematode, Sting nematodes are found commonly in pepper (American Phyto-Pathological society).

Among the major diseases of pepper caused by different pathogenic organisoms, the *Pepper leaf curl virus (PeLCV)* is common and destructive one. The virulence and the difficulty to control viral diseases is certainly related to their physio-pathology. Compared to phytophagous pathogens, viruses weaken the host innate immune system and subtilize the cell machinery to establish itself in plant tissues. As a matter of fact, the plant functioning, productivity and survival are seriously compromised (Li *et al*., 2014). This study unveils for the first time the suppression of *pepper leaf curl virus* using some selected endospheric bioagents inoculants namely *Trichoderma harzianum*, *Trichoderma viride*, *Metarhizium anisopliae*, *Verticillium lecanii, Beauveria bassiana* and botanicals Neem, Nishinda, Bhat and Lantana leaf extracts. Typical symptoms like leaves curling and yellowing, whole plant stunting, and eventually total dead were recorded in pepper plantlets/plants infested by whitefly in experiment plot in the framework of this study, marking a successful transfer of the virus from viruliferous insect vector (*Bemisia* sp) to the pepper plantlets/plants. In this study, the disease incidence and severity on the basis of observable symptoms of viral disease of pepper in response to different treatment were recorded at 25, 55, 85 DAT. All treatments reduced the disease incidence and severity of *PeLCV* over untreated control. Based on the disease incidence recorded in last observation at 85 DAT, the highest disease incidence (92.67%) was recorded in untreated control which is statistically similar to T_4 (Lantana) treatment. In other botanicals, better results in terms

of minimized the disease incidence which was statistically similar each and other. Among the selected endospheric bio-agents used in the study, the lowest disease incidence (63.00%) was recorded in T_7 (*Verticillium lecanii*) treatment which is statistically identical with other bio-agents treatments, but other bio-agents were statistically similar each and others. Based on the disease severity recorded at 85 DAT the highest disease severity (71.77%) was recorded in untreated control. Among the bio-agents used in the study, the lowest disease severity (42.23%) was recorded in T_7 (*Verticillium lecanii*) treatment and among the botanicals, the lowest disease severity (38.23%) was recorded in T_3 (Bhat) treatment which is statistically different from all treatments. Similar outputs were obtained by Muvea *et al.* (2018) in onion when infected with the *Iris yellow spot virus* (*IYSV*) vectored by Thrips tabaci and challenged with the beneficial fungus Hypocrea lixii. The authors pointed out the vector repellency and/or the antibiosis as probable mode of action underlying the disease suppression. It has indeed been demonstrated that promising biocontrol agents like *Trichoderma* species are endowed with potential to synthesize hundreds biologically active compounds like broad range of antimicrobial such as anti-bacterial *Trichodermin* (Leylaie and Zafari, 2018), antifungal Trichokonin (Shi *et al*., 2012) and antiviral Gliotoxin (Mukherjee *et al*., 2012). But, oftentimes, the elicitation of plant immune system is the major and the most probable route to reach the virus once upon host infestation. In this sense, our results have provided clear evidence of a shift in pepper metabolic signalling upon priming with bio-agents *Trichoderma harzianum, Trichoderma viride, Metarhizium anisopliae, Verticillium lecanii, Beauveria bassiana*. The so-called inductor has profusely been demonstrated to elicitate the systemic acquired resistance (SAR) of a wide range of host plant against majority of plant diseases (Dempsey and Klessig, 2017). It is likely that the *Trichoderma harzianum, Trichoderma viride, Metarhizium anisopliae, Verticillium lecanii, Beauveria bassiana* plantlets have produced some of them to avoid the vector (*Bemisia* sp.) to feed on the plantlets during infection. The

decrease in phenolic compounds in virus-infested plants only, (*Bemisia* sp) could thus be the result of viral-driven host defense-related genes repression as mentioned by (Markakis *et al*., 2010). It noteworthy that all the endospheric bio-agents and botanicals inoculation significantly improved all the evaluated plant growth parameters such as chlorophyll content, fruits/plant and individual fruit weight. For measurement the chlorophyll contents, fresh and infected leaves were used as tested samples. It was observed that chlorophyll contents was higher in the infected leave samples in all cases, due to high accumulation of chlorophyll in curled leaves. These findings go in the same line with that from (Eke *et al*., 2016) stipulating that single bio agent outcompeting with a pathogen is likely to fail compared to consortium application as the former are thought to reinforce each another leading and improved outcome. The performance of the treatments in respect of yield and yield contributing characters against *PeLCV* varied significantly. All the treatments effect was found effective in terms of fruits per plant and individual fruit weight, the highest number of fruits per plant was counted in T_7 (*Verticillium lecanii*) treatment which is statistically similer with T_3 (Bhat) treatment. On the base of individual fruit weight, the highest individual fruit weight was measured in T_7 (*Verticillium lecanii*) treatment which is statistically similer with T_3 (Bhat), T_4 (Lantana), T_6 (*Trichoderma viride*) and T_2 (Nishinda) treatments. These observations were also confirmed by the finding of De Palma *et al*. (2019) and Mayo *et al*. (2016) about the capacity of *Trichoderma* to fine-tuned gene expression of plant during infection for an efficient and strategic use of the plant defense machinery. The present study showed the capability of endospheric bio-agents to reduce *PeLCV* incidence and severity by enhancing systemic acquired resistances which was effective by the production of phenolic compounds. Overall, the bio-agents were the best and confirm the stipulation that single bio-agent outcompeting with a pathogen is likely to fail compared to botanical application.

SUMMARY AND CONCLUSION

A field experiment was conducted in the central farm of Sher-e-Bangla Agricultural University, Dhaka, to evaluate the selected endospheric bio-agents viz. *Trichoderma harzianum*, *Trichoderma viride*, *Metarhizium anisopliae, Verticillium lecanii, Beauveria bassiana* and botanicals namely Neem, Nishinda, Bhat and Lantana leaf extracts against the *Pepper leaf curl virus* (*PeLCV*). The selected bio-agents were cultured in artificial media PSA for multiplication and appearance of mycelium and spores also studied. Quality test performed through analytical method (pour plate method) for morphological and cultural variability test and counting the viable spores/colony forming units on selective medium using Hema-cytometer. The prepared soil and others selected inputes; cowdung and recommended fertilizers were collected to make bio-fortified soil media with bio-agents. Plant materials seeds were collected and sown in seed beds for seedling preparation. The experiment field were prepared with bio-fortified soil and normal soil for botanicals application.

For evaluation the selected bio-agents and botanicals in *in-vivo* management of *PeLCV*, in response to different selected treatments, percent disease incidence and disease severity on the basis of visible symptoms, chlorophyll content, yield and yield attributes were recorded. The data obtained for different characters were statistically analyzed to find out the significance of the difference among the treatments. An encouraging performances of the treatments used in the experiment was observed in reducing the disease incidence and severity in terms of plant infection and appearance the visible symptoms in leaves in comparison to manage at 25, 55, 85 days after transplanting (DAT) which was after 7 days of treatments application. At 25 DAT, among the treatments, the highest disease incidence (67.00%) was recorded in T_2 (Nishinda) which statistically similar with T_9 (*Beauveria*

bassiana, 63.33%) and identical with T_4 (Lantana, 59.33%). The lowest disease incidence (40.33%) was recorded in T_8 (*metarhizium anisopliae*) which was statistically identical with T_1 (Neem, 44.00%). In remaining treatments, the disease incidence was found moderate which was in range of 52.00% - 55.67%. At 55 DAT, the highest disease incidence (81.67%) was recorded in T_0 (Control) which statistically similar with T_2 (Nishinda) and T_4 (Lantana), the disease incidence was 78.00%. The lowest disease incidence (59.33%) was recorded in T_1 (Neem) which statistically similar with T_7 (*Verticillium lecanii,* 59.33%). In other treatments moderate disease incidence was found that was in range 67.00% -74.33 %. At 85 DAT, the highest disease incidence (92.67%) was recorded in T_0 (Control) which statistically similar with T_4 (Lantana), the disease incidence was 92.67%. The lowest disease incidence (63.00%) was recorded in T_7 (*Verticillium lecanii*) which statistically identical with T_1 (Neem), T_9 (*Beauveria bassiana*), T_2 (Nishinda) T_3 (Bhat), T_5 (*Trichoderma harzianum*), T_8 (*Metarhizium anisopliae*), T_6 (*T. viride*) which was 74.33%, 74.33%, 81.67%, 81.67%, 81.67%, 85.33%, 89.00% respectively.

The effect of different treatments on disease severity (%) of *Pepper leaf curl virus* (*PeCLV*) was observed based on visible symptoms. Disease severity was also recorded which was in the same date of disease incidence recorded. At 25 DAT, among the treatments, the highest disease severity (55.57%) was recorded in T_5 (*Trichoderma harzianum*) which was statistically identical with T_6 (*Trichoderma viride*) and T_8 (*Metarhizium anisopliae*) which was 50.87% and 51.78% respectively. The lowest disease severity (28.22%) was recorded in T_7 (*Vartecillium lecanii*) which was statistically identical with T_3 (Bhat) and T_9 (*Beauveria bassiana*) which was 36.33% and 37.11% respectively. In remaining treatments, the disease severity was found moderate that was in range of 41.97% - 43.89%. At 55 DAT, among the treatments, the highest disease severity (58.13%) was recorded in T_5 (*Trichoderma harzianum*) which was statistically similar with T_6 (*Trichoderma viride*, 58.13%) and identical with T_8 (*Metarhizium*

anisopliae, 53.11%). The lowest disease severity (33.13%) was recorded in T_7 (*Verticillium lecanii*) which is statistically identical with T_3 (Bhat, 37.00%). In other treatments moderate disease severity was found that was in range of 42.33% - 49.13%. At 85 DAT, the highest disease severity (71.77%) was recorded in T_0 (Control) which was statistically identical with T_5 (*Trichoderma harzianum*, 63.22%). The lowest disease incidence (38.23%) was recorded in T_3 (Bhat) which was statistically identical with T_7 (*Verticillium lecanii*), T_4 (Lantana), T_2 (Nishinda), T_1 (Neem), T_9 (*Beauveria bassiana*) and the disease severity was 42.23%, 48.43%, 49.89%, 52.33%, 55.67% respectively. In remaining treatments, T_6 (*Trichoderma viride*, 58.89%) and T_8 (*Metarhizium anisopliae*, 58.13 %) disease severity was found moderate which was statistically similar.

The effect of different treatments on chlorophyll content was tested based on visible symptoms in the field conditions. For measuring the chlorophyll content, fresh and diseased leaves were used as a tested samples. From the result it was found that among the treatments, the highest chlorophyll content was recorded in T_4 treatment (Lantana, 2.23 X 10^{-03} and 5.52 X 10^{-03}) and the lowest chlorophyll content in T_1 treatment (Neem, 0.51 X 10^{-03} and 1.65 X 10^{-03}) in case of both types (fresh and diseased) of samples. The effect of different treatments on yield and yield contributing characters also studied and significance variation was observed. In case of fruits per plant and individual fruit weight; the range of harvesting fruit per plant was 4.0533 to 8.1633 and the range of individual fruit weight was 69.38 to 100.07 gm.

Considering the overall results, application of bio-agent *Verticillium lecanii*, or *Beauveria bassiana* and the botanical neem or bhat leaf extracts may be recommended as ecofriendly approach for management of *Pepper leaf curl virus* (*PeCLV*). However, further investigation is needed to justify the present findings in different Agro Ecological Zones (AEZ) in the country for consecutive years through conducting the field experiments.

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APPENDIECS

Appendix I. Details of field experiment

1. Name of Crop	Capsicum
2. Variety	BU capsicum 1
3. Season	*Rabi* 2020-21
4. Design of Experiment	RCBD
5. No. of Treatments	10
6. No. of Replication	3
7. No. of Total Plots	30
8. Total Plot Size	306 m. sq.
9. Individual Plot Size	3 × 2m
10. Spacing	1m × 0.5m
11. Sowing methods	Line sowing
12. Date of Sowing	05-November-2020
13. Date of Transplanting	10-December-2020

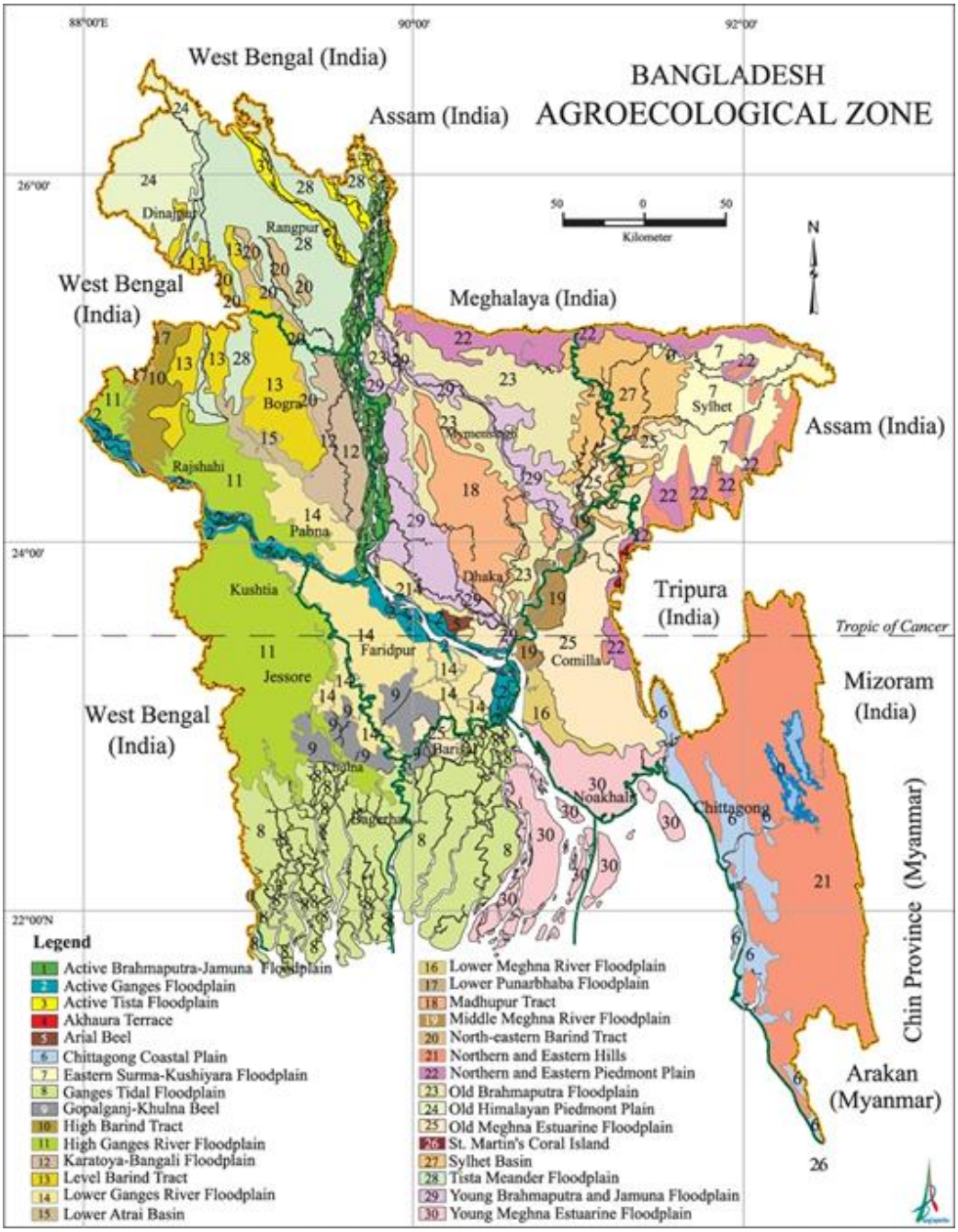
Appendix II. Map of the SAU farm land

Appendix III. Monthly maximum and minimum temperature, average relative humidity, rainfall, average sun hour and average wind speed of the experimental period (July 2020 to June 2021)

Year	Month	Average Temperatue (°C)		Average RH (%)	Average Rainfall (mm)	Average Sunshie hour	Averae wind speed (km/h)
		Min.	Max.				
	november	20.5	29.9	67	3.1	7.3	9.5
	December	17	26.4	60	1.5	7.4	7.4
	January	15.3	26	53	2	7.6	8.5
	February	17.4	29.8	45	10	7.5	8.3
	March	21.3	34	48	25	10.1	9.2

Source: Bangladesh Meteorological Department (Climate division), Agargaon, Dhaka-1207.

Appendix IV. AEZ of Bangladesh



Appendix V. Particulars of the Agro-ecological Zone of the Experimental site

Agro-ecological region	: Madhupur Tract (AEZ- 28)
Land Type	: Medium high land
General soil type	: Non- calcareous dark gray floodplain
soil Topography	: Up land
Soil series	: Tejgaon
Drainage	: Fairly good
Field level	: Above flood level

Source: Soil Resource Development Institute (SRDI)

Appendix VI. LSD value for different parameters at 5% level of significance

Parameters	$LSD_{(0.05)}$
Disease incidence at 25 DAT	16.399
Disease incidence at 55 DAT	17.172
Disease incidence at 85 DAT	28.298
Disease severity at 25 DAT	15.087
Disease severity at 55 DAT	15.310
Disease severity at 85 DAT	22.350
Chlorophyll Contain fresh leaf	2.927×10^{-05}
Chlorophyll Contain diseased leaf	3.004×10^{-05}
Fruits Per Plant	3.2075
Individual Fruit Weight (gm)	12.581

Appendix VII. Field view