

**Formulation of *Enterobacter cloacae* HSTUB 12 for  
the management of anthracnose of chilli (*Capsicum  
annuum* L.)**

**A THESIS**

**BY**

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**Student No. 1701167**

**Session: 2022-2023**

**MASTER OF SCIENCE (MS)**

**IN**

**PLANT PATHOLOGY**



**DEPARTMENT OF PLANT PATHOLOGY**

**HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY  
UNIVERSITY, DINAJPUR**

**December 2023**

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**DECEMBER 2023**



**Dedicated  
to My  
Beloved  
Parents**

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## ABSTRACT

A significant loss in yield is attributed to anthracnose of chili, which is caused by *Colletotrichum capsici*. The study aimed to develop a formulated bio-fungicide using *Enterobacter cloacae* HSTUB 12 and various substrate for the efficient and eco-friendly management of anthracnose of chilli under field condition. *Enterobacter cloacae* was formulated with charcoal, maize bran, wood dust, soybean bran and pea bran. Among all of the formulated bio-fungicides, charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension showed the maximum reduction of anthracnose incidence (17.2, 19.67%) and severity (22.33, 21.41%) in Bijlee plus and Roshni, respectively). Higher plant height (74.13 and 80.13 cm), maximum number of branch per plant (39 and 41), maximum number of flower per plant (102 and 103), highest number of fruit per plant (67 and 84), highest shoot weight (253 and 244 g), highest root length (26.70 and 28 cm), highest root weight (28.14, 36 g), highest yield per plant (2.5, 2.6 kg/plant), highest fruit length (13.17 and 14.13 cm), maximum fruit diameter (5.43 and 5.20 cm), higher accumulation of phenol content (4.65 and 4.78 mg/100g), higher content of total soluble solids (12.63 and 14.43 °Brix), higher content of ascorbic acid (11.82 and 8.3 mg/g) was exhibited by the implementation of charcoal-based formulated *E. cloacae* HSTUB 12 in Bijlee plus and Roshni, respectively. The prepared formulations were kept in two several storage environments: (A) a refrigerator(4<sup>0</sup>C), (B) a wooden shelve(at room temperature). By using serial dilution technique, charcoal-based formulation showed highest bacterial colony in refrigerator and pea bran-based formulation in wooden shelve which increased from 400 to 999 and from 265 to 712, respectively and this process was continued up to 240 days after incubation (DAI) at 30 days intervals. The study's finding showed that charcoal-based formulated *E. cloacae* HSTUB 12 could be used to manage anthracnose of chili in the field.

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# CHAPTER I

## INTRODUCTION

Bangladesh is an agriculture based country which dominated by crop production. Most of the inhabitants directly or indirectly are engaged in agricultural activities for their livelihood. Agriculture plays the key role in the economic growth of Bangladesh. One of the most significant revenue crops in Bangladesh is chilli (*Capsicum annum* L.), a desirable spice which belongs to the family Solanaceae. It is utilized as green, dried, and powdered forms. Chilli is considered as one of the commercial spice crops. Different varieties are cultivated for various uses like vegetable, pickles, spice and condiments. Due to the presence of phenolic compounds, carotenoid and capsaicinoid in peppers, it shows diverse prophylactic and therapeutic uses in allopathic and ayurvedic medicine (Hernández *et al.*, 2012). Chilli rich of nutrition like Beta Carotene, Capsaicin and vitamin A, C; Provitamin E, P, B<sub>1</sub> B<sub>2</sub> and B<sub>3</sub> (Reddy 2023). Chilli rich in medicinal value which control cholesterol, blood pressure, diabetes, blood clotting, cold, fever; and also improves digestion system. In addition, chilli can reduce the risk of cancer by preventing carcinogens from binding to DNA and reduce calorie intake by increasing thermogenesis (Lakshmi *et al.*, 2014).

In Bangladesh, chilli is usually cultivated during both Rabi and Kharif seasons and yielded 128602.24 MT under 50291.18 acres in Kharif and 496223.53 MT under 192709.69 acres in Rabi, respectively (BBS, 2022). The yield of chili in Bangladesh is far low in comparison to other neighbouring countries. Several factors *viz.* lack of high-yielding cultivar, low seed quality, diseases, and pests are responsible for the reduced yield. Among the factors, anthracnose disease caused by *Colletotrichum spp.* is one of the most severe and

important causes of yield losses in chili pepper ranging from 10 to 80% (Asare-Badiako *et al.*, 2015).

*Colletotrichum sp.* as a fungus that causes anthracnose disease, easily dispersed by the wind and capable to survive in the plants remain and in the soil for a relatively long time, so that anthracnose always accompanies each of chili plant; primarily *C. capsici* and *C. gloeosporioides* (Sattar *et al.*, 2016). Symptoms of anthracnose caused by *C. capsici* and *C. gloeosporioides* produce small, circular black spots or spots with concentric rings consisting of black aservulus body on the spot surface (Naznin *et al.*, 2016).

For the control of such kind of devastating diseases, farmers are habituated to using chemicals. However, if these activities are carried out continuously can cause resistance to the pathogen, therefore the fungicide does not work anymore. Moreover, intensive and unwise use of chemicals (pesticides) may leave toxic effects in the environment, and the breeding resistant genes have no long-lasting durability (Ji *et al.*, 2008), leading to a risk for public health, natural waters, and non-target organisms (Gikas *et al.*, 2022). To overcome the negative effect of chemical usage, use of different formulated biocontrol agents to control infection have become a solution. In 2021, it was estimated that the global market of biofertilizers and biopesticides will reach \$7.5 billion and \$12 billion, respectively, by 2031, according to their current increase rate in recent years.

Rhizobacteria also frequently possess the ability to dissolve phosphate, which can significantly boost plant development. In nature, *E. cloacae* is found in large quantities. It can be found in water, milk, dairy products, grains, plants, and the digestive tracts of humans and other animals (Breed RS *et al.*, 1957). This bacterium may be safe because it currently resides in the human digestive tract normally. The strain of *E. cloacae* MSR1 has the ability to solubilize insoluble phosphate into soluble form through which plant can easily uptake the phosphate (Li *et al.*, 2008). In *vivo*, in *vitro* and field trials, biocontrol agents

was reported to promote plant growth through production of hormones, volatile organic compounds (VOCs), siderophores, and increasing the availability of soil nutrients and suppressing soil pathogens (Shahid *et al.* 2021, Sheteiwy *et al.* 2021).

Many works are done with the genus of beneficial microbes throughout the world such as *Enterobacter* spp, *P. fluorescens* spp., *Acinetobacter* spp., *Bacillus* spp., *Pseudomonas putida*, *Paenibacillus macerans*, *Streptomyces*, and *Trichoderma* spp. as effective biocontrol agents against different plant diseases. *B. subtilis* has been used to control chilli diseases like fruit rot and die back (Jayalakshmi *et al.*, 1998). *Streptomyces* strains also have important applications in the agricultural field through their biological control potential against phytopathogens, particularly phytopathogenic fungi (González-Franco and Robles-Hernández, 2009). Wilt disease of some crops such as tomato, caused by *R. solanacearum*, has been controlled by beneficial bacteria: *Enterobacter cloacae*, *Pantoea ananatis*, *Pseudomonas oleovorans* (Upreti and Thomas ,2015). For example, (Götz *et al.*, 2006) used *Enterobacter cowanii* as biocontrol agents against bacterial wilt of tomato. *E. cloacae* was used successfully for controlling many plant diseases such as Fusarium wilt of spinach (Tsuda *et al.*, 2001), bacterial blight of rice (Yang *et al.*, 2000), dry rot of potato (Al-Mughrabi 2010), and damping-off of tomato (Yuliar *et al.*, 2019). So far in Bangladesh, these types of work are very rare or non-existent. So, our focus is on biological management of chilli disease by using developed formulated *Enterobacter cloacae*. Hence, the present study was aimed to develop and use of formulated bio-fungicide for the efficient and sustainable management of anthracnose of chilli with the following objectives:

## **Objectives**

1. To develop formulated bio-fungicide using *Enterobacter cloacae* HSTUB 12
2. To observe the efficacy of formulated bio-fungicide against anthracnose of chilli in field conditions
3. To observe the efficacy of formulated bio-fungicide in plant growth promotion of chilli
4. To observe shelf-life of the formulated bio-fungicide

## CHAPTER II

### REVIEW OF LITERATURE

The pathogen *Colletotrichum capsici* causing anthracnose of chilli has attracted the attention of plant pathologists and academic scholars worldwide. The management of the disease was attempted by the using of formulated beneficial bacteria using *Enterobacter cloacae* at field conditions. Some relevant literatures are reviewed as follows:

#### 2.1 Chilli anthracnose disease caused by *Colletotrichum capsici*

Sawant *et al.* (2023) chilli anthracnose caused by *Colletotrichum capsici* is the major constraint in chili production, the pathogen also has a wide host range. Under favourable environmental conditions, the pathogen manifest on the entire plant, including the fruit, causing total crop destruction. Under field conditions, management of the disease is challenging and most of the management practices are highly dependent on the use of chemicals. So, our study aimed at to develop the formulated beneficial bacteria.

Manda *et al.* (2020) presented that there are several diseases that damage chilli, among them chilli anthracnose being one of the most serious ones. It is also known as fruit rot or dieback. The causal organism of chilli anthracnose is *Colletotrichum capsici*. By damaging both mature and immature fruits, the destructive disease lowers the nutritional value and market value of chillies. The features of the pathogen, symptoms, and effective management techniques for treating chilli anthracnose are the main topics of this review study.

Kumar & Kerketta (2018) found that during chilli cultivation one of the main obstacle is anthracnose disease which is receiving a lot of attention towards the causes of damage. Both mature fruits in the field and those kept under ideal storage conditions suffer damage by this disease. This review gives

attention to identify the pathogen *Colletotrichum capsici* and various management strategies.

Oo *et al.* (2016) reported that Chilli is a crop that is frequently consumed worldwide. However, chilli anthracnose is a significant barrier to chilli production that causes enormous economic losses globally. *Colletotrichum* is a large genus of Ascomycete fungi, containing several species that cause anthracnose diseases on a variety of economically important crops.

Saxena *et al.* (2016) reported that the decline in chilli production has been connected to crop diseases like anthracnose or fruit rot, which account for a large portion of crop loss. Both mature fruits in the field and when they are stored under ideal conditions suffer severe damage which amplifies the loss in yield and finally the overall production of the crop. This review gives an account of the loss in production and yield in chilli cultivation due to anthracnose disease in Indian sub-continent, emphasis also given to the sustainable management strategies against the anthracnose disease.

Than *et al.* (2008) revealed that production of chillies is severely hampered economically by anthracnose disease, particularly in tropical and subtropical areas. Commercial cultivars of *Capsicum annum* which are resistant to the pathogens that cause chilli anthracnose have not yet been created, despite the fact that the management and control of anthracnose disease are still the subject of intensive research. This study examines the causes of chilli anthracnose, the progression of the disease, traditional methods for identifying the pathogen, and molecular techniques for identifying *Colletotrichum* species. Future developments leading to the disease management strategies are suggested.

## **2.2 Biological control of *Colletotrichum capsici***

Kelbessa *et al.* (2023) demonstrated that crop growth and total yield are negatively impacted by abiotic and biotic stresses throughout the world.

Abiotic and biotic stresses are thought to be responsible for global food crop losses of 50% and 30%, respectively. Numerous innovative agricultural technologies, like stress-tolerant varieties, smart irrigation systems, and shifting cropping calendars etc. have been used to both increase crop productivity and mitigate the effects of various stresses, however the majority of these techniques are time-consuming and expensive. By reducing the effects of various abiotic and biotic stresses associated with climate change plant growth-promoting rhizobacteria (PGPR) have the potential to boost agricultural yield. By using less harmful agrochemicals, which contribute to greenhouse gas emissions, this strategy can also increase the sustainability of agroecosystems.

May *et al.* (2023) performed an experiment where they used of indigenous bacterial community as inoculant for plant growth promotion in soybean cultivation. They found *Gammaproteo* bacteria (*Enterobacter*, *Pseudomonas*, and *Xanthomonas*) and other particular plant growth-promoting groups were enhanced in the resulting bacterial community of seedlings inoculated with bacterial consortium. The length of the radicle, the length of the hypocotyl, and the total dry weight of the seedlings were all enhanced by 44%, 30%, and 29%, respectively, by the application of bacterial consortia from the shoots of soybean plants grown in soil that had previously been used for soybean cultivation.

Kwon *et al.* (2022) showed that anthracnose is a fungus that harms various crops, including red pepper, and is brought on by *Colletotrichum* species. *Bacillus tequilensis* GYUN-300 (GYUN-300), which has antagonistic activity toward the fungus *Colletotrichum acutatum*, was used in this experiment. This pathogen produces anthracnose, which in red peppers primarily appears as a fruit rot. Under controlled condition GYUN-300 inhibited mycelial growth and spore germination of *C. acutatum*. GYUN-300 exhibited significant antagonistic characteristics, including the synthesis of siderophores, the solubilization of insoluble phosphate, and the creation of lytic

enzymes (cellulase, protease, and amylase). When red pepper fruits treated with GYUN-300 experienced preventive and curative benefits that were 66.6 and 38.3% as effective, respectively. Additionally, GYUN-300 proved resistant to a number of common fungicides, suggesting that the bacteria could be employed synergistically with fungicides to boost the effectiveness of biocontrol.

Ciofini *et al.* (2022) found that *Colletotrichum* spp. caused the severe disease named anthracnose on a variety of crop types. Both in the field and after harvest, fungal infections can happen, leading to serious fruit blemishes and financial losses. To manage the negative effects of anthracnose, physical treatments and synthetic fungicides have long been the preferred options; however, the urgent need to reduce the use of hazardous chemicals led to the investigation of creative and long-lasting defense strategies. Additionally biotechnology based strategies such as genome editing and RNA interference offering the potential to provide novel and secure techniques for managing anthracnose.

Wang *et al.* (2022) observed that for the management of pathogenic fungi, the *Bacillus amyloliquefaciens* group, which consists of the pathogenic fungus *B. amyloliquefaciens*, *B. velezensis*, *B. nakamurai*, and *B. siamensis*, has recently become an interesting source of biocontrol agents. In this review it is discussed that the antifungal mechanisms displayed by the *B. amyloliquefaciens* species included the generation of volatile organic compounds and antifungal lipopeptides, competition for nutrition, and the induction of disease resistance. Overall, research revealed that *B. amyloliquefaciens* species are an effective, ecologically acceptable substitute for the treatment of postharvest diseases.

Shi *et al.* (2021) demonstrated that anthracnose disease can be caused by the opportunistic plant pathogen *Colletotrichum* in a wide range of postharvest fruits. Although synthetic fungicides have traditionally been used to treat *Colletotrichum*, their detrimental effects on human health and the emergence of resistant strains have prompted the development of biocontrol techniques in

recent years. In this review for the first time they have analysed all previously published biocontrol techniques of *in vivo* control of *Colletotrichum* species in postharvest fruits.

Oleńska *et al.* (2020) concluded that to increase the production of plant biomass, more environmental friendly methods are needed. Beneficial plant growth-promoting (PGP) microorganisms can be used as effective and efficient biotechnological tools to enhance plant development including stressful–environments.

Prihatiningsih *et al.* (2019) conducted an experiment where *Bacillus subtilis* B298 strain was used as an antagonist of *Colletotrichum sp.* pathogens *in vitro* to evaluate the ability of microencapsulated *B. subtilis* B298 strain formula to suppress anthracnose disease of chilli *in vivo* condition. Spraying of microencapsulated *B. subtilis* B298 @ of  $2\text{gL}^{-1}$  was employed to combat anthracnose in the field. Among four treatments, results showed that *B. subtilis* B298 strain inhibited 56% growth of *Colletotrichum sp.* *in vitro*, micro capsulated *B. subtilis* B298 reduced disease intensity by 48% with infection rates of  $0,02 \text{ unitday}^{-1}$ . By increasing total phenol content on chilli plant microencapsulated *B. subtilis* B298 induced plant systemic disease resistance on chili.

Vejan *et al.* (2016) concluded that rhizobacteria that promote plant growth (PGPR) play a significant role in the sustainable agricultural sector. Nowadays it becomes a big challenge to meet up the demand for crop production with a reduced usage of synthetic chemical fertilizers and pesticides. By using of PGPR different plant diseases like anthracnose of chilli, fusarium wilt of tomato, white mold of tomato etc can be suppressed and that will be the environmentally responsible way to increase agricultural yields.

Seng Heng (2015) presented an experiment to investigate how well *Colletotrichum gloeosporioides* can be controlled in chilli fruits, *Streptomyces ambofaciens* S2 was used. Ten isolates of *Streptomyces* were tested for

antifungal activity, and each one produced an antifungal inhibitory zone ranging in size from 8 to 16 mm. Later, based on the widest antifungal inhibition zone exhibition (16 mm). *Streptomyces ambofaciens* S2 was then chosen for additional testing.

### **2.3 Beneficial microorganism against *Colletotrichum capsici***

Haron *et al.* (2023) reported that endophytic bacteria fungicides, offer safer substitutes for treating fungal diseases and a more environment friendly approach in comparison to chemical fungicides. This study's goal was to use an *in vitro* dual culture experiment to investigate the inhibitory effects of 14 isolates generated from shallot plants on *C. gloeosporioides*. The isolates were refined and classified as different species of the genus *Bacillus*, according to the findings, *B. velezensis* demonstrated the strongest antagonistic action (P 0.05), which reduced radial growth by 90% in comparison to the untreated control. *B. subtilis* came in second with an inhibition rate of 80%, followed by *B. cereus* (39%), *B. megaterium* (38%), and *B. licheniformis* (36%), which had the lowest inhibition rate.

Pupakdeepan *et al.* (2023) concluded that the strain named *Streptomyces* CINv1 was identified by analysis of its 16S rRNA gene sequences, and the result shows 100% similarity to *Streptomyces albus* CINv1. The CINv1 strain displayed high resistance (81.83%) against *Colletotrichum sp.* isolate CA0110, as tested by the dual culture technique. Spraying a spore suspension of the strain onto strawberry seedlings once a week resulted in a biological control trial in greenhouse conditions, which demonstrated a considerable decline in disease severity. CINv1 strain established a mutualistic interaction with the plant cells through colonization inter-and intracellularly in strawberry roots, leaves, and petioles.

Hiqmawati *et al.* (2023) performed a test which includes exploration and isolation, screening for antagonists, testing for inhibition, the activity of cell

wall degrading enzymes and volatile compounds. The outcomes demonstrated that ASR-3 (4) and ASR-7 (4) isolates were the best isolates with several antagonistic mechanisms, including the ability to inhibit the growth of *Colletotrichum sp.* through antibiosis, the ability to secrete extracellular enzymes that degrade cell walls in the form of proteases and amylases, as well as the ability to produce volatile compounds and have a very strong inhibition category with inhibition percentages that were, respectively, 51.11% , 50.54%, and 54.55%.

Le Thanh *et al.* (2023) observed that *B. subtilis* had an around 55% antagonistic percentage, however it had no effect on the germination rates, shoot height, or root length of chilli seedlings. On chilli leaves and fruits, the *Bacillus* treatment reduced anthracnose severity by 86-100% and 49-74%, respectively. *B. subtilis* increased the phenolic compound levels in the chilli plants' leaves and fruits to about 22 mg per 100 g fresh weight (FW) and 19 mg per 100 g FW, respectively.

Agnihotri *et al.* (2023) conducted an experiment to evaluate the isolates of *Trichoderma spp.*, isolated from rhizosphere soil of chilli experimental field. The experiment minimum radial growth of *Colletotrichum capsici* was measured by *Trichoderma* isolate Tr-8, followed by Tr-3 and Tr-1, with maximum growth inhibition as 62.60, 61.74, and 59.13 percent, respectively, over control. Inhibition percent of growth by various isolates of *T. harzianum* ranged between 46.96-54.78 percent of *C. capsici*.

Yadav *et al.* (2023) worked with *Trichoderma asperellum* and *T. harzianum* as potential biological controls against *Colletotrichum truncatum*. A hemibiotrophic fungus called *C. truncatum* affects the growth and fruit production of chilli plants by causing the anthracnose disease. Scanning electron microscope (SEM) technique demonstrated that the favorable interaction between chilli root-*Trichoderma spp.* inducing the plant growth promotion, mechanical barrier, and defensive network.

Nurbailis *et al.* (2023) stated that by using a group of endophytic bacteria the disease can be controlled in a different approach that is economical and eco-friendly. The aim of the study was to obtain the best consortium of endophytic bacteria to control anthracnose caused by *C. gloeosporioides* and improve the growth and yield of chili plants. This study used 28 species of endophytic bacteria, 14 treatments with 3 replications, among them all treatments of endophytic bacterial consortium showed the potentiality to suppress anthracnose disease caused by *C. gloeosporioides* and enhance the development and productivity of chili plants.

Alam *et al.* (2022) investigated to find out the natural management, a search for fungal bio-agents, plants and secondary metabolites of endophytic fungi against phytopathogens. The experiment resulted that highest inhibition 62.14 % was achieved when treated with bio agents *Trichoderma sp.* then 83.372% at 40% concentration when treated with plant extracts from Mehgoni seeds and finally the highest mycelial growth inhibition was found 91.525% by secondary metabolites of *Pestalotia sp.*

Pramudito *et al.* (2020) presented that PHP12 is a bacterial strain which is isolated from healthy oil palm rhizosphere and it is closely related to the recently described *Burkholderia stagnalis*, a member of the *Burkholderia cepacia* complex. This study aimed to identify the antifungal compounds produced by the strain and finally it was proved that fungal pathogens such as *Ganoderma boninense*, *Curvularia oryzae*, *Phellinus noxius*, and *Colletotrichum capsici* were all suppressed by PHP12.

Miljaković *et al.* (2020) discussed that by excreting extracellular metabolites such antibiotics, cell wall hydrolases, and siderophores, bacilli display antagonistic action and by triggering induced systemic resistance (ISR), plants become able to show response against pathogen. Antagonistic and plant growth-promoting strains of *Bacillus spp.* might be useful in formulating new preparations.

Thilagam and Hemalatha (2019) reported that actinobacterias were isolated from rhizosphere which were tested for antagonistic potentiality against pathogenic fungi *C. capsici*. Isolate BS-26 was identified as *Streptomyces violaceoruber* based on 16S rDNA sequencing that displayed highest antagonistic activity against the fungal pathogen. The application of *S. violaceoruber* fermentation broth resulted with a significant increase in germination %, total plant height, fresh weight and chlorophyll content over controls.

Jayapala *et al.* (2019) observed that anthracnose disease in the chili plant was suppressed by the beneficial rhizobacterium *Bacillus sp.* BSp.3/aM, which was tested for its capacity to enhance plant health. *Bacillus sp.* BSp.3/aM was discovered to be inhibitory to *Colletotrichum capsici* (6 mm) in the dual culture assay. Additionally, seed priming decreased the incidence of *C. capsici* in seeds by 2% and enhanced the vigor (vigor index: 1374 7.15) and germination (germination rate: 98 0.57%) of chili seedlings. By increasing the activity of defense-related enzymes and accumulation of phenolic compounds in the host plant, it is possible to induce resistance to invading pathogens. highest levels of phenolic and chitinase accumulation was observed in BSp.3/aM + pathogen treated seedlings 120 hpi (94.7 µg/g tissue) and at 96 hpi (9.36 units), respectively.

Lahkar *et al.* (2018) conducted an experiment to evaluate the antifungal efficacy of the biosurfactant produced by *Pseudomonas aeruginosa* JS29 strain for combating the fungal disease anthracnose of chilli. The results clearly demonstrated that the biosurfactant may successfully inhibit the growth of the pathogen's spore and mycelia.

Shafi *et al.* (2017) discussed that the primary risk to productive, profitable agriculture is posed by plant diseases. Therefore, the use of biological control agents such as *Bacillus*-based biocontrol agents play a fundamental role in the field of biopesticide, Many *Bacillus* species have been recognized as

plant growth promoter, systemic resistance inducer, and used for production of a broad range of antimicrobial compounds (lipopeptides, antibiotics and enzymes) and competitors for growth factors (space and nutrients) with other pathogenic microorganisms through colonization.

Basha and Ramanujam (2015) demonstrated that when chilli seeds were treated with the strain *Pichia guilliermondii* then increasing of seedling vigour index (55%), fresh weight (96%) and dry weight (45%) over untreated control were found. On the other hand, chilli seedlings challenge-inoculated with *Colletotrichum capsici* exhibited increased amounts of defence-related chemicals after seedlings dipped with *Hanseniaspora uvarum*. Under field conditions, foliar sprays and seedling dips with *H. uvarum* resulted in a 37–40% reduction in the incidence of chilli fruit rot.

Evangelista (2014) conducted an experiment where results showed morphological, physiological and biochemical characterization of six actinomycetes and their inhibitory activity against *Curvularia sp.*, *Aspergillus niger*, *Helminthosporium sp.* and *Fusarium sp.* With percentages ranging from 47 to 90%, one isolate, known as *Streptomyces sp.* CACIS-1.16CA, showed the ability to inhibit additional diseases such as *Colletotrichum sp.*, *Alternaria sp.*, *Phytophthora capsici*, and *Rhizoctonia sp.* The *Streptomyces* strain which is identified in this study has a wide range of antagonistic activity and this might be useful for the biocontrol of plant pathogenic fungi.

El-Mabrok *et al.* (2012) reported the efficiency of utilizing cultures of lactic acid bacteria (LAB) or their supernatant as a biological control against the fungus *Colletotrichum capsici* that causes the anthracnose disease in chillies. Seven isolates out of 324 lactic acid bacteria that were obtained from various sources by the dual culture method shown good inhibitory action against *C. capsici*. By using microtiter plates, the supernatant from LAB-C5 demonstrated a significant suppression of fungal growth. Seeds treated with LAB-C5 cells

after being infected with *C. capsici* had a higher rate of germination than seeds treated with supernatant.

Anand *et al.* (2010) presented that the turbidometric approach was used to determine the susceptibility of *P. fluorescens* to the fungicide azoxystrobin at various concentrations (100, 150, 200, 250, and 300 ppm). In two field trials, Pf1 tested in combination with reduced concentration of azoxystrobin was extremely effective in managing both chilli diseases named fruit rot and powdery mildew. The survival of Pf1 in the chilli phylloplane was considerably improved by adding *P. fluorescens* along with azoxystrobin.

#### **2.4 *Enterobacter cloacae* as a biocontrol agent**

Malek *et al.* (2023) discussed the objective of the current work was to isolate, assess, and identify possible endophytic bacteria to restrain *R. solanacearum* via in vitro antagonistic assay. Among 116 endophytic isolates, 55 exhibited abilities to restrain *R. solanacearum* in vitro and only two isolates named *Enterobacter tabaci* and *E. tabaci* showed the greatest potential against *R. solanacearum*.

Omotayo and Babalola (2023) showed that numerous plant-associated bacteria, particularly those connected to the rhizosphere niche, have been found to have antagonistic effects on *F. verticillioides*. The use of rhizosphere-associated biocontrol agents including *Enterobacter*, *Bacillus* spp., *Pseudomonas*, and *Microbacterium oleivorans* which forms part of the phytomicrobiome to prevent and control this toxicogenic fungus.

Parveen *et al.* (2023) demonstrated that in dual culture technique, among one hundred twenty-five endophytic microorganisms, strong antagonistic activity of five fungal (ranging from 89%–70%) and five bacterial (72%–61%) endophytes were observed. *Aspergillus flavus* and *Enterobacter cloacae* both exhibited growth-promoting characteristics, particularly in the formation of auxin, gibberellic acid, ammonia, and nitrogen fixation.

Maqbool *et al.* (2021) found that a plant growth promoting endophytic (PGPE) bacteria *Enterobacter cloacae* 2WC2 strain was investigated for its role in drought stress amelioration and plant growth promotion via ACC deaminase activity and enhanced levels of antioxidant enzymes in stressed plants like maize. *E. cloacae* showed tolerance to drought at various stress levels (10–40% PEG 6000) and resistance to 15 different antibiotics during *in vitro* testing.

Kazerooni *et al.* (2020) showed that the pathogen hyphae were deformed and shriveled by the bacterial strain named *Enterobacter cloacae*. It is most effective in suppressing the mycelial growth of *P. aphanidermatum*, by creating a 5mm inhibition zone. Furthermore in pot experiment, pre-treatment with the *E. cloacae* reduced disease incidence significantly by 63%.

Ji *et al.* (2020) demonstrated that after the inoculation of wheat with the *E. cloacae* HG-1 strain, we recorded increases in root length, plant height, fresh weight, and dry weight of 19.15%, 18.83%, 16.67%, and 17.96%, respectively, compared with uninoculated plants ( $P < 0.05$ ). Compared with the leaves of uninoculated plants, the proline concentration in the leaves of inoculated plants increased by 12.43% ( $P < 0.05$ ), the malondialdehyde level decreased by 27.26% ( $P < 0.05$ ),  $K^+$  increased by 20.69%,  $Ca^{2+}$  increased by 57.53% and  $Na^+$  decreased by 31.43% (all  $P < 0.05$ ).

Pattnaik *et al.* (2020) presented that under optimized condition the multi-metal resistant bacteria *Enterobacter cloacae* reduced 94% Cr (VI) within 92 h and Cr (VI) and promoted plant growth like  $N_2$  fixation; phosphate (146.87 ppm), potassium (12.55 ppm) and Zn solubilization; ammonification; IAA production ( $114 \mu g mL^{-1}$ ) and suppression of fungal phytopathogens such as *Rhizoctonia solani* (ITCC 2060) and *Pythium debaryanum* (ITCC 5488).

Damasceno *et al.* (2019) discussed that as an alternative to chemical control, biological control agents (BCAs) have been studied and used in this study to control banana anthracnose caused by *Colletotrichum musae*. For this purpose, *Enterobacter cloacae*, *Bacillus velezensis*, *Serratia marcescens* and

*Stenotrophomonas maltophilia*. *B. velezensis* were used and finally they showed similar performance as the Thiabendazole (Tecto SC®).

Bhise *et al.* (2017) investigated that among forty isolates, *Enterobacter cloacae* strain KBPD was the potent strain for maintaining ACC deaminase activity, phosphate solubilization, indole acetic acid, siderophore, ammonia, hydrogen cyanide and exopolysaccharide production under salt stress. In the pot study, maximum shoot length, root length, fresh and dry weights were recorded in *Vigna radiata* L. when supplemented with KBPD in the presence of 0, 50, 100 and 150 mM of NaCl. Proline content was high in salt-stressed plants but inoculation with *E. cloacae* KBPD reduced the concentration of proline.

Chen *et al.* (2016) presented that the volatile compounds produced by *E. cloacae* which inhibited conidial germination and hyphal elongation of *P. digitatum* and reduced green mold severity. *E. cloacae* cultured at temperatures ranging from 16°C to 28°C, at pH values  $\leq 6$ , or in a substrate carrier (sphagnum moss, vermiculite, or perlite) showed superior control against *P. digitatum*.

Swamy *et al.* (2016) found that among 46 lichenized bacterial isolates, only 11 fixed nitrogen also produced siderophore and 10 of them the IAA. Nine of these nitrogen fixers (NF) were also found to produce ammonia. These isolates were closely related to *Enterobacter cloacae*. This is the first record of *Enterobacter sp.* from lichens, which may naturally or artificially occupy lichen thalli.

Kryuchkova *et al.* (2014) analysed that *E. cloacae* K7 had a C-P lyase activity and degraded glyphosate to give sarcosine, which was then oxidized to glycine. Additionally, strain K7 inhabited the roots of sugar sorghum (*Sorghum saccharatum* Pers.) and common sunflower (*Helianthus annuus* L.), encouraging the growth and development of sunflower seedlings.

Saleh and Glick (2001) discussed that when seeds were exposed to *Enterobacter cloacae* CAL2, overproduction of either GacA or RpoS led in a

notable increase in the root lengths of canola seedlings, and overproduction of RpoS resulted in an increase in the shoot and root length.

Mayak *et al.* (2001) reported that when chilli seedlings were inoculated with bacterial suspension named *Enterobacter cloacae* and even in the presence of a nutrient solution, this bacterium can affect plant growth. Mineral analysis of the bacterial suspension indicated that minerals associated with the bacterium were the cause of the growth promotion effect.

## CHAPTER 3

### MATERIALS AND METHODS

The study was aimed for the eco-friendly management of anthracnose of chilli caused by *Colletotrichum capsici* by the use of the formulated bio-fungicide. The detailed methodology used to carry the experiments are as follows:

#### 3.1 Collection of beneficial bacteria

The beneficial bacteria namely *Enterobacter cloacae* HSTUB 12 were previously isolated and identified on the basis of molecular characterization and used in this study.

#### 3.2 Preparation of formulated bacteria

Bacterium (*E. cloacae* HSTUB 12) were cultured in nutrient broth media for 48 h. Maize bran, charcoal bran, soybean bran, wood dust and pea bran were used as the substrate for the development of the formulation. At first all the substrates were dried and blended followed by adding adhesive (white flour gum) at 1:10 ratio, the pH was kept at 7.4, and the autoclave was operated at about 15 pounds per square inch of pressure for 15 minutes in order to reach 121°C in the chamber. To hold things together functionally by surface contact that resists separation, adhesive was introduced. The mixture was autoclaved, plated, and then dried in a sterile environment. Mannitol (8.5 mL of 3%) was used for 100 g formulation. It was added to the mixture to increase its resistance to moisture. Formulation and bacteria were combined in a 10:1 ratio, and the mixture was dried for three days at room temperature in a sterile state. After that, the bacteria were prepared for field use. The formulated bacteria were kept fresh in polybags in the refrigerator at 4°C so that they could be used later.



**Fig. 1** A) Wood dust-based formulated *E. cloacae*; B) Pea bran-based formulated *E. cloacae*; C) Soybean bran-based formulated *E. cloacae*; D) Maize bran-based formulated *E. cloacae*; E) Charcoal-based formulated *E. cloacae*

### 3.3 Shelf-life of formulated *E. cloacae*

Serial dilution was used to count the viable cells in the prepared products at 30-day intervals until the last viable cell was found. This method involved repeated dilution using Nutrient Agar (NA) and Nutrient Broth (NB). For 10 minutes, both media were sterilized at 120°C. In this process, 1gm formulated bio-fungicide was suspended in 9 mL of sterile distilled water. This is then followed by the same procedure, where 1 mL from test tube 1 is added to 9 mL of tube 2, 1mL from tube 2 is added to 9 mL from tube 3, and so on until the desired concentration is reached. After that, the formulation solution was serially diluted to a dilution of  $10^{-9}$  times. Using the pour plate technique, 1 mL of the diluted suspension was mixed with each sample's nutrient agar (NA) and nutrient broth (NB) plates, and the samples were then incubated for 24 h at 28°C. From the initial culture plate, a single bacterial colony was isolated.

$$\text{Viable cell count (CFU/g formulation)} = \frac{\text{Number of colonies}}{\text{Volume of inoculum}} \times \text{Dilution factor}$$

### **3.4 Management of anthracnose of chilli by the application of formulated bio-fungicide composed with *E. cloacae* HSTUB 12**

The experiment was conducted at the research field under the Department of Plant Pathology, HSTU, Dinajpur, during the period from December 2022 to April 2023. Detailed of the field experiment are as follows:

#### **3.4.1 Location**

Geographically the experimental field was located at 25038/ N latitude and 88041/ E longitude, with a height of 38 m above the sea level belonging to Agro-ecological Zone-I (AEZ-I) named old himalayan piedmont plain.

#### **3.4.2 Soil**

The experimental field is a medium high land with sandy loam textured soil. The pH value of the soil is above 5.5.

#### **3.4.3 Experimental period**

The experiment was carried out during December, 2022 to March, 2023.

#### **3.4.4 Collection of chilli seeds and raising of seedlings**

Chilli seeds (Bijlee plus and Roshni) were collected from Bangladesh Agricultural Development Corporation (BADC), Dinajpur. The sterilized manure and soil were used to prepare the seed bed. After planting seeds at a depth of 2 to 3 cm, the soil was lightly moistened. The raised seedlings were prepared for transplanting after 35 days.

#### **3.4.5 Final land preparation**

A power tiller was used to clear the main land, and then laddering was done three times. The land's corner was levelled, and the larger clods that were

visible were pounded into tiny fragments. The land was free of any weeds or stubble.

### **3.4.6 Application of manure and fertilizers**

During the last stages of land preparation, well-decomposed cow dung was applied. According to Chowdhury and Hassan (2013), the sources of N, P, and K fertilizers were urea (550 kg/ha), TSP (450 kg/ha), and MP (250 kg/ha), in that order. Additionally, micronutrients were administered twice. During the last stages of land preparation, TSP and MP were administered in full, and urea was applied in two equal portions at 20 and 30 days following transplanting (DAT).

### **3.4.7 Combination of formulated bio-fungicide composed with *E. cloacae* HSTUB 12 as treatment**

Formulated bio-fungicides composed with *E. cloacae* HSTUB 12 were applied for the management of anthracnose of chilli. However, the experiment comprised of seven treatments with three replications and the treatment combinations are as follows:

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T <sub>0</sub> : Control
T <sub>1</sub> : Maize bran-based formulated <i>E. cloacae</i> HSTUB 12 followed by spraying of <i>E. cloacae</i> HSTUB 12 suspension.
T <sub>2</sub> : Pea bran-based formulated <i>Enterobacter cloacae</i> HSTUB 12 followed by spraying of <i>E. cloacae</i> HSTUB 12 suspension.
T <sub>3</sub> : Soybean bran-based formulated <i>Enterobacter cloacae</i> HSTUB 12 followed by spraying of <i>E. cloacae</i> HSTUB 12 suspension.
T <sub>4</sub> : Charcoal based-formulated <i>Enterobacter cloacae</i> HSTUB 12 followed by spraying of <i>E. cloacae</i> HSTUB 12 suspension.
T <sub>5</sub> : Wood dust-based formulated <i>Enterobacter cloacae</i> HSTUB 12 followed by spraying of <i>E. cloacae</i> HSTUB 12 suspension.
T <sub>6</sub> : Vulcan 50WP (Carbendazim)

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### **3.4.8 Design of experiment**

The experiment was laid out following Randomized Complete Block Design (RCBD) with three replications. There were 42 unit plots altogether in the experiment and the size of the unit plot were 60 cm x 60 cm and block-to-block distance was 50 cm.

### **3.4.9 Application of formulated bio-fungicide**

After preparation of soil bed, formulated bacterium *E. cloacae* @ 50g/plot was applied in before three days of transplantation and ten days of after transplantation with maintaining proper moisture.

### **3.4.10 Transplantation of seedlings**

Seedlings were transplanted in the main field on 29 November 2022. After transplantation, seedlings were watered regularly to make a firm relation with roots and soil to stand-alone.

### **3.4.11 Application of suspension of *E. cloacae* HSTUB 12**

*E. cloacae* HSTUB 12 suspension ( $10^8$  CFU/mL) was prepared in order to apply in the field. The beneficial bacterium was picked up from pure culture by the toothpick, inoculated in nutrient broth (8 mL/litter), and incubated at 28 °C in a shaking incubator for 24 h. The bacterial suspension were sprayed 2 times on the whole plant including soil and 2 times only whole plant started from 20 DAT at 25 days interval up to 90 DAT. Vulcane 50 WP was prepared @1 g/ litter and sprayed at the same day.

### **3.4.12 Irrigation**

After transplantation, the field was irrigated as per necessary, however, heavy rainfall occurred after one month of transplantation, so frequent irrigation was not required.

### **3.4.13 Weeding, thinning and gap filling**

There were different types of weeds, which were controlled effectively by hand weeding. Thinning and gap filling was also done after 10 DAT.

### **3.4.14 Application of pesticides**

Jago 70 WDG (Imidacloprid) @ 2g/L water was sprayed twice, 30 days apart, starting on 30 DAT, to avoid insect infestation. Hand weeding proved to be an excellent method of controlling several sorts of weeds.

## **3.5 Collection of data on different agronomical traits of chilli plant**

Data on different agronomic traits of chilli plants including plant height (cm), branches per plant, numbers of flower per plant, number of fruits per plant, and fruit weight (kg) per plant, shoot weight, shoot length and root weight, root length, were collected at the time of harvesting.

### **3.5.1 Plant height (cm)**

Plant height (cm) was measured from the ground to the tip of the leaves at 30 days interval started from 30 DAT up to 120 DAT.

### **3.5.2 Number of branches per plant**

The number of branches arising from the main stem above the ground was recorded started from 30 DAT at 30 days interval up to 120 DAT.

### **3.5.3 Number of flowers per plant**

The number of flowers was recorded at 60, 90, 120 DAT.

### **3.5.4 Number of fruits per plant**

Total number of marketable fruits were harvested from five plants. Total number of fruits was counted and the average number of fruits per plant was calculated. Fruits were picked on the basis of horticultural maturity, size, color and age. Picking was done throughout the harvesting period.

### **3.5.5 Yield (kg/plant)**

Yield (kg/plant) was recorded from every plant and total yield was calculated through average yield by multiplying plant population during harvest time up to 120 DAT.

### **3.5.6 Shoot weight (g) and shoot length (cm)**

The Shoot weight (g) and shoot length (cm) was recorded at harvesting 120 DAT.

### **3.5.7 Root weight (g) and root length (cm)**

The Root weight (g) and root length (cm) was recorded at harvesting 120 DAT.

## **3.6 Counting healthy and diseased plants**

The experimental plot was surveyed frequently to observe whether the plant became infected with anthracnose disease or not. However, the data was recorded at 60, 90, 120 DAT. The percentage of plant recovery and infected disease were also calculated. Anthracnose incidence (%) of leaves and fruits were recorded at 60 & 90 DAT and 90 & 120 DAT following the formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected leaves/fruits}}{\text{Total number of leaves/fruits}} \times 100$$

Anthracnose severity (%) of leaves and fruits: For recording the anthracnose severity (%), 0-5 disease grading scale was used.

**Table 1.** Chilli anthracnose disease severity grading scale

Plant parts	Grading	Tissue infected (%)
Leaves	0	No symptoms
	1	Small spots on leaves, less than 1 per cent of leaf area diseased
	2	Medium six spots on leaves covering 1-10 per cent infected area
	3	Spots big, coalescing, covering 11-25 per cent of leaf area.
	4	Spots large; coalescing covering 26-50 per cent of leaf area
	5	Spots on leaves cover above 51 per cent of the leaf area.
Fruits	0	No symptoms
	1	Slight to 10% infection
	2	11-25% infection
	3	26-50% infection
	4	51-75% infection
	5	Greater than 75% infection



**Fig. 2** Showing several portion of infection of chilli anthracnose severity disease; 0 (no infection), 1(slight-10%), 2(11-25%), 3(26-50%), 4(51-75%), 5(>75%) infection

The disease severity was calculated by following the formula:

$$\text{Disease Severity (\%)} = \frac{\text{Sum of total ratings}}{\text{Total number of observation} \times \text{Maximum grade}} \times 100$$

The percentage of reduction of disease incidence and severity over control were also calculated.

### 3.7 Biochemical analysis of fruit

Fruits of chilli were subjected to analysis for the total phenol contents (mg/100g), total soluble solids (TSS °Brix), ascorbic acid (mg/100g of fresh weight), in fruit.

#### 3.7.1 Estimation of total phenol (mg/100g)

The computation of total phenol was done by Saikia *et al.* (2012) and Singleton and Rossi (1965). In a nutshell, 0.5 mL of sample and 0.5 mL of Folin Ciocalteu's reagent were added to a 25 mL falcon tube and properly mixed. After adding 1 mL of 7.5% saturated sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) to the falcon tube for neutralization, the solution was allowed to react, and it was vortexed for 30 seconds. Following 35 minutes at ambient temperature in a dark environment, the mixture was centrifuged for 10 minutes at 4000g. An ultraviolet/visible spectrum (UV1800) measuring 725 nm was used to determine the sample's absorption. To perform a standard (calibration) curve, gallic acid was utilized. The findings have been shown to be equal mg/100 g of Gallic acid per 100 g of juice.

$$\text{Total phenol content (\%)} (\text{mg}/100\text{g sample}) = \frac{\text{Amount of phenol obtain}}{\text{Weight of sample}} \times 100$$

#### 3.7.2 Estimation of total soluble solids (°Brix)

Total soluble solids (TSS) were determined using refract meter and expressed as °Brix.

### 3.7.3 Estimation of antioxidant

Several types of chillies were tested for their antioxidant properties using the DPPH assay. From each dried and blended sample of chilli fruits, one milliliter was extracted. In order to prepare DPPH, methanol was used. The extracted sample-filled falcon tube was then filled with 2 mL of 0.004% w/v DPPH. Following that, the incubation process lasted for 30 minutes in a dark area. Ascorbic acid (20 ppm–0.156 ppm) standard solution was prepared from a 1000 ppm stock solution. The UV-Vis spectrophotometer was then used to measure absorbance at 517 nm. The percentage of antioxidants was calculated using the following formula, citing Baliyan *et al.* (2022):

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

Where,

Ac=Control reaction absorbance

As=Testing specimen absorbance

### 3.8 Statistical analysis

Data obtained from different parameters were statistically analyzed to find out the level of effectiveness of different treatments to control the Anthracnose of chilli. The analysis of variance was performed by using Statistix 10. The mean difference among the treatment were estimated by DMRT (Duncan`s Multiple Range Test) at 5% level of probability (Gomez and Gomez, 1984).

## CHAPTER IV

### RESULTS

An attempt was taken to identify a beneficial bacterium showing antibacterial potentiality against *Colletotrichum capsici* causing anthracnose disease in chilli by means of biochemical analysis. *Enterobacter cloacae* was applied as formulated and liquid state at the field alone or in combination at the field level for the eco-friendly management of anthracnose of chilli.

#### **4.1 Eco-friendly management of anthracnose of chilli using formulated bio-fungicide**

A field experiment was conducted during Robi-2022 on integrated management of anthracnose of chilli caused by *Colletotrichum capsici* with single treatment of *E. cloacae*. The results of the experiment were presented below

#### **4.2 Efficacy of formulated bio-fungicide against anthracnose incidence (%) of chilli at 120 DAT**

In Bijlee plus, T<sub>6</sub> showed lowest disease incidence (17.37%) followed by the treatment of T<sub>4</sub>, T<sub>5</sub>, T<sub>3</sub>, T<sub>2</sub>, and T<sub>1</sub> which showed 17.52 %, 26.44 %, 30.40 %, (33.38 %), and 35.87 % respectively. However, Plants without treatment T<sub>0</sub> (control) showed the highest anthracnose incidence (60.50 %) (Table 2).

In Roshni, T<sub>6</sub> showed lowest disease incidence (17.52 %) followed by the treatment of T<sub>4</sub>, T<sub>5</sub>, T<sub>3</sub>, T<sub>2</sub>, and T<sub>1</sub> (22.97%), (25.09 %), (29.40 %), (32.37 %) and (34.69 50 %) respectively. However, plants without treatment T<sub>0</sub> (control) showed the highest disease incidence (57.32 %) (Table 2).

**Table 2.** Efficacy of formulated bio-fungicide against anthracnose incidence (%) of chilli at 120 DAT

Variety	Treatments	Chilli anthracnose at 120 DAT	
		Incidence (%)	Reduction over control (%)
Bijlee plus	T <sub>0</sub>	60.50±1.02f	0.00
	T <sub>1</sub>	35.87±0.86e	40.71
	T <sub>2</sub>	33.38 ±0.62d	44.83
	T <sub>3</sub>	30.40±1.36c	49.75
	T <sub>4</sub>	17.52a±0.69a	71.04
	T <sub>5</sub>	26.44±0.65b	56.30
	T <sub>6</sub>	17.37±0.67a	71.29
	LSD	1.48	-
Roshni	T <sub>0</sub>	57.32±1.42e	0.00
	T <sub>1</sub>	34.69±1.96d	39.48
	T <sub>2</sub>	32.37±1.02d	43.53
	T <sub>3</sub>	29.40±0.43c	48.71
	T <sub>4</sub>	16.76±0.84a	70.76
	T <sub>5</sub>	25.09±0.42b	56.23
	T <sub>6</sub>	16.27±0.38a	71.62
	LSD	2.48	-

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: Vulcan 50WP (Carbendazim).

#### 4.3 Efficacy of formulated bio-fungicide against anthracnose severity (%) of chilli at 90 DAT.

In Bijlee plus, T<sub>6</sub> showed lowest disease severity (18.39 %). Second lowest disease severity showed by the treatment T<sub>5</sub> (19.77 %) followed by the treatment of T<sub>4</sub>, T<sub>1</sub>, T<sub>3</sub> and T<sub>2</sub> showed (21.82 %), (28.57 %), (31.71 %) and

(41.90 %) respectively. However, plants without treatment T<sub>0</sub> (Control) showed the highest disease severity (57.85 %) (Table 3).

In Roshni, T<sub>6</sub> showed lowest disease severity (17.74 %). Second lowest disease severity showed by the treatment of T<sub>4</sub> (18.57 %). Followed by the treatment of T<sub>5</sub>, T<sub>1</sub>, T<sub>3</sub> and T<sub>2</sub> showed (25.49 %), (28.94 %), (30.39 %) and (35.69 %) respectively. However, plants without treatment T<sub>0</sub> (Control) showed the highest disease Severity (56.39 %) (Table 3).

**Table 3.** Efficacy of formulated bio-fungicide against anthracnose severity (%) of chilli at 90 DAT.

Variety	Treatments	Anthracnose severity at 90 DAT	
		Severity (%)	Reduction over control (%)
Bijlee plus	T <sub>0</sub>	57.85±1.18f	0.00
	T <sub>1</sub>	28.57±0.68c	50.61
	T <sub>2</sub>	41.90±0.48e	27.57
	T <sub>3</sub>	31.71±0.48d	45.19
	T <sub>4</sub>	21.82±0.37b	62.28
	T <sub>5</sub>	19.77±0.70a	65.83
	T <sub>6</sub>	18.39±0.72a	68.21
	LSD	1.61	-
Roshni	T <sub>0</sub>	56.39±0.75e	0.00
	T <sub>1</sub>	28.94±1.44c	50.61
	T <sub>2</sub>	35.69±1.63d	27.57
	T <sub>3</sub>	30.39±0.44c	45.19
	T <sub>4</sub>	18.57±0.39a	67.07
	T <sub>5</sub>	25.49±0.77b	62.94
	T <sub>6</sub>	17.74±1.00a	68.54
	LSD	2.19	-

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: Vulcan 50WP (Carbendazim).

#### **4.4 Efficacy of formulated bio-fungicide against anthracnose severity (%) of chilli at 120 DAT.**

In Bijlee plus, T<sub>6</sub> showed lowest disease severity (20.82 %). Second lowest disease severity showed by the treatment of T<sub>4</sub> (22.33 %). Followed by the treatment of T<sub>5</sub>, T<sub>3</sub>, T<sub>1</sub> and T<sub>2</sub> showed (24.89 %), (32.37 %), (34.96 %) and (43.40 %) respectively. However, plants without treatment T<sub>0</sub> (Control) showed the highest disease Severity (64.72 %) (Table 4).

In Roshni, T<sub>6</sub> showed lowest disease severity (20.38 %). Second lowest disease severity showed by the treatment T<sub>4</sub> (21.41 %) followed by the treatment T<sub>5</sub>, T<sub>3</sub>, T<sub>1</sub> and T<sub>2</sub> showed (25.43%), (29.19 %), (33.00 %) and (40.18 %) respectively. However, plants without treatment T<sub>0</sub> (Control) showed the highest disease severity (60.67 %) (Table 4).

**Table 4.** Efficacy of formulated bio-fungicide against anthracnose severity (%) of chilli at 120 DAT.

Variety	Treatments	Anthracnose severity at 120 DAT	
		Severity (%)	Reduction over control (%)
Bijlee plus	T <sub>0</sub>	64.72±0.24f	0.00
	T <sub>1</sub>	32.37±1.18c	49.98
	T <sub>2</sub>	43.40±0.78e	32.94
	T <sub>3</sub>	34.96±0.60d	45.98
	T <sub>4</sub>	22.33±0.94a	65.50
	T <sub>5</sub>	24.89±1.40b	61.54
	T <sub>6</sub>	20.82±0.87a	67.83
	LSD	2.00	-
Roshni	T <sub>0</sub>	60.67±0.73f	0.00
	T <sub>1</sub>	29.19±0.38c	51.89
	T <sub>2</sub>	40.18±0.36e	33.77
	T <sub>3</sub>	33.00±0.50d	45.61
	T <sub>4</sub>	21.41±1.00a	64.71
	T <sub>5</sub>	25.43±0.73b	58.08
	T <sub>6</sub>	20.38±0.50a	67.73
	LSD	1.28	

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: Vulcan 50WP (Carbendazim).

#### 4.5 Interaction effect of formulated bio-fungicide on anthracnose severity (%) of chilli at 120 DAT.

Interaction of the variety and treatments revealed that, statistically similar and significant disease severity reduction (65.50 and 64.71 %) was obtained in both variety with the application of Charcoal-based *E. cloacae* over control (Table 5).

**Table 5.** Interaction effect of formulated bio-fungicide and variety of chilli on anthracnose severity (%) at 120 DAT.

Treatments × Variety	Anthracnose severity (%)	Reduction over control (%)
T <sub>0</sub> ×V <sub>1</sub>	4.72± 0.24	0.00
T <sub>1</sub> ×V <sub>1</sub>	32.37± 1.18	49.98
T <sub>2</sub> ×V <sub>1</sub>	43.40 ± 0.78	32.94
T <sub>3</sub> ×V <sub>1</sub>	34.96 ± 0.60	45.98
T <sub>4</sub> ×V <sub>1</sub>	22.33 ± 0.94	65.50
T <sub>5</sub> ×V <sub>1</sub>	24.89± 1.40	61.54
T <sub>6</sub> ×V <sub>1</sub>	20.82 ± 0.87	67.83
T <sub>0</sub> ×V <sub>2</sub>	60.67±0.73	0.00
T <sub>1</sub> ×V <sub>2</sub>	29.19 ± 0.38	51.89
T <sub>2</sub> ×V <sub>2</sub>	40.18 ± 0.36	33.77
T <sub>3</sub> ×V <sub>2</sub>	33.00 ± 0.50	45.61
T <sub>4</sub> ×V <sub>2</sub>	21.41 ± 1.00	64.71
T <sub>5</sub> ×V <sub>2</sub>	25.43 ± 0.73	58.08
T <sub>6</sub> ×V <sub>2</sub>	19.58± 0.56	67.73

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: Vulcan 50WP (Carbendazim).

#### 4.6 Efficacy of formulated bio-fungicide *E. cloacae* on growth attributes of chilli plant

The effect of all the treatments imposed for the management of Anthracnose of chilli causing *Colletotrichum capsici* and their influence on plant growth parameters viz., plant height, number of branches, number of flowers, number of fruits and yield (gm/plant) in chilli variety named Bijlee plus and Roshni was recorded as follows:

#### **4.6.1 Efficacy of formulated bio-fungicide on plant height (cm) of Bijlee plus at different DAT.**

AT 30 DAT in Bijlee plus, T<sub>5</sub> showed highest plant height (15.17 cm.) followed by the treatments T<sub>4</sub>, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>6</sub> showed 14.77, 14.16, 13.71, 13.27 and 12.96 cm. respectively. However, plants without treatment T<sub>0</sub> (control) showed the lowest plant height (12.00 cm.) (Table 5)

At 60 DAT in Bijlee plus, T<sub>4</sub> Showed highest plant height (45.03 cm.) followed by the treatments T<sub>5</sub>, T<sub>3</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>6</sub> showed plant height 44.07, 41.98 and 40.88, 28.03 and 24.57 cm. respectively. However, plants without treatment T<sub>0</sub> showed the lowest plant height (24.53 cm.) (Table 6).

AT 90 DAT in Bijlee plus, T<sub>4</sub> showed highest plant height (61.83cm.) followed by the treatments T<sub>5</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>6</sub> and T<sub>1</sub> showed the plant height 58.47, 51.50, 50.43, 39.97 and 38.40 cm. respectively. However, plants without treatment T<sub>0</sub> showed the lowest plant height (28.20 cm.) (Table 6).

At 120 DAT in Bijlee plus, T<sub>4</sub> showed highest plant height (79.77 cm.) and the second highest plant height T<sub>5</sub> showed (74.13 cm.) followed by the treatments T<sub>3</sub>, T<sub>6</sub>, T<sub>2</sub> and T<sub>1</sub> showed plant height 65.17, 61.03, 55.80 and 48.90cm. respectively. However, plants without treatment T<sub>0</sub> showed the lowest plant height (37.60 cm.) (Table 6).

**Table 6.** Efficacy of formulated bio-fungicide on plant height (cm) of Bijlee plus at different DAT.

Variety	Treatments	Plant height (cm)			
		30 DAT	60 DAT	90 DAT	120 DAT
Bijlee plus	T <sub>0</sub>	12.00±0.20c	24.53±0.80d	28.20±1.01e	37.60±0.70g
	T <sub>1</sub>	14.16±0.77a b	40.88±1.62b	38.40±0.87d	48.90±1.10d
	T <sub>2</sub>	13.71± 0.87bc	28.03± 0.81c	51.50±1.08c	55.80±0.85e
	T <sub>3</sub>	13.27±0.80b c	41.98±0.28b	50.43±1.91c	65.17±0.64c
	T <sub>4</sub>	14.77±0.71a	45.03±1.55a	61.83±1.29a	79.77±1.11a
	T <sub>5</sub>	15.17±1.35a	44.07±1.11a	58.47± 1.32b	74.13±0.42b
	T <sub>6</sub>	12.96±0.83a b	24.57±0.60d	39.97±1.46d	61.03±0.72f
	LSD (p ≤0.05)	1.47	2.00	2.53	1.60

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: Vulcan 50WP (Carbendazim).

#### 4.6.2 Efficacy of formulated bio-fungicide on plant height (cm) of Roshni at different DAT

At 30 DAT in Roshni, T<sub>1</sub> showed highest plant height (14.10 cm.) followed by the application of treatments T<sub>4</sub>, T<sub>3</sub>, T<sub>5</sub>, T<sub>2</sub> and T<sub>6</sub> showed the plant height 14.33, 13.63, 13.46, 13.26 and 13.15 cm. respectively. However, plants without treatment T<sub>0</sub> showed the lowest plant height (12.57 cm.) (Table 7).

At 60 DAT in Roshni, T<sub>4</sub> showed highest plant height (43.50 cm.) followed by the application of treatments T<sub>5</sub>, T<sub>2</sub>, T<sub>1</sub>, T<sub>3</sub> and T<sub>6</sub> showed the plant height 43.40, 42.03, 41.13, 38.93 and 37.14 cm. respectively. However, plants without treatment T<sub>0</sub> showed the lowest plant height (24.73 cm.) (Table 7).

At 90 DAT in Roshni, T<sub>5</sub> showed highest plant height (61.90 cm.) and T<sub>4</sub> showed second highest plant height (55.43 cm.) followed by the applied treatments T<sub>1</sub>, T<sub>3</sub>, T<sub>2</sub>, and T<sub>6</sub> showed plant height 54.50, 49.57, 47.77, and 44.57cm. respectively. However, plants without treatment T<sub>0</sub> showed the lowest plant height (33.93 cm.) (Table 7).

At 120 DAT in Roshni, T<sub>4</sub> showed highest plant height (80.13 cm.) and T<sub>5</sub> showed the second highest plant height (73.90 cm.) followed the treatment T<sub>3</sub>, T<sub>2</sub>, T<sub>6</sub> and T<sub>1</sub> showed plant height 63.57, 60.57, 56.70 and 50.07 cm. Respectively. However, plants without treatment T<sub>0</sub> (control) showed the lowest plant height (38.40 cm.) (Table 7).

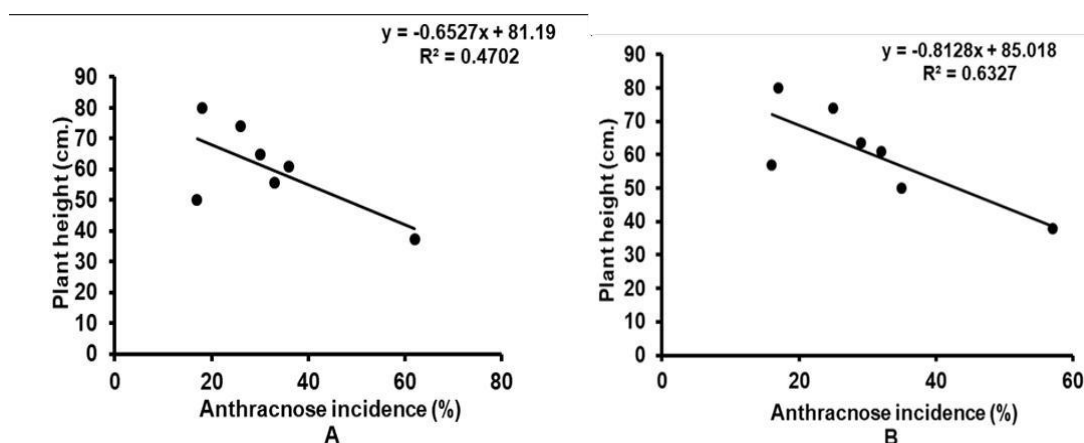
**Table 7.** Efficacy of formulated bio-fungicide on plant height (cm) of Roshni at different DAT.

Variety	Treatments	Plant height (cm)			
		30 DAT	60 DAT	90 DAT	120 DAT
Roshni	T <sub>0</sub>	12.57±1.16d	24.73±0.38d	33.93±1.59e	38.40±0.60g
	T <sub>1</sub>	14.33±0.76 a	41.13±1.33b	54.50±0.82b	50.07±0.40f
	T <sub>2</sub>	13.26±0.66bcd	42.03±1.10ab	47.77±0.35c	60.57±0.95d
	T <sub>3</sub>	13.63±0.55abc	38.93±1.38c	49.57±0.59c	63.57±1.05c
	T <sub>4</sub>	14.10±0.46ab	43.50±0.89a	55.43±1.21b	80.13±0.55a
	T <sub>5</sub>	13.46±0.29abc	43.40±0.80a	61.90±1.40a	73.90±0.30b
	T <sub>6</sub>	13.15±0.80cd	37.14±1.03c	44.57±0.96d	56.70±0.90e
	LSD (p≤0.05)	0.99	1.92	1.92	1.17

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: Vulcan 50WP (Carbendazim).

### 4.6.3 Relation between Anthracnose incidence (%) and plant height (cm) of chilli at 120 DAT

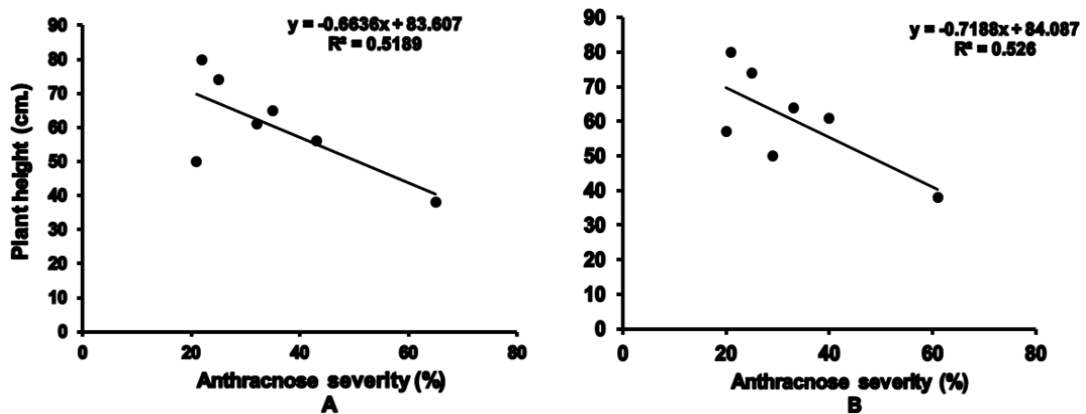
In the regression equation, disease incidence (%) was considered as independent and plant height (cm) as dependent variable. A strong negative relation was existing between the disease incidence and plant height. Due to anthracnose incidence maximum plant height (cm.) loss occurred in A. Bijlee plus (81.19%) and in B. Roshni (85.01%). The regression equation also showed that, for each unit increase in disease incidence (%), a plant height reduction of Bijlee plus (0.47 %) and Roshni (0.63%) will be occurred (Fig. 3).



**Fig. 3** Relation between Anthracnose incidence (%) and plant height (cm) of chilli at 120 DAT

### 4.6.4 Relation between Anthracnose severity (%) and plant height (cm) of chilli at 120 DAT

In the regression equation, disease severity (%) was considered as independent and plant height (cm) as dependent variable. A strong negative relation was existing between the disease incidence and plant height. Due to anthracnose severity maximum plant height (cm.) loss occurred in A. Bijlee plus (83.60%) and in B. Roshni (84.09%). The regression equation also showed that, for each unit increase in disease incidence (%), a plant height reduction of Bijlee plus (0.52 %) and Roshni (0.53%) will be occurred (Fig.4).



**Fig. 4** Relation between Anthracnose severity (%) and plant height (cm) of chilli at 120 DAT

#### **4.6.5 Efficacy of formulated bio-fungicide on number of branches/plant of chilli at 120 DAT.**

In Bijlee plus, T<sub>4</sub> showed highest number of branches per plant (31) and second highest number of branches per plant showed by T<sub>2</sub> (30). Followed by the treatments T<sub>5</sub>, T<sub>3</sub>, T<sub>1</sub> and T<sub>6</sub> showed the number of branches per plant (29, 27, 25, 24) respectively. However, the lowest number of branches showed by T<sub>0</sub> (20) (Table 8).

In Roshni, T<sub>4</sub> showed highest number of branches per plant (33) and second highest number of branches per plant was showed by T<sub>5</sub> (31) followed by the treatment T<sub>2</sub> (27). T<sub>1</sub> and T<sub>6</sub> showed the statistically similar number of branches per plant (26) respectively. However, the similar lowest number of branches was showed by T<sub>3</sub> and T<sub>0</sub> (control) (23) respectively (Table 8).

**Table 8.** Efficacy of formulated bio-fungicide on number of branch/plant of chilli at 120 DAT.

Treatments	Number of branch/plant	
	Bijlee plus	Roshni
T <sub>0</sub>	20± 1.53d	20± 1.00e
T <sub>1</sub>	25± 0.58c	26± 1.15c
T <sub>2</sub>	31±0.58b	27± 1.53c
T <sub>3</sub>	25± 2.00c	23± 1.73d
T <sub>4</sub>	39± 1.15a	41± 1.73a
T <sub>5</sub>	30± 1.00b	32± 1.15b
T <sub>6</sub>	20± 1.53d	25± 1.00cd
LSD (p≤0.05)	2.45	2.52

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: Vulcan 50WP (Carbendazim).

#### 4.6.6 Efficacy of formulated bio-fungicide on number of flowers of chilli at different DAT

At 60 DAT in Bijlee plus, T<sub>4</sub> showed highest number of flowers per plant (14) followed by the treatments T<sub>6</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> showed number of flowers 13, 9, 7 and 7 respectively. However, treatment T<sub>5</sub> and plants without treatment T<sub>0</sub>(control) showed the lowest and similar number of flowers per plant (6) (Table 9).

AT 90 DAT in Bijlee plus, T<sub>4</sub> showed highest number of flowers per plant (50) followed by the treatments T<sub>5</sub>, T<sub>3</sub>, and T<sub>2</sub> showed the number of flowers per plant 47, 46, and 41 respectively. T<sub>6</sub> and T<sub>1</sub> showed the similar number of flowers (36). However, plants without treatment T<sub>0</sub> (control) showed the lowest number of flowers per plant (25) (Table 9).

At 120 DAT in Bijlee plus, T<sub>4</sub> showed highest number of flowers per plant (102) and T<sub>5</sub> showed the second highest flower numbers (87) followed by the treatments T<sub>6</sub>, T<sub>2</sub>, T<sub>1</sub> and T<sub>3</sub> showed number of flowers per plant 84, 80, 66 and 59 respectively. However, plants without treatment T<sub>0</sub> (control) showed the lowest number of flowers per plant (47) (Table 9).

At 60 DAT in Roshni, T<sub>4</sub> showed highest number of flowers per plant (14) followed by the treatments T<sub>1</sub> and T<sub>5</sub> showed number of flowers per plant 11, 10 T<sub>6</sub> and T<sub>2</sub> showed similar number of flowers (9) respectively. However, treatment T<sub>5</sub> and plants without treatment T<sub>0</sub> (control) showed the lowest number of flowers per plant (6 and 4) (Table 9).

At 90 DAT in Roshni, T<sub>4</sub> showed highest number of flowers per plant (53) and second highest flowers number showed T<sub>5</sub> (46). T<sub>6</sub> and T<sub>3</sub> showed similar number of flowers (41) respectively followed by the treatments T<sub>2</sub> and T<sub>1</sub> showed number of flowers per plant 39 and 25. However, plants without treatment T<sub>0</sub> showed the lowest number of flowers per plant (24) (Table 9).

At 120 DAT in Roshni, T<sub>4</sub> showed highest number of flowers per plant (103) and second highest flowers number showed T<sub>6</sub> (93). Followed by the treatments T<sub>5</sub>, T<sub>2</sub>, T<sub>1</sub> and T<sub>3</sub> showed number of flowers per plant (90, 86, 67 and 61) respectively. However, plants without treatment T<sub>0</sub> (control) showed the lowest number of flowers per plant (46) (Table 9).

**Table 9.** Efficacy of formulated bio-fungicide on number of flowers of chilli at different DAT

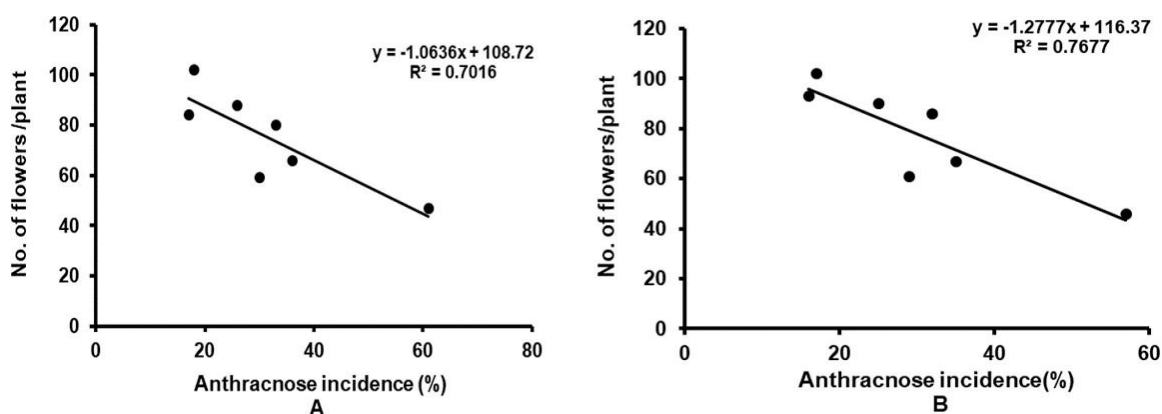
Variety	Treatments	Number of flowers/plant		
		60 DAT	90 DAT	120 DAT
Bijlee plus	T <sub>0</sub>	6.33±0.47c	25.00±1.63 e	47.00±1.41g
	T <sub>1</sub>	8.67±1.25b	36.67±1.25d	65.67±0.47e
	T <sub>2</sub>	7.33±0.94bc	41.00±0.82c	80.00±1.63d
	T <sub>3</sub>	7.00±0.82bc	46.33±1.25b	58.67±0.94f
	T <sub>4</sub>	14.00±0.82a	50.00±0.82a	102.00±0.82a
	T <sub>5</sub>	6.67±1.25c	46.67±0.47b	87.67±0.47b
	T <sub>6</sub>	13.67±0.47a	36.67±1.25d	84.00±0.82c
	LSD	1.72	2.35	1.45
Roshni	T <sub>0</sub>	4.33±0.47c	24.67±0.94e	46.33±0.94g
	T <sub>1</sub>	10.67±0.94b	25.33±0.47e	67.00±1.41e
	T <sub>2</sub>	9.00±0.82b	39.00±0.82d	85.67±1.70d
	T <sub>3</sub>	5.67±0.47c	41.00±0.82c	61.33±1.25f
	T <sub>4</sub>	14.00±1.63a	53.33±1.25a	102.67±1.70a
	T <sub>5</sub>	10.00±0.82b	46.00±0.82b	90.00±1.63c
	T <sub>6</sub>	9.00±1.63b	41.33±0.94c	93.00±0.82b
	LSD	2.08	1.82	1.58

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: Vulcan 50WP (Carbendazim).

#### 4.6.7 Relation between Anthracnose incidence (%) and number of flowers per plant of chilli at 120 DAT

In the regression equation, disease incidence (%) was considered as independent and number of flowers per plant as dependent variable. A strong negative relation was existing between the disease incidence and flower numbers. Due to anthracnose incidence, maximum number of flowers loss

occurred in A. Bijlee plus (81.19%) and in B. Roshni (85.01%). The regression equation also showed that, for each unit increase in disease incidence (%), a number of flowers reduction of Bijlee plus (0.47 %) and Roshni (0.63%) will be occurred (Fig. 5).



**Fig. 5** Relation between Anthracnose incidence (%) and number of flowers per plant of chilli at 120 DAT

#### 4.6.8 Efficacy of formulated bio-fungicide on plant shoot weight (g), root weight (g), root length (cm) of Bijlee plus at 120 DAT

In Bijlee plus, T<sub>4</sub> showed highest shoot weight (253.80 g) and T<sub>5</sub> showed the second highest shoot weight (227 g) followed by the treatments T<sub>1</sub>, T<sub>3</sub>, T<sub>2</sub> and T<sub>6</sub> showed the shoot weight 221.70, 184.10, 127.49 and 112.38 g respectively. However, T<sub>0</sub>(control) showed the lowest root weight (89.30 g) (Table 10).

In Bijlee plus, T<sub>4</sub> showed the highest root weight (28.14 cm) and T<sub>5</sub> showed the second highest root weight (24.45 g) followed by the applied treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>6</sub> showed the root weight 20.29, 20.07, 16.23 and 12.27 g respectively. However, T<sub>0</sub> (control) showed the lowest root weight (10.20 g) among all the treatments (Table 10).

In Bijlee plus, T<sub>4</sub> showed the highest root length (26.70 cm.) and T<sub>2</sub> showed the second highest root length (24.47 cm.) followed applied of treatments T<sub>1</sub>, T<sub>5</sub> and T<sub>3</sub> showed the root length 24.37, 22.07, 21.57, and 20.60

cm. respectively. However, the T<sub>0</sub> (control) showed the lowest root length (17.03 cm) among the all treatments (Table 10).

**Table 10.** Efficacy of formulated bio-fungicide on plant shoot weight (g), root weight (g), root length (cm.) of Bijlee plus at 120 DAT.

Treatments	Shoot Weight (g)	Root Weight (g)	Root Length (cm)
T <sub>0</sub>	89.30±0.86g	10.20±0.66f	17.03±0.41d
T <sub>1</sub>	221.70±1.16c	10.29±0.84f	24.37±1.07b
T <sub>2</sub>	127.49±0.73e	20.07±0.78c	24.47±1.18b
T <sub>3</sub>	184.10±1.35d	16.23±0.79d	20.60±0.50c
T <sub>4</sub>	253.80±1.31a	28.14±0.72a	26.70±0.78a
T <sub>5</sub>	227.00±1.15b	24.45±0.64b	22.07±1.07c
T <sub>6</sub>	112.38±1.82f	12.27±0.83e	21.57±1.27c
LSD (p≤0.05)	2.45	1.67	2.10

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: Vulcan 50WP (Carbendazim).



**Fig. 6** Showing plant root of Bijlee plus variety of chilli in different treatments

#### 4.6.9 Efficacy of formulated bio-fungicide on plant Shoot weight (g), Root Weight (g), Root Length (cm) of Roshni at 120 DAT

In Roshni, T<sub>5</sub> showed highest shoot weight (243.87 g) and T<sub>4</sub> showed the second highest shoot weight (232.67 g). According to the treatments T<sub>3</sub>, T<sub>2</sub>, T<sub>1</sub> and T<sub>6</sub> showed the shoot weight (221.70, 183.93, 135.17 and 123.27 g) respectively. However, T<sub>0</sub> (control) showed the lowest root weight (103.47 g) (Table 11).

In Roshni, T<sub>4</sub> showed highest root weight (36.27 g) and T<sub>5</sub> showed the second highest root length (33.30 g) followed by the applied of treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>6</sub> showed the root length 22.40, 20.17, 18.47 and 11.50 g respectively. However, the T<sub>0</sub>(control) showed the lowest root length among the all treatment (7.63 g) (Table 11).

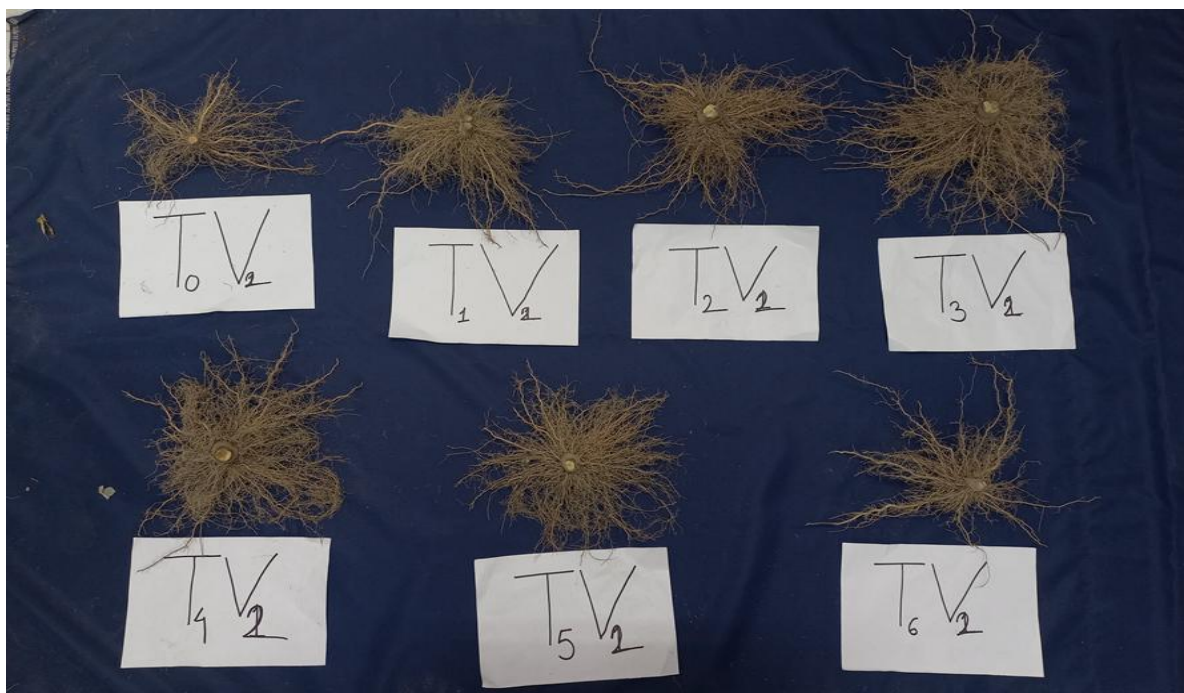
In Roshni, T<sub>4</sub> showed the highest root weight (27.93 cm) and second highest root length showed T<sub>2</sub> showed (25.13 cm) followed by the applied of treatments T<sub>1</sub>, T<sub>3</sub>, T<sub>5</sub> and T<sub>6</sub> showed the root weight 24.90, 23.37, 19.13 and 16.17 cm respectively. However, T<sub>0</sub> (control) showed the lowest root weight (11.17 cm) (Table 11).

**Table 11.** Efficacy of formulated bio-fungicide on plant shoot weight (g), root weight (g), root length (cm) of Roshni at 120 DAT

Treatments	Shoot Weight (g)	Root Weight (g)	Root Length (cm)
T <sub>0</sub>	103.47g±1.27g	7.63±0.54g	11.17±1.13f
T <sub>1</sub>	135.17±0.71e	22.40±0.54c	24.90±0.70bc
T <sub>2</sub>	183.93±1.06d	20.17±0.65d	25.13±0.56b
T <sub>3</sub>	221.70±1.71c	18.47±1.00e	23.37±0.62c
T <sub>4</sub>	232.67±1.89b	36.27±0.78a	27.93±0.82a
T <sub>5</sub>	243.87±1.11a	33.30±0.54b	19.13±0.78d
T <sub>6</sub>	123.27±1.76f	11.50±0.59f	16.17±0.83e
LSD (p≤0.05)	1.74	1.47	1.69

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12

suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: T<sub>6</sub>: Vulcan 50WP (Carbendazim).



**Fig. 7** Showing plant root of Roshni variety of chilli in different treatments

#### **4.7 Efficacy of formulated bio-fungicide on plant fruit Length (cm), fruit diameter (cm), no. of fruits/plant of Bijlee plus at 120 DAT**

In Bijlee plus, T<sub>4</sub> showed the maximum fruit length (12.83 cm). According to applied treatments T<sub>3</sub>, T<sub>1</sub>, T<sub>2</sub>, T<sub>5</sub> and T<sub>6</sub> showed the fruit length 11.67, 10.67, 10.5, 10.33 and 9.33 cm respectively. However, T<sub>0</sub> (control) showed the minimum fruit length (7.83 cm) (Table 12).

In Bijlee plus, T<sub>4</sub> showed the maximum fruit diameter (24 cm) followed by the applied treatments T<sub>3</sub>, T<sub>5</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>6</sub> showed fruit diameter 21.17,

20.67, 20.17, 19.17 and 17.67 cm. respectively. However, T<sub>0</sub>(control) showed the lowest fruit diameter (15.33 cm) (Table 12).

In Bijlee plus, T<sub>4</sub> gave the maximum number of fruits per plant (16) followed by the applied treatments T<sub>3</sub>, T<sub>2</sub>, T<sub>5</sub>, T<sub>1</sub> and T<sub>6</sub> showed the number of fruit 14, 13, 12, 10 and 9 respectively. However, T<sub>0</sub> (control) gave minimum number of fruits per plant (7) (Table 12).

**Table 12.** Efficacy of formulated bio-fungicide on plant fruit length (cm), fruit diameter (cm), no. of fruits/plant of Bijlee plus at 120 DAT.

Treatment	Fruit Length (cm)	Fruit Diameter (cm)	No. of Fruits/Plant
T <sub>0</sub>	8.33±1.01e	3.37± 0.31c	21.67±0.94e
T <sub>1</sub>	11.33±1.13ab	4.20±0.29b	61± 0.82b
T <sub>2</sub>	11.57±0.68ab	4.47 ±0.21b	51.67±0.47d
T <sub>3</sub>	10.47±0.61bc	4.23±0.2b1	50.33±1.25d
T <sub>4</sub>	13.17±1.26a	5.43±0.42a	66.67±1.25a
T <sub>5</sub>	12.20±0.73ab	4.17±0.21b	58.33±0.94c
T <sub>6</sub>	9.10±0.88cd	3.90±0.16bc	61.33±1.25b
LSD (p≤0.05)	2.00	0.63	2.39

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: Vulcan 50WP (Carbendazim).

#### 4.7.1 Efficacy of formulated bio-fungicide on plant fruit length (cm), fruit diameter (cm), no. of fruits/plant of Roshni at 120 DAT

In Roshni, T<sub>4</sub> showed maximum fruit length (33 cm.) followed treatments application T<sub>3</sub>, T<sub>5</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>6</sub> showed the fruit length 27.33, 25.67, 23.67, 23.33 and 20.83 cm. respectively. However, T<sub>0</sub> (control) showed minimum number of fruit length (18.67 cm) (Table 13).

In Roshni, T<sub>4</sub> showed maximum fruit diameter (11.67 c) followed by the applied treatment of T<sub>3</sub> Showed 10.17 cm of fruit diameter. T<sub>1</sub>, T<sub>5</sub> gave statistically similar number of fruit diameter (9.17 cm). According to application of treatments T<sub>2</sub> and T<sub>6</sub> showed fruit diameter 8.67 and 8.17 cm. respectively. However, T<sub>0</sub>(control) showed the lowest fruit diameter (6.33 cm) (Table 13).

In Roshni, T<sub>4</sub> showed the maximum number of fruits per plant (21) followed by the applied treatments T<sub>5</sub>, T<sub>3</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>6</sub> showed the number of fruits per plant 20, 18, 17, 16 and 16 respectively. However, T<sub>0</sub> (control) showed minimum number of fruits per plant (12) (Table 13).

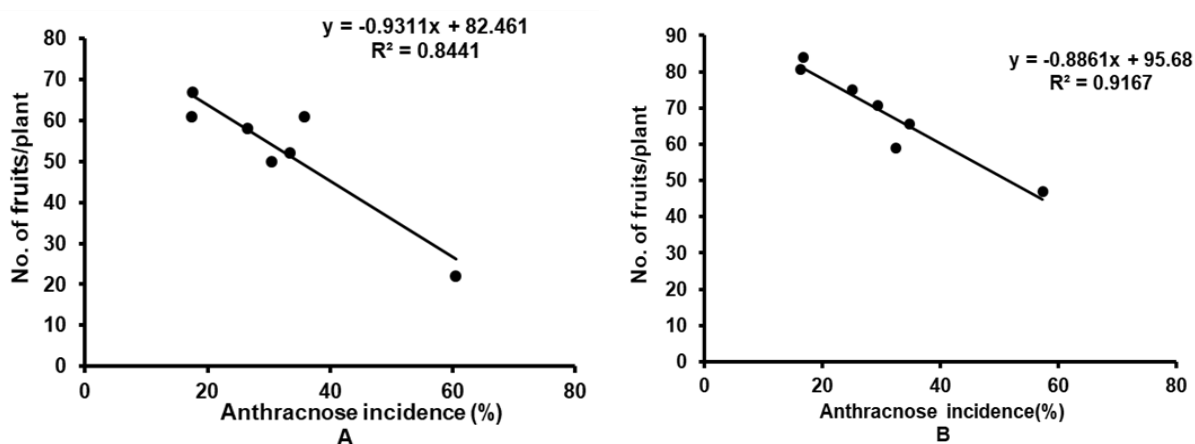
**Table 13.** Efficacy of formulated bio-fungicide on plant fruit weight/ fruit (g), fruit length (cm), fruit diameter (cm), no. of fruits/plant of Roshni at 120 DAT.

Treatments	Fruit Length (cm)	Fruit Diameter (cm)	No. of Fruits/Plant
T <sub>0</sub>	8.77±0.40d	3.30±0.24e	46.67±0.47g
T <sub>1</sub>	11.43±0.50b	4.77±0.21ab	65.67±0.47e
T <sub>2</sub>	11.13±1.18bc	4.03±0.12d	59±0.82f
T <sub>3</sub>	11.40±0.54b	4.55±0.19bc	70.67±1.70d
T <sub>4</sub>	14.13±0.69a	5.20±0.16a	84±1.63a
T <sub>5</sub>	11.97±0.86b	4.47±0.37bcd	75± 0.82c
T <sub>6</sub>	9.47±0.77cd	4.17±0.21cd	80.67±1.25b
LSD (p≤0.05)	1.68	0.50	2.39

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: Vulcan 50WP (Carbendazim).

#### 4.7.2 Relation between anthracnose incidence (%) and number of fruits per plant of chilli at 120 DAT

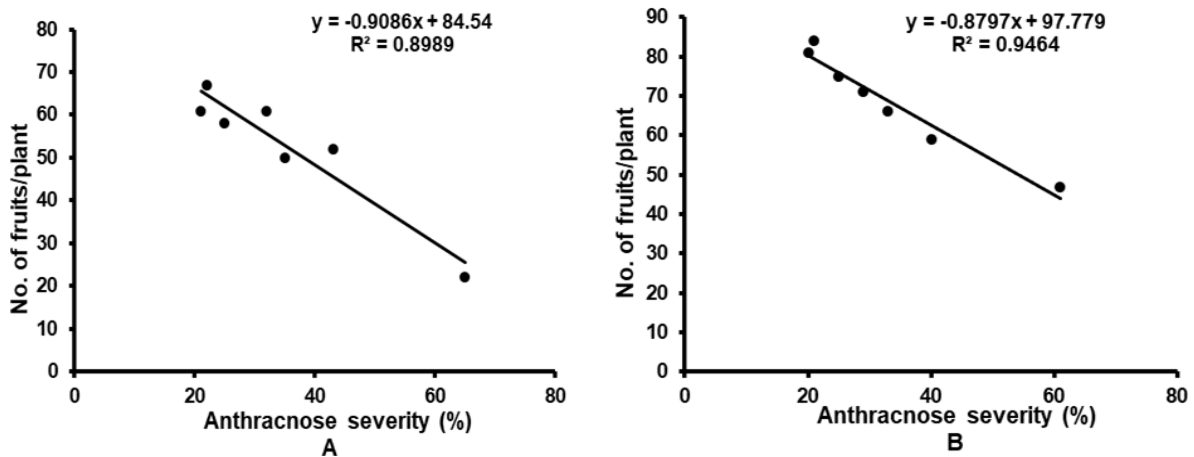
In the regression equation, disease incidence (%) was considered as independent and number of fruits per plant as dependent variable. A strong negative relation was existing between the disease incidence and fruit numbers. Due to anthracnose incidence maximum number of fruits loss occurred in A. Bijlee plus (82.46%) and in B. Roshni (95.68%). The regression equation also showed that, for each unit increase in disease incidence (%), a number of fruits reduction of Bijlee plus (0.84%) and Roshni (0.92%) will be occurred (Fig. 8)



**Fig 8.** Relation between Anthracnose incidence (%) and number of fruits per plant of chilli at 120 DAT

#### **4.7.3 Relation between anthracnose severity (%) and number of fruits per plant of chilli at 120 DAT**

In the regression equation, disease severity (%) was considered as independent and number of fruits per plant as dependent variable. A strong negative relation was existing between the disease severity and fruit numbers. Due to anthracnose severity maximum number of fruits loss occurred in A. Bijlee plus (82.46%) and in B. Roshni (95.68%). The regression equation also showed that, for each unit increase in disease severity (%), a number of fruits reduction of Bijlee plus( 0.84%) and Roshni (0.92%) will be occurred (Fig. 9)



**Fig. 9** Relation between Anthracnose severity (%) and number of fruits per plant of chilli at 120 DAT

#### 4.7.4 Efficacy of formulated bio-fungicide on yield (kg/plant) of chilli

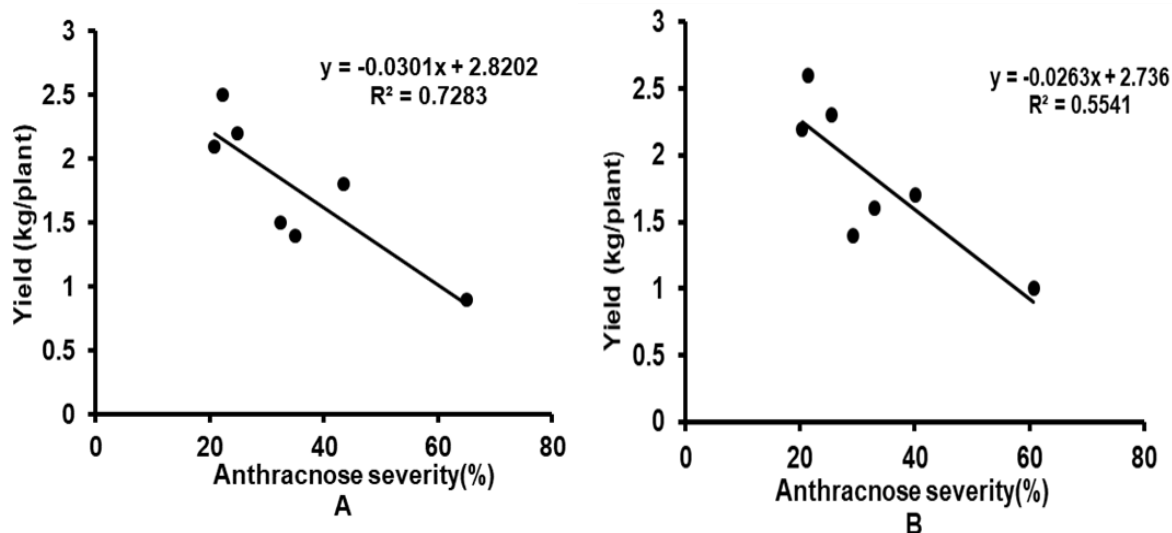
In Bijlee plus, T<sub>4</sub> showed the maximum yield per plant (2.5 kg/plant) followed by the applied treatments T<sub>5</sub>, T<sub>6</sub>, T<sub>2</sub>, T<sub>1</sub> and T<sub>3</sub> 2.2, 2.1, 1.8, 1.5 and kg/plant respectively. However, T<sub>0</sub> (control) showed lowest yield per plant (0.9 kg/plant).

In Roshni, T<sub>4</sub> showed the maximum yield per plant (2.6 kg/plant) followed by the applied treatments T<sub>5</sub>, T<sub>6</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>1</sub> yield per plant 2.3, 2.2, 1.7, 1.6 and 1.4kg/plant respectively. However, T<sub>0</sub> (control) showed lowest yield per plant (1 kg/plant).

#### 4.7.5 Relation between disease severity (%) and yield (kg/plant) at 120 DAT of chilli as an effect by different treatments.

In the regression, disease severity (%) was considered as independent and yield kg/plant as dependent variable. A strong negative relation was existing between the disease severity and yield kg/plant. Due to anthracnose disease, in case of Bijlee plus and Roshni 2.82% and 2.74 % loss in yield kg /plant was observed. The regression equation also showed that, for each unit

increase in disease severity, yield kg/plant reduction of (0.73 %) and(0.55 %) will be occurred (Fig. 10).



**Fig. 10** Relation between disease severity (%) and yield (kg/plant) at 120 DAT of chilli

#### **4.7.6 Efficacy of formulated bio-fungicide on total phenol content (mg/100g) in fruit, total soluble solids (°Brix.) content, ascorbic acid content of Bijli plus at 120 DAT**

In Bijlee plus, T<sub>4</sub> showed highest amount of phenol content in fruit (4.65 mg/100gm.) followed by the applied treatments T<sub>1</sub>, T<sub>5</sub>, T<sub>3</sub>, T<sub>6</sub>, and T<sub>2</sub> 3.91 3.84, 3.82, 3.52 and 5.65 mg/100gm. respectively. However, T<sub>0</sub> (control) showed minimum amount of phenol content in fruit (2.51 mg/100gm.) (Table 14).

In Bijlee plus, T<sub>4</sub> showed maximum percentage of TSS in fruit (12.63 %). Followed by the treatment application T<sub>6</sub>, T<sub>5</sub>, T<sub>4</sub>, T<sub>1</sub> and T<sub>2</sub> showed percentage of TSS in fruit (11.57, 11.02, 10.17, 8.17 and 7.10 %) respectively. However, T<sub>0</sub> showed lowest percentage of TSS in fruit (6.57 %) (Table 14).

In Bijlee plus, T<sub>4</sub> showed highest amount of ascorbic acid (11.82 mg/g) followed by the applied treatments T<sub>5</sub>, T<sub>4</sub>, T<sub>6</sub>, T<sub>1</sub> and T<sub>2</sub> showed the amount

9.26, 8.10, 7.94, 7.51 and 6.42 mg/g respectively. However, T<sub>0</sub> (control) showed lowest amount (5.32 mg/g) (Table 14).

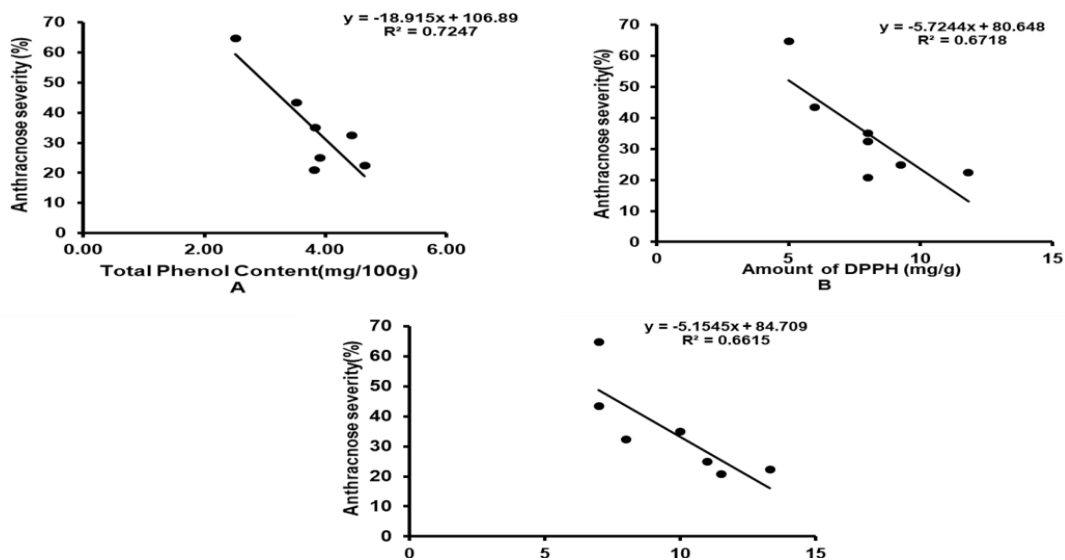
**Table 14.** Efficacy of formulated bio-fungicide on total phenol content (mg/100g) in fruit, total soluble solids (°Brix.) content, ascorbic acid content, chlorophyll content of Bijlee plus at 120 DAT

Treatments	Phenol(mg/100g)	TSS (%°Brix.)	DPPH(mg/g)
T <sub>0</sub>	2.51±0.47d	6.57± 0.62c	5.32±0.91e
T <sub>1</sub>	4.43±0.37ab	8.17±0.65c	7.51±0.64cd
T <sub>2</sub>	3.52±0.18c	7.10±0.54c	6.42±0.75de
T <sub>3</sub>	3.84±0.40bc	10.17±1.39b	8.10±0.28bc
T <sub>4</sub>	4.65±0.33a	12.63±0.61a	11.82± 0.51a
T <sub>5</sub>	3.91±0.36bc	11.02±0.77ab	9.26±0.46b
T <sub>6</sub>	3.82±0.24bc	11.57± 0.45ab	7.94±0.86bc
LSD (p≤0.05)	0.71	1.67	1.41

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: Vulcan 50WP (Carbendazim).

#### 4.7.7 Relation between disease severity (%) and total phenol content (mg/100g), TSS (°Brix.), DPPH (kg/min) of Bijlee plus at 120 DAT

In the regression, total phenol content (mg/100mg), total soluble solid (°Brix.) and ascorbic acid content (mg/g) was considered as independent and anthracnose severity (%) as dependent variable. A strong negative relation was existing between the disease severity and total phenol content, ascorbic acid content, total soluble solid (°Brix.). Due to disease, (106.89 %) phenol, (80.65 %) ascorbic acid and (84.71 %) total soluble solids loss was observed in Bijlee plus variety. The regression equation also showed that, for each unit increase in disease severity, reduction of phenol (0.73 %), ascorbic acid (0.67%) and total soluble solids (0.66%) will be occurred (Fig. 11)



**Fig. 11** Relation between disease severity (%) and total phenol content (mg/100g), TSS (°Brix.), DPPH (mg/g) of Bijlee plus at 120 DAT

#### 4.7.8 Efficacy of formulated bio-fungicide on total phenol content (mg/100g) in fruit, total soluble solids (°Brix.), ascorbic acid content of Roshni at 120 DAT

In Roshni, T<sub>4</sub> showed highest amount of phenol content in fruit (4.78 mg/100g) followed by the applied treatments T<sub>5</sub>, T<sub>6</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>1</sub> 4.48, 4.21, 3.94, 3.90 and 3.76 mg/100g respectively. However, T<sub>0</sub> (control) showed minimum amount of phenol content in fruit (3.40 mg/100g) (Table 15).

In Roshni, T<sub>4</sub> showed maximum percentage of TSS in fruit (14.43 %) followed by the applied treatments T<sub>5</sub>, T<sub>6</sub>, T<sub>2</sub>, T<sub>4</sub> and T<sub>1</sub> 13.13, 11.80, 11.40, 9.17 and 8.90 % respectively. However, T<sub>0</sub> (control) showed lowest percentage of TSS in fruit (7.93 %) (Table 15).

In Roshni, T<sub>4</sub> showed highest amount of ascorbic acid (10 mg/g) followed by the applied treatments T<sub>5</sub>, T<sub>1</sub>, T<sub>6</sub>, T<sub>3</sub> and T<sub>2</sub> 8.59, 8.15, 7.67, 7.19, and 6.87 mg/g respectively. However, T<sub>0</sub> (control) showed lowest amount (5.34 mg/g) (Table 15)

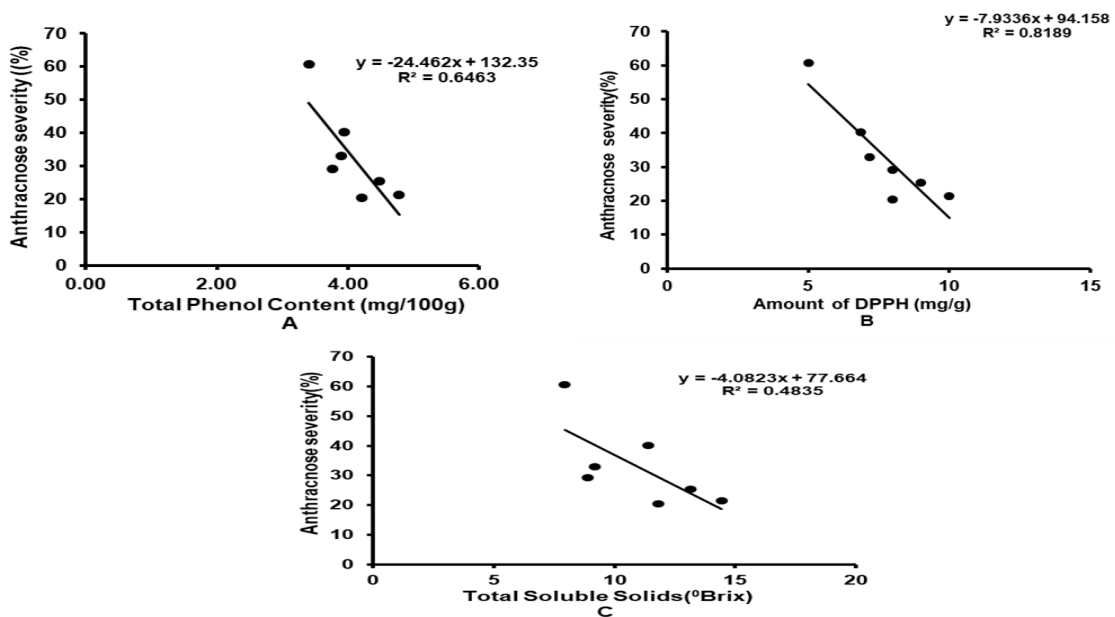
**Table 15.** Efficacy of formulated bio-fungicide on total phenol content (mg/100g) in fruit, total soluble solids (°Brix), ascorbic acid content, chlorophyll content of Roshni at 120 DAT.

Treatments	Phenol(mg/100g)	TSS (°Brix)	DPPH(mg/g)
T <sub>0</sub>	3.40±0.20d	7.93±0.96d	5.34±0.29d
T <sub>1</sub>	3.76±0.11cd	8.90±0.73d	8.15±0.66bc
T <sub>2</sub>	3.94±0.47c	11.40±0.45c	6.87±1.07cd
T <sub>3</sub>	3.90±0.17cd	9.17±0.92d	7.19±0.77bc
T <sub>4</sub>	4.78±0.09a	14.43±0.82a	10±0.25a
T <sub>5</sub>	4.48±0.20ab	13.13±0.58ab	8.39±1.11ab
T <sub>6</sub>	4.21±0.13bc	11.80±0.41bc	7.67±0.74bc
LSD (p≤0.05)	0.52	1.62	1.64

Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p=0.05. T<sub>0</sub>: Control; T<sub>1</sub>: Maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>2</sub>: Pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>3</sub>: Soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension; T<sub>4</sub>: Charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>5</sub>: Wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension ; T<sub>6</sub>: Vulcan 50WP (Carbendazim).

#### 4.7.9 Relation between disease severity (%) and total phenol content (mg/100g), TSS (°Brix.), DPPH (mg/g) of Bijlee plus at 120 DAT

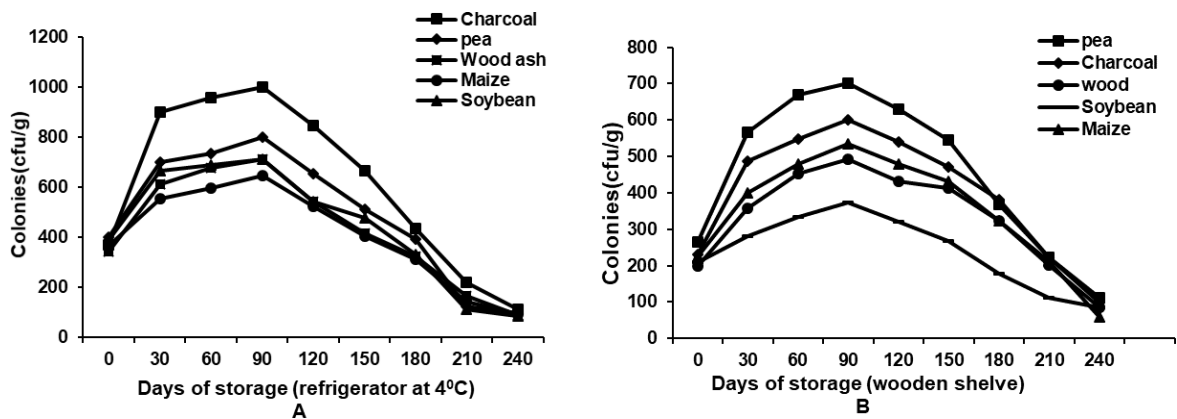
In the regression, total phenol content (mg/100g), Total Soluble Solid (°Brix.) and ascorbic acid content (mg/g) was considered as independent and anthracnose severity (%) as dependent variable. A strong negative relation was existing between the disease severity and total phenol content, ascorbic acid content, Total Soluble Solid (°Brix.). Due to disease, (132.35 %) phenol, (94.16 %) ascorbic acid and (77.66 %) total soluble solids loss was observed in Bijlee plus variety. The regression equation also showed that, for each unit increase in disease severity, reduction of phenol (0.65 %), ascorbic acid (0.82%) and total soluble solids (0.48%) will be occurred (Fig.12)



**Fig. 12** Relation between disease severity (%) and total phenol content (mg/100g), TSS (°Brix.), DPPH (mg/g) of Roshni at 120 DAT

#### 4.8 Shelf life of formulated bio-fungicide in different storage condition

The shelf life of charcoal-based, maize bran-based, wood dust-based, soybean bran-based and pea bran-based formulated native *E. cloacae* HSTUB 12 were evaluated in different storage conditions A. Refrigerator condition (4°C) C) B. Wooden shelve condition. In case of refrigerator storage condition, the bacterial colony density of all formulated products named charcoal, pea-bran, wood-dust, maize-bran and soybean-bran found to increase from its initial populations and reached at peak at 240 DAI which ranges (400-999, 369-800, 345-714, 367-645, 389-712) respectively. However, all the formulated products declines after reaching the peak at 240 DAI. Among all of the formulated products Charcoal-based formulated *E. cloacae* HSTUB 12 showed highest bacterial colony and maize-bran based formulated *E. cloacae* HSTUB 12 showed lowest bacterial colony (Fig. 13 A).



**Fig. 13** Shelf life of formulated products followed by *E. cloacae* in different stage condition:  
**A.** Refrigerator condition (4°C) **B.** Wooden shelf condition

In case of wooden shelf storage condition, the bacterial colony density of all formulated products named pea-bran, charcoal, wood-dust, soybean-bran and maize-bran found to increase from its initial populations and reached at peak at 240 DAI which ranges (265-700, 230-600, 200-491, 210-373, 225-535) respectively. However, all the formulated products declines after reaching the peak at 240 DAI. Among all of the formulated products pea-bran based formulated *E. cloacae* HSTUB 12 showed higher bacterial colony and maize-bran based formulated *E. cloacae* HSTUB 12 showed lowest bacterial colony (Fig. 13 B).

## CHAPTER V

### DISCUSSION

Chilli is a significant vegetable and spice crop grown in tropical and subtropical regions of the world which belongs to the Solanaceae family. *Colletotrichum* spp. caused anthracnose disease is one of the most serious and significant reasons for yield losses in chili peppers. Fungicide application is a typical part of field operations to combat anthracnose disease. Intensive and unwise use of chemicals may leave toxic effects in the environment, and the breeding resistant genes ultimately leading to a risk for public health, natural waters and non-target organisms (Gikas *et al.* 2022). In this concern, the use of plant-associated microorganisms as biological control agents of plant diseases may be an efficient and eco-friendly method (Xue *et al.* 2009). By managing plant diseases and improving plant growth, beneficial microorganisms may offer a sustainable agriculture. There are many evidences where the beneficial bacteria show better performances against plant diseases on several crops. In the field conditions, all the formulations using *E. cloacae* HSTUB 12 showed remarkable decline on anthracnose incidence and severity (%). Among them, charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension showed significant result through increasing all agronomic attributes *viz* height, branches, flowers, fruits, root weight, shoot weight, individual fruit weight, total yield including bio chemical parameter like total phenol content, ascorbic acid content, total soluble solid content in fruit. According to Hermosa *et al.* (2012), it was established that these microbes might promote plant development by controlling the plant hormone ethylene (ET) and inducing resistance in plants by producing salicylic acid (SA) and jasmonic acid (JA). Phenolic compounds are the secondary metabolites that have the capacity to donate hydrogen, quench singlet oxygen, and function as

metal chelators, all of which can make them antioxidants (Michalak A. 2006). Because these chemicals are harmful to pathogens, the build up of phenolic compounds in chili plants at the site of anthracnose disease infection was linked to the inhibition of pathogen development (Jayapala *et al.* 2019). The higher the phenol content in the plant, the more resistant to attack by pathogens, which is indicated by the decrease of disease intensity. Changes in the pH of the cytoplasm of plant cells can also boost resistance because they contain more phenolic acid, which prevents the growth of pathogens (Bereika 2008; Hassan and Abo-Elyousr 2013). Consuming foods and drinks which is rich in phenolics has been shown to protect against a number of diseases, including diabetes, cancer, heart disease, inflammation, arthritis, immune system-related illnesses, and neurodegenerative diseases (Chalise JP *et al.*, 2010). *E. cloacae* PS14 showed the highest level of peroxidase activity and it causes lignin biosynthesis that provides a physical barrier and/or limits the degree of pathogen invasion and spread throughout the plant (Vidhyasekaran 2008). *E. cloacae* PS14 significantly increased the activity of lipoxinase by preventing pathogen growth, phytoalexin accumulation, and signal transmission, LOX products support the defence response in plants-pathogen interactions (Mariutto *et al.*, 2011). It is important to emphasize that pathogenic fungus have the ability to boost host metabolic activity, such as the amount of photosynthetic pigments (Scholes and Farrar 1986; Roberts and Walters 1988). The K2 strain of *E. cloacae* is a good generator of HCN (Pramanik *et al.*, 2018). One of the known ways that antioxidants prevent lipid oxidation by the process of free-radical scavenging (Dvorakova M. *et al.*, 2008)

According to the results, managing chili anthracnose over control may be achieved more effectively by applying bio-control agents *E. cloacae* HSTUB 12 with charcoal-based formulation. Similar studies are also needed in the field experiment in order to utilize them efficiently as bio fertilizers and bio fungicides

## CHAPTER VI

### SUMMARY AND CONCLUSION

Because of its widespread usage throughout the world, chilli is a very significant spice cum vegetable. But this is severely affected by anthracnose disease which causes a huge economic loss. In this phenomena, use of chemicals is a widely used disease control strategy and a practical method to control anthracnose disease. But there are numerous undesirable effects of using chemicals such as economic loss, health hazardous for animals and other environmental concerns. To overcome the negative effect of chemical usage, use of formulated biocontrol agents to control infection have become a solution. The efficiency of *E. cloacae* HSTUB 12 against anthracnose of chilli as well as its shelf life as formulated products in different storage conditions was evaluated. Formulated *E. cloacae* HSTUB 12 were used in the field level for the management of anthracnose of chilli caused by *Colletotrichum capsici*. For the management of anthracnose of chilli, *E. cloacae* HSTUB 12 were formulated with charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension, maize bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension, wood dust-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension, pea bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension and soybean bran-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension. Among all the formulated products in the field, charcoal-based formulated *E. cloacae* HSTUB 12 followed by spraying of *E. cloacae* HSTUB 12 suspension showed the maximum reduction of anthracnose incidence (%) and severity (%) in both chilli varieties; maximum plant height; maximum shoot weight; higher root length; higher root weight also showed in

same formulated bio-fungicide; maximum number of branch/plant; highest number of fruit per plant; highest fruit length; maximum fruit diameter maximum yield; higher phenol content; higher total soluble solids; higher ascorbic acid content, higher chlorophyll content was demonstrated by the application of formulated *E. cloacae* HSTUB 12 in both variety named Bijlee plus and Roshni. The formulated bio-fungicide were packaged @ 50g/polyethylene bag and stored in different storage conditions viz., A: refrigerator (4<sup>0</sup> C), B: Wooden shelve. The initial populations of charcoal-based *E. cloacae* HSTUB 12 was recorded highest density and formulated soybean-based bio-fungicide gave lowest density of bacterial colony even after 90 days at Refrigerator (4<sup>0</sup>C) condition and counted up to 240 days after incubation (DAI) at 30-days intervals using serial dilution plate technique. Result revealed that wooden shelve at room temperature gave highest cfu at 60 DAI and the lowest viable bacterial colony were recorded in formulated maize-based at 240 DAI. The overall findings of the study demonstrated the potentiality of *E. cloacae* HSTUB 12 for the environment friendly control of anthracnose of chilli. Considering the eco-friendly management, the formulated *E. cloacae* HSTUB 12 as bio-fungicide could also be used in the farmer's field for the sustainable management of anthracnose of chilli and other vegetables.

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