FORMULATION OF *Bacillus cereus* HSTUB 17 FOR THE MANAGEMENT OF FUSARIUM WILT OF CHILLI

A THESIS

BY

SHARMIN AFROZ SHIMU

Student No: 1701243

Session: 2022-2023

MASTER OF SCIENCE (MS)

IN

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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DEDICATED TO MY BELOVED PARENTS

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The Author

ABSTRACT

Fusarium wilt of chilli caused by *Fusarium oxysporum* is a devastating disease responsible for huge yield loss in Bangladesh. The present study was aim to formulate a bio-fungicide composed with *Bacillus cereus* HSTUB 17 for the management of Fusarium wilt of chilli in field conditions as an alternative means of chemical control. Several substrate *viz*. charcoal, maize bran, wood dust, soybean bran, and pea bran were employed for the formulation. The prepared bio-fungicides were packaged and kept in two distinct storage environments: (A) a refrigerator (4^0C); (B) a wooden shelve (at room temperature). The initial populations of *B. cereus* HSTUB 17 with formulated bio-fungicides were counted up to 240 days after incubation at 30-day intervals. The greatest density of spores was reported even 90 days after storage in wood dust-based bio-fungicide. Among all the formulated bio-fungicides, wood dust-based bio-fungicide resulted higher reduction of Fusarium wilt incidence (%) and severity (%) (61.36% and 69.01%) (52.01% and 75.21%) in both Roshni and Bijlee plus, respectively. The same bio-fungicide also demonstrated maximum plant height (103.5 and 101.86 cm), highest shoot weight (420 g and 224 g), highest root length (29 and 28 cm), highest root weight (42.82 and 33 g), maximum number of branches/plant (180.90 and 229.29), highest number of fruit per plant (115.90 and 115.13), highest yield per plot (2.67 and 2.69 kg/plot), higher accumulation of phenol content (4.56 and 4.81 mg/100g), higher content of total soluble solids (13.77 and 13.23 °Brix), higher amount of antioxidant (9.15 and 8.13 mg/g), and higher amount of chlorophyll content (0.15 and 0.14 mg/mL) both Roshni and Bijlee plus, respectively. The findings of the study revealed the potentiality of the formulated wood dust-based bio-fungicide for the possible to management of Fusarium wilt of chili effectively.

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CHAPTER I

INTRODUCTION

A member of the Solanaceae plant family, chilli *(Capsicum annuum L.)* is one of the most significant vegetables and is farmed primarily for its pungency, color, and spicy flavor. Vitamins A and C (Rahman *et al*., 2011) are also abundant in chilli, which are utilized in drinks, cosmetics, and pharmaceuticals. It is an excellent source of vitamins including A, D, E, C, K, P, B2 and B12, (Ghoraba *et al*., 2013) as well as proteins, lipids, carbs, fiber, mineral salts (Ca, P, Fe), and a variety of dietary lipids. Ascorbic acid, carotenoids, tocopherols, flavonoids, and capsaicinoids, which are crucial in preventing chronic diseases like cancer, asthma, coughing up blood, sore throats, toothaches, diabetes, and cardiovascular diseases, are among the health-related phytochemical compounds that are abundant in chilli (Ghoraba *et al*., 2013). As a significant crop for both vegetables and spices, chillis are grown all throughout the world. It is offered and utilized in green, dried, and powdered forms. It serves as key component in Bangladeshi cuisine.

In Bangladesh, chilli is grown in two different crop seasons. In Kharif, 50671.78 acres produced 99126.30 MT (an approximate average yield of 1.96 MT per acre), while in Rabi, 196768.37 acres produced 393555.62 MT (an approximate average yield of 2 MT per acre) (BBS, 2022). According to the Bangladesh Bureau of Statistics, the country produced 0.624 millions tones of green chilli in 2022, with 0.496 million tones in winter and only 0.128 million tones in summer (BBS, 2022). The lack of high producing cultivars and their traditional cultivating methods with sparse irrigation facilities are the main causes of its poor acreage and yield, while there are other contributing aspects as well in compare to other countries. Modern high producing cultivars and the use of improved production technologies can boost the productivity of chilli. Therefore, in order to enhance the level of national production, it is essential to produce high yielding varieties with proper care of chillis. (Mehedi *et al.,* 2021).

Production of chillis has decreased significantly in recent years as a result of a number of factors, including persistent monoculture, poor cultivation practices, lack of healthy seeds, unfavorable weather, unfavorable soil conditions, pests, and various fungal, bacterial, and viral diseases (Monaim, 2012). Among all of these limitations, *Fusarium oxysporum* Schlecht. emend. Synd. and Hans. f. sp. *capsici* Riv.-caused wilt disease is one of the most damaging diseases in the world's chilli growing regions, resulting in yield losses up to 80% (Madhavi *et al.,* 2006). Fusarium wilts are frequent vascular diseases that obstruct and ultimately kill the water-conducting tissues, causing wilt symptoms and plant mortality (Gauthier *et al*., 2022).

The wilt fungus may from seedling to harvest, by primarily infecting the root system (Miller *et al.,* 1996). This prevents water and nutrient delivery and subsequently interferes with the physiological processes required for sufficient output and quality. (Morid *et al.,* 2012). Due to ineffective transport of conventional fungicides to the xylem vessels and challenges with soil treatments, there is currently no effective chemical control for Fusarium wilt of chilli. Chlamydospores are also extremely contagious and have a long survival time on plant detritus, which makes disease management extremely difficult (Cal *et al.,* 1997; Hou *et al.,* 2020)

To avoid the negative impact of chemical control, biological agents including beneficial bacteria might offer an alternative and durable management of wilt of chilli. Bacteria fight against various soil borne pathogen through a variety of mechanisms, including competition, parasitism, antibiosis, and induced resistance (Akram, *et al.,* 2016; Elanchezhiyan *et al.*, 2018; Bhattacharya *et al.*, 2019; Medeiros *et al.*, 2021; Zhang *et al.*, 2021). A number of species of *Bacillus* have emerged as promising bio-control agents to control chilli diseases (Jayalakshmi *et al.* 1998). *Bacillus* also have important applications in the agricultural field through their biological control potential against phytopathogens, particularly phytopathogenic fungi (González-Franco and Robles-Hernández, 2009). So far, the formulated bio-fungicide using *B. cereus* is very rare or non-existent in Bangladesh, therefore, our aim to formulate a bio-fungicide using our previous isolated and identified *B. cereus* HSTUB 17 for the eco-friendly and sustainable management of wilt of chilli for the first time in Bangladesh. Considering the bio-control ability of the formulated *Bacillus cereus* HSTUB 17 in controlling fusarium wilt of chilli and their shelf life, the present investigation is undertaken to achieve the following objectives:

i) To formulate a bio-fungicide using *Bacillus cereus* HSTUB 17.
ii) To observe the efficacy of the formulated bio-fungicide against fusarium wilt and agronomic attributes of chilli in field conditions.
iii) To study the shelf life of formulated bio-fungicide.

CHAPTER II

REVIEW OF LITERATURE

The study was aim to develop a formulated bio-fungicide using *Bacillus cereus* HSTUB 17 for the efficient and sustainable management of fusarium wilt of chilli in the field conditions. However, the relevant literature in regards to the efficacy in disease control and survival of the anti-fungal bacteria as a formulated bio-fungicide are collected and presented as follows:

2.1. Effect of beneficial bacteria to control fusarium wilt

Suryanto *et al.* (2010) reported that the capacity of chitinolytic bacteria to prevent Fusarium wilt in red chili (*Capsicum annuum L.*) seedlings through biocontrol. Red chili seeds were soaked in the bacterial isolate solution for 30 minutes before being planted in order to test the chitinolytic bacteria's capacity to suppress the disease. There was a noticeable decrease in seedling damping-off across all bacterial treatments, with a range of 28.57 to 60.71%. Next, out of all the chitinolytic isolates examined, BK08 showed the most potential as a biological control agent for Fusarium wilt in chili seedlings.

Bautista *et al*. (2016) reported that according to their findings, every bacterial strain exhibited a range of inhibitions in mycelial growth from 21.28 to 71.70%. Furthermore, the CBMT2 and CBMT51 strains had inhibition halos of 3.76 and 6.37 mm against *F. equiseti.* In habanero chilli seedlings, both viruses exhibited a 100% disease incidence rate. *F. solani* caused a 90% severity rating, while *F. equiseti* caused a 77.5% severity rating. We found that *B. subtilis* CBMT51 and *B. cereus* BL18 reduced the severity of disease caused by *F. equiseti* and BL18 strain for *F.*

solani in 47.7, 37.8, and 50.9% respectively at 28 days of evaluation. In resistance tests, four strains of *Bacillus* based on the antagonistic activity were used. Three inoculations were made in the base of stem 15, 28, and 35 days after germination.

Khan *et al*. (2017) examined that the developments in the study of the *Bacillus-Fusarium* interaction and concentrate on the principles and mechanisms of action among plant-growth promoting *Bacillus* species. The study's focus is on the multivariate interactions among plant-biocontrol agent-pathogen.They emphasize their use in preventing Fusarium infections and spread among economically significant crops.

Suryanto *et al*. (2018) examined that in order to less the amount of *Fusarium oxysporum*-caused chilli seedling-off, endophytic bacteria from red chili plants have been isolated and tested. A healthy red chili plant's stem, root, and leaves were used to isolate endophytic bacteria, and the sick root was used to isolate fungus. Dual culture was used to conduct antagonistic assays. Out of the five endophytic bacterial isolates, two (SDW1 and SDW2) shown greater ability to suppress *F. oxysporum* growth. It was shown that the isolates decreased chili seedling drop. Furthermore, as compared to a treatment without bacterial application, these two isolates improve the height and number of leaves on seedlings.

Yanti *et al*. (2017) reported that the goal of this study was to extract native rhizobacteria from the rhizosphere of healthy chili plants in order to produce more plant yields and effective control against the bacterial wilt disease. The rhizobacterial isolates were obtained from robust chili rhizospheres located in areas where bacterial wilt is endemic. Two rhizobacterial isolates from the rhizosphere of chili peppers (RZ.2.1.AG1 and RZ.1.3.AP1) demonstrated a strong capacity to reduce disease as well as boost the growth and yield of chili peppers.

2.2. *Bacillus cereus* against *Fusarium oxysporum*

Pelias *et al.* (2022) found that the development of plant pathogens was suppressed by volatile chemicals, with average inhibition rates of 55% against Fol and 17% against *Ascochyta*. This involves evaluating their capacity to promote the growth of their vegetative organs and the germination of chilli seeds. The treated seedlings significantly outperformed the controls, according to the data. The strains chosen for this investigation have the potential to be used as biocontrol agents, as demonstrated by all of these findings. To verify their actual potential, further thorough research is obviously required.

Ramirez *et al*. (2022) resulted that *B. cereus* MH778713 may be useful as a biocontrol agent since it assisted chilli seedlings manage Fusarium wilt through inoculation. These findings show the capacity of this metal-resistant microorganism to promote crop development and disease resistance, and they complement our earlier research on the chromium tolerance and bioremediation properties of *B. cereus* MH778713.

Pazarlar *et al.* (2022) suggested that EC9-mediated protection against Fol in tomatoes depends on JA/ET-dependent signaling. They showed that EC9 prepares tomato plants for higher proteinase inhibitor I (PI-I) and ethylene receptor 4 (ETR4) expression. In addition, they showed that EC9 causes tomato root callose deposition. Understanding the role of defense-related phytohormones in EC9-mediated resistance against Fusarium wilt has improved our understanding of how non-antifungal plant defense-inducing rhizobacteria interact with plants.

Venkataramanamma *et al.* (2022) resulted that ten *Bacillus* isolates were taken from healthy chickpea rhizosphere soil and characterized biochemically (up to species level) and Gram stained to determine their identity as *Bacillus*. Under in vitro testing, they were assessed against *Fusarium oxysporum f.sp. ciceris*. Based on an in vitro experiment, two possible Bacillus isolates were chosen out of a total of 10 and studied for characteristics that promote plant development. It was discovered that Bacillus-5 solubilizes phosphates, Bacillus-7 produces cellulases, and both create HCN. These possible competitors contain more sequences that resemble those of *Bacillus cereus*, according to 16S rDNA research. Future field effectiveness studies must also be conducted.

Malik *et al*. (2022) reported that these two bacterial isolates (TD11 and TD15) significantly reduced the disease rate in tomatoes brought on by the fungi *Fusarium* and *Rhizoctonia* in the pot experiments. Additionally, it was shown that *B. subtilis* (TD11) was the most promising putative biocontrol agent, 50% suppressing tomato fungal infections and displaying a wide range of antagonistic potential.

2.3. Biocontrol activity of *Bacillus cereus*

Joshi (2012) conducted a pathogenicity test on chilli. One isolate of *F. oxysporum* was found most virulent pathogen, while eleven isolates were non-pathogenic isolates. Isolate no. 65 was found most antagonistic towards *F. oxysporum,* under in-vitro dual culture assay. Thirty chilli varieties were screened for evaluation of resistance. Among these, two varieties were found resistant against the Fusarium wilt. The present investigation focused on recovery of antagonistic Fusarium and resistant varieties of chilli, for controlling and resisting wilt and improving the soil health.

Munene *et al*. (2023) resulted that seed treatment with rhizobacterial species of *P. fluorescens, Bacillus cereus,* and *Paenibacillus polymyxa,* along with foliar spray, greatly decreased the incidence and severity of Cff disease. As a result, using biocontrol techniques instead of pesticides to stop bean bacterial wilt may be a safe, efficient, and sustainable option.

Hernández-Huerta *et al*. (2023) revealed that Bc and Bt isolates were recognized. In vitro bioassays showed the bactericidal activity of Bc and Bt strains against Xe isolates, whereas formulations dramatically increased pepper development in seedbeds and pots. Furthermore, tests revealed that the Xe-resistant strains F-BC26, F-BC08, and F-BT24 significantly protected plants. According to the results, the formulations of the isolates F-BT24 and F-BC26 encouraged the development of pepper and shielded it from *Xanthomonas euvesicatoria.*

Khadiri *et al*. (2023) studied that the bacterium's capacity to make fengycin was shown by the molecular analysis. The main mechanism of action for this bacterium's biocontrol activity is more likely to include the production of this lipopeptide and lytic enzymes. Results showed that fruit quality measures under post-harvest pathogen challenge and B8W8 treatment were mostly very similar to those of the untreated control, indicating that B8W8 had no effect on fruit quality. It was therefore determined that B. cereus strain B8W8 might be a useful biological agent to control the primary fungi that cause disease in apples and citrus crops during the post-harvest period.

2.4. Shelf life study of formulated *Bacillus cereus*

Bayoï *et al*. (2021) studied that the amount of flavonoids was positively and substantially linked with the antibacterial activity of extracts against *Bacillus cereus*, *Bacillus subtilis*, and *Aspergillus* species. In contrast to control samples kept at room temperature, which went bad after one day, the "foléré" samples that had been created were still tasty after five days. According to this study, adding tamarind leaf extracts to native drinks may improve their quality and shelf life.

Yulensri *et al.* (2021) demonstrated that the ideal mixture is a mixture for compost and peat soils that also contains Molas, CMC, and Arginine. This formula meets the requirements for biological fertilizer as per Ministry of Agriculture has a shelf life of up to 6 months. It contains 4.64–4.67x 10 5 CFU/gram of bacterial consortium colony.

Shrestha and Harirum (2022) reported that a preliminary research in broth and existing prediction models revealed that *Bacillus spp*. might grow under the investigated circumstances. Uninoculated controls held at 4°C for 4 weeks and then at 7 or 10°C for 9 weeks did not show growth of total plate counts, presumed *Bacillus spp*., yeast, or mold counts. This study verified the inhibition of *B. weihenstephanensis* in the product for an extended shelf life of up to 13 weeks and evaluated the effectiveness of the maximum dose of nisin.

CHAPTER III

MATERIALS AND METHODS

The goal of the study was to develop a formulated bio-fungicide using *B. cereus* HSTUB 17 for the eco-friendly management of Fusarium wilt of chilli caused by *Fusarium oxysporum*. The following is a more thorough description of the experimental methodology:

3.1. Experimental site

The experiment was conducted at the research field under the Department of Plant Pathology, HSTU, Dinajpur.

3.2. Experimental period

The experiment was carried out during December 2022 to April 2023.

3.3. Soil type

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

3.4. Collection of Chilli seeds and raising of seedlings

Chilli seeds of two hybrid variety named Roshni and Bijlee plus were collected from Bangladesh Agricultural Development Corporation (BADC), Dinajpur. The seed bed was prepared using sterilized soil and manure. Seeds were sown at depth of 2-3 cm and covered with soil by light watering. After 8 weeks, the raised seedlings were ready for transplanting.

3.5. Final land preparation

A power tiller was used to prepare the main land, and it was then laddered three times. A visibly bigger clod was pounded into smaller pieces after being spaded into the corner of the ground. The land was completely cleared of weeds and leftover crop.

3.6. Application of manure and fertilizers

During final land preparation FYM or compost was applied. According to the land size well decomposed FYM was applied in the field. The source of N, P, K was Urea, TSP, MOP. The entire amounts of TSP, MP were applied during final land preparation where urea was applied in two equal installments at 20 and 30 days after transplanting (DAT).

3.7. Preparation of formulated bacteria

B. cereus HSTUB 17 was previously isolated and identified on the basis of molecular characterization in our lab. The bacterium was cultured in nutrient broth media for 48 h. Maize bran, charcoal bran, soybean bran, wood dust and pea bran were used for the development of bacterial formulation. At first all the substrates were dried and blended followed by added an adhesive (white flour gum) at 1:10 ratio by maintain pH 7.4. The substrate with adhesive was then autoclave under pressure of approximately 15 pounds per square inch to achieve a chamber temperature 121°C for 15 mins. Adhesive was added for holding materials together in a functional manner by surface attachment that resist separation. After autoclave, mixture was plated and dried in sterile condition followed by adding mannitol (8.5 mL of 3%) for 100 gm formulation. Mannitol was used for increasing protection against moisture in formulation. Finally, bacterial suspension was mixed with the formulating agents at 10:1 ratio and dried in room temperature in sterile condition for three days. The formulated *B. cereus* HSTUB 17 was preserved in polybag for further used in the refrigerator at 4° C.

3.8. Management of fusasrium wilt by the application of formulated bio-fungicide

For the eco-friendly management of Fusarium wilt of chilli, the formulated *B. cereus* HSTUB 17 was used in the following combinations.

T_0: Control

T_1*:* Soybean bran-based formulated *B. cereus* HSTUB 17+ *B. cereus* HSTUB 17 suspension

T_2: Charcoal bran-based formulated *B. cereus* HSTUB 17 + *B. cereus* HSTUB 17 suspension

T_3: Pea bran-based formulated *B.s cereus* HSTUB 17+ *B. cereus* HSTUB 17 suspension

T_4: Maize bran-based formulated *B. cereus* HSTUB 17+ *B. cereus* HSTUB 17 suspension

T_5: Wood Dust bran-based formulated *B. cereus* HSTUB 17 + *B. cereus* HSTUB 17 suspension

T_6: Autostin 50 WDG (Carbendazim)

3.9. Design of experiments

The experiment was laid out following Randomized Complete Block Design (RCBD) with three replications. There was 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.

3.10. Application of formulated bio-fungicide

After preparation of soil bed, formulated bio-fungicide @ 50g/plot was applied before three days of transplantation and ten days of after transplantation with the maintaining of proper moisture.

3.11. Transplantation of seedlings

Chilli seedlings of aged 45 days, transplanted in the main field. Following transplanting, seedlings were given continuous irrigation to form a strong bond between their roots and the soil and enable them to stand alone.

3.12. Application of *B. cereus* HSTUB 17 suspension

B. cereus HSTUB 17 suspension (10^8 CFU/mL) was prepared in order to apply in field. *B. cereus* HSTUB 17 was picked up from pure culture by the toothpick, inoculated in nutrient broth (8 mL/litter), and incubated at 28 ºC in a shaking incubator for 24 hrs. The bacterial suspension were sprayed 2 time on the whole plant including soil and 2 times only whole plant started from 20 DAT at 20 days interval up to 80 DAT. Autostin 50 WDG was prepared @2 gm/ litter at and sprayed at the same day.

3.13. Intercultural operations

Weeding, thinning and watering were done as when necessary.

3.14. Application of pesticides

In order to control aphid, Jago 70 WDG (Imidacloprid) 1g/5L was sprayed 3 times.

3.15. Recording of data

3.15.1. Counting healthy and wilted plants

The experimental plot was surveyed every day to observe whether the plant became infected with fusarium wilt disease or not. However, the data on rot and healthy plants were recorded in every 30, 60, 90 and 120 DAT. The percentage of plant recovery and infected disease were also calculated.

3.15.2. Assessment of fusarium wilt disease incidence (%) and fusarium wilt disease severity (%)

Disease incidence (%) and disease severity (%) of fusarium wilt disease was evaluated at 30, 60, and 90 DAT. Disease incidence was recorded as the number of plants infected by wilt disease out of total number of plants. The infected plants were identified by comparing their symptoms critically with those already published (El-Mohamedy *et al*., 2014). However, the incidence of the disease was calculated by the following formula (Bediako *et al*., 2015)

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

Disease severity (%) was scored according to the disease grading scale given by (Saha, 2001) is as follows:

Numeric scale	Symptoms
0	No wilted plants
1	0-10% plant wilted
2	11-25% plant wilted
3	26-50% plant wilted
4	51-70% plant wilted
5	70%< plant wilted

Disease severity of fusarium wilt was calculated by following the formula (Bediako *et al*., 2015)

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

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

3.16. 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, number of flower per plant, number of fruits per plant, and fruit weight (kg) per plot, shoot weight, shoot length and root weight, root length, were collected at the time of harvesting.

3.16.1. Plant height (cm)

The plant height (cm) was measured from ground level to tip of the plant with the help of a centimeter scale and mean was computed at 30, 60, 90 and 120 DAT.

3.16.2. Number of branches per plant

The number of branches arising from the main stem above the ground was recorded at 30, 60, 90 and 120 DAT.

3.16.3. Number of flowers per plant

The number of flowers was recorded at 30, 60, 90 and 120 DAT.

3.16.4. Number of fruits per plant

The number of fruits was recorded at 30, 60, 90 and 120 DAT. Total number of marketable fruits harvested from the 42 plots. Total number of fruits was counted and the average number of fruits per plot was calculated. Harvesting continued for about one month because fruits of different pots matured progressively at different dates and over long time. Fruits were picked on the basis of horticultural maturity, size, color and age being determined for the purpose of consumption as the fruit grew rapidly and soon get beyond the marketable stage. Picking was done throughout the harvesting period.

3.16.5. Weight of fruits (Kg) per plot

After harvest, weight of fruits (kg) per plot was recorded by using of an electrical balance and fruit yield was calculated using the weight of fruits and the mean was computed using an excel sheet.

3.16.6. Shoot weight (g) and shoot length (cm)

The shoot weight (g) and shoot length (cm) was recorded at harvesting 90 DAT.

3.16.7. Root weight (g) and root length (cm)

The root weight (g) and root length (cm) was recorded at harvesting 90 DAT.

3.17. Biochemical analysis of fruits

Fruits of chilli were subjected to analysis on the traits of TSS, total chlorophyll, total phenol, DPPH (antioxident) test.

3.17.1. Total Soluble Solid (^{0}Brix)

Total soluble solids (TSS) were determined for each sample fruit in 3 replications using an Atago DR-A1digital refractometer (Atago Co. Ld., Japan) at 20 °C and expressed as (%) ^{0}Brix (Ilić *et al*., 2015)

3.17.2. Estimation of total phenol (mg/100g)

The total phenol was calculated by (Singleton and Rossi 1965) and (Saikia *et al.* 2012). In brief, 0.5 mL chilli fruit sample along with 0.5 mL of Folin Ciocalteu's Reagent was taken in a 25 mL falcon tube and mixed thoroughly. The solution was allowed to react with 1 mL of 7.5% saturated sodium carbonate (Na_2CO_3) to the falcon tube for neutralization and then vortexed for 30s. After the mixture was allowed left in a dark place for 35 min at room temperature and centrifuged at 4000 rpm for 10 min. The absorption of the sample was read by a visible spectrometer (UV/VIS, UV1800) at 725 nm. Gallic acid was used to execute a standard (calibration) curve. The findings have been shown to be equal mg/100 g of Gallic acid per 100 g of juice.

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

3.17.3. Estimation of total chlorophyll content of leaves

Chlorophyll content of 2nd leaf from the top of each plant was estimated according to Witham *et al*. (1971). In brief, 0.25g leaf tissue from middle of the leaf was taken in brown bottles containing 25 mL of 80% aqueous acetone. The bottles were kept in dark for 48 hours. The optical density of the colored solutions was determined against 80% acetone as blank using spectrophotometer (SPECTRO UV-VIS RS Spectrophotometer, Labo Med, Inc.) at 645 nm and 663 nm. Total chlorophyll was measured using the fillowing formula:

$$\text{Total chlorophyll} = [20.2 * D_{(645nm)} + 8.02 * D_{(663nm)}] * [V/W * 1000]$$

Where,

V=Volume of 80% aqueous acetone (mL)

W=Weight of fresh leaf (g)

D_{645}=Absorbance at 645nm wave length

D_{663}= Absorbance at 645nm wave length

3.17.4. Estimation of antioxidant

The DPPH assay was used to measure the antioxidant activity of fruits of chilli. 1 mL sample was extracted from each of dried and blended sample of chilli fruits. DPPH was prepared with methanol solvent. Then 0.004% w/v DPPH (2 mL) was added to the falcon tube filled with extracted sample. After that incubation was done for 30 min in the dark place. A standard solution of Ascorbic acid (20 ppm-0.156 ppm) from 1000 ppm stock solution was made. Then absorbance were taken at 517 nm in UV-Vis spectrophotometer. According to Baliyan *et al.* (2022) the following formula was used to compute the percentage of antioxidants:

% of antioxidant activity= [(Ac−As) ÷Ac] × 100

Where,

Ac=Control reaction absorbance

As=Testing specimen absorbance

3.18. Shelf-life of formulated *B. cereus* HSTUB 17

The viable cell in the formulated bio-fungicide were counted by serial dilution at 30 days interval up to the last viable cell found. In this technique Nutrient Agar (NA) and Nutrient Broth (NB) Media were used for serial dilution. Both media were sterilized at 120 °C for 10 minutes. In this process 1g 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. The formulation suspension was then serially diluted to 10^{-9} dilution. Pour plate technique was used by mixing 1 mL of the diluted suspension on nutrient agar (NA) and Nutrient Broth NB) plates for each sample and incubated at 28°C for 24 h. Single bacterial colony was picked up from original culture plate.

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

3.19. Statistical analysis

Data obtained from different parameters were statistically analyzed to find out the level of effectiveness of different formulated bio-fungicides for the management of Fusarium wilt 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 develop a formulated bio-fungicide using *B. cereus* HSTUB 17. The developed bio-fungicide was further used for the environmental friendly management of fusarium wilt of chilli in field conditions. The potentiality of the developed formulated bio-fungicide to control the devastating disease fusarium wilt is described below:

4.1. Efficacy of formulated bio-fungicide to control fusarium wilt of chilli at field level

A field experiment was conducted during Robi-2022 on integrated management of fusarium wilt of chilli causing *F. osysporum* with formulated bio-fungicide. The results of the experiment were presented below:

4.1.1. Efficacy of formulated bio-fungicide against Fusarium wilt Incidence (%) of chilli at 60 DAT

In chilli variety Roshni, T_6 showed lowest statistical significant disease incidence (4.57 %) followed by T_5 (7.7 %), T_2 (9.45 %), T_4 (12.04 %), T_1 (13.17 %) and T_3 (15.99 %). However, T_0 (control) showed the highest disease incidence (18.22 %) (Table 1).

In chilli variety Bijlee plus, T_6 showed lowest statistical significant disease incidence (4.10 %) followed by T_5 (6.72 %), T_1 (9.84 %), T_3 (10.95 %), T_2 (13.56 %) and T_4 (14.31 %). However, T_0 (control) showed the highest disease incidence (16.59 %) (Table 1).

Table 1. Efficacy of formulated bio-fungicide against Fusarium wilt Incidence (%) of chilli at 60 DAT

Variety	**Formulated bio-fungicide**	**Fusarium wilt at 60 DAT**	
		Incidence (%)	**Reduction over control (%)**
Roshni	T_0	18.22 a ± 0.855	-
	T_1	13.17 c ± 1.026	27.72
	T_2	9.45 d ± 0.282	48.13
	T_3	15.99 b ± 0.501	12.24
	T_4	12.04 c ± 0.245	33.92
	T_5	7.7 e ± 0.843	57.77
	T_6	4.57 f ± 0.445	74.92
	LSD	1.27	-
Bijlee plus	T_0	16.59 a ± 0.307	-
	T_1	9.84 e ± 0.216	40.69
	T_2	13.56 c ± 0.600	18.26
	T_3	10.95 d ± 0.346	33.99
	T_4	14.31 b ± 0.251	13.8
	T_5	6.72 f ± 0.209	59.49
	T_6	4.11 g ± 0.696	75.22
	LSD	0.71	-

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17 + suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17 + suspension o*f Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.1.2. Efficacy of formulated bio-fungicide against Fusarium wilt Incidence (%) of chilli at 90 DAT

In chilli variety Roshni, T_6 showed lowest statistical significant disease incidence (3.67 %) followed by T_5 (5.53 %), T_4 (8.21 %), T_1 (10.60 %), T_2 (11.84 %) and T_3 (13.87 %). However, T_0 (control) showed the highest disease incidence (14.31 %) (Table 2).

In chilli variety Bijlee plus, T_6 showed lowest statistical significant disease incidence (4.52 %) followed by T_5 (6.54 %) T_2 (7.68 %), T_4 (9.77 %), T_1 (10.73 %) and T_3 (12.05 %). However, T_0 (control) showed the highest disease incidence (13.63 %) (Table 2).

Table 2. Efficacy of formulated bio-fungicide against Fusarium wilt Incidence (%) of chilli at 90 DAT

Variety	Formulated bio-fungicide	Fusarium wilt at 90 DAT	
		Incidence (%)	**Reduction over control (%)**
Roshni	T_0	14.31 a ± 0.341	-
	T_1	10.6 c ± 0.298	25.92
	T_2	11.84 b± 0.140	17.26
	T_3	13.86 a ± 0.811	3.07
	T_4	8.21 d ± 0.665	42.63
	T_5	5.52 e ± 0.323	61.36
	T_6	3.67 f ± 0.266	74.35
	LSD	0.71	-
Bijlee plus	T_0	13.63 a± 0.293	-
	T_1	10.73 c± 0.967	21.28
	T_2	7.68 e± 0.225	43.65
	T_3	12.05 b ± 0.04	11.59
	T_4	9.76 d± 0.152	28.39
	T_5	6.54 f± 0.15	52.01
	T_6	4.52 g± 0.235	66.83
	LSD	0.69	-

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17 + suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust-bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.1.3. Efficacy of formulated bio-fungicide against Fusarium wilt Severity (%) of chilli at 90 DAT

In chilli variety Roshni, T_6 showed lowest statistical significant disease incidence (2.49 %) followed by T_5 (3.25 %), T_4 (5.67 %), T_3 (7.6 %), T_1 (8.33 %) and T_2 (9.61 %). However, T_0 (control) showed the highest disease incidence (10.49 %) (Table 3).

In chilli variety Bijlee plus, T_6 showed lowest statistical significant disease incidence (2.17 %) followed by T_5 (3.16 %), T_1 (4.48 %), T_4 (7.35 %), T_3 (9.59 %) and T_2 (11.37 %). However, T_0 (control) showed the highest disease incidence (12.75 %) (Table 3).

Table 3. Efficacy of formulated bio-fungicide against Fusarium wilt Severity (%) of chilli at 90 DAT

Variety	**Formulated bio-fungicide**	**Fusarium wilt at 90 DAT**	
		Severity (%)	**Reduction over control (%)**
Roshni	T_0	10.49 a ± 0.211	-
	T_1	8.33 c ± 0.208	20.59
	T_2	9.61 b ± 0.222	8.38
	T_3	7.6 d ± 0.2	27.55
	T_4	5.66 e ± 0.251	46.04
	T_5	3.25 f ± 0.163	69.01
	T_6	2.49 g ± 0.362	76.35
	LSD	0.33	-
Bijlee plus	T_0	12.75 a ± 0.610	-
	T_1	4.48 e ± 0.205	64.86
	T_2	11.37 b ± 0.362	10.82
	T_3	9.59 c ± 0.351	24.78
	T_4	7.35 d ± 0.481	42.35
	T_5	3.16 f ± 0.958	75.21
	T_6	2.17 g ± 0.499	82.98
	LSD	0.93	-

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17+ suspension o*f Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.1.4. Interaction effect of formulated bio-fungicide and variety of chilli on disease severity (%)

Interaction of the variety and treatments revealed that, statistically similar and significant disease severity reduction (69.01 and 75.21%) was obtained in both the variety with the application of wood dust-based formulated bio-fungicide over control (Table 4).

Table 4. Interaction effects of formulated bio-fungicide and variety on fusarium wilt severity (%)

Formulated bio-fungicide×Variety	Severity (%)	Reduction (%) over control
T_0V_1	10.49 a ± 0.211	-
T_1V_1	8.33 c ± 0.208	20.59
T_2V_1	9.61 b ± 0.222	8.38
T_3V_1	7.6 d ± 0.2	27.55
T_4V_1	5.66 e ± 0.251	46.04
T_5V_1	3.25 f ± 0.163	69.01
T_6V_1	2.49 g ± 0.362	76.35
T_0V_2	12.75 a ± 0.610	-
T_1V_2	4.48 e ± 0.205	64.86
T_2V_2	11.37 b ± 0.362	10.82
T_3V_2	9.59 c ± 0.351	24.78
T_4V_2	7.35 d ± 0.481	42.35
T_5V_2	3.16 f ± 0.958	75.21
T_6V_2	2.17 g ± 0.499	82.98

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea-bran based formulated *Bacillus cereus* HSTUB 17+ suspension o*f Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim); V_1 =Roshni, V_2 =Bijlee plus.

4.2. Efficacy of formulated bio-fungicide on growth attributes of chilli plant

The effect of all the formulated bio-fungicides imposed for the management of fusarium wilt of chilli causing *Fusarium oxysporum* and their influence on plant growth parameters viz., plant height, number of branches, number of fruits, and yield (gm/plant) in chilli variety was recorded as follows:

4.2.1. Efficacy of formulated bio-fungicide on plant height (cm) of Roshni (V_1) at different DAT

At 30 DAT in Roshni, T_5 showed highest statistical significant plant height (17.79 cm.) and the second highest T3 showed (15.483 cm) followed by T_6 (15.05 cm), T_4 (14.49 cm), T_2 (14.21 cm) and T_1 (13.94 cm). However, only T_0 showed the lowest plant height (12.53 cm) (Table 5).

At 60 DAT in Roshni, T_5 showed highest statistical significant plant height (26.75 cm) and the second highest T_6 showed (23.053 cm) followed by T_1 (22.94 cm), T_2 (22.32 cm), T_4 (21.77 cm) and T_3 (21.52 cm). However, only T_0 showed the lowest plant height (19.04 cm) (Table 5).

At 90 DAT in Roshni, T_5 showed highest statistical significant plant height (46.26 cm) and the second highest T_4 showed (44.12 cm) followed by T_6 (43.77 cm), T_2 (43.22 cm), T_3 (39.59 cm) and T_1 (38.23 cm). However, only T_0 showed the lowest plant height (34.89 cm) (Table 5).

At 120 DAT in Roshni, T_5 showed highest statistical significant plant height (103.5 cm) and the second highest T_1 showed (97.13 cm) followed by T_6 (91.5 cm), T_2 (82.6 cm), T_4 (81.67 cm) and T_3 (80.53 cm). However, only T_0 showed the lowest plant height (64.67 cm) (Table 5).

Table 5. Efficacy of formulated bio-fungicide on plant height (cm) of Roshni (V_1) at different DAT

Formulated bio fungicide	Plant height (cm)			
	30 DAT	60 DAT	90 DAT	120 DAT
T_0	12.53 d±0.495	19.04 c±0.142	34.89 e±0.335	64.67 e±2.516
T_1	13.94 c±0.295	22.94 b±0.707	38.23 d±0.832	97.13 b±0.472
T_2	14.21 c±1.237	22.32 b±0.253	43.22 b±0.605	82.6 d±2.330
T_3	15.48 b±0.449	21.52 b±1.881	39.59 c±0.258	80.53 d±0.850
T_4	14.48 bc±0.704	21.77 b±1.905	44.12b±0.161	81.67 d±1.755
T_5	17.79 a±0.205	26.75 a±0.377	46.26 a±1.379	103.5 a±1.5
T_6	15.05 bc±0.476	23.05 b±0.670	43.77 b±0.125	91.5 c±0.608
LSD ($p \leq 0.05$)	1.18	1.86	1.12	2.53

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17+ suspension o*f Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.2.2. Efficacy of formulated bio-fungicide on plant height (cm) of Bijlee plus (V_2) at different DAT

At 30 DAT in Bijlee plus, T_5 showed highest statistical significant plant height (18.037 cm.) and the second highest T_6 showed (15.803 cm) followed by T_3 (14.13 cm), T_1 (14.05 cm), T_4 (13.33 cm) and T_2 (13.11 cm). However, only T_0 showed the lowest plant height (12.407 cm) (Table 6).

At 60 DAT in Bijlee plus, T_5 showed highest statistical significant plant height (26.663 cm) and the second highest T_4 showed (23.357 cm) followed by T_2 (22.39 cm), T_6 (21.30 cm), T_1 (20.52 cm) and T_3 (19.99 cm). However, only T_0 showed the lowest plant height (18.947 cm) (Table 6).

At 90 DAT in Bijlee plus, T_5 showed highest statistical significant plant height (46.563 cm) and the second highest T_3 showed (45.500 cm) followed by T_2 (43.55

cm), T_6 (42.11 cm), T_1 (40.86 cm) and T_4 (39.53 cm). However, only T_0 showed the lowest plant height (35.557 cm) (Table 6).

At 120 DAT in Bijlee plus, T_5 showed highest statistical significant plant height (101.86 cm) and the second highest T_4 showed (96.02 cm) followed by T_1 (92.00 cm), T_3 (91.33 cm), T_2 (87.50 cm) and T_6 (85.42 cm). However, only T_0 showed the lowest plant height (42.57 cm) (Table 6).

Table 6. Efficacy of formulated bio-fungicide on plant height (cm) of Bijlee plus (V_2) at different DAT

Formulated bio-fungicide	**Plant height (cm)**			
	30 DAT	**60 DAT**	**90 DAT**	**120 DAT**
T_0	12.40 e±0.155	18.94f±0.065	35.55g±0.413	42.56f±0.814
T_1	14.04 cd±1.497	20.51de±0.449	40.85e±0.558	92c±1.5
T_2	13.10 de±0.654	22.38c±0.635	43.54c±0.405	87.5d±0.5
T_3	14.12 c±0.200	19.99e±0.813	45.5b±0.519	91.33c±1.607
T_4	13.32cde±0.458	23.35b±0.262	39.52f±0.553	96.02b±0.522
T_5	18.03a±0.151	26.66a±0.377	46.56a±0.293	101.86a±0.691
T_6	15.80b±0.332	21.3d±0.248	42.11d±0.110	85.42e±0.530
LSD ($p \leq 0.05$)	0.95	0.83	0.82	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_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.2.3. Relation between Fusarium wilt incidence (%) and plant height (cm) of chilli

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 disease in Roshni and Bijlee plus, showed (107.14% and 115.9%) loss in plant height was observed. The regression equation also showed that, for each unit increase in disease incidence, a plant height reduction of (0.49% and 0.29%) will be occurred (Fig. 1).

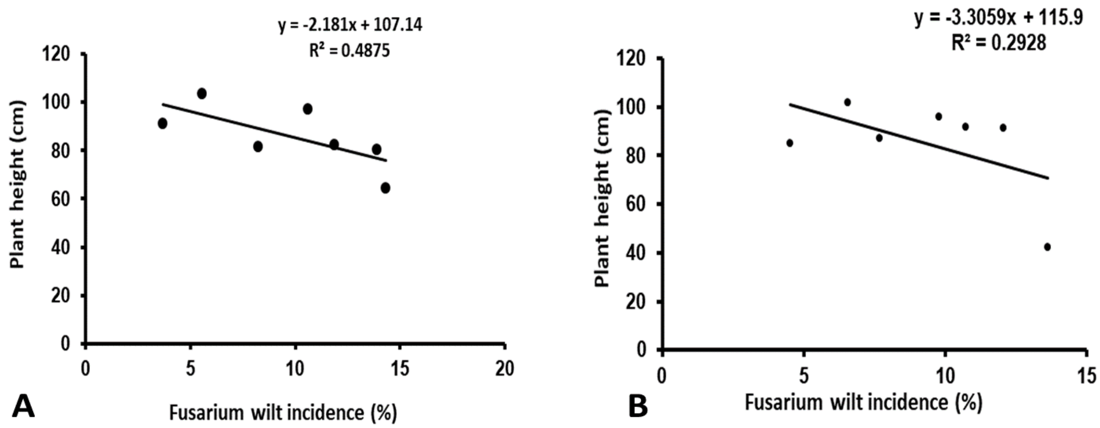

Fig. 1. Relation between Fusarium wilt incidence (%) and plant height (cm) of chilli.

4.2.4. Relation between Fusarium wilt severity (%) and plant height (cm) of chilli

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 severity and plant height. Due to fusarium wilt disease in Roshni and Bijlee plus (104.55% and 106.28%) loss in plant height was observed. The regression equation also showed that, for each unit increase in disease severity, a plant height reduction of (0.44% and 0.37%) will be occurred (Fig. 2).

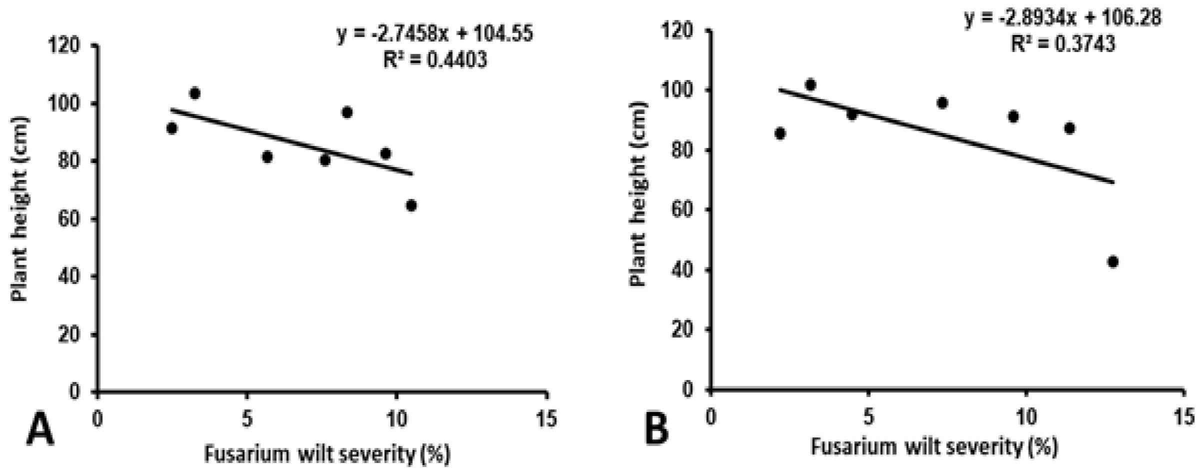

Fig. 2. Relation between Fusarium wilt severity (%) and plant height (cm) of chilli.

4.2.5. Efficacy of formulated bio-fungicide on number of branches per plant of Roshni (V_1) at different DAT

At 30 DAT in Roshni, T_5 showed maximum statistical significant number of branches (4.3) and the second maximum number of branches T_2 showed (2.33) followed by T_1 (1.66), T_3 (1.33), T_4 (1.33) and T_6 (0.67). However, only T_0 showed minimum number of branches (0.34) (Table 7).

At 60 DAT in Roshni, T_5 showed maximum statistical significant number of branches (10.22) and the second maximum number of branches T_3 showed (8.73) followed by T_6 (7.5), T_1 (6.53), T_2 (6.47) and T_4 (6.13). However, only T_0 showed minimum number of branches (5.53) (Table 7).

At 90 DAT in Roshni, T_5 showed maximum statistical significant number of branches (27.77) and the second maximum number of branches T_4 showed (25.03) followed by T_6 (24.50), T_1 (22.67), T_2 (22.54) and T_3 (22.33). However, only T_0 showed minimum number of branches (18.93) (Table 7).

At 120 DAT in Roshni, T_5 showed maximum statistical significant number of branches (180.90) and the second maximum number of branches T_3 showed (175.85) followed by T_6 (142.65), T_2 (133.80), T_1 (123.80) and T_4 (108.67). However, only T_0 showed minimum number of branches (72.33) (Table 7).

Table 7. Efficacy of formulated bio-fungicide on number of branches per plant of Roshni (V_1) at different DAT

Formulated bio-fungicide	Number of branches			
	30 DAT	**60 DAT**	**90 DAT**	**120 DAT**
T_0	0.33c ± 0.577	5.53e±0.321	18.93d±0.152	72.33g±0.577
T_1	1.67bc ± 0.577	6.53d±0.503	22.66c±0.503	123.18e±0.323
T_2	2.33b ± 0.577	6.46d±1.101	22.53c±1.225	133.8d±0.3
T_3	1.33bc± 0.577	8.73b±0.208	22.33c±0.208	175.85b±0.450
T_4	1.33bc ± 1.154	6.13de±0.230	25.03b±1.674	108.66f±0.577
T_5	4.33a ± 0.577	10.21a±0.104	27.76a±0.763	180.9a±0.360
T_6	0.66c± 1.154	7.5c±0.4	24.5b±0.529	142.64c±0.308
LSD ($p \leq 0.05$)	1.41	0.91	1.54	0.80

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.2.6. Efficacy of formulated bio-fungicide on number of branches per plant of Bijlee plus (V_2) at different DAT

At 30 DAT in Bijlee plus, T_5 showed maximum statistical significant number of branches (4.66) and the second maximum number of branches T_2 showed (2.66) followed by T_1 (1.66), T_6 (1.66), T_3 (1.33) and T_4 (1.00). However, only T_0 showed minimum number of branches (0.33) (Table 8).

At 60 DAT in Bijlee plus, T_5 showed maximum statistical significant number of branches (10.18) and the second maximum number of branches T_3 showed (9.53) followed by T_6 (8.50), T_1 (7.73), T_2 (6.27) and T_4 (5.87). However, only T_0 showed minimum number of branches (4.87) (Table 8).

At 90 DAT in Bijlee plus, T_5 showed maximum statistical significant number of branches (28.90) and the second maximum number of branches T_2 showed (25.60) followed by T_6 (23.77), T_3 (22.63), T_4 (21.37) and T_1 (20.53). However, only T_0 showed minimum number of branches (18.83) (Table 8).

At 120 DAT in Bijlee plus, T_5 showed maximum statistical significant number of branches (229.29) and the second maximum number of branches T_4 showed (223.67) followed by T_3 (215.70), T_1 (155.07), T_2 (147.50) and T_6 (141.67). However, only T_0 showed minimum number of branches (78.33) (Table 8).

Table 8. Efficacy of formulated bio-fungicide on number of branches per plant of Bijlee plus (V_2) at different DAT

Formulated bio-fungicide	Number of branches			
	30 DAT	**60 DAT**	**90 DAT**	**120 DAT**
T_0	0.33c±0.577	4.86d±0.057	18.83f±1.550	78.33g±0.577
T_1	1.66bc±1.527	7.73b±0.208	20.53ef±0.550	155.06d±0.404
T_2	2.66ab±0.577	6.26c±1.101	25.6b±0.435	147.5e±0.5
T_3	1.33bc±1.154	9.53a±0.404	22.63cd±1.357	215.7c±0.818
T_4	1bc±1	5.86c±0.057	21.36de±0.472	223.66b±0.585
T_5	4.66a±1.154	10.18a±0.166	28.9a±0.888	229.29a±0.527
T_6	1.66bc±1.527	8.5b±0.360	23.76c±0.472	141.66g±1.527
LSD ($p \leq 0.05$)	2.17	0.78	1.73	1.49

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB

17; T_5:Wood dust bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.2.7. Relation between Fusarium wilt incidence (%) and number of branches of chilli

In the regression equation, disease incidence (%) was considered as independent and number of branches as dependent variable. A strong negative relation was existing between the disease incidence and number of branches. Due to fusarium wilt disease, (165.99 % and 209.22 %) loss in number of branches was observed. The regression equation also showed that, for each unit increase in disease incidence, a number of branches reduction of (0.13% and 0.06%) will be occurred (Fig. 3).

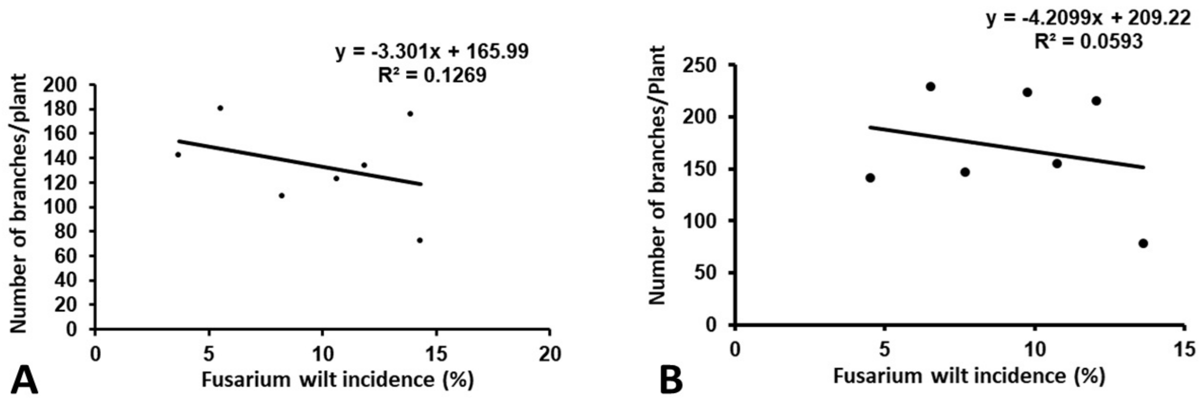

Fig. 3. Relation between Fusarium wilt incidence (%) and number of branches of chilli.

4.2.8. Relation between Fusarium wilt severity (%) and number of branches of chilli

In the regression equation, disease severity (%) was considered as independent and number of branches as dependent variable. A strong negative relation was existing between the disease severity and number of branches. Due to fusarium wilt disease in Roshni and Bijlee plus (179.84% and 208.46%) loss in number of branches was observed. The regression equation also showed that, for each unit increase in disease severity, number of branches reduction of (0.30% and 0.15%) will be occurred (Fig. 4).

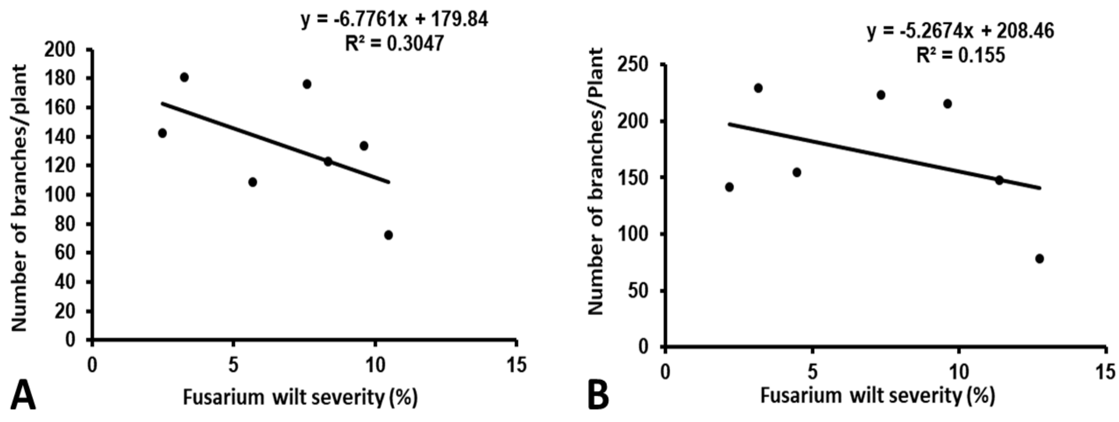

Fig. 4. Relation between Fusarium wilt severity (%) and number of branches of chilli

4.2.9. Efficacy of formulated bio-fungicide on number of flower per plant of Roshni (V_1) at different DAT

At 90 DAT in Roshni, T_5 showed maximum statistical significant number of flowers (15.23) and the second maximum number of flowers T_4 showed (12.57) followed by T_1 (11.30), T_6 (10.64), T_2 (8.88) and T_3 (8.27). However, only T_0 showed minimum number of flowers (6.53) (Table 9).

At 120 DAT in Roshni, T_5 showed maximum statistical significant number of flowers (176.60) and the second maximum number of flowers T_6 showed (143.89) followed by T_2 (135.71), T_4 (127.56), T_3 (117.28) and T_1 (75.89). However, only T_0 showed minimum number of flowers (67.38) (Table 9).

Table 9. Efficacy of formulated bio-fungicide on number of flowers per plant of Roshni (V_1) at different DAT

Formulated bio-fungicide	Number of flowers	
	90 DAT	120 DAT
T_0	6.53 e±0.416	67.38 g±0.440
T_1	11.3 c±0.458	75.88 f±0.179
T_2	8.88 d±0.557	135.71 c±0.245
T_3	8.26 d±0.208	117.28 e±0.602
T_4	12.57 b±0.455	127.56 d±0.550
T_5	15.23 a±0.404	176.6 a±0.793
T_6	10.64 c±0.217	143.89 b±0.810
LSD ($p \leq 0.05$)	0.77	0.91

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.2.10. Efficacy of formulated bio-fungicide on number of flower per plant of Bijlee plus (V_2) at different DAT

At 90 DAT in Bijlee plus, T_5 showed maximum statistical significant number of flowers (16.00) and the second maximum number of flowers T_6 showed (12.87) followed by T_2 (10.37), T_4 (8.50), T_1 (7.60) and T_3 (7.40). However, only T_0 showed minimum number of flowers (5.57) (Table 10).

At 120 DAT in Bijlee plus, T_5 showed maximum statistical significant number of flowers (175.67) and the second maximum number of flowers T_3 showed (145.92) followed by T_6 (137.22), T_4 (125.04), T_2 (114.96) and T_1 (108.41). However, only T_0 showed minimum number of flowers (52.67) (Table 10).

Table 10. Efficacy of formulated bio-fungicide on number of flowers per plant of Bijlee plus (V_2) at different DAT

Formulated bio-fungicide	Number of flowers	
	90 DAT	120 DAT
T_0	5.56 f±0.472	52.66 g±0.577
T_1	7.6 de±0.435	108.41 f±0.591
T_2	10.37 c±1.011	114.96 e±0.381
T_3	7.4 e±0.4	145.92 b±0.216
T_4	8.5 d±0.435	125.04 d±0.678
T_5	16 a±0.265	175.66 a±1.527
T_6	12.86 b±0.404	137.21 c±0.391
LSD p≤0.05)	1.02	1.38

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran based formulated *Bacillus cereus* HSTUB 17+ suspension o*f Bacillus cereus* HSTUB 17; T_4: Maize bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.2.11. Efficacy of formulated bio-fungicide on number of fruits per plant of Roshni (V_1) at different DAT

At 90 DAT in Roshni, T_5 showed maximum statistical significant number of fruits (15.57) and T_3 showed second maximum number of fruits (13.23) followed by T_6 (10.38), T_2 (9.27), T_4 (8.57) and T_1 (8.53). However, only T_0 showed minimum number of fruits (6.23) (Table 11).

At 120 DAT in Roshni, T_5 showed maximum statistical significant number of fruits (115.90) and the second maximum number of fruits T_6 showed (112.40) followed by T_3 (110.50), T_2 (108.83), T_1 (82.27) and T_4 (66.80). However, only T_0 showed minimum number of fruits (38.77) (Table 11).

Table 11. Efficacy of formulated bio-fungicide on number of fruits per plant of Roshni (V_1) at different DAT.

Formulated bio-fungicide	**Number of fruits**	
	90 DAT	**120 DAT**
T_0	6.23 f±0.057	38.77 g±0.907
T_1	8.53 e±0.404	82.27 e±1.755
T_2	9.26 d±0.208	108.83 d±0.763
T_3	13.23 b±0.152	110.5 c±0.458
T_4	8.56 e±0.550	66.8 f±0.793
T_5	15.56 a±0.351	115.9 a±0.781
T_6	10.38 c±0.171	112.4 b±0.529
LSD ($p \leq 0.05$)	0.44	1.43

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_4: Maize bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.2.12. Efficacy of formulated bio-fungicide on number of fruits per plant of Bijlee plus (V_2) at different DAT

At 90 DAT in Bijlee plus, T_5 showed maximum statistical significant number of fruits (16.17) and the second maximum number of fruits T_4 showed (12.93) followed by T_3 (11.57), T_6 (8.87), T_2 (7.80) and T_1 (6.63). However, only T_0 showed minimum number of fruits (5.70) (Table 12).

At 120 DAT in Bijlee plus, T_5 showed maximum statistical significant number of fruits (115.13) and the second maximum number of fruits T_3 showed (98.37) followed by T_6 (87.93), T_4 (76.17), T_2 (68.00) and T_1 (54.37). However, only T_0 showed minimum number of fruits (36.47) (Table 12).

Table 12. Efficacy of formulated bio-fungicide on number of fruits per plant of Bijlee plus (V_2) at different DAT.

Formulated bio-fungicide	**Number of fruits**	
	90 DAT	**120 DAT**
T_0	5.7 g±0.264	36.47 g±0.550
T_1	6.63 f±0.416	54.37 f±1.582
T_2	7.8 e±0.361	68 e±1
T_3	11.57 c±0.503	98.37 b±0.343
T_4	12.93 b±0.416	76.17 d±1.040
T_5	16.17 a±0.305	115.13 a±0.986
T_6	8.87 d±0.057	87.93 c±0.551
LSD ($p \leq 0.05$)	0.46	1.62

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17+ suspension o*f Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.2.13. Efficacy of formulated bio-fungicide on yield (kg/plot) of chilli variety

In chilli variety Roshni, T_5 showed the maximum yield per plot (2.67 kg/plot) followed by T_6 (2.27 kg/plot), T_1 (2.14 kg/plot), T_4 (1.94 kg/plot), T_3 (1.93 kg/plot) and T_2 (1.87 kg/plot). However, T_0 showed lowest yield per plot (1.5 kg/plot) (Fig. 5).

In chilli variety Bijlee plus, T_5 showed the maximum yield per plot (2.69 kg/plot) followed by T_6 (2.31 kg/plot), T_1 (2.31 kg/plot), T_3 (2.25 kg/plot), T_4 (2.03 kg/plot) and T_2 (1.92 kg/plot). However, T_0 showed lowest yield per plot (1.59 kg/plot) (Fig. 5).

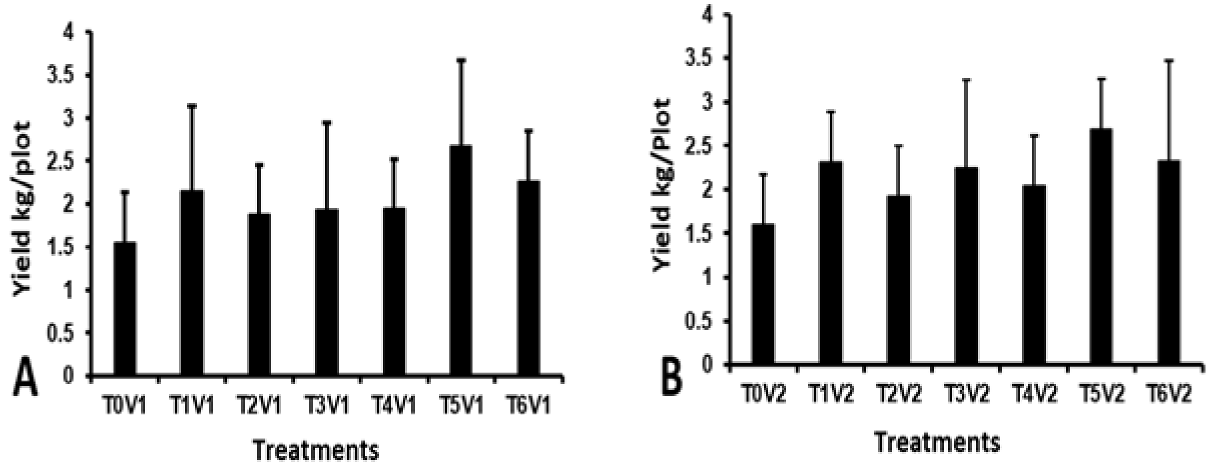

Fig. 5. Efficacy of formulated bio-fungicide on yield (kg/plot) of chilli variety.

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_4: Maize bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.2.14. Relation between Fusarium wilt incidence (%) and yield (kg/plot) of chilli

In the regression, disease incidence (%) was considered as independent and yield kg/plot as dependent variable. A strong negative relation was existing between the disease incidence and yield kg /plot. Due to disease, Roshni and Bijlee plus (2.72% and 2.74%) loss in yield kg /plot was observed. The regression equation also showed that, for each unit increase in disease incidence, yield kg/plot reduction of (0.62% and 0.33%) will be occurred (Fig. 6).

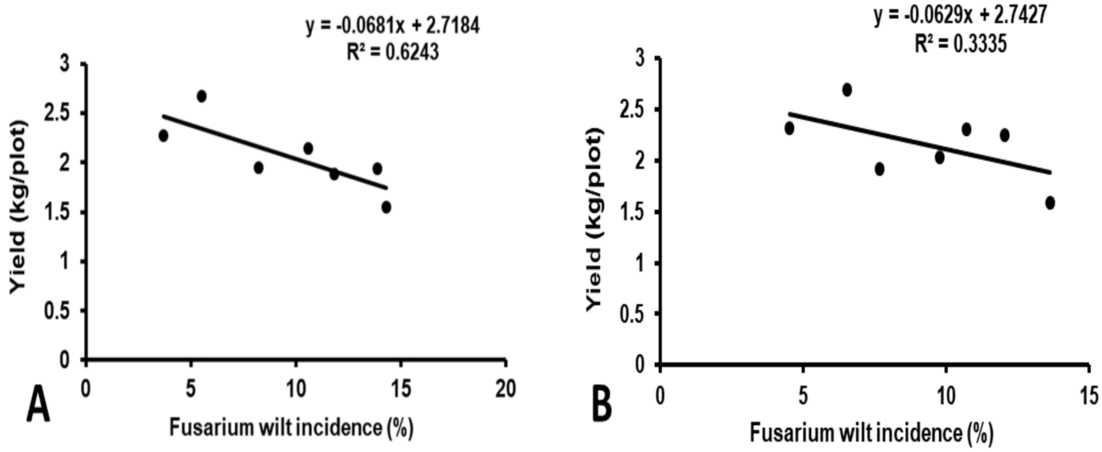

Fig. 6. Relation between Fusarium wilt incidence (%) and yield (kg/plot) of chilli.

4.2.15. Relation between Fusarium wilt severity (%) and yield (kg/plot) of chilli

In the regression, disease severity (%) was considered as independent and yield kg/plot as dependent variable. A strong negative relation was existing between the disease severity and yield kg /plot. Due to disease, Roshni and Bijlee plus (2.69% and 2.67%) loss in yield kg /plot was observed. The regression equation also showed that, for each unit increase in disease severity, yield kg/plot reduction of (0.66% and 0.71%) will be occurred (Fig. 7).

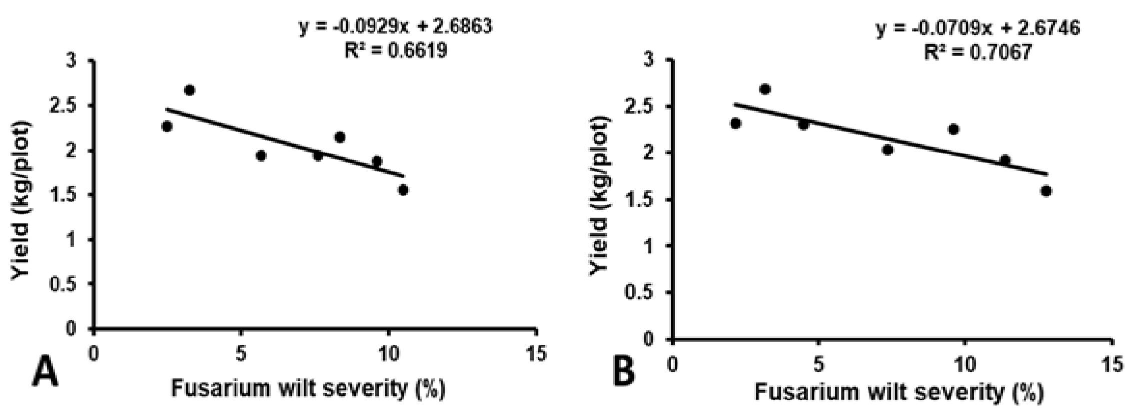

Fig. 7. Relation between Fusarium wilt severity (%) and yield (kg/plot) of chilli.

4.2.16. Interaction effect of formulated bio-fungicide on yield/plot with two chilli varieties at the time of harvesting

At the time of harvesting, Wood dust based formulated *B. cereus* HSTUB 17 alone gave significant and statistically similar result. Interaction between Treatment and variety on yield (kg/plot) against *Fusarium oxysporum* (Fig. 8).

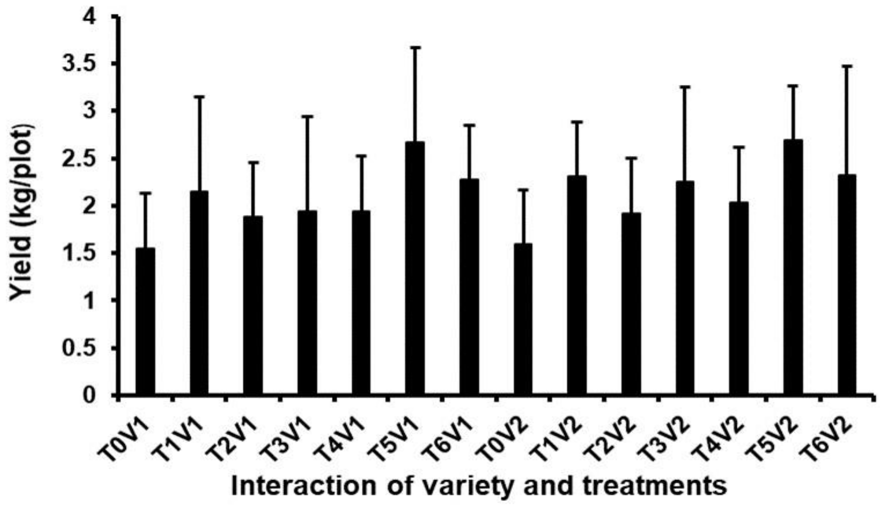

Fig. 8. T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17+ suspension o*f Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5: Wood dust-bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim). V_1=Roshni and V_2=Bijlee plus

4.2.17. Efficacy of formulated bio-fungicide on shoot length, shoot weight and root length, root weight of Roshni 120 DAT

In Roshni, T_5 showed highest shoot length (113 cm.) and T_6 showed the second highest shoot length (108 cm.) followed by T_3 (105 cm.), T_1 (103 cm.) T_2 (94 cm.) and T_4 (89 cm.). However, T_0 showed the lowest shoot length (74 cm.) among all the formulated bio-fungicide (Table 13).

In Roshni, T_5 showed the highest shoot weight (420 g) and T_6 showed the second highest shoot weight (320 g) followed by T_1 (206 g), T_2 (180 g), T_3 (153 g) and T_4 (130 g). However, the T_0 showed the lowest shoot weight (54 g) among all the formulated bio-fungicide (Table 13).

In Roshni, T_5 showed highest root length (29 cm.) and T_6 showed the second highest root length (27 cm.) followed by T_4 (26 cm.), T_1 (24 cm.), T_2 (23 cm.) and T_3 (22 cm.). However, T_0 showed the lowest root length (20 cm.) among all the formulated bio-fungicide (Table 13).

In Roshni, T_5 showed the highest root weight (42.82 g) and T_6 showed the second highest root weight (37.27 g) followed by T_2 (31.3 g), T_1 (23.2 g), T_4 (21.09 g) and T_3 (14.4 g). However, the T_0 showed the lowest root weight (10.09 g) among all the formulated bio-fungicide (Table 13).

Table 13. Efficacy of formulated bio-fungicide on shoot length, shoot weight and root length, root weight of Roshni at 120 DAT.

Formulated bio-fungicide	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
T_0	74	54	20	10.09
T_1	103	206	24	23.2
T_2	94	180	23	31.3
T_3	105	153	22	14.4
T_4	89	130	26	21.089
T_5	113	420	29	42.82
T_6	108	320	27	37.27

T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust-bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim). V_1=Roshni and V_2=Bijlee plus

Fig. 9. Showing plant roots of different treatments of chilli variety Roshni.

4.2.18. Efficacy of formulated bio-fungicide on shoot length, shoot weight and root length, root weight of Bijlee plus at 120 DAT

In Bijlee plus, T_5 showed highest shoot length (116 cm.) and T_1 showed the second highest shoot length (101 cm.) followed by T_6 (99 cm.), T_4 (93.5 cm.), T_3 (83 cm.) and T_2 (65.5 cm.). However, T_0 showed the lowest shoot length (44 cm.) among all the formulated bio-fungicide (Table 14).

In Bijlee plus, T_5 showed the highest shoot weight (224 g) and T_6 showed the second highest shoot weight (210 g) followed by T_4 (186 g), T_3 (164 g), T_2 (157 g) and T_1 (145 g). However, the T_0 showed the lowest shoot weight (17 g) among all the formulated bio-fungicide (Table 14).

In Bijlee plus, T_5 showed highest root length (28 cm.) and T_6 showed the second highest root length (26 cm.) followed by T_3 (25 cm.), T_2 (23 cm.), T_1 (22 cm.) and T_4 (21 cm.). However, T_0 showed the lowest root length (16 cm.) among all the formulated bio-fungicide (Table 14).

In Bijlee plus, T_5 showed the highest root weight (33g) and T_6 showed the second highest root weight (28 g) followed by T_2 (27 g), T_3 (26 g), T_4 (25 g) and T_1 (23 g). However, the T_0 showed the lowest root weight (9 g) among all the formulated formulated bio-fungicide (Table 14).

Table 14. Efficacy of formulated bio-fungicide on shoot length, shoot weight and root length, root weight of Bijlee plus at 120 DAT

Formulated bio-fungicide	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
T_0	44	17	16	9
T_1	101	145	22	23
T_2	65.5	157	23	27
T_3	83	164	25	26
T_4	93.5	186	21	25
T_5	116	224	28	33
T_6	99	210	26	28

T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17+ suspension o*f Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5: Wood dust-bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim). V_1=Roshni and V_2=Bijlee plus

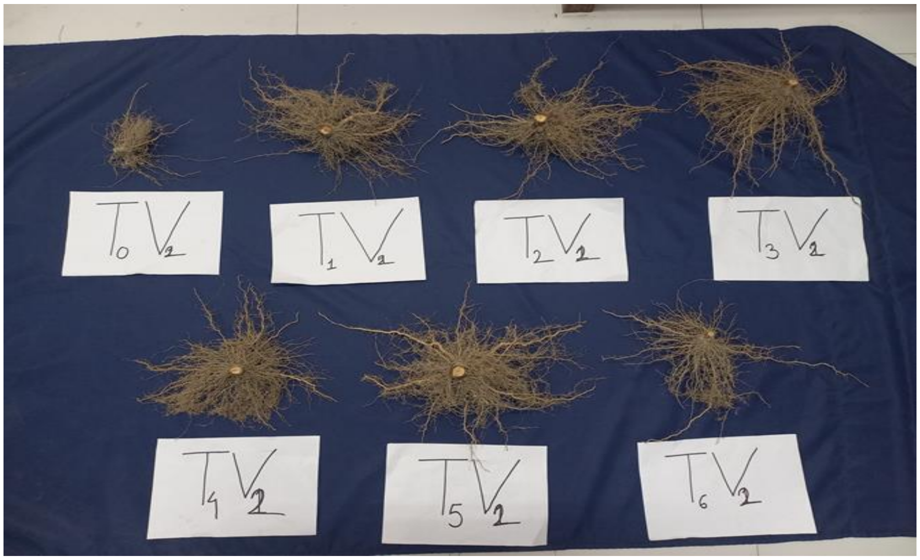

Fig. 10. Showing plant roots of different treatments of chilli variety Bijlee plus

4.3. Efficacy of formulated bio-fungicide on biochemical properties of chilli

4.3.1. Efficacy of formulated bio-fungicide on Total soluble solids (°Brix.) content in chilli variety Roshni and Bijlee plus at 120 DAT

In Roshni, T_5 showed maximum percentage of TSS in fruit (13.77 %) followed by T_6 (9.2 %), T_3 (8.77 %), T_2 (8.5 %), T_4 (8.4 %) and T_1 (8.1 %). However, T_0 showed lowest percentage of TSS in fruit (7.27 %) (Table 15).

In Bijlee plus, T_5 showed maximum percentage of TSS in fruit (13.23 %) followed by T_6 (10.26 %), T_2 (9.13 %), T_4 (8.57 %), T_3 (8.47 %) and T_1 (8.13 %). However, T_0 showed lowest percentage of TSS in fruit (7.13 %) (Table 15).

Table 15. Efficacy of formulated bio-fungicide on Total soluble solids (°Brix.) content in chilli variety Roshni and Bijlee plus at 120 DAT

Variety	Formulated bio-fungicide	TSS (%°Brix)
Roshni (V_1)	T_0	7.26 f ± 0.057
	T_1	8.1 e ± 0.1
	T_2	8.5 cd ± 0.3
	T_3	8.77 c ± 0.23
	T_4	8.4 de ± 0.3
	T_5	13.77 a ± 0.33
	T_6	9.2 b ± 0.26
	LSD ($p \leq 0.05$)	0.14
Bijlee plus (V_2)	T_0	7.13 d ± 0.15
	T_1	8.13 cd ± 0.42
	T_2	9.13 c± 0.57
	T_3	8.47 c ± 0.59
	T_4	8.56 c ± 0.57
	T_5	13.23 a ± 0.81
	T_6	10.26 b ± 0.57
	LSD ($p \leq 0.05$)	1.03

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust-bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.3.2. Relation between Fusarium wilt severity (%) and Total soluble solids (°Brix.) content in chilli

In the regression, Total Soluble Solid (%°Brix.) was considered as independent and Fusarium wilt severity (%) as dependent variable. A strong negative relation was existing between the disease severity and Total Soluble Solid (%°Brix.). Due to disease in Roshni and Bijlee plus, (15.69% and 19.94%) loss of TSS content was observed. The regression equation also showed that, for each unit increase in disease severity, TSS reduction of (0.45% and 0.43%) will be occurred (Fig. 11).

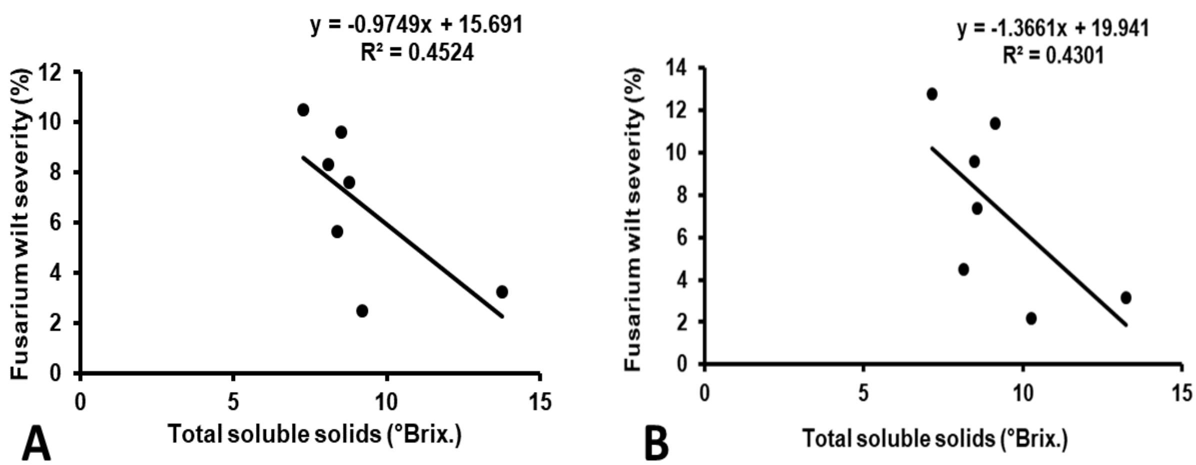

Fig. 11. Relation between Fusarium wilt severity (%) and Total soluble solids (°Brix.) content in chilli.

4.3.3. Efficacy of formulated bio-fungicide on Total chlorophyll (mg/mL) content in chilli variety Roshni and Bijlee plus at 120 DAT

In Roshni, T_5 showed maximum amount of chlorophyll in fruit (0.15 mg/ml) followed by T_1 (0.11 mg/mL), T_4 (0.027 mg/mL), T_2 (0.024 mg/mL), T_3 (0.036 mg/mL) and T_6 (0.02 mg/mL). However, T_0 showed lowest amount of chlorophyll in fruit (0.04 mg/mL) (Fig. 12).

In Bijlee plus, T_5 showed maximum amount of chlorophyll in fruit (0.143 mg/mL) followed by T_3 (0.083 mg/mL), T_2 (0.070 mg/mL), T_6 (0.066 mg/mL), T_4

(0.067 mg/mL) and T_1 (0.059 mg/mL). However, T_0 showed lowest amount of chlorophyll in fruit (0.058 mg/mL) (Fig. 12).

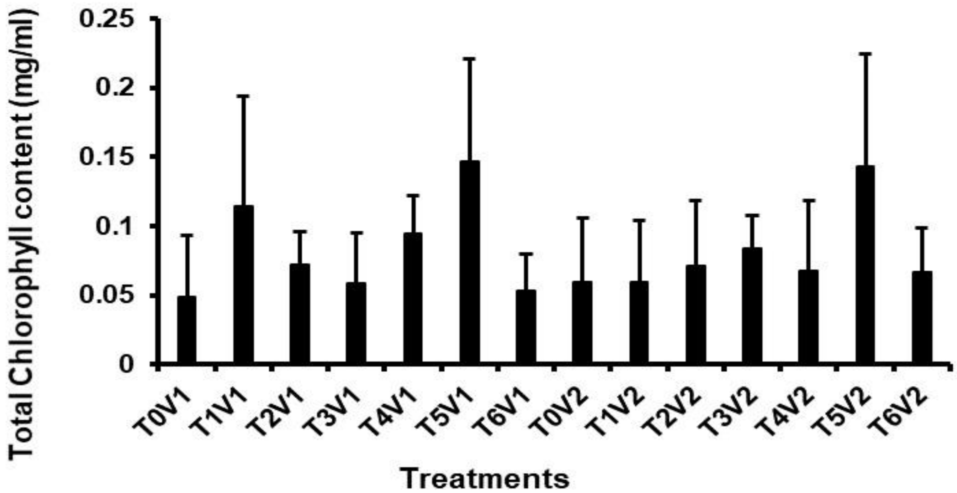

Fig. 12. Efficacy of formulated bio-fungicide on Total chlorophyll (mg/mL) content in chilli variety at 120 DAT

4.3.4. Efficacy of formulated bio-fungicide on Total phenol content (mg/100g) in chilli variety at 120 DAT

In Roshni, among all the formulated bio-fungicide, T_5 treated plants showed the higher amount of phenol content in fruit (4.56 mg/100g) followed by T_1 (4.0mg/100g), T_4 (3.85 mg/100g), T_6 (3.81 mg/100g), T_3 (3.69 mg/100g) and T_2 (3.57 mg/100g). However, only T_0 showed the lower amount of phenol content in fruit (3.20 mg/100g) (Table 16).

In Bijlee plus, among all the formulated bio-fungicide, T_5 treated plants showed the higher amount of phenol content in fruit (4.81 mg/100g) followed by T_2 (3.93 mg/100g), T_6 (3.89 mg/100g), T_4 (3.89 mg/100g), T_1 (3.84 mg/100g) and T_3 (3.38 mg/100g). However, only T_0 showed the lower amount of phenol content in fruit (3.38 mg/100g) (Table 16).

Table 16. Efficacy of formulated bio-fungicide on Total phenol content (mg/100g) in chilli variety at 120 DAT

Variety	Formulated bio-fungicide	Total Phenol Content(mg/100g)
Roshni (V_1)	T_0	3.2 d ±0.01
	T_1	4 b ±0.19
	T_2	3.58 c ±0.37
	T_3	3.69 c ±0.04
	T_4	3.85 bc ±0.04
	T_5	4.56 a ±0.04
	T_6	3.81 bc ±0.06
	LSD ($p \leq 0.05$)	0.27
Bijlee plus (V_2)	T_0	3.37 c ±0.44
	T_1	3.84 bc ±0.28
	T_2	3.93 b ±0.26
	T_3	3.71 bc ±0.16
	T_4	3.89 bc±0.39
	T_5	4.81 a ±0.072
	T_6	3.89 bc ±0.30
	LSD ($p \leq 0.05$)	0.53

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17 + suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17 + suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17 + suspension of *Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17 + suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust bran-based formulated *Bacillus cereus* HSTUB 17 + suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.3.5. Relation between Fusarium wilt severity (%) and Total phenol content (mg/100g) in chilli

In the regression, total phenol content (mg/100g) was considered as independent and fusarium wilt severity (%) as dependent variable. A strong negative relation was existing between the disease severity and total phenol content (mg/100g). Due to disease in Roshni and Bijlee plus, (26.60 % and 30.53 %) loss of total phenol content content (mg/100g) was observed. The regression equation also showed that, for each unit increase in disease severity, total phenol content (mg/100g) reduction of (0.49 % and 0.38 %) will be occurred (Fig. 13).

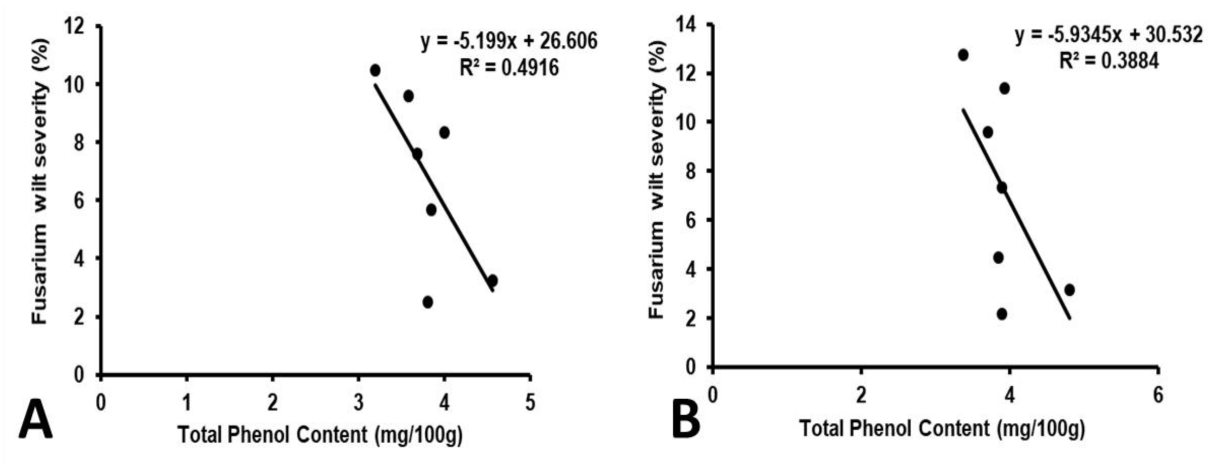

Fig. 13. Relation between Fusarium wilt severity (%) and Total phenol content (mg/100g) in chilli.

4.3.6. Efficacy of formulated bio-fungicide on Antioxidant (mg/g) in chilli variety at 120 DAT

In Roshni, among all the formulated bio-fungicide, T_5 treated plants showed the higher amount of antioxidant in fruit (9.15 mg/g) followed by T_4 (8.22 mg/g), T_3 (7.88 mg/g), T_2 (7.79 mg/g), T_6 (7.62 mg/g) and T_1 (6.78 mg/g). However, only T_0 showed the lower amount of antioxidant in fruit (6.25 mg/g) (Table 17).

In Bijlee plus, among all the formulated bio-fungicide, T_5 treated plants showed the higher amount of antioxidant in fruit (8.13 mg/g) followed by T_3 (7.69 mg/g), T_4 (7.63 mg/g), T_2 (7.61 mg/g), T_1 (7.59 mg/g) and T_6 (6.26 mg/g). However, only T_0 showed the lower amount of antioxidant in fruit (5.92 mg/g) (Table 17).

Table 17. Efficacy of formulated bio-fungicide on Antioxidant (mg/g) in chilli variety at 120 DAT

Variety	Formulated bio-fungicide	Antioxidant (mg/g)
Roshni (V_1)	T_0	6.25 f ±0.109
	T_1	6.78 e ±0.087
	T_2	7.78 c ±0.140
	T_3	7.88 c ±0.005
	T_4	8.23 b ±0.015
	T_5	9.15 a ±0.005
	T_6	7.62 d ±0.066
	LSD ($p \leq 0.05$)	0.133
Bijlee plus (V_2)	T_0	5.92 e ±0.040
	T_1	7.59 c ±0.040
	T_2	7.60 c ±0.075
	T_3	7.69 b ±0.040
	T_4	7.63 bc ±0.035
	T_5	8.13 a ±0.028
	T_6	6.26 d ±0.064
	LSD ($p \leq 0.05$)	0.084

Means followed by different letter(s) in the column are significantly different according to Duncans's multiple range test at p=0.05. T_0: Control; T_1: Soybean bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T2: Charcoal bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_3: Pea bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_4: Maize bran-based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_5:Wood dust-bran based formulated *Bacillus cereus* HSTUB 17+ suspension of *Bacillus cereus* HSTUB 17; T_6: Autostin 50 WDG (Carbendazim).

4.3.7. Relation between Fusarium wilt severity (%) and antioxidant (mg/g) in chilli

In the regression, antioxidant (mg/g) was considered as independent and fusarium wilt severity (%) as dependent variable. A strong negative relation was existing between the disease severity and antioxidant (mg/g). Due to disease in Roshni and Bijlee plus, (23.54 % and 14.86 %) loss of total phenol content content (mg/100g) was observed. The regression equation also showed that, for each unit increase in disease severity, antioxidant (mg/g) reduction of (0.45 % and 0.04 %) will be occurred (Fig. 14).

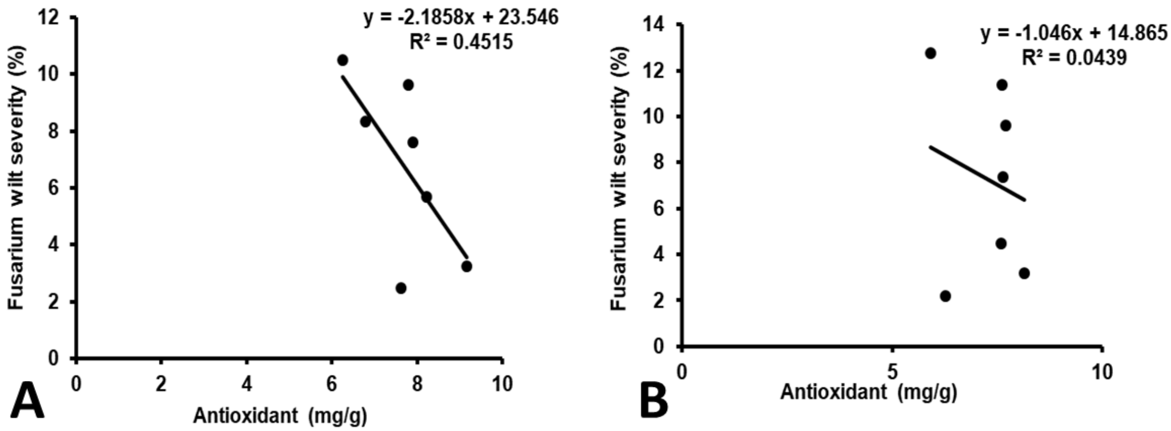

Fig. 14. Relation between Fusarium wilt severity (%) and antioxidant (mg/g) in chilli.

4.4. Shelf life of the developed bio-fungicides in different storage conditions

The shelf life of charcoal-based, maize bran-based, wood dust-based, soybean bran-based and pea bran-based formulated native *B. cereus* HSTUB 17 were evaluated in different stage conditions A. Wooden shelve condition (at room temperature) and B. Refrigerator condition (4^0 C). In case of wooden shelve storage conditions, the spore density of all formulated product were found to increase from its initial populations (95×10^9, 157×10^9, 251×10^9, 267×10^9, 212×10^9, 149.5×10^9, 142.5×10^9, 90×10^9, 47×10^9) reached at peak at 240 DAT charcoal-based with *B. cereus* HSTUB 17. The spore density of *B. cereus* HSTUB 17 with formulated soybean bran-based were found (78×10^9, 119×10^9, 187×10^9, 239×10^9, 188×10^9, 135×10^9, 127×10^9, 83×10^9, 28×10^9). The spore density of *B. cereus* HSTUB 17 with formulated wood dust-based were found (109×10^9, 253×10^9, 462.5×10^9, 518×10^9, 435×10^9, 328.5×10^9, 267.5×10^9, 150×10^9, 64.5×10^9). The spore density of *B. cereus* HSTUB 17 with formulated maize bran-based were found (65×10^9, 125×10^9, 289×10^9, 326×10^9, 289×10^9, 256×10^9, 191.5×10^9, 70×10^9, 30×10^9). The spore density of *B. cereus* HSTUB 17 with formulated pea bran-based were found (81×10^9, 188×10^9, 321.5×10^9, 375×10^9, 402×10^9, 305×10^9, 199×10^9, 140×10^9, 37.5×10^9)

reached at peak at 240 DAS. However, all the formulated products declines after reaching the peak and even after 240 DAS *B. cereus* HSTUB 17 with formulated wood dust-based was shown higher longevity (64.5×10^9) and soybean bran-based with *B. cereus* HSTUB 17 was shown lower longevity (28×10^9) at 240 DAS. (Fig. 15.A)

In case of refrigerator storage conditions, the spore density of all formulated product were found to increase from its initial populations (154×10^9, 245×10^9, 335×10^9, 402×10^9, 329×10^9, 188×10^9, 125×10^9, 91×10^9, 44×10^9) reached at peak at 240 DAT charcoal-based with *B. cereus* HSTUB 17. The spore density of *B. cereus* HSTUB 17 with formulated soybean bran-based were found (222×10^9, 589×10^9, 632×10^9, 716.5×10^9, 580×10^9, 446.5×10^9, 332.5×10^9, 250×10^9, 136×10^9). The spore density of *B. cereus* HSTUB 17 with formulated wood dust-based were found (288×10^9, 770.5×10^9, 816.5×10^9, 847.5×10^9, 724×10^9, 611×10^9, 456.5×10^9, 339.5×10^9, 253×10^9). The spore density of *B. cereus* HSTUB 17 with formulated maize bran-based were found (133×10^9, 452.5×10^9, 525.5×10^9, 557×10^9, 415×10^9, 319.5×10^9, 232×10^9, 181×10^9, 87.5×10^9). The spore density of *B. cereus* HSTUB 17 with formulated pea bran-based were found (105×10^9, 259×10^9, 435.5×10^9, 466×10^9, 387×10^9, 267×10^9, 216×10^9, 161×10^9, 76×10^9) reached at peak at 240 DAS. However, all the formulated products declines after reaching the peak and even after 240 DAS. *B. cereus* HSTUB 17 with formulated wood dust-based was shown higher longevity (253×10^9) and *B. cereus* HSTUB 17 with formulated charcoal based was shown lower longevity (44×10^9) at 240 DAS. (Fig. 15.B)

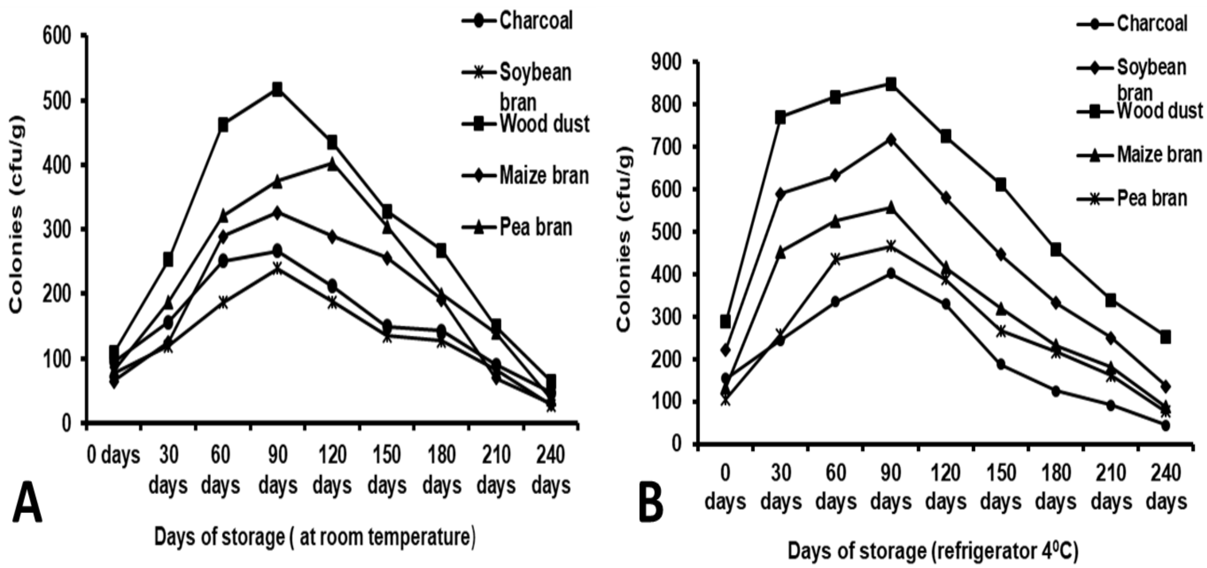

Fig. 15. Shelf life of formulated bio-fungicide in different stage condition: A. At room temperature B. Refrigerator condition (4^0C)

CHAPTER V

DISCUSSION

A significant cash crop grown all throughout the world, but especially in Asian nations, is chilli pepper. It is a member of the Solanaceae family, which is home to over 3000 different plant species. Chilli is susceptible to a range of diseases at different stages of development. One of the major diseases that is thought to be the most harmful to chilli (*Capsicum annuum* L.) is fusarium wilt, which is caused by *Fusarium oxysporum*. Bangladeshi people are losing a lot of money as a result of Fusarium wilt of chili. Thus, the use of pesticides by farmers is causing harm to several things. According to Jayaraj et al. (2016), microorganisms that are exposed to high amounts of chemicals are also developing resistance to such chemicals. Concerns over the toxic consequences of chemical pesticides, which are used to manage a variety of diseases and pests, are shared by people worldwide. Because of this, there is a growing interest in employing biological control techniques to stop the spread of dangerous microorganisms. These days, biological management techniques are being considered as viable substitutes for chemical treatments in the fight against crop disease. Plant infections can be effectively combated by Biological Control Agents (Reddy et al., 2018). The present investigations aim to develop a formulated bio-fungicide using a potential antifungal bacteria namely *B. cereus* HSTUB 17 for the eco-friendly management of wilt of chilli.

In the field conditions, all the formulated bio-fungicide reduced the Fusarium wilt severity (%). However, wood dust based formulated bio-fungicide followed by spraying of *B. cereus* HSTUB 17 suspension reduced maximum fusarium wilt severity (%). According to the findings of Yulmira Yanti *et. al.* (2021) introducing a solid *B. cereus* strain SLBE3.1AP formula to chili plants with varying storage durations indicated that each introduced formula was able to prevent the growth of

Fusarium wilt disease. The incubation period, disease incidence, and severity of Fusarium wilt disease in chili plants were all consistently suppressed by all formulations of *B. cereus* strain SLBE3.1AP. This is probably because the formula is made from endophytic bacteria, which have a lot of enzymes and transporters that carry nutrients necessary for bacteria to grow. It also has a long shelf life. The optimal formulation lasts for six weeks, during which *B. cereus* can generate a number of resistance molecules. This is consistent with Taghavi *et. al.* (2005) assertion in Yuniawati et. al. (2019) study that endophytic bacteria may create chemicals known to induce plant resistance, such as salicylic acid, ethylene, and enzymes.

The formulation of *B. cereus* HSTUB 17 introduced into chili seeds was able to increase chili growth in the seedling phase. The formulation was able to increase seedling field emergence, seedling height, number of seedling leaves, flower, branches and fruit number compared to control (without treatment). From the research results, the formulated bio-fungicide is increasing seedling growth with 100% effectiveness was the wood dust based formulated *B. cereus* HSTUB 17. This is apparently because the optimal formulation includes a mixture of carriers; the more carriers present in the formulation, the more nutrients it contains, boosting plant development and root fertility. *B. cereus* HSTUB 17 was added to all prepared items in this investigation to enhance the overall phenol content. The *B. cereus* HSTUB 17 product prepared with wood dust and sprayed with *B. cereus* HSTUB 17 suspension had the highest total phenol level of all the items. Higher phenol content is linked to increased antioxidant capacity and pest and disease resistance in a variety of crops, according to Kandoliya and Vakharia (2013). Furthermore, a number of studies have found a strong link between antioxidant activity and the total phenol content of plant extracts (Kandoliya et al. 2015; Kandoliya et al. 2016).

According to the Krishna et al. (2010) the antioxidants and antiradicals found in spices like pepper and chili are essential in eliminating harmful free radicals, which improves health and lowers the risk of chronic illnesses. Their unpublished results also showed a significant relationship between the total phenol concentration of spice extracts and their antioxidant activity. Thus, they have shown that spice extracts work effectively as radical scavengers in a variety of in vitro settings. Their next study will focus on the potential benefits of using spice extracts instead of synthetic antioxidants for oil and food preservation, as they are non-toxic and can stabilize oils.

CHAPTER VI

SUMMARY AND CONCLUSION

An essential vegetable crop in Bangladesh is chilli (*Capsicum annuum L.*). Because it can be produced and sold every week, chili is a terrific way to support the lives of small-farm owners. *Fusarium oxysporum*, the primary cause of fusarium wilt, is one of the many diseases that limit sustainable output. The disease's chemical control is primarily an expensive endeavor that also poses health risks to people and the environment. Due to its less expensive and environmentally friendly nature, biological management of plant diseases, especially those caused by fungi, has been seen as a promising substitute for chemical control.

As *B. cereus* HSTUB 17 showed potential antifungal efficacy against the causal agent of fusarium wilt if chilli, bacteria were used in the field level for the management of fusarium wilt. For the integrated management of fusarium wilt of chilli *B. cereus* HSTUB 17 were formulated with Charcoal bran-based formulated *B. cereus* HSTUB 17 + suspension of *B. cereus* HSTUB 17, Maize bran-based formulated *B. cereus* HSTUB 17 + suspension of *B. cereus* HSTUB 17, Wood dust bran-based formulated *B. cereus* HSTUB 17 + suspension of *B. cereus* HSTUB 17, pea bran-based formulated *B. cereus* HSTUB 17 + suspension of *B. cereus* HSTUB 17 and Soybean bran-based formulated *B. cereus* HSTUB 17 + suspension of *B. cereus* HSTUB 17. Among all the formulated products applied in the field, wood dust based formulated *B. cereus* HSTUB 17 + suspension of *B. cereus* HSTUB 17 showed reduction of disease severity (69.01%), (75.21%) in two variety. Wood dust based formulated *B. cereus* HSTUB 17 + suspension of *B. cereus* HSTUB 17 gave maximum yield (2.67 kg/plot and 2.69 kg/plot) in two variety. Total phenol content in two variety, wood dust based formulated *B. cereus* HSTUB 17 + suspension of *B. cereus* HSTUB 17 gave maximum phenol (4.56 mg/100g and 4.81 mg/100g, respectively) followed by the other formulated products. Total soluble solids (%), application of wood dust based formulated *B. cereus* HSTUB 17 + suspension of *B. cereus* HSTUB 17 gave highest soluble solids (13.77 % and 13.23 % respectively) in both variety. Total chlorophyll content in chilli fruits in two variety, application of wood dust based formulated *B. cereus* HSTUB 17 + suspension of *B. cereus* HSTUB 17 gave maximum amount (0.15 mg/ml and 0.14 mg/ml respectively). The antioxidant amount of chilli fruits in two variety, application of wood dust based formulated *B. cereus* HSTUB 17 + suspension of *B. cereus* HSTUB 17 gave maximum amount (9.15 mg/g and 8.13 mg/g respectively). The overall results of the study demonstrated the potentiality of *B. cereus* HSTUB 17 for the eco-friendly management of fusarium wilt of chilli. Considering the eco-friendly management, the wood dust based formulated bacteria and suspension as biocontrol agents could be used in the farmer's field for the sustainable management of fusarium wilt of chilli and other vegetables. However, before recommended both the bacteria as bio-fungicide, location specifies trial with different varieties of chilli is urgent.

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