RESEARCH ARTICLE

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SCREENHOUSE EVALUATION OF THE FUNGICIDES AND BIO-CONTROL AGENTS FOR THE MANAGEMENT OF FUSARIUM WILT (Foc Race 1) OF BANANA IN NEPAL

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ABSTRACT

Banana is affected by a wide number of diseases, of which, Fusarium wilt caused by Fusarium oxysporum f.sp. cubense has played a major role in devastating Malbhog (Silk) banana plantations in Nepal. The objective of this study was to evaluate the commonly available fungicides (Carbendazim 50%, Fenamidone 10%+ Mancozeb 50%, Chlorothalonil and biocontrol agents (Trichoderma sps., Bacillus thuringiensis and Pseudomonas species) for their efficacy against Foc. The effectiveness of fungicides and biocontrol agents to Foc was examined in screenhouse. The experiment was laid out in a completely randomized design with three replications and eight treatments. Among the chemicals, the most effective fungicide to reduce Fusarium wilt severity (root disease) was Bavistin with an efficacy of 55.33% followed by Sectin 46.33% and Chlorothalonil 35%. Similarly, maximum efficacy over control was recorded in Trichoderma harzianum with 41% and lowest was 32% in Pseudomonas sp. In the case of leaf disease, the lowest severity was 21.33% recorded in Bavistin and highest was 40.33% in Chlorothalonil. Similarly, among biocontrol agents, the lowest severity was found in Bacillus thuringiensis whereas highest was found in Trichoderma viride. A Suppression of disease by biocontrol agents might be due to their fungicidal activity, which means they produce a variety of antifungal compounds. These compounds induce plant defense mechanisms. Further field evaluations of the fungicides and biocontrol agents are required to determine if the effect observed in the screenhouse can be integrated into field management of Fusarium wilt.

1. INTRODUCTION

Banana (Musa spp.) is an important crop in many tropical and subtropical regions of the world, providing a source of food and income for millions of people. In terms of the potential growing area, production and domestic consumption, banana is one of the major fruit crops of Nepal. It is cultivated on 10,557 ha of land, with a total production of 204, 009,69 tons and productivity of 19.32 ton/ha (MoALD, 2020).

Malbhog is a variety of banana known for its sweet taste and juicy texture, making it a popular choice for both fresh eating and cooking. In Nepal, Malbhog bananas are typically grown in the terai region and are important crop for local farmers. Banana is also an important source of food and nutrition for many families in the country and suffer production losses due to *Fusarium oxysporum* f.sp. *cubense*. The disease is highly destructive to the Cavendish variety, which is the most widely grown banana variety in the world. The soil-borne fungus, *Fusarium oxysporum* f. sps. *cubense* causes the fusarium wilt of banana *(*Panamá disease*)* (E.F. Smith) Snyder and Hansen (*Foc*) (Stover, 1962). The fungus attacks the plants vascular system, causing wilting and eventually leading to plant death. This fungus also survives in plant debris and finds its way into the banana plant in most cases through damage to the roots. Fusarium wilt has been known to be a destructive disease all over the world where bananas are grown (Castle, 2009). Although, this disease probably originated in Southeast Asia (Ploetz & Pegg, 2000), it was first discovered in Australia in 1876. Recently most of the Malbhog banana growers in Nepal are switching to wilt resistant variety (Grand Nain) because of fear of

wilt disease (Paudel, 2020).

Panama wilt has been in existence since 19th Century, but its effect was noticed in the 20th Century where it wiped out plantations of crop in Panama and other parts of the world. The most popular variety in Panama: 'Gros Michel' comprised all the export production then. It's susceptibility to Fusarium wilt threatened complete wipe out of banana production (Castle, 2009). This disease is present in major banana growing regions of Nepal such as Chitwan, Nawalparasi, Bardia, Kailali and Kanchanpur district. Affected variety in Nepal is Malbhog (Silk, AAB) where upto 80% of the banana field may show symptoms of the disease (Personal communication with farmers).

Currently, there are three races of *F. oxysporum* f. sp. *cubense,* based on their banana hosts*,* Race 1 (Foc 1), Race 2 (FoC 2) and Race 4 (FoC 4). Race 4 has two strains that are TR4 (Tropical Race 4) and STR4 (Subtropical Race 4). TR4 (Tropical Race 4) is the most virulent and widespread race that affects Cavendish bananas and all the cultivars that are sensitive to other Races of *Foc* (Cheng *et al*., 2019a, 2019b). It is important to note that each race has different virulence factors and can cause different levels of damage to banana. Mainly, the severity of this disease is influenced by the level of stress, for example during the extended period of floods, imbalance of nutritional contents and limited or low temperatures and increased levels of salinity. The disease development is favored by the soil of warm, moist, and increased high levels of temperature with optimized soil temperatures for root infection being 30°C or above (Castle,2009).

Fusarium wilt is most serious problem to banana production in the Chitwan, Nawalparasi and Bara districts of Nepal (Personal communication with farmers) and has been causing destruction of banana fields. This has led to the abandonment of hectares of land. An irreversible and untreatable infection is caused by Tropical Race 4(TR4) of *Fusarium oxysporum* f.sp. *cubense.,* therefore its economic impact is profound. The global industry of banana is under serious threat due to the ease with which Fusarium wilt spread (Siamak & Zheng, 2018). Rhizome and infected suckers are the main source of disease spread. The spread may also occur through movement of infested soils adhering to vehicles, footwear, and animal feet over long distances. It can be spread within short distances through roots networks and in surface presence of run-off water (Daly & Walduck, 2006). From the discovery of Panama wilt disease, different control measures such as soil fumigation and Fungicides (Herbert & Marx, 1990); Crop rotation (Hwang, 1991; Su, Hwang, & Ko, 1986); Flood fallowing (Wardlaw, 1961) have been evolved and used, yet, the disease could not be controlled effectively except by planting of resistant varieties (Moore, Bentley, Pegg and Jones, 1995). Because of consumers preference, planting of resistant varieties also cannot be implemented (Viljoen, 2002). Under such circumstances, use of antagonistic microbes which protect and promote plant growth could be an alternative approach for the management of banana wilt. Several strategies have been tried on banana for the management of this disease, however, only a few strategies such as replacement of susceptible cultivar with resistant cultivar (Gand Nain) being the alternative control means in Nepal (Personal communication with farmers).

Trichoderma species are soil borne fungi and are known for their ability to produce various enzymes and secondary metabolites that have antifungal activity and can induce plant defense mechanisms. Pseudomonas and Bacillus species also produce a variety of antifungal compounds.

The aim of this study was to determine the efficacy of Fungicides and biocontrol agents in the management of *Fusarium oxysporum* f.sp. *cubense* race 1of banana in Screenhouse condition.

2. MATERIALS AND METHODS

An experiment was conducted during 2019/20 and 2020/21 at National Plant Pathology Research Center of Nepal Agricultural Research Council, Nepal to find out the effective fungicides and biocontrol agents against *Fusarium oxysporum* f. sp. *Cubense* race 1 *in-vivo*. The experiment was conducted in Complete Randomized design with three replications and eight treatments including control. Per treatments there were three plants.

2.1. Isolate used and inoculum preparation.

An isolate of *Foc* race-1 used in this study was isolated from diseased banana plants (Malbhog variety) collected from Nawalparasi distrit of Nepal. For the evaluation of fungicides and biocontrol agents, pathogenic isolate of *Foc* was grown for 7 days on PDA at 25-27°C in an incubator. Conidial spore suspension was prepared by transferring mycelia into 250ml conical flask containing water. The flasks were placed on a shaker operating at 170 rpm at 25°C for 5 days, after which the conidia

suspension was passed through muslin cloth to separate the mycelium from the spores. Concentration of spores in the remaining liquid was determined by using a hemocytometer, and diluted with sterile distilled water to a final concentration of 10^6 spores/ml.

2.2. Selection of fungicides and biocontrol agents

Three fungicides, named Carbendazim 50% (Bavistin), Fenamidone 10%+ Mancozeb 50%, (Sectin) and Chlorothalonil were selected for in vivo evaluation against *Foc*. These fungicides were selected based on their performance in the in vitro evaluation. Four biocontrol agents were also selected for evaluation against *Foc*. The name of different treatment used in this study were: T1-Bavistin, T2- Sectin, T3- *Trichoderma viride*., T4- *Trichoderma harzianum*, T5- Chlorothalonil, T6- *Bacillus thuringiensis*, T7- *Pseudomonas* sp. and T8- Water.

2.3. In- vivo evaluation of fungicides and biocontrol agents

Tissue cultured plantlets of banana of Malbhog variety were obtained from biotech banana nursery at Lalitpur. Banana plants with well-developed roots were selected for pot trial evaluation of fungicides and biocontrol agents against Fusarium wilt. The sand and soil combination were steam-sterilized before *Foc* inoculation. The potting mixture (sand, soil, and manure) was then deposited into pots. The Soil drenching method of application for disease inoculum and treatments (fungicides and biocontrol agents) was performed. Drenching for inoculum (100ml/ plant) was done after three days of planting. Four days after disease inoculation, drenching treatments with same dose was performed. Control plants were treated with 100ml of distilled water as a treatment. Disease development was estimated by observing wilt severity of external and internal appearance of plants. There was regular inspection of plants showing typical symptoms of wilting and leaf yellowing. The pseudostems of symptomatic individuals were further examined for the presence of the reddish-brown vascular discoloration characteristics of *Foc* infection. In the case of screenhouse experiments, when 50% of the control plants displayed leaf symptoms, then plants were considered ready for rating (Viljoen *et al*., 2017). Symptoms were recorded every 4 days until all the untreated control plantlets showed clear disease symptoms. Disease rating scale for external symptoms (leaf) was 1-5, where 1=No disease, 2= Little chlorosis and wilting with no petiole bending, 3= Moderate chlorosis and splitting of leaf base and wilting with some petiole bending, 4= Severe chlorosis and wilting, petiole bending and stunting of newly emerged leaf and 5= Dead.

(LDS) Leaf disease severity (%) = [Total sum of numerical rating/ (Total number of plants observed x Maximun category in the score chart)] ×100

Similarly, disease rating scale for internal symptoms or Rhizome discoloration was 1-6, where 1= No internal symptoms, 2= Few internal spots, 3= < 1/3 rhizome discolored, 4= 1/3 to 2/3 rhizome discolored, 5= > 1/3 discolored and 6= entire inner rhizome discolored (International Musa Testing Programme rating scale).

(RDS) Root disease severity (%) = [Total sum of numerical rating/ (Total number of plants observed x Maximun category in the score chart)] ×100

The efficacy of treatments was determined based on the severity of Fusarium wilt. Efficacy of the treatment was calculated using the formula as follow:

Efficacy = [(Disease severity of control - Disease severity of treatment)/ Disease severity of control] x 100

2.4. Inoculum preparation for trichoderma, bacillus and pseudomonas spp

Two Trichoderma isolate such as *Trichoderma Viride* and *Trichoderma harzianum* were obtained from Nepalese type culture collection of National Plant Pathology Research Center, Khumaltar, NARC. Inoculum was prepared by inoculating 5 days old culture of *Trichoderma* isolate in potato dextrose broth (Potato starch infusion 4gm/lit, and Dextrose 20gm/ lit) with subsequent incubation for 5 days at 28°C in an incubated shaker. Then the spore suspension was obtained by filtering the broth through muslin cloth. The spore density was determined by hemocytometer and filtrate was then diluted to 10^7 spores/ml.

Bacillus and *Pseudomonas* sp. used in this study were obtained from Central Department of Microbiology, Kritipur Kathmandu, Nepal. Formulation of *Bacillus* and *Pseudomonas* sp. was performed by submerged fermentation (SmF) /liquid fermentation (LF) technique as described by (Ralte, Nachimuthu, & Guruswami, 2016) with slight modification by inoculating the isolates in a 250ml conical flask containing sterilized Luria-Bertani (LB) broth (Tryptone 10g/lit, NaCl 10g/ lit, and yeast extract 5g/lit). The conical flask was

placed in a shaker water bath at 35°C for 90hrs until the colony forming unit (cfu/ml) was greater than $10^{9.}$ The inoculum was then adjusted to 10^{7} spores/ml.

2.5. Data Analysis

The statistical package IRRI STAR was used for the analysis of variance to test the significance of treatment effect on wilt severity of *Fusarium oxysporum* f.sp. *cubense*. Tukeys Honest Significant Difference test was used to compare the values of significant treatment means at 0.05% level of significance.

3. RESULTS AND DISCUSSION

An isolate of the pathogen was collected from banana fields in Nawalparasi in 2019. Pathogenicity test of the isolate was confirmed on cultivar Malbhog. Different chemicals and biocontrol agents were tested against *Fusarium oxysporum.* f.sp .*cubense* in pot trial condition. After 8 weeks of pathogen inoculation, the symptoms of Fusarium wilt disease were observed and recorded. The first indication of the disease was yellowing and drooping of lower leaves. Some plants exhibited dark brown vascular discoloration, splitting of pseudostem and death. Therefore, the pathogenicity of tested isolate on banana variety Malbhog was clearly distinct. The same fungus was reisolated from the discolored pseudostem and rhizome of the diseased plant.

Rating 1 Rating 2 Rating 3 Rating 4 Rating 5

Figure 1. Scoring of disease development according to the rhizome discoloration

Identification of *Fusarium oxysporum* f.sp. *cubense* was based on microscopic observation of different spores such as Microconidia, Macroconidia and Chlamydospores (Fourie, Steenkamp, Gordon, & Viljoen, 2009). The mycelial color of the pathogen was white cottony with purple tinge. Microconidia were borne on conidiophores and are small, thin walled. Macro- conidia were larger, thick walled and produced in chains and chlamydospores were thick-walled, spherical structures.

In our study, chemical fungicide Bavistin showed least leaf disease severity of 21.33%, which is consistent with the study of (Nel, Steinberg, Labuschagne, & Viljoen, 2007), that showed chemical fungicide belonging to the group of benzimidazole including carbendazim had limited success against Fusarium wilt. Similarly, Sectin and Chlorothalonil gave 29% and 35.67% wilt severity respectively. The results of the performed test indicated that Bavistin was the most effective fungicide with efficacy of 70.33% followed by Sectin 59.33% and Chlorothalonil 44%. However, the use of fungicide alone is not always sufficient to control Fusarium wilt, as the disease is often associated with soil borne pathogens that can persist in the soil for many years. Therefore, other control options such as the use of biocontrol agents are often recommended to manage Fusarium wilt in banana plants. Application of biocontrol agents as drenching after 4 days of disease inoculation significantly decreased mean leaf disease severity compared to *Foc* alone inoculated control plants. Among these isolates, *Trichoderma harzianum* and *Bacillus thuringiensis* gave the lowest wilt severity of 35.67%. A study carried out by ((Thangavelu & Mustaffa, 2012) showed that use of *Bacillus* strain,

KY-21 results in 35% wilt severity and 18.3% severe vascular discoloration. and *Trichoderma viride* showed wilt severity of 39.33% and *Pseudomonas* sp. showed highest wilt severity of 40%. In this study, biocontrol agents *Bacillus* and *Trichoderma harzianm* showed efficacy of 50%. According to (Bubici, Kaushal, Prigigallo, Gómez-Lama Cabanás, & Mercado-Blanco, 2019) 40-42% efficacy had been obtained by the application of *Bacillus* spp. in the field condition. An efficacy of 45.33% was found in *Pseudomonas* sp. applied treatment and 44% was found in *T. viride*. In the study of (Sivamani & Gnanamanickam, 1988), *P. fluorescens* showed less severe wilting and internal discoloration due to *Foc* in green house experiment in the seedlings of *Musa balbisiana*. Similarly in the case of root disease scoring, chemical fungicide Bavistin gave 29% severity followed by Sectin 34.33% and chlorothalonil 43.67%. Application of biocontrol agents such as *Pseudomonas* showed the highest wilt severity of 43.67% whereas *Trichoderma harzianum* gave 38% wilt severity. *Bacillus thuringiensis* and *Trichoderma viride* showed 42% wilt severity as compared to control. *Foc* alone- inoculated plants had a mean wilt severity of 64%. Among chemical fungicides, 55% efficacy was found in Bavistin applied treatment whereas lowest efficacy 33% was found in Chlorothalonil. Similarly, in the case of biocontrol agents, *Trichoderma harzianum* was found to be most efficient with efficacy of 41% followed by *Trichoderma viride* and *Bacillus thuringiensis* 35% and *Pseudomonas* sp. 32% as compared to control (Table 1).

Table 1. Effectiveness of Fungicides and biocontrol agents against *Fusarium oxysporum* f. sp. *cubense* (mean of first and second year)

	Internal scoring		**External scoring**	
Treatments	**RDS**	**Efficacy**	**LDS**	**Efficacy**
Bavistin	29.00e	55.00a	21.33e	70.33a
Sectin	34.33d	46.33b	29.00d	59.33b
T. *harzianum*	38.00cd	41.00bc	35.67c	50.00c
T. *viride*	42.00bc	35.00cd	40.00bc	44.00c
Chlorothalonil	43.67b	33.00d	40.33b	44.00c
Pseudomonas sp.	43.67b	32.00d	39.33bc	45.33c
Bacillus thuringiensis	42.00bc	35.00cd	35.67c	50.00c
Control	64.00a	0.00e	71.33a	0.00d
CV %	3.56	7.26	4.15	4.74
Tukey's HSD	4.24	7.09	4.58	6.07
P value	0.0000	0.0000	0.0000	0.0000

Disease severity is the mean of three replications of both years. Values in the same column followed by similar letters are not significantly different. HSD: Tukey's Honest significant difference

During 2020, the leaf disease severity was found lowest in Bavitin (22.33%) and highest in Chlorothalonil (46.67%) as compared to control. Similarly, the highest efficacy was found in Bavistin (74.33%) followed by Sectin (61.67%) and Chlorothalonil (46.33%) among fungicides. For biocontrol agents, *Trichoderma viride* showed the highest disease severity (49%) and lowest was found in *Bacillus thuringiensis* (35.65%). The maximum efficacy was found in Bt (59%) and least was found in *T. viride* (44%) as shown in Figure 2.

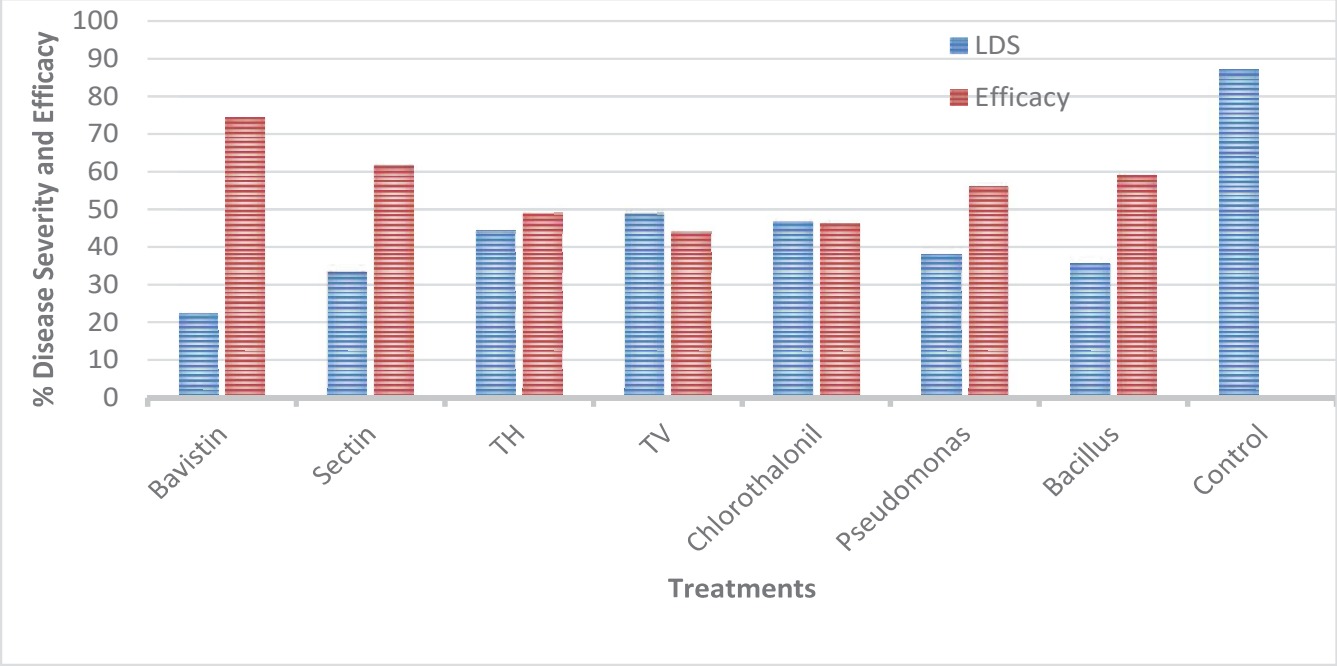

Figure 2. Leaf disease severity of *Fusarium* wilt and efficacy of different treatments during 2020 under screenhouse condition.

In the case of root disease severity, among fungicides, the highest severity was found in Chlorothalonil (48%) and lowest in Bavistin (31.33%). The highest efficacy was found in Bavistin (48.67%) and lowest was found in Chlorothalonil (21.33%). Similarly in the case of biocontrol agents, *T. viride* showed the maximum disease severity of (52%) and least was in Bt (42.67%). The maximum efficacy was shown by Bt (33.33%) and least was found in *T.viride* (14.67%) as shown in Figure 3.

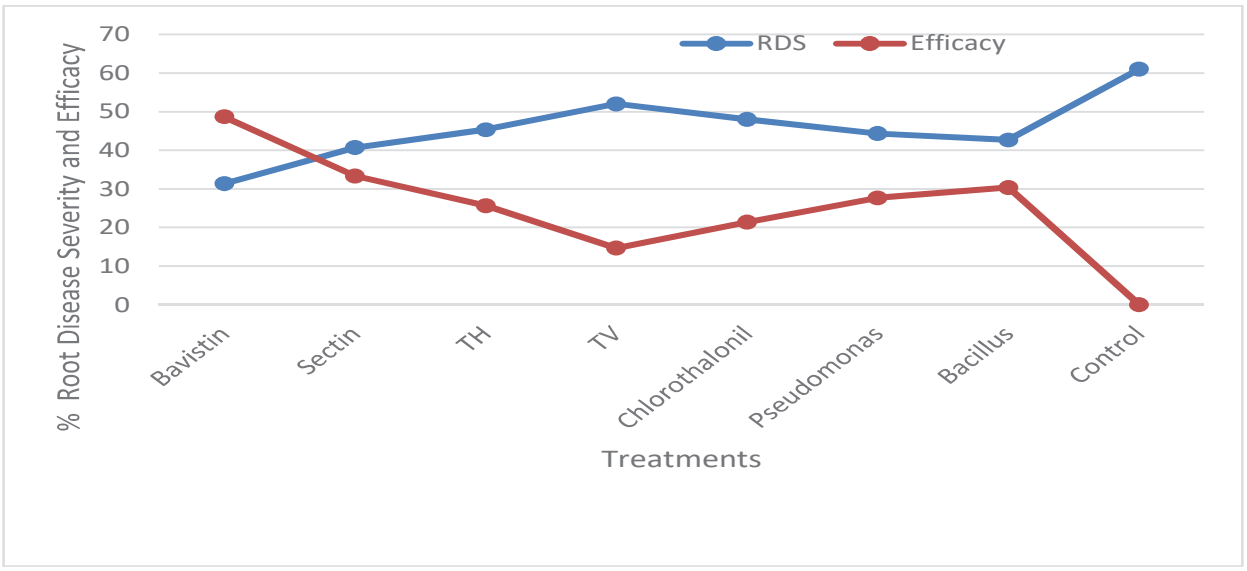

Figure 3. Root disease severity and efficacy of different treatments during 2020 under screenhouse condition

During 2021, the leaf disease severity was found least in Bavistin and highest in Chlorothalonil as compared to control whereas highest efficacy was found in Bavistin and lowest in Chlorothalonil. Similarly in the case of biocontrol agents, the maximum disease severity was found in *Pseudomonas* and least in *T. harzianum* whereas highest efficacy was recorded in *T. harzianum* and lowest was in *Pseudomonas* sp. as shown in Figure 4.

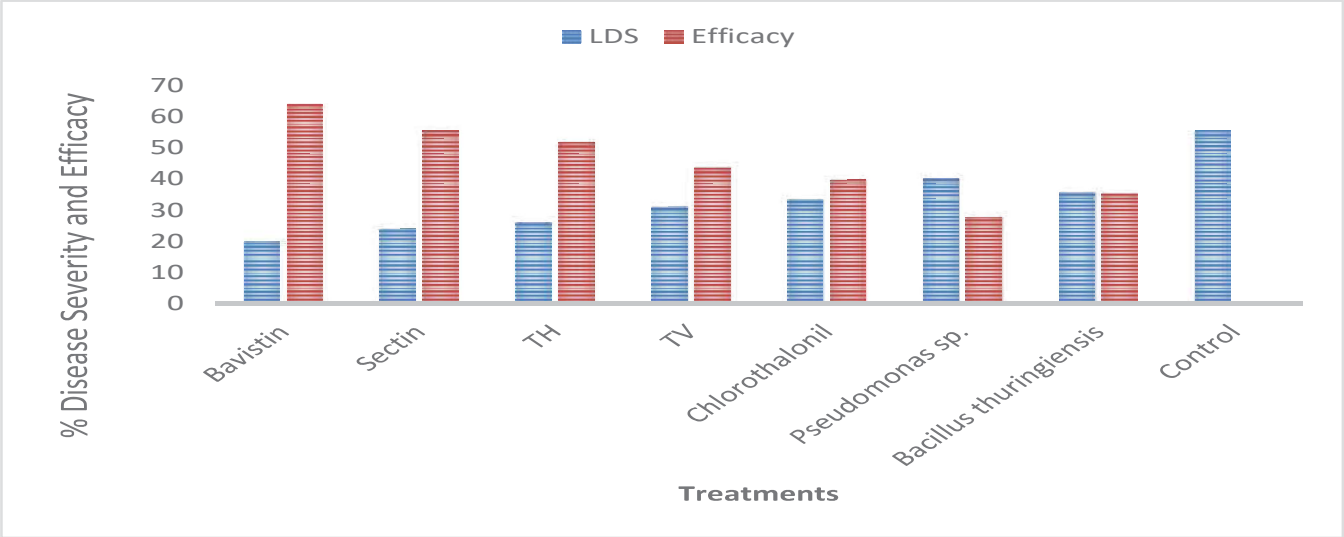

Figure 4. Leaf disease severity and efficacy of different treatments during 2021 under Screenhouse condition.

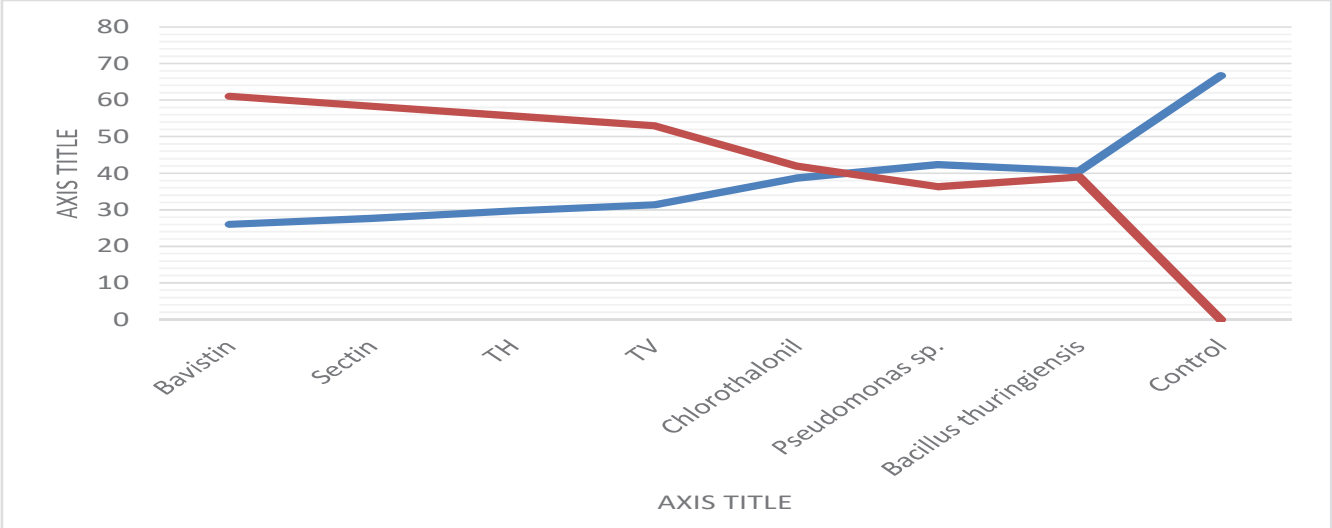

Figure 5. Root disease severity and efficacy of different treatments during 2021 under Screenhouse condition

Disease severity is mean of three replications. RDS= Root disease severity

In the case of root disease severity, the maximum severity was recorded in Chlorothalonil and least was in Bavistin whereas highest efficacy was recorded in Bavistin and lowest was in Chlorothalonil. Similarly for biocontrol agents, the highest severity was found in *Pseudomonas* sp. and lowest was in *T. harzianum* whereas highest efficacy was recorded in *T. harzianum* and lowest was in Pseudomonas sp. as shown in Figure 5.

4. CONCLUSION

Fusarium wilt of banana is a serious disease, caused by soil borne fungus *Fusarium oxysporum* f.sp. *cubense*. Although different Fundicides and biocontrol agents have been tried against Fusarium wilt disease, this lethal disease could still not be controlled completely. The biocontrol agents tested against Fusarium wilt of banana have not yet reached the banana growers,

because the biocontrol experiment was not conducted against Panama wilt in Nepal. This is mainly because of lack of confidence in the efficacy of biocontrol agents in controlling the disease. Therefore, effective biological control methods for the management of Fusarium wilt disease are a) Mixture of biocontrol agents along with or without fungicides or botanicals have to be tried to improve extent of disease control under different environmental and soil conditions b) Suitable and easy method of mass production and delivery system must be selected, c) Mass produced biocontrol-agents should be applied at right quantity (the initial inoculum level of bioagents should be more than the inoculum level of the pathogen) at the right place (at the soil around the rhizosphere) at the right time. For the management of this disease, fungicides such as Bavistin and Chlorothalonil are commonly used. This study evaluated the efficacy of different fungicides and biocontrol agents against *Foc* in banana plants under screenhouse conditions.

The results of the performed test showed that all the used fungicides were effective in reducing severity, but Bavistin was more effective than Sectin and Chlorothalonil (table 1.)

An effective and sustainable approach to control Fusarium wilt disease in banana plants is the use of biocontrol agents such as *Trichoderma, Pseudomonas* and *Bacillus* species. However, their effectiveness depends on several factors such as environmental conditions, banana variety and strain of the pathogen. In Nepal, Bt as a biocontrol agent against Fusarium wilt of banana is still in early stage of development. Further research is needed to optimize their use in banana farming.

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