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Biological Control of Damping off Caused by *Rhizoctonia solani* in Green Beans Crop.

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes in Egypt, consumed as animal protein especially for those who cannot afford in rural areas. It is important to note that damping-off disease caused by *Rhizoctonia solani* is one of the most severe diseases, causing significant damage to the green bean crop. Biological control is one of the most prominent and safest methods for managing various soil-borne diseases. In this study, the effect of nine treatments were conducted in green beans crop *Trichoderma sp.* for injection in soil and seed treatments, *Actinomycets sp.,* Vermi-compost, Bioactive Vermi-compost, Chitosan, Potassium permanganate, Potassium phosphite compared with negative control and Mon Cut® fungicide as chemical agents to control damping off caused by *Rhizoctonia solani*. Through this study, the seed treatments with *Trichoderma harzianum* recorded the highest survival rate in plants (71.8 %), followed by the Bioactive Vermi-compost treatment by concentration 5kg/m (66.2%) compared with negative control (49.4%) followed by Chitosan treatments (58.8 %). While, Mon Cut® chemical fungicide gave result (64.4%) in survival rate of plants. On the other hand, the effect of different treatment on plant growth parameters gave the highest rate by Bioactive Vermi-compost in plant height (65 cm) and number of leaves (17.6) while recorded seed treatment by *T. harzianum* the highest rate in root length (15.9 cm). In conclusion, this study showed that both Bioactive Vermi-compost and the microbes derived from it is capable of control soilborne diseases and promote the plant growth, in accordance with the bio farming system that meets export standards.

Keywords: Biological control; *Rhizoctonia solani*; green beans; damping-off disease; organic fertilizer; bioactive agent.

INTRODUCTION

Recently, green beans (*Phaseolus vulgaris L*.) are considered one of the most important leguminous crops grown to provide food for animals and humans (Peix et al., 2011). Root rot disease caused by *Rhizoctonia solani* is a chronic and serious disease that affects bean plants during the growing season, leading to shortage of plant health and negatively impacting the crop yield (Wen et al., 2005). Additionally, microbial-enriched compost is an effective and safe technology for reducing Sclerotium-producing pathogens and enhancing plant growth and yield. It has been shown that *Trichoderma* species are effective biological agents in controlling many soil-borne fungal pathogens.

Yang and Li (2012) found that *Rhizoctonia solani* affects a number of other important rotational crops and its inoculum can survive in their residues such as alfalfa, canola, peas, soybeans, sugar beet, red clover, tomato and potato. The significant increase in the global population and rapid climate change contributed to the wide gap between the request for food and its supply. To reduce this gap and meet the needs of the increasing population, the extreme use of chemicals has improved food production, but at the same time, it has contributed to the decline of soil health (Meemken and Qaim, 2018).

Organic fertilizers are a collection of natural products derived from organic materials that are added to the soil to enhance the plant growth. They are divided into two types: compost-based such as compost, Vermi-compost and compost tea and non-compost-based which include a variety of resources such as seaweed extracts, amino acids, plant parts, organic waste and some biofertilizers agents as mycorrhizal fungi and plant growth-promoting rhizobacteria (PGPR), and organic acids.,(Du Jardin, 2015 and Macdonald et al., 2018) .

Assefa and Tadesse (2019), showed that organic farming is known for its positive impact on soil structure and its related properties. Soils managed organically have improved characteristics such as higher porosity, increased moisture content, stable aggregate formation, and better nutrient availability, all of which contribute to a significant increase in microbial activity (Ayangbenro et al., 2022).

Vermi-composting is a process that uses earthworms to transform organic waste into a secondary product, which can be used as a fertilizer for crop production (Dominguez, 2004; Lim et al., 2016). Vermi-composting is the best method for recycling great amounts of organic waste and reduce the use of the mineral fertilizers and the chemical pesticides. The widespread use of Vermi-compost is an effective way to improve the organic matter content in the soil and increase its fertility (Dignac et al., 2017).

Actinobacteria, particularly the genus *Streptomyces*, are considered as a major source of bioactive metabolites and have shown significant effective role in controlling many plant pathogens (Wang et al., 2013)

The aim of this work was to investigate the potential of biological control against *Rhizoctonia solani* that cause damping-off disease Biocontrol forms (tea and wash) technology were used as a reliable and robust soil treatment processes to control diseases and pests and promote plant growth in case of green beans. Determine the most effective additives to vermicompost was investigated to get best control comparing with chemical pesticides and yield in selected three locations of Egypt.

MATERIAL AND METHODS

Laboratory experiments were conducted at the Environmental Biotechnology, Department of the Genetic Engineering and Biotechnology Research Institute, (GEBRI) at the University of Sadat City, Egypt. This study focused on biological control of *Rhizoctonia solani*, isolated from green bean plants (*Phaseolus vulgaris L.*) c.v. Paulista.

***In vitro* treatments:**

Isolation of pathogenic fungi:

Potato Dextrose Agar media (PDA) was used to isolate both of two fungi: *Rhizoctonia solani and Trichoderma sp. R. solani* was isolated from bean plants showing necrotic lesions on root and hypocotyls, collected from fields in three locations: El Behira, El Giza and El-Menofya governorate during 2022 growing season. Small pieces of infected root and hypocotyls were surface sterilized and placed on PDA medium. The plates were incubated at 25°C ± 1 for 72 hrs. After that, fungal hyphae from the edge 7 days culture was taken and transferred to a petri dish (Singh, 1988).

Identification of the fungal isolates using Biolog system:

The fungus isolated was identified in Agricultural Research Center according to Complete linkage rule and Euclidean distance measure as described by Druzhinina et al. (2006).

Pathogenicity Test of Rhizoctonia solani on bean plants:

Sterilized soil was distributed into pots, each one contains 1 kg / pot. *Rhizoctonia solani* grown on PDA medium was added to the soil at a 1% (w/w) ratio. Each pot was cultivated with one seed, while seeds sown in uncontaminated soil served as the control treatment. All plants were kept under greenhouse conditions. Three replicates were used for each treatment. The seed germination percentage was estimated five days after seedling emergence. Disease severity was recorded on a scale of 0 – 4, where 0 = healthy; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%, and 4 = 76–100% root area is infected (Aoyagi et al., 1998).

Vermi-compost production:

Red wiggler worms were obtained from the Central Laboratory for Agricultural Climate. A box containing 10 kg of animal waste was prepared, and the worms were added to it. Moisture was maintained daily or as needed until the worms finished feeding on the waste. This material was then transferred to another pile, and kitchen waste was added in small doses to allow the worms to adapt to it over a period of one and a half months. The appropriate amount of Vermi-compost was then obtained to start the experiment. Three to four days before separating the worms, feeding and moisture were stopped to allow the worms to migrate to the bottom. The Vermi-compost was then collected. (Nagavallemma et al., 2004).

Preparation of bioactive Vermi-compost:

The amount of Vermi-compost produced divided into two halves:

I. The first half was treated with 10% w/w superphosphate and wetted with **Nova Plus®** by the rate 2.5% for two weeks.
II. The other half was treated with shrimp shells and wetted with

Nova Plus® at a rate of 2.5% for two weeks as well (bioactive Vermi-compost). Following this treatment, the subsequent steps were taken:

***Nova Plus®**: A source of beneficial microorganisms used in mineral element analysis and raw material waste analysis.

Source of Actinomycets and Trichoderma:

Actinomycetes and Trichoderma were isolated from different types of Vermi-compost and bioactive Vermi-compost® (Vermi-compost enriched with biocontrol agents) using the serial dilution technique. One gram of soil from each sample was dissolved in 10 ml of distilled water, thoroughly mixed for three minutes, and then shaked well. The samples were serially diluted from 10^{-1} to 10^{-6}. The spread plate technique was then used to isolate the organisms from the diluted samples (Pangrikar et al., 2009).

Purification and morphological identification Trichoderma sp.

The macroscopic observations of Trichoderma strains were performed by using an interactive strains identifcation key given by (Jiang et al. 2016). Pure Trichoderma strains were recultured aseptically from the slant agar and transferred onto a fresh PDA agar Petridish and incubated at room temperature (25±2 °C) for 6 days. The experiment was performed with triplicates. Regular observations were made according to the following criteria: colony appearance and pigmentation, conidia and phialide size, and conidiophore branching patterns. Following the protocol of (Kubicek et al. 2019).

Effect of Trichoderma harzianum and Actinomycets sp. on Rhizoctonia solani:

Antagonism between *Actinomycetes sp.* and *T.harzianum* against the pathogenic fungi *Rhizoctonia solani* was assessed as follows: Stock cultures of *Rhizoctonia solani* was obtained from PDA slants stored at 4°C ±1 and transferred onto the surface of PDA plates. The cultures were then incubated at 25°C ±1 for 7 days. For the antagonistic effect, ½ cm diameter disc of the antagonist was placed adjacent to one 0.5 mm diameter disc of the pathogen at the edges of the PDA media. Control treatments followed the same method but included only the pathogen disc without the antagonist disc. Each treatment was replicated three times, and the plates were incubated at 25 ± 2°C in darkness for 7 days. The growth zone diameter of the pathogen was measured to assess antagonistic activity.

In vivo Experiments:

The trials were conducted at El -Hoda Farm in Sadat City during the 2022 season. A split-plot design experiments with three replicates each treatment includes 80 plants that were conducted to evaluate eight treatments in comparison to the control:

1. Traditional Vermi-compost at rate 1.5, 3 and 5 kg / m. (application before planting).
2. Bioactive Vermi-compost ® 1.5, 3 and 5 kg / m (application before planting).
3. *Trichoderma sp*. Seeds treatment before planting (100 ml/ 1Kg seeds).
4. *Trichoderma sp*. 0.5 ml, 1m and 2 ml / L (injection in soil after germination /m)
5. *Actinomycets sp*. 0.5 ml, 1m and 2 ml / L (injection in soil after germination /m)

6. All tested treatments combined with:
    - Potassium phosphite at 1 L / feddan.
    - Potassium permanganate at 250 g / feddan.
    - Chemical pesticide as Mon Cut® fungicide at 500 g / feddan.
    - Chitosan at 500 cm / feddan.
    - Negative control

Note: Rates used by Potassium phosphite, Potassium permanganate, Mon Cut® fungicide and chitosan according to the manufacturer's recommendation Since they are commercial products.

Disease assessment:

Following to the equations of Nutter *et al*. (1991), the disease incidence (DI %) was determined by recording the percentage of infection and healthy survival plants after 15 days planting, according to the following formulas (1-4):

Pre-emergence % =

$$\frac{\text{Total No. of un-germinated seeds x 100}}{\text{Total No. of planted seeds}}$$

Post-emergence % =

$$\frac{\text{Total No. of rotted seedlings x 100}}{\text{Total No. of planted seeds}}$$

Survived seedlings % =

$$\frac{\text{Total No. of survived seedlings x100}}{\text{Total No. of planted seeds}}$$

Reduction or increasing % =

$$\frac{\text{DI of Control - DI of treatment x100}}{\text{DI of Control}}$$

RESULTS

Pathogenicity tests:

The results that presented in (Figure 1) indicated all the isolates of *Rhizoctonia solani* collected from the regions of El Beheira (1), Giza (2), and El-Menoufia (3) showed the ability to infect bean plants, causing typical damping-off symptoms, albeit with varying degrees of severity. Isolate (3) exhibited the highest level of pathogenicity, leading to the most severe disease symptoms, while isolate (2) had the least effect on disease severity, followed by isolate (1). Based on these results, isolate (3) was selected for the subsequent in vitro experiments due to its high disease severity on bean plants, making it the most suitable isolate for studying the disease's effects under controlled laboratory conditions. Following to the equations of the disease incidence (DI %) and production% were determined by recording the percentage of infection and healthy as survival plants after 15 days planting, according to the following.

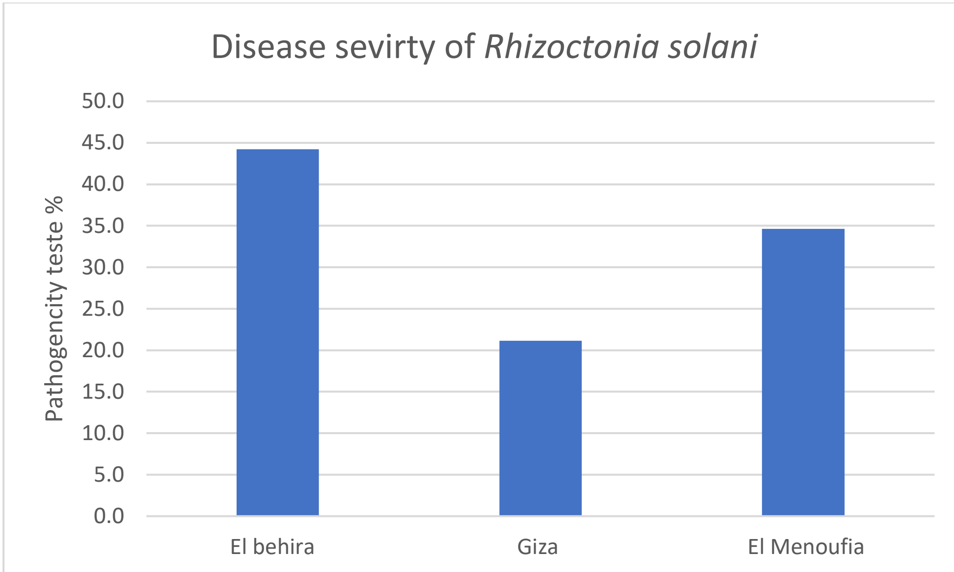


Fig. (1): Pathogenicity test of three isolates of *Rhizoctonia solani* from different location of Egypt.
(1) El behira (2) Giza (3) El Menoufia

Fig. (2): Pure culture of *Rhizoctonia solani* The causative of damping off disease

Fig. (3): The symptoms damping off disease on the green plants.

Fig. (4): The growth of *Actinomycets sp.* and *Trichoderma harzianum* on the liquid culture.

Figures (1) and (2), showed a pure culture of *Rhizoctonia solani* and the symptoms of damping off disease on green bean seedlings, while Figure (3) showed the growth of *Actinomycets sp.* and *Trichoderma harzianum* on liquid culture. Table (1) clear that the antagonistic effects of *Actinomycets* sp. and *Trichoderma harzianum* isolated from bioactive Vermi-compost against *Rhizoctonia solani*. Results provided the highest effect of suppression obtained by *T. harzinum* (92.2 %), followed by *Actinomycets* sp. (76.6%) in comparing with the control treatment.

Table (1): Effect of *Actinomycets sp.* and *Trichoderma harzianum* isolation on the Inhibition (%) of *Rhizoctonia solani*.

inhibitors	***Rhizoctonia solani***		
	Control	**Radial growth cm**	**Inhibition %**
Actinomycets sp.	9	4.3	52.2
Trichoderma harzianum	9	0.7	92.2

(a) **(b)**

Fig. (4): inhibition zone of *Actinomycets sp.* and *Trichoderma harzianum* on the growth of *Rhizoctonia solani*

In this study, the effect of using certain biocontrol agents on inhibiting the growth of the fungus *Rhizoctonia solani*, which causes seedling damping-off in beans, was measured. The treatment of seeds before planting with *Trichoderma harzianum* Figure (4), describe the inhibition zone resulted from interaction between *Actinomycets sp.* (a) and *Trichoderma harzianum (b)* and *Rhizoctonia solani*, there is a suppression effect of *Actinomycets sp.* and *Trichoderma harzianum* on *Rhizoctonia solani*. So, common beans (*Phaseolus vulgaris L.*) and its seeds and pods are rich in vitamins, proteins, mineral salts, calcium and many amino acids. However, green bean plants can be affected by several types of fungi, such as *Rhizoctonia solani* during the growing season and/or during storage. The plant densities are facing disease affecting and are leading to significant losses and negatively impacting the Marketing and export.

***In vivo* Experiments:**

Data in **table (2)** cleared that, seeds treated with *Trichoderma sp*. before planting recorded the highest plant survival rate 71.8 %, followed by Bioactive Vermi-compost 66.2 % compared to *Actinomycets sp.* at concentration 0.5 ml / m which recorded the lowest survival rate (58%) and negative control 49.4%.

Table 2; Effect of seeds treated with different methods before planting of beans.

Effects / Treatments	Damping- off caused by *Rhizoctonia solani*				
	Pre-emergence		Post- emergence		Survival plants %
	Incidence %	Reduction %	Incidence %	Reduction %	
Vermi-compost 1.5 kg	19	13.6	21.4	25.2	59.6
Vermi-compost 3 kg	18.6	15.6	20.6	27.8	60.8
Vermi-compost 5 kg	**18**	**18.2**	**20**	**30**	**62**
Bioactive Vermi-compost 1.5 kg	17.4	20.9	19.4	32.1	63.2
Bioactive Vermi-compost 3 kg	16.6	24.5	18.8	34.3	64.6
Bioactive Vermi-compost 5 kg	**16**	**27.3**	**17.8**	**37.7**	**66.2**
***T.harzianum* (seeds treatment)**	**12.6**	**42.7**	**15.6**	**45.5**	**71.8**
***T.harzianum* 0.5 ml**	21.2	3.6	20	30	58.8
***T.harzianum* 1 ml**	20.6	6.4	19.2	32.3	60.2
***T.harzianum* 2 ml**	**19.6**	**10.9**	**18.6**	**35**	**61.8**
***Actinomycetes sp.* 0.5 ml**	21.4	2.7	20.6	28	58
***Actinomycetes sp.* 1 ml**	20.4	7.3	19.4	32.2	60.2
***Actinomycetes sp.* 2 ml**	**20**	**9**	**19**	**33.7**	**61**
Chitosan	**21**	**4.5**	**20.2**	**29.4**	**58.8**
Potassium permanganate	21.8	9	17.4	39	60.8
Potassium phosphite	21.4	2.7	18.6	35	60
Mon Cut® fungicide	21	4.5	14.6	49	64.4
Negative control	**22**	**0**	**28.6**	**0**	**49.4**

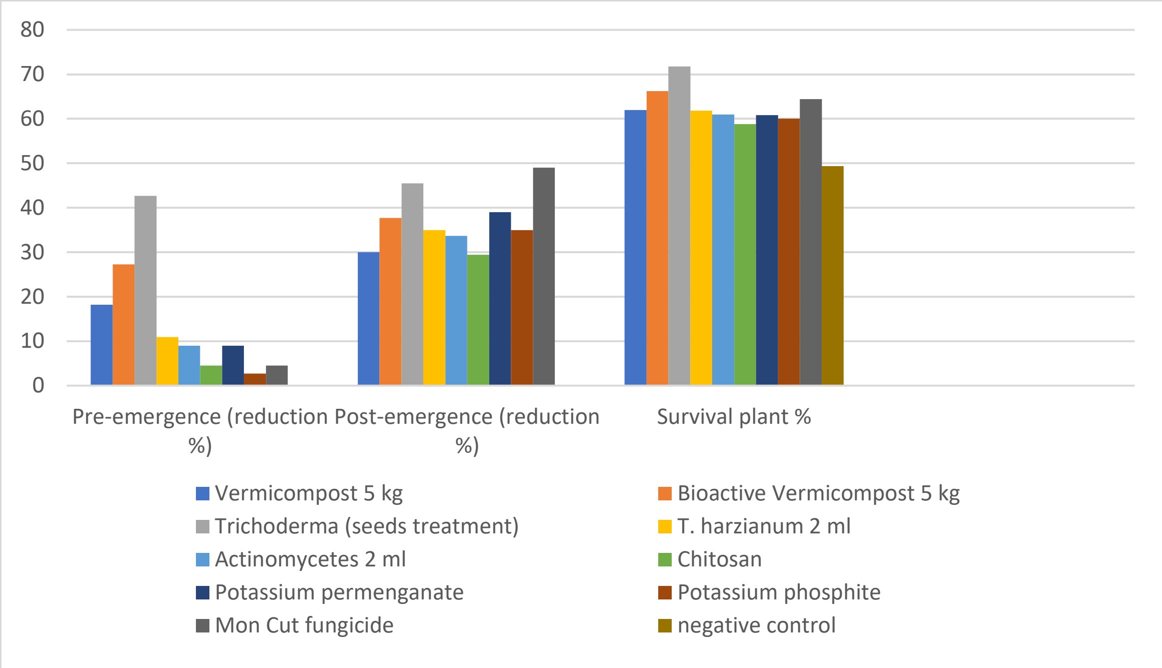


Figure (5): Effect of different treatments on the disease reduction and plant survival.

Figure (6), indicated that, the highest percentage in plant height was obtained in Bioactive Vermi-compost treatment at the rate 5 kg/ feddan (65 cm) followed by seed treatments with *T. harizianum*, (63 cm), compared to negative control, which recorded the lowest growth rate (26.7cm) followed by Mon Cut® fungicide (38 cm).

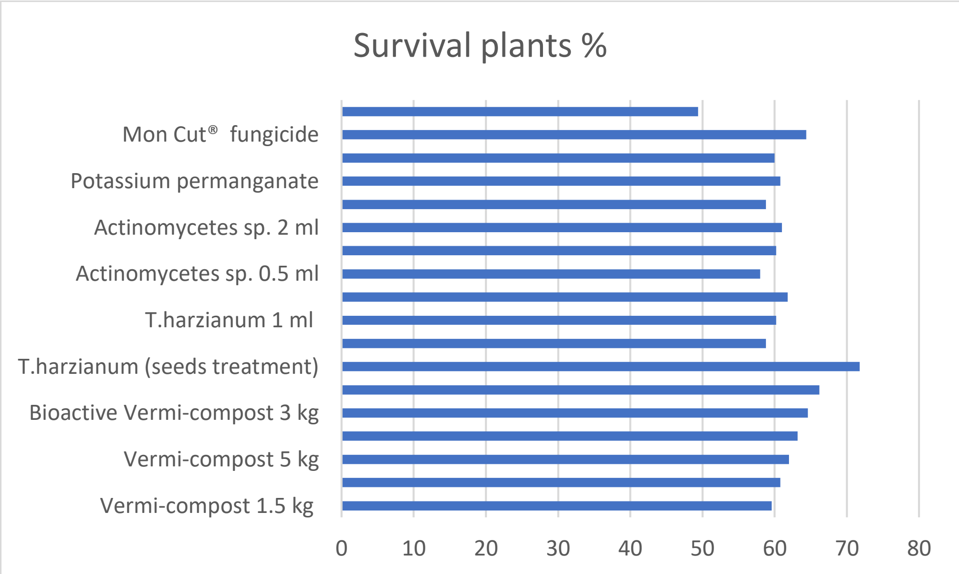
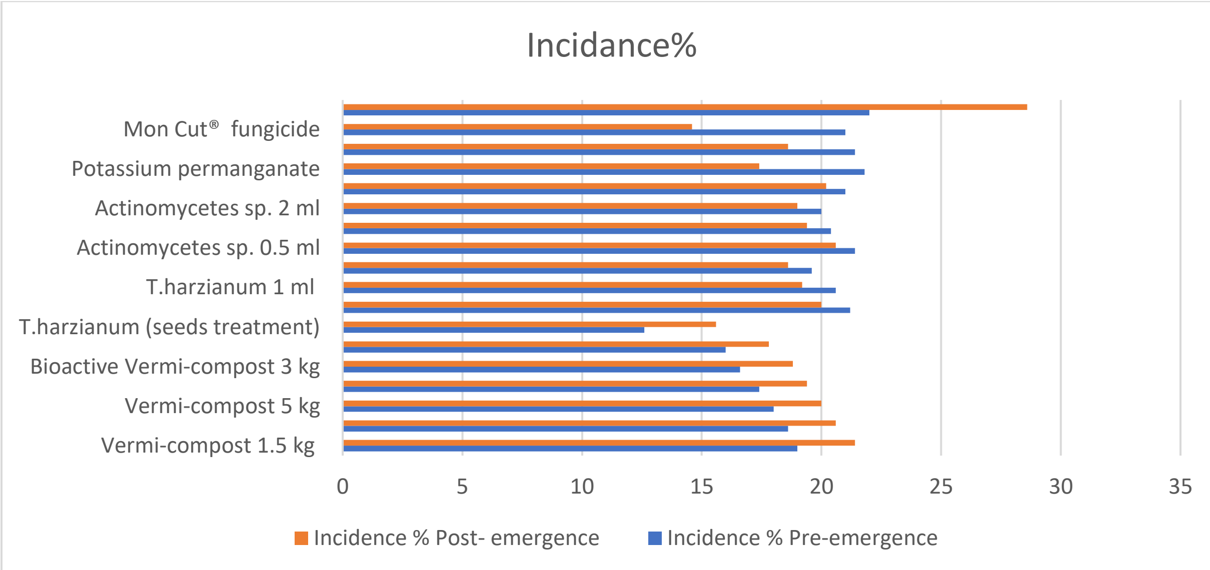


Figure (6): Survival plants as effect of different treatments on the disease reduction and plant survival.



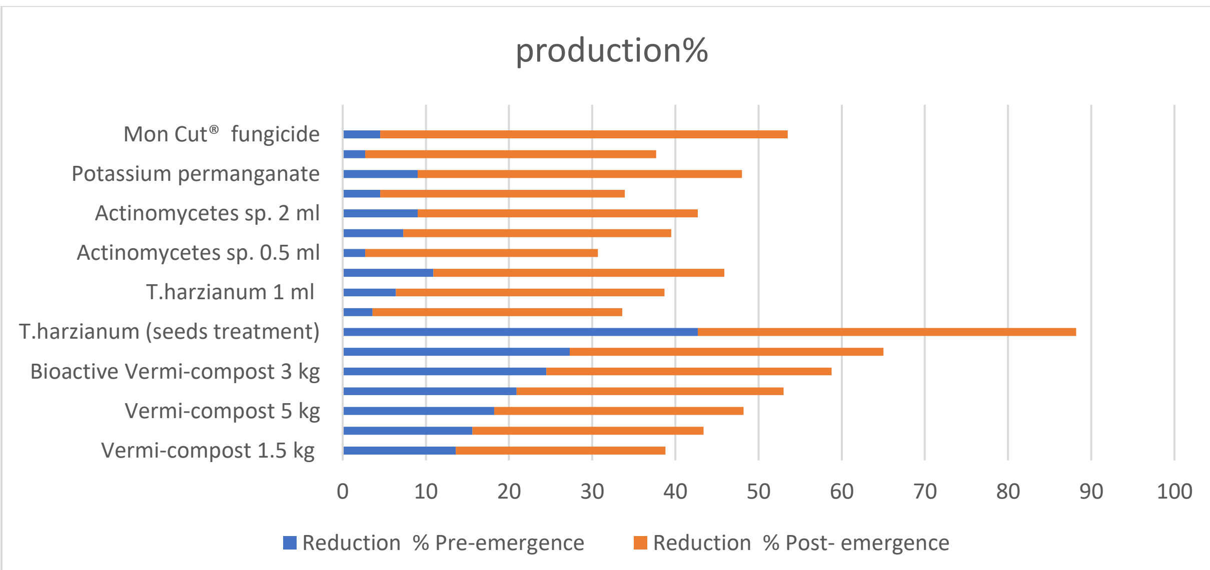


Figure (6): Effect of different treatments on the plant height after 30 days

Following to the equations of Nutter *et al*. (1991), the disease incidence (DI %) was determined by recording the percentage of infection and healthy survival plants after 15 days planting, according to the following formulas (1-4): Fig. (7): Cleared that, the highest rate in root length was obtained on seeds treated with *Trichoderma* sp. (15.9 cm) followed by bioactive Vermi-compost at the rate 5 kg/ feddan (15.7 cm), compared to negative control, which recorded the lowest growth rate (7.3 cm) followed by potassium phosphite (11 cm) and Mon Cut® fungicide (11.1 cm).

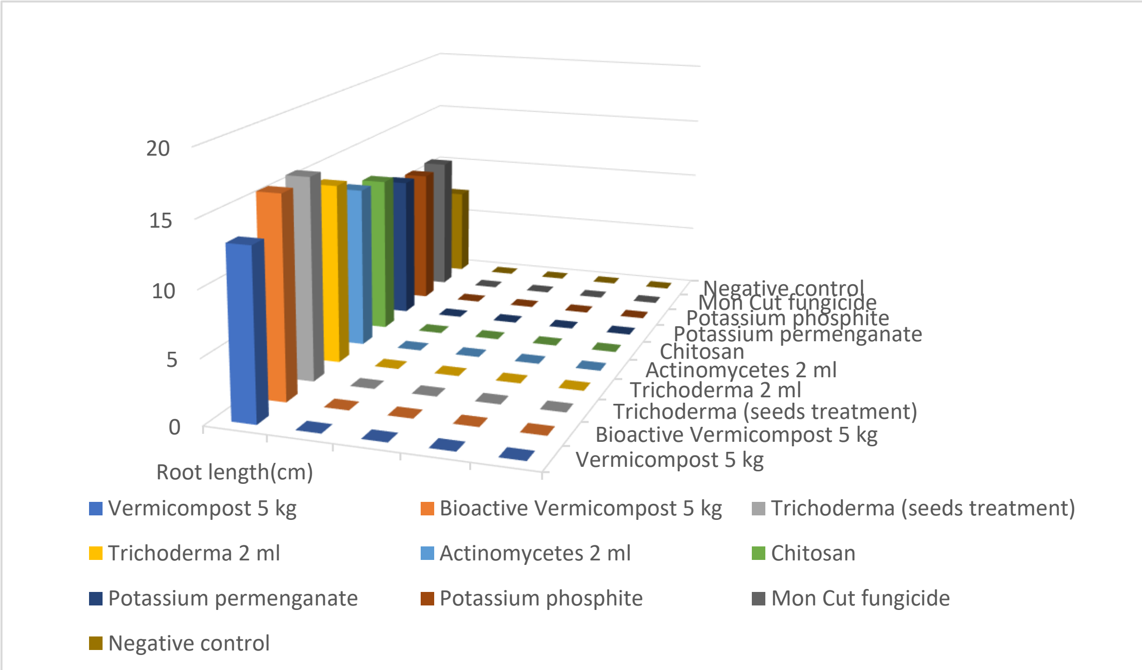


Fig. (7): Effect of different treatments on the root length after 30 days

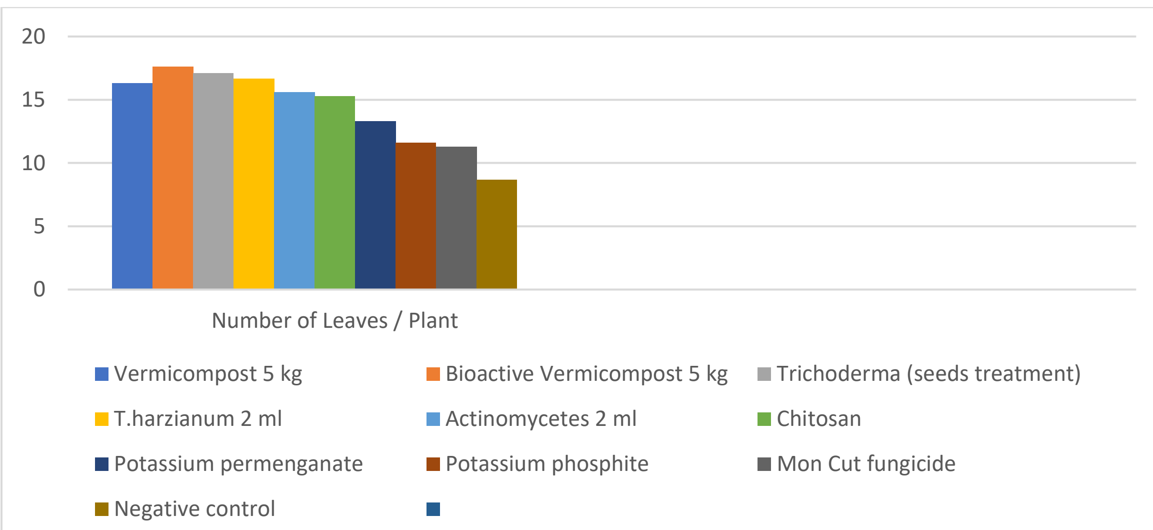


Fig. (8): Effect of different treatments on the number of leaves /plant after 30 days

Due to the ongoing spread of fungal disease, many researchers have focused on how to control it specially in the case of certified organic agriculture systems, aiming to find sustainable solutions to mitigate its negative effects on crops and protect the environment. Of the results, Figure (8) showed that the highest leaves number was with Vermi-compost treatment followed by *Trichoderma harizianum* compared with the control treatment while Mon Cut® fungicides had the lowest values in both cases of incidence and production%. In the recent decade, with the impact of climate change, charcoal rot has become a year-round issue, particularly with its effects intensifying during the nursery period, stages when plants are exposed to environmental conditions that favor the spread of the fungus.

DISCUSSION

Eight experimental groups were conducted in order to investigate the impact of different treatments on reduction of charcoal red disease in common beans (*Phaseolus vulgaris* L.). Our results show that, the seed treatments with *Trichoderma harzianum* recorded the highest survival rate in plants (71.8 %), followed by the Bioactive Vermi-compost treatment by concentration 5kg/m (66.2%) compared with negative control (49.4%) followed by Chitosan treatments (58.8 %). While Mon Cut® chemical fungicide gave result (64.4%) in survival rate of plants. On the other hand, the effect of different treatment on plant growth parameters gave the highest rate by Bioactive Vermi-compost in plant height (65 cm) and number of leaves (17.6) while recorded seed treatment by *T. harzianum* the highest rate in root length (15.9 cm). Vermi-compost has an important role as a control process of OM degradation, during which earthworms were added at an early stage of decomposition as resulted by Lim et al. (2016)

Vermi-composting is a biotechnology tool that uses the complex symbiotic interaction of earthworms and microorganisms to convert organic waste into useful nutrient-rich products (Domínguez et al., 2019). In this study, the effect of using certain biocontrol agents on inhibiting the growth of the fungus *Rhizoctonia solani*, which causes seedling damping-off in beans, was measured. The treatment of seeds before planting with *Trichoderma harzianum* (isolated from Bioactive Vermi-compost) recorded the highest plant survival rate, followed by the treatment protocols with Bioactive Vermi-compost. Both treatments also showed the highest efficiency in plant growth parameters. The results indicated that, the treatment with *Trichoderma sp.* recorded the highest survival rate in plants, followed by the Bioactive Vermi-compost treatment compared to the control, followed by Chitosan treatment. Additionally, the highest rate of Pre-emergence damping off disease reduction was observed in this treatment. The results were in agreement with Vukovic et al. (2021); Ansari et al. (2020); Indrani et al. (2019).

The effect of different treatment on plant growth parameters. plant height, root length and amount of leave/ plant. The highest results were observed for Bioactive Vermi-compost and seed treatment with *Trichoderma sp.* In the objective of Gudeta et al. *(*2021); Yatoo et al. (2021); Sulaiman and Mohamad (2020), all the treatments have a suppression effect on damping off disease caused by *Rhizoctonia solani*, Also, this result was in agreement with. (Yatoo et al. 2021; Machfudz et al. 2020; Reddy et al. 2012; Simsek-Ersahin 2011).

In conclusion, Biocontrol using bioactive Vermi-compost and the microorganisms isolated from it showed the highest rate of disease inhibition as well as the highest rate of plant growth parameters. Therefore, using this type of Vermi-compost as a biofertilizer and biocontrol agent, or isolating and culturing the antagonistic microorganisms from it, will be promising approaches for agriculture in the future. Bioactive vermicompost will be a promising approach for sustainable agriculture sustainable research and support SDGs.

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