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Biological Control of Charcoal Rot Caused by *Macrophomina phaseolina* Through The Suppressive Role of Bioactive Vermicompost Compared With Chemical Control.

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ABSTRACT

In recent years, the use of vermicompost as organic fertilizer which produced by earthworms has become increasingly popular in many fields that could inhibit most fungal diseases. In this study, the effect of seven treatments were conducted in strawberry nursery, Actinomycetes, Trichoderma, bioactive vermicompost, bioactive vermi-wash, chitosan in addition two chemical treatments (potassium permanganate and potassium phosphite) compared to a control one. Ridomil Gold Plus fungicide is used to control charcoal rot caused by *Macrophomina phaseolina*. Through this study, the treatment of Trichoderma and Actinomycetes in concentration 3L/feddan extracted from bio active vermicompost has the best results (61.2 and 54.7 %) respectively compared with the other biocontrol treatments, Chitosan (52.2%), Bio active vermi-wash (50.1 %) and Bio active Vermicompost tea (35%) but the chemical control it is heigh results in all treatment to control of pathogen only Potassium permanganate (69.3%), Ridomil Gold Plus(chemical fungicide) (66%) and Potassium phosphite (63.7%). However, Trichoderma and Actinomycetes recorded the best results in growth parameters (20.1 %) and (19.8), respectively in plant height followed by Bio active Vermi-wash 19.5 %, chitosan 19%, Bio active Vermicompost tea (16.1%), Potassium phosphite (16%), Potassium permanganate (15.7 %), Ridomil Gold Plus (15%) and control (11.3%). Inconclusion, this study has shown that the treatment with microbes derived from bioactive vermicompost is capable of combating *Macrophomina phaseolina* diseases while promoting plant growth, in accordance with the bio farming system that meets export standards.

Keywords: biological control; *Macrophomina phaseolina*; organic fertilizer; vermi-wash; bioactive agent.

INTRODUCTION

Recently, Strawberry (*Fragaria×ananassa* Duch.) which is very important crop, and is cultivated on approximately 372,000 hectares worldwide, (FAO STAT, 2018). Strawberry production faces challenges from various plant pathogens, with soil-borne pathogens increasingly recognized as among the most limiting diseases in strawberry crops today (Holmes et al., 2020). *Macrophomina phaseolina* is a ubiquitous soil-borne fungus with a broad global distribution, capable of affecting over 500 plant species across more than 100 plant families. It is known to cause various diseases, including stem and root rot, charcoal rot, and seedling blight Ghosh et al. (2018).

Bortolotti et al. (2018) showed that decentralized management of urban organic waste to produce simple organic matters (OM) is a promising alternative to centralized agricultural activity facilities. There are many techniques for recovering organic waste that can be adapted at different scales, converting it into a valuable resource. Lohri et al. (2017) classified these into four different categories as direct use (direct land application), (ii) biological treatment (composting, vermicomposting), (iii) physicochemical treatment (transesterification, densification), and (iv) thermo-chemical treatment (pyrolysis, liquefaction, gasification). Among the products produced from these techniques, compost (from composting and vermicomposting and biochar from pyrolysis could be used in agriculture.

In addition, for transforming organic wastes into beneficial soil amendments, composting and vermicomposting are popular methods. Traditional composting refers to the managed aerobic breakdown of raw materials. In contrast, vermicomposting relies on earthworms and microorganisms working together to bio-oxidize and stabilize organic materials (Dominguez and Edwards, 2011). While microorganism additions are primarily responsible for the biochemical breakdown process of organic matter, and thereby significantly boosting microbial activities during the processes. The application of vermicompost derived from many sources not only provides crop plants with beneficial microorganisms that aid in nutrient mobilization and uptake but also promotes plant growth and inhibits many plants pathogenic microorganisms. The efficacy of Jatropha, Annona, and Parthenium vermi-washes were shown to inhibit *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Fusarium oxysporum f. sp. ciceri* (Gopalakrishnan et al., 2010).

Now a day, vermicompost is an effective way to improve soil quality, control diseases and pests and promote different plants growth. Vermicompost contains beneficial microbes compared to the chemical fungicides (Yatoo et al., 2021). The uses of vermicompost are environmentally friend and support organic farming. Also, it enhances the crop production without pollution. Under conditions of moderate temperatures (30–35°C) with low soil moisture (below 60%), *M. phaseolina* significantly contributes to yield losses in crops such as soybean and sorghum, thereby affecting farmers' incomes (Kaur et al., 2012). *Macrophomina phaseolina* can survive for several years (2–15 years) as microsclerotia on plant residue or in soil, depending on environmental conditions and the mechanisms of pathogen dispersal. Microsclerotia can germinate and infect root tissues within a

temperature range of 20°C to 40°C. (Resnikov et al., 2020).

Vermicomposting processes are a controlled OM degradation based on the addition of earthworms to accelerate the stabilization processes upstream of the decomposition (Lim et al., 2016). The main objective is to stabilize and degrade OM to produce a humus-like material, called vermicompost (Doan et al., 2015).

The aim of this work was to investigate the potential of vermicompost and its forms (tea and wash) technology as a reliable and robust soil treatment processes to control diseases and pests and promote plant growth in case of strawberry nursery. Determine the most effective additives to vermicompost was investigated to get best control and yield in selected three locations of Egypt.

MATERIALS AND METHODS

1- Laboratory experiments:

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. The study focused on biological control of *Macrophomina phaseolina*, isolated from Strawberries.

Media used for In vitro treatment:

Using potato Dextrose Agar PDA for fungus Isolation, two hundred grams of potatoes were extracted by boiling for 25 minutes in 1000 ml of distilled water. The volume was then adjusted to 1000 ml, and 15 g of agar and 20 g of dextrose were added. The medium was autoclaved for 20 minutes at 121°C and 15 psi, and then allowed to cool before use.

Strawberry sampling and assessment:

The incidence of disease was 30% among the sampled plants, which were randomly collected from three fields surveyed in June and July 2020. Infected plants were carefully washed under running tap water to remove soil. The severity of strawberry decline was assessed as follows: crown and root disease were separately assessed based on a 0–4 disease severity scale, according to the following **Table (1)**:

disease severity scale	symptoms
0	no crown/root tissue discolored
1	<25% crown/root tissue discolored
2	≥25, <50% crown/root tissue discolored
3	≥ 0, <75% crown/root tissue discolored
4	≥75% crown/root tissue discolored

Macrophomina phaseolina isolation and purification:

For *Macrophomina phaseolina* isolation, Crowns and roots of all infected plants were surface sterilized in 1.25% sodium hypochlorite for 30 seconds (for roots and crowns), rinsed three times in sterile distilled water, and air-dried on sterilized paper towels in a laminar flow cabinet for 15 minutes. Fifteen pieces of crown and root tissues (each measuring 0.3–0.5 cm) were separately placed onto PDA medium for each plant. A disc from the edge of 7 days fungal culture was put at the center of petri dishes on agar PDA media and incubated for 7 days at 25°C±1 ºC (Singh, 1988).

Identification of the fungal isolates using Biolog system

The isolated fungus was identified in Agricultural Research Center according to this protocol: BiologTM micro-plates (Biolog, Inc., 3938 Trust way, Hayward, CA 94545, USA) test the ability of microorganisms to utilize a preselected panel of different carbon sources and amino acids by using FF Micro Plates as a Metabolic fingerprint of the inoculated organism. The FF microplate test panel comprises of 96 wells with different nutrients and test reagents of carbon sources and amino acids, and one well with water. The inoculated microplates were incubated at 26 ºC for 24-96 hours. The microplates were examined using the Biolog micro-station ™ reader beginning 24 hours after inoculation. Complete linkage rule and Euclidean distance measure as described by Druzhinina et al. (2006).

Pathogenicity Test of Macrophomina phaseolina on strawberry plants.

Pathogenicity tests of *Macrophomina phaseolina* isolates were conducted under greenhouse conditions during the 2020-2021 experiments at the Environmental Biotechnology Department of GEBRI, University of Sadat City, Egypt. Pathogen inoculum was prepared using cultures of *Macrophomina phaseolina* grown for 10 days in potato dextrose broth medium at 25°±1 °C. After full growth, the mycelium suspension was triturated with sterilized distilled water. Strawberry plants (c.v. Festival) were cultivated in plastic pots containing 3 kg of sterilized sandy soil mixed with commercial local compost (4:1). Inoculation was carried out 15 days after planting. The suspension was applied under each plant, with 25 ml/plant. The control treatment received 25 ml of sterilized distilled water without the inoculum of *Macrophomina phaseolina*.

The symptoms of disease caused by the pathogen isolates were evaluated at 15, 30, and 45 days after inoculation, consisting of observing typical symptoms according to (liu et al., 1995). Who used 0-5 scale where 0 = no visible symptoms, 1= 1-25 %, 2 = 26-50%, 3 = 51-75%, 4 = 76-100% of stem rot area and 5 = dead plants. All plants were kept under greenhouse conditions, and the experimental design was completely randomized with three replicates for each treatment.

Production of Vermicompost:

Worms were obtained from the Central Laboratory for Agricultural Climate. Half a kilogram of worms, along with some raw material (animal waste), was placed in a box. Kitchen waste was added to feed the worms until vermicompost was produced (Aslam et al., 2020). One month after warms feeding, the quantity was divided into two halves:

I. The first half was treated with 10% w/w superphosphate and wetted with **Nova Plus®** as at a rate of 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 vermicompost). 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.

Preparation of bioactive vermicompost tea:

One hundred liters of vermicompost tea were prepared according to (Arancon et al., 2019) by:

1. 10 kg of bioactive vermicompost in a fine mesh bag were added in a tank of water and mixed with 1 kg of molasses and completed to 100 L.
2. An air pump was installed to continuously aerate the water and vermicompost mixture for 24 hours.
3. Vermicompost tea was allowed to aerate before use.

Preparation of bioactive vermi-wash:

During the production of bioactive vermicompost, we incorporated a slight 10 cm slope on the bottom surface of the vermicompost bed. An exit pipe, 10 cm in diameter, was inserted into the outer wall by making a hole. The mouth of the exit pipe was directed into a plastic pot to collect the liquid that accumulated at the bottom of the vermicompost bed, allowing it to flow into the pot through the pipe (Gudeta et al., 2021).

Characterization of bioactive vermicomposts effects:

1- Chemical analysis of each type of vermicompost was conducted at the Soil and Water Laboratory of the Genetic Engineering and Biotechnology Research Institute (GEBRI). This included analysis of the initial vermicompost, vermicompost amended with specific feeding types, vermicompost amended with superphosphate, and bioactive vermicompost.
2- The isolation of total microbes, Actinomycetes, and Trichoderma from each type of vermicompost was conducted using the serial dilution technique. One gram of soil from each sample was suspended in 10 ml of distilled water and thoroughly mixed for 3 minutes, followed by vortexing. Each suspension was then serially diluted from 10^{-1} to 10^{-6}. The spread plate technique was employed to isolate organisms from the diluted samples.
3- Antagonism between Actinomycetes and Trichoderma against the pathogenic fungus *Macrophomina phaseolina* was assessed as follows: Stock cultures of *Macrophomina phaseolina* were obtained from PDA slants stored at 4°C and transferred onto the surface of PDA plates. The cultures were then incubated at 25°C for 7 days.

For the antagonistic effect, one 5 mm diameter disc of the antagonist was placed adjacent to one 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.

Open field trials of strawberries:

The trials were conducted at ELSHROUK Farm in Sadat City during the 2021 season. A split-plot design with four replicates was conducted to evaluate eight treatments in comparison to the control:

1. Liquid Actinomycetes isolated from Bioactive vermicompost tea at three doses: 1 L, 2 L, and 3 L per feddan)
2. Liquid Trichoderma isolated from Bioactive vermicompost tea at three doses: 1 L, 2 L, and 3 L per feddan.
3. Treatment with Bioactive vermicompost tea in three doses 1 L, 2 L, and 3 L per feddan.
4. Treatment with Bioactive vermi-wash (an agent of disease and pest control in soil) in three doses 1 L, 2 L, and 3 L per feddan.
5. Four tested chemical treatments combined with:
   - Potassium phosphite at 1 L per feddan.
   - Potassium permanganate at 250 g per feddan.
   - Chemical pesticide Rhidomil Gold Plus at 500 g per feddan
   - Chitosan at 500 cm per feddan.
6- Control without any treatment.

Note: Application of Potassium phosphite, Potassium permanganate, Rhidomil Gold Plus and Chitosan 5% according to traditional application rates (commercial products) following instruction of manufacturers.

Disease assessment:

Following to (Nutter et al., 1991), the disease incidence (DI %) was determined by recording the percentage of infection and healthy survival plants after 30 days planting, according to the following formulas:

$$\text{Disease incidence \%} = \frac{\text{Number of dead plants} \times 100}{\text{Total number of plants in a plot}}$$

$$\text{Survived plants \%} = \frac{\text{Total No. of survived plants} \times 100}{\text{Total No. of planted in a plot.}}$$

$$\text{Reduction or Increasing \%} = \frac{\text{DI of Control - DI of treatment} \times 100}{\text{DI of Control}}$$

RESULTS

Production vermicompost and its chemical analysis:

Four types of vermicomposts were prepared and produced in Lab following the steps of Arancon et al. (2019). Chemical analyses of different vermicompost's were conducted in Environmental and food biotechnology laboratory (EFBL), USC. The data in table (1) indicated that the highest-level phosphorus, magnesium, potassium and calcium in super vermicompost followed by bioactive vermicompost, amended vermicompost, traditional vermicompost, respectively. Both super and bioactive vermicompost had phosphorous and total elements content.

Table 1: Chemical analysis of different types of vermicompost.

Element	Unit	Traditional vermicompost	Amended Vermicompost	Super vermicompost	Bioactive Vermicompost
N	ppm	1480.9	19.84	21.61	21.07
P	ppm	832.1	17153.08	28557.98	18953.06
K	ppm	494.24	638.11	1277.87	674.15
Ca	ppm	1079.05	2126.89	3149.66	2360.31
Mg	ppm	1083.72	2113.61	5357.65	2696.21
Mn	ppm	13.67	171.10	185.18	189.23
Fe	ppm	215.72	893.06	1092.11	943.52
Cu	ppm	44.44	72.99	121.99	74.71
Zn	ppm	137.89	159.19	410.57	230.06

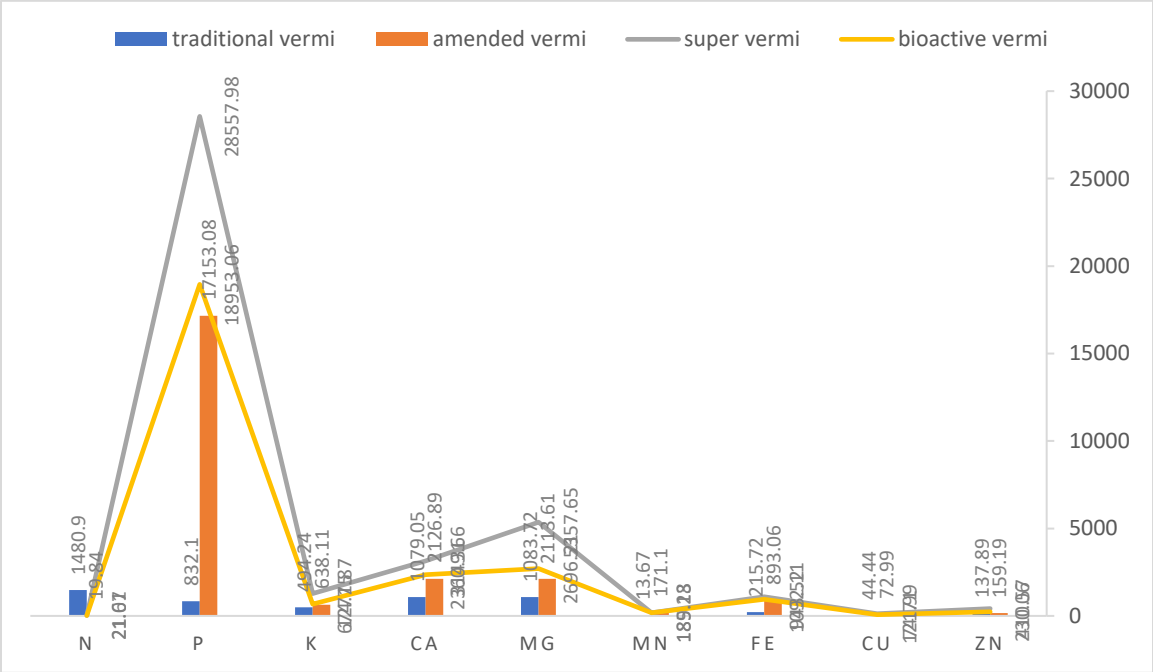

Fig. (1): Chemical analysis of different types of vermicompost.

In EFBL laboratory, total microbial counts were conduct under septic conditions. The filtration systems were used after addition of certain amount of sterile water.

Data in table (2) indicated that the highest microbial population in bioactive vermi-compost was total microbes (20.3×10^6 CFU/g dwt) Actinomycets (11.6×10^6 CFU/g dwt) and Trichoderma (1×10^2 CFU/g dwt) followed by super vermi-compost, amended vermic-ompost, and traditional vermi-compost total microbes (17.3×10^6, 12.5×10^6 and 4.7×10^6 CFU/total) and actinomycets (8.7×10^6, 5.6×10^6 and 2.1×10^6

CFU/total) respectively, but noted that bioactive vermicompost only contains of Trichoderma.

Table 2: Microbial population of different types of vermicompost:

Types of vermicompost	Unit	Total microbes	Actinomycets	Trichoderma
Traditional vermicompost	**(CFU/g dwt)**	4.7×10^6	2.1×10^6	ND
Amended vermicompost	**(CFU/g dwt)**	12.5×10^6	5.6×10^6	ND
Super vermicompost	**(CFU/g dwt)**	17.3×10^6	8.2×10^6	ND
Bioactive vermicompost	**(CFU/g dwt)**	20.3×10^6	11.6×10^6	1×10^2

ND: not detected

Fig. (2): Pure culture of Actinomycets

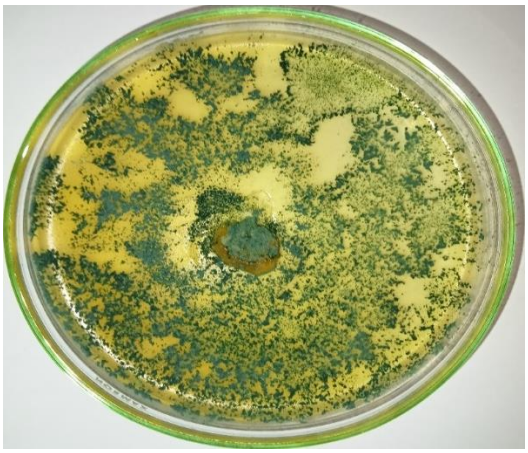

Fig. (3): Pure culture of Trichoderma

A **B**

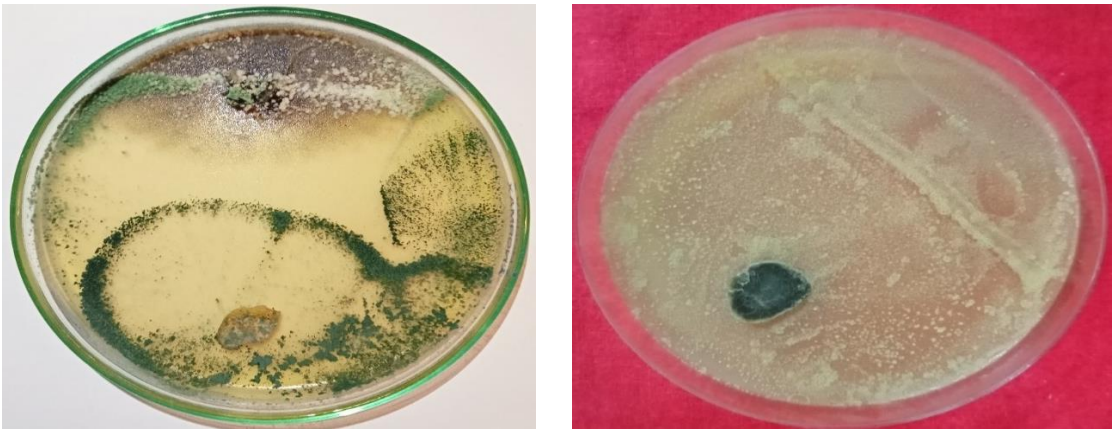

Fig.(4): Effect of beneficial microbes on *Macrophomina phaseolina* with Actinomycets (A) and with Trichoderma (B).

Pathogenicity tests of Macrophomina phaseolina:

Results shown in (Fig.4) indicate that all the tested isolates of *Macrophomina*

phaseolina obtained from El Behira (1), El-Qalyubia (2) and El-Ismailia (3), were able to infect strawberry plants causing typical charcoal rot with different degrees of disease severity. Data indicate that isolate (1) was the highly pathogenic and caused the highest disease severity. While Isolate (3) was the lowest in disease severity on strawberry plants followed by isolate (2). Based on this result, isolate (1) was used in the following *in vitro* experiments according to its highly disease severity. Figure 5 show the pure culture of *Actinomycets* spp. and *Trichoderma harzianum* that isolated and prepared in liquid culture for application purposes in soil.

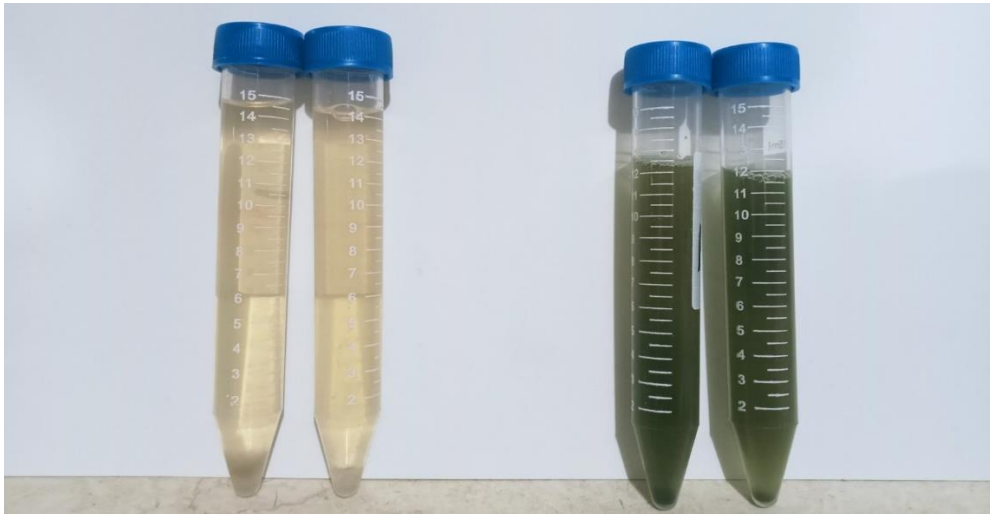

Fig. (5): liquid culture from *Actinomycets* spp. and *Trichoderma harzianum* for application in soil.

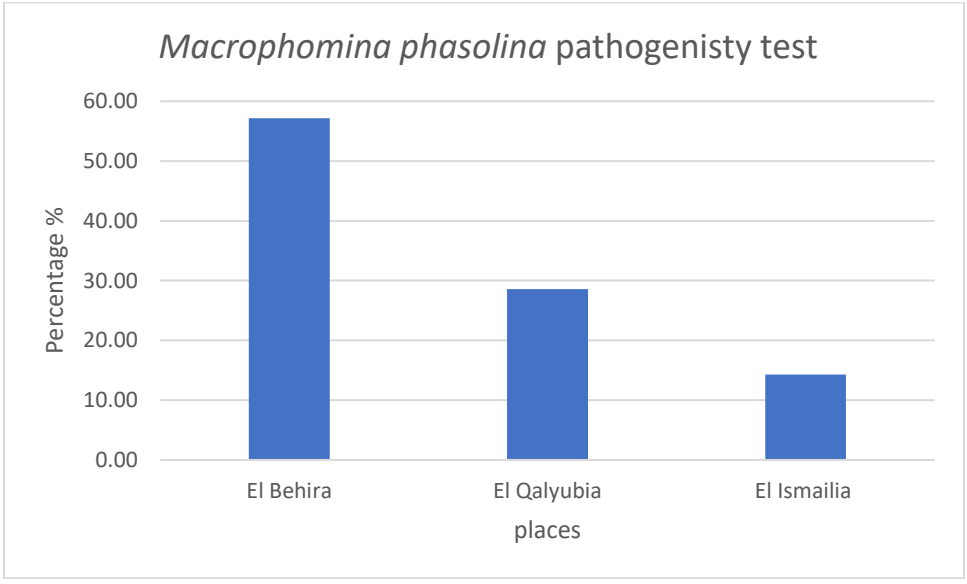

Fig. (6): Pathogenicity test of three isolates of *Macrophomina phaseolina* from different location of Egypt.

Table (3) shows the running experiments that were conducted in EFBL where the effect of Actinomycets and Trichoderma isolation on the Inhibition (%) of *Macrophomina phaseolina* were tested. Our results cleared the antagonistic effects of *Actinomycets* spp. and *Trichoderma harzianum* against *Macrophomina phaseolina*. Results provided the highest effect of suppression obtained by *Actinomycets sp.* (76.7 %), followed by *Trichoderma harzianum* (70 %).

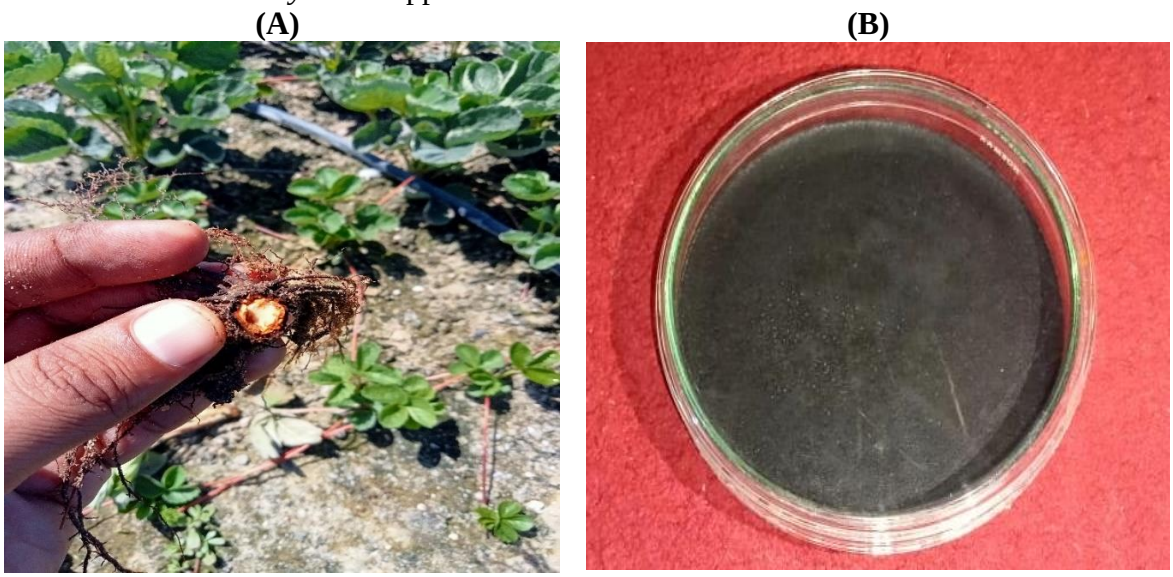

Fig.(7): Charcoal rot caused by of *Macrophomina phaseolina* (A) and its pure culture (B)

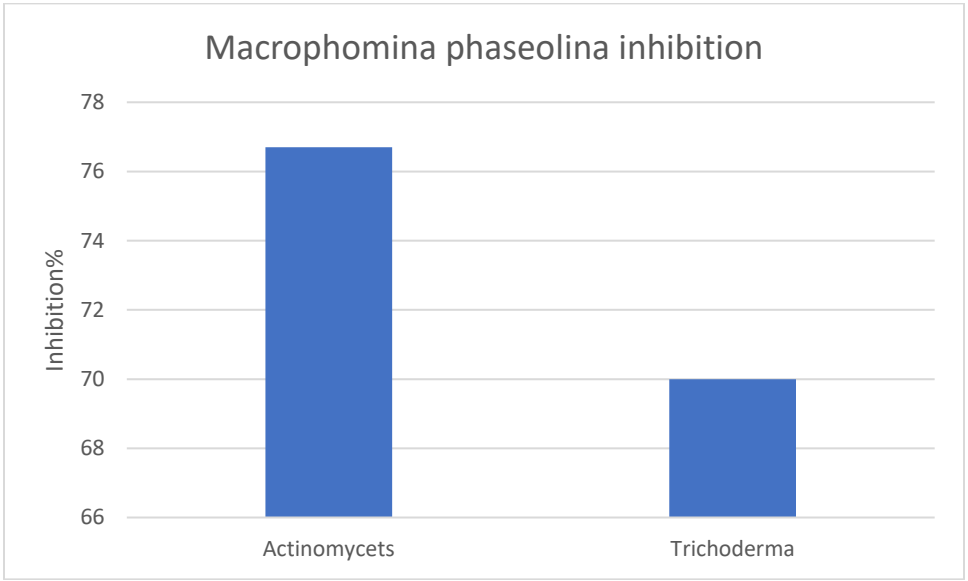

Fig. (8) Effect of Actinomycets and Trichoderma isolation on the Inhibition (%) of *Macrophomina phaseolina*

Table (3): Effect of Actinomycets and Trichoderma isolates on the Inhibition (%) of *Macrophomina phaseolina*

Biocontrol agent	**unit**	**Control**	***Inhibition effects on Macrophomina phaseolina***
Actinomycets spp.	%	9.0	76.7
Trichoderma harzianum	%		70.0

Open field experiments:

Data in **Table (4)** clear that the highest percentage in plant height was obtained on Trichoderma in concentration 3L/ feddan followed by Actinomycets, bio active Vermi-wash, chitosan, bio active Vermicompost tea, potassium phosphite, potassium permanganate and Rhidomil Gold plus compared with control.

Table (4): Effect of different treatment on (growth parameters) in strawberry nursery**.**

Growth parameter / Treatment	**Plant height (cm)**	**Number of runners / plant (1st week after treatments)**	**Number of runners / plants (2nd week after treatments)**	**Number of runners / plants (3rd week after treatments)**
Actinomycets 1L / feddan	18	81.25	99	101.5
Actinomycets 2L / feddan	19.1	83.25	103.75	106.75
Actinomycets 3L / feddan	**19.8**	**86.75**	**104**	**113**
Trichoderma 1L / feddan	18.5	82.5	103.25	109.5
Trichoderma 2L / feddan	18.7	84.25	107.5	114
Trichoderma 3L / feddan	**20.1**	**89**	**111.25**	**120**
Bio active Vermicompost tea 1L / feddan	15.1	55	79.5	82
Bio active Vermicompost tea 2L / feddan	15.7	55.75	83.25	83.5
Bio active Vermicompost tea 3L / feddan	**16.1**	**60.75**	**85**	**86.75**
Bio active Vermi-wash 1L / feddan	18.1	67	99	102.25
Bio active Vermi-wash 2L / feddan	18.5	68.75	101.25	103.5
Bio active Vermi-wash 3L / feddan	**19.5**	**70.5**	104.25	110.25
Chitosan	**19**	**66**	**95.5**	**111.75**
Potassium permenganate	15.7	66.6	95.25	108.75
Potassium phsphite	16	60.25	86	99.5
Ridomil Gold Plus	15	59.75	81	89
Control	**11.3**	**29.75**	**44.25**	**19.75**

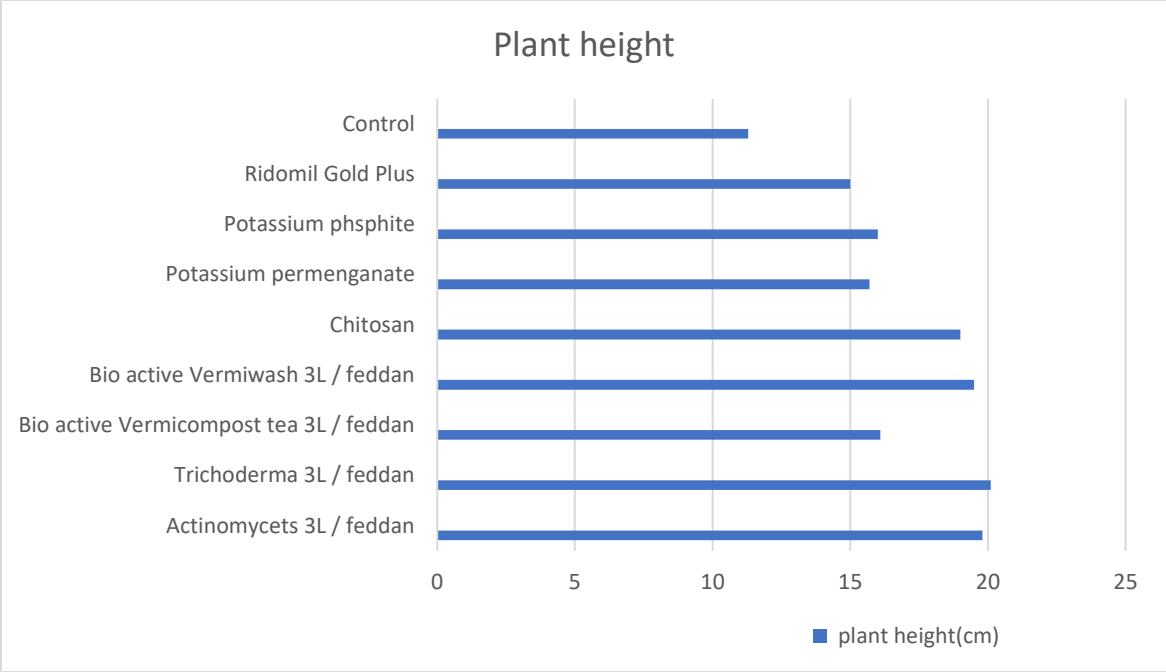

Fig. (9): Effect of different treatment on (growth parameters) in strawberry nursery**.**

An open field experiments, the trials were conducted at ELSHROUK Farm in Sadat City, Egypt during the 2021 season. A split-plot design with four replicates was conducted to evaluate eight treatments in comparison to the control as shown in Table 5. The main factors affect in were used as bioactive tea, Liquid Actinomycetes, vermicompost, vermi-wash compared with chemical agents. The chemical pesticides were included Potassium phosphite, Potassium permanganate, Rhidomil Gold Plus and Chitosan. Data in **Table (5)** clear that, the highest percentage in disease reduction was obtained on Potassium permenganat (69.3%) followed by Ridomil Gold Plus and Potassium phsphite in chemical treatment but Trichoderma (61.2) the highest percentage in biocontrol treatment followed by Actinomycets and chitosan.

Table (5): Effect of field treatments on the percentage of charcoal rot disease under field condition plants after 30 days of planting.

Treatment	Disease Incidence %	Disease reduction (%)	Survival plants %
Actinomycets 1L / feddan	15.7	48.1	68.6
Actinomycets 2L / feddan	16	47.2	68
Actinomycets 3L / feddan	**13.7**	**54.7**	**72.6**
Trichoderma 1L / feddan	17.3	42.9	65.4
Trichoderma 2L / feddan	14	53.7	72
Trichoderma 3L / feddan	**11.7**	**61.2**	**76.7**
Bio active Vermicompost tea 1L / feddan	25	17.4	50
Bio active Vermicompost tea 2L / feddan	21.3	29.7	57.4
Bio active Vermicompost tea 3L / feddan	**19.7**	**35**	**60.6**
Bio active vermi-wash 1L / feddan	16.3	42.2	67.4
Bio active vermi-wash 2L / feddan	16	47.2	68
Bio active vermi-wash 3L / feddan	**15**	**50.1**	**70**
Chitosan 5%	**14.3**	**52.1**	**71.4**
Potassium permenganat	**9.3**	**69.3**	**81.4**
Potassium phsphite 42%	**11**	**63.7**	**78**
Ridomil Gold Plus (chemical fungicide)	**10.3**	**66**	**79.4**
Control	**30.3**	**0**	**39.4**

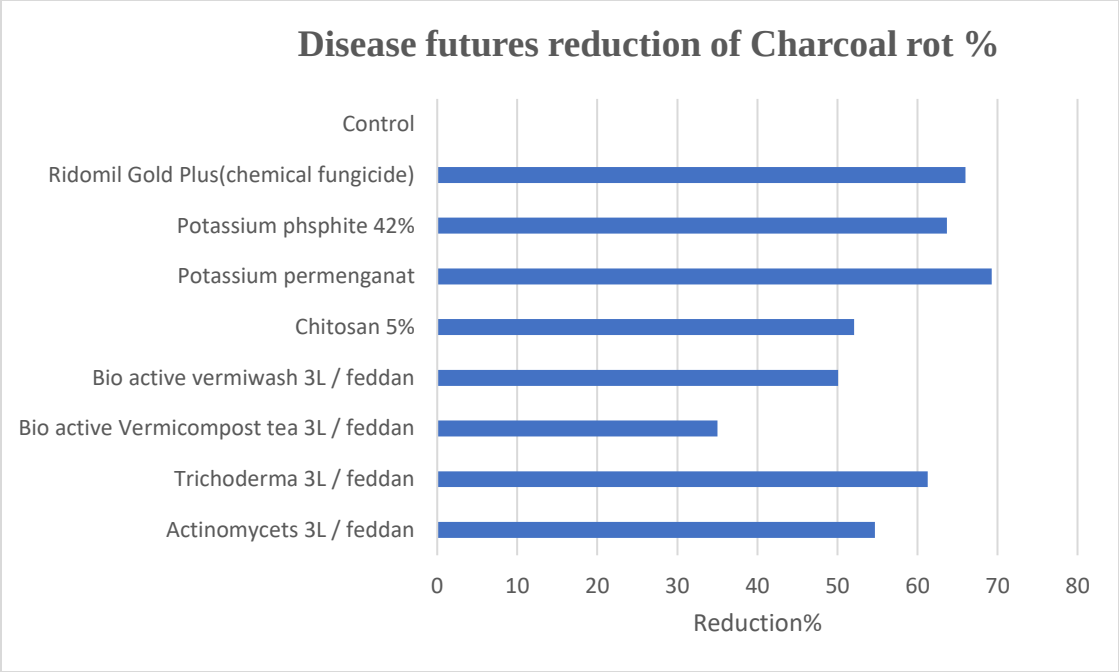

Fig. (10): Effect of different treatment on disease future reduction of charcoal rot in strawberry nursery.

DISCUSSION

Eight experimental groups were conducted in order to investigate the impact of different treatments on reduction of charcoal red disease in strawberry nursery. Through this study, the treatment of Trichoderma and Actinomycetes in concentration 3L/feddan extracted from bio active vermicompost has the best results (61.2 and 54.7 %), respectively compared with the other biocontrol treatments, Chitosan (52.2%), Bio active vermi-wash (50.1 %) and Bio active Vermicompost tea (35%) but the chemical control it is heigh results in all treatment to control of pathogen only Potassium permanganate (69.3%), Ridomil Gold Plus(chemical fungicide) (66%) and Potassium phosphite (63.7%).

Our results cleared that, bio-active vermicompost (vermicompost with some enhancing additions) gave the best results in terms of total microorganisms compared to all types of vermicompost used and it is the only one that contains Trichoderma. Vermicompost resulted from manure increases crop yield and total biomasses (Blouin et al., 2019). Our research cleared that, bio-active vermicompost with some additives. Vermicompost additions enhance the plants and get the best results in terms of total microorganism counts compared to all vermicompost types that are used, and it is the one that contains Trichoderma sp. According to many fields and laboratories trials, the vermicompost has been found to suppress most soil borne diseases (Yasir et al., 2009). Pathma and Sakthivel (2013) observed that, about 96 bacterial strains were also gained from vermicompost showed that antagonistic potential against phytopathogenic fungus was obtained.

According to many laboratory and field trials, vermicompost has been found to inhibit most of soil borne diseases (Yasir et al., 2009). Pathma and Sakthivel (2013) observed that, a total of 96 bacterial strains, which were also isolated from vermicompost, demonstrated antagonistic potential against phytopathogenic fungus. In this study, two biocontrol agents (*Trichoderma harzianum* and *Actinomycets sp*.) isolated from bioactive vermicompost by using the conventional method. These strains shown a promising potential for biological control against charcoal rot caused by *Macrophomina phaseolina* in strawberry nursery followed by other biocontrol treatment (bio active vermi-wash, chitosan and bio active vermicompost tea respectively)but the chemical treatment recorded heigh results in all treatment. On the other hand, the treatments with (*Trichoderma harzianum* and *Actinomycets sp*.) recorded heigh results in growth parameters plant height and number of runners.

The used tow biocontrol agents such as Trichoderma and Actinomycets that isolated from bioactive vermicompost using the conventional method. This strains exhibited a promising potential for biological control against of charcoal rot caused by *Macrophomina phaseolina* in strawberry nursery followed by other biocontrol treatment (bio active vermi-wash , chitosan and bio active vermicompost tea respectively) but the chemical treatment recorded heigh results in all treatment. On the other hand, the treatments with (Trichoderma and Actinomycets) recorded heigh results in growth parameters plant height and number of runners.

Inconclusion, the microbes derived from vermicompost enhanced with certain biological agents additives, known as bioactive vermicompost, exhibit antimicrobial effects through biological interactions controlled by secreted chemical metabolites. Since these microbes from bio active vermicompost can increase soil fertility, promote plant growth, and suppress pathogenic diseases, using bio active vermicompost extract as a biofertilizer and biocontrol agent, or isolating and culturing antagonistic microorganisms from bio active vermicompost, will be promising approaches for sustainable agriculture researches in the future and support SDGs.

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