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Management of root and crown-rot of strawberry by Bacillus Bioagents, Bion (BTH) and vermicompost in Libya

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إدارة تعفن الجذور والتاج في الفراولة باستخدام عوامل الباسيلس الحيوية، والبيون (*BTH*)، وسماد الكمبوست الدودي في ليبيا

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Abstract:

Many fungal isolates *i.e., Fusarium* spp., *F.solani* , *Macrophomina phaseolina* the pycnidial state of *Sclrotium bataticola*) , the fungus like *Phytophthora cactorum, Rhizoctonia solani* the imperfect state of *Thanatephorus cucumeris*), *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* (the sclerotial state of *Athelia rolfsii*) were isolated from strawberry roots existing mainly root and crown -rot symptoms collected from different fields at Tagoraa,Trablus, Libya. The pathogenicity of these isolates manifested that they all caused root and crown-rot symptoms. *M.phaseolina* and R.*solani* were the most pathogenic ones. Six Bacillus bioagents *i.e.*, were isolated from the rhizospheric soil of strawberry plants apparently free from root and crown-rot infection. These bacteria were evaluated for their antagonistic capability for *M.phaseolina* isolate-3 and *R.solani* isolate-2 both *in vitro* and *in vivo*. Both *B.subtilis* and *B. thuringiensis* exerted the highest antagonism against the linear growth of both pathogenic fungi. Sterilized tea filtrate of the tested vermicompost caused significant inhibition to the linear growth of the tested two pathogenic fungi in comparison with control treatment. This inhibition of both *B.subtilis* , *B. thuringiensis* and vermicompost was gradually increased significantly by increasing their concentration. The combination among the bioagents *B.subtilis* and *B. thuringiensis* ,the inducer resistance elicitor Bion (BTH) and Vermicompost significantly reduced strawberry root and-crown-rot with significant increase to the produced fruits yield and their total soluble solids (T.S.S.) , when each of them was used alone or in their different combinations, compared with control treatment . Furthermore, vermicompost was the most efficient treatment in this respect in comparison with the other three items of disease management. Moreover, no apparent infection (dead plants) was detected when *B.subtilis* , *B. thuringiensis* , BTH and vermicompost were used together, which resulted in producing the highest fruit yield of high T.S.S., firmness and total ascorbic acid (vitamin-c), to somewhat, nearly similar to control treatment (un-infested soil with the any of the causal fungi).

Keywords: Strawberry, Bacillus bioagents, Disease management, Firmness, Fruit yield, Total soluble solids (T.S.S), Vermicompost.

الملخص

تم عزل العديد من العزلات الفطرية، وهي*Fusarium spp.* :، و*F. solani*، و *Macrophomina phaseolina*الطور البكنيدي للفطر *Sclerotium bataticola*، والفطر الشبيه بالفطريات *Phytophthora cactorum*، و) *Rhizoctonia solani*الطور الناقص للفطر *Thanatephorus cucumeris*، و*Sclerotinia sclerotiorum*، و) *Sclerotium rolfsii*الطور السكليروتيني للفطر (*Athelia rolfsii*، وذلك من جذور نباتات الفراولة التي تظهر عليها أعراض تعفن الجذور والتاج، والتي جُمعت من حقول مختلفة في منطقة تاجوراء، طرابلس، ليبيا. أظهرت اختبارات القدرة الإمراضية أن جميع هذه العزلات تسببت في أعراض تعفن الجذور والتاج، وكان الفطران *M. phaseolina*و *R. solani* هما الأكثر ضراوة. تم عزل ستة عوامل حيوية من جنس *Bacillus*من التربة المحيطة بجذور نباتات الفراولة التي بدت خالية من الإصابة بتعفن الجذور والتاج. قُيمت هذه البكتيريا لقدرتها التضادية ضد عزلة *M. phaseolina*)رقم 3) وعزلة *R. solani*) رقم 2) في المختبر (in vitro) وفي الحقل .(in vivo) أظهرت كل من بكتيريا *B. subtilis*وبكتيريا *B. thuringiensis*أعلى قدرة تضادية ضد النمو الخطي لكلا الفطرين الممرضين. كما تسبب الراشح المعقم (شاي الكمبوست) للسماد العضوي الدودي (Vermicompost)المستخدم في تثبيط معنوي للنمو الخطي للفطرين الممرضين مقارنة بمعاملة المقارنة .(Control)وقد ازداد هذا التثبيط بواسطة *B. subtilis* و*B. thuringiensis*والسماد الدودي بشكل تدريجي ومعنوي بزيادة تركيزاتها. أدى الجمع بين العوامل الحيوية *B. subtilis*) و(*B. thuringiensis*، ومحفز المقاومة(Bion/BTH) ، والسماد الدودي إلى خفض معنوي في نسبة الإصابة بتعفن جذور وتاج الفراولة، مع زيادة معنوية في محصول الثمار ومحتوى المواد الصلبة الذائبة الكلية(T.S.S) ، سواء عند استخدام كل منها على حدة أو في تركيبات مختلفة، مقارنة بمعاملة المقارنة. علاوة على ذلك، كان السماد الدودي هو المعاملة الأكثر كفاءة في هذا الصدد مقارنة بعوامل إدارة المرض الثلاثة الأخرى. إضافة إلى ذلك، لم تُسجل أي إصابات ظاهرة (نباتات ميتة) عند استخدام *B. subtilis* و*B. thuringiensis* و (BTH)والسماد الدودي معاً، مما أدى إلى الحصول على أعلى محصول من الثمار ذات الصلابة العالية، والمحتوى العالي من المواد الصلبة الذائبة الكلية، وحمض الأسكوربيك الكلي) فيتامين(C ، بنسبة تقارب إلى حد ما النتائج المتحصل عليها في معاملة المقارنة (التربة غير الملوثة بأي من الفطريات المسببة للمرض).

الكلمات المفتاحية :الفراولة، عوامل *Bacillus*الحيوية، إدارة الأمراض، الصلابة، محصول الثمار، المواد الصلبة الذائبة الكلية(T.S.S) ، السماد العضوي الدودي.(Vermicompost)

Introduction

Strawberry (*Fragaria* ananassa) is become one of the most important and delicious untraditional crops of a high economic value in Libya. On the other hand, root and-crown-rot poses a serious threat to commercial strawberry production worldwide, which causes severe economic losses. Many complains were received from strawberry growers during the last decade due to infection by root and crown-rot caused by *Macrophomina phaseolina* , which causes great mortality to the growing plants with high reduction to the produced fruit yield (Abied and Duzan,2020). Strawberry is grown in most arable regions of the world including West Libya.

The infection by strawberry root and-crown-rot is responsible for causing great mortality to the growing plants with high yield loss in commercial strawberry fields (Mertely,2005; Fang *et al.*, 2011; Juber *et al.*,2014; Sánchez *et al.* ; 2016, Abied and Duzan,2020 and Abd-El-Kareem

et al.,2022). Symptoms of the infection by *Macrophomina phaseolina* consisted of wilting of foliage, drying and death of older leaves, plant stunting, and eventual collapse and death of plants (Koike, 2008 and . Abied and Duzan,2020). When plant crowns were dissected, internal vascular and cortex tissues were dark brown to orange brown. When signs are absent, incubating symptomatic stems with the top of the root system for 24 to 48 hours in a moist chamber usually results in abundant vegetative growth of the fungus and later, production of sclerotia . *Rhizoctonia solani* causes black root rot and crown rot in strawberries, leading to stunted growth, leaf yellowing, vascular browning, and plant collapse. Symptoms include dry, sunken, reddish-brown to black lesions on roots and crowns, often appearing in patches within fields, particularly in warm temperatures or stressed conditions (Abd-El-Kareem *et al.*,2022)

The early detection and diagnosis of the infection by the causals of root and-crown-rot in the transplants are very essential for development of an effective disease management strategy. In recent years, biological control has become a promising safer and ecologically acceptable alternative to fungicides control in the management of many soil-borne diseases (Ragab *et al.*,2015 and Abada *et al.*,2025). Among Baciullus bioagents, both *B.subtilis* and *B. thuringiensis* received more attention than many other bacterial bioagents (Santoyo *et al.*, 2012 and Abada *et al.*,2018).

The major approach to managing root and-crown-rot in strawberry has relied upon the use of synthetic fungicides. However, fungicide applications bring a range of issues such as accumulation of developing acquired resistance in the targeted pathogens, toxic residue on the fruits, with drawl of the chemical products from the market, and negative impact on the environment and human health (Iqbal *et al.,* 2019) .There is a growing interest in utilizing plant extracts and bioagents as sustainable, environmentally friendly alternatives to conventional chemical pesticides for disease management (Salem et al., 2023; Salem, 2026). Recent research has demonstrated the efficacy of various bioactive compounds in inhibiting the growth of phytopathogens and bacterial strains (Alshawish et al., 2025; Salem, 2025).

Disease management rather than chemical management must be done due to strawberry fruits are consumed mainly as fresh or canned. In this respect, biological management has emerged as an alternative safety and most promising means of the management of plant pathogens. Biocontrol of strawberry root and-crown-rot caused by root and-crown-rot can be accomplished by either promoting the native antagonists such as that found in vermicompost to reach a density sufficient to suppress the pathogen(s) or by introducing alien antagonists. Among the several antagonists tested by various scientists, genus *Bacillus* have been found effective in inhibiting the causal of many soil-borne pathogens (Fang *et al.*,2011 ; Juber *et al.*, 2014; Abada *et al.*, 2018 and2025). Therefore, introducing several antagonists against the causal of root and-crown-rot of strawberry *seems* to hold great promise to managing the infection by fungal root and-crown-rot.

Bion (BTH) is become a one of the common approaches used in managing plant diseases , which is of much less harmful to the environment and plant products in comparison to the harmful of agrochemicals that used for managing plant diseases (Yan *et al.,* 2003 and Carrion *et al.*,2019).

This work aimed to investigate the role of Bion (BTH), *Bacillus subtilis, B.thuringiensis* and vermicompost, each alone or in different combinations in management of root and-crown-rot of strawberry caused by *M.phaseolina* and *R.solani*. In addition, evaluate the reflect of these items on the produced fruits yield and their T.S.S., firmness and total ascorbic acid (vitamin-c).

Materials and Methods

1. Isolation, Purification and Identification of the Associated Fungi to Strawberry Root and-Crown-Rot:

At the end of the growing season (April-May) collapsed and dying strawberry plants were observed in several fields at Tagoraa, Tripoli, Libya. Strawberry plants showing characteristic root and-crown-rot symptoms were collected. The diseased crown and root samples were thoroughly washed by running tap water and cut into small segments (0.5 cm) with lesion having half healthy and half diseased tissue. The segments were surface sterilized with 2 % sodium hypochlorite for two minutes. The sterilized segments were subsequently washed in three changes of sterilized distilled water to eliminate excess sodium chlorite and then the pieces were transferred onto PDA medium amended with 250 mg L^{-1} of chloramphenicol in Petri-dishes. The dishes were incubated at 25 ± 1°C and observed periodically for the fungal growth. Axenic culture of the isolated fungi was obtained by hyphal tip technique and / or single spore method and maintained on PDA slants throughout the investigation. The emerged fungi were identified depending on the basis of their morphological features and the description of Gilman (1957); Booth (1971) and Domsch *et al.* (1980).

2. Isolation, Purification and Identification of The Bacillus Bioagents:

Rhizospheric soil of strawberry plants grown in a field have severe infection by root and-crown-rot, were collected to isolate Bacillus antagonists. Serial of dilution plate technique (Johnson and Curl, 1959) was used to isolate native antagonistic *Bacillus* spp. on nutrient agar medium (Oedjijono and Dragar,1993). The isolated bacteria were then purified and identified depending on the description of Parry *et al.* (1983) and Holt and Krieg (1984).

3. Pathogenicity Test of the Isolated Fungi:

Formalin disinfested clay soil was infested by 2 % inoculum level of any of the isolated fungi *i.e.*, each alone and distributed in Plastic post (25 cm in diameter). Sweet Charlie strawberry cv. transplants were dipped in 1% of the Maxim fungicide (Fludioxonil belongs to the chemical class of phenylpyrazoles) for 30 minutes to make sour that the transplants were not infested with any fungi then 2 transplants (Sweet Charlie cv.) were transplanted in each pot. Transplants transplanted in un-infested soil were prepared as control. Five pots were prepared for each treatment and the control. The incidence and severity of root and-crown-rot were assessed using the devised scale (0.0-5) by Fang *et al.* (2011). Total soluble solids (TSS) and firmness of the fruits were estimated and recorded (Kafkas e*t al*.,2007). Also, total ascorbic acid (TAA) was estimated spectrophotometrically using a procedure described by Hodges *et al.* (2001)

4. Effect of the Bioagents *Bacillus subtilis* and *B. B.thurengensis* on The Linear Growth of The Pathogenic Two Fungi:

The efficacy of both *B. subtilis* and *B.thurengensis* on the linear growth of *M.phaseolina* isolate-3and *R.solani* isolate-1 was evaluated as a method given by Dennis and Webster (1971).

One hundred ml. of nutrient medium were put in each 250 ml conical flask and sterilized by the autoclave. The medium was inoculated with a loop of the *B. subtilis* and *B.thurengensis* taken from two days-old culture. Inoculated flasks were incubated on a rotary shaker at 200 rpm for 3 days at 30 ± 2°C . The growth of any of the tested bacterial bioagents (heated to about 35ºC) was sterilized using 0.25 µm syringe filter then mixed with the component of PDA medium in different proportion (10, 25 and 50 %) , just before solidification, and poured into Petri-dishes (20 ml $plate^{-1}$).

After solidification the Petri-dishes were carefully inoculated with 5 mm. discs of any of the test two pathogens cut from the five day old culture. PDA dishes inoculated with the test pathogen, but not amended with culture filtrate of the bacterial bioagents were maintained as control. Plates were then incubated in an incubator at 30±2°C. Each treatment was repeated five times. Observations on the inhibition were periodically done on the linear growth of both fungi then recorded when control plates of complete growth. Inhibition percentage of the mycelial growth of both pathogens was calculated by the formula:

$$I = (C- T)/C X100$$

Where;

I = Percentage of inhibition in the growth of test pathogen,

C = Linear growth of pathogen (mm) in control and

T = Linear growth of pathogen (mm) in treatment.

5. Effect of The Filtrate of Vermicompost Tea on The Linear Growth of The Causal Two Pathogens:

One kg virmicompost were soaked overnight in three-liter water then filtrate through two layers of Whatman 1 filter paper. The filtrate was sterilized using 0.25 µm syringe filter. Concentrations of 10, 25 and 50% of the filtrate (heated to about 35ºC) were added to the calculated amount of PDA medium, just before solidification and poured in sterilized Petri-dishes. After solidification the Petri-dishes were carefully inoculated with 5 mm. discs of any of the two pathogenic pathogens cut from the five-day old culture. PDA plates inoculated with any of both pathogens, but not amended with vermicompost filtrate (normal PDA) were maintained as control (check). Dishes were then incubated in an incubator at 30±2°C. Five replications were prepared for each treatment. Periodic examination to the linear growth of both fungi were recorded. Inhibition percentage of the mycelial growth of both pathogens was calculated as mentioned before.

6. Effect of Combination among *B.subtilis, B.thurengensis* , BTH, Vermicompost, on Management of root and-crown-rot:

In the present investigation, transplants of Sweet Charlie strawberry cv. were used. The two pathogens were isolated from strawberry crowns by tissue segment method on PDA medium. BTH, *B.subtilis* and *B.thurengensis* and Vermicompost were tested to evaluate their efficiency against the two pathogenic fungi *i.e.,M.phaseolina* and *R.solani* , each alone or in different combinations, *in vivo* (Table, 4).

The soil was divided in into four groups. The first group was infested with the inoculum of the fungus *M.phaseolina* (grown on corn-sand medium) at the rate of 2% inoculum level and did not treated with another treatments. The second group was infested with the fungus *R.solani* at the rate of 2% inoculum level and did not treated with another treatments. The third group was infested with the fungus *M.phaseolina* at the rate of 2% inoculum level and the following treatments were carried out (a) strawberry transplants soaked in 40 mM BTH for 30 mint. just before sowing, (b) the bioagents *B.subtilis* and *B.thurengensis* ($1x10^6$ cfu L $water^{-1}$) were added to each pot, each alone, at the rate of 100 ml pot^{-1}, and (c) 100 g vermicompost were added to each pot (mixed thoroughly with the soil of each pot), each alone or in different combinations. The fourth group was done for the pathogen *R.solani* as in the third group (Table,4).

In all cases strawberry transplants (Sweet Charle Frigo transplants cv.) were dipped in 1% of the fungicide Maxim fungicide for 30 minutes just before transplanting to make sour that the transplants were un-infested with any fungal pathogen.

Recommended agricultural practices *i.e.*, irrigated, fertilization was done. The incidence and severity of root and-crown-rot were assessed as shown under disease assessment.

The produced mature fruits were harvested periodically, weighed and the average was of the produced yield was recorded.

7. Effect of The Tested Treatments on Fruit Firmness, Total Soluble Solids (TSS) and Total Ascorbic Acid (Vitamin-C) :

Twenty-five fruits taken randomly per each treatment were used to measure fruit firmness using a digital penetrometer (model 53205, TR). The total soluble solids content was measured using the juice of randomly 25 fruits, by a digital table refractometer (HI96811; Hanna instruments) (Kafkas *et al.*,2007).

Also, twenty-five fruits taken randomly were used the assess total ascorbic acid (TAA) by spectrophotometrically using a procedure described by Hodges *et al.* (2001). Fresh fruit tissues (25 g) were weighed and placed in a 250 ml centrifuge tube and 100 ml of ice-cold 5% (w/v) metaphosphoric acid was added. This followed by homogenization at the speed of 15,000 rpm for 2 min in an ice-water bath by the aid of a homogenizer. The homogenized tissues were centrifuged at 7000 rpm for 15 min at 4 °C. The supernatant was filtered through filter paper (Whatman No. 1). The obtained filtrate was used for calculating total ascorbic acid (TAA) by converting dehydro-ascorbic acid (DAA) to FAA with dithiothreitol. Finally, TAA were estimated spectrophotometrically at 525 nm. The concentrations of TAA were assessed by using the standard curve (all $R\ 2 \geq 0.99$) of L-ascorbic acid and their distinction was equal to the concentration of DAA.

8. Disease Assessment:

Disease incidence (DI%) for root and crown-rot was determined 8-weeks after transplanting as dead plants.

Disease severity was estimated according to symptoms on the aerial part of the plants and damage observed in the crown and roots. The symptomatology of the aerial part was monitored weekly during 10 weeks after inoculation, and was scored based on severity of symptoms according to a scale from 0.0 to 5 as described by Fang *et al.* (**2011)** . Also, the severity of tissue damage in the crowns and on roots was assessed at the end of the experiment, by making longitudinal cuts of the crowns and the rate of rotten of the roots (0.0-5). Tissue necrosis rotten were scored based on an arbitrary visual scale using the following formula:

$DS\% = \Sigma d / (d\ max \times n) \times 100$.

Where:

(d)= Disease rating of each plant,

(d) =Means of the maximum disease rating (5) and

(n)= Represents the total number of plants tested in each replicate.

9. Statistical Analysis:

Data were statistically analyzed using the standard procedures for complete randomized block, split and split split designs as reported by Snedecor and Cochran (1989). The averages were compared at 0.05 level using least significant differences (L.S.D) according to Fisher (1948).

3. RESULTS

3.1. Isolation, Purification and Identification of The Associated Fungi to Root and-Crown-Rot:

Many fungal isolates were isolated from strawberry plants (Sweet Charlie cv.) showing characteristic symptoms of root and-crown-rot collected from different areas at Tagoraa, Tripoli, Libya. The isolated fungi were purified and identified as *Fusarium* spp. (5 isolates) , *F.solani*) 4 isolates) , *Macrophomina phaseolina* (the pycnidial state of *Sclerotium bataticila*) (6 isolates) , the fungus like *Phytophthora cactorum* (4 isolates), *Rhizoctonia solani* (the imperfect state of *Thanatephorus cucumeris*) (7 isolates), and *Sclerotium rolfsii* (the sclerotial state of *Athelia rolfsii*) (3 isolates).

Pathogenicity **T**est of and *R.solani M.phaseolina* Isolates:

Pathogenicity test of *M.phaseolina* and *R.solani* isolates revealed that both fungi were pathogenic to Sweet Charlie strawberry cv. and showing typical root and-crown-rot

symptoms on the foliage growth, roots and the crowns. Results, also, reveal that *M.phaseolina* isolate-3 and *R.solani* isolate-2 were the most pathogenic ones than the other isolates and resulted in the lowest figures of fruits weight (g) plant^{-1} , % total soluble solids (T.S.S.), fruits firmness and total ascorbic acid. Re-isolated from diseased strawberry plants proved their pathogenicity. Therefore, both *M.phaseolina* isolate-3 and *R.solani* isolate-2 were selected to carry out this investigation due to their high virulence.

Table 1. Pathogenicity test of isolates of *M.phaseolina* isolate-3 and *R.solani* isolate-2 using Frigo transplants of strawberry (Sweet Charlie cv.), pot experiment.

Isolates	% Root and-crown-rot incidence	% Root and-crown-rot severity	Average weight of fruits (g) plant^{-1}	% Total soluble solids	Fruits firmness *	Total ascorbic acid
M.phaseolina-isolate-1	10	38.8	210.6	9.32	1.11	34.7
M.phaseolina isolate-2	30	37.4	278.7	9.26	1.16	36.6
M.phaseolina isolate-3	50	45.3	230.3	9.12	1.01	30.8
M.phaseolina isolate-4	10	41.2	240.7	9.24	1.16	34.7
M.phaseolina isolate-5	40	43.4	240.2	9.22	1.12	33.2
M.phaseolina isolate-6	30	44.2	254.0	9.23	1.09	32.0
R.solani isolate-1	40	43.5	243.3	9.21	1.11	33.3
R.solani isolate-2	60	57.4	228.7	9.18	0.98	29.6
R.solani isolate-2	20	45.0	250.3	9.26	1.18	33.6
R.solani isolate-4	40	42.6	239.5	9.28	1.08	34.8
R.solani isolate-5	50	41.4	240.2	9.29	1.10	34.6
R.solani isolate-6	40	46.2	254.3	9.26	1.12	33.2
R.solani isolate-7	30	45.2	244.8	9.25	1.07	35.0
Control	0.0	0.0	488.5	10.87	1.54	52.4

3.2. Isolation, Purification and Identification of The *Bacillus* spp.:

Isolation trial from the rhizospheric soil of healthy strawberry roots grown in a field have severe infection by root and-crown-rot at Tagoraa, Tripoli , Libya on nutrient agar medium yielded many Bacterial species. Six species belonging to genus *Bacillus* were isolated , purified and identified as: *Bacillus chitinosporus, B. coagulans, B.humilus, B megaterium ,B. subtilis* and *B. thuringiensis* addition to unknown genera. The isolated bacteria were used for testing their antagonistic action against *M.phaseolina isolate-3* and *R.solani isolate-2 in vitro.*

Results shown in Table (2) indicate that all the tested six isolates of *Bacillus* spp. resulted in significant reduction to the linear growth of both of *M.phaseolina* isolate-3 and *R.solani* isolate-2 , 5 days after incubation at 30±1°C compared with control treatment. Linear growth reduction was gradually increased significantly by increasing the concentration of incorporated culture filtrate of the tested Bacillus bioagents in comparison with the control treatment. Meantime, both, *B.subilis* and *B. thuringiensisi* were the most affected ones in this regard, being 27.7 and 29.1 mm linear growth, on the average. Meanwhile, *B.chitinosporus* was the lowest affected one, being 39.4 mm linear growth, on the average.

Table 2. Effect of six *Bacillus* spp. culture filtrate *in vitro* on the linear growth of *M.phaseolina* isolate-3 and *R.solani* isolate-2 , 5 days after incubation at 30±1°C.

Bacillus bioagents	Pathogens	Linear growth (mm) at conc. (%)			Mean	General mean
		10	25	50		
B.chitinosporus	*M.phaseolina*-3	66.2	37.0	16.4	39.9	39.4
	R.solani-2	63.6	34.4	15.0	38.8	
B.coagulans	*M.phaseolina*-3	65.6	36.8	16.0	39.5	38.7
	R.solani-2	63.0	34.8	15.6	37.8	
B.humilus	*M.phaseolina*-3	65.6	36.4	16.0	39.3	38.5
	R.solani-2	62.0	34.8	14.6	37.7	
B.megaterium	*M.phaseolina*-3	63.0	33.4	14.8	37.8	36.8
	R.solani-2	61.6	32.2	13.2	35.7	
B.subtilis	*M.phaseolina*-3	56.2	25.4	0.0	27.2	27.7
	R.solani-2	58.0	26.2	0.0	28.1	
B. thuringiensis	*M.phaseolina*-3	58.8	26.2	0.0	28.3	29.1
	R.solani-2	60.6	28.8	0.0	29.8	
Control *	*M.phaseolina*-3	90.0	90.0	90.0	90.0	90.0
	R.solani-2	90.0	90.0	90.0	90.0	
Mean	*M.phaseolina*-3	66.5	40.7	21.9	42.1	
	R.solani-2	65.5	40.2	21.2	42.8	-----
General mean		66.0	40.5	21.6	----	-----

L.S.D. at 0.05 for : Bacterial bioagents (B) = 3.4, Pathogens (P) = n.s, Concentrations (C) = 4.1. B × P = 3.0, BxC = 3.3, PxC= 3.2 and BxPxC= 3.1 .

3.3. Effect of vermiompost Tea Filtrate on The Linear Growth of Both Pathogens:

Table (3) shows that vermicompost tea filtrate caused significant inhibition to the linear growth of both *M.phaseolina* isolate-3 and *R.solani* isolate-2 , five days after incubation at 30±1^{O}C compared with control treatment. This inhibition was gradually increased significantly by increasing the concentration, being 72.7 and 32.4 mm at the concentrations 10 25 %, respectively . In addition, both pathogenic fungi failed to grow on the concentration of 50 %.

Table 3. Effect of vermicompost tea filtrate on the linear growth of *M.phaseolina* isolate-3 and *R.solani* isolate-2 , five days after incubation at 30±1^{O}C.

Concentration (%)	Linear growth (mm) of		Mean
	M.phaseolina isolate-3	*R.solani* isolate-2	
10	71.6	74.8	72.7
25	29.8	34.0	32.4
50	0.0	0.0	0.0
Control *	90.0	90.0	90.0
Mean	47.9	49.7	

L.S.D. at 0.05 for: Concentration (C) = 3.3, Pathogens (P) = 1.4, C × P = 3.8.

3.4. Effect of *B.subtilis,B.thurengensis,* BTH and Vermicompost , each alone or in Different Combinations, on Management of Strawberry Root and Crown-Rot Caused by and *M.phaseolina* isolate-3 and *R.solani* isolate-2:

The obtained resulted (Table 4) revealed that the combination among the bacterial bioagents *B.subtilis* and *B. thuringiensisi* , BTH and vermicompost caused significant reduction to the incidence and severity of strawberry root and-crown-rot. The superior management of strawberry root and-crown-rot was obtained from the combination among bacterial bioagents *B.subtilis* and *B. thuringiensisi* , BTH and vermicompost, where no incidence of the disease was occurred and low disease severity were recorded for both pathogens *i.e.,* 2.4 and 3.0%, on the average. In addition, The bi- combination between two treatments of *B.subtilis* and *B. thuringiensisi* , BTH and vermicompost was more efficient in managing root and crown-rot compared with using each of them alone. The fungus *R.solani* isolate-2 was , to somewhat, aggressive than the fungus *M.phaseolina* isolate-3.

Table 4. Effect of *B.subtilis, B. thuringiensis,* BTH and Vermicompost , each alone or in different combinations, on the incidence and severity of the infection by *M.phaseolina* isolate-3 and *R.solani* isolate-2, the causal of root and-crown-rot of strawberry (Sweet Charlie cv.), plot experiment.

Treatments	% Root and-crown-rot incidence for		Mean	% Root and-crown-rot severity for		Mean
	M.phaseolina isolate-3	*R.solani* isolate-2		*M.phaseolina* isolate-3	*R.solani* isolate-2	
B.subtilis (BS)	30	20	25	16.6	14.2	15.4
B. thuringiensis (BT)	30	20	25	15.8	13.0	14.4
BTH	40	20	30	17.2	15.8	16.5
Vermicompost (V)	30	20	25	14.6	13.0	13.8
BS+BT	20	10	15	12.8	12.2	12.5
BS+BTH	20	10	15	11.2	10.6	10.9
BS+V	20	10	15	11.2	10.0	10.6
BT+BTH	10	10	10	11.8	10.4	11.1
BT+V	10	10	10	9.4	8.8	9.1
BTH+V	10	10	10	10.0	9.6	9.8
BS+BT+BTH+V	0.0	0.0	0.0	2.4	3.0	2.7
Control	60.0	40.0	50	48.4	41.6	45.0
Mean	18.3	11.7	---	15.1	13.5	----

L.S.D. at 0.05 for:

Treatments (T) =	3.2	2.7
Pathogens (P) =	2.9	1.3
TxP =	3.5	3.7

respectively. No apparent disease incidence was detected when the combination among the bioagents *B.subtilis* and *B.thurengensis* , BTH, and vericompost was performed and the lowest root and-crown-rot severity were recorded compared with the other treatments and the control.

3.5.Effect of *B.subtilis,B.thurengensis,* BTH and Vermicompost , Each Alone or in Different Combinations, on Fruit Yield and it's T.S.S , Firmness and Total Ascorbic Acid (Vitamin-C):

Data presented in Table (5) reveal that *B.subtilis,B.thurengensis***,** BTH and vermicompost resulted in considerable increase to the produced fruits and their total soluble solids (T.S.S.), firmness and total ascorbic acid (vitamin-c), either each of them was used alone or in their different combinations, compared with control treatment (infested with any of the two causal fungi). In general, the highest figures of the produced fruits and their total soluble solids (T.S.S.), firmness and total ascorbic acid (vitamin-c) were obtained from the combination among *B.subtilis,B.thurengensis*, BTH and Vermicompost . The bi combination between two treatments of *B.subtilis* and *B. thuringiensisi* , BTH and vermicompost was more efficient in recorded intermediate values compared with using each of them alone. In addition, vermcompost was the most efficient one in this regard when used alone compared with the other three items of disease management *i.e.,* the bacterial bioagents *B.subtilis* and *P.fluorescens* and BTH when each of them was used alone. The fungus *R.solani* resulted in considerable reduction to the assessed items than the fungus *R.solani.*

Table 5. Effect of combination among Vermicompost, SA and the Bacterial Bioagents *B.subtilis* and *B.thurengensis* on fruit yield and it's T.S.S , firmness and total ascorbic acid

Treatments	Average of fruit yield(g) plant^{-1}		% Total soluble solids		Fruits firmness for*		Total ascorbic acid**	
	MF***	RS****	MF	RS	MF	RS	MF	RS
B.subtilis (BS)	410.3	408.6	9.15	9.14	1.82	1.80	47.8	46.8
B.thurengensis (BT)	412.6	410.5	9.17	9.16	1.81	1.80	45.6	43.1
BTH	401.8	400.9	9.20	9.18	1.86	1.285	45.0	44.8
Vermicompost (V)	454.4	448.4	10.87	10.84	1.95	1.92	48.1	47.0
BS+BT	426.1	422.0	10.18	10.15	1.88	1.86	50.1	49.8
BS+BTH	430.5	430.7	10.17	10.15	1.92	1.90	49.3	49.0
BS+V	460.0	455.5	11.18	11.16	2.12	2.10	52.6	51.2
BT+BTH	429.8	425.5	10.79	10.76	1.98	1.96	49.3	48.5
BT+V	479.8	473.3	10.88	10.85	2.13	2.11	56.8	55.4
BTH+V	470.6	365.8	10.95	10.92	2.19	2.17	57.0	56.7
V+SA+BS+PF	545.8	540.5	11.40	11.38	2.32	2.30	59.4	58.3
Control	556.4	550.6	11.65	11.61	2.38	2.36	62.8	61.4
L.S.D. at 0.05	3.8	3.6	1.4	1.4	0.8	0.7	2.9	3.0

*Maximum value = 0.41and minimum value=3.19, ** mg 100g fruit^{-1} fresh weight. ***=*M.*phaseolina-3 and ***= *R.solani*-2.

Values are the average of five replicates. Different letters in each row show the significant statistical difference ($p < 0.05$) between the samples.

3. **Discussion**

Globally, farmers are interested in lowering their dependence on agrochemicals input for agricultural production and pests managements in order to produce healthier products. So, rather than chemical control such as agriculture practices, sanitation, biological control, resistant cvs. , soil solarization …ect could be exploded to play an important role in Integrated Pest Management (IPM) systems, especially in case of vegetables production. A model describing the several steps required for a successful IPM has been developed by Mc Spadden and Fravel (2002). In addition, soil infestation with plant pathogens in strawberry fields are amongst the most limiting factors in production system.

Many fungal isolates were isolated from strawberry plants showing mainly root and-crown-rot symptoms grown at different location in Tagoraa, Tripoli, Libya. They purified and identified as: *Fusarium* spp. , *F. solani* , *Macrophomina phaseolina* (the pycnidial state of *R.solani*),

Phytophthora cactorum (fungus like), *Rhizoctonia solani* (imperfect state of (*Thanatephorus cucumeris*) and *Sclerotium rolfsii* (the imperfect state of *Athelia rolfsii*). These fungi were previously isolated from strawberry plants showing root and crown- rot by Golzar *et al.*(2007) ; Fang *et al.* (2011; Hajlaoui *et al.*(2015; Awad, 2016; Sánchez *et al.* (2016); Attia (2019); and Hafez *et al.*,2025). Pathogenicity test of the isolates of *M.phaseolina* revealed that they all caused root and-crown-rot symptoms. *M.phaseolina* isolate -3 and *R.solani* isolate-1 were the most pathogenic ones>

The tested culture filtrate of the six *Bacillus* spp., resulted in different degrees of inhibitory effect to the linear growth of both *M.phaseolina* isolate-3 and *R.solani* isolate-2, 5 days after incubation at 30±1°C compared with control treatment. In this respect, both fungi failed to grow on the concentration of 50 % culture filtrate of both *B.subtilis* and *B.thurnengsis* . Meanwhile, *B.chitinosporus* was the lowest effective one in reducing the linear growth of the two causal pathogens.

Scientists toward to application induction of systemic resistance for protection from plants diseases as a new approach, where this is much less harmful to the environment in comparison with many agrochemicals applied to management plant diseases (Kloepper *et al.*,2004).

Jacobsen *et al.* (2004) mentioned that Bacillus-based biological control agents (BCAs) have great potential in integrated pest management (IPM) systems; however, relatively little work has been published on integration with other IPM management tools. Unfortunately, most research has focused on BCAs as alternatives to synthetic chemical fungicides or bactericides and not as part of an integrated management system.

Sterilized aqueous filtrate of the tested vermicompost resulted in significant reduction to the linear growth of the tested two fungi compared with control treatment. This reduction was gradually increased by increasing it′s concentration. Compost and/or vermicompost have, also, been shown to suppress several plant diseases in the field. The suppressive effect of compost and/or vermicompost generally, increased with rate of application. Integrated pest management (IPM) is a sustainable approach to managing pests by combining many items viz. bioagents, organic manure (cattle and poultry manure, compost and/or vermicompost) resistant cultivars, cultural and sanitary practices, physical and chemical tools in a way that minimizing environmental risks with economic return to the farmers in order to fructification healthy agricultural products (Noble and Coventry ,2003) . So, the present work evaluated the integrated use of vermicompost with another disease management including two efficient bacterial bioagents and BTH. This integration is important because the consistency and degree of disease management by the both bioagents , BTH and vermicompost, could be equal to the management afforded by the best fungicides. Therefore, integration of several tools of disease management brings stability to the disease management programs .

Using of bacterial bioagents (*B.subtilis* and *B.thurengensis*), BTH and vermicompost caused significant reduction to the severity of the infection by the two pathogenic fungi with significant increase to the produced fruits yields and their total soluble solids (T.S.S.), firmness and total ascorbic acid (vitamin-c) in comparison with control treatment. In addition, the combination among the two bacterial bioagents, BTH and vermicompost, was more efficient in reducing disease severity and increasing fruit yield and it′s T.S.S, firmness and total ascorbic acid (vitamin-c) than when each of them was used alone. Moreover, the combination among the the two bacterial, BTH and vermicmpost was the superior treatment in this regard , which no apparent infection by the root and-crown-rot was detected and the highest fruit yield and it′s T.S.S, firmness and total ascorbic acid (vitamin-c) were achieved . The highest efficiency of this combination may be greatly due to the drastic effect of the two bacterial bioagents (*B.subtilis* and *B.thurengensis*) on the propagules of both pathogenic fungi

in addition to induce acquired resistance by these bioagents and BTH. Furthermore, vermicompost can play a suitable medium for reproduction and establishment of the added bacterial bioagents and saprophytic microbes in the soil. In this regard, Noble and Coventry (2003) reported that composts have also been shown to suppress several diseases in the field, although the effects have been generally smaller and more variable than in container experiments. The disease suppressive effect of compost generally increased with rate of application. Compost inclusion rates of at least 20% (v/v) are normally required to consistently obtain a disease suppressive effect, particularly in peat-based media, but significant disease suppression has been found at lower inclusion rates in soil.

Management of soil-borne plant diseases in strawberry is challenging because the causal pathogens, can survive for long periods on many hosts and / or as Sclerotia and fruit bodies. The management of root and-crown-rot is currently accomplished primarily through the use of soil treatment with fumigants, fungicides , biological control and , to somewhat , resistant cvs. However, the frequent and discriminate use of soil fumigation and fungicides leads to atmosphere pollution and create imbalance in the microbial community, which maybe unfavorable to the reproduction and survival of beneficial micoflora and may lead to development of resistance strains of the pathogens (Attia, 2019).

Kwok (1987) mentioned that copiotrophic bacteria recolonize composts most rapidly (24-48 h) after peak heating of compost. He added that the predominant biocontrol agents in this group include strains of *Bacillus* species. In addition, edaphic microorganisms stimulated by compost amendments contribute to the suppressive activity of the amended soil through four control mechanisms *i.e.,* antibiosis , hyperparasitism , competition and the induction of systemic acquired resistance in the host plant (Lockwood,1988).

It has been reported that *Bacillus* spp. could be have diverse plant response involved in synthesis and accumulation of antimicrobial phytoalexins (Hammond-Kosack and Jones,1996),), production of activated oxygen species (Baker *et al.*,1993), induction of hypersensitive response (He *et al.*,1993, modification of plant cell wall by deposition of callose (Veit *et al.*,2001) and production of defense-related proteins (Yu *et al.*,2011).

Kloepper *et al.*(2004) mentioned that various species of the genus *Bacillus* can induce a distinct broad-spectrum resistance response in both below- and above-ground parts of the plant. This mode of disease resistance is named as induced systemic resistance (De Vleesschauwer *et al.*,2009). The application of some *Bacillus* strains to the seedlings has been found effective for suppressing soil-borne plant diseases and has successfully induced systemic resistance in the treated plants (Kloepper *et al.,* 2004 and Szczech and Shoda, 2007). BCAs may offer more durable and fungicidal effect without any toxic/unsafe residues in human food chain (Martín and Bull, 2002; Choudhary and Johri 2009 and Abada *et al*.,2025). PGPR can produce a variety of antibiotics often associated with the ability of the bacteria to prevent proliferation of plant pathogens. Meantime, a number of PGPRs can generate enzymes such as proteases, chitinases, glucanases, and lipases that can lyse a portion of the cell walls of many pathogenic fungi (Majeed *et al.,* 2015). The mechanisms by which these BCAs suppress many types of pathogens differ among species/strains and therefore can be used in IPM (Lucy *et al*., 2004 and Remans *et al.*, 2008).

Bargabus *et al*.(2004) reported that for a long time, phytopathologists have begun to characterize the determinants and pathways of induced resistance stimulated by bioagents and other non-pathogenic microbes.

The application of vermicmpost alone or in combination with BTH, the two bioagents increased the produced fruits and their T.S.S., firmness and total ascorbic acid (vitamin-c) compared with the control. This enhancement may be due to the beneficial properties of

these treatments. The incorporating vermicompost with plant growth promoting bacteria resulted in significant increases in tomato fruit yield and quality (Ruiz and Salas Sanjuan ,2022). Also, Maas (1998) revealed that mineral nutrients in plant-available forms, a hormone-like effect on plant growth, and stimulation of plant mineral nutrition are all potential beneficial mechanisms of vermicompost on plants. Mahadeen (2009) determined that organic farming improves fruit´s soluble solids content of strawberries. The observed inhibition of the pathogenic fungi, *M. phaseolina* and *R. solani*, is consistent with previous findings regarding the broad-spectrum antimicrobial activity of natural extracts and their potential to combat resistant strains through diverse mechanisms (Salem & Salem, 2024; Salem, 2025).

4. Conclusions

Among the isolated fungi from the rotted roots and crowns of strawberry plants, both *M.phaseolina* and *R.solani* where the most dominant and virulence to strawberry plants. In addition, *B. subtilis* and *B. B.thurengensis* , BTH and vermicompost showed different degrees of inhibition to the main causals of strawberry root and-crown-rot *i.e.,M.phaseolina* and *R.solani*. Pot experiment revealed that applying *B. subtilis* , *B. B.thurengensis* , BTH and vermicompost, each alone or in different combination, resulted in considerable reduction to the infection by root and-crown-rot of strawberry (Sweet Charlie cv.) caused by *M.phaseolina* and *R.solani* . This reduction in the infection by root and-crown-rot resulted in considerable increase in the produced fruits and their T.S.S., firmness and total ascorbic acid (vitamin-C). The use of vermicompost in combination with plant growth promoting rhizobacteria (PGPR) and inducer resistance chemicals as alternative eco-friendly environment for disease management is required for obtaining healthy agricultural production.
This achieve needs to apply under field conditions in order to confirm the efficiency of these items in management of root and-crown-rot of strawberry**.**

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The authors declare that they have no conflict of interest.

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