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Effect of combination among vermicompost, salicylic acid and bacterial bioagents on management of Rhizoctonia crown and root-rot of strawberry

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ABSTRACT

Isolation from the roots of strawberry plants appearing mainly root-rot signs collected from Ismailia, Sharkia, Kalubia in addition to Giza governorates resulted in *Macrophomina phaseolina, Fusarium oxysporum, Pythium ultimum* (fungus-like), *F. solani, Phytophthora cactorum* (fungus-like), *Rhizoctonia fragariae, Sclerotium rolfsii* in addition to *R. solani*. Pathogenicity test of the isolates proved that they prompted root-rot signs as well. Both *R. fragariae* in addition to *R. solani* were the most malignant ones. Four isolates of *Bacillus* spp., i.e., *Bacillus cerous, B. humilus, B. megaterium, B. subtils* in addition to *Pseudomonas fluorescens* and *P. putida* were also isolated from the rhizospheric soil of strawberry plants cultivating in a field exhibiting a serious illness by root-rot. They were assessed for their inhibitory effect towards both *R. fragariae* and *R. solani in vitro* or *in vivo*. On the whole, *P. fluorescens* accompanied by *Bacillus subtilis* were the foremost efficacious in lowering the linear outgrowth of both pathogenic fungi. Sanitized aqueous filtrate of the examined vermicompost leaded to substantial drop in the linear outgrowth of the examined two fungi in comparison with untreated group. This drop was progressively raised by elevating its concentration. The combination among vermicomposting, salicylic acid (SA) or the bioagents *B. subtilis* or *P. fluorescens* leaded to substantial drop in strawberry root-rot with substantial elevation in the produced fruits in addition to their total soluble solids (TSS), either every of them was utilized only or in their diverse arrangements, in comparison to control treatment (infested with any of causative two fungi). On the opposite side, vermicompost was the most efficacious in this case in comparison with the remaining three illness management elements, i.e., SA in addition to the biologic agents *B. subtilis* or *P. fluorescens* when every of them was utilized only. Furthermore, no obvious infection was found when vermicompost, SA, the biologic agents *B. subtilis* or *P. fluorescens*, in addition to soil solarization were applied together. Then, the yielded fruits were obtained with a high TSS, firmness or total ascorbic acid (vitamin-c), to some extent, comparable to untreated group (un-infested soil with the any of causative fungi).

Keywords: Strawberry, Bacterial bio agents, Fruit yield, Rhizoctonia, Total soluble solids, Vermicompost.
Article type: Research Article.

INTRODUCTION

Strawberry, (*Fragaria ananassa*) is among the foremost crucial in addition to flavorful unusual crops, which is of a high economic value. The crop is favorable as well as appreciated by millions of people around the globe. It is susceptible to infection by an array of soil-borne diseases. Yet, crown or root-rot prompted by genus *Rhizoctonia* creates a major danger to the global commercial strawberry crop then substantial financial consequences are the result. Strawberry is cultivated in most fertile areas of the planet. Over the previous few centuries several complains have been submitted by strawberry farmers regarding the loss of strawberry plants resulting from crown and root-rot infection shortly after planting the plants till the conclusion of the cultivating season. Rhizoctonia infection of strawberry caused by *R. fragariae* and *R. solani* holds responsibility for triggering large output damages in the manufacturing of commercial strawberries (Fahim *et al.* 1989; Fang *et al.*

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2011; Juber *et al.* 2014; Abd-El-Kareem *et al.* 2022). The early recognition in addition to diagnostic of the infection by both pathogens in plants then infested soils are very crucial for creation of a successful disease prevention plan. Biological management has recently emerged as a potential, safer, and more sustainable substitute to chemical control in the treatment of a variety of soil-borne diseases (Ragab *et al.* 2015; Abada & Hassan 2017; Amini *et al.* 2023; Mukhambetov *et al.* 2023). Genera *Bacillus* in addition to *Pseudomonas* got more focus than most other bacterial groups among the bacterial bioagents (Santoyo *et al.* 2012; Utama *et al.* 2021). In most cases, the major approach to control crown and root-rot in strawberry cultivation has dependent on injecting artificial fungicides (Coque *et al.* 2020). However, the usage application of fungicides raises a number of problems, including the buildup of resistance in the diseases that are being targeted, harmful residue on fruits, the removal of chemical items from the market, and adverse outcomes on the earth as well as society (Iqbal *et al.* 2019; Mheidi *et al.* 2023 Mheidi *et al.* 2023). Since strawberry fruits are primarily utilized cool or preserved, disease administration must be utilized instead of chemical control. In this aspect, biological control has evolved into a more efficacious as well as substitute way to handle plant infections. Biocontrol of strawberry root rot triggered by the *Rhizoctonia* genus can be accomplished by either boosting native antagonists, like those present in vermicompost, to a density adequate for suppression of pathogen(s) or inserting alien antagonists. Amid the many antagonists investigated by scientists are the *Bacillus* in addition to *Pseudomonas* genera, which have been proven to be beneficial for lowering the spread of numerous soil-borne diseases (Fang *et al.* 2011; Juber *et al.* 2014; Abada & Hassan 2017; Attia 2019). The development of many antagonists towards the genus *Rhizoctonia* appears to hold great potential in preventing root-rot triggered by any of *R. fragariae* and *R. solani* and has been proved successful in limiting the spread of these fungi during *in vitro* conditions. Initiation of systemic resistance towards plant pathogens has become one of the most common approaches utilized for plant disease management, and it is considerably less detrimental to the environment and plant products than dangerous agrochemicals utilized in plant disease management (Yan *et al.* 2003; Carrion *et al.* 2019; Syman *et al.* 2023). The current inquiry seeks to look into the impact of vermicompost, SA, and two bacterial bioagents in multiple combinations in the treatment of strawberry root rot triggered by *R. fragariae* or *R. solani*. The effects of these items of disease management on the producing fruits and their T.S.S, firmness and total ascorbic acid (vitamin-c) were estimated.

MATERIALS AND METHODS

Isolation, purification as well as identification of the associated fungi to strawberry root-rot

Strawberry plants with distinctive root-rot signs were gathered from Ismailia, Sharkia, Kalubia and Giza governorates, Egypt. The infected crown and root samples, each was carefully cleansed with flowing tap water before being sliced into short segments (0.5-1.0 cm) with the lesion comprising half normal and half ill tissue. The segments were surface sanitized for two minutes utilizing 2% sodium hypochlorite. The sanitized segments were afterwards rinsed in three variations of sanitized distilled water to get rid of extra sodium chlorite before being placed to PDA medium in Petri dishes. The dishes were incubated at 25 ± 1 ºC and fungal outgrowth was monitored on a regular basis. Axenic cultures of the isolated fungus were generated utilizing the single spore approach or the hyphal tip approach then cultivated on PDA slants during the examination. The fungi that appeared were recognized based on their morphological traits and the identifications keys provided by Booth (1971) & Domsch *et al.* (1980).

Isolation, purification as well as identification of the bacterial antagonists

Soil samples acquired from the root zone soil of plant expanding in a field that had a substantial root-rot infection were utilized to pick up the antagonists. The serial dilution plate approach (Johnson & Curl 1959) was employed to pick up colony native antagonistic *Pseudomonas fluorescens* and *Bacillus* spp. on dietary agar medium (Oedjijono & Dragar 1993). Bacteria were subsequently cleansed and recognized utilizing the details given by Parry *et al*. (1983) and Holt & Krieg (1984). The identity was validated utilizing the Biolog System approach (Biocontrol of faba bean chocolate spot disease project, Plant Pathology Research Institute, ARC, Giza, Egypt).

Pathogenicity test of *R. fragariae* and *R. solani* isolates

Formalin-treated clay soil had been contaminated with 2% inoculum levels of *Rhizoctonia fragariae* and *R. solani*, every by itself, and spread in a 25-cm diameter plastic post. Festival strawberry cv. transplants were immersed in 1% Uniform 390 SE fungicide (mefenoxam along with azoxy strobin) for 3% minutes in order to ensure that they

were free of any fungal pathogens, and then two transplantations were planted in every pot. Transplantation placed in un-infested soil served as untreated group. The prevalence as well as severity of Rhizoctonia crown or root-rot were assessed utilizing the devised scale (0.0-5) by Fang *et al.* (2011). Total soluble solids (TSS) as well as fruit hardness were taken into account then captured (Kafkas *et al.* 2007). In addition, total ascorbic acid (TAA) was measured spectrophotometrically utilizing an approach reported by Hodges *et al.* (2001).

Impact of the culture filtrate of the bacterial biologic agents on the linear growth of the examined two pathogens

The impacts of the culture filtration of the 4 isolates of *Bacillus* spp. or *Pseudomonas fluorescens* on the outgrowth of *R. fragariae*, or *R. solani* were examined as an approach described by Denni & Webster (1971). Every 250 mL flask was filled with 100 mL nutritional medium then sanitized utilizing a steamer for three days in a row. The medium was injected utilizing a loop of the biologic agents from a culture that had been cultivated for two days. Flasks with inoculum were incubated at 200 rpm for three days at 30 ± 2 ºC. The culture filtrate was gathered in a flask after being passed via Whatman No.1 filter paper. The culture filtrate (heated to around 40 ºC) of any of the examined bacterial bioagents was combined in various amounts (25, 50 or 75%) with the ingredient of PDA medium. The medium was next steam sanitized for 3 days then placed into Petri plates (20 mL plate^{-1}). Following solidification, the Petri-dishes were meticulously contaminated with 5 mL discs of the examined pathogen sliced from 5- day-old culture. PDA plates contaminated with the examined pathogen yet not altered with bioagent culture filtrate served as controls. The plates were placed in an incubator set to 30 ± 2 ºC. For every treatment, 5 replicates were preserved. Periodic assessments on the linear outgrowth of the examined fungus were conducted. Inhibition rate (%) of the mycelial outgrowth of examined pathogens was estimated by the following equations:

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

where:

I = Percent of inhibition in growth of examined pathogen,

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

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

Impact of vermicompost tea on the linear outgrowth of the causative two pathogens

One kilogram of vermicompost was submerged overnight in 3-L water, afterwards, it passed via two sections of Whatman 1 filter paper. The filtrate was sanitized subsequently and the estimated concentrations of 25, 50 and 75% of the filtrate (heated to approximately 40 ºC) were incorporated to the prescribed amount of PDA medium, and shortly before solidification, were transferred into sanitized Petri dishes. After solidification, the Petri-dishes were properly contaminated with 5 mL discs of both of the two pathogens examined, sliced from a 5-day-old culture. PDA plates injected with any of the pathogens examined unfortunately not altered with vermicompost filtrate (normal PDA) served as controls. Plates were incubated in an incubator at 30 ± 2 ºC. For every treatment, five replications were preserved. Periodic inspection of the examined two fungi's linear outgrowth was documented. Inhibition rate (%) of the mycelial outgrowth of examined two pathogens was investigated as aforementioned.

Impact of combination among vermicompost, salicylic acid and two bacterial bioagents *B. subtilis* in addition to *P. flurescens* on management of root-rot along with some crop parameters

Vermicompost, SA also, the great antagonistic bacterial biologic agents *B. subtilis* or *P. flurescens* were employed to evaluate their efficiency towards the examined two pathogens, every alone or in diverse combinations, *in vivo* (Table 4). The soil was divided in four groups:

The first group was infested with the fungus *R. fragraiae* 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 an average of 2% inoculum level and did not treated with another treatments.

The third group was infested with the fungus*R. fragraiae* at the rate of 2% inoculum level. The following treatments were carried out: (a) strawberry transplants soaked in 50 mM salicylic acid just before sowing: (b) the bioagent *P. fluorescens* (1×10^6 CFU L water^{-1}) was inoculated to every pot at the rate of 100 mL pot^{-1}: and (c) 50

g vermicompost were added to each pot (mixed thoroughly with soil of each pot), each alone or in different combinations. The fourth group was infested with *R. fragraiae* at the rate of 2% inoculum level. The following treatments were carried out: (a) strawberry transplants soaked in 50 mM salicylic acid just before sowing: (b) the bioagent *B. subtilis* (1×10^6 CFU L water^{-1}) was inoculated to every pot at the rate of 100 mL pot^{-1}: and (c) 50 g vermicompost were added to each pot (mixed thoroughly with soil of each pot), each alone or in different combinations (Table 4). In all cases strawberry grafts (Frigo transplants) were immersed in 1% fungicide Uniform 390 SE minutes before transplantation (mid-October, 2021) to ensure that the transplants were free of any fungal disease. The pots were watered as needed and fertilized with the proper doses as suggested by the Ministry of Agriculture and Land Reclamation. The prevalence or seriousness of crown and also root-rot were assessed as shown under disease assessment. The plots were then watered for three days before being planted with Festival strawberry cv. In addition, the mature fruits were taken on a regular basis, weighed, and the average was documented.

Measurement of fruit firmness, total soluble solids (TSS) along with total ascorbic acid (vitamin C)

Twentyfive fruits per each treatment were utilized to determine fruit firmness. Fruit firmness was assessed at the equator at two opposite spots, utilizing a digital penetrometer (model 53205, TR). A digital table refractometer was utilized to figure out the total soluble solids content of the juice from the 25 fruits (HI96811; Hanna instruments; Kafkas *et al.* 2007). Total ascorbic acid (TAA) was determined spectrophotometrically utilizing an approach reported by Hodges *et al.* (2001). Fresh fruit tissues (25 g) were weighed and deposited in a 250 mL centrifuge tube before being combined with 100 mLice-cold 5% (weight per volume) metaphosphoric acid. This accompanied with blending at the speed of fifteen thousand rpm for two min in an ice−water bath utilizing a homogenizer. The blended tissues were centrifuged at 7000 rpm for 15 min at 4 ^{o}C. The supernatant was passed via utilizing Whatman No. 4 filter paper. The resulting filtrate was utilized to calculate total ascorbic acid (TAA) via the transformation of dehydro-ascorbic acid (DAA) to FAA with dithiothreitol. Finally, TAA was calculated utilizing spectrophotometry at 525 nm. TAA concentrations were determined utilizing the standard curve (all $R^2 \geq 0.99$) of L-ascorbic acid, and the variation was equivalent to DAA concentration.

Disease assessment

The disease prevalence (DI%) of total crown and root rot illnesses were assessed according to the following equation 10 weeks after transplanting:

DI (%) of crown and root rot = Number of plants of rotted crowns and roots /Total number of examined plants ×100. The seriousness of the disease was assessed based on signs on the plants' aerial parts and damage observed on the crown and roots. The symptomatology of the airborne component was assessed weekly for ten weeks after inoculation and was scored on a scale of 0.0 to 5 outlined by Fang *et al.* (2011) with slight adjustments. At the end of the experiment, the severity of tissue destruction in the crowns and the roots was also measured through carrying out longitudinal crown incisions, as well as calculating the rate of rotten roots (0.0-5). Tissue necrosis was graded on an arbitrary visual scale utilizing the equation given:

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

where:

(d)= Disease rating of every plant,

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

(n) = Indicates the total number of plants examined in every replicate.

Statistical analysis

Details were evaluated statistically utilizing the usual methods for fully randomized block, split, as well as splitting split designs outlined by Snedecor & Cochran (1989). The mean values were considered significant at the 0.05 level using Fisher's (1948) least significant difference (LSD).

RESULTS

Isolation, purification and identification of the associated fungi to crown along with root-rot

Isolation examines from strawberry plants (Festival cv.) with root-rot signs gathered from Ismailia, Sharkia, Kalubia, and Giza governorates yielded an abundance of fungal isolates. The isolated fungi underwent

purification and recognized as *Fusarium oxysporum* (4 isolates), *F. solani* (4 isolates), *Pythium ultimum* (3 isolates), *Phytophthora cactorum* (4 isolates), *Rhizontonia fragariae* (3 isolates), *R. solani* (4 isolates), and *Sclerotium rolfsii* (3 isolates). The two *R. fragariae* and *R.solani* isolates were selected to carry out their pathogenic potentiality.

Pathogenicity test of the isolates *of R. fragariae* and *R. solani*

Pathogenicity testing of *R. fragariae* (3 isolates) and *R. solani* (4 isolates) isolates proved that both fungi were detrimental to festival strawberry cv. along with displayed usual crown or root-rot signs on leaf growth, roots, or crowns. The results additionally revealed that *R. fragariae* isolates from Ismailia governorate and R. solani isolates from Kalubia governorate were the most aggressive ones examined, as a result, were utilized for the subsequent tests.

Table 1. Pathogenicity test of *R. fragariae* and *R. solani* isolates using Frigo transplants of strawberry (Festival cv.) pot experiment.

Isolates	Disease incidence (%)	Crown and root-rot severity (%)	Average weight of fruits (g)plant^{-1})	Total soluble solids (%)	Fruits firmness *	Total ascorbic acid
R. fragariae (Ismailia)	10.8	38.5	400.6	9.18	1.10	35.8
R. fragariae (Sharkia)	8.6	35.4	378.7	9.26	1.16	36.6
R. fragariae (Kalubia)	8.8	35.8	380.3	9.28	1.08	36.8
R. solani (Ismailia)	12.6	41.1	340.7	9.26	1.06	34.6
R. solani (Sharkia)	13.2	43.4	330.2	9.22	1.02	33.2
R. solani (Kalubia)	14.0	44.2	324.0	9.20	1.00	32.0
R. solani (Giza)	13.3	43.5	323.3	9.21	1.01	33.3
Control	0.0	0.0	588.0	10.60	1.34	52.34

Note: * Maximum = 0.41 and minimum = 3.19.

***In Vitro* impact of three *Bacillus* spp., *P. fluorescens* and. *putida* on the linear outgrowth of the two examined pathogens**

Bacillus cereus, *B. megaterium*, *B. sunltis*, *Pseudomonas fluorescens*, and *Pseudomonas putida* were the bacterial colonies cultured from the rhizospheric soil containing wholesome strawberry roots, together with unknown species. The isolated bacteria were cleansed and used for testing their antagonistic action against *Rhizoctonia fragariae* then *R. solani in vitro* and *in vivo*. The results provided in Table (2) depict that all three *Bacillus* spp. isolates examined, *P. fluorescens* or *P. putida*, demonstrated a substantial decrease in the linear development of both *R. fragariae* and *R. solani*, 5 days after incubation at 28± 1 °C in comparison with untreated group. The decline was progressively augmented through raising the concentrations of integrated culture filtrate of the investigated biologic agents in comparison with untreated group.

Impact of filtrate of vermicompost on the linear outgrowth of both pathogens

The findings presented in Table 3 reveal that the vermicompost filtrate substantially lowered the linear outgrowth of *R. fragariae* then *R. solani*, five days after incubation at 28 ± 1 ℃ in comparison with control treatment. The decline was gradually accelerated through raising the concentration. Furthermore, both fungi were unable to develop at a concentration of 75%.

Impact of combination among vermicompost, SA and the bacterial biologic agents, *B. subtilis* or *P. fluorescens*

As shown in Table (4), a combination of vermicompost, SA, or the bacterial bioagents *B. subtilis* and *P. fluorescens* contributed to a substantial decrease in the prevalence and severity of strawberry *Rhizoctonia* crown and root-rot, followed by a substantial rise in the amount of generated fruits or their total soluble solids (TSS), firmness, and total ascorbic acid (vitamin C), when utilized alone or in multiple sets, in comparison

with untreated group (infested with any of the two causal fungi). The superior management of strawberry *Rhizoctonia* crown then root-rot was obtained from the set among vermicompost, SA, the bacterial biologic agents *B. subtilis* and *P .fluorescens*, where no incidence of the disease was occurred and low disease severity was recorded (2.9%, on the average) followed by the combination among vermicompost + SA + *P. fluorescens*, being 3.3 and 5.4%, on the average, respectively then the combination among vermicompost +SA+ *B .subtilis* being 3.3 and 5.6%, on the average, respectively. The bi-combinations between vermicompost, SA, *B. subtilis* and *P. fluorescens* recorded intermediate values of prevalence in addition to seriousness of the disease. In addition, when SA, the bacterial bioagents *B. subtilis* and *P. fluorescens* were each administered separately, vermicompost was the most efficacious in this aspect in comparison with the other three disease control tools, being 7.9 and 15.1%, on the average, respectively. The fungus *F. solani* was to somewhat, aggressive compared to *R. solani.*

Table 2. *In vitro* impact of three *Bacillus* spp., *P. fluorescens* or *P. putida* culture filtrate on the linear outgrowth of *R. fragariae* and *R. solani*, 5 days after incubation at 28 ± 1°C.

Bioagents	Pathogens	Linear growth (mm) at conc. of (%)			Mean	General mean
		25	50	75		
B. cereus	*R. fragariae*	84.2	45.2	20.6	50.0	50.6
	R. solani	85.0	46.0	22.4	51.1	
B. megaterium	*R. fragariae*	83.4	41.6	16.2	47.1	47.4
	R. solani	84.0	42.0	17.2	47.7	
B. subtilis	*R. fragariae*	80.0	33.4	0.0	37.8	38.3
	R. solani	81.6	34.2	0.0	38.6	
P. fluorescens	*R. fragariae*	79.2	31.4	0.0	36.9	37.1
	R. solani	80.0	32.0	0.0	37.3	
P. putida	*R. fragariae*	82.8	33.2	0.0	38.7	38.9
	R. solani	83.2	34.0	0.0	39.1	
Control *	*R. fragariae*	90.0	90.0	90.0	90.0	90.0
	R. solani	90.0	90.0	90.0	90.0	
Mean	*R. fragariae*	81.9	36.8	7.4	42.1	
	R. solani	82.8	37.6	7.7	42.8	-----
General mean		82.4	37.2	7.6	----	-----

Note: * Control not included in the mean; L.S.D. at 0.05 for: Bioagents (B) = 3.2, Pathogens (P) = n's; Concentrations (C) = 4.8 B × P = 4.0, B ×C = 3.6, P ×C = 3.9 and B ×P ×= 4.2.

Table 3. Impact of vermicompost tea on the linear outgrowth of *R. fragariae* and *R.solani*, 5 days after incubation at 28 ± 1°C.

Conc. (%)	Linear growth (mm) of		Mean
	R. fragariae	*R. solani*	
25	74.6	76.8	75.7
50	40.2	44.0	42.1
75	0.0	0.0	0.0
Control *	90.0	90.0	90.0
Mean	38.3	40.3	

Note: * Control not concluded in the mean LSD at 0.05 for: Concentration (C) = 3.9, Pathogens (P) = 1.6, C × P = 4.8.

No apparent disease incidence was observed when the set among vermicompost, salicylic acid and the bioagents *B. subtilis* and *P. fluorescens* was used and the lowest crown or root-rot severity was found in comparison with the other treatment and control treatment.

Impact of combination among vermicompost, SA and the bacterial biologic agents, i.e., *B. subtilis* or *P. fluorescens* on fruit output along with its T.S.S, firmness and total ascorbic acid (vitamin C)

Details shown in Table (5) indicate that the applying SA, vermicompost, and the bacterial bioagents, *B. subtilis* or *P. fluorescens* in set substantially raised the amount of total ascorbic acid (vitamin C), total soluble solids

(T.S.S), then firmness in the fruits generated in comparison with the untreated group (infested with either of the two responsible fungi). In general, the highest figures of the generated fruits and their total soluble solids (T.S.S.), firmness in addition to total ascorbic acid (vitamin C) were obtained from the set among vermicompost, SA, the bacterial biologic agents *B. subtilis* and *P. fluorescens*, followed by the combination among vermicompost + SA + *P. fluorescens* then the combination among vermicompost + SA + *B. subtilis*. The bi-combinations between vermicompost, SA, *B. subtilis* and *P. fluorescens* recorded intermediate values. However, vermicompost was the most efficacious one in this respect, in comparison with the other three illness management aspects *i.e.,* SA, the bacterial biologic agent *B. subtilis* and *P. fluorescens* when each were employed alone. The fungus *F. solani* dropped the appraised items substantially more than the fungus *R. solani*

Table 4. Impact of set among vermicompost, SA and the bacterial bioagents *B. subtilis* in addition to *P. fluorescens*, each only or in diverse sets, on the incidence and seriousness of *Rhizoctonia* crown along with root-rot of strawberry (Festival cv.), plot experiment.

Treatments	Disease incidence (%) for		Mean	Crown and Root-rot severity (%) for		Mean
	R. fragariae	*R. solani*		*R. fragariae*	*R. solani*	
Vermicompost (V)	7.4	8.4	7.9	14.6	15.5	15.1
Salicylic acid (SA)	9.4	10.0	9.7	17.0	18.1	16.6
B. subtilis (BS)	8.6	9.2	8.9	15.6	16.0	15.8
P. fluorescens (PF)	8.2	8.6	8.4	15.6	15.8	15.7
V+SA	6.6	7.0	6.8	13.0	13.8	13.4
V+BS	6.2	6.8	6.5	13.0	14.0	13.5
V+PF	6.0	6.6	6.3	12.8	13.2	13.0
SA+BS	7.8	8.2	8.0	13.2	13.6	13.4
SA+PF	7.6	8.2	7.9	13.2	14.0	13.6
BS+PF	7.6	8.0	7.8	11.2	11.4	11.3
V+SA+BS	3.0	3.6	3.3	5.4	5.8	5.6
V+SA+PF	3.0	3.6	3.3	5.2	5.6	5.4
V+SA+BS+PF	0.0	0.0	0.0	2.8	3.0	2.9
Control	24.0	26.0	25.0	41.4	43.0	42.2
Mean	6.3	6.8	---	11.7	12.3	----
Treatments (T) =			3.1		2.7	
Pathogens (P) =			n.s		n.s	
T ×P =			3.2		3.9	

Table 5. Impacts of combination among vermicompost, SA and the bacterial biologic agents *P. fluorescens* or *B. subtilis* on fruit output along with its 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 for**	
	RF***	RS****	RF	RS	RF	RS	RF	RS
Vermicompost (V)	440.3 ^{f}	432.8 ^{f}	10.15 ^{a}	10.13 ^{a}	1.32^{b}	1.38^{b}	47.8 ^{c}	46.9 ^{c}
Salicylic acid (SA)	410.6 ^{g}	405.5 ^{g}	9.87 ^{a}	9.86 ^{a}	1.95 ^{a}	1.98 ^{a}	42.6 d	42.1 ^{e}
B. subtilis (BS)	401.8 ^{h}	395.9 ^{h}	9.86 ^{a}	9.84 ^{a}	1.86 ^{a}	1.95 ^{a}	43.0 ^{d}	42.8 ^{e}
P. fluorescens (PF)	404.4 ^{h}	400.4 ^{g}	9.87 ^{a}	9.84 ^{a}	1.87 ^{a}	1.96 ^{a}	43.1 ^{d}	43.0 ^{e}
V + SA	456.1 ^{d}	450.0 ^{d}	10.18 ^{a}	10.15 ^{a}	1.24^{b}	1.18	50.1 ^{b}	49.8 ^{b}
V + BS	450.5 ^{e}	440.7 ^{e}	10.17 ^{a}	10.15 ^{a}	1.22^{bc}	1.28 ^{c}	49.3 ^{b}	49.0 ^{b}
V + PF	452.0 ^{e}	442.5 ^{e}	10.18 ^{a}	10.16 ^{a}	1.22 ^{bc}	1.29 ^{c}	49.6 ^{b}	49.2 ^{b}
SA + BS	406.8 ^{f}	400.5 ^{g}	9.89 ^{a}	9.86 ^{a}	1.95 ^{a}	1.98 ^{a}	45.8 ^{c}	45.5 ^{d}
SA + PF	409.8 ^{g}	403.3 ^{g}	9.88 ^{a}	9.85 ^{a}	1.95 ^{a}	1.97 ^{a}	45.8 ^{c}	45.5 ^{d}
BS + PF	400.6 ^{h}	390.8 ^{h}	9.65 ^{a}	9.62 ^{a}	1.89 ^{a}	1.97 ^{a}	45.0 ^{c}	44.8 ^{c}
V + SA + BS	485.2 ^{c}	475.8 ^{c}	10.22 ^{a}	10.20 ^{a}	1.18 ^{bc}	1.21 ^{c}	51.8 ^{b}	51.4 ^{b}
V + SA + PF	490.2 ^{b}	480.9 ^{b}	10.23 ^{a}	10.21 ^{a}	1.20 ^{bc}	1.27 ^{c}	51.9 ^{b}	51.5 ^{b}
V+ SA + BS + PF	510.8 ^{a}	500.5 ^{a}	10.30 ^{a}	10.28 ^{a}	1.12 ^{bc}	1.28 ^{c}	53.4 ^{a}	53.1 ^{a}
Control	150.4^{i}	140.6 ^{i}	6.55 ^{b}	6.35 ^{b}	1.26^{b}	1.30 ^{b}	36.8 ^{e}	36.1 ^{f}

Note: *Maximum = 0.41and minimum = 3.19; ** mg 100g fruit^{-1} fresh weight; *** = *R. fragariae* and **** =*R. solani;* Values are the average of five replicates. Diverse letters in every row display the significant statistical variance ($p < 0.05$) among the samples.

DISCUSSION

Farmers are becoming more intrigued by lowering their reliance on pesticide inputs for crop cultivation in order to generate more nutritious goods. So, instead of chemical control, agricultural techniques, soil solarization, resistant, biological control, sanitation could be extended to serve a crucial part in Integrated Pest Management systems, particularly for vegetable output. A framework explaining the various phases needed for a an effective

system has been generated by Spadden & 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 developed in Ismailia, Sharkia, Kalubia, and Giza governorates, like *Pythium ultimum* (fungus-like), *Fusarium oxysporum*, *R. solani*, *Macrophomina phaseolinae*, *Phytophthora cactorum* (fungus-like), *F. solani*, *Sclerotium rolfsii* or *Rhizoctonia fragariae*. Fungi were already picked up from bacterial colony by Fahim *et al.* (1989), Mass (1998), Golzar *et al.* (2007), Fang *et al.* (2011); Abada *et al.* (2014); Awad, (2016) and Attia, (2019). Pathogenicity test of the isolates of *R. fragariae* and *R. solani* revealed crown and root-rot symptoms. *R. fragariae* isolate from Ismailia governorate and *R. isolate* isolate from Kalubia governorate were the foremost aggressive ones. Culture filtrate of three *Bacillus* spp., *P. fluorescens* and *P. putida* hindered the linear outgrowth of both *R. fragariae* and. *solani*, 5 days after incubation at 28 ± 1 °C in comparison with untreated group. In this case, both fungi struggled to develop on 100% culture filtrate of *B. subtilis* or *P. fluorescens*. Yet, *P. fluorescens* exhibited the foremost efficacious at minimizing the linear development of the causative fungus, accompanied with a *B. subtilis* isolate. In the meantime, *B. cereus* was the least effectiveness in minimizing the linear outgrowth of the two causative pathogens, accompanied with isolates of *B. pumilus* and *B. megaterium*. According to Ramamoorthy *et al.* (2001), the biopreparation treatment triggers systemic resistance as the primary form of action on a plant. This could be as *P. fluorescens* generates multiple kinds of antibiotics, involving active 2, 4 diacetyl-phloroglucinole, that regulate illnesses, since *P. fluorescens* uses multiple approaches to dominate the disease, like the output of antifungal components involving siderophore output, nutrient competition and the stimulation of systemic resistance. In addition, Meena *et al.* (2006) pointed out that the decline in plant pathogen infection along with a rise in plant length and fresh weight of the treated plants could be attributed to *P. fluorescens* generating indole acetic acid as a growth regulator in addition to some antibiotics like pyrrolnitrin, pyoluterin, or 2, 4 diacetyl phloroglucino. In the past few decades, a novel approach to disease protection has been developed through the advancement of systemic resistance in plants. This is substantially fewer destructive to the globe than the lethal agrochemicals utilized to dominate plant diseases (Kloepper *et al.* 2004). In accordance with Jacobsen *et al.* (2004), Bacillus-based biocontrol agents offer significant potential in integrated pest management systems; yet, not many studies on integration with other management approaches has been documented. Unluckily, the majority of studies on BCAs have concentrated on them as substitutes to synthetic chemical fungicides or bactericides rather than as part of an integrated management strategy. In comparison with control treatment, the disinfected aqueous filtrate of the examined vermicompost substantially lowered the linear outgrowth of the two examined fungus. By progressively raising its concentration, this decline was accelerated. Noble &Coventry (2003) noted that composts had discovered to hinder diverse illnesses in the field, however their impacts were being less also, greater variability than in container tests. In general, the disease repressive impact of compost rose with application rate. Integrated pest management (IPM) is a sustainable technique to administrating pests by blending 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. So, the present work evaluated the integrated utility of vermicompost with another disease management involving BTH and two efficient bacterial bioagents. This integration is crucial due to the consistency and degree of disease management by compost, BTH the both bioagents could be equivalent to the management supplied by the best fungicides. In theory, incorporating distinctive disease management methods enhances the stability of disease management programs. Employing vermicompost, SA, or two bacterial biologic agents (*B. subtilis* or *P. fluorescens*) brought about a substantial decrease in the seriousness of Rhizoctonia root-rot of strawberry with a substantial rise in the fruits and their total soluble solids (TSS), firmness, along with total ascorbic acid (vitamin C) compared to the control treatment. Furthermore, combining at least two vermicompost, SA, and the two bacterial bioagents was of greater benefit in lowering the incidence of disease and boosting fruit output, T.S.S, firmness, or total ascorbic acid (vitamin C) than utilizing any of them alone. Moreover, the combination among the vermicompost. SA and the two bacterial was the superior treatment in this case, No obvious infection by the illnesses was observed, albeit the greatest fruit output along with its T.S.S, firmness and also vitamin C were acquired. The greatest effectiveness of this combination could be attributed to the profound impact of the two bacterial biologic agents (*P. fluorescens* and *B. subtilis*) on the propagules of both fungi, and the ability of SA to promote acquired resistance In addition, vermicompost serves as a suitable medium for the development then generating the additional bacterial bioagents

along with saprophytic microbes in the soil. In this regard, Noble &Coventry (2003) observed that composts were proven to hinder various illnesses in the field, yet their impacts had frequently utilized fewer also, greater variability than in container tests. Compost generally had a disease-suppressing impact that grew with application rate. Particularly in peat-based media, inclusion averages of at least 20% (v/v) of compost are typically necessary to reliably generate illness-suppressing impact; nevertheless, significant disease repression has been observed in soil at lower inclusion rates. Managing soil-borne illnesses in strawberries is difficult due to the pathogens involved, can survive for long periods on many hosts and / or as resistant structures *i.e.*, chlamydospores, sclerotia and fruit bodies. It propagated through a variety of mechanisms, such as wind, soil, organic manure, and contaminated plant materials. Rhizoctonia disease is now managed mostly via soil treatment with fumigants, fungicides, biological control, and, to a lesser extent, resistant cysts. Yet, the extensive and selective utilization of soil fumigation and fungicides pollutes the environment and creates discrepancy in the organisms, which may be detrimental to the actions of helpful micoflora then might serve to boost growth of pathogen resistant strains (Martin & Bull 2002; Attia 2019). In accordance with Kwok (1987), copiotrophic bacteria recolonize composts most quickly (24- 48h) after peak heating. He went on to say that the most common biologic agents in this group are *Pantoea*, *Pseudomonas*, and *Bacillus* strains. Furthermore, Lockwood (1988) stated that compost-stimulated edaphic microorganisms give to the repressive behavior of the soil via four control systems, namely predation hyperparasitism, competition, antibiosis and the establishment of systemic gained resistance in the host plant. *Bacillus* spp. is thought to have a unique plant reaction associated with the manufacturing and storage of antibacterial phytoalexins (Hammond-Kosack & Jones1996), stimulation of hypersensitivity responder (He *et al.* 1993), manufacturing proteins involved in defensive (Yu 1995; Yu *et al.* 2011), manufacturing oxygen types that have been triggered (Baker *et al.* 1993), then callose precipitation modifies the plant cell wall (Veit *et al.* 2001). Nonpathogenic bacteria, like *Bacillus* species (Kloepper *et al.* 2004), can elicit a unique broad-spectrum immunity action across beneath- or outside-ground parts of plants. This mode of disease resistance induced systemic resistance (van Loon 2007; De Vleesschauwer *et al.* 2009). The treatment of some Bacillus strains to seedlings was discovered to be useful in controlling soil-borne illnesses then in inducing systemic resistance in the treated plants (Kloepper *et al.* 2004; Szczech & Shoda 2007). BCAs may provide a more long-lasting and fungicidal impact while leaving no toxic/unsafe residues in the human food chain (Choudhary & Johri 2009; Ahmed 2013; Abd-El Kareem *et al.* 2022). PGPR can create several kinds of antibiotics, which are frequently related to the bacterial capacity to suppress plant pathogen development. Meanwhile, a variety of PGPRs can produce enzymes like proteases, chitinases, glucanases, and lipases, which can lyse a section of the cell walls of numerous dangerous fungi (Majeed *et al.* 2015). The processes by which these BCAs reduce various types of infections differ between species/strains then can thus be exploited in IPM (Lucy *et al.* 2004; Remans *et al.* 2008). Phytopathologists are now starting to identify factors and processes of induced resistance caused by biologic agents and non-pathogenic bacteria (Park 1995; Bargabus *et al* 2004) known as systemic acquired resistance, is triggered by salicylic acid, a substance that is often produced during pathogen infection and usually results in the production of proteins involved in pathogenicity. These proteins contain a range of enzymes, a few of them may lyse intruding cells, strengthen cell wall borders to withstand infections, or induce localized cell destruction. A second one, known as induced systemic resistance, is mediated by jasmonic acid and/or ethylene, both of which are produced after the administration of some nonpathogenic rhizobacteria. The application of vermicompost alone or in combination with the bioagents and SA increased the generated fruits along with their TSS, firmness and total ascorbic acid (vitamin C) in comparison with the control. This enhancement may be due to that vermicompost has beneficial properties. Ruiz & Salas Sanjuan (2022) discovered that incorporating vermicompost with plant outgrowth encouraging bacteria resulted in significant increases in tomato fruit yield and quality. Mineral nutrients in plant-available forms, a hormone- like impact on plant growth, and stimulation of plant mineral nutrition are all possible enthusiastic mechanisms of vermicompost on plants (Zhang *et al.* 2015). Mahadeen (2009) revealed that organic cultivation induces the strawberries soluble solids content.

CONCLUSION

Pot experiment revealed that applying vermicompost, salicylic acid (SA) and two bacterial bioagents *i.e.*, *B. subtilis* and *P. fluorescens,* alone or in different set, leaded to considerable lowering the infection by crown or root-rot of strawberry (Festival cv.) prompted by *R. fragariae* and *R. solani*. The decline in the infection by crown and root-rot was reflected on the manufactured fruit output and their total soluble solids, firmness and total

ascorbic acid (vitamin), where substantial rise in the generated fruits along with their T.S.S., firmness and total ascorbic acid (vitamin C) were occurred. This experiment needs to apply under field circumstances to be able to confirm the efficiency of these items in dominating crown or root-rot of strawberry. The utilize of vermicompost in set with plant outgrowth stimulating rhizobacteria (PGPR) and inducer resistance chemicals as alternative eco-friendly environment for disease management is required for obtaining healthy agricultural production.

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