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Research Article

Efficacy of different bioagents in suppressing *Meloidogyne incognita*, and evaluation of some physio-biochemical changes in *Phaseolus vulgaris* L.

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Abstract

Plant parasitic nematodes cause severe damage, reducing plant production. The ability of four various biocontrol agents was surveyed for effectiveness in inhibiting J_2 of *Meloidogyne incognita in vitro*. The study aims to explore the impact of different bio-agents (*Bacillus cereus* 54-1, *Streptomyces erythrogriesus* sub sp. 2, *Pleurotus ostreatus*, and *Spirulina platensis*) on the root-knot-nematode, *M. incognita* reproduction, and their influence on plant growth as well as physiological and biochemical parameters in *Phaseolus vulgaris* L. plants under greenhouse conditions. Effective inoculation of four bio-control agents on growth and physio-biochemical parameters of bean plants infected with root-knot-nematode was also investigated. After 48 hours of exposure to bioagents, mortality was caused by *M. incognita* J_2s. Mortality ranged between 67.3 and 89%. Under experimental conditions, further validating the relative efficacy of different bioagents in control *M. incognita* on common bean in two successive seasons. All pageants were efficient in preventing nematode reproduction, but with varying efficacy. Oxamyl (Nematicide) was an extremely effective treatment for suppressing total nematode populations. Nevertheless, the second most effective treatment for reducing *M. incognita* in roots and soil was *B. cereus*. All treatments significantly enhanced growth as compared to the control. Treatments with four bioagents significantly reduced H_2O_2 and malondialdehyde levels. While it significantly raised the activity of peroxidase, polyphenol-oxidase, and superoxide dismutase, in addition to raising the content of phenolics and flavonoids in the infected common bean. The tested bioagents were efficient in preventing nematode reproduction, but at various levels of efficacy. In addition, all treatments significantly enhanced common bean growth parameters and reduced the levels of both H_2O_2 and MDA.

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While it raised the activity of POD, PPO, SOD, and contents of phenolics and flavonoids in the infected common bean. These results highlight the value of bioagents as a promising biocontrol technique to manage root-knot-nematodes in common beans.

Keywords: biocontrol agent; biological control; J_2; *Meloidogyne incognita*; nematodes; *Phaseolus vulgaris*

Introduction

The common bean (*Phaseolus vulgaris* L.) is an essential vegetable crop worldwide. The crop provides essential nutrients, e.g., proteins, vitamins, antioxidants, and minerals (Karavidas *et al*., 2022). Additionally, the shoot is consumed as livestock feed. As a legume plant, it improves soil fertility by atmospherically fixing nitrogen (Mao *et al*., 2022).

Plant parasitic nematodes (PPNs) are pervasive and have the potential to seriously harm and reduce the output of a variety of agricultural plants (Sasanelli *et al*., 2019). PPNs cause annual losses of about 12 to 25% of the production of the most economically essential crops worldwide, amounting to about USD 80 billion (Agrios, 2005; Nicol *et al*., 2011). Root-knot-nematodes are major plant pathogens that can cause serious damage to crops worldwide (Onkendi *et al*., 2014). There are around 5500 plant species in this genus's host area, which is shared by its many species. There are several plant parasitic nematodes linked with common beans, but *Meloidogyne* spp. root-knot-nematodes are important economically. A root-knot-nematode infestation could trigger up to 60% yield loss in common bean productivity (Adomako *et al*., 2022). Due to the wide-host range, short life cycle, rapid reproduction rate, and endoparasitic nature, *Meloidogyne* spp. can be controlled (Sivasubramaniam *et al*., 2020).

Alternative management methods for defending crops from root-knot-nematode infestations are currently being investigated because of a greater understanding of the detrimental effects of chemical pesticides and an alteration in the public's perception of environmental pollution (Mendoza-de Gives, 2022). Therefore, it is urgent to find alternative nematode control strategies. The biological management of soil-borne diseases can enhance soil health for the best possible functioning of ecosystems, is comparatively safe, friendly to the environment, and has no known negative effects on human or animal health (Sulaiman and Bello, 2024).

Nematodes and bacteria are closely related constituents of the soil biota in the natural world. Some bacteria are naturally occurring nematode killers, synthesizing poisonous, antibiotic, or inhibitory products of soil nematodes and serving as soil nematode regulators in nature. In contrast, some nematodes primarily feed on bacteria (Mendoza-de Gives, 2022). RKN is reported to be extremely virulent to rhizobacteria like the Bacillus group isolates, including *B. cereus*, from various soils (Xiao *et al*., 2018). *B. cereus* enhanced the quantities of specific defensive chemicals (Siahpoush *et al*., 2011) or enhanced systemic resistance (ISR) (Niu *et al*., 2012) in plants to control RKN and other pathogens. Abdel-Baset and Kandil (2023), showed that *B. cereus* caused a reduction in the nematode population around wheat root.

Streptomyces is the chief genus of actinomycetes that has been established to be active against nematodes by antagonism or parasitism in numerous investigations (Yoon *et al*., 2012; Kaur and Manhas, 2014). In addition, it generates over 7,500 bioactive compounds containing anticancer agents, vitamins, and antibiotic compounds (Manivasagan *et al*., 2014). Numerous strains of *Streptomyces* were effectively tested as phytopathogen biocontrol agents (Law *et al*., 2017). According to Park *et al.* (2020) it was reported that *the Streptomyces* strains caused a decline in the number of nematodes in the soil and the number of egg masses in the roots of red peppers.

One of the primary natural antagonistic groups of nematodes is fungi, which also function as soil-based bio-regulators of nematode populations (van Ooij, 2011). Nematophagous fungi are a broad class of fungi that

are thought to be the primary nematode natural enemies (Moosavi & Zare, 2020). Certain edible mushrooms have evolved complex defense systems against nematodes. For instance, one of the genera of edible mushrooms with nematocidal qualities that has been explored the most is *Pleurotus*. *Pleurotus ostreatus* produced a nematocidal toxin that rendered 95% of the free-living worm *Panagrellus redivivus* immobile (Karakas, 2020).

On the other side, application of some blue-green algae (cyanobacteria) in soil may impede PPN infestation (Holajjer *et al*., 2012) by generating hydrolytic enzymes and secondary compounds known as cyanotoxins (Gupta *et al*., 2013). Notably, *Spirulina platensis* is a potent inhibitor of root-knot nematodes as well as effectively stimulating plant growth (Hamouda *et al*., 2019).

Reactive oxygen species (ROS) production was accelerated by nematode infection. During RKN infection, chemical barriers, which comprise enzymes or secondary metabolites, are one of the plant's defence mechanisms (Jones and Dangl, 2006). Defense-related enzymes such as PPO, POD, and SOD were triggered to boost the resistance of plants, according to several studies (Waewthongrak *et al*., 2015). The primary enzyme for phenolic compound oxidation is PPO, and plant disease resistance has been positively associated with PPO activity (Quarta *et al*., 2013). The improvement in POD and PPO activity was linked to the decrease in root-knot-nematodes (RKN) (Guimarães *et al*., 2010). Certain plant secondary metabolites, such as phenols and flavonoids, are employed as plant anti-nematode phytochemicals (ANPs). According to Hajam *et al*. (2024), phenolic compounds significantly contribute to plant resistance to numerous pests and diseases. With a wide range of plant-nematode combinations, it has been discovered that higher baseline and/or induced quantities of phenolic mixtures correlate with nematode resistance (Dhakshinamoorthy *et al*., 2014). Regarding PPN resistance, flavonoids are among the most prevalent plant secondary metabolites (Hajam *et al*., 2024).

The principal aim of this study was to explore the impact of different bio-agents (*Bacillus cereus* 54-1, *Streptomyces erythrogriesus* sub sp. 2, *Pleurotus ostreatus*, and *Spirulina platensis*) on the root-knot-nematode, *M. incognita* reproduction, and their influence on plant growth as well as physiological and biochemical parameters in *Phaseolus vulgaris* L. plants under greenhouse conditions.

Materials and Methods

Isolation of bio-agents

Streptomyces erythrogresious subsp. 2 isolated from soil according to (Hammad *et al*., 2022), was plated in a liquid starch-casein-nitrate medium (Tadashi, 1975) The flasks were incubated on a rotary checker for 7-14 days at 28 °C. This strain was previously tested for its ability to combat pathogenic, negative, and positive gram bacteria as well as harmful fungi. It demonstrated antimicrobial action and identified active ingredients (Hammad *et al*., 2022).

Bacillus cereus 54-1 isolated from soil according to (Hammad *et al*., 2022), was plated in a liquid nutrient medium. For 24-48 hours, the flasks were incubated at 37 °C. This strain also has antibacterial properties against a variety of pathogenic, gram-negative, and positive bacteria, and substance-producing bacteria were identified as bacteriocen (Hammad *et al*., 2022).

Spirulina platensis, offered by the Microbiology Department of the Agriculture Research Centre's Soils, Water, and Environment Research Institute. It was raised and spread using the Zarrouk medium (Zarrouk, 1966).

Pleurotus ostreatus, kindly offered by the Microbiology Department of the Agriculture Research Centre's Soils, Water, and Environment Research Institute. It was raised on (PDA).

Nematode inoculum preparation

M. incognita must be used to start and grow a pure culture of the root-knot-nematode. The galled roots of a highly infected common bean (*Phaseolus vulgaris* L) were collected. A single egg mass was utilized to protect tomatoes (*Solanum lycopersicum* L) cv. 'Supervet', which were grown in greenhouse environments. The perpetual pattern of females was identified, according to (Taylor *et al*., 1955). After 2 months, re-injection on new tomato seedlings was carried out continuously in preparing pure egg mass culture of *M. incognita*. All subsequent studies were conducted using eggs and the nematode J_2s.

In vitro assay

Effect of bio-agent isolates on mortality of juveniles

The capability of bacterial isolates to inhibit *M. incognita's* second juvenile (J_2s) growth was tested. Mortality was calculated according to (AbdelRazek and Yaseen, 2020) Concerning the mortality test, 2 ml of fresh hatched J_2s of *M. incognita*, composed of 100±10 juveniles/ml was located in every Petri dish and 2 ml of bacterial culture from each isolate containing 1×10^8 (cfu/ml for Actinomycetes and blue-green algae) and 1×10^8 (sores /ml for fungus) were supplemented individually. Petri- dishes with nematodes and without culture were treated as controls. All dishes were incubated at 25 ± 2 °C. After 48 hours, the J_2s were counted for mortality under a stereoscope. The percentage of nematode mortality was determined using the following formula, according to Abbott (1987).

Mortality (%) = [(% of mortality in treatment – % of mortality in control)/ (100 –mortality % in control)] × 100.

Greenhouse experiments

The experiments were conducted at Ismailia Agriculture Research Station during two successive seasons, the first (15th September to 6th December 2021), and the second (15th September to 6th December 2022).

Test plants

Clean clay pots (25 cm diameter) were filled with 3 kg of sterilized clay and sand mixture (4:1 w/w). Five common bean seeds were then planted in the pots. (cv. 'Paulista'). After a week of emergence, by one seedling per pot, the seedlings were thinned. The experiment complied with relevant institutional, national, and international guidelines and legislation.

Treatments and design

Four different bio-agents (*Bacillus cereus* 54-1, *Streptomyces erythrogriesus* sub sp. 2, *Pleurotus ostreatus*, and *Spirulina platensis*) were compared in an experiment with greenhouse pots to handle *M. incognita*. In a nutrient broth medium, the test bioagents were cultivated. The mass of the bacterial microorganism was obtained by centrifugation and then suspended in the saline buffer. Spectrophotometry was subsequently used to organize each isolate to 1×108 cfu/ml, and 1×108 spore/ml; at λ=600 nm before greenhouse examinations. As a soil treatment, the bacterial microorganism inocula were injected at a rate of 25 ml of bacterial suspension per plant. A comparable treatment containing the nematicide Oxamyl (24% L), Vydate® (N', N'- dimethyl-N-((methyl carbamoyl) oxy)-1- thiooxamimidate) was added at the rate of 4 L/Fed (0. 2 ml /plant) as recommended. Untreated-infected plants are considered control. One-week-old common bean seedling cultivar 'Paulista' plants were treated with a bacterial suspension. At ten days old, each seedling was inoculated with 3,000 eggs/pot (1 egg /g soil) by drilling three holes with varying depths (2-3 cm) surrounding the roots. Following 7, and 14 days from infection, microbial inoculants were re-supplemented at the same rate. Every three days, pots were watered intermittently and kept at 30±2 °C. Thus, a fully randomized design with six treatments and four replicates was applied. Experiments were completed after 60 days of nematode inoculation.

Growth parameters included shoot and root lengths (cm), weights of the fresh shoot (SFW) and root (RFW/ g), number of branches per plant, the number of pods per plant, and pod weights per plant. Comparing the experimental treatment to the control treatment, increasing percentages of plant FW and pod weights (yield) were calculated. In addition, the biomass allocation in terms of shoot/root ratio (SRR; $g.g^{-1}$) is the ratio of the aboveground biomass to the belowground biomass. The parameter that most directly reflects biomass allocation by plants, either above or below ground, was calculated by dividing shoot/root fresh weights.

Nematode populations in each pot's soil were calculated following to (Hooper *et al*., 2005). Roots were dyed with acetic acid and acid fuchsin according to (Bybd *et al*., 1983) to investigate No. of developmental stages, females/root, No. of galls, and egg masses. Eggs/egg mass per plant were extracted by utilizing sodium hypochlorite (NaOCl) as explained by (Hussey and Barker, 1973).

The ultimate nematode populations (Pf) and reproduction factor (RF) were estimated according to Oostenbrink (1966).

Biochemical determinations

Determination of total soluble protein: The concentration of total soluble protein was calculated in different samples as mg g1 FW using the bovine serum albumin standard. According to Bradford (Bradford, 1976), 2 ml of Bradford reagent was added to 200 µl of leaf extract, and the resulting blue color was measured at 595 nm.

Non-enzymatic antioxidant

Total flavonoid contents (mg/100g FW) were determined using the aluminium chloride colorimetric method (Chang *et al*., 2002).

Total phenolic compounds (mg/100g FW) were assessed by a moderated Folin-Ciocalteu method and assessed at 650 nm depending on (Horwitz *et al*., 1970).

Enzymatic antioxidant

The extract of the enzyme was done according to Urbanek *et al*. (1991).

Peroxidase (POD) activity has been determined depending on (Allam and Hollis, 1972). POD activity was expressed as the change in absorbance each 0.5 min at 425 nm using a spectrophotometer.

Polyphenol oxidase (PPO) activity was determined according to (Matta and Dimond, 1963). PPO activity was expressed as the change in absorbance each 0.5 min at 430 nm using a spectrophotometer.

Superoxide dismutase (SOD) activity: the capacity of extracts to prevent the reduction of nitro blue tetrazolium (NBT) at 560 nm was used to measure its activity (Beauchamp and Fridovich, 1971).

Hydrogen peroxide (H_2O_2) concentration: The H_2O_2 concentration was estimated depending on the method described by (Shi *et al*., 2007). Fresh leaves were extracted with trichloroacetic acid in an ice bath. The supernatant was added to potassium phosphate buffer (pH 7) and potassium iodide. The concentration of H_2O_2 was expressed as mmol/g FW by using a standard H_2O_2 solution at 390 nm.

Malondialdehyde (MDA) concentration: MDA was determined by the thiobarbituric acid (TBA) reaction (Gallego et al., 1996). The MDA concentration was determined as (E532–E600), and the results were expressed as $\mu mol.g^{-1}$ FW.

Statistical analysis

Data were collected, checked, revised, and organized in tables and figures and were subjected to outliers' detection and handling. Normality testing is used to detect whether the data are parametric or nonparametric using Shapiro-Wilk and/or Kolmogorov-Smirnov at the 0.05 level. The data were presented as means and standard deviations. Differences between treatment groups were performed using two-way repeated measure analysis of variance at 0.05, 0.01 and 0.001 levels. ANOVA was followed by Duncan's Multiple Range Test

(DMRT) to compare between treatment groups at the 0.05 level. Interaction between variables was performed using Pearson's correlation. A heatmap was performed using PAST statistical software version 4.04. All statistical analyses were carried out using the computer software Statistical Package for Social Science SPSS (IBM-SPSS ver. 29.0 for Mac OS) (Knapp, 2023).

Results

The shoot fresh weight (g) showed in treatment groups *Bacillus cereus, Streptomyces erythrogriseus, Spirulina platensis, Pleurotus ostreatus*, Control (Nematode only), and Oxamyl (Vydate) in season 1 showed an average (±SD) of 24.10±1.01, 22.00±1.00, 19.67±0.58, 18.33±1.15, 12.33±2.08 and 26.80±1.31 g; respectively, where the difference between groups was highly significant as revealed by one way ANOVA (p<0.001). Moreover, the shoot fresh weights in season 2 were 21.77±1.08, 19.83±0.29, 18.33±0.58, 16.00±1.00, 13.00±3.00, and 24.47±1.50; respectively, and the difference between groups in season 2 was highly significant (p<0.001). SFW results showed a significant elevation in oxyamyl (Vydate group) over control, which showed the lowest values in seasons 1 and season 2. Treatment with *Bacillus cereus* significantly enhanced the shoot fresh weight over the control and was non-significant with the oxamyl group (Figure 1).

The root fresh weight (g/plant) in Season 1 showed an average (±SD) of 5.33±0.58, 4.00±0.00, 4.00±0.00, 4.00±0.00, 2.87±0.40, and 6.00±1.00; respectively. However, season-2 showed an average (±SD) of 5.00±0.00, 4.00±0.00, 3.67±0.58, 3.33±0.58, 3.00±0.00, and 5.33±0.58; respectively, and the difference between groups in season-1 and 2 was highly significant (p<0.001). RFW results showed a significant elevation in oxamyl (Vydate group) over control, which showed the lowest values in seasons 1 and season 2. Treatment with *Bacillus cereus* significantly enhanced the root fresh weight over the control and was non-significant with the oxamyl group (Figure 1; Table 1).

Shoot:root ratio (SRR, $g.g^{-1}$) in treatment groups in Season-1 showed an average (±SD) of 4.55±0.48, 5.50±0.25, 4.92±0.14, 4.58±0.29, 4.41±1.20, and 4.56±0.92; respectively, and the difference between groups was significant. However, Shoot:root ratio ($g.g^{-1}$) in Season-2 showed an average (±SD) of 4.35±0.22, 4.96±0.07, 5.08±0.80, 4.89±0.84, 4.33±1.00, and 4.62±0.57; respectively. and the difference between groups was significant.

Table 1. Multivariate analysis of variance (MANOVA) presenting the effect of different treatment groups, including treatments, seasons, and interactions between treatments and seasons

Dependent Variable	Source							
	Corrected Model		Treatment		Season		Treat. * Season	
	F	Sig.	F	Sig.	F	Sig.	F	Sig.
POD	61.9	<.001***	94.5	<.001***	150.1	<.001***	11.6	<.001***
PPO	34.2	<.001***	69.0	<.001***	18.8	<.001***	2.6	0.051 ns
SOD	20.4	<.001***	41.8	<.001***	4.1	0.053 ns	2.2	0.093 ns
Phenol	29.3	<.001***	59.8	<.001***	2.4	0.138 ns	4.3	0.007**
Flavonoid	18.8	<.001***	37.3	<.001***	7.5	0.012*	2.5	0.056 ns
Protein	9.1	<.001***	19.7	<.001***	0.0	0.967 ns	0.4	0.867 ns
MDA	12.7	<.001***	27.4	<.001***	1.5	0.240 ns	0.3	0.931 ns
H_2O_2	50.9	<.001***	102.4	<.001***	13.0	0.001***	7.0	<.001***
Shoot weight	30.3	<.001***	63.2	<.001***	12.3	0.002**	1.1	0.392 ns
Shoot length	30.2	<.001***	64.0	<.001***	4.2	0.052 ns	1.6	0.204ns
Root weight	14.2	<.001***	29.7	<.001***	4.2	0.052 ns	0.8	0.56 ns
Root length	110.4	<.001***	242.0	<.001***	1.0	0.337 ns	0.5	0.746 ns
Pod number	17.8	<.001***	38.7	<.001***	0.0	>0.999 ns	0.5	0.784 ns

Pod weight	69.6	<.001***	141.4	<.001***	26.1	<.001***	6.5	<.001***
Branch n	40.3	<.001***	85.1	<.001***	0.5	0.479 ns	3.4	0.019*
Galls	525.1	<.001***	1154.5	<.001***	2.2	0.148 ns	0.2	0.956 ns
J_2	155.7	<.001***	342.1	<.001***	0.9	0.344 ns	0.3	0.897 ns
Developmental	60.7	<.001***	132.3	<.001***	3.0	0.097 ns	0.7	0.650 ns
Females	137.7	<.001***	300.2	<.001***	3.7	0.067 ns	2.0	0.115 ns
Eggs/ root	176.3	<.001***	387.2	<.001***	1.7	0.208 ns	0.2	0.938 ns
Egg-mass	239.7	<.001***	526.8	<.001***	2.0	0.173 ns	0.2	0.960 ns
Eggs/egg mass	23.3	<.001***	50.9	<.001***	1.2	0.286 ns	0.2	0.960 ns
24-hours	856.3	<.001***	1883.9	<.001***	0.0	>0.999 ns	0.0	>0.999 ns
48-hours	1035.1	<.001***	2277.3	<.001***	0.0	>0.999 ns	0.0	>0.999 ns

*, **, *** Significant at p<0.05, <0.01, <0.001; ns: non-significant at p>0.05.

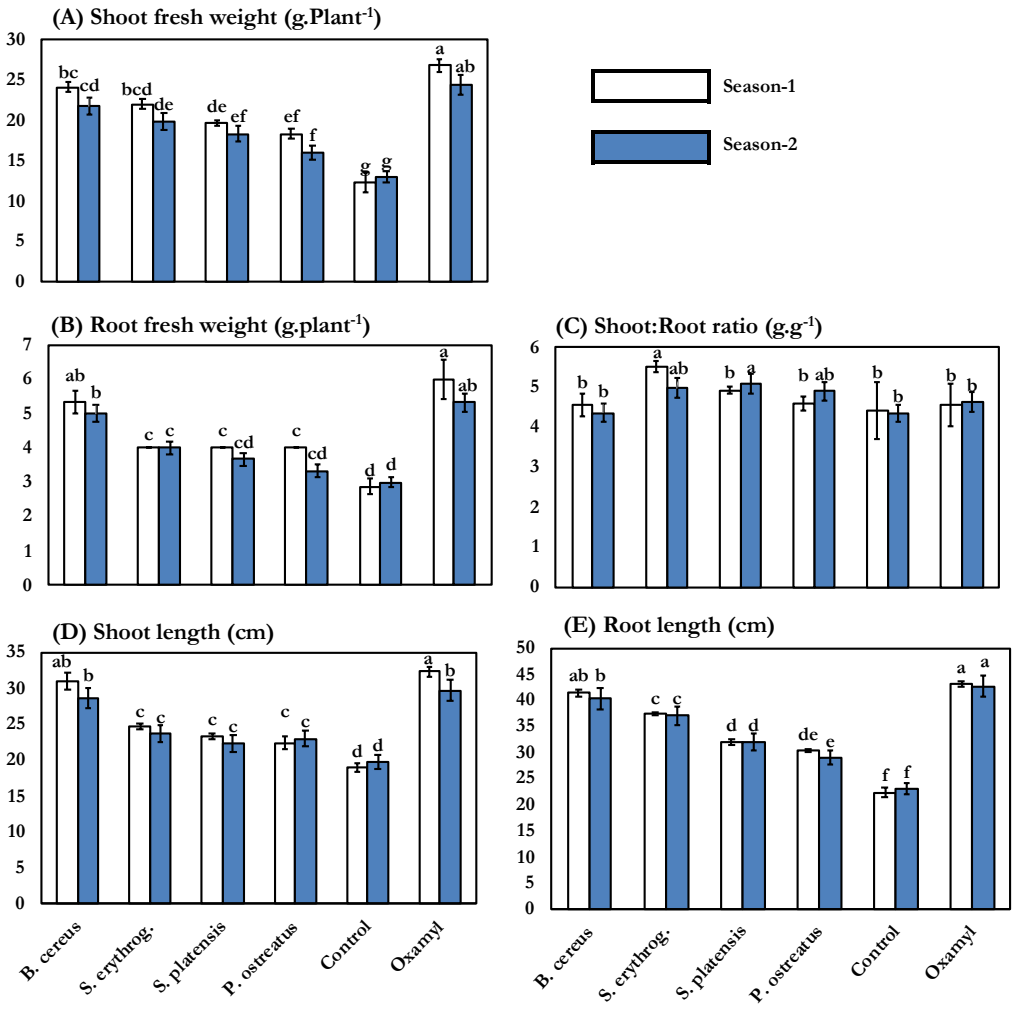

Figure 1. Various growth parameters at different treatment groups including; *Bacillus cereus, Streptomyces erythrogriseus, Spirulina platensis, Pleurotus ostreatus*, Control (Nematode only), and Oxamyl (Vydate). Bars followed by different letters are significantly different according to DMRTs (p value at 0.05 level)

Shoot length (cm) in treatment groups in season 1 showed an average (±SD) of 31.00±2.00, 24.67±0.58, 23.33±0.58, 22.33±1.53, 19.00±1.00, and 32.33±1.15; respectively, and the difference between groups was significant. However, shoot length (cm) in treatment groups in season-2 showed an average (±SD) of 28.67±1.15, 23.67±1.53, 22.33±0.58, 23.00±2.65, 19.67±0.58, and 29.67±1.53; respectively, and the difference between groups was significant. Root length (cm) in season-1 showed an average (±SD) of 41.33±1.15, 37.33±0.58, 32.00±1.00, 30.33±0.58, 22.33±1.53, and 43.00±1.00; respectively, and the difference between groups was significant. However, season 2 showed an average (±SD) of 40.33±2.08, 37.00±1.00, 32.00±1.00, 29.00±1.00, 23.00±1.00, and 42.67±1.53; respectively, and the difference between groups was significant.

The oxidative stress was evaluated in terms of hydrogen peroxide accumulation and lipid peroxidation (malondialdehyde) in various treatment groups. The cellular H_2O_2 level in treatment groups *Bacillus cereus, Streptomyces erythrogriseus, Spirulina platensis, Pleurotus ostreatus*, Control (Nematode only), and Oxamyl (Vydate) in Season-1 recorded an average (±SD) of 6.90±0.30, 8.45±0.57, 9.45±0.43, 9.44±0.31, 8.56±0.53, and 14.07±0.96; respectively, and the difference between groups was significant. However, Season-2 showed an average (±SD) of 8.30±0.27, 8.12±0.13, 8.35±0.39, 7.93±0.79, 7.92±0.24, and 12.59±0.53; respectively, and the difference between groups was significant (Figure 2).

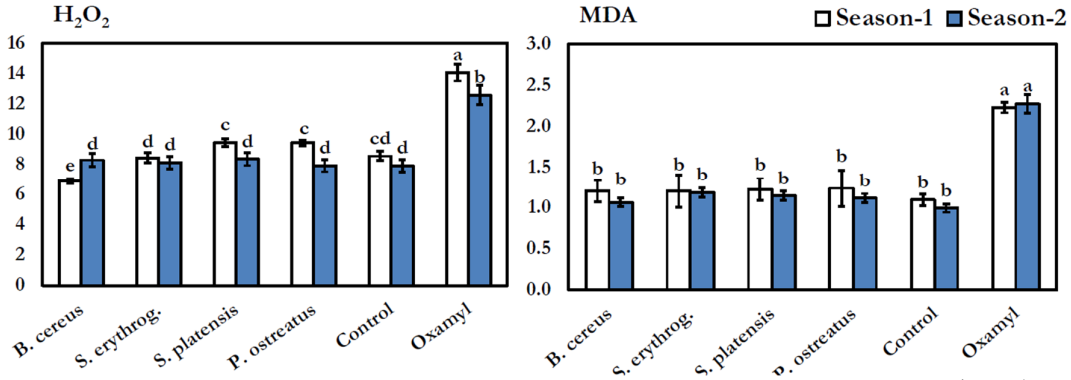

Figure 2. Oxidative damage in terms of hydrogen peroxide accumulation and lipid peroxidation (MDA) at different treatment groups including *Bacillus cereus, Streptomyces erythrogriseus, Spirulina platensis, Pleurotus ostreatus,* Control (Nematode only), and Oxamyl (Vydate). Bars followed by letters are significantly different according to DMRTs at 0.05 level

The oxidative damage in terms of lipid peroxidation (MDA) in season 1 recorded an average (±SD) of 1.20±0.23, 1.21±0.34, 1.23±0.23, 1.24±0.39, 1.10±0.12, and 2.23±0.11; respectively, and the difference between groups was significant. However, season 2 showed an average (±SD) of 1.07±0.07, 1.19±0.15, 1.15±0.05, 1.12±0.12, 0.99±0.02, and 2.27±0.16; respectively, and the difference between groups was significant (Figure 2).

The total protein contents in treatment groups in Season 1 recorded an average (±SD) of 31.50±1.61, 31.77±0.90, 31.20±1.25, 31.26±1.40, 30.56±1.23, and 23.65±1.20; respectively, and the difference between groups was significant. However, protein contents in Season-2 showed an average (±SD) of 32.10±2.54, 31.87±2.46, 30.59±2.13, 31.11±1.40, 29.55±1.21, and 24.82±0.44; respectively, and the difference between groups was significant. Total phenol contents in Season-1 showed an average (±SD) of 5.42±0.78, 3.88±0.39, 3.35±0.46, 4.13±0.19, 2.52±0.33, and 2.59±0.53; respectively, and the difference between groups was significant. However, phenol in Season 2 showed an average (±SD) of 6.24±0.68, 5.24±0.42, 3.17±0.19, 4.26±0.26, 2.02±0.28, and 2.29±0.27; respectively, and the difference between groups was significant (Figure 3).

Total flavonoid levels in treatment groups in Season 1 showed an average (±SD) of 3.08±0.18, 2.62±0.33, 1.97±0.35, 2.69±0.16, 1.04±0.07, and 1.42±0.10; respectively, and the difference between groups was significant. However, Season-2 showed an average (±SD) of 2.40±0.42, 2.32±0.11, 1.91±0.12, 2.20±0.23, 1.29±0.41, and 1.30±0.27; respectively, where the difference between groups was significant (Figure 3).

Polyphenol oxidase (PPO) is an important cellular enzyme. The level of PPO in treatment groups *Bacillus cereus, Streptomyces erythrogriseus, Spirulina platensis, Pleurotus ostreatus*, Control (Nematode only), and Oxamyl (Vydate) in Season 1 showed an average (±SD) of 1.74±0.31, 1.61±0.24, 0.99±0.09, 1.61±0.34, 0.44±0.05, and 0.55±0.05; respectively, and the difference between groups was significant. However, PPO Season-2 showed an average (±SD) of 2.05±0.05, 1.97±0.11, 1.65±0.29, 1.88±0.15, 0.44±0.08, and 0.55±0.08; respectively, and the difference between groups was significant (Figure 3).

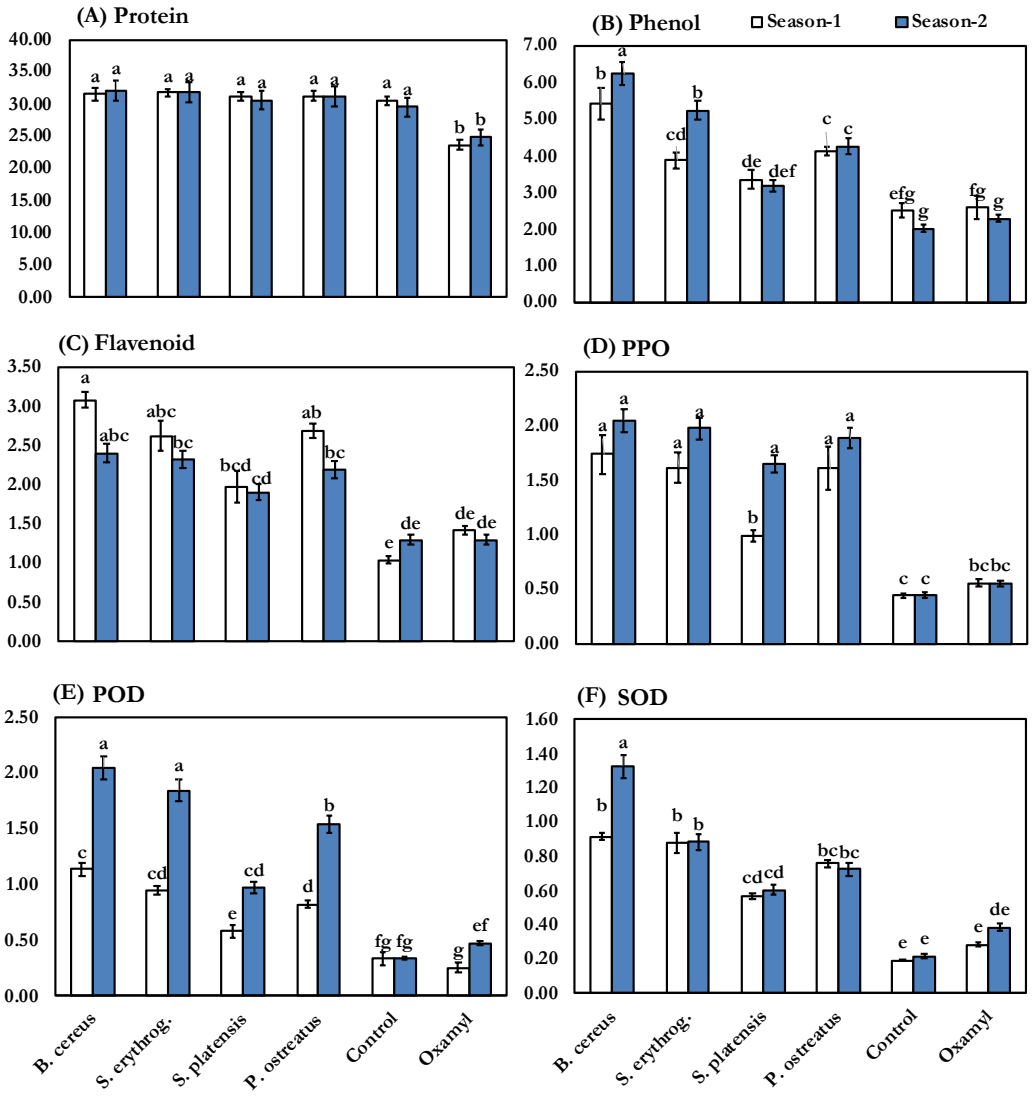

Figure 3. Macromolecules (proteins, phenols, and flavonoids) and metabolic enzymes (PPO, POD, and SOD) at different treatment groups including *Bacillus cereus, Streptomyces erythrogriseus, Spirulina platensis, Pleurotus ostreatus,* Control (Nematode only), and Oxamyl (Vydate). Bars followed by different letters are significantly different according to DMRTs at 0.05 level

The cellular antioxidant enzyme peroxidase (POD) in Season-1 showed an average (±SD) of 1.14±0.10, 0.95±0.06, 0.59±0.10, 0.82±0.06, 0.34±0.10, and 0.25±0.08; respectively, and the difference between groups was significant. However, in Season-2 recorded and average (±SD) of 2.06±0.07, 1.85±0.17, 0.98±0.08, 1.55±0.32, 0.34±0.09, and 0.48±0.02; respectively. and the difference between groups was significant (Figure 3).

The superoxide dismutase (SOD) activities in season 1 showed an average (±SD) of 0.91±0.04, 0.88±0.10, 0.57±0.03, 0.76±0.04, 0.19±0.01, and 0.28±0.02; respectively, and the difference between groups was significant. However, SOD activity in Season-2 showed an average (±SD) of 1.33±0.40, 0.88±0.11, 0.60±0.02, 0.72±0.07, 0.22±0.02, and 0.39±0.01; respectively, where the difference between groups was significant (Figure 3).

The number of pods per plant in Season 1 showed an average (±SD) of 14.00±2.65, 12.00±1.73, 10.33±0.58, 10.33±0.58, 4.33±0.58, and 14.67±2.52; respectively, and the difference between groups was significant. However, Season-2 showed an average (±SD) of 15.00±2.65, 12.00±1.00, 10.00±1.00, 9.00±1.00, 4.00±1.00, and 15.67±1.15; respectively, where the difference between groups was significant (Figure 4).

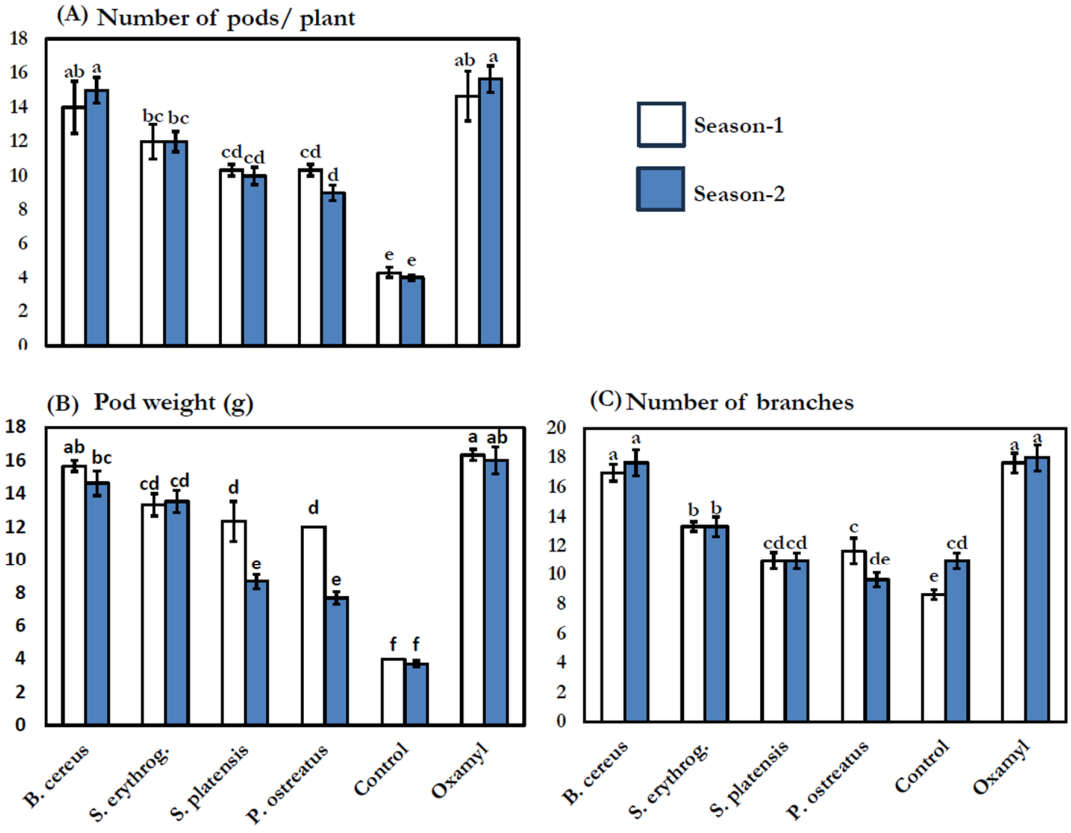

Figure 4. Number of pods per plant, pod weight, and number of branches at different treatment groups including *Bacillus cereus, Streptomyces erythrogriseus, Spirulina platensis, Pleurotus ostreatus,* Control (Nematode only), and Oxamyl (Vydate). Bars followed by different letters are significantly different according to DMRTs at 0.05 level

The average (±SD) pod weight (g) in Season 1 was 15.67±0.58, 13.33±1.15, 12.33±2.08, 12.00±0.00, 4.00±0.00, and 16.33±0.58; respectively, and the difference between groups was significant. However, in

Season-2, it was 14.63±1.01, 13.53±0.45, 8.67±1.15, 7.67±0.58, 3.70±0.26, and 16.00±1.00; respectively, and the difference between groups was significant (Figure 4).

The average total number of branches in Season-1 showed an average (±SD) of 17.00±1.00, 13.33±0.58, 11.00±1.00, 11.67±1.53, 8.67±0.58, and 17.67±1.15; respectively, and the difference between groups was significant. However, in were 17.67±0.58, 13.33±0.58, 11.00±1.00, 9.67±0.58, 11.00±1.00, and 18.00±1.00; respectively, and the difference between groups was significant (Figure 4).

The average (±SD) galls in Season 1 were 22.33±1.53, 33.33±5.51, 47.33±8.39, 48.00±1.73, 224.00±13.08, and 11.67±1.15; respectively, and the difference between groups was significant. However, in Season-2 were 26.00±1.00, 35.00±4.58, 50.00±6.56, 50.33±2.08, 230.67±7.37, and 12.00±1.00; respectively, the difference between groups was significant (Figure 5A).

Final nematode population in soil (second stage juveniles, J_2) in treatment groups in Season-1 showed an average (±SD) of 72.67±6.43, 116.67±25.17, 193.33±11.55, 240.00±20.00, 606.67±64.29, and 53.33±11.55; respectively, and the difference between groups was significant. However, Season 2 showed an average (±SD) of 76.67±5.77, 120.00±20.00, 206.67±11.55, 240.00±20.00, 640.00±52.92, and 53.33±11.55; respectively. The (developmental stages) in treatment groups in Season 1 showed an average (±SD) of 2.67±0.58, 4.67±1.15, 7.00±2.00, 8.33±2.52, 27.67±3.51, and 1.33±0.58; respectively, and the difference between groups was significant. However, in Season 2, the average (±SD) was 3.00±1.00, 5.00±1.00, 8.67±0.58, 9.67±1.15, 31.67±5.51, and 1.33±0.58; respectively. and the difference between groups was significant (Figure 5).

The number of females/roots in Season 1 showed an average (±SD) of 9.00±1.00, 14.00±1.00, 16.33±0.58, 19.33±1.15, 37.33±2.52, and 5.42±0.38; respectively, and the difference between groups was significant. However, Season 2 showed an average (±SD) of 10.00±1.73, 15.00±1.00, 17.00±1.00, 20.00±0.00, 42.00±4.36, and 4.00±1.00; respectively. and the difference between groups was significant (Figure 5).

The number of eggs on roots in Season 1 was recorded at an average (±SD) of 552.67±99.61, 875.00±189.01, 1,016.33±98.52, 1,149.67±79.60, 5,211.00±444.53, and 225.00±13.08; respectively, and the difference between groups was significant. However, Season 2 showed an average (±SD) of 691.67±113.38, 1,028.33±247.76, 1,061.33±164.79, 1,149.67±79.60, 5,457.67±538.42, and 247.00±25.87; respectively. and the difference between groups was significant. Moreover, the average (±SD) egg- masses in Season 1 were 6.00±1.00, 8.33±1.53, 10.00±1.00, 11.00±1.00, 39.33±2.08, and 3.33±0.58; respectively, and the difference between groups was significant. However, in Season 2, they were 7.00±1.00, 9.33±1.53, 10.33±1.53, 11.00±1.00, 40.67±2.52, and 3.67±1.15; respectively, and the difference between groups was significant. Furthermore, average (±SD) eggs/egg-mass in Season-1 showed an average (±SD) of 92.00±2.00, 104.67±4.51, 101.67±1.53, 104.67±3.21, 132.33±4.51, and 69.00±13.45; respectively, and the difference between groups was significant. However, Season 2 showed an average (±SD) of 98.67±3.51, 109.33±8.39, 102.67±2.31, 104.67±3.21, 134.00±5.00, and 70.33±15.18; respectively, and the difference between groups was significant.

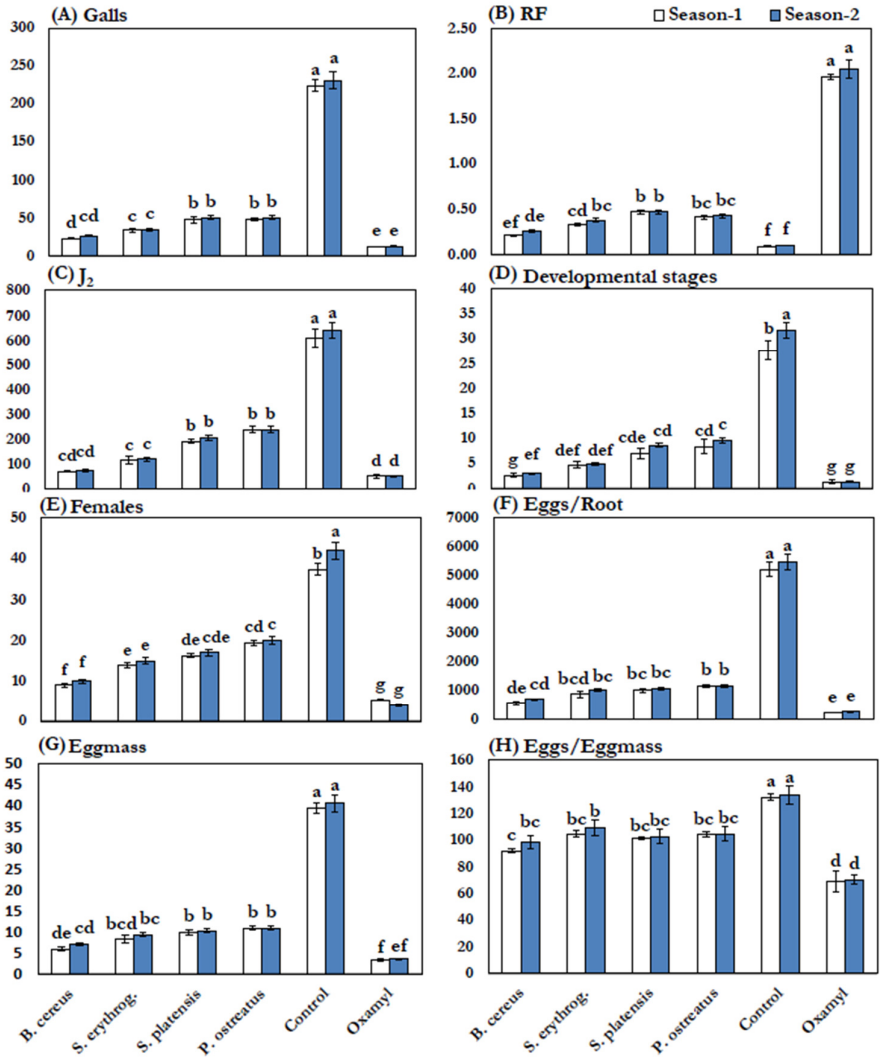

Figure 5. Galls count of second-stage juveniles, (J_2)/ pot, developmental stages, females/root, egg/root, egg-mass, eggs/egg-mass at different treatment groups including *Bacillus cereus, Streptomyces erythrogriseus, Spirulina platensis, Pleurotus ostreatus,* Control (Nematode only), and Oxamyl (Vydate). Bars followed by different letters are significantly different according to DMRTs at 0.05 level

Figure 6 represents a red-blue correlation heatmap, which is a graphical representation used to visualize the correlation between pairs of variables in a dataset. It's often used in data analysis to quickly identify patterns of positive and negative correlations among variables. The color scale typically ranges from red (indicating negative correlation) through white (indicating no correlation) to blue (indicating positive correlation). Furthermore, the intensity of the color in a cell indicates the strength of the correlation. The blue-red heatmap presents the interaction and correlation between all study variables, in which POD, PPO, SOD, phenolic compounds, flavonoids, and proteins show a significant negative correlation with treatment. However, MDA, H_2O_2 Galls, J_2, and development show a significant direct correlation with treatment. The season of investigation clearly showed a significant positive correlation with POD. Moreover, according to the heatmap, the oxidative damage in terms of MDA and H_2O_2 showed a significant negative correlation with antioxidants, e.g., POD, PPO, SOD, phenolics, and flavonoids.

In addition, the overall effect and interaction between the studies were evaluated using the multivariate analysis of variance (MANOVA) presented in Table 1.

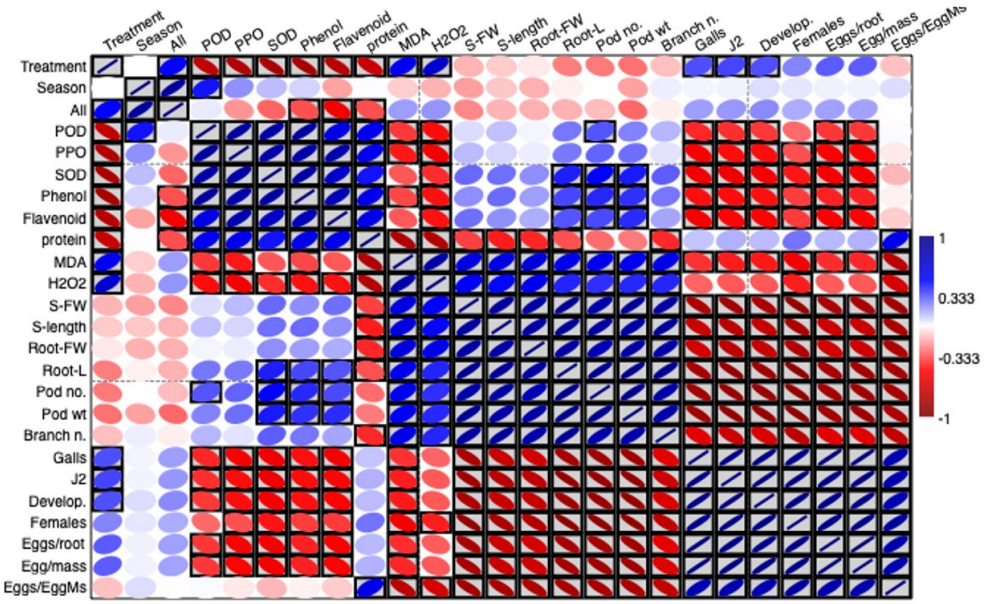

Figure 6. Heatmap presenting the interaction between study variables
Blue color indicates positive correlation, red color for negative correlation and white for no correlation. boxed colors indicate significant correlation.

Discussion

Plant parasitic nematodes are controlled with several chemical nematicides, have negative consequences, have high costs, and are restricted (Rockström *et al*., 2017). As a result, to produce natural chemicals that influence pathogenic pests, including plant parasitic nematodes in various crops, yield production is necessary. The most effective method for protecting human health and the environment against plant parasitic nematodes is biological control (Gawade *et al*., 2017).

In the current study, the efficacy of biocontrol agents (*B. cereus* 54-1, *S. erythrogriesus* sub sp. 2, *P. ostreatus*, and *S. platensis*) on mortality of second-stage juvenile J_2 of root-knot-nematode *M. incognita* was studied *in vitro*. Results implied that after 48 hours of exposure, all bio-agent cultures caused significant mortality in *M. incognita* J_2s. *B. cereus* 54-1 revealed the highest percentage (89% of mortality), followed by *S. erythrogriesus* subsp. 2 which recorded 81%. While the lowest P. ostreatus was recorded at 67.3%. These results are in line with (Hu *et al*., 2021), who reported that Streptomyces erythrogriesus strain DN41 and *S. fimicarius* strain D153 had exceeded mortality by 82% after 12 hours and 96% after 48 hours of *M. incognita* in vitro. This highlights that bioagents have a better impact on the control of *M. incognita* in vitro; the same results were obtained in greenhouse experiments in two successive seasons.

Based on the no. of galls, egg-masses/root, and nematode, nematicide treatment Oxamyl 24% L was the most operative treatment in suppressing total nematode populations; the second most effective treatment in reducing *M. incognita* in roots and soil was noticed in *B. cereus* with (RF). Followed by *S. erythrogriesus* subsp. 2, and *S. platensis*. The same results were obtained previously by Xiao *et al*. (2018) who reported that the use of *B. cereus* strain Jdm1 decreased the number. of galls on tomato roots infected with *M. incognita* in the pot test. Also, results from Siahpoush *et al*. (2011) revealed that *B. cereus* decreased *M. javanica* galling, egg masses, and egg numbers on the roots of cucumber plants. Similarly, Hu *et al*. (2021) showed that *S. albogriseolus* strain DN41 and *S. fimicarius* strain D153 had potential agents for the control of *M. incognita* in tomato plants.

The egg and second-stage juvenile phases of plant-parasitic nematodes are the most delicate stages of biological control. The existence of these life stages outside of the plant on soil particles covered in a water film gives competing microorganisms the chance to interact with the nematodes, infect them, and parasitize them. If these two plant-parasitic nematode stages are managed, the nematode's life cycle will be disrupted, which will lead to a lower population density and successful management. The biological management of diseases requires rhizoplane bacteria to colonize the roots (Weng *et al*., 2013). Some bacteria can produce biofilms on root surfaces, which are communities of microorganisms and individual cells bound by an endogenous extracellular matrix (O'Toole *et al*., 2000). Effective root colonization is essential for the development of concurrent screening of biocontrol agents (BCAs) on plant roots and, subsequently, for biocontrol diseases (Weng *et al*., 2013). Root metabolite production and root secretion are directly impacted by *B. cereus* colonization of the root surface, strengthening the host defensive system and reducing RKN invasion (Hashem and Abo-Elyousr, 2011). Hence, *Spirulina platensis*, a cyanobacterium, has various mechanisms that prevent the growth of RKNs. For example, numerous cyanobacterial species are known to produce harmful substances such as nodularin, microcystins, and neurotoxins (Gaget *et al*., 2017). Additionally, microalgae create benzoic acid (Uzair et al., 2018). By disrupting the cytoplasmic membrane and inhibiting the production of proteins, cyanobacteria can also reduce the activity of pathogenic organisms (Swain *et al*., 2017). The most significant microbial resources are actinomycetes, which produce bioactive compounds that can inhibit or even kill nematodes (Zeng *et al*., 2013). The majority of actinomycetes that exhibit activity against plant parasitic nematodes by generating extracellular enzymes and other toxic substances are *Streptomyces* spp. The establishment of environmentally friendly and secure integrated crop management is becoming more interesting with the secondary metabolites from *Streptomyces*.

In this study, in addition to bio-controlling *M. incognita* in common bean plants, bioagents were also able to stimulate plant growth (Li *et al*., 2019) and reported that *B. cereus* BCM2 enhanced tomato shoot length and fresh weight. Applied bioagents could not only accelerate plant development but also enhance the nutritional value of fruits by boosting their glucose, protein, and vitamin contents (Rashid *et al*., 2016). Different species and strains of bioagents have different mechanisms for influencing plant growth. However, bioagents can positively affect plant growth by reducing some of the negative effects of a pathogenic organism by creating antagonistic chemicals. To be effective, biological control mechanisms must either directly affect the pathogen (direct antagonism) or use the host as an intermediary (Xiang *et al*., 2018). The biological control of plant pathogens has been controlled by two primary mechanisms: antagonism (antibiosis, competition for nutrients or niche exclusion, and siderophore-mediated suppression) and induced resistance (systemic acquired resistance or SAR and induced systemic resistance or ISR) (Patel *et al*., 2016).

As hazardous by-products of plant metabolic pathways reach levels above the optimal limits on exposure to parasitic nematodes, oxidative stress indicators and free radicals begin to accumulate (Chavan and Deshpande, 2013). The findings corroborated those of (Khanna *et al*., 2019), who discovered that plant parasitic nematodes (PPNs) caused an increase in H_2O_2 and MDA levels in tomato seedlings. This relates to the oxidative damage that parasitic nematodes, which produce reactive oxygen species (ROS), cause to plants (ROS). Furthermore, Gupta *et al*. (2017) observed that the *Bacopa monnieri* L. infected with *M. incognita* had greater levels of total reactive oxygen species and H_2O_2 than healthy plants and discovered that nematode-infested plants injected with Streptomyces sp. reduced hydrogen peroxide and lipid peroxidation levels. (Vos *et al*., 2013) hypothesized that the inoculating agents stimulated defensive mechanisms by lowering the amount of free radicals produced and boosting their immune systems. Also, they reinforce the cell membrane of plant tissues to lessen the impact on biomolecules and to stop nematodes from moving to further parts of the plant. Our results showed that nematode infection decreased protein levels, which could be linked to the development of ROS that specifically targets proteins that may be carbonylated because of the oxidation of amino acid chains (Gill and Tuteja, 2010). Yet, in two seasons, the common bean's protein content under nematode infection

was improved using bioagents. According to Singh *et al*. (2016), the presence of microbial agents decreased the overproduction of ROS that was brought on by pathogens. To prevent ROS damage, both enzymatic and non-enzymatic antioxidants must be developed. The current study found an increase in the activities of both enzymes and non-enzymatic antioxidants when treating nematode-infected plants with bioagents.

According to our results, bioagents demonstrated strong activity of the protective enzymes peroxidase (POD), polyphenol oxidase (PPO), and superoxide dismutase (SOD) in two seasons. In two seasons, *B. cereus* had the highest enzyme activity. POD, PPO, and SOD activity increased by 4.5, 3.1, and 3.2 times, respectively, in season 1 as compared to control and were followed by *S. erythrogriesus* and *S. platensis*. Plant defence enzymes PPO, POD, and SOD are essential and positively connected with a plant's systemic resistance to infections (Choudhary *et al*., 2007). According to Moghbeli *et al*. (2017), POD and PPO have a clear connection to their role in giving plant tissues structural stiffness through increased lignin production in cell walls to stop plant parasitic nematode penetration in plants. SOD, a first line of defence against ROS, is produced to dismutate H_2O_2 into non-toxic forms (Clarke and Vinatzer, 2017). Lahlali *et al*. (2022) demonstrated how rhizosphere bacterial interactions with plants might indubitably increase the plant's overall disease resistance. According to Gao *et al*. (2016), treatment with B. cereus increases the POD and PPO activities in tomato seedlings for the biocontrol of M. incognita. Under the effect of *Streptomyces* sp., nematode-infested *B. monnieri* L. plants have shown increased SOD activity (Gupta *et al*., 2017). Streptomyces hydrogenans treatment increased the POD, PPO, and SOD activities in tomato plants infected with *M. incognita,* according to Sharma *et al*. (2022). Also, results showed that the levels of phenolic compounds and flavonoids were further increased when infected plants were treated with bioagents throughout both seasons. In comparison to the control, *B. cereus* enhanced the level of flavonoid and phenolic compounds by 2.2 and 2.1 times, respectively, in season 1 and by 1.8 and 2.7 times, respectively, in season-2. After root-knot-nematode, *M. incognita* inoculation (Gálvez *et al*., 2019) showed elevated levels of total phenolics in *Capsicum annuum* to develop plant resistance. Phenolic compounds induce lignification in the epidermal regions of plants as a constitutive and inducible post-penetration method to give resistance against nematode infection (Galeng-Lawilao *et al*., 2019). Additionally, it was attributed to the activation of the shikimate biosynthetic pathway, which led to the overproduction of metabolites involved in the resistance mechanisms (Nunes Da Silva *et al*., 2019). According to Chin (Chin, 2019), flavonoids operate as signalling molecules that regulate auxin transport during gall growth and plant defence against PPNs in the rhizosphere. The overall phenolic content of *M. incognita*-infected cowpea plants increased because of interactions with diverse rhizobacterial strains, root-knot nematodes, and phenolic-rich nematodes (Abd-El-Khair *et al*., 2019). The quantity of phenolic and flavonoid increased in nematode-infested plants treated with Streptomyces hydrogenans, according to Sharma *et al*. (2022).

Conclusions

The obtained results proved the efficacy of 4 various biocontrol agents against the root-knot nematode (*M. incognita*) under in vitro and greenhouse conditions in two successive seasons. The tested bioagents were efficient in preventing nematode reproduction, but at various levels of efficacy. In addition, all treatments significantly enhanced common bean growth parameters and reduced the levels of both H_2O_2 and MDA. While it raised the activity of POD, PPO, SOD, and contents of phenolics and flavonoids in the infected common bean.

Authors' Contributions

S.H.A., E.A., and A.M.H. proposed the concept and designed the experiments. S.H.A., E.A., and A.M.A. conceived the study and wrote the manuscript. E.A. performed all plant measurements. S.H.A. performed all nematode experiments and measurements. A.M.H. and A-S.M.A. prepare bio-agents and experimental materials. A.M.A. analysed the data. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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