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ORIGINAL ARTICLE

Bacillus safensis Strain OM1 Mediate Growth Promotion and *Meloidogyne incognita* Suppression on Potato (*Solanum tuberosum*) Plants

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ABSTRACT

Being effective and environmentally safe, biocontrol of root-knot nematodes became a global demand. *Bacillus safensis* strain OM1 showed nematicidal properties against *Meloidogyne incognita* increasing J2 mortality (75%) and decreased egg hatching by 78.2% % after 48 h of incubation compared to the control. Greenhouse obtained data showed significant reduction in gall numbers, egg masses and soil population (J2/250 g soil) by 69.8%, 72.99, and 49.39%, respectively. Moreover, *M. incognita*-infested potato plants inoculated with OM1 showed a significant increase in growth and photosynthetic pigments. Total chlorophyll (1.05 mg/g FW, 88.1%) and carotenoid contents (2.72 mg/g FW, 70.23%) were ameliorated compared to infested plants. Peroxidase (POD) activity of root was significantly reduced after OM1 co-culturing, nevertheless shoot content (18.74 U/mg protein /min) was higher than *M. incognita* infected plants. *B. safensis* co-culturing treatment significantly increased polyphenol oxidase (PPO) activities in root and shoot up to 18.73 U/mg protein /min (71.11%) and 32.48 U/mg protein /min (28.2%), respectively compared to *M. incognita* infested plants. Treatments elevated superoxide dismutase (SOD) activity in both root and shoot, maximizing after *B. safensis* treatment (0.95 U/mg protein /min) for both. The findings obtained from this work inform the efficiency of *B. safensis* in potato growth and the amendment of physicochemical characteristics to mitigate *M. incognita* infection stress.

Key words: Potato, *Meloidogyne incognita*, *B. safensis* strain OM1, Bacteria, Culture filtrate and growth promotion.

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important cultivated crops in terms of export for hard currency, manufacturing, and food safety in Egypt. It can be challenged by several nematode species such as *Meloidogyne* spp., *Pratylenchus* spp., *Globodera* sp., *Rotylenchulus reniformis*, *Ditylenchus dipsaci*, *Ditylenchus destructor*, *and Nacobbus* spp. However, *Meloidogyne* spp. are the widest deleterious among all plant parasitic nematodes in potato (Khan *et al*., 2023).

Root knot nematode (RKN) infecting potato can significantly limit plant growth and reduce crop yield; considering *Meloidogyne incognita*, the aggressive phytopathogen infecting potato plants exhibits noticeable visible galls or swellings in the roots, leading to diminished root development, stunted growth, and wilting of the plants. In cases of severe infestation, the malformation of tubers can occur, rendering them unsuitable for both consumption and storage (Mitiku 2018, Wilschut and Geisen 2021).

Employing commercial synthetic nematicides for managing root-knot nematodes was the most common strategy. However, it became limited with the availability of active ingredients, nematode resistance, and caused negative

environmental and public health impacts (El-Nagdi *et al.,* 2023). Therefore, there is a need to provide novel, feasible, and environment-friendly strategies for the control of nematode infections within plants. Biological agents have become vital in crop protection due to their various positive characteristics, including low toxicity or even non-toxicity to host plants (Kalele *et al.,* 2010). Among the range of biopesticides, endophytes and soil-dwelling microorganisms like bacteria and fungi have been effectively utilized as biological agents against plant-parasitic nematodes. They offer a promising alternative strategy for managing root-knot nematodes (Berg *et al.,* 2017). Plant growth-promoting bacteria (PGPB) can associate with plant roots, leaves, and flowers, or even exist within plant tissues. These bacteria not only enhance plant growth but also trigger defense mechanisms, ultimately increasing the plants' tolerance to both biotic and abiotic stresses.

Bacillus spp. is abundant in natural environments, especially in the root system of plants, and is reported for their antagonistic potential against *M. incognita* on potato and eggplants, achieving various degrees of control, as well as promoting growth and yield measurements under field conditions (El-Nagdi and Abd-El-Khair 2019, El-Nagdi *et al*., 2023). Growth-promoting bacilli can directly suppress RKN by rhizosphere colonization, parasitism, and antibiosis; indirect mechanisms include siderophores, hormone production, phosphate solubilization, nitrogen fixation, and transformation of bacterial microbiome (Fatma *et al*., 2014, Lopes *et al*., 2019). *B. safensis* strains' potency in biotic and abiotic stress attenuation and plant growth promotion has been widely explored. Formulated *B. safensis* significantly ameliorated drought and salinity-induced stresses; enhanced growth and quality of *Brassica juncea*, *Oryzae sativa*, and *Cicer arietinum* (Khan *et al*., 2017, Bibi *et al.*, 2024, Karimian *et al*., 2025). Complex cyclic hydrocarbons and heavy metal bioremediation efficiency of *B. safensis* strains were reported to induce stress tolerance (Wu *et al*., 2019, Nazli *et al.*, 2021). The biocontrol potential of *B. safensis* has been well documented in several reports; whereas *B. safensis* T052-76 obviously decreased foot rot disease of sweet potato infected plants by regulating bacilysin, bacillibactin, fengycin, lichenysin, and surfactin antimicrobial molecules coding genes (Mateus *et al.*, 2021). *Bacillus safensis* strain Bsa27 reduced the *Heterodra glycines* population density on soybean plants (*Glycine max*) at 60 days after planting in the field trials (Xiang *et al.*, 2017).

The current study was designed to investigate the toxicity of a culture filtrate of *Bacillus safensis* strain OM1 on *M. incognita* J2 mortality and egg hatching under *in vitro* conditions. It also studied the growth promotion and induced resistance in potato plants inoculated with *M. incognita* and treated with a bacterial bioagent.

Materials and Methods

Nematode inoculum preparation

Meloidogyne incognita was obtained from potato growing fields in Alexandria Governorate. Uniform-sized egg masses were manually collected from the roots. The surface was treated with 0.1% sodium hypochlorite for 10 seconds and then rinsed five times with sterile water and incubated in distilled water to allow the hatching of J2. Freshly hatched J2 were used in *in vitro* toxicity assessment and *in vivo* experiments to infect potato plants.

Bacterial isolation and identification

Cicer (*Cicer arietinum* L) plants exhibiting vigorous healthy phenomena cultivated in a field with a history of nematode infection disease were sampled. Root, shoot, and seeds collected were immediately transported to physiological diseases laboratory, department of plant pathology, faculty of agriculture, Alexandria University, Egypt. Samples were sterilized by soaking in 96% ethanol for 10 seconds, then treated with a 1% sodium hypochlorite solution for 3 minutes. After that, they were

washed three times with sterile distilled water, each wash lasting one minute, all of which took place in a laminar flow cabinet. The plates containing nutrient agar were incubated at 25 °C for 2-3 days. Single colonies were picked up and spread once again (Wicaksono *et al.,* 2018). Bacterial genomic DNA was extracted using the cells lysis protocol to amplify 16S rRNA gene using the universal bacterial primers FD1 (5'AGAGTTTGATCC TGGCTCAG3') and RP2 (5'ACGGTTACCTTGTTACGACTT3'). A total volume of 50 µl reaction mixture was prepared, adhering to the following thermal cycling conditions: an initial denaturation at 94 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 50 seconds, annealing at 55 °C for 45 seconds, and extension at 72 °C for 90 seconds. A final extension was performed at 72 °C for 3 minutes (Keegan *et al*., 2005). The amplicon was sequenced, and the results were blasted and submitted to the National Center for Biotechnology Information database (www.ncbi.nlm.nih.gov) to secure an accession number.

In *vitro* nematicidal potential of bacterial culture filtrate

The bacteria were cultured in a 250 ml conical flasks that held 100 ml of nutrient broth medium at 28 °C and 170 rpm agitation for 48 h. The suspension was centrifuged at 8000 rpm for 15 minutes. The supernatant concentration was adjusted to 1.2×10^7 CFU /ml as a stock solution. Freshly hatched *M. incognita* suspension (100 J2) was exposed to gradient bacterial filtrate concentrations 25, 50, and 100 %. The count of dead juveniles was noted at both 48 and 72 hours of exposure. Juveniles treated with sterilized distilled water were served as a negative control. Mortality rate of J2 was calculated using the formula admitted by Abbot (Abbott 1925).

Mortality (%) of J2= [(m – n)/(100-n)] X 100

Where m represents the percentage of mortality observed in the treatment group, while n refers to the mortality percentage in the control group.

Hatchability was assessed at 48 and 72 h of exposure at 25°C utilizing a light microscope. A juvenile is considered hatched when it has entirely emerged from the egg. The percentage of hatched eggs was calculated using the following formula:

Percentage of hatching suppression = [1-(Ht/ Hc)] ×100

Where Ht represents the number of juveniles that hatched in the treatment, while Hc indicates the number of juveniles that hatched in the control.

Greenhouse experiment

The experiment was conducted to evaluate the efficacy of isolated bioagent against *M. incognita* on potato plants *cv*. Metro In *Vivo*. Three weeks old potato seedlings of uniform size were individually transplanted into 25 cm diameter plastic pots containing 3 kg of autoclaved sandy: clay mixture (2:1). The experiment was designed as randomized complete block design on benches in the greenhouse at 18 -25 °C with four treatment groups. Bacterial suspension was added to the pots by pipetting 20 ml of bacterial suspension (1.2×10^7 CFU). One week later, 4000 eggs of *M. incognita* were added by pipette into four holes around the growing plant. Pots were irrigated and fertilized as needed. Each treatment was replicated five times. Forty-five days after inoculation, plants were uprooted, washed under tap water, and growth parameters were recorded. Numbers of root galls, egg-masses /root system, and J2/250 g soil were detected. Also, dry weights of the shoot, root systems were determined.

Biochemical Analysis

Photosynthetic pigments content

A 0.2 g of 50 days old potato fresh leaves were sampled to determine chlorophyll contents. Chlorophyll a, chlorophyll b, and carotenoid pigments contents were extracted in 10 ml of 80% acetone solution. The absorbance of the obtained extract was measured using a spectrometer to indicate absorbency (A663, A646, and A470), and concentration (mg/g fresh weight) was assessed according to Lichtenthaler and Wellburn (1983).

Crude extract and total protein determination

Potato leaf and root samples (0.2 g) were initially powdered in liquid nitrogen and homogenized in 1.8 ml extraction buffer, which contained 50 mM Tris-HCl solution (pH 8), 1% Triton X-100, and 0.1% mercaptoethanol. The solution was centrifuged at 15000 g for 15 min at 4 °C. The extract was used to determine the total protein content and enzyme activity (Rodrıguez-Rosales *et al*., 1999). The total protein of treatments was assayed using the Bradford buffer solution (Bradford 1976). A 40-µl crude extract was mixed with 940 µl of Bradford solution, and the absorbance was measured at 595 nm. The standard curve was constructed using bovine serum albumin. The estimated protein concentration was employed to determine the activity of antioxidants enzymes activity.

Polyphenol Oxidase (PPO) Activity

The reaction mixture was prepared by combining 200 µl of the enzyme extract with 1.5 ml of 0.1 M sodium phosphate buffer at pH 6.5. To initiate the reaction, 200 µl of 0.01 M catechol was added. The enzyme activity was measured as changes in absorbance at 495 nm (Cheema and Sommerhalter, 2015).

Peroxidase (POD) Activity

The guaiacol peroxidase assay was conducted using a mixture of 50 mM phosphate buffer (pH 7), 8.26 mM hydrogen peroxidase, and 100 µl of enzyme extract (Cakmak and Marschner, 1992). Absorption changes at 470 nm.

Superoxide dismutase assay (SOD)

The reaction mixture (5 mL) consisted of 50 mM potassium phosphate buffer, 0.1 µM EDTA, 0.013 mM methionine, two µM riboflavin, and 50 µL protein extract. This mixture was exposed to moderate light intensity (300 µmol m-2 s-1). In this experiment, a 50% decrease in NBT optical absorption at 560 nm relative to the control was considered equivalent to one enzyme unit (Beauchamp and Fridovich, 1971).

Statistical analysis

All statistical analyses were performed using the SPSS statistical package (SPSS Version 16.0). Analysis of variance (ANOVA) was performed using one-way ANOVA followed by a post hoc Tuky test to analyze the significant difference among treatments. A p-value of≤0.05 was considered statistically significant.

Results

The characterization and identification of endophytic bacteria

The bacterium strain OM1 colony was appeared creamy white and exhibited a round to oval shape, classified as gram-positive. The individual bacteria were rod-shaped and motile. The 16s gene sequence alignment showed 96.73% similarity to *Bacillus safensis* sub species safensis strain EG128 (accession number, MN704543.1). Thus, strain OM1 (accession number: PV791787.1) was identified as *B. safensis*. A phylogenetic tree based on the 16S rDNA sequences was constructed using MEGA 12 (Fig.1).

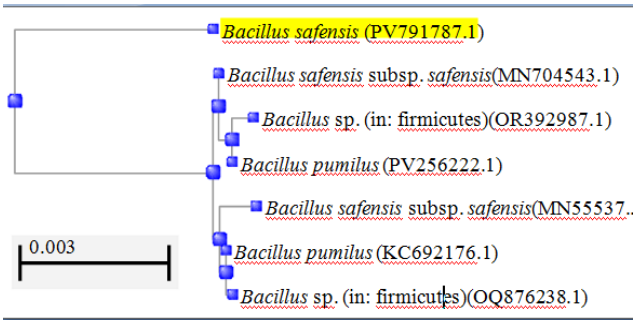

Fig. 1 Phylogenetic clustering based on the 16S rDNA gene sequence of strain OM1 constructed by the neighbor-joining method

***B. safensis* OM1 filtrate effect on *M. incognita* mortality and hatchability**

The impact of *B. safensis* strain OM1 culture filtrate concentrations on *M. incognita* egg hatching and mortality considering time incubation was investigated**.** The obtained data exhibited significant differences between concentrations for both mortality and

hatching inhibition over time (Tables 1 and 2). *B. safensis* filtrate significantly increased mortality percentage in *M. incognita* in concentration dependent manner ranging 25%-75%; whereas 100% filtrate was the most significant by 55 and 75% at 24 and 48 h of exposure, respectively. Meanwhile, 50% culture filtrate treatment showed moderate mortality by 43% and 57% at 24h and 48h, respectively. On the other hand, 25% filtrate treatment was the least significant in this respect at 24 and 48 h by 25% and 44%, respectively (Table, 1).

Table 1: Effect of *B. safensis* OM1 culture filtrate on the mortality of *M. incognita* at 24h and 48h of incubation

Time	Treatment %	Mortality %
24h	0	00±00 [d]
	25	25±2.23 [c]
	50	43±4.18 [b]
	100	55 +5.24[a]
48h	0	00±00 [d]
	25	44±4.06 [c]
	50	57±5.24 [b]
	100	75+3.80[a]

Values are the mean ± Standard Error of three replicates. The same letter in a column within same time does not differ significantly according to Tukey's test at 5% probability.

Throughout the experiment, the number of hatched J_2 significantly decreased as a result of *B. safensis* strain OM1 filtrate exposure over time (Table 2). All concentrations significantly inhibited hatching, whereas the concentration 100% was the most significant by 70% and 78% at 24h and 72h, respectively, followed by 50% filtrate (40% and 68%, respectively). Meanwhile, 25% filtrate was the least significance of hatching inhibition by 20% and 33% at 24h and 48h, respectively.

Table 2: Effect of *B. safensis* strain OM1 culture filtrate on the hatching of *M. incognita* J_2 at 24h and 48h of incubation

Time	Treatment %	Inhibition of hatching %
24h	0	00±00 [e]
	25	20±2.91 [d]
	50	40±3.80 [c]
	100	70±2.70 [b]
48h	0	00±00 [e]
	25	33.0±2.2 [d]
	50	68.0±2.91 [c]
	100	78.2±1.78 [b]

Values are the mean ± Standard Error of three replicates. The same letter in a column within same time does not differ significantly according to Tukey's test at 5% probability.

In *vitro* nematicidal activity of *B. safensis* strain OM1 against *M. incognita*

M. incognita induced stress of poatao infected root was significantly mitigated by *B. safensis* and Oxamyl treatment (Table3). *B. safensis* treatment significantly reduced the number of galls, egg masses, and population by 69.81%, 72.99% and 49.39%, respectively, compared to *M. incognita* infested plants. Considering Oxamyl, the commercial nematicide, *B. safensis* is an effective bioagent in root nematode management.

Growth promotion of potato plants infested with *M. incognita*

M. incognita infested potato plants exhibited significant reduction of root fresh weigh (67.6%) and shoot fresh weight (51.35%) compared to control, *B. safensis* stain OM1 treatment mitigated fresh weights reduction up to 16.27% and 21%, respectively and increased root fresh weight by 72.1% and shoot fresh weight by 143.53% compared to infected plants (Fig. 2 A&B). Dry weights of root and shoot exhibited significant reduction by 63.2% and 67.43%, respectively compared to healthy plants, nevertheless reduction was ameliorated by *B. safensis* co-culturing up to 15.25% and 11.66%, as the treatment increased root and shoot dry weights by 130.31% and 171.23% compared to infected plants treatment. *B. safensis* OM1 exhibiting high efficient compare to Oxamyl, the standard commercial nematicide (Fig. 2 C&D).

Root and shoot lengths significantly reduced after *M. incognita* infection by 47% and 43.34%, respectively, while *B. Safensis* starin OM1 treatment increased the infected plant root length by 67.74% and shoot length by 57.57% compared to *M. incognita* infected plants, reducing the reduction by 11% and 10.72% (Fig. 3 A&B).

Table 3: Effect of *B. safensis* OM1 and Oxamyl treatments on *M. incognita* potato root colonization and rhizosphere establishment

Treatments	Root-galling		Egg-masses		*M. incognita* population	
	Number of galls	Reduction (%)	Number of egg masses	Reduction (%)	No. of J2/ 250 g soil	Reduction (%)
CK	0.0+ 00 [d]	-	0.0+ 00 [d]	-	0.0+ 00 [d]	00
Mi	287.6 + 6.63 [a]	0	294.8+ 7.19 [a]	-	1276.6+ 37.68 [a]	-
B.s+Mi	86.8+ 4.14 [b]	69.81	79.6+ 3.61 [b]	72.99	646.0+ 38.45 [b]	49.39
Oxa+Mi	16.4+ 1.32 [c]	94.29	18.6+ 1.43 [c]	93.69	145.0+ 12.33 [c]	88.64

Values are the mean ± Standard Error of five replicates. Data with the same letter (s) are not significantly different at $P \leq 0.05$ using the Tukey's test. Mi, indicates *Meloidogyne incognita*; B.s indicates *Bacillus safensis* and Oxa, indicates oxamyl nematicide.

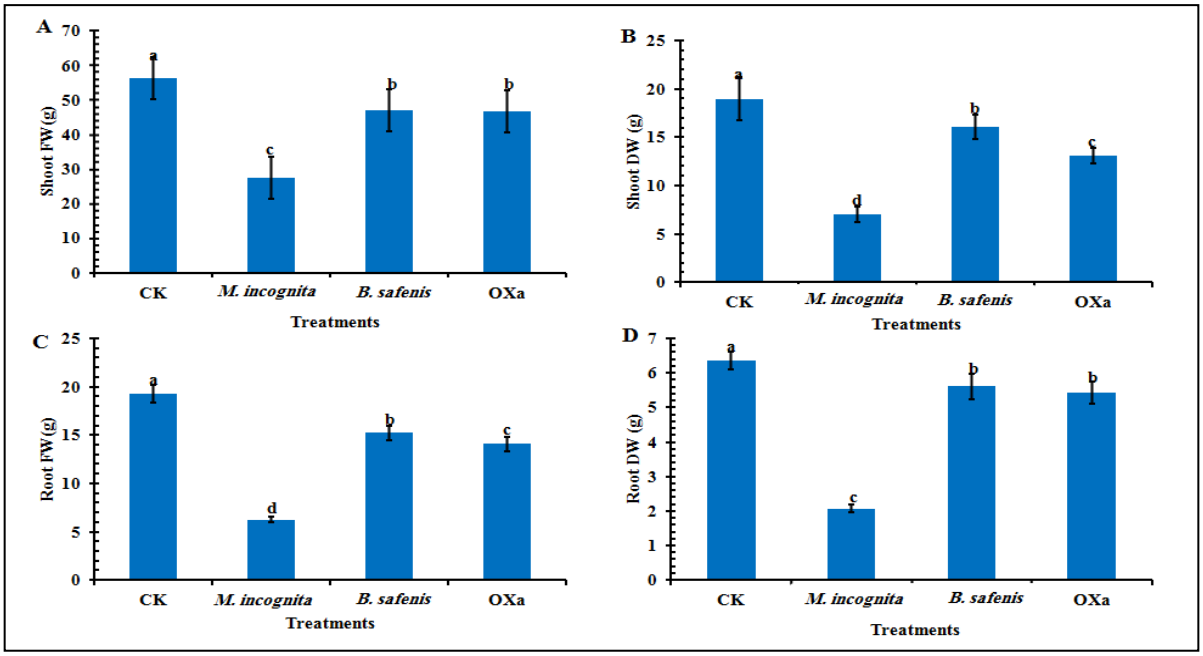

Fig. 2. Effect of *B. safensis* ,OM1 co-culturing and Oxamyl treatments on shoot fresh weights (A), shoot dry weights (B), root fresh weights (C) and root dry weights (D) of 45 days old *M. incognita* infested potato plants. Values are means of five replicates (N=5). Values with different letters show a significant difference ($p \leq 0.05$) in the mean value of replicates as determined by the Tukey's test.

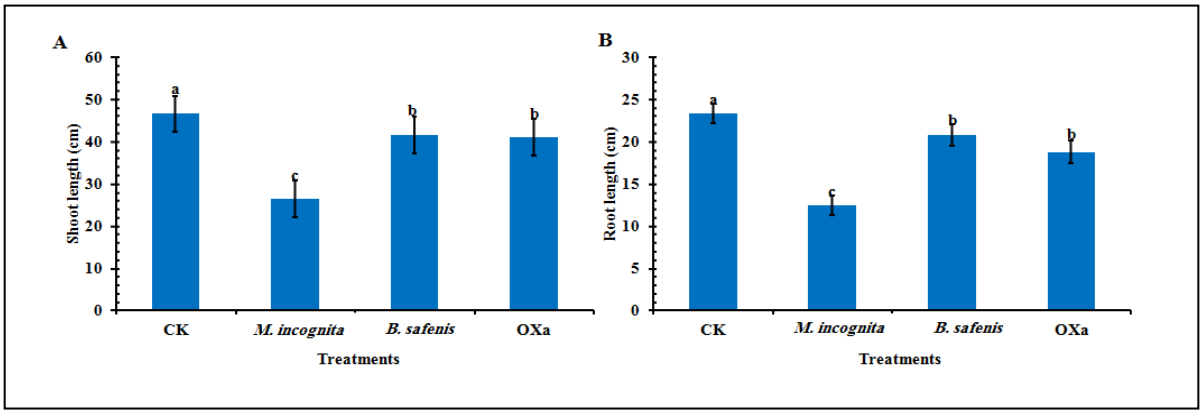

Fig. 3. Effect of *B. safensis* strain OM1co-culturing and Oxamyl treatments on shoot (A) and root (B) lengths of 45days old M. incognita infested potato plants. Values are means of five replicates (N=5). Values with different letters show a significant difference ($p \leq 0.05$) in the mean value of replicates as determined by the Tukey's test.

Effect of *B. safensis* on physicochemical attributes

Photosynthesis pigments contents

Photosynthetic pigments were drastically decreased in *M. incognita* infested potato plants compared to healthy plants (Fig. 4A&B). Total chlorophyll contents (0.57 mg/g FW) and carotenoids (2.54 mg/g FW) of *M. incognita* infested potato plants were the least among treatments, exhibiting 45% and 36.53% reduction, respectively, compared to healthy plants. *B. safensis* co-culturing treatment increased both TCC and CC contents of *M. incognita* infested plants up to 1.045 mg/g FW and 2.74 mg/g FW, respectively, indicating an enhancement in TCC (88.1%) and CC (70.23%) compared to infected plants exhibiting more efficient rather than Oxamyl in this respect.

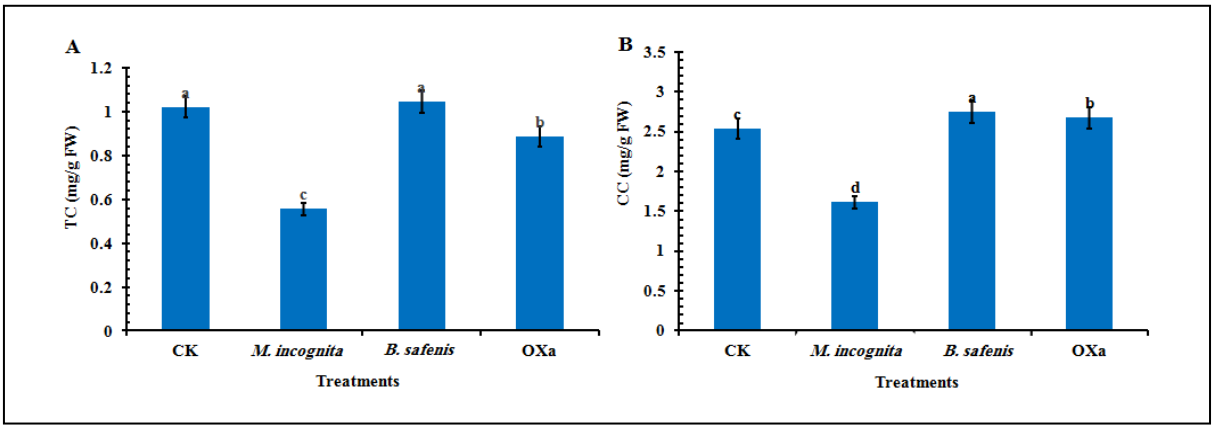

Fig. 4. Total chlorophyll (A) and carotenoids (B) contents of *B. safensis* co-cultured and Oxamyl treated *M. incognita* infested plants. Values are means of five replicates (N=5). Values with different letters show a significant difference ($p \leq 0.05$) in the mean value of replicates as determined by the Tukey's test.

Antioxidant enzymes

Root POD activity was the maximum in response to *M. incognita* infection (106.37 U/mg protein/min); meanwhile, the activity was downregulated after *B. safensis* co-culturing up to 82.96 U/mg protein/min (22% reduction) and Oxamyl (72.62 U/mg protein. min, 29.84% reduction) compared to *M. incognita* infected plants (Fig. 5A). *M. incognita* infested potato roots exhibited a reduction of PPO except, Oxamyl treated plants (31.53 U/mg protein/min) which exhibited 2.88 and 1.68-fold activity of *B. safensis* and *M. incognita* treatments, respectively (Fig. 5B). Root SOD activity was the maximum (0.947 U/mg protein/min) in *B. safensis* co-cultured infected plants, exhibiting a 76% increase compared to the positive control (Fig. 5C).

POD (peroxidase) activity was significantly increased in Oxamyl treated *M. incognita* infected potato shoot (43.58 U/mg protein. min, 35.58%) and oxamyl treated plants (42.39 U/mg protein. min, 31.86%), meanwhile *B. safensis* co-culturing treatment downregulated the activity up to 31.9 U/mg protein/min (Fig. 6A). However; *M. incognita* infested potato shoot exhibited reduced PPO activity (18.32 U/mg protein/ min, 6.43%), the treatments (*B. safensis* and Oxamyl) increased the activity up to 23.48 and 20.97 U/mg protein/ min indicating 28.2% and 14.48% increase, respectively (Fig. 6B). *B. safensis* co-culturing treatment significantly induced shoot SOD activity (0.94 U/mg protein. min, 20%) followed by Oxamyl treated plants (0.88 U/mg protein/ min, 12%) compared to *M. incognita* infested plants treatment (Fig. 6C).

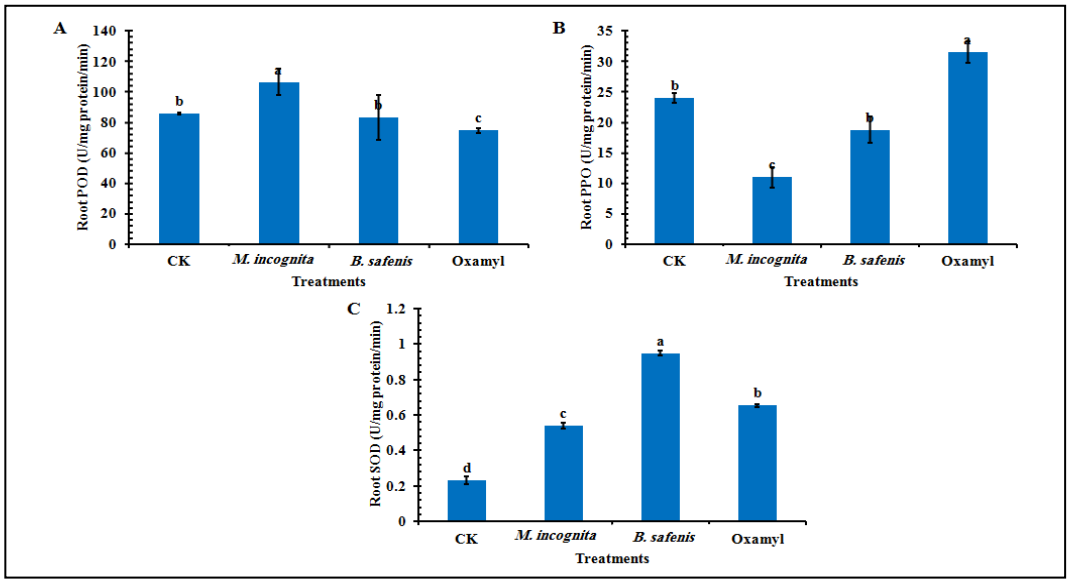

Fig. 5. Antioxidant enzyme activities of *B. safensis* co-culturing and Oxamyl treatments of 45 days old *M. incognita* infested potato roots. PPO (polyphenol oxidase) and SOD (superoxide dismutase). This means that sharing the same letters is not significantly different ($p \leq 0.05$). Means are compared using Tuky test.

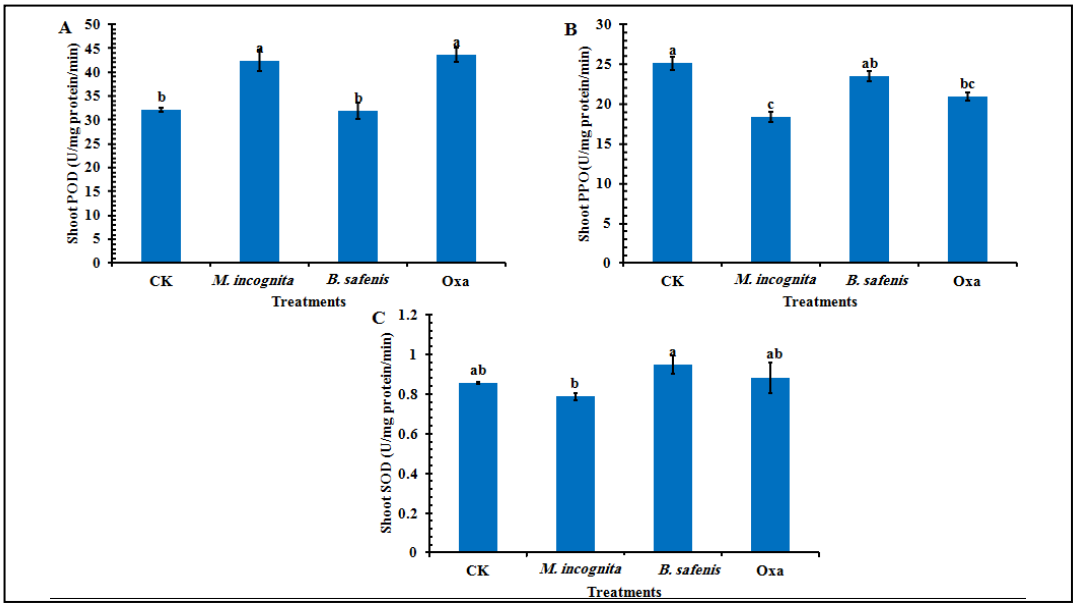

Fig. 6. Antioxidant enzyme activities of *B. safensis* co-cultured and Oxamyl of 45 days old *M. incognita* infested potato shoot. POD (peroxidase), PPO (polyphenol oxidase) and SOD (superoxide dismutase). This means that sharing the same letters is not significantly different ($p \leq 0.05$). Means are compared using Tuky.

DISCUSSION

M. incognita, the destructive soil-borne and wide host range pathogen, is of significant interest to many researchers around the world. The application of chemicals continues to be the primary tactic to mitigate crop diseases but pose a risk to humans and the environment. Therefore, employing alternative eco-friendly management strategies is in great demand. In addition to the growth-promoting features, some plant growth-promoting rhizobacteria (PGPR) have demonstrated nematicidal effects against root-knot nematodes (RKN).

These beneficial bacteria can colonize the plant rhizosphere and help suppress the disease through various mechanisms, including antibiosis, competition, myco-parasitism, and cell wall degradation. They can also induce resistance in plants and produce substances that promote plant growth (El-Nagdi and Abd-El-Khair 2019).

Investigating the *in vitro* nematicidal capacity of *B. safensis* strain OM1 against *M. incognita* using different culture filtrate concentrations indicated that the mortality of treated J2 significantly increased, and the hatching was decreased as well. Several reports indicated the impact of *Bacillus* spp. filtrate on J2 mortality and eggs hatchability (Abo-Elyousr *et al.,* 2010 and Das *et al.*, 2021). Culture filtrates from various strains of *Bacillus* spp. significantly increased the mortality of J2 and reduced the hatch rate of root-knot nematodes (RKN) through antibiosis by producing volatile organic compounds, toxins, and diffusible antibiotics (Ab Rahman *et al.,* 2018 and Jamily *et al*. 2018).

The nematicidal potential of *B. safensis* strain OM1 against *M. ingonita* and stress alleviation of infested potato, considering growth promotion, and physicochemical attributes boosting, was studied under greenhouse conditions. Our findings indicated that *B. safensis* strain OM1 treatment reduced gall numbers and J2/250 g soil of *M. incognita* infested potato roots and the surrounding rhizosphere colonization by 69.8% and 49.39%, respectively. Our results are in agreement with previous reports indicating the antagonistic properties of *B. safensis* to phytopathogens (Altimira *et al.*, 2022 and Nour *et al.,* 2024). Plant growth-promoting rhizobacteria (PGPR) isolates have various mechanisms that suppress plant-parasitic nematodes in the rhizosphere. These mechanisms include the production of enzymes, the release of toxins, and other metabolic products. *B. safensis* can produce antimicrobial molecules such as bacilysin, lichenysin, bacillibactin, fengycin, and surfactin (Teixeira *et al.,* 2021).

RKN infection negatively impacted potato growth and physicochemical attributes, where the common growth indicators and photosynthetic pigment exhibited significant reduction in agreement with previous reports (Mitiku 2018, Wilschut and Geisen 2021). The application of *Bacillus* to the soil proved high significant efficiency in root-knot nematode management under greenhouse conditions, promoting plant growth and increasing yield (Du *et al*., 2022 and Vasantha-Srinivasan *et al.,* 2025). Our results showed the potential of *B. safensis* in ameliorating the stress of *M. incognita*-infested potato and increasing growth measurements compared to infected plants. These findings are align with the observations on radish, *Stevia rebaudiana* and leafy vegetables that indicated the observed improvements in plant height and fresh weight can largely be attributed to the plant growth-promoting (PGP) properties of the bacterial strain, which include the production of indole-3-acetic acid (IAA), phosphate solubilization, hydrogen cyanide (HCN), ammonia production, nutrient uptake, and siderophore production (Prakash and Arora 2020 and Chebotar *et al.,* 2023).

Photosynthetic pigments are greatly affected by nematode infection due to the oxidative stress and membrane injury, where chloroplasts are the primary site of reactive oxygen species (ROS) generation (Wise *et al*., 2004 and Hasan *et al*., 2024). In our observations, *M. incognita*-infested potato plants exhibited significant reduction in TCC and CC contents; meanwhile, *B. safensis* co-culturing minimized the stress and lessened chlorophyll reduction compared to infected plants. Our obtained results are in harmony with previous studies conducted on wheat and lettuce (Sarkar *et al.,* 2018 and Zhang *et al.,* 2023).

Investigating induction of oxidative antioxidant enzymes production of treated plants showed a sharp increase in root and shoot POD enzyme contents against nematode infection; meanwhile, *B. safensis* co-cultured plants showed reduced activity. These findings were in agreement with (Van Staden *et al.,* (2006) and Kang *et al*., (2015). Our findings indicated significant reduction of PPO activity of root and shoot of *M.*

inocgnita infested potato plants, meanwhile *B. safensis* co-cultured plants showed *B. safensis* co-cultured plants showed a significant sharp increase in SOD activity of both root and shoot, which is compatible with previous research (Sarkar *et al.,* 2018). Reactive oxygen species such as hydrogen peroxide, superoxide, and singlet oxygen, are frequently produced by nematode infections, resulting in oxidative stress, these signaling molecules trigger the antioxidant response, interact with lipids and polyunsaturated fatty acids through peroxidation, and impair the stability of cell membranes by upsetting the secondary and tertiary structures of protein molecules found in membranes. They also speed up molecular transport across membranes, which increases electrolyte leakage (Hasan *et al.,* 2024).

PGPR priming can help regulate oxidative stress by reducing the activities of peroxidase (POD), superoxide dismutase (SOD), and polyphenol oxidase (PPO) compared to the control (Van Staden *et al*. 2006 and Kang *et al*. 2015). In response to nematode-induced stress, enzymatic antioxidants exhibited increased activity. Additionally, rhizobacteria alleviated stress indicators, strengthened antioxidant defenses, and boosted metabolite production.

CONCLUSION

B. safensis strain OM1showed significant nematicidal activity against *M. incognita in vitro* where culture filtrate inhibited egg hatching and increased J2 mortality and *in vivo* reduced gall numbers, egg masses and J2/250 g soil. *B. safensis* strain OM1 significantly promoted *M. incognita* infested potato plants growth parameters such as weights and plant height. Photosynthetic pigment contents of *M. incognita* infested plants significantly ameliorated compared to infested plants. *B. safensis* strain OM1co-culturing regulated antioxidant enzymes (POD, PPO and SOD) of shoot and root in response to *M. incognita* infection.

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Majority contribution for the whole article belongs to the author(s). The authors read and approved the final manuscript.

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The authors declare that they have no competing interests. The contents of the manuscript have neither been published nor under consideration for publication elsewhere.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)
The author (s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

STATEMENT AND ETHICS DECLARATIONS
The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this article. Ethical approval was not required for this study.

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