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RESEARCH ARTICLE

Characterization of plant growth-promoting rhizobacteria from black pepper rhizosphere: Antinomic activity and field efficacy of *Pseudomonas* isolate KBPf16

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Abstract

A random survey was undertaken to collect the rhizosphere soil samples from major black (*Piper nigrum*) pepper growing in highly elevated areas of Tamil Nadu to isolate and evaluate the native strains of Plant Growth Promoting Rhizobacteria (PGPR), with a focus on their antinomic activity and potential to enhance crop growth. 100 rhizobacterial strains were isolated, of which six were identified as *Pseudomonas* spp. (KBPf23, KBPf16, BBPf16, BBPf22, YBPf17 and TBPf21), demonstrating significant growth-promoting and antinomic properties. Among these, the strain KBPf16 showed the highest antagonistic activity against *Meloidogyne incognita*, a root-knot nematode. Under pot culture conditions, the talc-based formulation of native *Pseudomonas* isolate KBPf16 recorded a significant increase in plant growth parameters, viz., plant height, shoot weight, root length and root weight by checking nematode population in root and soil. The result of the field trials confirmed that KBPf16, applied as a talc-based formulation (20 g/vein in two splits), significantly reduced nematode populations and enhanced yield parameters in black pepper, increasing the yield to 3578.7 g/vein compared to the chemical control (2456.2 g/vein). Additionally, plants treated with KBPf16 exhibited elevated levels of defense-related enzymes, including peroxidase, phenols, polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL), along with enhanced lignification in roots. These findings suggest that the cold-tolerant strain KBPf16 holds great potential as a biocontrol agent for sustainable nematode management and growth promotion in black pepper cultivation.

Keywords: antagonism; antinomic property; black pepper; PGPR; root-knot nematode

Introduction

Black pepper (*Piper nigrum* L.) is an essential spice crop in India, cultivated across approximately 137,378 hectares, with an estimated production of 61000 tons in 2019-2020 (1). However, black pepper cultivation faces significant challenges, particularly from parasitic nematodes, which contribute to reduced productivity and yield losses through both biotic and abiotic stresses (2,3). Among the most problematic nematodes affecting black pepper are the burrowing nematode (*Radopholus similis*) and root-knot nematode (*Meloidogyne incognita*), both of which cause considerable damage to the crop (4, 5). Infestation by these nematodes often leads to symptoms like yellowing, stunted growth, wilting and root damage, ultimately resulting in plant death (6-8). Globally, root-knot nematodes were recognized as major pests, affecting over 90 species in the genus *Meloidogyne*, with *M. javanica*, *M. incognita*, *M. arenaria* and *M. hapla* being particularly widespread (9,10).

Cultural practices, resistant cultivars and chemical nematicides (Phorate 10 G @ 30 g or Carbofuran 3 G @ 100g) are examples of traditional nematode management methods. However, continuous reliance on chemical nematicides poses environmental hazards and can lead to toxicity issues. Biological control has consequently become a sustainable alternative that is frequently incorporated into Integrated Pest Management (IPM) strategies. Plant-parasitic nematodes are suppressed by using antagonistic fungi and bacteria in biological control (10). Microbial-based methods for nematode management have

gained attention due to their environmental safety, sustainability and cost-effectiveness (11,12).

Among various microorganisms, Plant growth-promoting rhizobacteria (PGPR) have garnered interest for suppressing pathogens and promoting plant health. Free-living bacteria known as PGPR colonize the rhizosphere, enhancing root growth and providing a dual benefit of promoting growth and disease suppression. Species like *Bacillus subtilis*, *Bacillus sphaericus* and *Pseudomonas fluorescens* were widely studied for their antagonistic properties against plant-parasitic nematodes (13). In particular, *Pseudomonas fluorescens* is recognized as an effective biocontrol agent, colonizing seeds and soil to suppress nematode populations (14,15). These rhizobacteria employ multiple antagonistic mechanisms, including the production of siderophores, antibiotics, volatile compounds as well as hydrolytic enzymes, to compete with pathogens and protect plants (16,17).

Furthermore, PGPRs support plant health by producing phytohormones that promote plant growth while inhibiting pathogens, like gibberellins, cytokinins and indole acetic acid (18 -23). Antagonistic bacteria, such as *Pseudomonas fluorescens*, have also been demonstrated to produce secondary metabolites, enzymes and toxins that disrupt nematode development by inhibiting egg hatching and reducing infective juvenile populations (24-26). This study focuses on the isolation and evaluation of PGPR strains, particularly *Pseudomonas fluorescens* KBPf16, for their ability to manage *Meloidogyne incognita* in black pepper under field conditions. The objective is to determine the potential of these strains to reduce nematode infestation and improve crop yield, thus offering a sustainable approach to black pepper cultivation.

Materials and Methods

Survey and collection of rhizospheric soil samples

A comprehensive survey was conducted from 2012 to 2013 across key black pepper-growing regions in Tamil Nadu, including Ooty, Kodaikanal, Yercaud and Kolli Hills. The purpose of the survey was to isolate *Pseudomonas* strains from the black pepper (*Piper nigrum* L.) rhizosphere. With an emphasis on the rhizosphere zone, 100 soil samples were systematically gathered from ten different villages within each region. Each sample comprised approximately 200 cc of moist soil, ensuring an accurate representation of the microbial population. Sampling was done using sterile tools to prevent contamination and stored in a refrigerator at 4 °C for further processing.

Isolation of rhizobacterial strains

Rhizobacteria were isolated using a serial dilution method, a well-established procedure for bacterial isolation. 100 mL of sterile distilled water was used to suspend 1 g of soil from each sample in a 250 mL conical flask. The suspension was thoroughly agitated on a rotary shaker for 15 min to ensure uniform distribution of bacteria. Dilutions ranging from 10^{-5} to 10^{-6} were prepared and 1 mL aliquots of these dilutions were spread on sterile Petri dishes containing King's B agar medium, a selective medium for *Pseudomonas* isolates. The plates were incubated at 28 ± 2ºC for 24 to 48 hr. Colonies exhibiting typical fluorescence under UV light were isolated, purified through streaking on fresh King's B plates and sub-cultured to obtain the pure cultures. Fluorescence and colony morphology were confirmed for the preliminary identification of *Pseudomonas* spp. and further biochemical confirmation was carried out by (27).

Preparation of bacterial inoculum

For the preparation of bacterial inoculum, the *Pseudomonas* isolates were cultivated in King's B broth with continuous shaking at 150 rpm at 28 ± 2ºC for 48 hr. Following the incubation period, the bacterial cells were harvested by centrifugation at 6000 rpm for 15 minutes and the resulting pellet was then resuspended in 0.01M phosphate buffer (pH 7.0). A spectrophotometer adjusted the bacterial suspension's concentration to 10^8 CFU/mL (OD595 = 0.3). For long-term storage, the bacterial cultures were preserved at -80ºC in 44% glycerol. For experimental use, the stored cultures were revived by inoculating one loopful of culture into 100 mL of King's B broth in a 250 mL conical flask, which was incubated at 28 ± 2ºC for 48 hr under shaking conditions at 150 rpm. Sub-culturing was performed monthly to maintain the bacterial strains throughout the experiment.

Plant growth-promoting assays

Seed germination and seedling vigor

To evaluate the plant growth-promoting potential of the *Pseudomonas* isolates, seed germination and seedling vigor assay were conducted using the roll towel method. The seeds (Rice var. IR 20) were soaked in 10 mL of the bacterial suspension (10^8 cfu/mL) with continuous shaking at 150rpm at 24 hr and the seeds were blot dried, placed in wet blotters and incubated in a growth chamber for ten days. The seeds which were treated with sterile water acted as the control. Following ten days of incubation, the percentage of germination was noted and the following formula was employed to compute the seedling vigor index by (28):

Vigor index = Germination percentage × Seedling length (root length + shoot length) (Eqn.1)

This approach provided a quantitative measure of the influence of the bacterial isolates on early plant growth.

Biochemical characterization of *Pseudomonas* isolates

Biochemical characterization of the *Pseudomonas* isolates was carried out to confirm their identity and determine their metabolic capabilities. The isolates were examined for their ability to grow at various temperatures and to use different carbon sources as their exclusive energy sources. Additionally, biochemical tests specific to Gram-negative bacteria were performed using the HiMedia KB002 Hi Assorted™ Biochemical Test Kit [Kit contains sterile media for Citrate utilization, Lysine utilization and Ornithine utilization, Urease detection, Phenylalanine Deamination Test, Nitrate reduction, H_2S production test and five different carbohydrates for utilization test - Glucose, Adonitol, Lactose, Arabinose, Sorbitol] (HiMedia Laboratories Pvt. Ltd.). The findings of these tests were compared with the diagnostic scheme outlined previously to group the isolates based on their biochemical responses (27).

Catalase test

Pure cultures (18-24 hr) of bacteria and 3 % hydrogen

peroxidase (H_2O_2) were used to observe the production of gas bubbles (positive reaction) (29).

Starch hydrolysis

Starch was mixed with water until creamy and molten nutrient agar was added. The mixture was autoclaved for 15 min and dispensed into sterilized Petri dishes. The isolate was streaked on starch agar Plates and incubated for three days. The Plates were flooded with Lugol's iodine solution and observed (30).

Carbon source utilization

Native bacterial isolates were characterized based on standard biochemical tests. Characterization included growth at different temperatures and the ability to utilize different substrates as a sole carbon source. In addition, the bacterial biochemical response was tested by using a ready biochemical kit for the specific identification of gram-negative rods (Rapid biochemical identification test kits-KB002 HiAssortedTM Biochemical test, HiMedia Laboratories Pvt. Ltd). The results of these tests were scored either as positive or negative and grouped with the aid of a determinative scheme developed by earlier workers (27).

Indole Acetic Acid (IAA) production

The fluorescent *Pseudomonas* isolates were cultured in King's B broth supplemented with 100µg/mL of L-tryptophan to evaluate their capacity to produce IAA, a well-known plant growth regulator. After 42 hr of incubation, bacterial cells were removed by centrifugation at 5000 rpm for 10 min and the IAA concentration in the supernatant was measured using the Salkowski reagent. In brief, 4 mL of Salkowski reagent (150 mL concentrated H_2SO_4, 250 mL distilled water, 7.5 mL 0.5 M $FeCl_3.6H_2O$) was combined with 1 mL of the supernatant and the mixture was incubated for 20 min at room temperature. The absorbance of the resulting mixture was measured at 535nm and the concentration of IAA was assessed by comparing it to a standard curve prepared with IAA concentrations that were known. This assay provided a quantitative assessment of the plant hormone production potential of the isolates.

In vitro efficacy of Pseudomonas isolates against Meloidogyne incognita

A cell-free filtrate of the bacterial cultures was prepared to assess the effectiveness of different *Pseudomonas* isolates against the root-knot nematode *Meloidogyne incognita*. A single colony from each isolate was grown in King's B broth in a 250 mL flask and incubated for 72 hr at 28°C on a shaker at 100 rpm. Sterilized Whatman filter papers (Nos. 1 and 42 sequentially) filtered the bacterial cultures. The final cell-free filtrate was obtained by passing the supernatant through a 0.22 µm Millipore filter after the filtrates were concentrated by centrifugation at 6000 rpm for 10 minutes. This filtrate was used at different concentrations (100%, 75%, 50% and 25%) to test its nematicidal activity.

Nematode mortality test

The nematicidal effect of the *Pseudomonas* isolates was assessed using the prepared bacterial cell-free filtrates. One mL of each filtrate concentration (100%, 75%, 50% and 25%) was added to separate Syracuse dishes. After 100 second-stage juveniles (J2) of *M. incognita* were introduced to each plate in 0.1 mL of sterile distilled water, the plates were incubated at 27 ± 1°C. Each treatment was replicated 3 times. Mortality was noted at 24, 48 and 72 hr post-incubation. To ensure permanent nematode mortality, the inactive nematodes were transferred to sterile distilled water and incubated overnight. A sterile blank and King's B broth were included as control treatments. Mortality percentages were calculated based on the observed inactive nematodes.

Preparation of talc-based formulation of *Pseudomonas* isolates

The effective *Pseudomonas* isolates were prepared by growing the bacterial cultures in King's B broth at room temperature (28±2°C) for 48 hr at 150 rpm to prepare a talc-based formulation. The bacterial population in the broth was adjusted to 9×10^8 cfu/mL. To produce a talc-based formulation (1:2.5), 400mL of the bacterial culture was then mixed with 1 kilogram of sterile talc powder, 15g of calcium carbonate (to bring the pH to neutral) and 10g of carboxymethyl cellulose (CMC) as an adhesive. After lowering the mixture's moisture content to less than 20% through shade drying, it was sealed and placed within polypropylene bags. The bacterial population in the talc formulation was measured at 2.5-3 × 10^8 cfu/g at the time of application.

Pot Culture evaluation of *Pseudomonas* isolates against *M. incognita*

To assess the efficacy of the *Pseudomonas* isolates *in vivo*, a pot culture experiment was performed by employing rooted cuttings of black pepper cv. Panniyur 1, obtained from the Horticultural Research Station, Yercaud. A steam-sterilized potting mixture (2:1:1 ratio of red soil, sand and farmyard manure) was placed in 10-kg pots with the cuttings planted in them. To inoculate the root zone with nematodes at a rate of one nematode per gram of soil, three holes were made around the plant five days after planting and covered with sterilized soil.

The following treatments were applied at 20 g/pot for each isolate and three replications of a completely randomized design (CRD) were used to arrange the pots:

Sl. No.	Treatment details
1.	T1-KBPf23(20g/pot)
2.	T2-KBPf16(20g/ pot)
3.	T3-BBPf16(20g/ pot)
4.	T4-BBPf22(20g/ pot)
5.	T5-YBPf17(20g/ pot)
6.	T6-TBPf21(20g/ pot)
7.	T7-Pf1(TNAU)(20g/ pot)
8.	T8-Carbofuran 3G@1kga.i./ha
9.	T9-Control

Observations on plant height, number of leaves per plant, root length, root weight, shoot weight, lesion index and number of nematodes / 5g root were recorded 90 days after treatment. The root lesion index was measured using the INIBAP technical guidelines by (31), which classify root lesions on a scale from 0 (no root rot) to 4 (100% root rot).

Per cent	Index
No root rot/lesion/root-knot.	0
Up to 25% lesion/root-knot.	1
Up to 50% lesion / root lesion/root-knot.	2
Up to 75% lesion/root-knot	3
100% lesion/root-knot.	4

Field evaluation of *Pseudomonas* isolates against *M. incognita*

Field trials were conducted during the 2023-2024 growing season at two locations, Pattipadi (Yercaud Taluk, Salem district) and Semmedu (Kolli hill, Namakkal district), in fields naturally infested with *M. incognita* at a density of over one nematode per gram of soil. Five replications of each treatment were included in the randomized block design (RBD) utilized in the experiment. Five plants were observed per replication. The

Sl. No.	Treatment details
1	T1-KBPf23(20g/plant)
2	T2-KBPf16(20g/ plant)
3	T3-BBPf16(20g/ plant)
4	T4-BBPf22(20g/ plant)
5	T5-YBPf17(20g/ plant)
6	T6-TBPf21(20g/ plant)
7	T7-Pf1(TNAU)(20g/ plant)
8	T8-Carbofuran 3G@1kga.i./ha
9	T9-Control

treatments were as follows:

Standard agronomic practices were followed as recommended by Tamil Nadu Agricultural University, Coimbatore. The bacterial formulations were applied around the rhizosphere, 10 inches from the base of the plant, before the flowering and maturation stages. Data were collected from both sites and pooled for analysis.

Enzyme assays

Peroxidase (PO) activity

To determine peroxidase (PO) activity, 1g of plant tissue was ground in 2 mL of 0.1M sodium phosphate buffer (pH 7.0) under chilled conditions (4°C) to ensure enzyme stability. After centrifuging the homogenate at 16,000g for 15 min at 4°C, the clear supernatant was utilized as an enzyme source. The reaction mixture comprised 1.5 mL of 0.05M pyrogallol, 0.5 mL of the enzyme extract and 0.5 mL of 1% hydrogen peroxide (H_2O_2). The mixture was incubated at room temperature (28 ± 2°C) and a spectrophotometer was used to detect the change in absorbance at 420nm at 30-sec intervals during 3 min. Through the calculation of the change in absorbance per minute per gram of fresh weight ($min^{-1}g^{-1}$), the expression for the enzyme activity was found.

Polyphenol Oxidase (PPO) activity

To prevent enzyme degradation for the polyphenol oxidase (PPO) assay, 1g of tissue was homogenized in 2 mL of 0.1M sodium phosphate buffer (pH 6.5) under ice-cold conditions. The homogenate was centrifuged at 16,000g for 15 min at 4°C and the supernatant was used as an enzyme source. The reaction mixture consisted of 1.5 mL of sodium phosphate buffer (pH 6.5) and 200 µL of enzyme extract. To initiate the reaction, 200 µL of 0.01 M catechol was added. The PPO activity was calculated as the change in absorbance per minute per gram of fresh weight ($min^{-1}g^{-1}$), using the observed absorbance change at 495 nm.

Phenylalanine Ammonia-Lyase (PAL) activity

PAL activity was measured by determining the conversion of L-phenylalanine to trans-cinnamic acid. To prevent the oxidation and polymerization of phenolics, 1 gram of plant tissue was homogenized in 3ml of ice-cold 0.1M sodium borate buffer (pH 7.0), which also contained 0.1g of insoluble polyvinylpyrrolidone and 1.4 mM 2-mercaptoethanol. After the extract was passed through cheesecloth, the filtrate was centrifuged for 15 min at 16,000 g. The supernatant was the source of the enzyme. The reaction mixture contained 0.4 mL of enzyme extract, 0.5 mL of 0.1M sodium borate buffer (pH 8.8) and 0.5 mL of 12 mM L-phenylalanine in the same buffer. After 30 min of incubation at 30°C, the mixture's amount of trans-cinnamic acid was measured utilizing the absorbance at 290 nm. PAL activity was presented as nmol trans-cinnamic acid per minute per gram of fresh weight ($nmol\ min^{-1}g^{-1}$).

Statistical analysis

To determine the importance of treatment effects, data from field experiments and pot culture were analysed using analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was used to assess treatment differences at a 5% significance level ($p \leq 0.05$). All statistical analyses were performed using IBM SPSS version 22.0 (Armonk, NY, USA). The outcomes were reported as mean values with standard errors and graphs were generated to visualize treatment comparisons.

Results

In vitro, the efficacy of *Pseudomonas* isolates against *M. incognita*

100 bacterial isolates were collected from pepper-growing regions of Tamil Nadu, India (Table 1). These isolates were initially screened for colony morphology and antinomic activity to evaluate their potential against *Meloidogyne incognita* under *in vitro* conditions. Although many isolates showed low antagonistic effects, six isolates-YBPf17, KB Pf16, KB Pf23, TBPf21, BBPf16 and BPf22 were selected for further evaluation based on their superior nematicidal activity. These were compared to a control broth and the reference strain *Pseudomonas fluorescens* Pf1 from TNAU.

Among these, KBPf16 exhibited the highest nematicidal activity, with a mortality rate of 73.61% at 100% culture filtrate concentration, followed by YBPf17 with 70.48% mortality (Table 2, Fig. 2). This trend was consistent across lower concentrations (75%, 50% and 25%), where KBPf16 consistently outperformed the other isolates. The lowest mortality, 6.77%, was observed in the control treatment. These results indicate that KBPf16 has a high degree of nematicidal activity against *M. incognita* juveniles, making it a promising candidate for further studies.

Plant growth-promoting assays

Seed germination and seedling vigor

The growth-promoting abilities of the 100 *Pseudomonas* isolates were evaluated using rice seeds (variety IR 20) in a roll towel method. Of these, seven isolates-KBPf23, KBPf16, BBPf16, BBPf22, YBPf17, Pf1 (TNAU) and TBPf21-showed

Table 1. Soil samples collected from black pepper rhizosphere in various hilly regions of Tamil Nadu

Name of the region	Name of the villages	Total number of samples collected	Name of the region	Name of the villages	Total number of samples collected
Yercaud	Athiyur	3	Burliyar	Kolikarai	3
	Asambur	2		Sembukarai	1
	Pattipadi	3		Pudhupattu	4
	Vellore	3		Karanci	1
	Nadur	2		Marakanam	1
	Manjakuttai	2		Sirumuni	5
	Myilampatti	1		Chimapattu	4
	Vellakadai	4		Siladi	1
	Coffee board	4		Palladi	3
	HRS	3		Moodurai	2
Kolli hills	Semmedu	1	Thadiyankudisai	Thandikudi	4
	Solakadu	2		Pannaikadu	2
	Valavandhinadu	4		Pattalankadu	4
	Periya salakalani	1		Kamanur	2
	Oorpuramkalyanai	2		Manjalparappu	1
	Adkampatty	1		Panrimalai	4
	Vendalapadi	3		Perumparai	1
	Pathiyapatti	2		K.C.Patti	2
	Aipilankadu	2		Aadalur	3
	Melkalingam	4		Pachillaur	3

significant enhancement in seed germination rates, as well as improved shoot and root growth, leading to increased seedling vigor. Among these, KBPf16 demonstrated the highest vigor index (4401.67), followed closely by TBPf21 (4277.1) (Fig. 1). These results suggest that KBPf16 is not only effective against nematodes but also promotes plant growth.

Carbon source utilization by Pseudomonas isolates

Six *Pseudomonas* isolates, including KBPf16, were tested for their metabolic versatility using a result interpretation chart (HIMEDIA). The strains were subjected to various biochemical tests, including citrate utilization, phenylalanine deamination, urease activity, lysine and ornithine decarboxylation, H_2S production, nitrate reduction and carbohydrate fermentation tests (lactose, glucose, arabinose, adonitol and sorbitol). The reference strain Pf1, along with the six isolates, displayed positive reactions for most of the tests, indicating a wide metabolic potential and adaptability of these strains to different environmental conditions (Table 3).

Production of Indole-3-Acetic Acid (IAA)

Six selected *Pseudomonas* isolates were tested for their ability to produce IAA, a key phytohormone involved in plant growth promotion. All isolates produced detectable levels of IAA, as quantified using the Salkowski reagent. The highest IAA production (40 µg/mL) was observed in KBPf16, followed by BBPf16 (29 µg/mL), BBPf22 (21 µg/ml) and KBPf23 (20 µg/mL). The lowest IAA concentration was recorded in TBPf21 (18 µg/mL) (Table 4, Fig. 3a). This suggests that KBPf16 not only promotes plant growth through enhanced seedling vigor but also synthesizes significant amounts of IAA, which may contribute to its plant growth-promoting properties.

Estimation of nematode population in soil

Two separate pot culture experiments were carried out under glasshouse situations to validate the efficacy of talc formulations of the *Pseudomonas* isolates (KBPf23, KBPf16, BBPf16, BBPf22, YBPf17, Pf1 and TBPf21) against *M. incognita* in black pepper (*Piper nigrum*) var. Panniyur 1. The results from both experiments were pooled and analysed statistically. Soil samples (200 cc) from each treatment were evaluated using Cobb's sieving and decanting method and the Modified Baermann's funnel technique to assess the nematode population. The data indicated a significant decrease in *M. incognita* populations in treatments with KBPf16,

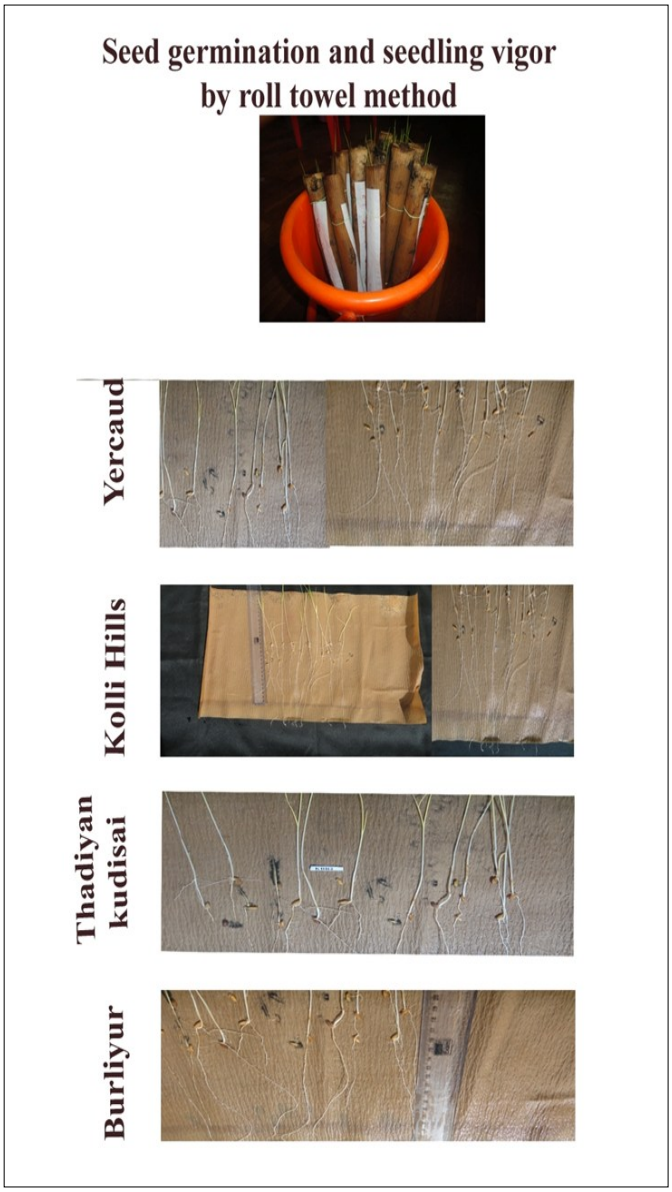
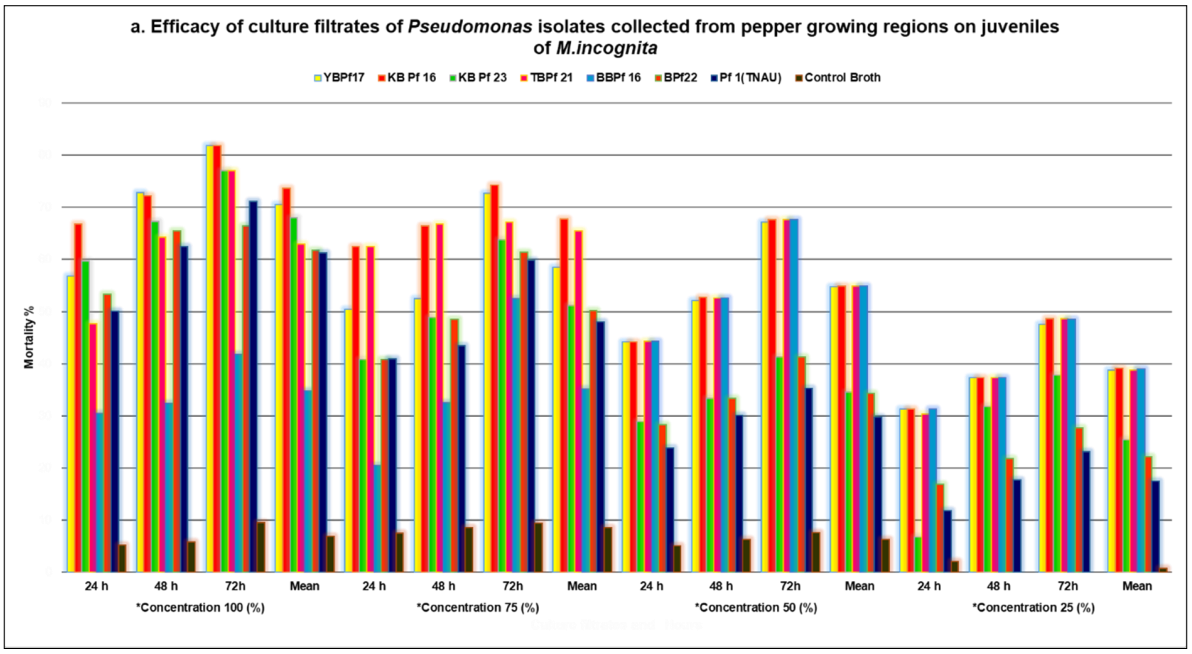


Fig. 1. Effect of selected *Pseudomonas* isolates on seed germination and seedling vigor under laboratory conditions

Table 2. Efficacy of culture filtrates of *Pseudomonas* isolates collected from pepper growing regions on juveniles of *M. incognita,*

Mortality of *M.incognita* at different concentration(%) and intervals(h)																
Culture filtrates	***Concentration 100 (%)**				***Concentration 75 (%)**				***Concentration 50 (%)**				***Concentration 25 (%)**			
	24 h	48 h	72h	Mean	24 h	48 h	72h	Mean	24 h	48 h	72h	Mean	24 h	48 h	72h	Mean
YBPf17	56.85	72.83	81.77	70.48	50.43	52.42	72.69	58.51	44.13	52.14	67.12	54.80	31.36	37.32	47.52	38.73
KB Pf 16	66.83	72.23	81.78	73.61	62.43	66.47	74.17	67.69	44.23	52.65	67.67	54.85	31.34	37.36	48.62	39.11
KB Pf 23	59.62	67.25	76.98	67.95	40.83	48.83	63.81	51.16	28.85	33.31	41.33	34.50	6.83	31.73	37.74	25.43
TBPf 21	47.62	64.25	76.98	62.95	62.43	66.77	67.17	65.46	44.26	52.64	67.62	54.84	30.34	37.36	48.62	38.77
BBPf 16	30.42	32.35	41.72	34.83	20.44	32.54	52.41	35.13	44.26	52.64	67.62	54.84	31.32	37.34	48.52	39.06
BPf22	53.36	65.44	66.45	61.75	40.84	48.45	61.41	50.23	28.26	33.41	41.23	34.30	16.84	21.83	27.73	22.13
Pf 1(TNAU)	50.11	62.41	71.11	61.21	40.87	43.44	59.81	48.04	23.81	30.11	35.30	29.74	11.81	17.72	23.10	17.54
Control Broth	5.27	5.83	9.50	6.87	7.54	8.61	9.48	8.54	5.04	6.34	7.62	6.33	2.13	0.00	0.00	0.71
SEd	0.425	0.698	0.206	0.443	0.425	0.512	0.547	0.494	1.242	0.387	0.413	0.680	0.321	0.581	0.413	0.438
CD (P=0.05	0.912	1.498	0.443	0.951	0.912	1.099	1.173	1.061	0.579	0.831	0.886	0.765	0.689	1.247	0.886	0.940

*Mean of 3 replications



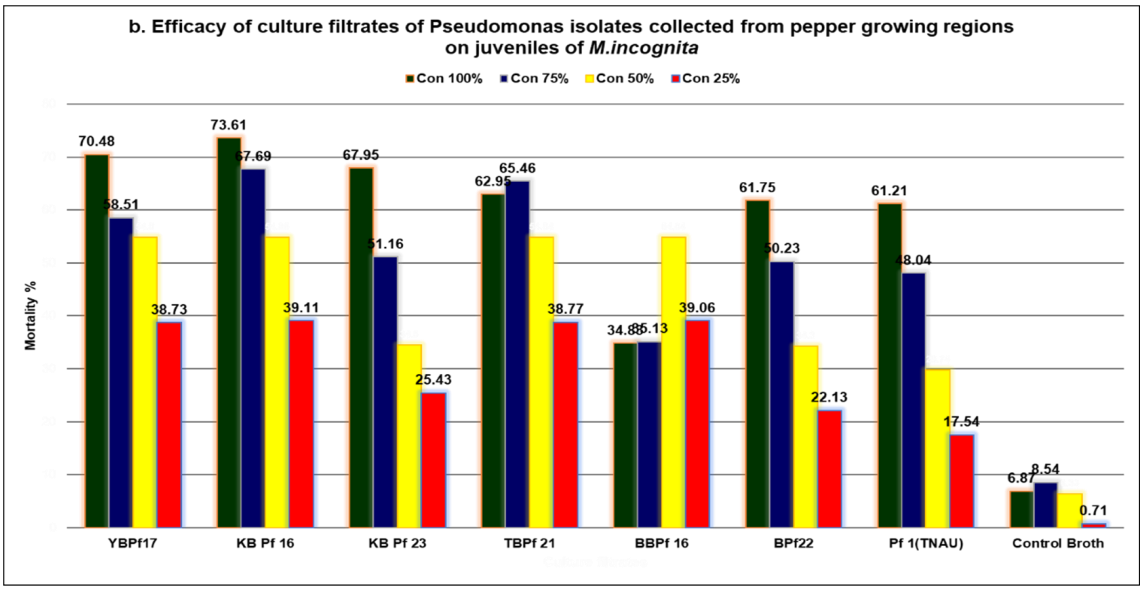


Fig. 2. Efficacy of culture isolates of Pseudomonas isolates from pepper growing regions against M. incogmita (a) DAT (b) Mean values

demonstrating its potential as an efficient biocontrol agent against root-knot nematodes.

Nematode population in black pepper under pot culture conditions

Pot culture experiments revealed that all *Pseudomonas* isolates substantially decreased nematode populations in both roots and soil. Among the tested isolates, KBPf16 was particularly effective, reducing the root nematode population by 73.62% compared to the untreated control, with the population dropping to 62.55 nematodes per 5 g of root tissue. In contrast, untreated plants exhibited the highest population at 108.60 nematodes per 5 g of root tissue. A similar trend was observed in the soil population, where KBPf16-treated plants showed a nematode population of 155.21 per 200 cc soil, representing a 92.27% reduction compared to the control,

Table 3. Carbon source utilization of selected Pseudomonas isolates using biochemical kit

Test	Isolates							
	Pf1 (TNAU)	KBPf23	KBPf6	BBPf16	BBPf22	YBPf17	TBPf21	Control
Citrate Utilization	+++	+++	++	++	++	++	+	-
Lysine Utilization	++	++	++	+	+	+	+	-
Ornithine Utilization	++	+	+	++	++	++	+	-
Urease	+	++	+	+	++	+	+	-
Phenylalanine Deamination	+	+	+	+	+	+	++	-
Nitrate reduction	+++	+	++	+	++	++	-	-
H_2S production	+	-	+	-	-	-	-	-
Glucose	-	+	-	-	-	-	-	-
Adonitol	-	-	-	-	-	+	-	-
Lactose	+++	-	-	-	-	-	-	-
Arabinose	++	-	+	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-

\+ Low production, ++ Medium production, +++ Strong production, - No production

Table 4. Production of Indole 3 Acetic Acid (IAA) by the selected *Pseudomonas* isolates

S. No.	*Pseudomonas* isolates	IAA (µg/ml of culture filtrate)
1.	KBPf23	20
2.	KBPf16	40
3.	BBPf16	29
4.	BBPf22	21
5.	YBPf17	14
6.	TBPf21	18
7.	Pf1(TNAU)	30
8.	Control	0

which recorded 298.43 nematodes. Additionally, root lesion indices, an indicator of nematode damage, were significantly lower in KBPf16-treated plants (1.24), while untreated control plants had a root lesion index 4.00. These results demonstrate that KBPf16 was highly effective in reducing nematode populations and mitigating nematode-induced damage in black pepper under controlled conditions (Table 5, Fig. 3b).

Plant growth characteristics of black pepper under pot culture conditions

The impact of *Pseudomonas* isolates on the growth parameters of black pepper plants was also assessed under pot culture conditions. Among the isolates, KBPf16 exhibited the highest efficacy in promoting plant growth, with a notable rise in root and shoot weights, plant height and root length. The KBPf16-treated plants showed a maximum height of 55.13 cm, significantly higher than the untreated control plants. Similarly, shoot weight was significantly enhanced in the KBPf16-treated plants, recording 110.37 g, an increase of 79.90% over the control. Root length was also markedly improved in KBPf16-treated plants, with an average root length of 49.83 cm, reflecting a 97.42% increase compared to the control plants, which had an average root length of only 25.24 cm. Furthermore, the number of leaves per plant was also considerably higher in KBPf16-treated plants, with 11.21 leaves per plant, accounting for an 80.52% increase over the control plants (Table 6, Fig. 4a).

Root population and lesion index of *Meloidogyne incognita* in black pepper

The nematode population in the black pepper plants' roots was further evaluated 180 days after treatment (DAT). The KBPf16 isolate recorded the lowest root nematode population of 102.54 nematodes per 5 g of root, resulting in a 96.55% reduction compared to the control plants, which recorded 201.54 nematodes per 5 g of root. This was comparable to the performance of the chemical control (carbofuran) treatment. Moreover, the root lesion index in KBPf16-treated plants was significantly lower at 1.10, compared to 4.00 in the untreated control plants. These findings indicate that KBPf16 is highly effective in reducing nematode populations and minimizing root damage caused by *Meloidogyne incognita* (Table 7, Fig. 4b).

Yield of black pepper

Various yield-related parameters of black pepper were assessed at the time of harvest, including the number of berries per spike, berry set percentage, spike length and dry pepper yield. The KBPf16 treatment significantly enhanced the yield parameters compared to the untreated control. The highest number of berries per spike was observed in KBPf16-treated plants, with an average of 120.5 berries per spike, compared to only 65.4 berries per spike in the control plants. Similarly, the berry set percentage was highest in KBPf16-treated plants (95.4%) compared to the control (48.5%). Spike length was also significantly improved, with KBPf16-treated plants recording an average spike length of 19.2 cm, compared to just 5.22 cm in the untreated plants. Regarding dry pepper yield, KBPf16-treated plants documented the highest yield of 3578.7kg/ha, which was a 34.77% increase over the control. The treatments with KBPf23 and carbofuran also enhanced the pepper yield, recording 3300.1 kg/ha and 3200.4 kg/ha, respectively. The untreated control plants produced the lowest yield of 2655.4kg/ha. The benefit-to-cost (B: C) ratio was highest in the KBPf16-treated plants, with a ratio of 1:18.93, compared to 1:11.17 in the carbofuran-treated plants (Table 8, Fig. 5).

Peroxidase activity

Peroxidase (PO) activity in pepper plants significantly increased with applying *Pseudomonas* isolates. KBPf16-treated plants exhibited the highest level of PO activity in leaf and root samples, with a value of 1.75 in leaves and 1.45 in roots. On the other hand, the roots of the untreated control plants had the lowest PO activity, with a value of 0.78. This suggests that applying *Pseudomonas* isolates, particularly KBPf16, plays a crucial part in enhancing plant defense responses by increasing peroxidase activity (Table 9, Fig. 6a).

Polyphenol oxidase activity

In addition, PPO activity was considerably higher in the plants treated with P. fluorescens than in the control group. With values of 1.95 in the leaves and 2.85 in the roots, KBPf16-treated plants have the highest PPO activity. In contrast, the untreated control plants demonstrated the lowest PPO

Table 5. Efficacy of talc formulations of *Pseudomonas* isolates on *M. incognita,* infestation in black pepper under pot culture condition at HRS, Yercaud

Treatments	Root population (5g)	Per cent decrease over control	Soil population (200cc)	Per cent decrease over control	Root lesion Index
KBPf23(10g/pot)	77.99	39.24	158.33	88.49	2.12
KBPf16(10g/ pot)	62.55	73.62	155.21	92.27	1.24
BBPf16(10g/ pot)	87.43	24.21	225.00	32.64	3.44
BBPf22(10g/ pot)	94.53	14.88	211.00	41.44	3.22
YBPf17(10g/ pot)	81.76	32.83	208.76	42.95	2.42
TBPf21(10g/ pot)	96.04	13.08	159.32	87.31	3.12
Pf 1(TNAU)(10g/ pot)	80.62	34.71	228.65	30.52	2.44
Carbofuran 3G 1kgai/ha)	67.21	61.58	167.24	78.44	2.00
Control	108.60		298.43		4.00
CD(p=0.05)	3.27		3.96		

*Pooled analysis of data gathered from two pot culture experiments

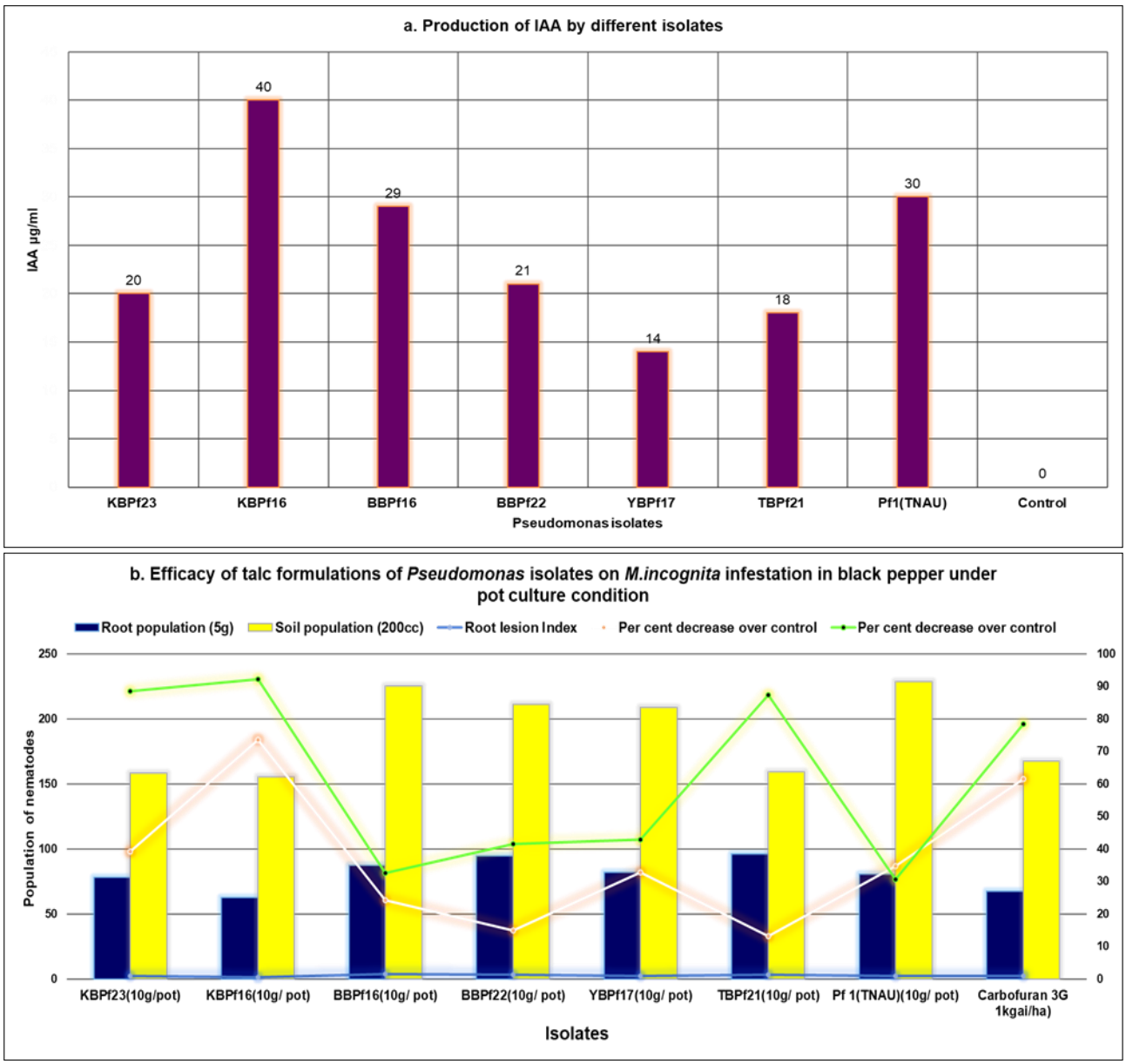


Fig. 3. Efficacy of *Pseudomonas isolates* against M. *incognita* (a) Production of IAA (b) Efficacy of talc formulations of *Pseudomonas isolates* against M. *incognita*

activity, particularly in the roots (1.8). These results indicate that KBPf16 not only promotes plant growth but also boosts the activity of PPO, which is associated with plant defense mechanisms against pathogens (Table 9, Fig. 6b).

Phenylalanine ammonia-lyase (PAL) activity

Plants treated with P. fluorescens isolates exhibited considerably higher levels of PAL activity, a critical enzyme involved in plant defense. KBPf16-treated plants exhibited the highest PAL activity in leaf and root samples, with values of 3.21 in leaves and 4.38 in roots. On the contrary, the plants in the untreated control group had the lowest PAL activity, especially in the roots (2.46). These results imply that applying KBPf16 increases the overall plant defense system,

Table 6. Efficacy of talc formulations of *Pseudomonas* isolates on the growth of Black Pepper at pot culture condition at Horticultural Research Station, Yercaud.

Treatments	Plant height (cm)	Per cent increase over control	Shoot weight (g)	Per cent increase over control	Root length (cm)	Per cent increase over control	Root weight(g)	Per cent increase over control	No. of leaves	Per cent increase over control
KBPf23 (10g/pot)	46.61	63.89	94.33	53.76	38.75	53.53	81.68	65.75	9.74	56.84
KBPf16 (10g/ pot)	55.13	93.85	110.37	79.90	49.83	97.42	97.35	97.54	11.21	80.52
BBPf16 (10g/ pot)	36.44	28.13	99.39	62.00	45.97	82.13	79.67	61.67	8.21	32.21
BBPf22 (10g/ pot)	31.58	11.04	75.48	23.03	36.07	42.91	56.12	13.88	8.97	44.44
YBPf17 (10g/ pot)	39.67	39.49	71.6	16.71	37.83	49.88	75.63	53.47	9.35	50.56
TBPf21 (10g/ pot)	44.41	56.15	81.87	33.45	35.12	39.14	59.63	21.00	8.37	34.78
Pf 1 (TNAU) (10g/ pot)	38.47	35.26	101.34	65.18	49.91	97.41	81.61	65.60	9.21	48.43
Carbofuran 3G 1kg bnnnai/ha)	33.83	18.95	101.21	64.97	32.48	28.68	81.63	65.65	10.12	62.96
Control	28.44	-	61.35	-	25.24	-	49.28	-	6.21	-
CD(P=0.05)	4.19		3.13		4.84		3.00		1.75	

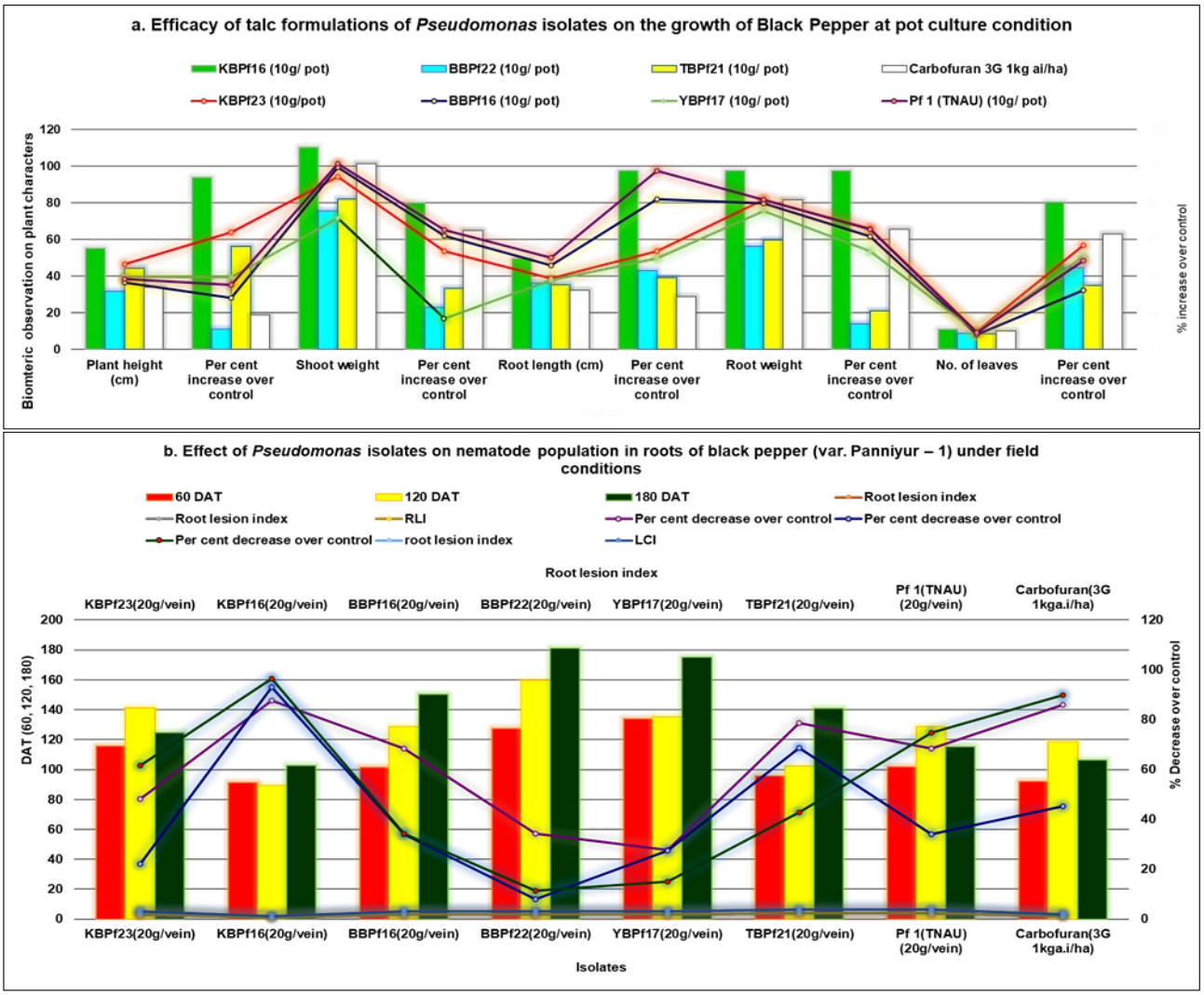


Fig. 4. Efficacy of talc formulation of Pseudomonas isolates (a) Pseudomonas isolates on growth parameters of black pepper

(b) Pseudomonas isolates on nematode population

Table 7. Effect of *Pseudomonas* isolates on nematode population in roots of black pepper (var. Panniyur – 1) under field conditions

Treatments	60 DAT	Per cent decrease over control	120 DAT	Per cent decrease over control	180 DAT	Per cent decrease over control	Root lesion index
KBPf23(20g/vein)	115.47	48.30	141.24	22.03	124.65	61.68	3.20
KBPf16(20g/vein)	91.24	87.68	89.32	92.97	102.54	96.55	1.10
BBPf16(20g/vein)	101.61	68.53	128.47	34.16	150.12	34.25	3.14
BBPf22(20g/vein)	127.61	34.19	159.47	8.08	181.11	11.28	2.97
YBPf17(20g/vein)	134.1	27.70	135.12	27.56	175.11	15.09	3.12
TBPf21(20g/vein)	95.89	78.58	102.25	68.57	141.01	42.93	3.87
Pf 1(TNAU) (20g/vein)	101.68	68.41	128.54	34.09	115.35	74.72	3.91
Carbofuran(3G 1kga.i/ha)	92.03	86.07	118.61	45.32	106.11	89.93	1.97
Control	171.24		172.36		201.54		4.00
CD(P=0.05)	3.74		9.72		7.47		

Pooled analysis of experimental data collected from difference location of Pattipadi Vellore, Yercaud and Semmedu, Kolli hills Nammakal dt.

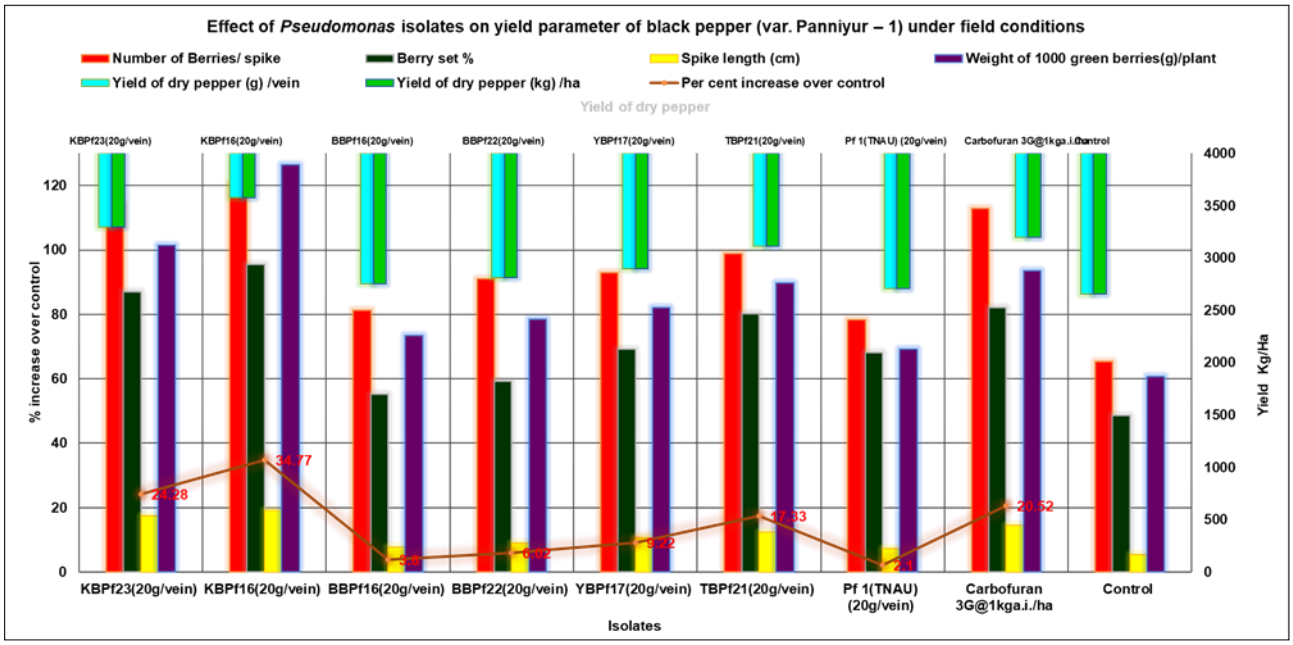
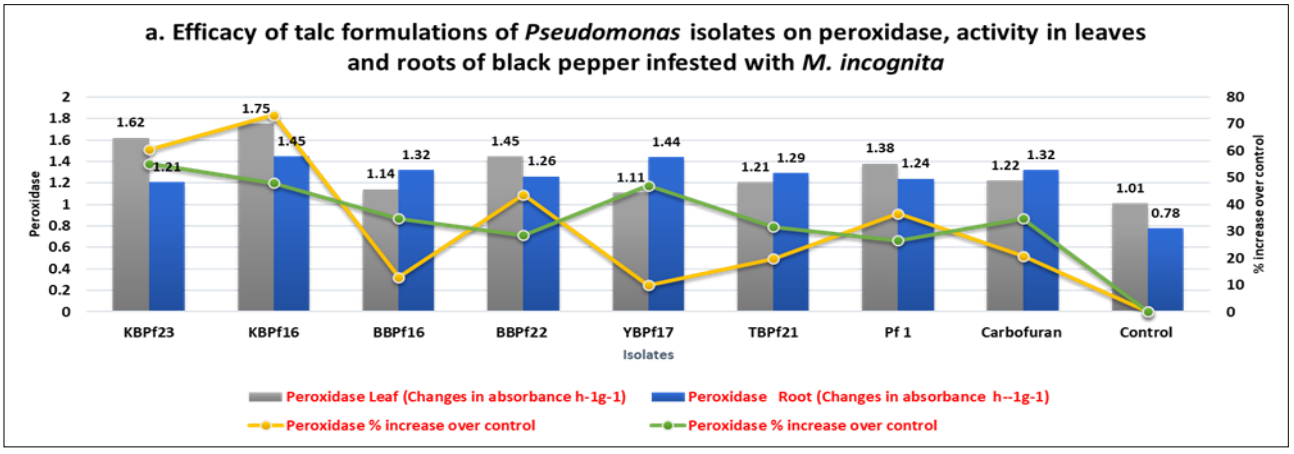
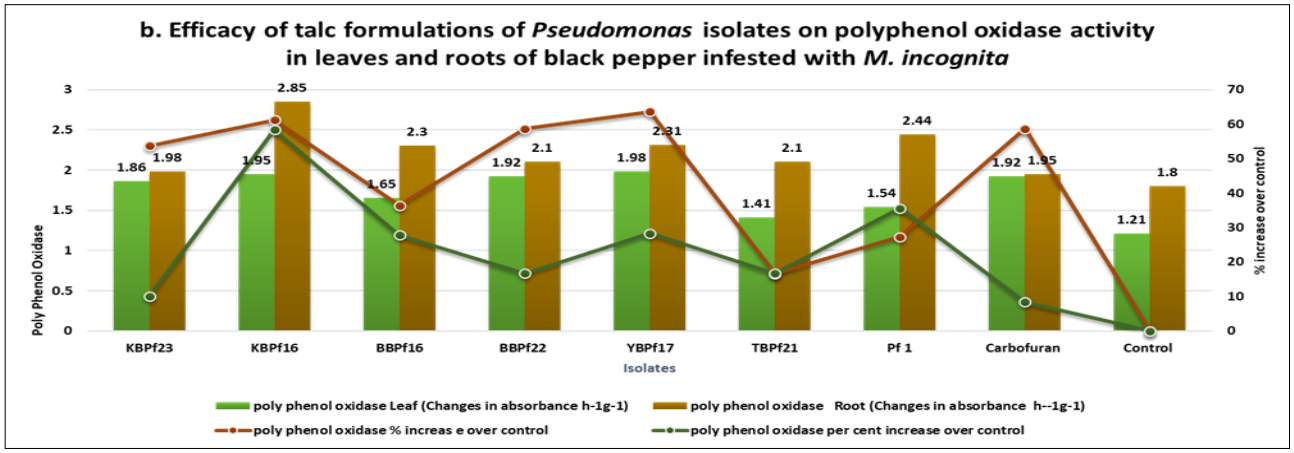


Fig. 5. Effect of native isolates on yield parameters of black pepper

Table 8. Effect of *Pseudomonas* isolates on yield parameter of black pepper (var. Panniyur – 1) under field conditions

Treatments	Number of Berries/ spike	Berry set %	Spike length (cm)	Weight of 1000 green berries(g)/plant	Yield of dry pepper (g) / vein	Yield of dry pepper (kg) /ha	Per cent increase over control	B:C ratio
KBPf23(20g/vein)	114.3 (10.69)	87.1 (8.54)	17.32 (3.91)	101.45 (9.97)	3300.1 (53.82)	3300.1	24.28	1:13.22
KBPf16(20g/vein)	120.5 (11.26)	95.4 (10.05)	19.20 (4.26)	126.4 (10.82)	3578.7 (59.82)	3578.7	34.77	1:18.93
BBPf16(20g/vein)	81.2 (9.99)	55.2 (8.36)	7.55 (4.03)	73.37 (7.45)	2756.2 (57.66)	2756.2	3.80	1:2.07
BBPf22(20g/vein)	91.0 (10.09)	59.2 (8.72)	8.92 (4.37)	78.54 (9.97)	2815.2 (54.80)	2815.2	6.02	1:3.28
YBPf17(20g/vein)	93.0 (9.58)	69.2 (10.05)	10.65 (3.89)	82.21 (8.21)	2900.3 (53.43)	2900.3	9.22	1:5.02
TBPf21(20g/vein)	98.9 (8.96)	80.3 (9.06)	12.35 (4.26)	89.65 (9.35)	3115.5 (35.43)	3115.5	17.33	1:9.43
Pf 1(TNAU) (20g/vein)	78.4 (9.05)	68.1 (8.67)	7.24 (4.07)	69.21 (9.38)	2711.1 (48.64)	2711.1	2.10	1:1.14
Carbofuran 3G@1kga.i. /ha	112.9 (11.26)	82.1 (9.90)	14.50 (4.26)	93.54 (10.82)	3200.4 (59.82)	3200.4	20.52	1:11.17
Control	65.4 (8.14)	48.5 (8.27)	5.22 (3.89)	60.63 (8.37)	2655.4 (54.46)	2655.4		
CD(P=0.05)	3.23	1.34	NS	3.46	6.24			





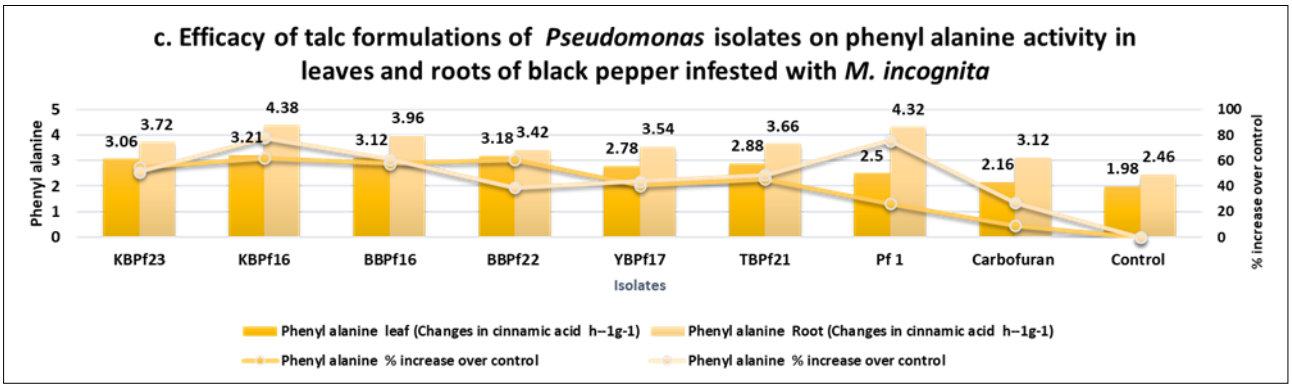


Fig. 6. Efficacy of talc formulation of *Pseudomonas* isolates (a) Peroxidase (b) Polyphenol Oxidase (c) Phenylalanine

Table 9. Efficacy of talc formulations of *Pseudomonas* isolates on peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase activity in leaves and roots of black pepper infested with *M. incognita**

Treatments	PO				PPO				PAL			
	(Changes in absorbance $h^{-1}g^{-1}$) Leaf	% increase over control	(Changes in absorbance $h^{-1}g^{-1}$) Root	% increase over control	(Changes in absorbance $h^{-1}g^{-1}$) Leaf	% increase over control	(Changes in absorbance $h^{-1}g^{-1}$) Root	per cent increase over control	(Changes in cinnamic acid $h^{-1}g^{-1}$) Leaf	% increase over control	(Changes in cinnamic acid $h^{-1}g^{-1}$) Root	% increase over control
KBPf23(20g/vein)	1.62	60.40	1.21	55.13	1.86	53.72	1.98	10.00	3.06	54.55	3.72	51.2
KBPf16(20g/vein)	1.75	73.27	1.45	47.96	1.95	61.16	2.85	58.33	3.21	62.12	4.38	78.0
BBPf16(20g/vein)	1.14	12.87	1.32	34.69	1.65	36.36	2.3	27.78	3.12	57.58	3.96	61.0
BBPf22(20g/vein)	1.45	43.56	1.26	28.57	1.92	58.68	2.1	16.67	3.18	60.61	3.42	39.0
YBPf17(20g/vein)	1.11	9.90	1.44	46.94	1.98	63.64	2.31	28.33	2.78	40.40	3.54	43.9
TBPf21(20g/vein)	1.21	19.80	1.29	31.63	1.41	16.53	2.1	16.67	2.88	45.45	3.66	48.8
Pf 1(TNAU)(20g/vein)	1.38	36.63	1.24	26.53	1.54	27.27	2.44	35.56	2.5	26.26	4.32	75.6
Carbofuran 3G@1kga.i./ha	1.22	20.79	1.32	34.69	1.92	58.68	1.95	8.33	2.16	9.09	3.12	26.8
Control	1.01		0.78		1.21		1.8		1.98		2.46	
CD (P = 0.05)	0.21		0.23		0.42		0.46		0.57		0.68	

contributing to its effectiveness in managing nematode infections (Table 9, Fig. 6c).

Discussion

PGPR plays a crucial role in enhancing crop health by promoting growth and suppressing a wide range of pathogens. Their significance has been extensively recognized in recent years, especially due to their multifaceted functions, including improving soil texture, facilitating nutrient acquisition and secreting vital extracellular compounds such as antibiotics, enzymes and hormones (32). These beneficial microorganisms -fungi and bacteria-have increasingly been used as biological agents to control plant diseases, especially soil-borne pathogens. Fungi and endophytic bacteria, in particular, can colonize the rhizosphere and plant tissues (endorhiza), thereby improving the defense mechanisms of plants against root-knot nematodes (33). These microbes not only improve plant nutrition and general growth but also increase plant resistance to pathogens (34, 35).

In our investigation, we isolated cold-tolerant isolates of *Pseudomonas* from the rhizosphere of black pepper plants in the hilly regions of Tamil Nadu, including Yercaud, Kolli Hills, Thadiyankudisai and Burliyar. Through biochemical analyses, these isolates were identified and their efficacy in managing plant-parasitic nematodes, particularly *Meloidogyne incognita*, was evaluated. The results indicate that these cold-tolerant strains, particularly KBPf16, performed better than the existing isolate Pf1 (TNAU), isolated from the plains.

One of the most remarkable findings was strain KBPf16's capacity to promote plant growth. This was demonstrated through an increase in the germination percentage and root and shoot length of rice plants, indicating its potential as a plant growth promoter. Further testing in black pepper under pot culture conditions confirmed these findings, significantly improving plant morphometric characteristics such as shoot length, root length and overall biomass.

In addition to growth promotion, strain KBPf16 showed strong antinomic activity. When applied in a talc-based formulation at the flowering and maturation stages of black pepper, the strain significantly reduced nematode infestation in the roots and soil. This reduction in nematode population, along with a rise in plant growth parameters, highlights the dual benefit of using PGPR strains like KBPf16 in nematode management and growth promotion.

This study underscores the potential of cold-tolerant rhizobacteria like KBPf16 as a valuable tool in managing nematodes in crops grown in hilly regions. As plant-parasitic nematodes can severely impact crop yield and quality, finding biological solutions tailored to specific agro-climatic conditions is crucial. The results of this research open the door for further investigations into the use of cold-tolerant PGPR for sustainable nematode management in a range of crops beyond black pepper.

Similar findings were reported in the literature. For example, it was demonstrated that certain species of *Bacillus* and *Pseudomonas* have antagonistic properties against soil-borne pathogens, especially in the rhizosphere of solanaceous crops (36). In another study, 34 rhizobacterial isolates were evaluated for their plant growth-promoting characteristics, including the production of siderophores, ammonia and IAA, as well as phosphate solubilization (37). These beneficial traits were found to be widespread among the isolates, with 79.41% of them showing phosphate solubilization activity and 67.75% exhibiting IAA production, among other traits. This demonstrates the broad applicability of PGPR in promoting plant health across different crop systems.

Our study also supports previous findings on the role of PGPR in modulating plant defence mechanisms via the activation of oxidative enzymes like polyphenol oxidase (PPO) and peroxidase (PO). These enzymes are critical in forming lignin and other phenolic compounds, which serve as structural barriers against pathogens (38). In particular, the combined uses of *Bacillus subtilis* and *Pseudomonas fluorescens* in other crops were shown to increase the activity of PPO, PO and other defence-related enzymes, resulting in enhanced plant resistance (37).

Furthermore, our study found that the strain KBPf16 increased the activity of key defence enzymes, including PAL, peroxidase and polyphenol oxidase, in black pepper plants. These enzymes have significant roles in the plant's defence response and their increased activity suggests that KBPf16 can not only reduce nematode infestation but also strengthen the plant's internal defence systems. These findings align with previous research, showing that PGPR-induced defence enzymes contribute significantly to plant pathogen resistance.

Additionally, several investigations have emphasized the significance of incorporating biological amendments like PGPR into crop management strategies. For example, the application of *Bacillus pumilus* strain ZHA90 in another study enhanced plant growth parameters and decreased root galling resulting from root-knot nematodes (10). In brinjal, a gradual reduction in gall formation was observed with increased amendments of neem sawdust, with complete gall suppression achieved at 70-100% amendment levels (39). This highlights the potential of biological agents like PGPR in integrating sustainable pest and disease management practices into agricultural systems.

The cold-tolerant strain KBPf16 of *Pseudomonas* has demonstrated significant potential in managing *M. incognita* in black pepper while enhancing plant growth and yield. The findings from this study imply that this strain could be an effective component of IPM strategies, particularly in hilly regions where cold tolerance is necessary for optimal microbial performance. Future research should focus on expanding the use of such cold-tolerant strains across different crops and environments and exploring their potential in combination with other biocontrol agents and organic amendments to further enhance their efficacy.

Conclusion

In this study, the fluorescent *Pseudomonas* strain KBPf16, isolated from the rhizosphere of black pepper in cold, hilly regions, demonstrated significant plant growth-promoting and antinomic activity. Applying this strain not only enhanced the growth parameters of black pepper but also effectively reduced root-knot nematode (*Meloidogyne incognita*) populations. Compared to existing biocontrol agents, KBPf16 exhibited superior efficacy in promoting plant health and mitigating nematode infestations under field conditions. These findings suggest that the cold-tolerant strain KBPf16 holds great potential as a biocontrol agent for managing plant-parasitic nematodes in black pepper, particularly in regions with challenging agro-climatic conditions. Moreover, the strain's ability to enhance plant growth underscores its dual role in improving crop yield and suppressing nematode populations. Given its efficacy, KBPf16 could become a vital component in integrated nematode management strategies, offering a sustainable and eco-friendly alternative to chemical nematicides. Future research should explore its application across different crops and environments to fully harness its potential in sustainable agriculture.

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Authors' contributions

All the authors contributed to the manuscript's writing, editing, methodology and corrections. SP Carried out the research work, DM wrote the manuscript, SK wrote and edited the methodology, JS edited the manuscript, SM and SB made a graphical presentation, AP carried out correction and alignment, GK wrote the discussion, KM overall corrected and edited the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

Declaration of generative AI and AI-assisted technologies in the writing process

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators were used during the writing or editing of manuscripts.

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