Verdant Legacy

Journal of Applied Botany and Green Economy

1(1): 2026

Research article

Benzoic Acid Influenced *Phaseolus vulgaris* L. Rhizospheric Soil Fungal Community and Crop Quality

Nadia Saleh[1], Abdul Nasir Khalid[2], Saman Khaliq[3]*

[1]Department of Botany, Lahore College for Women University, Lahore, Pakistan.nadiaslaeh14@yahoo.com
[2]Department of Botany, University of the Punjab, Lahore, Pakistan.
[3]Department of Plant Breeding and Genetics, PMAS Arid Agriculture University, Rawalpindi, Pakistan.

*Corresponding Email: khaliqsaman98@gmail.com

Abstract

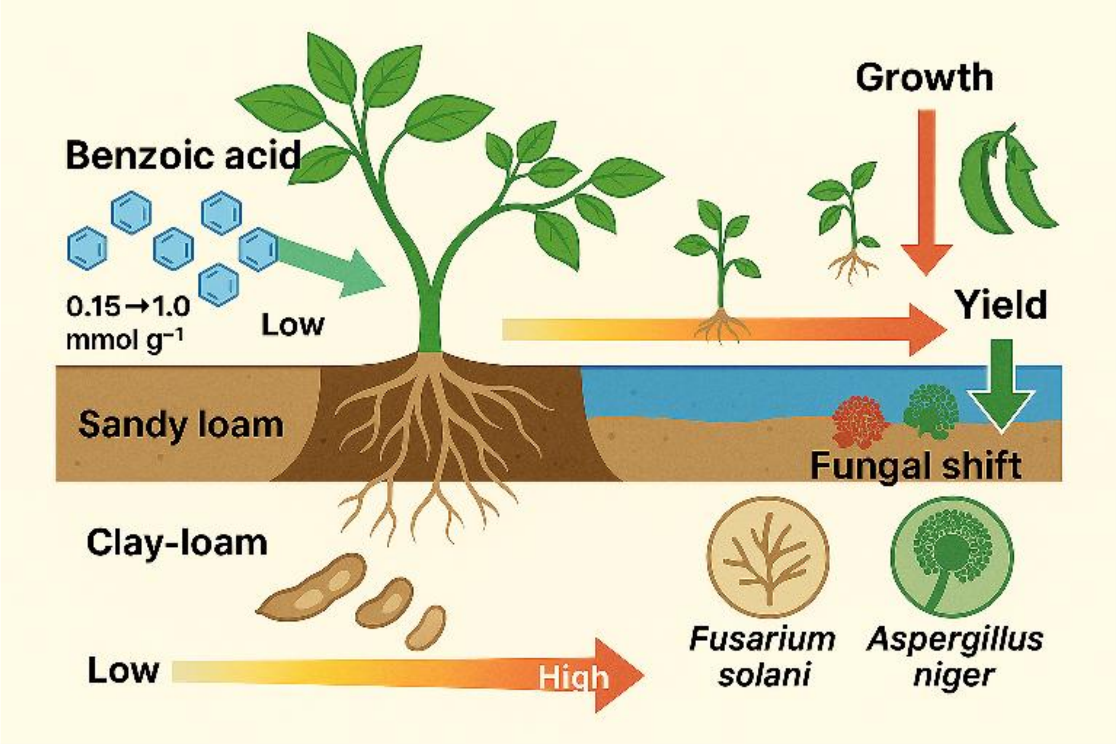
The exudation of allelochemicals by root of legumes can lead to soil fatigue through the accumulation of the allelochemicals in the rhizosphere following repeated release and slow decomposition rates by microbes inhabiting the soil. Phaseolus vulgaris L. exuded benzoic acid, which is one of the key phenolic compounds and is said to have strong phytotoxic effects. The purpose of the study was to assess the impact of benzoic acid on the growth of P. vulgaris and yield, as well as to determine the influence of benzoic acid on the population of rhizospheric fungi. Sandyloam and clay loamy soils that were sprayed with benzoic acid at 0.15, 0.25, 0.5 and 1.0 mmol g-1 soil were used to grow plants. The percent germinating, biomass, the number of pods, and yield of the seed were determined and the rhizospheric fungi were determined by using standard culture methods. The findings indicated that the growth of plants and their yield were inhibited by benzoic acid in a concentration-dependent way, and the maximum inhibition was observed when the concentration was 1.0 µmolg-1. The abundance of culturable fungi reduced substantially with benzoic acid concentration; whereas intermediate concentrations promoted the proliferation of certain fungi, including *Fusarium solani* and *Aspergillus niger*. These results prove that the presence of benzoic acid in the rhizosphere negatively impacts plant functions and fungal communities of the soil. The allelochemical accumulation in the continuous cropping systems is hence an issue of concern in terms of the balance of microbes of the soil and enhancing crop yield.

Keywords: Benzoic acid; Monocropping; *Phaseolus vulgaris*; Allelochemicals; Pathogenic fungi; Rizosphere

Received: 02 December 2025 **Accepted**: 14 January 2026 date: **Published:** 30 January 2026

Citation: Saleh N., A. N. Khalid, S. Khaliq (2026). Benzoic Acid Influenced Phaseolus Vulgaris L. Rhizospheric Soil Fungal Community and Crop Quality. Journal of Applied Botany and Green Economy (JABGE). 1(1):1-16

Graphical Abstract



1: Introduction

One of the most popular legume crops that has been grown throughout the world is *Phaseolus vulgaris*, also referred to as the common bean. It is a very crucial source of protein, vitamins, as well as minerals and it means a lot to the economy of most developing and developed nations both locally and internationally. Regardless of its significance, cultivation of *P. vulgaris* is associated with various difficulties and some of them are soil-based stresses that may restrict its growth, productivity and general health of the crop (Osogo *et al*., 2025). Sustainable and eco-friendly agricultural production is the necessity of the present era to fulfill the human needs in safe environment. The production of sustainable crops is immensely affected by the soil type, plant species and soil microbial community. The relationship between plants and soil microbes is both positive and negative, based on soil and environmental conditions. To its advantage, legumes are symbiotic to nitrogen-fixing rhizobia that convert atmospheric nitrogen into plant-usable forms thereby improving plant growth and soil fertility (Goyal *et al*., 2023). Plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) also aid in nutrient uptake, phytohormone synthesis and resistance to pathogens (Laishram *et al*., 2025).Nonetheless, other interactions are destructive: pathogenic fungi like Fusarium and allelochemicals exuded by roots may suppress plant growth, as well as alter useful communities of microbes (Tharanath *et al*., 2024) . Allelochemicals are specialized plant metabolites among the compounds that are released by the plant roots and these compounds mediate the interactions that exist between plants and their immediate soil environment. The phenolic compound (benzoic acid) produced by P. vulgaris roots has been known to affect plant development as well as the rhizosphere microbial communities. It has been demonstrated that phenolic acids can inhibit or moderate soil fungi and thereby change nutrient availability, pathogen suppression and the health of the soil (Osogo *et al*., 2025).

The effects are affected by the characteristics of the soil, the availability of nutrients and the level of moisture and therefore, the processes of interaction between plant-microbe and soil are dynamic and context-specific (Cao *et al*., 2024). There is a complex interaction between plant, soil and microbes (Garbeva *et al*., 2004). The autotoxic chemicals are released from plants into the rhizospheric soil, through various mechanisms such as leachation (Scavo *et al*., 2019), volatilization, root exudation, and crop residue decomposition (Rice, 2012). The phenomena decrease the availability of mineral nutrients and promote the growth of pathogenic fungi and bacteria in soil(Cheng & Cheng, 2015). Two main groups of microbes present in soil are saprophytic and symbiotic microbes. They can either be detrimental, neutral or beneficial. The detrimental microorganisms include deleterious rhizospheric organisms, major plant. Rhizosphere is the area around plant roots, which contains a wide range of microbial community, which is vital in the development of plants and the health of soil. Whereas a number of microorganisms, e.g. nitrogen- fixing bacteria and arbuscular mycorrhizal fungi, develop positive interactions that improve nutrient status and plant health, other soil-dwelling symbionts are harmful, inducing diseases or suppressing growth. Pathogens that may affect root functioning, decrease nutrient uptake, and cause enormous yield losses in many crops have been shown to be soil-borne, such as fungi such as Fusarium solani, Pythium s*pp*, and *Rhizoctonia spp*, bacteria such as *Ralstonia solanacearum*, and *Agrobacterium tumefaciens* (Prasad *et al*., 2017). Besides the threat of microbes, plants also secrete allelochemicals, including benzoic acid, into the rhizosphere by root exudation, leaching, volatilization, and breakdown of residues. Such compounds may have phytotoxic effects, disrupt microbial communities in soils, suppressing the growth of plants, and causing such phenomena as soil fatigue in the conditions of constant cultivation. It is important to understand the intricate interaction of the plants, allelichemicals and rhizospheric microbes so as to reduce autotoxicity, restore soil microbial balance and enhance crop yield (Mendes *et al*., 2013).

The microbial community structure in soil is determined by the two key factors, one is the soil type with variation in soil mineral nutrients and other is the presence of phenolic compounds, released by the plant in the soil. The phytotoxic phenolic compounds are responsible for the changes in biological activities of microorganisms. Under the influence of phenolic compound, microorganisms can inhibit or delay the growth and germination of the donor plant (Miller, 1996; Garbeva *et al*., 2004). In some cases, due to these phytotoxic phenolic compounds neighboring plants suffer the cost and show a reduction in crop yield and its quality.

In presence of phytotoxic phenolic compound, plant microbial interaction may lead toward the soil sickness and negatively affect the crop yield, replantation and regeneration in case of successive crop and their cultivation(Mondal *et al*., 2015). The soil sickness is because of enhanced growth of soil-borne pathogens or microbes, deterioration of soil properties, nutrient availability imbalance, and accumulation of autotoxic substances due to monoculturing (Yu *et al*., 2000; Huang *et al*., 2006). With the increase in phytotoxic allelochemicals, soil becomes mineral deficient and leads to soil stress due to increase. In agriculture system where leguminous crops are grown continuously, such as mono cropping practices, the repeated release of these allelochemicals exacerbates soil stress and and contribute a phenomena call Soil sickness.

Soil sickness due to monoculturing of the leguminous plant is common all over the world and causes more than 50% crop losses. The successive cropping of beans results in the accumulation of phytotoxic phenolic compounds in the soil and suppress or delay germination. The phytotoxic phenolic compounds have been reported from legumes (*Lens culinaris, Phaseolus vulgaris, Glycine max, Lupinus spp. Pisum sativum, Vicia faba* and *Cicer arietinum*) are benzoic acid, *p*-hydroxybenzoic acid, salisylic acid, vanillic acid, adipid acid and *p*-hydroxyphenylacetic acid ((Asaduzzaman & Asao, 2012;Mondal *et al*., 2015). Common beans (*Phaseolus vulgaris L.*) is one of the most valuable legume crops world, accounts for its high proportion of daily protein intake in several countries. The common beans are known for their high quality nutrients, vitamins, dietary fibers, unsaturated fatty acids rich grains (Assefa *et al*., 2019). However, the crop is currently facing wide range of biotic and abiotic challenges. The biotic stresses include fungal, bacterial and viral diseases that occur possibly due to the change in secondary metabolites in the rhizospheric soil by continuous monocropping (yang *et al*., 2013). The effect of benzoic acid and trans-cinnamic acid on mineral composition, growth and chlorophyll content has been studied in soybean. It was found that quantity of magnesium, manganese, phosphorus and potassium has significantly reduced due to buildup of pathogenic microbes in soil (Baziramakenga *et al*., 1994) Most common soil-borne pathogenic fungi of legumes are *Fusarium oxysporum* and *F. solani* which cause *Fusarium* wilt in plants (Pouralibaba *et al*., 2015). The symptoms of the disease are necrotic leaves lead to foliar wilting and eventually cause the death of the plant after a few days or weeks. The pathogen causes premature leaf, damping off, stunting and browning of vascular system or vascular wilt ((Minuto *et al*., 2006; Ye *et al*., 2004) . Therefore, the goal of present study was to investigate the the impact that benzoic acid has on the rhizospheric fungal community of *P. vulgaris* and to determine the possible implication of this effect on plant development and quality of crops. The study will offer understanding of the interactions between plants and soils and microbes with the aim of guiding sustainable production and soil management systems of this valuable legume.

2: Materials and Methods

The experiment was conducted partly in Fungal Biosystematics Lab., Department of Botany, University of the Punjab, Lahore and Molecular Taxonomy Lab, Department of Botany, Lahore College for Women University, Jail Road Lahore during August 2016- Septemeber 2017. The experimental design was randomized complete block design (RCBD) for field trials and split block randomized complete design (RCD) for Lab experimentation.

2.1: Germination Experiment

To check germination percentage under the influence of various concentrations of benzoic acid seeds of *P. vulgaris* were germinated on sand. Germination test was performed at four different concentrations (0.15, 0.25, 0.5, 1.0 μmol/ml) of Benzoic acid according to optimized protocol. All concentrations were maintained at pH=7. The experiment was conducted in triplicates per treatment. Each replicate consisted of 10 seeds. Seven days after sowing seedlings were sampled. Seed germination percentage, plant length and dry weight were measured. Leaf surface area was calculated by leaf destructive method using ImageJ software. The concentrations to calculate germination percentage were selected using previous studies (Peters, 2000).

2.2: Seedling Experiment

Two types of soils (clayey and sandy loamy) were collected from open fields of University of the Punjab, Lahore and PCSIR Phase II, Johar Town Lahore. These soil samples were used for seedling experiment. Seedlings of *P. vulgaris* at three leaf stage were transplanted in the pots containing 200g of soil. Seedlings were treated with different concentrations of Benzoic acid after every 48 hour to achieve final concentration of 0.15, 0.25, 0.5, 1.0 μmol/g soil (makes total 30 μM, 50 μM, 100 μM and 200 μM of benzoic acid in each pot) (Zhou *et al*., 2014). For treatments pH of Benzoic acid was maintained to 7.0 with the help of 0.1 mol/L NaOH solution. Each treatment consisted of 5 replicates. After 20 days plants were harvested and plant length, dry weight, leaf surface area and percentage disease indices were measured ((Zhou & Wu, 2012). Disease indices percentage (DI %) was calculated by following formula.

$$\text{DI}\ \% = \frac{\text{Obtained disease Indices}}{\text{Total possible disease Indices}} \times 100$$

2.3: Collection of Rhizosphere Soil

After experimentation, the soil samples were collected from the rhizosphere of plants by shaking and sieving it with 1 mm size strainer. The samples were analyzed for EC, pH, Organic matter percentage, available phosphorous, potassium, carbon, saturation percentage and texture form Soil Fertility Research Laboratory, Lahore.

2.4: Determination of Available Phosphorus, Potassium, and Carbon

The Olsen method was used to determine the concentration of available phosphorus, whereby soil samples were extracted in 0.5 M sodium bicarbonate (pH 8.5), and phosphorus concentration was determined in a spectrophotometer colorimetrically. Potassium available was removed using 1 N ammonium acetate and measured using a flame photometer. The Walkley and Black dichromate oxidation method was used to determine soil organic carbon.

2.5: Isolation of Micromycetes from Soil

Malt Extract Agar is an excellent isolating media for broad range isolation of environmental samples especially fungi (Kinnunen *et al*., 2017). To prepare 2% Malt Extract Agar media, 2g Malt Extract and 2g Agar was dissolved in 100 ml of distilled water. Media was autoclaved at 121 ºC at 15 lb/ inch^2 for fungal culturing. In direct plate method, approximately 20 ml of MEA media was poured to each petri plate (90 mm), antibacterial vibramycin capsule was used to avoid bacterial contamination. .disperse soil in the medium. The plates were incubated at 25±2 ºC for 7-10 days. In dilution plate method, 1% dilutions of soil were prepared. Further, serial dilutions of soil samples were made up to 10^{-9}. The petri plates containing 20 ml of MEA sterile media was inoculated with 1 ml of soil suspension. The petri plates were incubated at 25±2 ºC for 7-10 days. After 10 days' colonies were observed macro morphologically and micromorphologically. Pure cultures were stored at 4 ºC in refrigerator for long term use.

2.6: DNA isolation by General CTAB protocol

CTAB protocol followed by (Gardes & Bruns, 1993) was used for extraction of DNA from fresh pure fungal cultures. Fresh pure fungal culture was transferred to autoclaved eppendorf tube using sterile needle. The eppendorf tube was properly labeled for specimen identification. 300 µl of 2% CTAB isolation buffer (1M Tris-HCL, 0.5 M EDTA, 5M NaCl, CTAB, B- Mercaptoethanol) was added to eppendorf and tubes were freezed and thawed for three times. After freezing and thawing, the fungal culture was crushed with an autoclaved micropestle. After crushing, the eppendorf tube was incubated at 65 ºC for 30 minutes in water bath the tubes were inverted after every five minutes. The eppendorf tubes were taken out of water bath and 300 µl of isoamylalcohol: chloroform (1:24) was added, mixed and vortexed for the removal of cellular junk or proteins. The mixture was clarified by centrifugation for 10 minutes at maximum speed (13200 rpm) and 200 µl of supernatant was taken in autoclaved and labeled eppendorf tube. In supernatant 133 µl ice cold isopropanol was added and the eppendorf tube was incubated in freezer overnight. Next day, the solution in the eppendorf tube was centrifuged for 15-20 minutes at 13200 rpm to pellet down the DNA. The supernatant was discarded after centrifugation and 200 µl 70% ethanol was added to eppendorf for washing of pellet (mixing, vortexing and centrifugation at 13200 rpm for 10 minutes). The step was repeated and after draining the buffer pellet was allowed to dry. Dried pellet was stored in 50 µl TE buffer or deionized water in eppendorf tube and stored at 4 ºC. Samples were sent for molecular identification of fungal species. ITSF1/ITS4 were used as primer to obtain PCR product. The specimens were sent to China for sequencing and were submitted to NCBI genbank after phylogenetic analysis https://www.ncbi.nlm.nih.gov/genbank/.

2.7: Mycelial Growth Experiment under the influence of Benzoic acid concentrations

Fusarium solani, Aspergillus niger, Talaromyces pinophilus and *Trichoderma erinaceus* strains were isolated from rhizosphere soil of *P. vulgaris*. MEA medium was prepared and autoclaved at 121 ºC for 15 minutes (Zhao *et al.*, 2010). Benzoic acid solution was added to MEA media after cooling it down (at 53 ºC) to make up final concentrations 1 µmol/ml, 0.5 µmol/ml, 0.25 µmol/ml, and 0.15 umol/ml. The pH was maintained at 7 and media was poured in the petri plates. Vibramycin was used as antibacterial capsule. Afterwards, strains were inoculated in the center of petri plates. The experiment was performed in triplicates. After 7-10 days colony diameter and growth rate were measured.

2.8: Seed Infestation Experiment

Culture suspensions were prepared from pure cultures plate of *Fusarium solani*, *Aspergillus niger, Talaromyces pinophilus* and *Trichoderma erinaceus* by scraping the strains from media and, mixing and vortexing it with sterile distilled water. Seeds of *P. vulgaris* were infested separately with culture suspensions of *Fusarium solani Aspergillus niger, Talaromyces pinophilus* and *Trichoderma erinaceus* for 1 h and 6 hrs. The infested seed were grown in sand boxes. After 10 days, germination percentage and plant dry weight were calculated (Murali *et al*., 2012).

$$\text{Germination }\% = \frac{\text{No. of seed germinated}}{\text{Total no. of seed sown}} \times 100$$

2.9: Statistical Analysis

Statistical test was applied to record data using SPSS software Inc. 16.0. The test was performed to analyze the values using Duncan's multiple range test and standard deviation was calculated. Significance level was used P = 0.05.

3: Results

3.1: Seed Germination

Benzoic acid did not significantly inhibit the germination of *P. vulgaris* seed at all treatments (Table 1). The maximum inhibition percentage was 20% at 0.15 µmol/ml and 1.0 µmol/ml concentrations. Although, the germination reduction was not significant. Similarly, seedling length was also significantly reduced at 1.0 µmol/ml concentration. The maximum reduction in seedling length was 5.9 cm and plant dry weight was 830 mg at 1.0 µmol/ml of Benzoic acid solution. However, leaf area was also reduced significantly at 1.0 µmol/ml concentration (Leaf Surface area= 11.8 cm^2) (Table 1).

3.2: Seedling Growth

3.2.1: Seedling Growth in Sandy Loamy soil

Seedling growth was not significantly reduced in sandy loamy soil at all treatments of Benzoic acid (0.15, 0.25, 0.5, 1.0 µmol/g soil) except plant length at 0.15 µmol/g soil. The inhibition in plant length was 27 cm at this concentration. Although, there was no significant difference in leaf surface area and plant dry weight (P=0.05) but addition of Benzoic acid in soil increased the disease indices and plant loss percentage. The percentage plant loss (27 %) and disease indices (50-53 %) were maximum at 0.5 and 1.0 µmol/g soil (Table 1).

3.2.2: Seedling Growth in Clayey Loamy soil

In clayey loamy soil, no significant reduction in plant length, leaf surface area or plant dry weight was observed at all treatments of Benzoic acid solution in soil. However, plant loss percentage was increased in clayey loamy soil (60 % at 1.0 µmol /g soil) in comparison to plant loss percentage in sandy loamy soil. Likewise, the disease indices percentage was maximum at higher concentration of Benzoic acid concentration in soil (Table 1).

3.2.3: Available Phosphorous, Potassium and Carbon

There was a prominent difference in the quantity of phosphorous in sandy loamy soil as compared to clayey loamy soil by increasing the concentration of benzoic acid in soil. The quantity of phosphorus was significantly reduced at 0.25 and 1.0 µmol/g (of clayey loamy soil) concentration of benzoic acid whereas no significant difference recorded in sandy loamy soil. Minimum quantity of available potassium was observed at 0.5 and 1.0 µmol/g (of sandy loamy soil) and at 0.15, 0.5, 1.0 µmol/g (of clayey loamy soil). (Fig. 1).

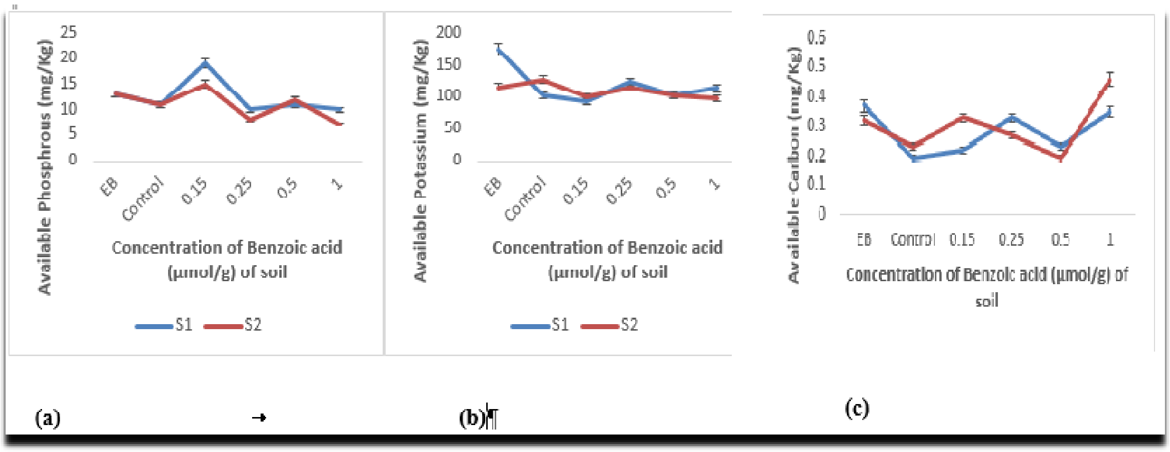


Figure 1. Amount of available Phosphorous (a), Potassium (b), Carbon (c) in sandy loamy and clayey soil (mg/Kg)

Table 1. Effect of different concentrations of Benzoic acid (0.15, 0.25, 0.5, 1.0 µmol/ml) on seed and seedlings of *Phaseolus vulgaris* L. on germination, Seedling/ Plant growth and biomass.

Effect on Germination

Concentrations (µmol/ml)	**Germination percentage %**	**Dry weight (mg)**	**Seedling length (cm)**	**Leaf Surface area/ plant (cm^2)**
Control	60±26.45 a	1100±0.62 a	11.66±6.90 a	35.13±16.53 a
0.15	40±17.32 a	1020±0.63 a	9.46±6.24 ab	37.53±6.94 a
0.25	43±20.81 a	1110±0.58 a	9.35±5.04 ab	41.17±19.47 a
0.5	46±5.77 a	6700±0.23 a	8.28±2.42 ab	21.83±1.67 a
1.0	40±20.00 a	830±0.96 a	5.91±6.00 b	11.8±0.96 a
P value	.404	0.522	0.039	0.061

Effect on Seedling

Concentrations (µmol/g of soil)	**Plant length (cm)**	**Dry Weight (mg/plant)**	**Leaf Surface area/plant (cm^2)**	**Plant loss percentage %**	**Disease Indices %**
0	38.58±10.67 a	585±216 a	20.19±7.59 a	0	0
0.15	27.00±16.31 a	384±145 a	15.24±8.86 a	20	16
0.25	33.90±14.1 a	581±346 a	16.55±10.63 a	13	46
0.5	33.90±18.45 a	564±309 a	17.20±13.57 a	27	53
1.0	37.73±6.57 a	537±286 a	17.14±10.42 a	27	50
P value	0.177	0.269	0.784		

Effect on plant growth and biomass

Concentrations (µmol/g soil)	**Plant length (cm)**	**Dry Weight (mg/plant)**	**Leaf Surface area/plant (cm^2)**	**Plant loss %**	**Disease Indices %**
0	11.6±3.36 a	272±151.2 a	14.72±12.93 a	0	0
0.15	10.8±2.86 a	262±69.78 a	9.68±6.72 a	40	26
0.25	13.20±3.88 a	246±140.1 a	10.88±12.16 a	20	30
0.5	10.80±6.37 a	266±122.8 a	9.86±10.11 a	40	20
1	10.80±6.37 a	282±193.0 a	8.28±8.12 a	60	40
P value	0.872	0.996	0.887		

Duncan's multiple range test applied on the data; Mean±Standard deviation; $P \leq 0.05$

3.2.4: Isolation of micromycetes from rhizospheric soil

Total nine micromycetes were isolated from rhizospheric soil of *P. vulgaris*. The mycomycetes were analyzed macro-morphologically and micro-morphologically. The fungi were identified named *Lasiodiplodia theobromae*, *Paecilomyces lilacinus*, *Fusarium solani*, *Talaromyces pinophilus*, *Aspergillus niger*, *Trichoderma virens*, *Aspergillus fumigatus*, *Trichoderma erinaceus*, *Penicillium javanicum*. The accession numbers assigned to the specimens were MF565837, MF589642, MF589618, MF589638, MF589636, MF589640, MF565840, MF589616 and MF574327 respectively.

3.2.5: Mycelial Colony Growth

In the petri plate experiment, Benzoic acid significantly increased *Fusarium solani* colony diameter (32.5 cm, 28 cm and 43.1 cm) at 0.15 µmol/ml, 0.25 µmol/ml and 0.5 µmol/ml concentrations respectively. The increment in mycelial colony growth was maximum at 0.5 µmol/ml concentration of Benzoic acid in media. *Talaromyces pinophilus* mycelial colony growth was significantly reduced with increase in concentration of concentration of Benzoic acid. The minimum mycelial growth was recorded 8.3 cm at 0.25 µmol/ml concentration. In presence of *Aspergillus niger*, the mycelial colony diameter growth was significantly decreased at 0.15 µmol/ml of Benzoic acid. However, no significant difference was observed in mycelial colony diameter of *Trichoderma erinaceus* at all treatments (Figure 2; Table 2).

.

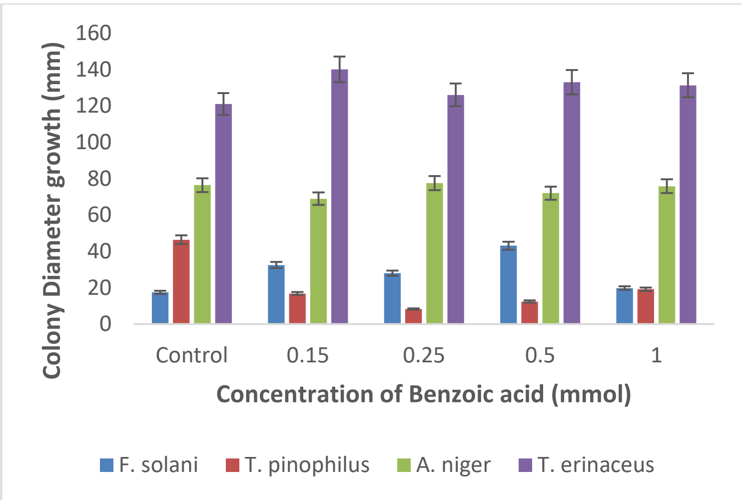


Figure 2. Comparative effect of different concentration of benzoic acid on colony diameter growth of *Fusarium solani*, *Trichoderma pinophilus*, *Aspergillus niger* and *Trichoderma erinaceus*. grown on MEA medium

Benzoic acid had a concentration-dependent effect on the growth of fungi. A statistical analysis revealed a significant difference ($P > 0.05$) between benzoic acid and the control treatment at the selected concentrations only, with no difference observed in other concentrations. The reaction pattern was different in the case of the species, which showed species-specific

.

Table 2. Effect of different concentration (0.15, 0.25, 0.5, 1.0 µmol/ml) of Benzoic acid on the growth of micromycetes isolated from the rhizosphere soil of *P. vulgaris*

Name	Fungal Isolates	Concentration					P value
		Controlled	0.15	0.25	0.5	1.0	
F-33	*Fusarium solani*	17.5±3.9 [c]	32.5±1.5 [b]	28±7.3 [b]	43.1±2.0 [a]	19.8±7.0 [c]	0.00
G2	*Talaromyces pinophilus*	46.4±26.4 [a]	16.8±5.9 [b]	8.3±2.0 [b]	12.4±3.0 [b]	19.2±11.1 [b]	0.038
A1	*Aspergillus niger*	76.1±3.5 [a]	68.9±2.9 [b]	77.5±4.1 [a]	72.0±4.9 [ab]	75.8±1.5 [a]	0.083
W2	*Trichoderma erinaceus*	121±16.3 [a]	140±0.0 [a]	126±22.6 [a]	133±6.0 [a]	131.3±1.5 [a]	0.598

Table 3. Effect of different culturable fungal isolates on the growth of *P. vulgaris*

Talaromyces pinophilus	**Plant length**	**Surface Area / plant (cm^2)**	**Germination %**	**Dry weight (mg)**
Controlled	55.48±4.54 a	47.02±10.73 a	67	5230
1 h	61.69±16.61 a	29.27±12.62 a	40	4970
6 h	28.13±9.14 b	26.22±7.21 a	27	2480
P-value	0.023	0.099		
Fusarium solani				
Controlled	55.48±4.54 a	47.02±10.73 a	67	5230
1 h	46.61±5.98 b	47.62±26.30 a	47	4260
6 h	0.00±0.00 c	0.00±0.00 b	0	0
P-value	0.00	0.019		
Aspergillus niger				
Controlled	55.48±4.54 a	47.02±10.73 a	67	5230
1 h	28.81±10.24 b	7.36±5.12 b	33	1610
6 h	23.12±3.16 b	16.35±3.46 b	13	2430
P-value	0.002	0.001		
Trichoderma erinaceus				
Controlled	55.48±4.54 a	47.02±10.73 a	**67**	5230
1 h	72.57±19.65 a	40.14±10.38 a	**67**	5750
6 h	4.18±4.08 b	0.33±0.57 b	**20**	90
P-value	0.001	0.001		

3.3: Effect of seed treatment with fungal micromycetes *Fusarium solani, Talaromyces pinophilus, Aspergillus niger* and *Trichoderma erinaceus*

The treatment of conidial suspension of four fungal micromycetes on *P. vulgaris* seed were investigated for germination percentage, plant length, leaf surface area and plant dry weight after 1 hr and 6 hr seed infestation (Table 3). *F. solani* immensely effected the growth of *P. vulgaris.* The plant length was significantly reduced in result of 1 h exposure of conidial suspension. There was recorded 46.61 plant length, 47.62 leaf surface area, 47% germination percentage and 4260 mg plant dry weight. After 6 hr infestation of seed with conidial suspension of *F. solani* seed germination was completely retarded

There were observed maximum decrease in germination percentage (27%), plant length (28.13 cm) and plant dry weight (2480 mg) after 6 hrs infestation of *T. pinophilus* conidial suspension in seed in comparison to 1 hr exposure of conidial suspension. There was significant reduction in plant length after 6 hr fungal infestation. *A. niger* also significantly reduced the seed germination, plant length, leaf surface area and plant dry weight after 1 hr and 6 hr infestation of conidial suspension in seed. Although, *T. erinaceus* did not affect the plant growth after 1 hrs infestation of its conidial suspension however, was significantly reduced at 6 hrs exposure of conidial suspension (Table 4).

4: Discussion

Phenolic compounds rapidly diminish in soil solution due to uptake of plant roots, microbial degradation, and adsorption onto soil particles (Berge *et al*., 2009). Among thes benzoic acid one is a key phenolic compound that can influence plant growth by modulating soil physiological processes. Therefore, this study was designed to determine the phytotoxic effects of Benzoic acid on crops and to evaluate its impact soil-dwelling micromycete (Table 4). Benzoic acid has a concentration-dependent physiological impact on *P. vulgaris* affecting root elongation, shoot growth, and the overall growth of the plant. The phytotoxic and allelopathic effect of benzoic acid mostly transpire in the range of 50-400 mM. High (50-250 mM) concentrations of benzoic acid have been reported to be inhibitory to the growth and development of seedlings in quack grass.(Asaduzzaman and Asao, 2012). Hence the quantity of benzoic acid for plant treatment was selected 0.15, 0.25, 0.5 and 1.0 μmol/g soil by following previous studies (Zhou *et al*., 2014, Zhou and Wu, 2012, Zhou *et al*., 2012).

The strongest inhibitory effect on the plant growth in sandy loamy soil was 0.15 $\mu molg^{-1}$ benzoic acid (corresponding to the equivalent of 30mM) which indicated the existence of a dose-dependent phytotoxic effect. Past studies on rhizosphere allelochemicals have demonstrated that phenols like benzoic acid can affect plant growth by disrupting growth activities like root and shoot growth, nutrient acquisition, and physiological activities that result in biomass growth. Indicatively, recent research indicates that phenolic compounds have been shown to prevent seed germination, modify photosynthetic activity and seedling growth in a range of crops by interfering with cellular metabolic and nutrient uptake pathways. These allelopathic effects are concentration and context dependent on soil and a significant cause of lower growth responses in legumes and other crops exposed to benzoic acid and other phenolic acids (Kanjana *et al*., 2024).

Table 4. Effect of Benzoic acid on the growth of *P. vulgaris* and rhizospheric micromycetes

Fungal Isolates	Accession Number	Growth Correlation	Effect on NPK in soil	Effect on Plant
Fusarium solani	MF589618	Increased	Antagonistic effect on N, P, K in soil especially P (Yergeau *et al*., 2006)	Cause Fusarium wilt ((Cha *et al*., 2016)
Talaromyces pinophilus	MF589638	Decrease	Phosphate solubilizing fungi (Sembiring *et al*., 2015)	Positive effect on plant growth ((Sembiring *et al*., 2015)
Aspergillus niger	MF589636	Decreased	Phosphate solubilizing fungi (Whitelaw, 1999)	Root rot, Deterioration of crop by mycotoxins (Amusa *et al*., 2003)
Trichoderma erinaceus	MF589616	No effect	Positive correlation with K (Liu *et al*., 2008)	Enhances plant growth due to chitinase activity (Sunar *et al*., 2014)
Trichoderma virens	MF589640	-	Positive correlation with K (Liu *et al*., 2008)	Antagonistic to soil borne pathogens and enhance plant growth (Dubey *et al*., 2007).
Purpureocillium lilacinum	MF589642	-	No significant effect on nutrient profile in soil (Hernández-Leal *et al*., 2016)	Mutual relationship with plant due to antifungal (Lan *et al*., 2017)
Aspergillus fumigatus	MF565840	-	Phosphate solubilizing fungi (Whitelaw, 1999)	Enhances plant growth ((Kaur *et al*., 2009)
Penicillium javanicum	MF574327	-	Phosphate stabilizing genera (Umechuruba & Nwachukwu, 1994)	Seldom Retardation in plant growth (Yuen & Schroth, 1986)
Lasiodiplodia Theobromae	MF565837	-	Cause deficiency in soil nutrients, Antagonistic to N in soil ((McMahon, 2012)	Reduces plant growth, crop quality and root function ((Umechuruba & Nwachukwu, 1994)

Additionally, released into the soil phenolic compounds may modify the structure and the activity of the soil microbial community, impacting the availability of nutrients in the soil and further debilitating phytotoxic stress on growing plants (e.g., phenolic acids in the rhizosphere can change the microbial communities and soil growth responses). These effects are essential to know since phenolic allelochemicals are concentrated in periods of continuous crop farming and will pose a risk to soil health degradation, decrease in crop yield, and problematic sustainable farming system.(Ahmed *et al*., 2014; Kaur *et al*., 2009). The factors directly affect the microbial level in rhizospheric soil which in result mediates the plant growth. Benzoic acid is the bioactive compound responsible for weathering and assimilation in soil. The higher level of the phenolic compound directly targets the mineral nutrients and biological activities in soil. However, the phytotoxic and allelopathic effect of benzoic acid varies with soil type, due to difference in adsorption, microbial degradation, and nutrient availability (Zhang *et al*., 2010). The decrease in availab potassium was more pronounced in clay loamy soil, whereas the reduction was greater in sandy loamy soil. Potassium and phosphorus are essential macro nutrient necessary for the leguminous plant growth. It is reported that benzoic acid hinders the uptake of P by plants (Ohno, 2001; Asaduzzaman and Asao, 2012).

Our data show that Benzoic acid accumulation in soil alleviates the growth of fungal pathogens (*F. solani*) in soil. These fungal pathogens consume the available mineral nutrients present in soil and create a stress for the growth of healthy crop. The Benzoic acid in soil effect the plant root and shoot biomass, reduce the growth and mineral nutrients deficiency (such as P, K and Mg) in rhizospheric soil (Zhou *et al*., 2012; Baziramakenga *et al*., 1994). Similar effect of *Medicago sativa* L. allelochemical residues was observed on *Vicia faba* L. plant growth in different textures of soil due to deficiency of mineral nutrients in soil (Salama *et al*., 2014). Various micromycetes were isolated from soil to investigate their pathogenicity on *P. vulgaris* growth. The growth of *Fusarium solani* was increased with increase in concentration of Benzoic acid. It is one of the most lethal pathogen, cause root rot and wilt of beans (Marcenaro & Valkonen, 2016). The infestation of *F. solani* in the *P. vulgaris,* reduced the germination percentage and seedling growth after 1 hr and the complete loss of seedling was observed after 6 hr infestation of pathogen. Previous studies showed 30 to 70% yield loss due to soil borne pathogen *F. solani* in beans, pea, wheat, corn and rice (Saremi *et al*., 2011). Several studies demonstrated that phenolic compounds related to benzoic acid can increase the susceptibility of plants to soil borne *Fusarium* sp(Zheng *et al*., 2023).

1. Conclusion

It can be concluded from the experiment that Benzoic acid released from leguminous roots alters the microbial community structure and function in rhizospheric soil. Benzoic acid accumulation in soil enhances the assimilation and weathering process in soil. The process results in decrease in available phosphorous and potassium in soil. These soil mineral nutrients regulate the fungal community in soil. The microbial community consequently regulate the plant growth and crop quality. The effect on crop quality (such as plant disease indices and plant loss percentage) was most prominent in clayey loamy soil as compared to sandy loamy soil. In future, there is a need to optimize the level of phytotoxic allelochemicals in soil and to control the growth of pathogenic microbial community in soil for higher crop yield.

Author Contributions:

Conceptualization, SK; methodology, NS. Software, SK and ANK. Validation, NS and ANK. . Formal analysis, SK; investigation, NS. Resources, ANK. Data curation, NS. Writing—original draft preparation, NS. Writing—review and editing, SK and ANK; visualization, SK.; supervision, ANK. Project administration, ANK.. All authors have read and agreed to the published version of the manuscript

Funding: The author states that no external funding was received for this research.

Conflicts of Interest: The authors declare no conflicts of interest

References

Ahmed, H. A., El-Darier, S. M., & El-Dien, M. H. Z. (2014). Role of soil moisture in allelopathic activity of medicago sativa l. Residues in Zea mays l. Fields. *Journal of Global Biosciences*, **3(**5), 835–847.

Amusa, N. A., Ashaye, O. A., & Oladapo, M. O. (2003). Biodeterioration of the African star apple (Chrysophylum albidum) in storage and the effect on its food value. *African Journal of Biotechnology*, **2**(3), 56–59.

Asaduzzaman, M., & Asao, T. (2012). Autotoxicity in beans and their allelochemicals. *Scientia Horticulturae*, **134,** 26–31.

Baziramakenga, R., Simard, R. R., & Leroux, G. D. (1994). Effects of benzoic and cinnamic acids on growth, mineral composition, and chlorophyll content of soybean. *Journal of Chemical Ecology*, **20**(11), 2821–2833. https://doi.org/10.1007/BF02098391

Berge, O., Lodhi, A., Brandelet, G., Santaella, C., Roncato, M.-A., Christen, R., Heulin, T., & Achouak, W. (2009). Rhizobium alamii sp. Nov., an exopolysaccharide-producing species isolated from legume and non-legume rhizospheres. *International Journal Of Systematic And Evolutionary Microbiology*, *59*(2), 367–372. https: // doi.org/ 10.1099 /ijs.0.000521-0.

Cao, Y., Shen, Z., Zhang, N., Deng, X., Thomashow, L. S., Lidbury, I., Liu, H., Li, R., Shen, Q., & Kowalchuk, G. A. (2024). Phosphorus availability influences disease-suppressive soil microbiome through plant-microbe interactions. *Microbiome*, **12**(1), 185. https://doi.org/10.1186/s40168-024-01906-w

Cha, J.-Y., Han, S., Hong, H.-J., Cho, H., Kim, D., Kwon, Y., Kwon, S.-K., Crüsemann, M., Bok Lee, Y., & Kim, J. F. (2016). Microbial and biochemical basis of a Fusarium wilt-suppressive soil. *The ISME Journal*, **10**(1), 119–129.

Cheng, F., & Cheng, Z. (2015). Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science*, **6,** 1020.

Dubey, S. C., Suresh, M., & Singh, B. (2007). Evaluation of Trichoderma species against Fusarium oxysporum f. Sp. Ciceris for integrated management of chickpea wilt. *Biological Control*, **40**(1), 118–127.

Gallegos, J. A., Beaver, J., Brick, M., Brown, J. K., Cichy, K., Debouck, M. D., CIAT, C. A. D.-S., de Jensen, C. E., Karasev, A. V., & Kelly, J. D. (n.d.). *Phaseolus Beans–Crop Vulnerability Statement (September 2020)*. Retrieved December **24,** 2025, from https://www.ars-grin.gov/documents/cgc/cvs/2020-Phaseolus%20Crop%20Vulnerability%20Statement.pdf

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology*, **2**(2), 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Goyal, R. K., Habtewold, J. Z., Goyal, R. K., & Habtewold, J. Z. (2023). Evaluation of Legume–Rhizobial Symbiotic Interactions Beyond Nitrogen Fixation That Help the Host Survival and Diversification in Hostile Environments. *Microorganisms*, **11**(6). https://doi.org/10.3390/microorganisms11061454

Huang, H. C., Chou, C. H., & Erickson, R. S. (2006). *Soil sickness and its control.* https://www.cabidigitallibrary.org/doi/full/10.5555/20063152307

Kanjana, N., Li, Y., Shen, Z., Mao, J., & Zhang, L. (2024). Effect of phenolics on soil microbe distribution, plant growth, and gall formation. *Science of The Total Environment*, **924**, 171329. https://doi.org/10.1016/j.scitotenv.2024.171329

Kaur, R., Kaur, S., & Callaway, R. M. (2009). Impact of (±)-catechin on soil microbial communities. *Communicative & Integrative Biology*, **2**(2), 127–129. https://doi.org/10.4161/cib.7744

Kinnunen, A., Maijala, P., Jarvinen, P., & Hatakka, A. (2017). Improved Efficiency in Screening for Lignin-Modifying Peroxidases and Laccases of Basidiomycetes. *Current Biotechnology*, **6**(2), 105–115. https://doi.org/10.2174/2211550105666160330205138

Laishram, B., Devi, O. R., Dutta, R., Senthilkumar, T., Goyal, G., Paliwal, D. K., Panotra, N., & Rasool, A. (2025). Plant-microbe interactions: PGPM as microbial inoculants/biofertilizers for sustaining crop productivity and soil fertility. *Current Research in Microbial Sciences*, *8*, 100333. https://doi.org/10.1016/j.crmicr.2024.100333

Lan, X., Zhang, J., Zong, Z., Ma, Q., & Wang, Y. (2017). Evaluation of the Biocontrol Potential of *Purpureocillium lilacinum* QLP12 against *Verticillium dahliae* in Eggplant. *BioMed Research International*, *2017*, 1–8. https://doi.org/10.1155/2017/4101357

Liu, B., Glenn, D., & Buckley, K. (2008). Trichoderma communities in soils from organic, sustainable, and conventional farms, and their relation with Southern blight of tomato. *Soil Biology and Biochemistry*, **40**(5), 1124–1136.

Marcenaro, D., & Valkonen, J. P. (2016). Seedborne pathogenic fungi in common bean (Phaseolus vulgaris cv. INTA Rojo) in Nicaragua. *PLoS One*, **11**(12), e0168662.

McMahon, P. (2012). Effect of nutrition and soil function on pathogens of tropical tree crops. *Plant Pathology*, 243–272.

Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews*, *37*(5), 634–663. https://doi.org/10.1111/1574-6976.12028

Miller, D. A. (1996). Allelopathy in Forage Crop Systems. *Agronomy Journal*, **88**(6), 854–859. https://doi.org/10.2134/agronj1996.00021962003600060003x

Minuto, A., Spadaro, D., Garibaldi, A., & Gullino, M. L. (2006). Control of soilborne pathogens of tomato using a commercial formulation of Streptomyces griseoviridis and solarization. *Crop Protection*, **25**(5), 468–475.

Mondal, M. F., Asaduzzaman, M. D., & Asao, T. (2015). Adverse effects of allelopathy from legume crops and its possible avoidance. *American Journal of Plant Sciences*, **6**(6), 804–810.

Murali, M., Amruthesh, K. N., Sudisha, J., Niranjana, S. R., & Shetty, H. S. (2012). *Screening for plant growth promoting fungi and their ability for growth promotion and* *induction of resistance in pearl millet against downy mildew disease.* https://www.cabidigitallibrary.org/doi/full/10.5555/20133364588

Ohno, T. (2001). Oxidation of Phenolic Acid Derivatives by Soil and Its Relevance to Allelopathic Activity. *Journal of Environmental Quality*, **30**(5), 1631–1635. https://doi.org/10.2134/jeq2001.3051631x

Osogo, A. K., Muyekho, F., Were, H., & Okoth, P. (2025). Deciphering common bean (*Phaseolus Vulgaris L.*) microbiome assemblages reveal mechanistic insights into host-pathogen-microbiome interactions. *Genomics*, **117**(4), 111064. https://doi.org/10.1016/j.ygeno.2025.111064

Peters, J. (2000). *Tetrazolium testing handbook*. Contribution.

Pouralibaba, H. R., Rubiales, D., & Fondevilla, S. (2015). Identification of resistance to Fusarium oxysporum f.sp. Lentis in Spanish lentil germplasm. *European Journal of Plant Pathology*, **143**(2), 399–405. https://doi.org/10.1007/s10658-015-0692-x

Prasad, R. M., Sagar, B. V., Devi, G. U., Triveni, S., Rao, S. K., & Chari, D. K. (2017). Isolation and screening of bacterial and fungal isolates for plant growth promoting properties from tomato (Lycopersicon esculentum Mill.). *Int. J. Curr. Microbiol. App. Sci*, **6**(8), 753–761.

Rice, E. L. (2012). *Allelopathy*. https://books.google.com/books?hl=en&lr=&id=uqcH7AOXMCUC&oi=fnd&pg=PP1&dq=RICE,+E.+L.+2012.+Allelopathy,+Academic+press.&ots=BYbrFhqEYC&sig=kQQmGC5K5qiQ8g-idXvcpppOQYI

Saremi, H., Okhovvat, S. M., & Ashrafi, S. J. (2011). Fusarium diseases as the main soil borne fungal pathogen on plants and their control management with soil solarization in Iran. *African Journal of Biotechnology*, **10**(80), 18391–18398.

Scavo, A., Abbate, C., & Mauromicale, G. (2019). Plant allelochemicals: Agronomic, nutritional and ecological relevance in the soil system. *Plant and Soil*, **442**(1), 23–48. https://doi.org/10.1007/s11104-019-04190-y

Sembiring, M., Elfiati, D., Sutarta, E. S., & Sabrina, T. (2015). *Effect of Talaromyces pinophilus and SP36 on phosphate available and Potato (Solanum tuberosum L) production on andisol impacted by Mount Sinabung Eruption, North Sumatera, Indonesia*.

Sunar, K., Chakraborty, U., & Chakraborty, B. N. (2014). Exploitation of native microflora of Darjeeling hills for sclerotial rot management and growth promotion in pulses. *Indian Phytopathol*, **67**, 59–69.

Tharanath, A. C., Upendra, R. S., Rajendra, K., Tharanath, A. C., Upendra, R. S., & Rajendra, K. (2024). Soil Symphony: A Comprehensive Overview of Plant–Microbe Interactions in Agricultural Systems. *Applied Microbiology*,

4(4), 1549–1567. https://doi.org/10.3390/applmicrobiol4040106

Umechuruba, C. I., & Nwachukwu, E. O. (1994). Efficacy of certain fungicides against seed-borne fungi of African yam bean *(Sphenostylis stenocarpa* (Hochst ex A. Rich) Harms) seeds. *International Journal of Pest Management*, **40**(2), 126–131. https://doi.org/10.1080/09670879409371869

Whitelaw, M. A. (1999). Growth promotion of plants inoculated with phosphate-solubilizing fungi. *Advances in Agronomy*, **69**, 99–151.

Ye, S. F., Yu, J. Q., Peng, Y. H., Zheng, J. H., & Zou, L. Y. (2004). Incidence of Fusarium wilt in Cucumis sativus L. is promoted by cinnamic acid, an autotoxin in root exudates. *Plant and Soil*, **263**(1), 143–150. https://doi.org/10.1023/B:PLSO.0000047721.78555.dc

Yergeau, E., Sommerville, D. W., Maheux, E., Vujanovic, V., Hamel, C., Whalen, J. K., & St-Arnaud, M. (2006). Relationships between Fusarium population structure, soil nutrient status and disease incidence in field-grown asparagus. *FEMS Microbiology Ecology*, **58**(3), 394–403.

Yu, J. Q., Shou, S. Y., Qian, Y. R., Zhu, Z. J., & Hu, W. H. (2000). Autotoxic potential of cucurbit crops. *Plant and Soil*, **223**(1–2), 149–153.

Yuen, G. Y., & Schroth, M. N. (1986). Interactions of Pseudomonas fluorescens strain E 6 with ornamental plants and its effect on the composition of root-colonizing microflora. *Phytopathology*, **76**(2), 176–180.

Zhang, E.-P., Zhang, S.-H., Zhang, W. B., Li, L. L., & Li, T. L. (2010). Effects of exogenic benzoic acid and cinnamic acid on the root oxidative damage of tomato seedlings. *J. Hortic. For*, **2**(2), 22–29.

Zheng, Y., Guo, Y., Lv, J., Dong, K., & Dong, Y. (2023). Faba Bean-Wheat Intercropping Can Control the Occurrence of Faba Bean Fusarium Wilt by Alleviating the Inhibitory Effect of Benzoic Acid on Disease Resistance Metabolism and the Expression of Resistance Genes. *ACS Omega*, **8**(3), 2897–2906.

Zhou, X., Gao, D., Liu, J., Qiao, P., Zhou, X., Lu, H., Wu, X., Liu, D., Jin, X., & Wu, F. (2014). Changes in rhizosphere soil microbial communities in a continuously monocropped cucumber (Cucumis sativus L.) system. *European Journal of Soil Biology*, **60**, 1–8.

Zhou, X., & Wu, F. (2012). Dynamics of the diversity of fungal and Fusarium communities during continuous cropping of cucumber in the greenhouse. *FEMS Microbiology Ecology*, **80**(2), 469–478.

Zhou, X., Yu, G., & Wu, F. (2012). Responses of Soil Microbial Communities in the Rhizosphere of Cucumber (Cucumis sativus L.) to Exogenously Applied p-Hydroxybenzoic Acid. *Journal of Chemical Ecology*, **38**(8), 975–983.