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Evaluating *Rhizobium* and *Bradyrhizobium* species as potential biocontrol agents for root rot fungi in soybean seedlings

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Abstract

Soybean (*Glycine max*) is among the legumes which are highly prone to soil-borne pathogens which causes root-rot diseases limiting the growth and development resulting to low yield of plants. This study was conducted to test the ability of three rhizobia strains, *Rhizobium sp*. TZSR12C, *Rhizobium sp*. TZSR25B and *Bradyrhizobium sp*. TZSR41A, in comparison with the commercial biocontrol (*Trichoderma harzianum* in suppressing the growth of root rot fungal pathogens (*Fusarium solani*, *Rhizoctonia solani*, *Fusarium oxyporum*, and *Macrophominaphaseolina*) under *in vitro* and greenhouse conditions. The rhizobium cell filtrates were used in testing their activities against fungal pathogens under *in vitro* while the solid biofertilizer formulations containing the respective rhizobia inoculants were used to inoculate the soybean seeds sown in pathogen contaminated soil under greenhouse conditions. Results showed that all rhizobia isolates and *T. harzianum* were capable of suppressing the fungal pathogens both under *in vitro* and greenhouse conditions with the highest inhibition zone (8.3 mm) and colony diameter (25.0 mm) being in *Rhizobium sp*. TZSR25B against *F. oxyporum* under *in vitro* conditions. Under greenhouse experiment, *Rhizobium sp*. TZSR12C had the highest performance in inhibiting the infection of plant up to 27.78% with severity of 5.56% in roots and 0.00% infection in foliage against the combination of *F. solani*, *R. solani*, *F. oxyporum*, and *M. phaseolina*. We found that, on their performance, the tested rhizobia strains can potentially be utilized as biocontrol agents against the fungal pathogens in the rhizosphere of soybean plants.

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Introduction

Rhizobia are widely known for their ability fix nitrogen in symbiotic association with plants especially legumes. Nitrogen fixation occurs symbiotically through the formation of root nodules in leguminous plants which is induced by the production of nod factors[1]. In symbiotic nodules, the fixed nitrogen is converted to ammonia for uptake by the host plants[2]. This is a natural process which reduces the need and use synthetic fertilizers resulting to reduced cost of production[3].

Apart from their well-known ability of fixing nitrogen, rhizobia have a good ability of suppressing soil-borne pathogens which affects plants[4,5]. Some rhizobia species such as *R. japonicum, S. meliloti*, *B. japonicum*, and *R. leguminosarum* have been observed to protect the plants from infection by below root-rot disease causing fungal pathogens such as *Phytophthora clandestine*, *Pythium ultimum*, *Fusarium solani*, *F. oxysporum*, *Pythium sp.*, *Rhizoctonia bataticola*, and *Macrophomina phaseolina*[1,6,7]. As other legumes, soybean plants are highly prone to soil-borne pathogens which causes root-rot diseases which limits the growth and development resulting to low yield of plants[8,9]. *Macrophomina phaseolina*, *F. solani*, *F. oxyporum* and *R. solani* are among the most significant soybean root pathogens[6,10]. Some of the soybean nodulating rhizobial species, *Bradyrhizobium sp*, *S. meliloti,* and *Rhizobium sp*, have been witnessed to suppress *F. solani*, *M. phaseolina*, *F. oxysporum*, and *R. solani* in soybean rhizosphere[10].

Several mechanisms such as production of hydrocyanic acid, antibiotics, and mycolytic enzymes have been observed to limit the growth of soil pathogens in the rhizosphere of leguminous plants including soybean[11]. The particular species of rhizobia have been observed to be effective in suppression *F. solani*, *M. phaseolina*, *F. oxysporum*, and *R. solani* in the rhizosphere of soybean plants[1,11]. Furthermore, *Trichoderma harzianum* has been witnessed to be effective in the biocontrol of root rot fungal pathogens[12] and hence used as an control organism in different *in vitro* and greenhouse experiments. However, rhizobia are affected by some agro-ecological factors including management practices[13,14] therefore, multi-location on farm trials are needs to be conducted to test the biocontrol effectiveness of particular rhizobia strains.

Root rot diseases are among the major factors which contributes in the low yield of soybean, although the impact is neglected as they are difficult to evaluate. The pathogens causing the diseases in the roots are the inhabitants of soil. For this case, the plant, may be infected by more than one disease causing pathogen, hence causing a huge plant and yield loss. Various studies have demonstrated the effectiveness of rhizobia as biocontrol agents in suppressing root rot fungi; nonetheless, their usage receives limited attention, particularly when dealing with complicated effect of root rot diseases caused by more than one fungal pathogen. Therefore, exploration of the new rhizobia species capable of suppressing the root rot fungi is essential in reducing the loss of yield due to their infection in plants. Furthermore, the soil can be colonized by more than one pathogenic fungi. It is also worth testing the ability of rhizobia in suppressing the combined effect of fungal pathogen. This study aimed at testing the ability of *Bradyrhizo-*

bium sp. TZSR41A and *Rhizobium sp*. TZSR12C and TZSR25B from the soils of Tanzania in supressing the growth of *F. solani*, *M. phaseolina*, *F. oxysporum*, and *R. solani*. The findings of this study will contribute to enriching the knowledge which the researchers could tap into, for the benefit of further studies the use of rhizobia inoculant in suppressing the growth of root rot fungi and their effectiveness in controlling the complex infection caused by more than one pathogen.

Materials and methods

Testing the ability of soybean rhizobial strains in suppressing root fungi under *in vitro* conditions

Collection of infected plant samples

The fungal pathogens, *Rhizoctonia solani*, *Fusarium oxyporum* and *Fusarium solani* were isolated from the roots of infected soybean plants during the growing season[15]. *Macrophomina phaseolina* was isolated from the infected soybean seeds. The identification and sampling of infected soybean plants was done in the farmers' fields at Mbeya city council. The plants were sampled basing on the visible symptoms on the shoots of plants. Some specific symptoms such as blighted leaves by *Rhizoctonia solani*, stunted growth, yellowing and death of older plants by *Fusarium solani* and *Fusarium oxyporum* and premature death with leaves attached associated with the gray or silver on lower stem and tap root for *Microphomina phaseolina* were observed during sampling. The sampling was done at seedling (Fig. 1a), flowering (Fig. 1b) and maturity (Fig. 1c) stage before the drying of plants. *Microphomina phaseolina* symptoms are mostly visible at maturity and it is spread to seeds.

Isolation of fungi from infected roots and seeds

After sampling, the plants were brought to the Laboratory of Seed Health Center at Sokoine University of Agriculture for isolation of fungal pathogens. The isolation of fungi from roots was done as per protocol explained by[10,11]. First, the roots of plant samples were cut at the crown and then washed with tap water to remove the soil debris and seeds from the soybean plants infected with *M. phaseolina* were prepared. The roots and seeds were dipped in 1% sodium hypochlorite (NaOCl) for 2 minutes. Then the samples were dipped in sterile distilled water for 5 minutes to allow the removal of disinfectant. After 5 minutes, the samples were placed on sterile filter papers to allow drying.

After drying, the roots were cut to small pieces of about 2–3 mm while concentrating on the infected portions with the disease symptoms such as rusty-brown and dry sunken lesions for *R. solani* and dark brown discoloration and rot of tap roots for *F. solani* and *F. oxyporum*. Afterwards, the roots and seeds were placed by punching in a sterile PDA in plates. The plates were kept in the incubator at 28 °C for 3–5 days. The growth of fungi was observed daily. The purification of cultures was done by cutting and picking a small piece from the mycelial edge for each fungi and then inoculated on a fresh PDA. Further purification was done by picking a single spore isolation under microscope (Olympus CX23 at x20 magnification) placed in aseptic condition under biosafety cabinet system (BSC-IIA2-2F). The observation of the features of *R. solani*, *F. oxyporum*, *F. solania* and *M. phaseolina* was recorded under microscope. The plates were then incubated at 28 °C for 7 days to allow the growth of fungal pure colonies for test of antifungal activity of rhizobia under controlled conditions.

Preparation of Rhizobia cell-free filtrates

The fresh cultures of rhizobia isolates were prepared by recovering them through spot inoculation on a YEMA plates and then incubated at 28 °C for 72 hours to allow the growth of colonies. Then the cultures were transferred in 4 ml of sterile YEMB in 15 ml falcon tubes, followed by incubation at 28 °C in orbital incubator at the speed of 180 rpm for 72 h to allow the multiplication of cells. After incubation, 1 ml of the broth was transferred in 1.5 ml Eppendorf tubes, followed by two times centrifugation at 3,000 rpm for 20 min to obtain the culture filtrates. The supernatants with rhizobial cells were transferred in new Eppendorf tubes for use in testing *in vitro* antifungal activity[1] and the pellets were discarded.

Preparation of Czapek's Dox Agar and thick filter paper discs

Czapek's Dox Agar medium containing 30.0 g of sucrose ($C_{12}H_{22}O_{11}$), 2.0 g of sodium nitrate ($NaNO_3$), 1.0 g of dipotassium phosphate (K_2HPO_4), 0.50 g of Magnesium sulphate ($MgSO_4$), 0.5 g of potassium chloride (KCl), 0.010 g of ferrous sulphate ($FeSO_4$) and 15.0 g of Agar was dissolved in 1000 ml of distilled water. The final pH of the medium was adjusted to 7.2 at room temperature. The 5 mm discs of the thick filter paper

Fig. 1 The soybean rot symptoms observed on (a) seedling, (b) flowering and (c) matured plants

were prepared by punching with a paper punching machine and then placed in a beaker covered with aluminium foil. Both the medium and the discs were sterilized by autoclaving at 15 pound-force per square inch (lbs) and 121 ℃ for 15 min.

In vitro antifungal activity of cell-free cultures of rhizobial filtrates

The inhibition of root-infecting fungi was tested by growing rhizobial strains and 5 mm discs of fungi on Czapek's Dox Agar. The previously sterilized filter paper discs were placed by using a neo syringe, one at the center of the medium and three on the sides at equal dimensions in petri dish plates. The fungal mycelial were inoculated on the disc and were loaded with 10 µl of the rhizobial cell filtrates[16]. Each test was in three replicates and the Petri dishes were incubated at 28 ℃ for 7 days. The distance between the fungal colony and the disc will be considered as an inhibition zone which was measured in mm to obtain the average for three replicates[1,17].

Testing the activity of rhizobia against fungal pathogens under greenhouse conditions

Preparation of fungal inoculum for inoculation in experimental soil

The fungal inocula were prepared on some sterilized wheat groats. About 2 kg of wheat groats were divided in 4 portions of 0.5 kg and were soaked in water for 60 minutes. After soaking, the portions were placed in the separate clean sulphate bags and then sterilized in an autoclave at 15 pound-force per square inch (lbs) and 121 ℃ for 15 minutes and then allowed to cool at room temperature under aseptic condition. Each portion of the sterile wheat groats was then inoculated with a respective fresh and actively growing pure cultures of fungi, *F. oxyporum*, *F. solani*, *R. solani* and *M. phomina* and then incubated at the room temperature 25–29 ℃ for 14 days to allow the growth of cultures on wheat groats before inoculating the experimental soils.

Rhizobium antagonism against fungal pathogens under greenhouse

For experimentation under greenhouse, three soil samples were collected at the depth of 0–30 cm uncultivated area at the vicinity of NM-AIST banana farm and CREATES green houses. A composite sample was prepared through mixing and removing the roots and crumps. The sample was air dried, ground then sieved with 2 mm mesh. After preparation, fertility status of the sample was evaluated for its for its suitability in supporting rhizobia activities and growth of soybean plants for optimum yield. According to[18] the wet digestion (oxidation) method of Walkley-Black was used to characterize soil organic carbon. As per description by Thomas, the pH of the soil was measured electrochemically using a 1:2.5 (w/v) water suspension in a potentiometric manner. According to[19], total nitrogen was determined using the micro-Kjeldahl digestion-distillation method. As the soil sample's pH was less than seven, the extraction of P was determined using the Bray 1 technique[20]. A 1 M solution of ammonium acetate (NH4OAc) was used to determine the cation exchange capacity at pH 7. By using a flame photometer, the exchangeable cations potassium (K^+) and sodium (Na^+) were identified. Atomic absorption spectrophotometer measurements were made for the exchangeable bases calcium (Ca^{2+}) and magnesium (Mg^{2+}), as well as the micronutrients iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn)[19,21]. Exchangeable Na by CEC (× 100) was divided to calculate the exchangeable sodium percentage (ESP)[22]. The rankings of physico-chemical parameters were according to[19,22].

Prior to sowing of rhizobia inoculated seeds, the soil for pot experiment was sterilized by oven drying and at 70 ºC for 48 hours. After cooling to room temperature under aseptic conditions, the soils were filled in 2 L plastic pots which were previously sterilized with 70% ethanol for 1 minute and rinsed with 0.25% NaClO solution[23]. The sterile soils in pots were watered and then inoculated/contaminated with 4 g of wheat groats containing actively growing fungal cultures and left to acclimatize for 14 days before sowing the soybean seeds. During the 14 days of acclimatization, the contaminated soils were sprayed with sterile distilled water to provide the optimum moisture for the growth of fungi. Before sowing, the seeds were sterilized by soaking in 70% ethanol for 1 min and 0.25% NaClO solution for 3 minutes followed by washing with distilled water five times to remove the disinfectants[24] before sowing. The solid Biofertilizers formulations containing the two *Rhizobium sp.*, TZSR12C, TZSR25B and one *Bradyrhizobium sp.* TZSR41A were used. The seeds were inoculated with each respective rhizobia inoculant through damp inoculation by adding about 0.5 ml of sugar solution as a sticking agent, followed by addition of inoculum and then thorough mixing again to ensure all seeds are coated. After inoculation, seeds were left to air dry under the shade for about 30–60 min. Then the seeds were sown at the depth of 2.5 cm in the respective plots as designed.

The experiment was set in RCBD layout and the plan for layout was generated in Genstat software 15th Edition. The treatments included the fungal cultures alone (FC), rhizobia as biocontrol (BC) + fungi, rhizobia (BC) alone, no contamination and biocontrol (NOB) and, control fungi alone, control fungi + fungal cultures, rhizobia (BC) and combination of fungal cultures and control fungi and combination of fungal pathogens alone as well as effective rhizobia with the combinations of biocontrol with effective rhizobia and fungal cultures (Table 1). For each treatment, 10 seeds were sown. To estimate the efficiency of rhizobia biocontrols, *T. harzianum* (commercial biocontrol) was used as a positive control.

The data for germination was collected 7 days after emergence and for disease incidence (DI) and disease severity (DS) (Fig. 2) on seedlings the data was collected at 14 days after emergence. The disease incidence was determined by uprooting 6 seedlings and then counting the seedlings with symptoms of diseases out of 6. The disease severity was determined by using a six points scoring scale of 0–5 (where 0 = no symptoms and 5 = plant wilted or dead) as previously described by[25].

Statistical data analysis

GenStat 15th Edition was used to statistically evaluate the germination data, and Excel 2016 running on Windows 10 was used to create the graphs. Using Jamovi version 2.3.2.0, the mean and standard errors within the treatments for fungal diseases and biocontrols were computed. The mean separation between the treatments was calculated using a one-way analysis of variance (ANOVA) that was applied to the factor effect model as displayed in Equation 1. The 15th Edition of GenStat was used to apply the Tukey's-HSD multiple comparison test at a threshold of 5% to distinguish between mean values among replications of fungal pathogens and biocontrols. As a result, sample replications were treated as a random

Table 1. *In vitro* growth inhibition of root-rot fungal pathogens by cell free culture filtrates of rhizobial strains in agar disc-diffusion assay.

Treatment	Diameters of inhibition zones (mm)				Colony diameters (mm)			
	FS	MP	RS	FO	FS	MP	RS	FO
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
*Rhizobium sp.*TZSR12C	5.0	1.3	2.3	***	8.7	6.3	23.7	22.7
*Rhizobium sp.*TZSR25B	8.3	1.7	***	***	5.3	4.7	24.0	25.0
*Bradyrhizobium sp.*TZSR41A	5.7	2.3	***	***	7.7	4.0	23.0	23.7

*** Growth inhibited but no zone of inhibition was formed. FS = *F. solani*, Mp = *M. phaseolina*, RS = *R. solani* and FO = *F. oxyporum*.

Fig. 2 Various disease symptoms observed and used in scoring disease incidence and symptoms.

effect, and only one factor the treatment with various biocontrols and fungal pathogens was considered the fixed main effect.

$$Y_i = \mu + \alpha_i + \varepsilon_i \quad (1)$$

Where *Yi* is the observed response variable in the i^{th} factor; μ is the overall (grand) mean; α_i is the main effect of the treatment; ε_i is the random error associated with the observation of response variable in the i^{th} factor.

Results

Inhibition of root rot fungal pathogens under *in vitro* conditions by rhizobia strains

Out of 25 rhizobia strains, only three of them, *Rhizobium sp.* TZSR12C, TZSR25B and *Bradyrhizobium sp.* TZSR41A, were capable of suppressing the growth of four fungal pathogens which are *F. solani*, *M. phaseolin*, *R. solni* and *F. oxyporum* under *in vitro* conditions (Fig. 3; Table 1). Interestingly, each of the three strains of rhizobia were able to suppress the growth of all four fungal pathogens, though, in different capacities. Also, modes of suppression included the formation or no formation of inhibition zone, depending on the fungal isolate. The largest diameter (8.3 mm) of inhibition zone was formed by *Rhizobium sp.* TZSR25B against *F. oxyporum* which is followed by (5.7 mm) in *Bradyrhizobium sp.* TZSR41A whereby the smallest (1.3 mm) was in *Rhizobium sp.* TZSR12C against *M. phaseolina*. *Rhizobium sp.* TZSR12C was capable of forming clear zones in three fungi, *F. solani*, *M. phaseolina* and *R. solani* while *Rhizobium sp.* TZSR25B and *Bardyrhizobium sp.* TZSR41A were able to form the inhibition zones on only two fungi which are *F. solani* and *M. phaseolina*. On the other hand, the largest colony of 25.0 mm against *F. oxyporum*, and closely followed by 25 mm against *R. solani* while the smallest colony of 4.7 mm against *M. phaseolina* were formed by *Rhizobum sp.* TZSR25B. Interestingly, the largest colonies are found in fungal growth inhibition with smallest of without the formation of inhibition zones.

The physico-chemical properties of the soil used for potting experiment

The soil used in the pot experiment had its physical and chemical characteristics evaluated, and the results are shown in Table 2. The soil's texture was sandy clay loam, and its pH ranged from neutral (pH = 6.93 in water). The proportion of organic carbon (OC) was 3.42 (high), and the total nitrogen content was 0.39 (medium). The soil had very high levels of copper (2.34 $mg{\cdot}kg^{-1}$), zinc (8.17 $mg{\cdot}kg^{-1}$), manganese (11.77 $mg{\cdot}kg^{-1}$), and iron (9.98 $mg{\cdot}kg^{-1}$) as well as very high levels of extractable phosphorus (75.64 $mg{\cdot}kg^{-1}$). The soil showed very high concentrations of the exchangeable bases calcium (13.95 $cmol_{(+)}\ kg^{-1}$) and magnesium (2.99 $cmol_{(+)}\ kg^{-1}$), medium concentrations of potassium (0.43 $cmol_{(+)}\ kg^{-1}$) and very low concentrations of sodium (0.07 $cmol_{(+)}\ kg^{-1}$). Furthermore, cation exchange capacity-CEC of an experimental soil was 18.22 $cmol_{(+)}\ kg^{-1}$ which is rated as medium.

The influence of rhizobia isolates and *Trichoderma harzianum* on germination of soybean seeds in the soils contaminated with fungal pathogens under greenhouse conditions

The ability and efficiency of rhizobia isolates and *T. harzianum* in enhancing the germination of seeds in the soils contaminated with fungal pathogens was tested under greenhouse conditions. The test was biocontrols (rhizobia or *T. harzianum*) without fungal pathogens (Fig. 4c), with one (Fig. 4d), two (Fig. 4a), three (Fig. 4e) and four (Fig. 4b) pathogens. Rhizobia isolates and *T. harzianum* were observed to possess different capacities in enhancing the germination of soybean seeds, in the soils without and those with contamination of different fungal pathogens under greenhouse conditions.

In the soils without fungal pathogens, *Rhizobium sp.* TZSR25B yielded the highest germination of 96.67% which was closely followed by 93.33% in *Rhizobium sp.* TZSR12C and soils without biocontrol and then 90% in *Bradyrhizobium sp*TZSR41A, whilst the lowest germination of 70% was observed in *T. harzianum*. In the test of rhizobia against one fungal pathogen, the highest (86.67%) germination was observed in *F. solani*, which is closely followed by 83.33% in *F. oxyporum* both by *Rhizobium sp.*TZSR12C, and then 83.33% by *Rhizobium sp.*TZSR25B. In this group, the lowest germination 33.33% was observed in *M. phaseolina*, followed by 53.33% germination in *F. oxyporum*, *F.*

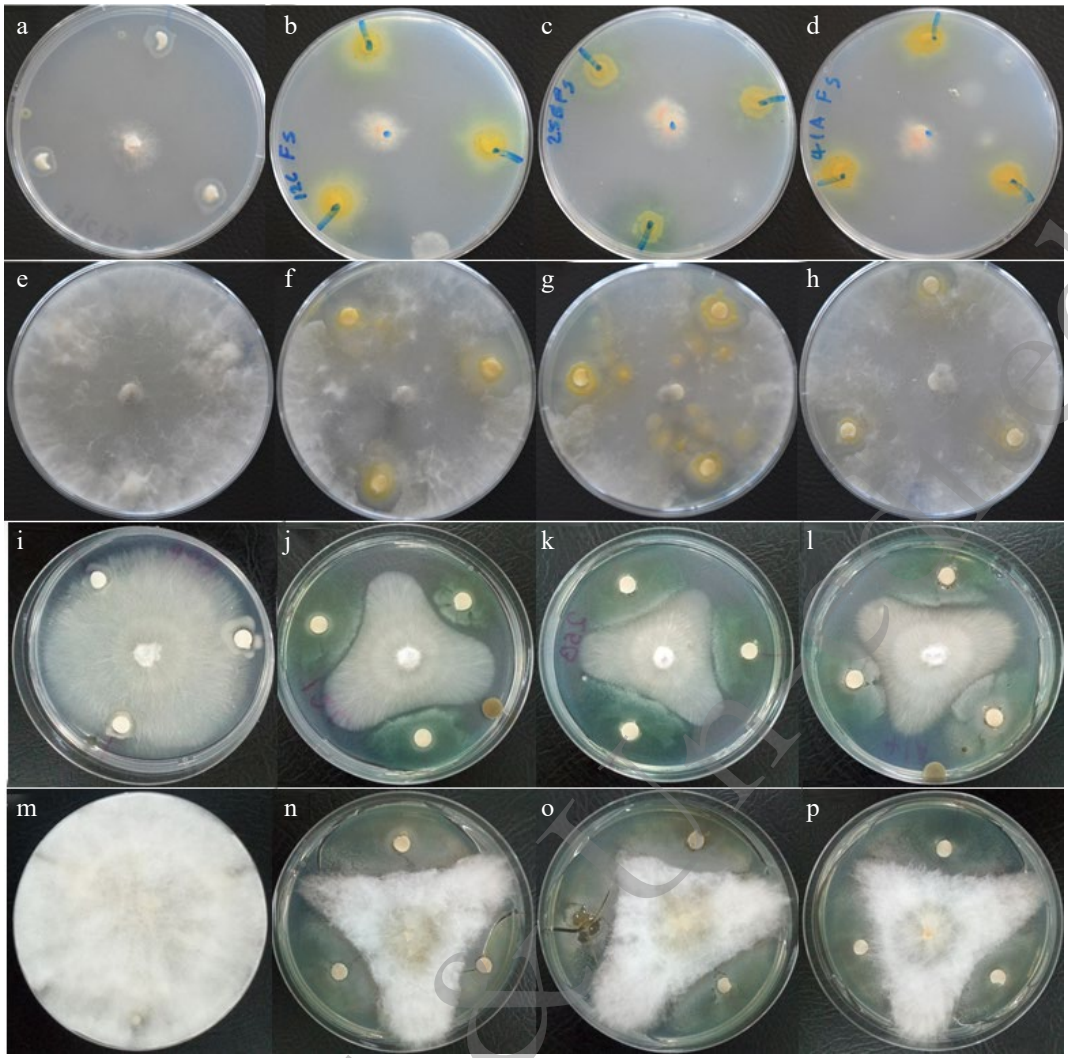

Fig. 3 Fungal growth inhibition by cell free culture filtrates of rhizobial strains in agar disc-diffusion assay. No inhibition of (a) *F. solani*, (e) *M. phaseolina*, (i) *R. solani* and (m) *F. phaseolina* by *B. japonicum* TZSR39C, inhibition of (b) *F. solani*, (f) *M. phaseolina*, (j) *R. solani* and (n) *F. oxyporum* by *Rhizobium sp.* TZSR12C, inhibition of (c) *F. solani*, (g) *M. phaseolina*, (k) *R. solani* and (o) *F. oxyporum* by *Rhizobium sp.* TZSR25B and, inhibition of (d) *F. solani*, (h) *M. phaseolina*, (l) *R. solani* and (p) *F. oxyporum* by *Bradyrhizobium sp.* TZSR41A.

Table 2. Physicochemical parameters of an experimental soil.

Parameter	Mean	Ratings	SD
Soil pH (H_2O)	6.60	N	0.01
Electrical Conductivity (dS/m)	0.22	NYR	0.04
Organic Carbon (%)	3.20	VH	0.07
Total Nitrogen (%)	0.36	M	0.1
Extractable Phosphorus (mg kg^{-1})	83.95	VH	0.63
Copper (mg kg^{-1})	2.28	H	0.05
Zinc (mg kg^{-1})	8.09	VH	0.01
Manganese (mg kg^{-1})	10.96	VH	0.36
Iron (mg kg^{-1})	10.53	VH	0.09
Exchangeable Calcium ($cmol_{(+)}$ kg^{-1})	14.05	VH	0.01
Exchangeable Magnesium ($cmol_{(+)}$ kg^{-1})	3.12	VH	0.09
Exchangeable Sodium ($cmol_{(+)}$ kg^{-1})	0.09	VL	0.01
Exchangeable Potassium ($cmol_{(+)}$ kg^{-1})	0.40	M	0.03
Cation Exchange Capacity ($cmol_{(+)}$ kg^{-1})	19.12	M	0.56
Exchangeable Sodium Percentage (%)	0.56	M	0.05
Texture	Sandy Clay Loam		

Key: VH = very high, H = high, M = medium, VL = very low, N = neutral and NYR = no yield reduction. S.D. = Standard Deviation (source[26])

solani and *R. solani*, all without biocontrol. In the combination of two fungal pathogens, *Rhizobium sp.* TZSR12C exhibited the highest germination of 83.33% in combination of *R. solani* with *F. oxyporum*, this was followed by 76.67% and two combinations of *F. solani* with *F. oxyporum* and, *F. oxyporum* with *M. phaseolina* in *Rhizobium sp.* TZSR25B. For the combination of two fungi, the lowest germination of 26.67% was observed in *F. oxyporum* and *M. phaseolina*, followed by 33.33% in both *F. solani* with *R. solani* and, *R. solani* with *M. phaseolina*, all without any biocontrol.

The highest germination (93.33%) was observed in *Rhizobium sp.*TZSR12C against the combination of three fungal pathogens, *F. solani*, *R. solani* and *M. phaseolina* which was distantly followed by 80% in *Rhizobium sp.*TZSR25B and 76.67% in *Rhizobium sp.*TZSR12C against *R. solani*, *F. oxyporum* and *M. phaseolina*. On the other hand, the three lowest germination were 30% in *F. solani*, *F. oxyporum* and *M. phaseolina*, 40% in *F. solani*, *R. solani* and *F. oxyporum* and 43.33% in *R. solani*, *F. oxyporum* and *M. phaseolina*, all without biocontrol. In the combination of four fungal pathogens, the highest germina-

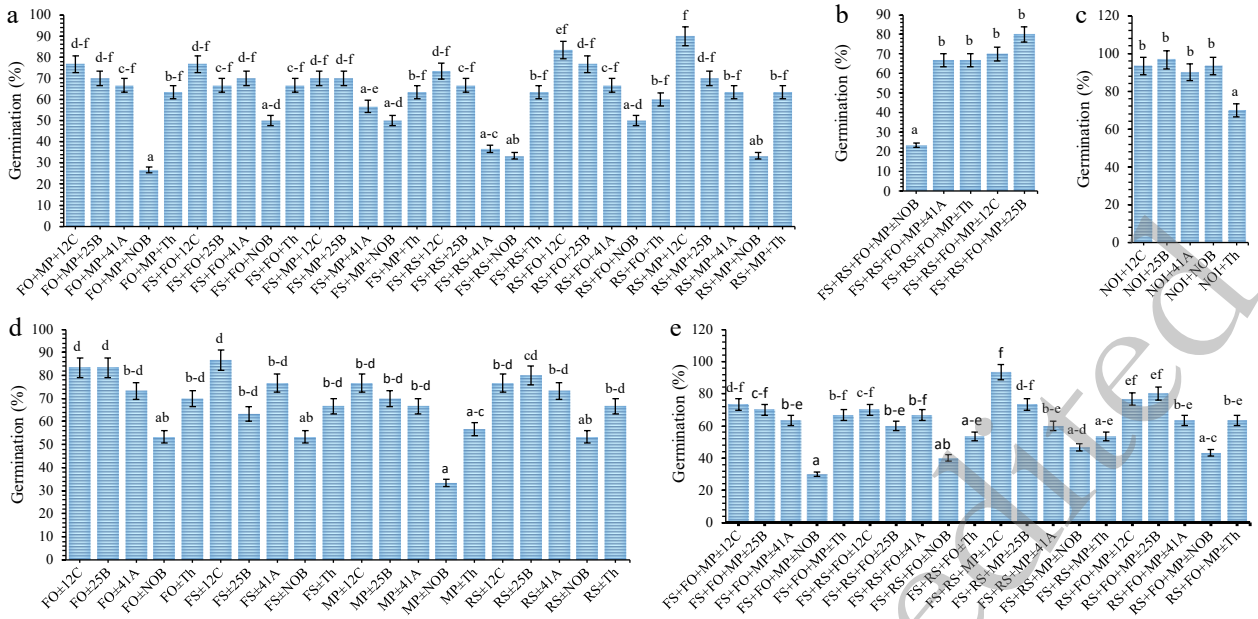

Fig. 4 The effect of rhozibia species and *Trichoderma harzianum* on germination of soybean seeds in the soils having root rot fungi contaminats. Infront of each ID numbers, 12C, 25B and 41A there is common ID TZSR. FS = *F. solani*, MP = *M. phaseolina*, RS = *R. solani* and FO = *F. oxyporum*. The X-axis is biocontrol agents, in front of each ID numbers, 12C, 25B and 41A there is common ID TZSR. The labels (a, b, c, d, and e) are biocontrols with (a) combination of two fungi, (b) four fungi, (c) without fungi, (d) one pathogen, and (e) three pathogens.

tion of 80% was attained by *Rhizobium sp.* TZSR25B, followed by 70% in *Rhizobium sp.* TZSR12C and 67% in *Bradyrhizobium sp.* TZSR41A and *T. harziunum*, all against *F. solani*, *R. solan*, *F. oxyporum* and *M. phaseolina*. The lowest germination of 23% was observed in the soil contaminated with all four fungi without any biocontrol agent. In all treatments, *Rhizobium sp.* TZSR12C and TZSR25B were observed to performed well.

Ability of rhizobia in supressing the root rot fungal pathogens under greenhouse

Antagonistic activity of rhizobia and Trichoderma harzianum against individual fungal pathogens

The strains of rhizobia and *T. harzianum* exhibited the better (0.00%) performance in suppressing the individual fungal pathogen in the rhizosphere of soybean plants under greenhouse conditions (Table 3). All the biocontrol rhizobia were observed to be effective in suppressing *F. oxyporum* and *F. solani* with 0.00% infection in both the roots and foliage. Conversely, in the treatments of rhizobia isolates and *T. harzianum*, the highest infection of 16.67% with severity of 5.33% in roots was observed in *Rhizobium sp.* TZSR12C against *F. solani*. For the case of treatments without biocontrols, *R.*

Table 3. The effect of rhizobia species and *Trichoderma harzianum* as biocontrol inoculants on single root rot fungi of soybean plants.

Treatment	RDI (%)	RDS (%)	FDI (%)	FDS (%)
FO+*Rhizobum sp.*TZSR12C	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FO+*Rhizobum sp.*TZSR25B	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FO+*Bradyrhizobium sp.*TZSR41A	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FO+NOB	61.33 ± 5.67^{b}	20.00 ± 4.04^{ab}	50.00 ± 9.81^{b}	10.00 ± 1.73^{b}
FO+*T. harzianum*	11.33 ± 5.67^{a}	2.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS+*Rhizobum sp.*TZSR12C	16.67 ± 9.53^{a}	5.33 ± 3.93^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS+*Rhizobum sp.*TZSR25B	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS+*Bradyrhizobium sp.*TZSR41A	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	5.67 ± 5.67^{a}	0.00 ± 0.00^{a}
FS+NOB	61.33 ± 5.67^{b}	29.00 ± 5.86^{ab}	38.67 ± 5.67^{b}	8.00 ± 1.00^{b}
FS+*T. harzianum*	5.67 ± 5.67^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
MP+*Rhizobum sp.*TZSR12C	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
MP+*Rhizobum sp.*TZSR25B	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
MP+*Bradyrhizobium sp.*TZSR41A	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
MP+NOB	5.67 ± 0.00^{a}	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
MP+*T. harzianum*	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
RS+*Rhizobum sp.*TZSR12C	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
RS+*Rhizobum sp.*TZSR25B	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
RS+*Bradyrhizobium sp.*TZSR41A	11.00 ± 11.00^{a}	2.33 ± 2.33^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
RS+NOB	66.67 ± 9.53^{b}	38.67 ± 22.26^{b}	39.00 ± 11.00^{b}	7.67 ± 2.33^{b}
RS+*T. harzianum*	16.67 ± 9.53^{a}	3.33 ± 2.03^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
p-Value	<0.001	<0.001	<0.001	<0.001

solani caused the highest infection of 66.67% with severity of 38.67% in roots, this resulted to the infection of 39.00% with severity of 7.67% in plant foliage. Despite the minor infections and severity of *F. solani* in *Rhizobum sp.* TZSR12C, *R. solani* in *Bradyrhizobium sp.* TZSR41A and, *F. oxyporum*, *F. solani* and *R. solani* in *T. harzianum*, no infection was spread in the foliage parts of the plants. Furthermore, *Rhizobium sp.* TZSR25B was observed to perform better by completely suppressing *F. oxyporum*, *R. solani* and *M. phaseolina* with very minimum infection of 5.67% and severity of 1.00% by *F. solani* in the roots.

Antagonistic activity of rhizobia and T. harzianum against combination of two fungal pathogens

Rhizobia and *T. harzianum* acted differently in suppressing the growth of combined two fungal pathogens under greenhouse conditions (Table 4). The highest (100%) infection with 33.33% severity in roots, and 77.67% infection with 25.67% severity in foliage was observed in the combination of *R. solani* with *F. oxyporum* in contaminated soils without biocontrol rhizobia and *T. harzianum*. In the plants treated with biocontrols, *Rhizobum sp.* TZSR12C cleared the infection up to 0.00%, in both roots and foliage of plants, in the soils contaminated with combinations of *F. oxyporum* and *M. phaseolina*, *R. solani* and *F. oxyporum* and *R. solani* and *M. phaseolina*. On the other hand, the highest infection (22.33%) and severity (6.33%) in roots, with no infection on the foliage parts for *Bradyrhizobium sp.* TZSR41A against *F. solani* and *R. solani* was noticed. Rhizobia strains and *T. harzianum* in most treatments were able to reduce infection and severity in roots up to either 1.0% or 0.00% infection in foliage parts.

Antagonistic activity of rhizobia and Trichoderma harzianum against combination of three fungal pathogens

Rhizobia strains and *T. harzianum* possessed different abilities in suppressing the growth of combined three fungal pathogens in the rhizosphere of plants (Table 5). For the contaminated soils without biocontrol, the highest (100.00%) infection with severity of 40.00% in roots, and 83.00% infection with severity of 27.67% in foliage was determined in combination of *R. solani*, *F. oxyporum* and *M. phaseolina*. For the plants treated with biocontrols (rhizobia and *T. harzianum*), the highest (33.33%) infection with severity of 10.00% in roots and 0.00% infection in foliage was observed in *T. harzianum* against the combination of *R. solani*, *F. oxyporum* and *M. phaseolina*. Except for *Rhizobum sp.* TZSR25B against the combination of *F. solani*, *R. solani* and *F. oxyporum* having the infection of 5.67% and severity of 1.00%, the two *Rhizobium sp.* TZSR12C and TZSR25B had cleared infection in the foliage for all treatments.

Antagonistic activity of rhizobia and Trichoderma harzianum against combination of four fungal pathogens

Table 4. The effect of rhizobia species and *Trichoderma harzianum* as biocontrol inoculants on combination of two root rot fungi of soybean plants.

Treatment	RDI (%)	RDS (%)	FDI (%)	FDS (%)
FO+MP+*Rhizobum sp.*TZSR12C	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FO+MP+*Rhizobum sp.*TZSR25B	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FO+MP+*Bradyrhizobium sp.*TZSR41A	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FO+MP+NOB	72.33 ± 14.68^{b}	32.33 ± 3.93^{b}	39 ± 14.74^{b}	7.67 ± 2.91^{a}
FO+MP+*T. harzianum*	22.33 ± 5.33^{a}	6.33 ± 3.33^{a}	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}
FS+FO+*Rhizobum sp.*TZSR12C	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS+FO+*Rhizobum sp.*TZSR25B	11.33 ± 5.67^{a}	2.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS+FO+*Bradyrhizobium sp.*TZSR41A	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}
FS+FO+NOB	83.33 ± 16.67^{b}	43.33 ± 8.82^{b}	61 ± 14.74^{bc}	22.33 ± 7.86^{bc}
FS+FO+*T. harzianum*	16.67 ± 9.53^{a}	3.33 ± 2.03^{a}	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}
FS+MP+1*Rhizobum sp.*TZSR12C	11.33 ± 5.67^{a}	2.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS+MP+*Rhizobum sp.*TZSR25B	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}
FS+MP+*Bradyrhizobium sp.*TZSR41A	17.00 ± 0.00^{a}	3.00 ± 0.00^{a}	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}
FS+MP+NOB	88.67 ± 5.67^{b}	30.00 ± 6.81^{b}	72.33 ± 5.33^{c}	24.33 ± 5.93^{bc}
FS+MP+*T. harzianum*	11.33 ± 5.67^{a}	2.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS+RS+*Rhizobum sp.*TZSR12C	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS+RS+*Rhizobum sp.*TZSR25B	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}
FS+RS+*Bradyrhizobium sp.*TZSR41A	22.33 ± 5.33^{a}	6.33 ± 3.33^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS+RS+NOB	83.33 ± 9.53^{b}	33.33 ± 3.76^{b}	61.33 ± 5.67^{bc}	33.33 ± 6.67^{c}
FS+RS+*T. harzianum*	11.33 ± 5.67^{a}	2.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
RS+FO+*Rhizobum sp.*TZSR12C	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
RS+FO+*Rhizobum sp.*TZSR25B	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
RS+FO+*Bradyrhizobium sp.*TZSR41A	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
RS+FO+NOB	100 ± 0.00^{b}	33.33 ± 6.67^{b}	77.67 ± 5.33^{c}	25.67 ± 4.67^{bc}
RS+FO+*T. harzianum*	11.33 ± 5.67^{a}	2.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
RS+MP+*Rhizobum sp.*TZSR12C	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
RS+MP+*Rhizobum sp.*TZSR25B	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
RS+MP+*Bradyrhizobium sp.*TZSR41A	11.33 ± 5.67^{a}	2.00 ± 1.00^{a}	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}
RS+MP+NOB	88.67 ± 5.67^{b}	28.67 ± 4.33^{b}	67.00 ± 0.00^{bc}	13.00 ± 0.00^{ab}
RS+MP+*T. harzianum*	11.33 ± 5.67^{a}	2.00 ± 1.00^{a}	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}
p-Value	<0.001	<0.001	<0.001	<0.001

*FS = *F. solani*, MP = *M. phaseolina*, RS = *R. solani* and FO = *F. oxyporum*, NOB = no biocontrol and TZSR = Tanzania soybean rhizobia, RDS = root disease severity, FDI = foliar disease incidence and FDS = foliar disease severity.

Table 5. The effect of rhizobia species and *Trichoderma harzianum* as biocontrol inoculants on combination of three root rot fungi of soybean plants.

Treatment	RDI (%)	RDS (%)	FDI (%)	FDS (%)
FS + FO + MP + *Rhizobum sp.*TZSR12C	22.33 ± 5.33^{a}	4.33 ± 1.33^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS + FO + MP + *Rhizobum sp.*TZSR25B	17.00 ± 0.00^{a}	3.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS + FO + MP + *Bradyrhizobium sp.*TZSR41A	22.33 ± 5.33^{a}	4.33 ± 1.33^{a}	11.33 ± 5.67^{a}	2.00 ± 1.00^{a}
FS + FO + MP + NOB	89.00 ± 11.00^{b}	46.67 ± 6.67^{b}	44.33 ± 5.67^{bc}	17.67 ± 2.33^{b}
FS + FO + MP + *T. harzianum*	22.33 ± 5.33^{a}	4.33 ± 1.33^{a}	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}
FS + RS + FO + *Rhizobum sp.*TZSR12C	17.00 ± 0.00^{a}	3.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS + RS + FO + *Rhizobum sp.*TZSR25B	17.00 ± 0.00^{a}	3.00 ± 0.00^{a}	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}
FS + RS + FO + *Bradyrhizobium sp.*TZSR41A	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS + RS + FO + NOB	100.00 ± 0.00^{b}	40.00 ± 11.55^{b}	72.33 ± 5.33^{cd}	29.00 ± 2.00^{c}
FS + RS + FO + *T. harzianum*	22.33 ± 14.68^{a}	7.67 ± 6.23^{a}	16.67 ± 16.67^{ab}	3.33 ± 3.33^{a}
FS + RS + MP + *Rhizobum sp.*TZSR12C	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS + RS + MP + *Rhizobum sp.*TZSR25B	11.33 ± 5.67^{a}	2.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS + RS + MP + *Bradyrhizobium sp.*TZSR41A	22.33 ± 5.33^{a}	6.33 ± 3.33^{a}	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}
FS + RS + MP + NOB	100.00 ± 0.00^{b}	40 ± 11.55^{b}	83.00 ± 0.00^{d}	27.67 ± 5.33^{c}
FS + RS + MP + *T. harzianum*	22.33 ± 14.68^{a}	4.33 ± 2.96^{a}	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}
RS + FO + MP + *Rhizobum sp.*TZSR12C	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
RS + FO + MP + *Rhizobum sp.*TZSR25B	11.33 ± 5.67^{a}	2.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
RS + FO + MP + *Bradyrhizobium sp.*TZSR41A	11.33 ± 5.67^{a}	2.00 ± 1.00^{a}	17.00 ± 0.00^{ab}	3.00 ± 0.00^{a}
RS + FO + MP + NOB	94.33 ± 5.67^{b}	43.33 ± 3.33^{b}	72.33 ± 5.33^{cd}	29.00 ± 2.00^{c}
RS + FO + MP + *T. harzianum*	33.33 ± 16.67^{a}	10.00 ± 5.77^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
p-Value	<0.001	<0.001	<0.001	<0.001

* FS = *F. solani*, MP = *M. phaseolina*, RS = *R. solani* and FO = *F. oxyporum*, NOB = no biocontrol and TZSR = Tanzania soybean rhizobia, RDS = root disease severity, FDI = foliar disease incidence and FDS = foliar disease severity.

The ability of rhizobia strains and *T. harzianum* against the combination of four fungal pathogens is presented in Table 6. The highest infection of 77.78% with severity of 46.67% in roots, and infection of 72.22% with severity 40.00% in foliage was observed in the treatment without any biological control agents. This was distantly followed by 38.89% of infection with 7.78% severity in roots, and 5.67% infection with 1.00% severity in *T. harzianum* against the combination of four fungal pathogens. The lowest infection (27.78%) with 5.56% severity in roots and without any spread of infection in foliage was observed in *Rhizobium sp.* TZSR12C against the combination of four fungal pathogens.

Discussion

Testing the ability of effective rhizobia strains suppressing the root rot fungal pathogens under *in vitro* and greenhouse conditions

Antagonistic activities of rhizobia strains under in vitro conditions

In this study, *Bradyrhizobium sp.* TZSR41A and two *Rhizobium sp.* TZSR12C and TZSR25B have been observed to be capable of suppressing the selected root rot fungi (*F. solani*, *F. oxyporum*, *R. solani* and *M. phaseolina*) both under *in vitro* conditions. Similarly, Parveen et al.[1] identified the ability of *Rhizobium sp.* and *Bradyrhizobium sp.* in inhibiting the growth of particular fungal pathogens under *in vitro* conditions. The strains of rhizobia were observed to produce different colours in inhibiting the growth of different fungal pathogens. The changes in the colour of colonies in an antagonistic activity against fungi indicates the production of antibiotics, HCN or mycolytic enzymes[11,27].

Experimental soil's ability to support microbial activities and growth of soybeans

An experimental soil was examined for a variety of physico-chemical traits prior to validation of the ability of rhizobia isolates to prevent the infection of seedlings by fungal pathogens under greenhouse conditions. According to[28], the soil's neutral pH and medium Sandy Clay Loam (SCL) texture indicate that it is suitable for a different rhizobia populations and their activities. According to[29,30], soil pH is the best indicator for determining the availability of macro- and micronutrients that support plant growth and development for optimum yield. The very high percentage of soil organic carbon and the moderate total nitrogen levels found in this study suggest that the soil has been enriched with organic matter[29,31,32] . Higher

Table 6. The effect of rhizobia species and *Trichoderma harzianum* as biocontrol inoculants on combination of four root rot fungi of soybean plants.

Treatment	RDI (%)	RDS (%)	FDI (%)	FDS (%)
FS + RS + FO + MP + *Rhizobium sp.*TZSR12C	27.78 ± 5.56^{a}	5.56 ± 1.11^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FS + RS + FO + MP + *Rhizobium sp.*TZSR25B	27.78 ± 5.56^{a}	7.78 ± 2.94^{a}	5.56 ± 5.56^{a}	1.111 ± 1.11^{a}
FS + RS + FO + MP + *Bradyrhizobium sp.*TZSR41A	27.78 ± 5.56^{a}	10.00 ± 3.33^{a}	11.11 ± 5.56^{a}	2.222 ± 1.11^{a}
FS + RS + FO + MP + NOB	77.78 ± 14.7^{b}	46.67 ± 8.82^{b}	72.22 ± 11.11^{b}	40.00 ± 10.00^{b}
FS + RS + FO + MP + *T. harzianum*	38.89 ± 5.56^{ab}	7.78 ± 1.11^{a}	5.67 ± 5.67^{a}	1.00 ± 1.00^{a}
p-Value	0.02	<0.001	<0.001	<0.001

* FS = *F. solani*, MP = *M. phaseolina*, RS = *R. solani* and FO = *F. oxyporum*, NOB = no biocontrol and TZSR = Tanzania soybean rhizobia, RDS = root disease severity, FDI = foliar disease incidence and FDS = foliar disease severity.

carbon levels are crucial for supplying energy for rhizobial activities, while medium to low nitrogen levels are enough for the biological nitrogen fixation process[31,32]. A very high concentration of available P in an experimental soil notifies its sufficiency for energy acquisition, storage, and utilization in microbial activities[33,34]. Very higher levels of micronutrients Zn, Mn and Fe, and higher level of Cu suggests the availability of particular nutrients for uptake by plants uptake and serving various functions for the growth of plants and microbial interactions[30–33].

Exchangeable bases, Ca, Mg, and K serves different essential roles in soils to support the activities rhizobia and the growth and development of plants. The higher level of calcium in this study will promote the abundance of rhizobia, development of roots, hence enhance the access of rhizobia to root hairs, root infection by rhizobia and hence nodulation[38–42]. Magnesium, is important in stabilization of cell membrane, nucleic acids, and ribosomes of the microbes[43]. Additionally, the higher concentrations of potassium will help in the regulation of water by the plant and influence the growth of roots. Furthermore, the CEC of an experimental soil was medium, indicating the balance of the soil's exchangeable bases and acid forming cations, Al^{3+} and H^{+}[44]. The very low exchangeable Na is however, required for microbial activities to avoid salinity stress. Exchangeable potassium can replaced by the presence of potassium[38,45]. In general, physico-chemical properties of soil used in this study was suitable in supporting rhizobia activities.

Influence of rhizobia strains in enhancing the germination of inoculated seeds

The strains of rhizobia, in this study were observed to enhance the germination of soybean seeds in the soils inoculated with sole or combination of different fungal pathogens. The ability of rhizobia in enhancing the germination of legumes including soybean have been demonstrated by different studies[27,46]. In this study, *Rhizobium sp.* TZSR12C improved the germination up to 93.33% in soils inoculated with *F. solani*, *R. solani* and *M. phaseolina* and up to 96.67% by *Rhizobium sp.* TZSR25B in non-contaminated soils however, the seeds inoculated with *T. harzianum* had the least germination of 70%. The improvement percentages germination by rhizobia strains in this study are distantly, higher than 83.3% obtained in the study by[6,47], suggesting the higher efficiency of the tested rhizobia species in influencing the germination of seeds.

Antagonistic activities of rhizobia strains under greenhouse conditions

The ability of rhizobia in suppressing root rot disease causing pathogens such as *Fusarium solani F. oxysporum*, *Macrophomina phaseolina* and *F. solani* have been observed in different studies[1,4,6,48]. Different strains which forms symbiotic nodules and fix nitrogen in soybean have been studied on their abilities of performing the dual purpose of fixing nitrogen as well as suppressing the root rot diseases[1,6,49,50]. In this study, *Bradyrhizobium sp.* TZSR41A and two *Rhizobium sp.* TZSR12C and TZSR25B as well as *T. harzianum* have been observed to be capable of suppressing root rot fungi (*F. solani*, *F. oxyporum*, *R. solani* and *M. phaseolina*) in soybean seedlings under greenhouse conditions. Similarly, some studies[10,16,51,52] identified the ability of *Bradyrhizobium sp*. and *Rhizobium sp.* in suppressing *F. oxyporum*, *F. solani*, *R. solani* and *M. phaseolina* in the roots of soybean plants.

On the other hand, the study by[6,53], demonstrated the ability and efficiency of *R. japonicum* in inhibiting the infection of soybean roots and foliage parts. In this study, the rhizobia were capable completely inhibiting the infection and severity to 0.00% which is equivalent to 100% inhibition for the occurrence of single pathogen. The ability of *Rhizobium sp.*TZSR12C in clearing the infection to 0.00% in *F. solani* with *F. oxyporum*, *R. solani* with *F. oxyporum* and *R. solani* with *M. phaseolina* indicates the highest efficiency of the strain against the particular pathogens[6,54]. However, there is limited report on the ability of rhizobia in suppressing the combination of three or four fungal pathogens in the rhizosphere of plants. Being capable of suppressing the combination of different fungal pathogens is an indication of the highest efficiency of inhibiting the growth and protecting the plant roots from infection by pathogens. However, this needs the confirmation of their abilities under field conditions.

Conclusions

This study identified three stains of rhizobia, *Rhizobium sp*. TZSR12C, *Rhizobium sp*. TZSR12C and *Bradyrhizobium sp.* TZSR41A, which are capable of suppressing the common species of root rot fungal pathogens affecting the soybean, both under *in vitro* and greenhouse conditions. Under *in vitro* conditions, *Rhizobium sp.* TZSR25B had the highest performance by forming largest colony diameter and inhibition zone against *F. oxyporum*. Under greenhouse conditions, *Rhizobium sp.* TZSR12C and TZSR25B had the better performance in enhancing the germination of seeds in the soils contaminated with fungal pathogens. *Rhizobium sp.* TZSR25B possessed the highest performance in suppressing the infection against individual fungi and in enhancing the germination in most of the treatments while *Rhizobum sp.* TZSR12C had the highest performance in combination of two, three and four pathogens. The results of this study gives an insight of the suitability of the tested rhizobia species as effective biocontrol agents for the selected root rot fungi. However, the test was conducted only under *in vitro* and greenhouse conditions hence, future research should focus on on-farm trials to validate the biocontrol potential of the identified rhizobia strains in real-world agricultural settings. Additionally, further investigations into the mechanisms of interaction between the rhizobia strains and specific fungal pathogens would contribute to a more comprehensive understanding of their biocontrol capabilities.

Author contributions

The authors confirm contribution to the paper as follows: conceptualization and methodology: Nakei MD, Ndakidemi PA, Venkataramana PB; original draft preparation: Nakei MD; review and editing: Ndakidemi PA, Venkataramana PB. All authors have read and agreed to the published version of the manuscript.

Data availability

The datasets generated or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest

The authors declare that they have no conflict of interest.

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