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ORIGINAL PAPER

Beyond fungicides: embracing bioformulation innovation in mitigating white root rot disease impact on rubber plantations

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Abstract

The white root rot (WRR) disease poses a formidable economic challenge to rubber plantations globally, with Malaysia particularly hard-hit. This disease is attributed to *Rigidoporus microporus*. This glasshouse experiment investigated the effects of a stored, peat moss-based formulation containing silicon (Si), *Glomus mosseae*, and *Enterobacter* sp. UPMSSB7 on combatting WRR and promoting the growth of rubber plants. Compared to the positive control, the experimental bioformulation significantly reduced disease incidence ($P < 0.0001$), with efficacy comparable to the propiconazole fungicide. Furthermore, the bioformulation and fungicide treatments demonstrated superior disease mitigation compared to the positive control 24 weeks after *R. microporus*-inoculation. The bioformulation treatment not only reduced disease incidence and mitigated foliar and root rot symptoms, but it also resulted in a lower disease progressive curve and reduced *R. microporus* colonisation. Additionally, bioformulation significantly increased ($P < 0.001$) plant growth parameters 24 weeks after *R. microporus* inoculation. These parameters included stem height, girth size, chlorophyll content, leaf area, root and shoot dry weight, root volume, total root length, and root surface area. These effects surpassed those observed in fungicide and control treatments. The Si content in shoot and root and leaf N, P, and K nutrient contents were also significantly ($P < 0.001$) increased in the *R. microporus*-inoculated plants with the tested bioformulation than the fungicide and control. In the case of *R. microporus*-inoculated plants of bioformulation treatment, there was a significant ($P < 0.001$) increase in the population density of *Enterobacter* sp. (1.5×10^8 cfu g^{-1} soil), surpassing the levels observed in non-inoculated plants of bioformulation and inoculants with Si, with or without *R. microporus*-inoculation. Moreover, bioformulation treatments improved ($P < 0.001$) root colonisation as well as spore density of *G. mosseae* after *R. microporus*-inoculation than control and fungicide. This study suggests that a peat-based bioformulation containing *G. mosseae*, *Enterobacter* sp., and Si could be an effective strategy for both enhancing plant growth and mitigating WRR in rubber plants.

Keywords White root rot · *Hevea brasiliensis* · Bioformulation · Disease severity · Propiconazole · Growth promotion

Introduction

The scientifically known *Hevea brasiliensis*, or rubber plant, serves as a crucial industrial crop, offering an economically sustainable source of natural rubber [1]. White root rot (WRR) disease, attributed to the fungus *Rigidoporus microporus*, presents a substantial worldwide menace to rubber plants [2]. The fungus spreads from infected trees to healthy ones through highly branching rhizomorphs, resulting in substantial economic losses within rubber plantations [3]. Traditionally, chemical fungicides have been employed to control *R. microporus*, but their widespread use raises environmental and human health concerns [4]. Additionally, some fungal pathogens have developed resistance to these fungicides [5].

On the contrary, there have been documented instances of various biocontrol agents effectively managing pathogens, offering a potentially eco-friendly solution and enhancing plant growth as well [6, 7]. These agents, which encompass bacteria, fungi, and actinomycetes, are readily found in the natural environment [8]. Biocontrol agents are common in agricultural soils around the world [9]. Several

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bacterial strains belonging to the genera *Rhizobia*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, and *Bacillus* have been identified as capable of releasing silicon (Si) through silicates and stimulating plant germination [10]. Multiple biocontrol agents have distinct advantages over single-agent approaches for pathogen suppression [11]. The combination of plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) achieved more efficient WRR control than a single agent [12].

Silicon (Si) is a beneficial element that has been shown to improve plant growth [13] as well as alleviate plant stress [14]. Si predominantly exists in the soil in an insoluble state, but microbial activity or rock weathering processes can transform it into a soluble form. Specific silicate solubilising bacteria (SSB), recognised as PGPR, not only have the capacity to solubilise insoluble silicates, however, it can also activate defence systems against various infections [15, 16]. In pepper plants, the *Enterobacter* species exhibited resistance to *Solani stemphylium* [17]. *Enterobacter* has been shown to increase plant development in soybean [18] and maize [19].

The effectiveness of arbuscular mycorrhizal fungi (AMF) in outcompeting soil-borne pathogens surpasses that of fungicide treatment [20]. Recognised for their roles as bio-enhancers, biostimulants, and agents for biocontrol [21], certain AMF species, such as *Glomus mosseae*, have demonstrated a significant reduction in root disease symptoms in the trees tomato [22]. *G. mosseae* has been found to reduce the root rot disease in chickpeas [23]. Additionally, the introduction of native AMF has been associated with enhanced growth and increased phosphorus content in rubber seedlings [24].

Biological formulations involve extensively utilised globally to boost plant growth and counteract pathogens [25]. Effectively addressing diverse fungal infections and fostering plant growth has been highlighted in various studies [26, 27 and 28]. The conversion of indigenous microbial agents into user-friendly and economically viable bioformulation products for phytopathogen management is crucial [29].

Traditionally, peat moss has functioned as a carrier for plant growth-promoting rhizobacteria (PGPR) [30]. The application of diverse peat formulations of PGPR proved successful in managing root and soil diseases [31]. Microbes can be used to combat WRR in rubber plants [32]. However, investigations on the effectiveness of bioformulation consisting of *Enterobacter* sp., *G. mosseae* and Si for the treatment of WRR in rubber plants are lacking. Consequently, this study aims to assess the effectiveness of 36-week stored bioformulation in curtailing WRR and fostering the growth of rubber plants with a comparison to an antifungal chemical in glasshouse.

Materials and methods

Microbes and the formulation

The rubber plants of the PB-350 clone were selected for this glasshouse trial. The rubber plants, aged two months with two whorl leaves, were utilised in this study. We employed *Enterobacter* sp. UPMSSB7, was identified as a silicate-solubilising bacterium and previously isolated from the rhizosphere soil of rubber plants [33]. This bacterium was examined for its ability to solubilise silicate using a modified procedure on glucose agar media supplemented with magnesium trisilicate (0.25%) and tested on potato dextrose agar (PDA) for antagonistic effect against *R. microporus* [34]. The specified strain has been officially deposited and designated the accession number UPMC1340 in the Institute of Bioscience, Universiti Putra Malaysia (UPM).

Glomus mosseae was provided by the Soil Microbiology Laboratory, Agriculture Faculty, Universiti Putra Malaysia. The *G. mosseae* was multiplied in sterilised sand using corn as host plants to produce its inoculum. Following 10 weeks post-*G. mosseae* inoculation, corn plants were harvested, and *G. mosseae* spores were extracted from the sand. The inoculum was determined to consist of 250 to 300 spores per 10 g of dried sand. Calcium silicate was employed as Si source. For this study, peat moss was purchased from Agroniche, Pvt. Ltd., Serdang, Malaysia. To create a fine powder, peat moss underwent crushing using a disk mill, ensuring it could pass through a 1 mm sieve. Subsequently, 500 g of this finely powdered peat was packed into polyethylene bags (30 cm x 40 cm) and sterilised at 121 ˚C for 2 h.

A slightly modified protocol was employed to produce a peat-based bioformulation [35]. The strain *Enterobacter* sp. was cultured on Luria-Bertani (LB) broth, with subsequent cell harvesting via a centrifugator (Sigma 3K30, Germany) at 10,000 rpm for 15 min at 30 °C. A suspension was then prepared using sterilised water. An estimation of the bacterium's concentration in the suspension using the plating with dilution method on LB agar [36, 37]. The 20 mL of the *Enterobacter* sp. isolate at 10^7-10^8 cfu mL^{-1} and sterilised peat moss (500 g) were added into LB broth (250 mL). This peat, enriched with the bacterium, was dried to achieve a moisture content of 15–20% using a laminar flow hood and subsequently transferred to a sterilised polyethylene bag [38]. Lastly, the bioformulation was added with 4 g of calcium silicate and *G. mosseae* inoculum (50 g sand with 250–300 spores for 10 g of sand), in a polyethylene bag. After preparation of this peat-based bioformulation was stored for 36 weeks at temperature 25 ± 2 °C and then used in this study.

Experimental design

The rubber plants were first transplanted into the polybag (50 cm x 50 cm, Agroniche, Pvt. Ltd., Serdang, Malaysia) added with about 20 kg sterilised soil of Munchong soil series, and inoculated with microbial agents. For microbial inoculation without formulation, firstly *G. mosseae* inoculum (250 to 300 spores per 10 g dry sand) was added as a layer around the roots at 50 g per plant. Then *Enterobacter* sp. was inoculated 7 days post-inoculation with *G. mosseae*. The *Enterobacter* sp. (10^8 cfu mL^{-1}) was applied near roots at 250 mL suspension per plant by drenching the soil. Immediately after *Enterobacter* sp. application, Si was added around the plant's roots at 4 g per plant. On the other hand, the 36-week stored bioformulation was applied at 500 g per plant on the roots at a depth of 2 cm and about 5 cm apart from the stem in a circular furrow. This study was carried out in a glasshouse at Agriculture Faculty, UPM. This experiment was arranged in randomised complete block design (RCBD) having 5 replications. Two plants were grown in each replication under the glasshouse at 35 to 37 ˚C. The treatments include:

T1 (Negative control − R): Plants inoculated with sterilised peat moss and water,

T2 (Positive control + R): Plants inoculated with sterilised peat moss and water and then, 6 weeks later, plants were infected with *R. microporus*,

T3 (Inoculants + Si– R): Inoculation with *G. mosseae* was followed by the application of *Enterobacter* sp. and Si 7 days after *G. mosseae*-inoculation,

T4 (Inoculants + Si + R): Inoculation with *G. mosseae* was followed by the application of *Enterobacter* sp. and Si 7 days after *G. mosseae*-inoculation, and finally, 6 weeks later, plants were infected with *R. microporus*,

T5 (Bioformulation– R): Application of a peat-based bioformulation containing *G. mosseae*, *Enterobacter* sp., and Si,

T6 (Bioformulation + R): Application of bioformulation and then, 6 weeks later, plants were infected with *R. microporus*,

T7 (Fungicide + R): Plants were infected with *R. microporus* and then, 3 days later, plants were applied with propiconazole.

This study was conducted for 24 weeks after artificially infecting plants with pathogens. Each plant received 300 ml of water daily via an automated drip irrigation system. The plants were fertilized with RISDA 1 fertilizer (N-P-K = 10.7-16.6-9.5 + Mg = 2.4) twice at a rate of 75 g per plant per application, following the recommendations of RISDA, Malaysia. Plants in the bioformulation and inoculant treatments received 67% of the recommended fertilizer dose (100 g per plant). This reduced rate reflects the known ability of microbial agents to decrease plant fertilizer requirements by up to 40%. In contrast, plants in other treatments received the full recommended fertilizer dose.

Rubber wood blocks colonisation

A 7-day-old culture of *R. microporus* was acquired from the Mycology Laboratory, Agriculture Faculty, Universiti Putra Malaysia. A method was used to colonise the rubber wood blocks with a fresh culture of *R. microporus* and incubated at 30 ± 2 °C for 6 days [38]. Rubber wood blocks measuring 5 cm × 5 cm × 10 cm were cleaned and then sterilised for 2 h at 121 °C. A polypropylene bag (12 cm x 25 cm x 0.1 mm, Malaysia Plastic, Sdn. Bhd., Malaysia) was used to keep each block in it. The malt extract agar (MEA, Sigma-Aldrich, Darmstadt, Germany) of 100 mL was added in moulted form into each polypropylene bag and then the rubber wood block was autoclaved again at 121 °C for 45 min. After sterilisation, the block was allowed to cool down until agar was solidified, and then inoculated with mycelial plugs (5 mm) of a 5-day-old colony of *R. microporus*. These blocks were then incubated at 30 ± 2 °C for 3 to 5 weeks in a dark chamber until all sides of block were completely covered with fungal mycelium.

Inducing artificial infection of plants with *R. microporus* and fungicide application

A slightly modified method was utilised for the artificial infection of plants with *R. microporus* using pre-colonised rubber blocks [39]. For treatments T2, T4, T6, and T7, artificial inoculation occurred four weeks after the initial inoculation of plants with microbial agents, with or without bioformulation. This required exposing the roots of the plant and positioning a colonised rubber block next to them. Un-colonised blocks were positioned near the roots of treatments T1, T3, and T5. In Malaysia, propiconazole, a triazole fungicide is being used to prevent WRR [40]. Three days after being inoculated with *R. microporus*, plants were applied with propiconazole (30 mL mixed with 1 L of water for each plant).

Disease assessment

The assessment of WRR involved evaluating the disease incidence (DI), disease severity of foliar (DSF) symptoms, disease severity of root rot (DSR) symptoms, pathogen colonisation, area under disease progress curve (AUDPC), and disease reduction [41]. To assess DSF symptoms over 24 weeks at four-week intervals, an adapted scale was used: 0 = healthy; 1 = rhizomorphs forming at the base and lower leaves are yellowing; 2 = button-like sporophore appears at

the base and lower leaves are necrotic; 3 = basidiocarp forms at the base and more than 50% of the leaves are necrotic; and 4 = dead plant [42]. The DSF and DSR were calculated using the following formula [43]:

$$\text{DSF/DSR}\,(\%) = \frac{\text{Number of plants in the rating X rating number}}{\text{Total number of plants assessed x highest rating}} \times 100$$

Total number of plants assessed x highest rating.

The disease progress curve was constructed by analysing disease severity data from various treatments in comparison to the control. The progression of the disease was quantified by calculating the AUDPC, which relied on DSF, following the methodology outlined by [41]:

$$AUDPC = \sum_{i=1}^{n_{i-1}} \frac{(y_i + y_{i+1})}{2} + (t_{i+1} - t_i)$$

Where "n" represents the quantity of assessments, "Y" denotes the degree of disease severity in foliar symptoms, and "t" corresponds to the observation time.

The progression of WRR was monitored by tracking the DI (in percentage) at four-week intervals throughout twenty-four weeks. Disease incidence shows the count of plants that displayed disease symptoms, including chlorosis and/or leaf necrosis, with or without the presence of fruiting bodies. The DI calculation is [41]:

$$\text{DI}\,(\%) = \frac{\text{Number of affected plants}}{\text{Total number of evaluated plants}} \times 100$$

Total number of evaluated plants.

The degree of pathogenic colonisation within the taproot was determined by calculating the root length covered by *R. microporus* mycelium, dividing it by the overall length of the root, and multiplying the resulting ratio by a factor of hundred.

Following a twenty-four-week exposure to *R. microporus*, the roots underwent a longitudinal fracture to visibly evaluate any signs of root rot. These assessments were made using a slightly adapted scale: 0 indicated healthy roots, 1 indicated 1 to 20% rotting of root tissues, 2 indicated 21 to 50% rotting of root tissues, 3 indicated 51 to 90% root rotting, and 4 indicated more than 90% rotting of root tissues [42].

An equation that was significantly adjusted and taken was used to calculate disease reduction [44]:

$$\text{Disease reduction}\,(\%) = [(\text{DSR in treated/DSR in control}) - 1] \times 100$$

Analysis of growth characters and nutrient contents in plants

The experiment involved the measurement of various plant growth parameters. A measuring tape and a vernier calliper were used to assess the girth size and stem height, respectively. Total chlorophyll content was determined using a SPAD meter and a leaf area meter was used to calculate leaf area. Using a root scanning device, EPSON Perfection V700 Photo, Canada, the root growth characteristics were measured. The Si in shoot and root was determined through the autoclaved induced digestion (AID) procedure [45]. The leaf N, P, and K contents were calculated based on recommended method described by Rubber Research Institute, Malaysia [46]. A CNS analyser was used for the N analysis, and the dry ash technique was used to prepare P and K. The final filtrates were subjected to examination using an auto analyser for P determination and a spectrophotometer (Perkin Elmer, Model AAS 3110) for K content.

Enterobacter sp. population, *G. mosseae* spores as well as root colonisation

The bacterial density for *Enterobacter* sp. (expressed as cfu g^{-1} soil) was conducted utilising a serial dilution technique. This was accomplished by employing a glucose agar media enriched with magnesium trisilicate (0.25%), following the procedure outlined by Vasanthi et al. [34].

A wet sieving and decanting technique were used to measure the spore density of *G. mosseae* in each treatment [47]. The count of *G. mosseae* spores per 10 g of dry soil was used to express the results. Furthermore, fresh root samples taken at harvest were examined to evaluate the root colonisation of *G. mosseae* [48]. The fine lateral roots (2 g) were randomly picked and cut into pieces before being cleaned with tap water. These sections were subsequently placed in a 25 mL MacCartney bottle and immersed in a KOH solution (10%) for a duration of 3 days (with the KOH solution being replaced after 24 h). After that, the material was heated for an hour in a water bath that was set at 90 ˚C. After carefully cleaning the root samples with tap water, they were dyed with a lacto-glycerol solution (a mixture of distilled water, glycerol, and lactic acid) containing 0.05% Trypan blue. The dyed root segments were seen under a microscope (Leica DM5000B, Wetzlar, Germany) at magnifications of 100–400x. Subsequently, the percentage of root colonisation by *G. mosseae* was calculated using the following method:

$$\text{Root colonisation}\,(\%) = (\text{No. of positive sections of G. mosseae/Total no. of sections}) \times 100$$

Statistical analysis

Statistical Analysis System Ver. 9.4 (SAS Institute Inc., Cary, NC, USA, 2013) was used for data analysis. One-way ANOVA was used for data analysis, and differences between means were assessed using the least significant difference (LSD) test at a significance level of $P \leq 0.05$.

Results

Evaluation of disease

Disease incidence (DI)

No disease incidence was observed in the T1, T3 and T5 treatments without *R. microporus* infection. Under pathogen-inoculated positive control treatment (T2), the disease development in rubber plants was constant over several weeks. On the other hand, inoculants + Si and bioformulation treatments during a 24-week period showed a much-delayed development of the disease (Fig. 1). The DI for T2 (Positive control + R) plants was first assessed in week 8, it was found to be 12%. The DI for T2 had risen to 30% by week 12. At week 16, DI started to show results in T4 (Inoculants + Si + R), T6 (Bioformulation + R), and T7 (Fungicide + R). The respective values were 10%, 6%, and 12%. At week 16, T2 had a DI of 48%. As of week 20, DI for T2 had increased to 68%, whereas for T4, T6, and T7 (16%, 12%, and 20%, respectively) were lower ($P < 0.001$) than T2. When compared to T4, T6, and T7, which had lower values at 22%, 20%, and 26%, respectively, and were not statistically different from one another, T2 showed the quickest DI escalation after 24 weeks, achieving the greatest DI at 82%.

Disease severity of foliar (DSF)

Over a period of 24 weeks, disease progression in T4, T6, and T7 plants happened at a slower pace ($P < 0.001$) than in T2 plants (Table 1; Fig. 2). When the DSF of T2 plants was first assessed in week 8, it registered at 8%. T2 and T7 plants had DSF of 30% and 8%, respectively, by week 12. At week 16, DSF was first measured in T4, T6, and T7 plants. These plants showed DSF values of 10%, 8%, and 14%, respectively, which were lower ($P < 0.001$) than T2 plants (48%). T2 plants had the greatest DSF value (74%) at week 20, whereas for T4, T6, and T7 plants (DSF values at 14%, 12%, and 16%, respectively) and didn't have any significant difference among these. Following a 24-week period, T2 plants exhibited the maximum DSF value of 86%. In contrast, T4, T6, and T7 plants had lower values of 18%, 16%, and 24%, respectively, with no significant difference among them.

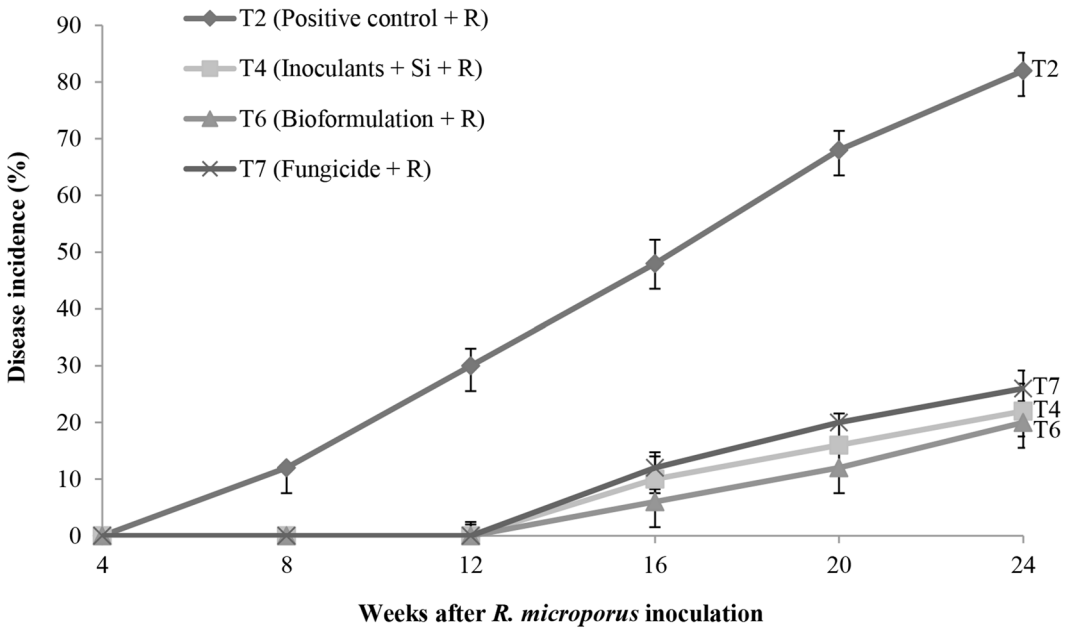

Fig. 1 Effect of bioformulation on disease incidence in rubber plants (PB-350) 24 weeks after *R. microporus*-inoculation in the glasshouse. + R: Rubber plants with *R. microporus* inoculation, Inoculants: *Enterobacter* sp. + *G. mosseae*, Si: Calcium silicate, Bioformulation: formulation based on peat moss consists of *Enterobacter* sp., *G. mosseae* and Si, Fungicide: Propiconazole. Each value is mean of 5 replicates ± SE. Values followed by different letters are significantly different at $P \leq 0.05$ by LSD

Table 1 Effect of bioformulation on WRR development in rubber plants (PB-350) 24 weeks after *R. microporus*-inoculation in the glasshouse

Treatment	Disease severity (%)		AUDPC (units^2)	Pathogen colonisation (%)	DR (%)
	Foliar (DSF)	Root (DSR)			
T1 (Negative control – R)	Nd	Nd	Nd	Nd	Nd
T2 (Positive control + R)	86 ± 2.44 a	84 ± 2.44 a	920 ± 61.31 a	88.94 ± 2.95 a	0.00
T3 (Inoculants + Si – R)	Nd	Nd	Nd	Nd	Nd
T4 (Inoculants + Si + R)	18 ± 3.74 b	21 ± 1.22 b	106 ± 8.12 b	15.71 ± 2.09 b	63.55 ± 7.75 b
T5 (Bioformulation – R)	Nd	Nd	Nd	Nd	Nd
T6 (Bioformulation + R)	16 ± 2.44 b	19 ± 2.44 b	90 ± 6.32 b	11.37 ± 3.23 b	79.22 ± 1.37 a
T7 (Fungicide + R)	24 ± 2.44 b	23 ± 1.22 b	132 ± 13.92 b	13.36 ± 3.57 b	70.27 ± 1.47 ab

Note: − R: Rubber plants without *R. microporus* inoculation, + R: Rubber plants with *R. microporus* inoculation, Inoculants: *Enterobacter* sp. + *G. mosseae*, Si: Calcium silicate, Bioformulation: formulation based on peat moss consists of *Enterobacter* sp., *G. mosseae* and Si, Fungicide: Propiconazole, DSF: Disease severity of foliar, DSR: Disease severity of root rot, AUDPC: Area under disease progress curve and DR: Disease reduction and Nd = Not detected. Each value is mean of 5 replicates ± SE. Values followed by different letters are significantly different at $P \leq 0.05$ by LSD

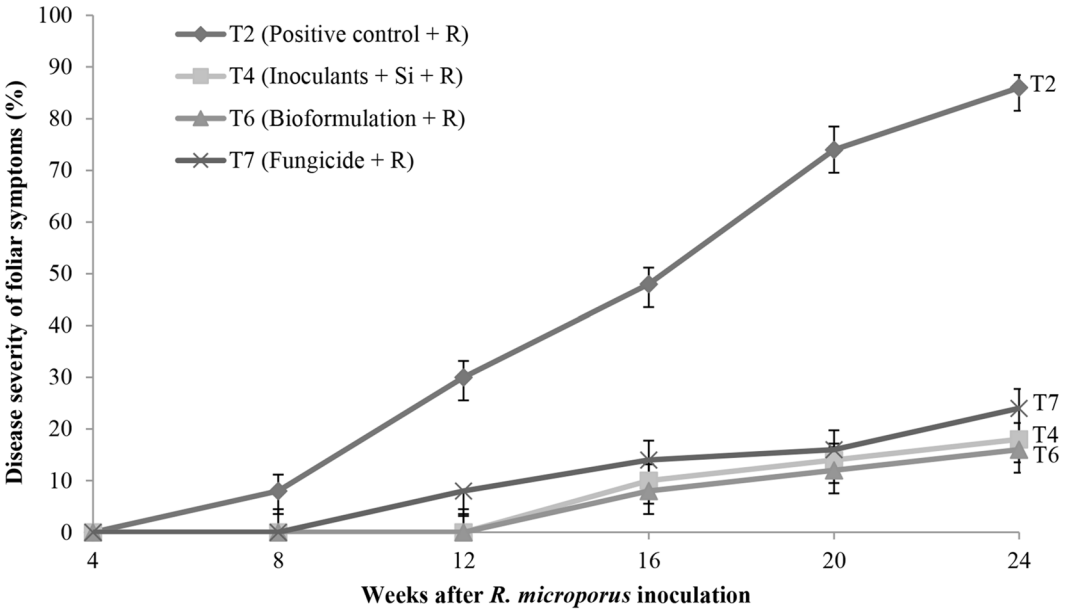

Fig. 2 Effect of bioformulation on disease severity of foliar symptoms in rubber plants (PB-350) 24 weeks after *R. microporus*-inoculation in the glasshouse. + R: Rubber plants with *R. microporus* inoculation, Inoculants: *Enterobacter* sp. + *G. mosseae*, Si: Calcium silicate, Bioformulation: formulation based on peat moss consists of *Enterobacter* sp., *G. mosseae* and Si, Fungicide: Propiconazole. Each value is mean of 5 replicates ± SE. Values followed by different letters are significantly different at $P \leq 0.05$ by LSD

Disease severity of root rot (DSR)

Rubber plants subjected to foliar desiccation after 24 weeks showed signs of severe internal root rotting (Table 1; Fig. 3). Healthy rubber plants exhibited no evidence of internal root rot. Compared to the *R. microporus*-inoculated positive control, DSR value was lower ($P < 0.0001$) in T4, T6 and T7. The T2 plants exhibited the highest DSR value (84%), whereas T4, T6, and T7 plants displayed lower values at 21%, 19%, and 23%, respectively, and didn't have any significant difference among these.

AUDPC, *R. microporus* colonisation, and disease reduction

At the end of the 24-week period, T4, T6, and T7 exhibited AUDPC values of 106 unit², 90 unit², and 132 unit², respectively, without any significant differences among these. T2 plants displayed the highest AUDPC value at 920 unit². There were no significant differences in pathogen colonisation on roots across T4, T6, and T7 plant treatments (15.71%, 11.37%, and 13.36%, respectively) (Table 1). The maximum pathogen colonisation (88.94%) observed in T2 plants. T4, T6, and T7 treatments significantly ($P < 0.0001$) reduced the disease by 63.55%, 79.22%, and 70.27%,

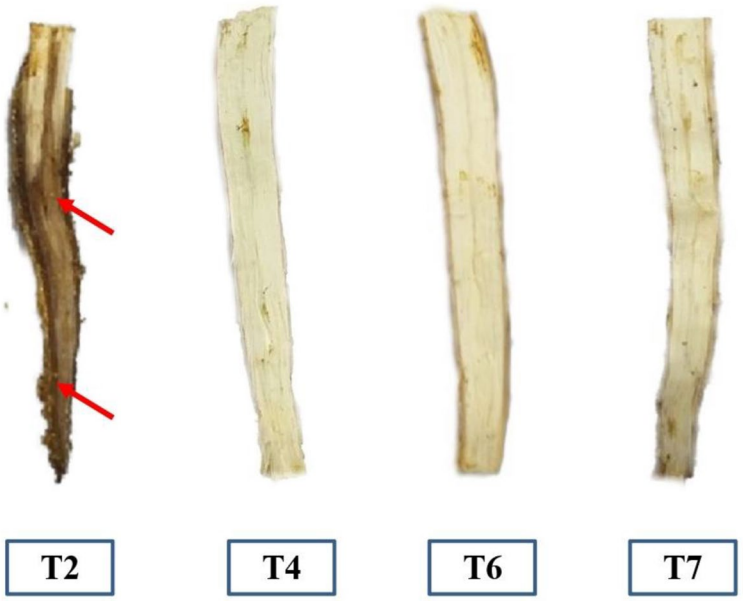

Fig. 3 Effect of bioformulation on disease severity of root rot symptoms (arrows) in rubber plants (PB-350) 24 weeks after *R. microporus*-inoculation in the glasshouse. Note: T2 (Positive control + R), T4 (Inoculants + Si + R), T6 (Bioformulation + R) and T7 (Fungicide + R). + R: Rubber plants with *R. microporus* inoculation, Inoculants: *Enterobacter* sp. + *G. mosseae*, Si: Calcium silicate, Bioformulation: formulation based on peat moss consists of *Enterobacter* sp., *G. mosseae* and Si, Fungicide: Propiconazole

Table 2 Effect of bioformulation on growth of rubber plants (PB-350) 24 weeks after *R. microporus*-inoculation in the glasshouse

Treatment	Stem height (cm)	Girth size (mm)	Chlorophyll content (SPAD value)	Leaf area (cm^2)	Dry weight (g $plant^{-1}$)	
					Root	Shoot
T1 (Negative control– R)	78 ± 3.35 d	6.24 ± 0.16 e	47.38 ± 0.67 e	2240 ± 215 d	23.25 ± 0.93 d	28.14 ± 2.52 d
T2 (Positive control + R)	45 ± 2.15 e	5.22 ± 0.16 f	35.32 ± 1.09 f	850 ± 40 e	14.98 ± 0.55 e	14.45 ± 1.18 e
T3 (Inoculants + Si– R)	101 ± 3.99 ab	7.88 ± 0.23 bc	54.86 ± 0.71 bc	3397 ± 257 ab	34.71 ± 0.89 ab	37.26 ± 1.29 ab
T4 (Inoculants + Si + R)	94 ± 2.59 bc	7.26 ± 0.42 cd	52.98 ± 0.91 cd	3122 ± 161 bc	33.63 ± 1.33 b	33.91 ± 2.62 bc
T5 (Bioformulation– R)	109 ± 3.82 a	8.64 ± 0.16 a	58.72 ± 0.77 a	3822 ± 257 a	37.37 ± 0.89 a	40.72 ± 1.58 a
T6 (Bioformulation + R)	102 ± 1.77 ab	8.22 ± 0.2 ab	55.90 ± 0.89 ab	3547 ± 161 ab	37.19 ± 1.18 a	35.93 ± 2.59 ab
T7 (Fungicide + R)	87 ± 3.72 cd	6.75 ± 0.21 de	50.35 ± 1.88 de	2622 ± 114.57 cd	29.05 ± 1.25 c	30.13 ± 2.63 cd

Note: − R: Rubber plants without *R. microporus* inoculation, + R: Rubber plants with *R. microporus* inoculation, Inoculants: *Enterobacter* sp. + *G. mosseae*, Si: Calcium silicate, Bioformulation: formulation based on peat moss consists of *Enterobacter* sp., *G. mosseae* and Si, Fungicide: Propiconazole. Each value is mean of 5 replicates ± SE. Values followed by different letters are significantly different at $P \leq 0.05$ by LSD

respectively, than T2 treatment. However, no significant differences were observed among T4, T6 and T7.

Plant growth performance

There was an increase ($P < 0.001$) in the growth characteristics of rubber plants over a 24-week period in T3, T5, and T6 in comparison to T1, T2, T4, as well as T7. The T3, T5, and T6 exhibited a significant ($P < 0.001$) increase in stem height, leaf area, shoot and root dry weight, root length and root surface area when compared to T1, T2, T4, and T7, except for chlorophyll content, girth size, and root volume. The T1 and T7 had no significant difference between them, but both showed an increase ($P < 0.001$) in all growth parameters than the T2, except for root dry weight. When compared to all other treatments, the T2 showed the lowest growth parameters (Tables 2 and 3).

Analysis of plant nutritional value (Si, N, P, and K)

When comparing bioformulation-treated plants to other treatments, the plant contents of Si, N, P, and K shown a considerable increase (Table 4). T6 plants had higher ($P < 0.001$) root Si content than all other treatment after 24 weeks, while both T4 and T6 plants had significantly increased Si content in shoots than all other treatments. The T7 plants had higher Si content in both roots and shoots than T1 and T2. The Si content in roots and shoots of T1 and T2 plants did not differ significantly. After 24 weeks, T5 plants displayed significantly higher N and P contents than all other treatments, while both T5 and T6 observed significantly higher K content than all other treatments, with no significant differences between them (Table 4). The T7 plants had higher N and P contents than T1 and T2, while T1, T2, T4, and T7 did not show significant differences in K content. T2 treatment recorded the lowest N content, while

Table 3 Effect of bioformulation on root growth of rubber plants (PB-350) 24 weeks after *R. microporus*-inoculation in the glasshouse

Treatment	Root volume (cm^3)	Root length (cm)	Root surface area (cm^2)
T1 (Negative control– R)	15.79 ± 0.72 c	855 ± 14.56 d	245 ± 15.06 c
T2 (Positive control + R)	7.31 ± 0.58 d	495 ± 18.74 e	90 ± 5.73 d
T3 (Inoculants + Si– R)	23.92 ± 1.69 b	1571 ± 69.38 ab	367 ± 32.03 ab
T4 (Inoculants + Si + R)	21.98 ± 0.7 b	1433 ± 40.72 b	344 ± 7.62 b
T5 (Bioformulation– R)	26.96 ± 1.69 a	1662 ± 83.21 a	403 ± 36.59 a
T6 (Bioformulation + R)	23.28 ± 0.7 b	1583 ± 40.72 a	384 ± 7.52 ab
T7 (Fungicide + R)	15.48 ± 0.56 c	1075 ± 54.79 c	290 ± 18.85 c

Note − R: Rubber plants without *R. microporus* inoculation, + R: Rubber plants with *R. microporus* inoculation, Inoculants: *Enterobacter* sp. + *G. mosseae*, Si: Calcium silicate, Bioformulation: formulation based on peat moss consists of *Enterobacter* sp., *G. mosseae* and Si, Fungicide: Propiconazole. Each value is mean of 5 replicates ± SE. Values followed by different letters are significantly different at $P \leq 0.05$ by LSD

Table 4 Effect of bioformulation on nutrient contents of rubber plants (PB-350) 24 weeks after *R. microporus*-inoculation in the glasshouse

	Si content (g kg^{-1} of dry weight)		Leaf nutrient content (% of dry weight)		
Treatments	Shoot	Root	*N*	*P*	K
T1 (Negative control– R)	4.80 ± 0.71 e	4.16 ± 0.81 e	2.67 ± 0.11 e	0.10 ± 0.002 f	0.83 ± 0.01 d
T2 (Positive control + R)	6.59 ± 0.83 e	4.33 ± 0.39 e	2.26 ± 0.07 f	0.10 ± 0.001 f	0.84 ± 0.02 d
T3 (Inoculants + Si– R)	20.61 ± 2.15 c	13.68 ± 0.65 c	3.64 ± 0.03 b	0.16 ± 0.004 c	0.97 ± 0.06 bc
T4 (Inoculants + Si + R)	24.95 ± 1.36 ab	16.39 ± 0.32 b	3.25 ± 0.06 c	0.13 ± 0.005 d	0.94 ± 0.06 cd
T5 (Bioformulation– R)	22.5 ± 2.32 bc	15.68 ± 0.65 b	4.21 ± 0.03 a	0.22 ± 0.004 a	1.13 ± 0.06 a
T6 (Bioformulation + R)	26.85 ± 1.55 a	18.19 ± 0.28 a	3.65 ± 0.06 b	0.17 ± 0.005 b	1.09 ± 0.06 ab
T7 (Fungicide + R)	13.74 ± 1.47 d	9.01 ± 0.57 d	2.94 ± 0.11 d	0.12 ± 0.002 e	0.91 ± 0.02 cd

Note − R: Rubber plants without *R. microporus* inoculation, + R: Rubber plants with *R. microporus* inoculation, Inoculants: *Enterobacter* sp. + *G. mosseae*, Si: Calcium silicate, Bioformulation: formulation based on peat moss consists of *Enterobacter* sp., *G. mosseae* and Si, Fungicide: Propiconazole. Each value is mean of 5 replicates ± SE. Values followed by different letters are significantly different at $P \leq 0.05$ by LSD

Table 5 Effects of bioformulation on population density of *Enterobacter* sp., spore density and root colonisation of *G. Mosseae* 24 weeks after *R. microporus*-inoculation in the glasshouse

Treatment	Population density of Enterobacter sp. (cfu g^{-1} soil)	Spore density of G. mosseae (per 10 g soil)	Root colonisation of G. mosseae (%)
T1 (Negative control– R)	Nd	4 ± 0.54 d	4.00 ± 0.66 c
T2 (Positive control + R)	Nd	3 ± 0.51 d	2.00 ± 0.81 c
T3 (Inoculants + Si– R)	9.6×10^6 c	78 ± 2.39 b	46 ± 2.66 ab
T4 (Inoculants + Si + R)	8.6×10^6 c	69 ± 5.47 c	42.60 ± 3.44 b
T5 (Bioformulation– R)	1.5×10^8 a	86 ± 3.89 ab	51.33 ± 1.33 a
T6 (Bioformulation + R)	8.6×10^7 b	88 ± 2.67 a	49.33 ± 1.24 a
T7 (Fungicide + R)	Nd	2 ± 0.24 d	1.33 ± 0.81 c

Note − R: Rubber plants without *R. microporus* inoculation, + R: Rubber plants with *R. microporus* inoculation, Inoculants: *Enterobacter* sp. + *G. mosseae*, Si: Calcium silicate, Bioformulation: formulation based on peat moss consists of *Enterobacter* sp., *G. mosseae* and Si, Fungicide: Propiconazole; Nd = Not detected. Each value is mean of 5 replicates ± SE. Values followed by different letters are significantly different at $P \leq 0.05$ by LSD

T1 as well as T2 had the lower P and K contents than all other treatments.

Population density of *Enterobacter* sp., *G. Mosseae* spore density, and root colonisation

The rhizosphere of T5 plants recorded the *Enterobacter* sp. population (1.5×10^8 cfu g^{-1} soil) significantly ($P < 0.001$) greater than in the T3, T4, and T6 treatments (Table 5). In contrast, the rhizosphere of T6 (8.6×10^7 cfu g^{-1} soil) had a significantly higher population than that of T3 and T4. *Enterobacter* sp. population was not found in any of the other treatments. Bioformulations treatments with and without *R. microporus* infection showed a significant ($P < 0.01$) increase in spore population density and root colonisation of *G. mosseae* than other treatments. *G. mosseae* spore density was significantly greater in T6 and T5 compared to other treatments but was not significantly different. T1,

T2, and T7 treatments observed lower *G. mosseae* spore densities, with no significant difference amongst them. The presence of *G. mosseae* spore particles significantly ($P < 0.01$) increased its root colonisation. The root colonisation was increased ($P < 0.01$) in T5 as well as T6 (51.33% and 49.33%, respectively) compared to other treatments, but was not significantly different between them. The T1, T2, and T7 treatments had no significant difference among them.

Discussion

The findings of the study emphasise the effectiveness of bioformulation containing *Enterobacter* sp., *G. mosseae*, and Si in suppressing WRR as well as promoting the growth of rubber plants in a glasshouse environment. This study demonstrates that bioformulation reduced disease incidence as efficiently as liquid culture of inoculants and propiconazole fungicide. The propiconazole, applied in this study, has previously been recommended in Malaysia for managing WRR disease in rubber trees [40]. The propiconazole, inoculants with Si, as well as the tested bioformulation treatments, exhibited lower DI, DSF, DSR, and AUDPC and less pathogen colonisation than a positive control that was inoculated with *R. microporus* 24 weeks post-inoculation. The study suggests that the bioformulation effectiveness in reducing WRR was on par with the propiconazole. Research has revealed that *Enterobacter* strains can produce phytohormones as well as siderophores, which can contribute to plant disease resistance [16, 49, 50]. Studies have demonstrated that *Glomus mosseae* and *Glomus intraradices* enhance plant resistance to fungal pathogens [51]. Peat-based formulations containing bacteria like *Pseudomonas fluorescens* have been used in cotton successfully against fungal pathogens [52]. A bioformulation comprising of *Trichoderma viride* and *Pseudomonas fluorescens* lowered the rice sheath blight incidence [53]. Intentional use of AMF and PGPR into the soil, along with the natural presence of these microbes in the soil or rhizosphere, has the potential to trigger disease resistance [54].

Furthermore, results indicated that 24 weeks after *R. microporus*-inoculation, bioformulation improved growth parameters compared to control, the inoculants with Si and propiconazole. The bioformulation treatments showed no significant differences in growth parameters, while these treatments showed improved growth compared to the inoculants with Si and propiconazole with *R. microporus*-inoculation. This suggests that the bioformulation product could be a promising pre-treatment to prevent stunting of plants after pathogen infection. The improved growth may be attributed to growth-improving characters induced by the inoculants added in the formulation [38]. *Enterobacter* is one of several bacteria recognised as PGPR [55]. AMF are symbiotic root partners that mutually benefit plants by improving nutrient and water absorption from the soil and providing protection against fungal pathogens [56]. A bioformulation based on peat moss containing different antagonistic bacteria improved the plant growth of cucumber [38]. Additionally, the study revealed that significantly higher nutrient contents (N, P, K and Si) in plants were recorded in bioformulation treatment than control, the inoculants with Si and propiconazole. The mixture of AMF-PGPR-rhizobia increased the nutrient (N, P and K) contents in wheat and faba bean [57]. The biomass and N and P accumulation were significantly increased in co-inoculation with *Bacillus subtilis* and *Glomus intraradices* in onion plants [58].

Lastly, the study also indicated that the *Enterobacter* sp. isolate's bacterial density in the rhizosphere was significantly increased in the bioformulation with *R. microporus*-inoculation than in bioformulation without *R. microporus*-inoculation, as well as inoculants + Si with or without *R. microporus*-inoculation. Moreover, the study found that the bioformulation had significantly higher spore density as well as root colonisation of *G. mosseae* than without adding *G. mosseae*. Co-inoculation of *Pseudomonas* sp. F113 and *G. mosseae* improved tomato root colonisation of *G. mosseae* spores [11]. The term "mycorrhiza helper bacteria (MHBs)" was coined to refer to bacteria that stimulate mycelial formation of mycorrhizal fungi [59]. *Enterobacter* sp. with *G. intraradices* acted as a MHB by improving root colonisation [58]. Many studies have indicated variations in the effectiveness of different microbial inoculants when used in field conditions, where they may be completely ineffective or less effective than in controlled conditions due to climatic variations (humidity and temperature etc.), ecological competence of microbial (colonisation ability or survival), or product stability issues [59].

Conclusion

In conclusion, the bioformulation based on peat moss consisting of *Enterobacter* sp., *G. mosseae* and Si reduced the development of WRR 24 weeks after *R. microporus*-inoculation. It was observed in terms of reduction in disease incidence and severity symptoms, AUDPC and pathogen root colonisation in bioformulation treatment than positive control. Bioformulation exhibited a level of effectiveness in reducing disease progression that was on par with that of a treatment involving propiconazole fungicide. The tested bioformulation could be a promising alternative method to fungicides for effectively managing WRR disease and improving plant growth of rubber plants at nursery stages.

The findings of this study suggest that it appears worthwhile to consider further research and potential field applications of the tested bioformulation product to enhance growth and suppress WRR disease of rubber plants.

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Data availability The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors have no conflict of interest.

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