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Mechanisms of biochar and vermicompost in suppression of root rot fungal disease of common bean (*Phaseolus vulgaris* L)

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Abstract

Composts and biochar improve soil fertility and also suppress fungal soil-borne diseases through their ability to promote beneficial microbial communities. The study sought to determine the mechanisms through which biochar and vermicompost suppress root rot pathogens. Extracts of biochar and vermicompost were used for spore germination, mycelial growth tests at different concentrations. To assess the ability of biochar and vermicompost to adsorb signaling molecules from bean, root and seed exudates were filtered through biochar and vermicompost. Antagonistic activity of *Trichoderma harzianum* and *Penicillium* spp against root rot pathogens was also assessed. Germination of *Pythium ultimum* sporangia and *Fusarium solani* macroconidia was significantly inhibited by various concentrations of water extractible substances from biochar and vermicompost. Water extracts from biochar and vermicompost inhibited the germination of root rot propagules and reduced the ability of root and seed exudates to induce sporangial and conidial germination. *Trichoderma harzianum* and *Penicillium* spp significantly inhibited the growth of *Pythium ultimum* sporangia and *Fusarium solani*. The water extracts significantly reduced the growth of root rot colonies on PDA plates. Biochar and Vermicompost greatly inhibit germination of spores and mycelial growth of root rot pathogens.

Keywords: *Germination inhibition, Signal adsorption, Competition, organic amendments*

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1. Introduction

Bean root rot has been cited as a major constraint in common bean production leading to production losses of up to 70% in some bean varieties such as Rosecoco in Kenya (MOA, 2011). This has led to reduced yield despite the increase in demand due to the ever growing population (Katungi *et al.,* 2009). There are various options available for managing the root rot complex of beans but some are of questionable efficacy after planting (Abawi and Pastor-corrales, 1990). The use of options known to effectively control root rot such as the broad spectrum soil fumigants including metham sodium are restricted by their toxicity to man and environment as well as high costs (United Nations Environmental Protection Agency, 2008). Applications of organic amendments such as compost, farmyard manure and biochar have been shown to have positive effects on root disease dynamics and yield increase (Jaiswal, 2013; and Ruano-Rosa and Mercado-Blanco 2015). They reduce disease inoculum density in the soil as well as creating conditions favourable for development of beneficial microorganisms such as *Trichoderma harzianum*, *Penicillium* spp., *Athrobotrys* spp. and *Paeciliomyces* spp. that are antagonistic to plant pathogens (Were, 2019).

Composts and biochar have been reported to improve the soil fertility and also suppress fungal soil-borne diseases (Mehta *et al.,* 2014). The suppressiveness of compost and biochar has been attributed to their ability to promote beneficial microbial communities, improve plant growth and vigour, increase availability of plant nutrients, induce systemic resistance and / or fungistatic abilities that compost and biochar amendments may express (Meller Harel *et al.,* 2012; and Graber *et al*., 2014). Populations of *Trichoderma* and *Penicillium* spp have been observed to increase following application of biochar and vermicompost and have also been reported to have antagonistic effect on root rot fungal pathogens (Sreevidya and Gopalakrishnan, 2016; and El-Sheshtawi *et al.,* 2014).

The objective of this study was to elucidate the mechanism(s) exhibited by biochar and vermicompost in suppression of common bean root rot. We hypothesized that (i) vermicompost and biochar-derived water extracts inhibit germination and growth of root rot pathogens (*Fusarium spp., Pythium spp* and *Rhizoctonia spp.*); (ii) biochar and vermicompost adsorbs and alters availability of plant root exudates leading to reduced spore germination resulting to reduced infection of beans by pathogenic soil-borne fungi; and (iii) duration of exposure of biochar to air has an effect on its ability to suppress the growth of root rot pathogens.

2. Materials and methods

2.1. Production and characterization of vermicompost and biochar

Plant residues, sugarcane bagasse were sourced from Kibos Sugar Factory in Kisumu Kenya and sun dried. The bagasse was pyrolised to produce biochar (Laird, 2008) using a metallic production kiln with a perforation at the base to allow for air flow and a chimney to expel the burning gases. The resultant biochar was weighed and packed into 6 kg gunny bags. The biochar was analyzed for chemical properties at Crop Nutrition Laboratories Nairobi, Kenya. Vermicompost was produced at Dudutech, Naivasha, Kenya from vegetable residue. The debris were chopped and air dried for 7-10 days then placed in 30 cm deep rectangular troughs containing 6,000 earth worms (*Eisinia andrei*) in 40 kg of pre decomposed crop material and soil mixture. The crop residue was spread evenly on the surface of the trough where it was decomposed by earth worms feeding on the plant debris for a period of 6 weeks. The resultant worm casting referred to as vermicompost was then analyzed for nutrition and chemical content at Dudutech Naivasha, Kenya. The vermicompost was packed in gunny bags and stored before application into the fields.

2.2. Antagonistic activity of Trichoderma harzianum and Penicillium spp against root rot pathogens

Trichoderma harzianum and *Penicillium* spp were tested against the isolated pathogenic *F. solani, F. oxysporum, P. ultimum* and *M. phaseolina in vitro* using the dual culture technique. Each pathogen was inoculated on sterilized PDA and grown for a period of seven days at 25 °C. The antagonist was inoculated on the plate with the established pathogen using a 5 mm culture disc. Plates inoculated with pure cultures of root rot pathogens were used as controls. The plates were then incubated at room temperature for a period of nine days. Each treatment was replicated three times. Observations of the plates was done daily and the growth antagonism ratings were recorded using the modified scale of class 1 to 5 (Bell *et al.,* 1982) where; Class 1 (R1) = the antagonist completely overgrows the pathogen. Class 2 (R2) = the antagonist overgrows at least ¾ of pathogen surface. Class 3 (R3) = the antagonist colonized at least half of the pathogen. Class 4 (R4) = the pathogen locked at point of contact with the antagonist. Class 5 = the antagonist overgrown by the pathogen. Percent inhibition

on growth of the pathogenic fungi by the antagonist was then calculated after measuring the radial growth of the test pathogens in control, as well as in dual culture plates. The formula used was:

$$Percent\ inhibition\ radial\ growth\ of\ pathogen(\%) = \frac{(c - T)}{c} \times 100$$

where *C* is radial growth of the pathogen (mm) in control; *T* is Radial growth of the pathogen (mm) in the treatment

2.3. Antagonistic effect of *Trichoderma harzianum and Penicillium spp against root rot pathogens on a slide culture*

A clean slide was placed on a v-shaped glass rod and autoclaved. Inside a 9 cm petri dish, a thin film of molten water agar was evenly spread on the slide leaving one end free of medium to enable handling. The slide with the medium was inoculated at a distance of 1 cm with the pathogen and a bio control agent separately following the technique by Sivakumar *et al.* (2000). Sterile water was then added to the petri dish at a rate 1 mL to prevent drying during incubation at 25 °C for seven days. On incubation, the regions where the two fungi met was observed under microscope at the end of the incubation period and pictures taken of wall disintegration of the pathogen which was indicated by crumbling of the mycelium or coiling structures produced by the antagonist. The treatments were replicated three times.

2.4. Effect of water extracts of biochar and vermicompost on root rot pathogens

Extraction of biochar water-extractable substances (BWES) from biochar was done according to Smith *et al.* (2012). Fifty grams of each biochar was soaked separately in 200 mL of nano pure water in 500 mL conical flasks. The biochar and water mixture was shaken at 100 rpm for 24 h on a mechanical shaker. The mixture was transferred to 12 cm diameter Buchner funnel lined with Whatman Grade-1 qualitative filter paper (11μm pore size) and vacuum filtered. The collected filtrate was frozen and lyophilized to obtain and determine the volume of BWES in a dry state. This also was to enable preparation of known concentrations of stock solutions. The remaining biochar solid was stored for further testing for adsorption of seed exudates. The BWES were redissolved in sterile nano pure water to make the stock solution from which different concentrations of total BWES were made and used for experiment. Vermicompost extract was obtained after mixing 50 g vermicompost with 100 mL of sterile distilled water. The mixture was then shaken for 5 min on a mechanical shaker and left to stand at room temperature for an hour. This extract was then obtained by filtration using a Whatman filter paper, frozen and then lyophilized to determine the volume of the extract in dry state. The filtrate was stored at –20 °C until it was used for the assay on their effect on spore germination and mycelial growth. The pH of the water extracts of both biochar and vermicompost was measured at the aqueous state of the extracts which was done in triplicates following filtration.

2.5. Effect of water extracts of biochar and vermicompost on spore germination of root rot pathogens

Bioassays of the effect of BWES on spore germination were conducted for *Fusarium solani* and *Pythium ultimum* on water agar and SM+ Lecithin discs respectively due to their ability to produce spores. The germination experiments were conducted with BWES from the two biochars (sugarcane bagasse and rice husks). The spore and sporangia germination for the two fungi was done using the different concentrations of BWES (0%, 0.5%, 1%, 3%, 10% and 20%). A solution of root exudates collected from bean plants (extraction has been described below) and sterile water were used as the positive and negative control respectively. A volume of 200 μL of BWES was added to 200 μL of fungal spore suspension adjusted to 1 x 10^7 spores' ml^{-1} containing both macro and micro conidia of *F. solani* and sporangia of *P. ultimum* on SM+L discs were used for the assay. These were incubated at 25°C in a moist chamber. The experiment was conducted twice with five replicates. After 24 h, lacto phenol cotton blue and 0.03% acid fuchsin in 85% lactic acid were used to stain the conidia and sporangia so as to determine the germination microscopically by counting 200 microconidia per slide (Steinkellner *et al.*, 2008) and 200 sporangia on SM+L discs. A conidium / sporangium was considered germinated if the germ tube is visible under the microscope. The spore / sporangial germination / inhibition was calculated using the formula given below:

$$Percent\ Spore\ Germination = \frac{Number\ of\ spore\ germinated}{Total\ number\ of\ spores\ examined} \times 100$$

$$Percent\ inhibition\ in\ spore\ germination = \frac{SG}{SG - TSG} \times 100$$

where, *SG* = number of spores germinated in the positive control, *TSG* = total number of spores germinated in treatment

2.6. Effect of water extracts of vermicompost and biochar on mycelial growth of root rot pathogens

Four wells were cut in PDA media using a 5 mm diameter cork borer before introduction of 1 ml of biochar water extracts and vermicompost water extracts into two opposite 5 mm wells in separate plates. One milliliter of sterile distilled water which served as the negative control was introduced into the other two wells on each plate. A disc measuring 5 mm cut from a 5-day old culture of *F. solani, P. ultimum* and *R. solani* were placed at center of PDA plates separately. Evaluation of the plates was done after incubation of the five replicates for seven days at 25 ºC. Vermicompost and biochar extracts filtered using 0.2 μm pore Syringe filters were also evaluated for their effects on mycelium growth of *F. solani, P. ultimum* and *R. solani* based on the method by Szczech (1999) with minor modifications where biochar and vermicompost extracts were introduced into 5mm cored wells on the PDA as opposed to addition flooding the surface of the media with the extracts. The effect of water extracts was determined by the presence of an inhibition zone around the wells infused with the extracts. Diameters of the zones of inhibition were measured using a vernier caliper.

2.7. Effect of time of maturation of biochars on fungal growth

Biochars produced from sugarcane bagasse and rice husks were used together with sand at a ratio of 1:1 w / w for this assay translating to 2.5 g of biochar mixed with 2 g of sand. Biochars produced as described above were left to mature for 5 days, 10 days and 15 days at room temperature in glass jars under sterile conditions. Sand was wet sieved to 0.5 to 1 mm diameter, oven dried at 65 °C for 24 h, then autoclaved at 121 °C for 30 min at 15 psi. Five treatments were set up as outlined below to test both *F. solani* and *P. ultimum* for inhibition or promotion of mycelial growth when exposed to biochar. Treatments of biochar; biochar moistened with 5 mL of water; biochar mixed with sand; biochar and sand moistened; sand; moistened sand and PDA. These treatments were used for each batch of biochar exposed for varying period of time to air. All the treatments were replicated three times. Five grams of biochar was placed on a petri dish lid for the biochar and sand individual treatments while 2.5 g of sand and 2.5 g of biochar for biochar and sand treatments. Five millimeter agar plugs from vigorously growing 3 to 5 d-old-cultures of *F. solani* and *P. ultimum* were inoculated on petri plates with PDA media. Each inoculated plate was inverted and incubated for eight days at a temperature of 25 °C in sterile incubation boxes. The germinating cultures were observed and the diameters of the developing cultures were measured every two days from the day of plating up to the 8th day. Completely randomized design was used for the experiment which was run twice with three replications.

2.8. Effect of filtering bean seed and root exudates through biochar and vermicompost on root rot pathogen spore germination

Rosecoco bean seeds were used to extract seed exudates based on the method by Tambalo *et al.* (2014) with minor modifications. Seeds were sorted and only those with no visible cracks utilized for the experiment. A total of 80 seeds were weighed separately in four batches of 20. Each batch of seeds was surface sterilized in 2.5% Sodium hypochlorite for 3 min followed by washing with 70% ethanol for 3 min. The seeds were rinsed with sterile nano pure water three times. To each set of twenty (20) seeds that were surface sterilized seeds, sterile nano pure water was added at a ratio of 2 mL g^{-1} of seed. These were then shaken on a rotary shaker (120 rpm) at a temperature of 22°C ± 2 for 8 h. Seed exudates from different batches were then collected separately. The seeds were then transferred into 150 cm^3 sterile 2 mm acid washed glass beads at 60% moisture content for an additional 48 h before being removed from the glass bead matrix. Harvesting of the root exudates was carried out by rinsing with 400 mL of sterile nano pure water, and strained through four layers of sterile cheesecloth or whatman filter paper No. 1 and then lyophilized. The lyophilized exudates were weighed, reconstituted in 15 mL sterile water then filtered using a 2 μm with sterile cellulose acetate syringe filters and relyophilized for a second time. The resulting powder was stored at –80 °C and reconstituted prior to use in the bioassays described below. Spore germination was conducted using filtrates from the biochars and vermicompost with water, the seed and root exudates being the control.

2.9. Adsorption of bean seed and root exudates by biochar and vermicompost

Adsorption of bean seed and root exudates by biochar was done by filling 25 g of each biochar and vermicompost as substrates into separate 12 cm diameter Buchner funnels. Sterile distilled water was added to the substrates in the funnels up to field capacity and the excess water let to drain for 2 h. Bean seed exudates were then added to the moist substrates in the funnels at a volume of 50 mL. The biochars and vermicompost were then covered with a perforated parafilm to create moist chamber conditions in the funnel. These were then incubated for

12 h. The substrates were then flooded with sterile distilled water and left to sit for 15 min after which the funnels were drained. The water collected was strained through whatman filter paper No. 1 and lyophilized. The bean seed and root exudates were assessed separately for presence of phytochemicals in the fresh exudates and in the filtrate collected after straining the exudate through biochar and vermicompost to determine presence of alkaloids, flavonoids, fatty acids, phenols and amino acids.

Alkaloids were tested by mixing 2 mL of each test sample with 1 mL dilute hydrochloric acid and 1 mL Mayer's reagent. The formation of a white precipitate indicated the presence of alkaloids. Presence of flavonoids was tested by mixing 2 mL of the extracts with magnesium granules (100 mg) in 0.5 mL of concentrated HCl. Presence of the flavonoids was denoted by the appearance of a red color within 2 min of mixing all the reagents (Markham, 1982). The exudates were tested for phenols by adding a few drops of ferric chloride solution to 2 mL to the test solution. A bluish green or red color indicated the presence of phenols (Kardong *et al.*, 2013).

3. Data collection and analysis

Data on inhibition of fungal growth (mm) and spore germination was collected ten days after incubation using a vernier caliper and counting microscope respectively. Dry weight of water extracts of biochar and vermicompost as well as that of seed and root exudates was measured after their lyophilization. Counts on pathogen spore and sporangial germination was recorded at intervals of 12 h. The presence or absence of phytochemicals in the seed and root exudates was determined by the color change in the test samples. The data of all variables measured were averaged and the ANOVA ($p < 0.05$) conducted using GENTSTAT 15th Edition. Means for treatments were separated by LSD (Least Significant Difference) following Tukey multiple dispersal range.

4. Results

4.1. Antagonism of Trichoderma harzianum and Penicillium spp on root rot pathogens of common bean

There was significant difference ($p < 0.05$) in inhibition of mycelial growth of all the test fungi in dual culture with *Trichoderma harzianum* and *Penicillium* spp (Table 1; Plate 1, 3, 5, 7). The highest inhibition was recorded for *T. harzianum* against *F. oxysporum* while the lowest inhibition was observed for *Penicillium* spp against *R. solani*. Significant difference ($p < 0.05$) was observed in percent inhibition of *Trichoderma* spp against all the test pathogens. *T. harzianum* recorded the highest inhibition against *F. oxysporum* at 67.7% while the lowest inhibition by *T. harzianum* was recorded in *M. phaseolina* at 45.3%. Percent inhibition in *Penicillium* spp dual plates was highest on *M. phaseolina* at 39.9% and the lowest on *R. solani* at 35.8% though the differences were not significant (Table 1; Plate 1, 2, 3, 4, 5, 6).

Table 1: Antagonism of *Trichoderma harzianum* and *Penicillium* spp on root rot pathogens of common bean

Pathogen	Radial mycelial growth (mm)			Percent inhibition (%)	
	Control	*T. h*	*P. s*	*T. h*	*P. s*
F. oxysporum	68.4b	22.1d	43.3b	67.7a	36.6a
F. solani	67.1b	31.8c	42.9b	52.7b	36.1a
M. phaseolina	66.6b	36.3b	41.8b	45.3c	39.9a
P. ultimum	85.9a	39.1a	52.1a	54.4b	39.4a
R. solani	79.3a	35.0b	49.4a	55.8b	35.8a
LSD	6.5	2.0	2.8	5.3	4.8
%CV	4.7	3.2	3.3	5.1	6.8
F.Pr	<0.001	<0.001	<0.001	<0.001	0.247

Note: Means with same letter(s) within a column not are significantly different at $p \leq 0.05$. *T. h – Trichoderma harzianum, P. s – Penicillium* spp, LSD – Least significant difference at 5% level, and CV – Coefficient of variation.

Table 2: Effect of *Trichoderma harzianum* and *Penicillium* spp on mycelial growth of bean root rot pathogens

Pathogen	Time taken to contact (days)		Time taken to overlap (days)		Bell's ranking (class)	
	T. h	***P.* spp**	*T. h*	***P.* spp**	***T. h***	***P.* spp**
F. oxysporum	3.3a	4.7a	4.3a	Loc	R2	R4
F. solani	3.3a	4.7a	4.3a	Loc	R2	R4
M. phaseolina	3.0a	0.0c	0.0c (Loc)	Loc	R4	R4
P. ultimum	2.3ab	3.3b	2.3b	Loc	R3	R4
R. solani	1.3b	3.7ab	3.7ab	Loc	R2	R4
LSD	1.3	1.0	1.1			
%CV	25.6	15.8	19.2			
F.Pr	0.031	<0.001	<0.001			

Note: Means with same letter(s) within a column are not significantly different at $p \leq 0.05$. *T. h – Trichoderma harzianum, P. s – Penicillium* spp, Loc – Locked at point of contact; LSD – Least significant difference at 5% level, and CV – Coefficient of variation.

Plate 1: Dual plate culture of *F. solani* and *Penicillium* sp. on PDA

Plate 2: Mycelial growth of *F. solani* on PDA (control)

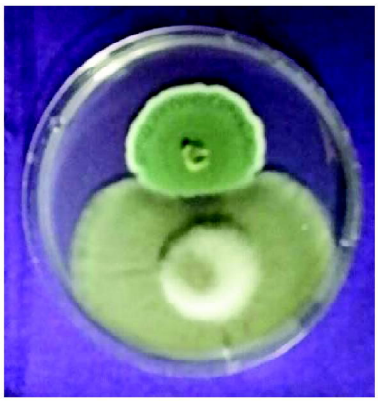

Plate 3: Dual plate culture of *Fusarium oxysporum* and *Pencillium* sp on PDA

Plate 4: Mycelial growth of *Fusarium oxysporum* on PDA (control)

Plate 5: Dual plate culture of *Macrophomina phaseolina* and *Penicillium* spp on PDA

Plate 6: Mycelial growth of *Macrophomina phaseolina* on PDA (control)

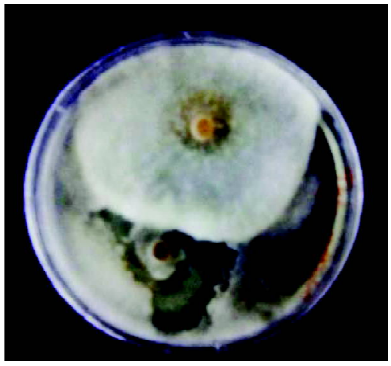

Plate 7: Dual plate culture of *P. ultimum* and *Trichoderma harzianum* (Magnification X 100)

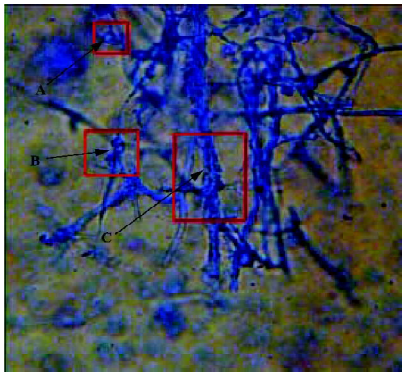

Plate 8: Mycelia of Trichoderma harzianum parasitizing *M. phaseolina* A: Production of an appresorium by *Trichoderma harzianum* upon contact with *M. phaseolina*, B: *Trichoderma* spp mycelium twisting around *M. phaseolina mycelium*, C: Lysed mycelium of *M. phaseolina* after parasitization (Magnification X 100)

There was a significant difference ($p < 0.05$) in the time taken for the antagonists to contact the pathogens in the dual plates. The shortest period to establish contact was recorded for *T. harzianum* against *R. solani* while the longest period was recorded for *Penicillium spp* against the two *Fusarium* spp (Table 2). *Penicillium* spp were observed to be locked with all the test pathogens upon contact after 10 days of incubation thereby being rated R4 according to the ranking by Bell for all the test pathogens (Table 2; Plate 1, 3 and 5). *T. harzianum* on the other hand was observed to overlap the test pathogens upon contact and achieved 75% overgrowth on *F. solani, F. oxysporum* and *R. solani* as a result being ranked R2. *T. harzianum* antagonism was however ranked R3 against *P. ultimum* when it was observed to achieve 50% overgrowth following 10 days of incubation. It was however observed to be locked upon contact with *M. phaseolina* and was rated as R4. Observations made from the slide culture showed *Trichoderma harzianum* coiling around the mycelium of *Macrophomina phaseolina* and production of appresorium after five days of incubation (Plate 8). The disintegration of the pathogen observed indicated by crumbling of the mycelial walls.

4.2. Quantity and pH of water extracts from biochar and vermicompost

Significant differences ($p < 0.05$) were observed in the water extractible substances from the organic amendments both in trial one and two (Table 3). Sugarcane bagasse biochar BWES and rice husks BWES had alkaline pH though the sugarcane bagasse was highest of the two. Vermicompost on the other hand had a near neutral pH. Concentration of water extractable substances was significantly different ($p < 0.001$) across all the three samples. Vermicompost had the highest concentration in both experiment 1 and 2 translating to 35% higher than rice husks BWES and 20% higher than sugarcane bagasse BWES.

4.3. Effect of water extracts of vermicompost and biochars on spore germination

There was a significant difference ($p < 0.001$) in the germination of *F. solani* spores (Plate 9) and *Pythium ultimum* sporangia (Plate 10) across all the concentrations of water-extractable substances and seed exudates (Table 4).

Table 3: Quantity, concentration and pH of water extracts of different biochars and vermicompost

Sample	Experiment 1			Experiment 2		
	pH	WES wt/ 250 g	Conc. WES (gm/L)	pH	WES wt/ 250 g	Conc. WES (gm/L)
Sugarcane bagasse biochar	9.33a	0.06b	0.64b	9.30a	0.07a	0.70b
Rice husks biochar	8.47b	0.05c	0.52c	8.48b	0.05b	0.50c
Vermicompost	6.92c	0.08a	0.81a	6.84c	0.08a	0.80a
MEAN	**8.24**	**0.07**	**0.66**	**8.21**	**0.07**	**0.67**
LSD	0.16	0.01	0.08	0.18	0.01	0.08
%CV	1.6	9.7	9.7	1.8	10.2	10.2
F pr.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Means with different letter(s) within each column are significantly different at $p \leq 0.05$. Conc. WES – concentration of water-extractable substances, SB – sugarcane bagasse, RH – rice husks, LSD – Least significant difference at 5% level, and CV – Coefficient of variation.

Spores of *F. solani* incubated in seed exudates recorded the highest germination percentage in seed exudates (SE). The highest was achieved in 20% concentration of SE. Significant inhibition of *F. solani* spores was observed in biochar water extractible substances (BWES) across all concentration gradients. Highest inhibition was observed in 20% rice husks BWES where 100% inhibition was achieved. However these differences in germination inhibition of *F. solani* spores in rice husks BWES were not significant from sugarcane bagasse BWES and vermicompost water extract. Similar observations were made for *F. solani* spore inhibition during the second experiment (Table 4).

Significant differences ($p < 0.05$) were also observed in sporangial germination of *P. ultimum* germination during the first experiment (Table 4). *Pythium ultimum* sporangia incubated in seed exudates had the highest germination which was more than 80% higher than other treatments. Root exudates on the other hand resulted in 50% higher germination of *P. ultimum* sporangia than water, vermicompost and biochar WES. The greatest inhibition in sporangia germination was observed in the rice husks BWES. It represented an almost 100% inhibition in 20% concentration of rice husks BWES. This was however not significantly different from sugarcane

Plate 9: Germinating Macro conidia of *F. solani* in seed exudates X100, GM: Germinating macro-conidium; GT: germ tube of *F. solani*

Table 4: Effect of different water extracts of biochars, vermicompost and bean root and seed exudates on spore germination of *F. solani* and *P. ultimum*

Treatment	Experiment 1						Experiment 2					
	F. solani spores			*P. ultimum* sporangia			*F. solani spores*			*P. ultimum sporangia*		
Conc.	3%	10%	20%	3%	10%	20%	3%	10%	20%	3%	10%	20%
R. husks biochar WES	1.0d	0.3d	0.0d	0.4d	0.3d	0.1d	1.3c	0.5c	0.2d	0.9c	0.7d	0.6d
Root exudates	18.9b	29.9b	36.4b	13.1b	24.9b	34.3b	16.3b	27.4b	34.3b	12.7b	26.9b	35.2b
S. bagasse biochar WES	0.2d	0.1d	0.1d	0.9d	0.4d	0.2d	2.9bc	1.0c	1.2d	1.7c	0.9d	0.4d
Seed exudate	53.8a	59.2a	69.5a	68.6a	72.1a	84.3a	56.3a	63.2a	78.2a	63.2a	70.1a	83.5a
Vermicompost WES	1.7d	0.8d	0.1d	1.0d	0.9d	0.3d	2.1c	0.6c	0.4d	1.1c	0.4d	0.2d
Water	8.2c	7.5c	8.0c	6.5c	6.1c	6.1c	16.7b	16.5b	16.1c	12.7b	12.0c	11.6c
LSD	4.5	3.5	6.0	5.0	5.0	5.2	13.9	14.2	14.1	9.7	8.8	10.3
%CV	24.8	16.4	24.2	25.2	22.1	19.1	66.9	60.0	49.5	48.3	36.3	35.9
F pr.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Means with same letter(s) within a column are not significantly different at $p \leq 0.05$. Conc. WES- concentration of water-extractable substances, S bagasse WES-sugarcane bagasse biochar water-extractable substances, R husks WES-rice husks biochar water-extractable substances, Vermicompost WES – Vermicompost water-extractable substances, LSD – Least significant difference at 5% level, and CV – Coefficient of variation.

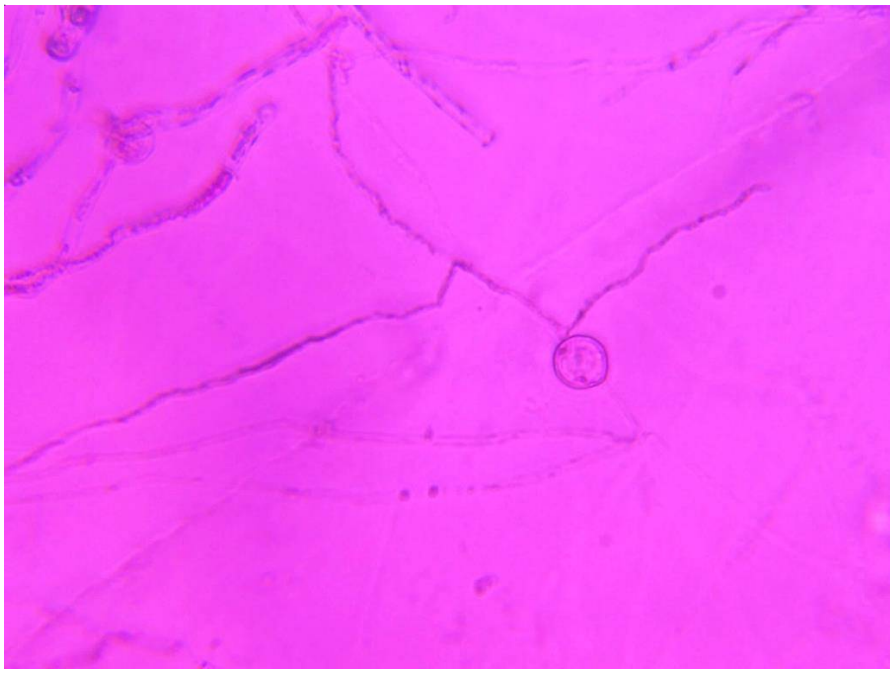

Plate 10: Sporangia of Pythium ultimum germinating in seed exudate X100 GS: Germinating sporangium of *P. ultimum*; EG: Elongating germ tube of *P. ultimum*

bagasse BWES and vermicompost WES which also inhibited sporangia germination significantly ($p < 0.05$) at all the concentration gradients. Similar observations were made in the repeat experiments where biochar and vermicompost WES significantly ($p < 0.05$) inhibited *F. solani* spore and *P. ultimum* sporangia germination (Table 6.4). Consequently, seed exudates did enhance the germination of the spores and sporangia significantly ($p < 0.05$) in all the concentrations by between 57% and 79%.

4.4. Effect of time of maturation of sugarcane bagasse biochar on fungal growth

Colony growth of *F. solani* and *P. ultimum* were affected when grown inverted above biochar exposed to air over different periods of time after production. There was a significant difference ($p < 0.05$) in the growth of *F. solani* across all the treatments of sugarcane bagasse (SB) biochar (Table 5). The SB biochar exposed to air for 15 days and slightly moistened had the highest colony growth on the second day after incubation. The lowest growth at the same period was recorded in SB biochar exposed to air for 5 days that was moistened representing a 26% inhibition of growth. After eight days of incubation, the greatest inhibition of *F. solani* growth was observed in SB biochar exposed to air for 5 days as compared to the one exposed for 15 days with the differences being significant ($p < 0.05$). Similar observations were made in the second experiment where the 5 day exposed biochar resulted in a 14% inhibition of *F. solani* growth (Table 6).

There was a significant difference ($p < 0.001$) in growth of *P. ultimum* colonies over the eight day period when incubated inverted over plates with SB biochar (Table 5). The greatest inhibition was observed in SB biochar exposed to air for five days. This represented a 12% inhibition of *P. ultimum.* Biochar exposed to air for 15 days resulted in the lowest inhibition of 1% which was not significantly different from the control. Similar observations were made in the repeat experiments where 15% inhibition of *P. ultimum* growth was observed in freshly produced SB biochar (Table 6).

4.5. Effect of time of maturation rice husks biochar on growth of Fusarium solani and Pythium ultimum

Significant differences ($p < 0.05$) in growth of *F. solani* and *P. ultimum* were also observed in the trials with rice husks (RH) biochar (Table 7). Significant difference ($p < 0.05$) in the diameter of *F. solani* colonies was observed across all the treatments with the exception of the fourth day after incubation. At the sixth day of growth, the highest growth was recorded in plates incubated over sand while the lowest was recorded in plates incubated over freshly produced RH biochar. Upon termination of the experiment on the eighth day, the lowest growth was recorded in freshly produced RH biochar while colonies incubated over RH biochar exposed for 15 days recorded the highest growth. This translated to a growth inhibition of 19%. Similar observations were made in the repeat experiment where significant differences ($p < 0.05$) were observed in *F. solani* colonies across all treatments (Table 10). *Fusarium solani* colonies that were incubated inverted over freshly produced RH biochar had the least growth. The highest growth of *F. solani* colony was recorded in RH biochar that had been exposed to air for 15 days. The reduction reflects an 18% growth inhibition.

Significant differences ($p < 0.05$) were also recorded in the growth of *P. ultimum* colonies across all treatments (Table 7). Control plates with PDA recorded the highest growth across the 8-day period with diameters ranging from 16.5 mm to 78.8 mm. These were not significantly different from the growth of colonies incubated over RH

Table 5: Effect of time of maturation of sugarcane bagasse biochar on colony growth (mm) of *Fusarium solani* and *Pythium ultimum* – experiment 1

Treatment	*Fusarium solani* colony growth (mm) over 8days				*Pythium ultimum* colony growth (mm) over 8 days			
	Day 2	Day 4	Day 6	Day 8	Day 2	Day 4	Day 6	Day 8
PDA (Control)	13.4def	31.2abc	43.0ab	53.6a	16.6a	34.3a	53.9a	78.8a
Sand	13.4def	32.2a	44.1a	52.3bcd	16.2ab	33.7ab	53.6a	77.4ab
Sand + W	13.3def	31.8ab	43.6a	52.4bc	15.7ab	33.5abc	53.7a	78.2a
Sugarcane bagasse biochar fpd	12.5f	27.4fg	38.3g	48.9g	10.3f	24.5ij	44.4de	69.0d

Table 5 (Cont.)

Treatment	*Fusarium solani* colony growth (mm) over 8days				*Pythium ultimum* colony growth (mm) over 8 days			
	Day 2	Day 4	Day 6	Day 8	Day 2	Day 4	Day 6	Day 8
Sugarcane bagasse biochar +W fpd	13.7def	27.2g	38.4g	49.6fg	11.7def	25.1hij	44.7d	70.2d
Sugarcane bagasse biochar +S fpd	12.3g	27.2g	40.6de	51.3de	11.9def	25.9ghi	42.1e	70.4cd
Sugarcane bagasse biochar +S+W fpd	12.0g	29.5cde	40.2def	51.4cde	13.4cd	23.8j	43.7d	68.6d
Sugarcane bagasse biochar 10d	13.3def	27.6fg	38.8fg	49.4fg	10.6f	26.3fgh	48.0b	74.1bc
Sugarcane bagasse biochar +W 10d	15.6ab	29.5cde	40.0efg	49.6fg	13.2cd	30.8d	45.6cd	76.3ab
Sugarcane bagasse biochar +S 10d	12.9efg	29.0def	41.3cde	52.2bcd	12.7cde	30.6d	45.7bcd	76.5ab
Sugarcane bagasse biochar +S+W 10d	14.1cd	30.1bcd	41.3cde	51.8bcd	14.4bc	27.4efg	45.4cd	77.5ab
Sugarcane bagasse biochar 15d	14.6c	28.3efg	39.7efg	49.9fg	11.2ef	27.7ef	52.6a	77.3ab
Sugarcane bagasse biochar +W 15d	16.4a	30.0cde	41.2cde	50.4ef	13.5cd	32.0bcd	48.0b	77.1ab
Sugarcane bagasse biochar +S 15d	14.7bc	30.1bcd	42.6abc	52.4bc	14.2c	31.8cd	47.4bc	78.1a
Sugarcane bagasse biochar +S+W 15d	14.9bc	30.9abc	41.7bcd	52.6ab	15.7ab	28.1e	47.1bc	77.3ab
LSD	0.9	1.7	1.6	1.0	1.9	1.7	2.3	3.8
%CV	3.9	3.4	2.3	1.2	8.4	3.5	2.9	3.1
F pr.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Means with same letter(s) within a column are significantly different at $p \leq 0.05$. fpd-freshly produced biochar, 10d – exposed to air for 10 days after production, 15d – exposed to air for 15 days after production, +S – addition of sand, +W – addition of water, PDA – potato dextrose agar, LSD – Least significant difference at 5% level, and CV – Coefficient of variation.

Table 6: Effect of time of maturation of sugarcane bagasse biochar on colony growth (mm) of *Fusarium solani* and *Pythium ultimum* - experiment 2

Treatment	*Fusarium solani* colony growth (mm) over 8days				*Pythium ultimum* colony growth (mm) over 8 days			
	Day2	Day4	Day6	Day8	Day2	Day4	Day6	Day8
PDA (Control)	18.3abc	31.9a	43.2a	55.0a	17.6a	36.7a	52.3ab	76.2a
Sand	18.5ab	31.6a	42.1ab	51.2bc	18.5a	31.6bcd	52.4ab	75.8ab
Sand +W	18.6a	29.8abc	42.0ab	51.4bc	18.6a	29.8cde	53.0a	75.4abc
Sugarcane bagasse biochar fpd	13.5gh	27.1bc	39.3de	48.9de	11.2e	22.8gh	46.4d	69.3fg
Sugarcane bagasse biochar +W fpd	14.1g	26.9c	39.7cd	50.3cd	12.2de	26.1efg	45.4de	69.3fg
Sugarcane bagasse biochar +S fpd	12.7h	29.5abc	39.9cd	47.3e	12.9cd	24.8fgh	37.0g	67.9g
Sugarcane bagasse biochar +S+W fpd	13.7gh	27.9bc	37.8e	47.0e	13.1cd	22.1h	37.7fg	64.2h
Sugarcane bagasse biochar 10d	14.9efg	27.8bc	40.3bcd	50.4bcd	12.6de	28.9de	48.3bcd	73.0cde
Sugarcane bagasse biochar +W 10d	14.9efg	28.2bc	40.4bcd	51.2bc	13.4cd	30.0cd	48.0cd	72.3de
Sugarcane bagasse biochar +S 10d	15.9de	29.0bc	40.9bcd	50.5bcd	13.7cd	28.5def	38.7fg	70.5ef
Sugarcane bagasse biochar +S+W 10d	14.8fg	30.1ab	41.3bc	50.4bcd	14.3bc	26.1efg	39.4fg	70.6ef
Sugarcane bagasse biochar 15d	15.8def	28.3bc	42.1ab	51.6bc	13.8cd	34.7ab	51.9abc	75.9ab
Sugarcane bagasse biochar +W 15d	16.4de	29.7abc	41.8ab	52.4b	14.3bc	33.4abc	51.1abc	74.7abcd
Sugarcane bagasse biochar +S 15d	17.1abc	29.4abc	41.9ab	51.1bc	15.6b	30.1cd	41.4ef	73.4bcd
Sugarcane bagasse biochar +S+W 15d	16.9cd	27.6bc	41.8ab	51.6bc	15.9b	29.3de	41.1fg	72.6de

Table 6 (Cont.)

Treatment	*Fusarium solani* colony growth (mm) over 8days				*Pythium ultimum* colony growth (mm) over 8 days			
	Day2	Day4	Day6	Day8	Day2	Day4	Day6	Day8
LSD	1.5	3.0	1.8	2.0	1.6	3.7	4.1	2.5
%CV	5.9	6.2	2.7	2.4	6.5	7.8	5.4	2.1
F pr.	<0.001	0.045	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Means with same letter(s) within a column are significantly different at $p \leq 0.05$. fpd-freshly produced biochar, 10d-exposed to air for 10 days after production, 15d exposed to air for 15 days after production, +S- addition of sand, +W-addition of water, PDA- potato dextrose agar, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

Table 7: Effect of time of maturation rice husks biochar on colony growth (mm) of *Fusarium solani* and *Pythium ultimum* - experiment 1

Treatment	*Fusarium solani* colony growth (mm) over 8days				*Pythium ultimum* colony growth (mm) over 8 days			
	Day2	Day4	Day6	Day8	Day2	Day4	Day6	Day8
PDA (control)	13.4	31.2	43.0ab	53.6ab	16.6a	34.3a	53.9a	78.8a
Sand	13.4	32.2	44.1a	52.3bcd	16.2a	33.7a	53.6a	77.4ab
Sand + W	13.3	31.8	43.6a	52.4bcd	15.7ab	33.5a	53.7a	78.2a
Rice husks biochar fpd	12.1	24.7	32.0g	43.9g	10.8de	27.0e	45.9cde	71.3cd
Rice husks biochar + W fpd	12.0	28.4	35.5f	45.2g	12.1cde	27.1e	44.5e	70.8cd
Rice husks biochar + Sand fpd	12.6	27.0	39.3e	50.9cdef	10.5e	24.6f	43.7e	71.3cd
Rice husks biochar + Sand +W fpd	12.3	26.5	39.4e	51.8bcde		11.3cde	23.6f	42.7e 68.8d
Rice husks biochar 10d	13.0	28.8	40.7bcde	48.9f	12.7bc	28.9cd	48.9bc	73.6bc
Rice husks biochar +W 10d	12.4	26.3	40.5cde	49.6ef	13.0bc	28.6de	51.9ab	73.7bc
Rice husks biochar + Sand 10d	13.0	30.5	41.2bcde	52.2bcd	12.4bcd	28.3de	44.8e	74.7abc

Table 7 (Cont.)

Treatment	*Fusarium solani* colony growth (mm) over 8days				*Pythium ultimum* colony growth (mm) over 8 days			
	Day2	Day4	Day6	Day8	Day2	Day4	Day6	Day8
Rice husks biochar + Sand +W 10d	12.7	29.1	40.2de	52.0bcd	12.6bc	30.5bc	44.9e	73.2bc
Rice husks biochar 15d	13.2	29.2	41.2bcde	50.2def	13.0bc	30.8bc	52.2ab	77.3ab
Rice husks biochar + W 15d	12.8	28.5	41.0bcde	52.0bcd	14.0b	30.5bc	52.7a	76.2ab
Rice husks biochar + Sand 15d	13.3	31.2	42.8abc	54.7a	13.0bc	29.6cd	45.3de	76.9ab
Rice husks biochar + Sand +W 15d	13.2	31.0	41.9abcd	53.1abc	12.9bc	31.8b	48.4cd	75.7ab
LSD	NS	NS	2.3	2.2	1.7	1.7	3.4	4.2
%CV	4.7	13.2	3.5	2.5	7.7	3.4	4.3	3.4
F pr.	0.1	0.469	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Means with same letter(s) within a column are significantly different at $p \leq 0.05$. fpd-freshly produced biochar, 10d-exposed to air for 10 days after production, 15d exposed to air for 15 days after production, +S- addition of sand, +W-addition of water, PDA- potato dextrose agar, LSD: Least significant difference at 5% level, CV: Coefficient of variation.

biochar exposed for 15 days to air. The least growth was however recorded in plates inverted over RH biochar exposed to air for five days, being significantly different ($p < 0.05$) from those grown over RH biochar exposed for 10 days and 15 days. This indicated a growth inhibition of 12% for colonies incubated over freshly produced RH biochar. Similar observations were made during the follow up experiments where significant differences ($p < 0.05$) were recorded across all the treatments (Table 8). The lowest growth was recorded for plates incubated over freshly produced RH biochar while those incubated over RH biochar exposed for 15 days had the highest diameter. This difference in growth represents an inhibition of 33%.

4.6. Adsorption of bean seed exudates by biochar and vermicompost

Quantities of seed and root exudates varied amongst the different seed weights and seed sets of Rose coco beans though the differences were not significant (Table 9). The highest yield was recorded as 7.8 mg of seed exudate and 6.7 mg of root exudates per gram seed though the difference was not significant($p < 0.05$). Phytochemical analysis of the seed exudates indicated high concentration of flavonoids and phenols in bean seed exudates (Table 10; Plate 13; Plate 14). Only phenols were detected in the root exudates. Other phytochemicals such as alkaloids, fatty acids and amino acids were not detected in both the seed and root exudates (Plate 12; Plate 15). Upon passing the exudates through moistened biochar and vermicompost, there was no detection of flavonoids and phenols for exudates passed through SB biochar and RH biochar. However phenols were detected in root exudates passed through vermicompost (Table 10; Plate 14). These results indicated that the phytochemicals had not passed through the substrates (Table 10).

Table 8: Effect of time of maturation rice husks biochar on colony growth (mm) of *Fusarium solani* and *Pythium ultimum* - experiment 2

Treatment	*Fusarium solani* colony growth (mm) over 8days				*Pythium ultimum* colony growth (mm) over 8 days			
	Day 2	Day 4	Day 6	Day 8	Day 2	Day 4	Day 6	Day 8
PDA (Control)	18.3a	31.9a	43.2a	55.0a	17.6ab	36.7a	52.3a	76.2a
Sand	18.5a	31.6a	42.1ab	51.2bcd	18.5ab	31.6bcd	52.4a	75.8a
Sand +W	18.6a	29.8ab	42.0ab	51.4bcd	18.6a	29.8cde	53.0a	75.4ab
Rice husks biochar fpd	12.1c	24.9d	37.0ef	44.6h	10.4i	27.7def	36.4g	67.0g
Rice husks biochar +W fpd	12.0c	26.7bcd	36.2f	46.9g	12.1fghi	27.4efg	37.0fg	69.6ef
Rice husks biochar +Sand fpd	12.3c	26.4bcd	39.6bcde	48.9ef	11.2hi	21.9h	39.4ef	59.9h
Rice husks biochar +Sand +W fpd	12.3c	25.2cd	37.4def	46.7g	11.3ghi	21.5h	40.5e	50.5i
Rice husks biochar 10d	13.8c	29.5ab	39.9bcd	50.2de	12.1fghi	29.9cde	43.9d	72.9bcd
Rice husks biochar +W 10d	12.5c	28.3abcd	38.8cde	48.3fg	13.1efg	29.6cde	43.6d	72.5cd
Rice husks biochar +Sand 10d	13.8c	29.3abc	41.6a	50.8cd	13.4def	24.6fgh	43.4d	66.0g
Rice husks biochar +Sand +W 10d	13.1c	28.1abcd	40.2bc	50.6de	13.0efgh	23.6gh	43.3d	67.1fg
Rice husks biochar 15d	14.2bc	30.9a	41.0abc	52.5bc	14.0de	31.8bc	48.9b	75.2ab
Rice husks biochar +W 15d	14.4bc	30.2ab	40.6abc	51.4bcd	15.2cd	34.1ab	47.4bc	75.0abc
Rice husks biochar +Sand 15d	16.3ab	31.0a	41.9ab	53.0b	16.7bc	29.6cde	45.6cd	68.9ef
Rice husks biochar +Sand +W 15d	13.4c	29.6ab	42.1ab	51.4bcd	16.9abc	28.2cdef	48.4b	71.1de
LSD	2.4	4.1	2.7	1.8	1.8	3.9	2.6	2.6
%CV	9.9	8.5	4.0	2.1	7.7	8.2	3.5	2.2
F pr.	<0.001	0.018	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Means with same letter(s) within a column are significantly different at $p \leq 0.05$. fpd-freshly produced biochar, 10d – exposed to air for 10 days after production, 15d – exposed to air for 15 days after production, +S – addition of sand, +W – addition of water, PDA – potato dextrose agar, LSD – Least significant difference at 5% level, and CV – Coefficient of variation.

4.7. Effect of filtered bean seed exudates through biochar and vermicompost on spore germination of root rot pathogens

Significant differences ($p < 0.05$) were observed in conidial and sporangial germination of *F. solani* and *P. ultimum* respectively in the root and seed exudates filtered through the SB biochar, RH biochar and vermicompost (Table 11). The highest percent spore germination was observed in *F. solani* incubated in fresh seed exudates while the lowest was recorded in vermicompost strained seed exudates. There was a 96% to 99% and 49% to 97% inhibition of germination *F. solani* for seed and for root exudates respectively. Similar observations were made in the repeat experiment.

Germination of sporangia of *P. ultimum* in seed exudates recorded the highest percentage which was significantly different ($p < 0.05$) from other treatments (Table 12). Filtering the exudates through vermicompost and biochar resulted in significant inhibition of germination. Highest inhibition to germination was observed

Table 9: Quantities of seed and root exudates of different batches of Rosecoco bean seeds

Seed batch	Weight of seed (g)	Liquid seed exudate (mL)	Lyophilized seed exudate (g)	Liquid root exudates (mL)	Lyophilized root exudate (g)
1	9.32a	9.90a	0.06a	18.06a	0.06a
2	8.99a	9.40a	0.07a	18.18a	0.06a
3	8.02a	8.83a	0.05a	15.55a	0.05a
4	9.03a	9.63a	0.06a	17.24a	0.06a
LSD	2.29	1.69	0.03	4.28	0.05
%CV	8.1	5.6	15.8	7.8	26.9
F pr.	0.433	0.382	0.314	0.349	0.858

Note: Means with the same letter(s) within each column are not significantly different at $p \leq 0.05$; LSD – Least significant difference at 5% level, and CV – Coefficient of variation.

Table 10: Presence of different phytochemicals in bean seed and root exudates

Treatment	Alkaloids	Flavonoids	Phenols	Fatty acids	Amino acids
Seed exudate (SE)	–	++	++	–	–
Root exudates (RE)	–	–	+	–	–
Water	–	–	–	–	–
SE through S. bagasse	–	–	–	–	–
SE through R. husks	–	–	–	–	–
SE through vermicompost	–	–	–	–	–
RE through S. bagasse	–	–	–	–	–
RE through R. husks	–	–	–	–	–
RE through vermicompost	–	–	+	–	–

Note: – = Absent, + = Present, and ++ = Present in high concentration.

in seed exudates filtered through vermicompost at 99% inhibition. This was however not significantly different from filtering through the two biochars which resulted in a 97% inhibition of sporangial germination. Filtering of exudates resulted in a germination inhibition of between 50% and 80%, the greatest inhibition being observed in seed exudates strained through vermicompost. Similar observations were in the repeat experiments (Table 11).

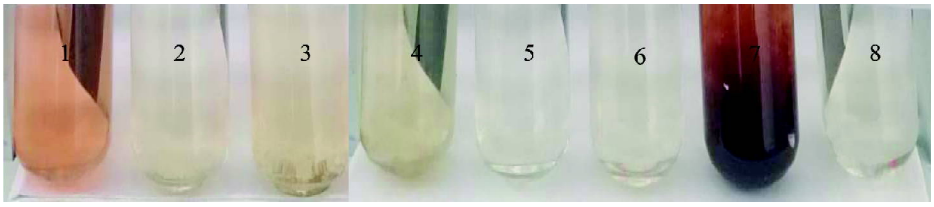

Plate 12: Presence of alkaloids in bean seed and root exudates

Note: 1-fresh seed exudate; 2-seed exudate passed through rice husks biochar; 3-seed exudates passed through sugarcane bagasse biochar; 4-fresh root exudates; 5-root exudates passed through sugarcane bagasse biochar; 6-root exudate passed through rice husks biochar; 7-root exudates passed though vermicompost; and 8-blank.

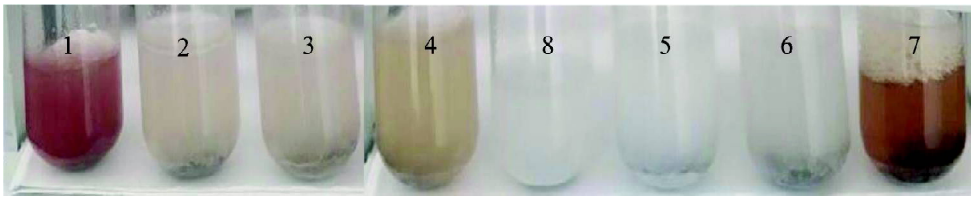

Plate 13: Presence of flavonoids in bean seed and root exudates

Note: 1-fresh seed exudate; 2-seed exudate passed through rice husks biochar; 3-seed exudates passed through sugarcane bagasse biochar; 4-fresh root exudates; 5-root exudates passed through sugarcane bagasse biochar; 6-root exudate passed through rice husks biochar; 7-root exudates passed though vermicompost; and 8-blank.

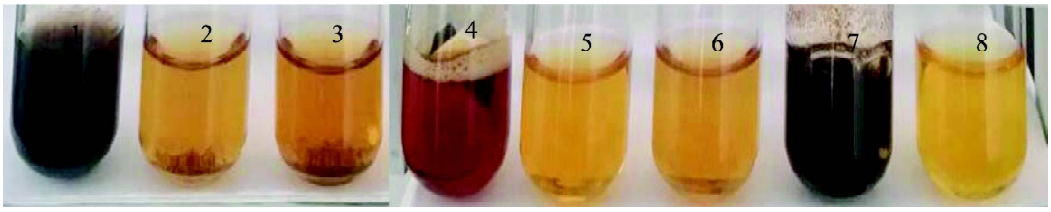

Plate 14: Presence of phenols in bean seed and root exudates

Note: 1-fresh seed exudate; 2-seed exudate passed through rice husks biochar; 3-seed exudates passed through sugarcane bagasse biochar; 4-fresh root exudates; 5-root exudates passed through sugarcane bagasse biochar; 6-root exudate passed through rice husks biochar; 7-root exudates passed though vermicompost; and 8-water.

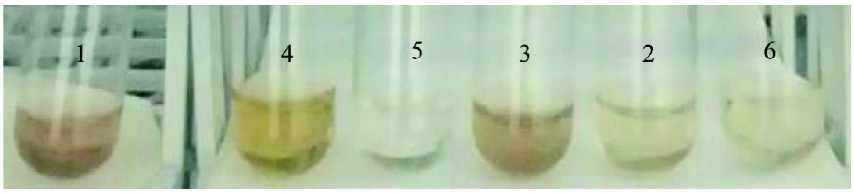

Plate 15: Presence of amino acids in bean seed and root exudates

Note: 1-fresh seed exudate; 2-seed exudate passed through rice husks biochar; 3-seed exudates passed through sugarcane bagasse biochar; 4-fresh root exudates; 5-root exudates passed through sugarcane bagasse biochar; and 6-root exudate passed through rice husks biochar.

5. Discussion

The increase in number of soil beneficial microorganisms following application of biochar and vermicompost in a study by Were [7] contributed to the decline in population of pathogenic fungi. The microorganisms that were observed to increase in number included *Trichoderma, Penicillium, Aspergillus, Paeciliomyces* and *Athrobotrys* spp. The decline in pathogenic microbes was due to competition for space as well as the production of toxic

Table 11: Effect of filtering bean seed and root exudates through vermicompost, sugarcane bagasse and rice husks biochars on spore germination of *F. solani* and *P. ultimum*

Treatment	Experiment 1 (% germination)		Experiment 2 (% germination)	
	F. solani spores (%)	*P. ultimum* sporangia (%)	*F. solani* spores (%)	*P. ultimum* sporangia (%)
Seed exudate (control)	53.8a	68.6a	56.3a	63.2a
Seed exudate through vermicompost	0.3d	0.5d	0.4c	0.6c
Seed exudate through RH biochar	1.9d	2.0d	1.0c	1.5c
Seed exudate through SB biochar	1.3d	1.4d	1.0c	0.8c
Root exudates (control)	18.9b	13.1b	16.3b	12.7b
Root exudates through vermicompost	9.6c	6.6c	11.4b	8.5b
Root exudates through RH biochar	0.4d	1.5d	0.5c	0.9c
Root exudates through SB biochar	0.5d	1.1d	0.6c	0.8c
LSD	7.9	8.4	12.3	9.5
%CV	28.1	27.6	43.6	33
Fpr	<0.001	<0.001	<0.001	<0.001

Note: Means with different letter(s) within each column are significantly different at $p \leq 0.05$. % – percent germination, SB Biochar – sugarcane bagasse biochar, RH Biochar – rice husks biochar, LSD – Least significance difference at 5% level, and CV – Coefficient of variation.

metabolites including antibiotics by the beneficial microorganisms. *In vitro* inhibition of the bean root rot fungi by antagonistic *Trichoderma spp* and *Penicillium spp* in dual culture pointed to different mechanisms for the two antagonists. These include production of fungal growth inhibitory substance(s) by the *Penicillium spp* (Sreevidya and Gopalakrishnan, 2016) and mycoparasitism and direct competition by *Trichoderma spp* (El-Sheshtawi *et al.*, 2014).

Growth inhibitory substances produced by the antagonists may diffuse through the media affecting growth and sporulation of the pathogen. This was observed in the plates with *Penicillium* spp which reduced the growth of *F. solani, F. oxysporum, P. ultimum, M. phaseolina* and *R. solani*. This resulted in a 35 to 40% inhibition of growth of all the five pathogens. This has been observed in previous studies by Graber *et al.* (2014) and Kolton *et al.* (2011) who reported on soil microorganisms that are promoted by biochar additions and thrive on biochar residues. They observed that these microorganisms compete with pathogens for resources, produce compounds that are toxic to pathogens, or parasitize pathogens. Graber *et al.* (2010) also observed that beneficial microorganisms promoted by biochar enhance plant growth directly, thus affecting plant susceptibility or resistance to disease. The findings of this study are similar to previous findings by Sreevidya and Gopalakrishnan (2016) reported on the control of *Macrophomina phaseolina* in sorghum using *Penicillium citrinum.* They observed that citrinin which is produced by many species of *Penicillium* was responsible for growth inhibition of *M. phaseolina*. Other studies by Melouk and Akem (1987) reported on the antagonistic activity of citrinin against soil borne plant pathogenic fungi such as *Rhizoctonia solani.*

Other mechanisms such as mycoparasitism and competition were also observed in this study and may be responsible for pathogen growth inhibition by the antagonists. The observed interaction between the mycelia of *Trichoderma* spp and *M. phaseolina* gives credence to mycoparasitism mechanism. In this study, *Trichoderma* spp mycelium was observed twisting around the mycelia of *M. phaseolina.* It was also observed to grow over test pathogens upon contact covering 75% of colony surfaces of *F. solani, F. oxysporum* and *R. solani*. Similar observations have been made in previous studies by El-Sheshtawi *et al.* (2014) who reported 50% reduction of *F. solani* f.sp *cucurbitae* colony diameter by *Trichoderma* spp. Ahmad and Baker (1987) and Boughalleb *et al.*

(2018) have also pointed to direct parasitism of *Trichoderma* on hyphae of other fungi. They observed that control of many soil borne plant pathogens occurs by production of extracellular lytic enzymes which degrade pathogen cell walls. Ramaraju *et al.* (2016) also reported on the mechanism of *Trichoderma* antagonism by coiling around fungal mycelia which they parasitize. They eventually produce penetration pegs that are haustoria like knobs which penetrate the host dissolving the protoplasm. This in turn may lead to shrinking of mycelia and eventual lysis thus achieving control.

Fusarium solani spores and *P. ultimum* sporangia germination were greatly inhibited by three concentrations of biochar and vermicompost water extracts as compared to seed exudates and root exudates. The water extractible substances reduced the germination of *F. solani* conidia and *P. ultimum* sporangia by more than 80%. The results are similar to those of Smith *et al.* (2016) who reported on growth inhibition of blueHgreen algae (*Synechococcus elongates*) and the eukaryotic alga (*Desmodesmus*) in biochar water extracts derived from pine wood biochar. Jack (2012) also reported the inhibition of *Pythium aphanidermatum* zoospores in vermicompost extract. The antifungal potential of biochar could be due to the water soluble organic compounds found in biochar which can affect soil and aquatic microorganisms (Graber *et al.*, 2014; and Fabbri *et al.*, 2012). Graber *et al.* (2010) identified these compounds known to adversely affect microbial growth and survival as ethylene glycol and propylene glycol, hydroxypropionic and hydroxybutyric acids, benzoic acid and *o*-cresol, quinones and 2-phenoxyethanol. Klinke *et al.* (2004) also reported of other biochar associated compounds known to inhibit microbial activity to include carboxylic acids, furans and ketones.

When the biochar and vermicompost water-extractable substances were tested on mycelia growth using the well diffusion technique, no inhibition of growth was observed at all the three concentrations. This pointed to the fact that the biochar water extracts are effective in inhibiting spores and sporangia germination but not on the somatic growth of the pathogens. Consequently, sterile vermicompost extract was also not found to have an effect on mycelia growth. Similar findings have been reported by Ersahin *et al.* (2009) on the loss of vermicomposts' inhibitory effect on mycelial growth following sterilization.

This study tested the effect of exposing biochar to air at staggered durations after production. This was then tested for its effect on colony growth of *F. solani* and *P. ultimum.* Radial growths of the microorganisms were significantly ($p < 0.05$) reduced when they were incubated over biochar. Freshly produced sugarcane bagasse biochar slightly moistened recorded significantly lower radial growth of *P. ultimum* as did the freshly produced rice husks biochar on growth of *F. solani* colony. This was in comparison to biochar exposed to air for 10 days and 15 days. When the freshly produced rice husks biochar was mixed with sand and slightly moistened it also significantly reduced radial growth of *P. ultimum.* Similar findings were observed in the repeat experiments. These findings point to production of compounds biochar that have inhibitory effects on fungal colony growth. This study showed that the effectiveness of these compounds wanes with prolonged exposure of biochar after production. No previous findings have reported on this phenomenon of the effect on fungal pathogens in biochar. However previous studies by Ghidotti *et al.* (2016) profiled the volatile organic compounds in biochar and reported of some harmful compounds such as benzene, ethylbenzene, xylenes and polycyclic aromatic hydrocarbons present in biochar. They reported on the compound not being released at ambient temperature when biochar is produced at temperatures greater than 400 ºC. These volatile organic compounds were however observed not be harmful to cress seed germination.

This study established adsorption of phytochemicals such as flavonoids and phenols present in seed exudates by vermicompost, rice husks and sugarcane bagasse biochar. This was achieved by straining seed exudates and root exudates through pre moistened biochar. When the rinsate was passed through biochars and vermicompost negative results for all the phytochemicals tested were obtained save for phenols which were positive in rinsate through vermicompost.

When the rinsate were tested for their effect on germination of *Pythium* sporangia and *Fusarium* conidia, it was observed to be greatly reduced as compared to that of fresh seed and root exudates. These findings point to the fact that the pathogen germination signaling molecules of *Pythium* and *Fusarium* were not present in the rinsates. These results give credence to suppositions by Masiello *et al.* (2015) and Graber *et al.* (2014) of fractional adsorption of signaling molecules by biochar. This in turn modifies the communication among soil biota leading to disease suppression by biochar. It is also important to note that biochar may have possibly changed the chemistry of the root exudates resulting in reduced spore and sporangia germination. According to findings by Silber *et al.* (2010), increased adsorption may be linked to the cation exchange capacity (CEC) of the biochar surface.

In this study, flavonoids and phenols were observed to be absorbed by biochar and vermicompost. This is of importance since flavonoids and phenols have been shown to be efficient anti-microbial agents which inhibit several root pathogens, especially fungal ones (Makoi and Ndakidemi, 2007). Importance of flavonoids in development of plant resistance to pathogenic microorganisms has been previously stated by Mierziak *et al.* (2014). They reported that these molecules' anti pathogenic activities can be nonspecific but result from their anti-oxidative properties. Dai *et al.* (1996) and Blount *et al.* (1992) also observed that these molecules lead to quenching of reactive oxygen species generated by both the pathogens and plants as a result of infection leading to control of disease.

By biochar and vermicompost exhibiting adsorption capabilities of the phytochemicals including flavonoids and phenols, points to enhanced concentration of these phytochemicals around the germinating plants. These may in turn enhance the protection of the plants both in a specific and a nonspecific manner. Previous studies by Beckman (2000) and Skadhauge *et al.* (1997) have also reported that flavonoids may contribute to the tightening of the plant tissues and structures by controlling the activity of auxin (IAA) in plants. This may lead to the differentiation of tissues and promotion of tylose, callus formation and closure of vascular system preventing pathogen infection. They also reported on other mechanisms such as inhibition of pathogen xylanases, pectinases and cellulases. Other studies by Treutter (2005) have shown that these phytochemical compounds may directly inhibit pathogen enzymes that digest plant cell wall. This they do by chelating metals that are required for the enzyme activity. Previous studies by Matern and Kneusel (1988) suggested that the rapid accumulation of phenols at infection site serves to slow and halt the growth of pathogens. This therefore acts as the first line of plant defense thereby allowing the plant to activate secondary mechanisms of pathogen control.

6. Conclusion

Biochar and Vermicompost greatly inhibit germination of spores and growth of root rot pathogens thus may end up protecting plants from pathogenic attack. The organic amendments therefore have the potential to suppress pathogenic microorganisms directly in the soil. The induced resistant microbial communities suppress pathogens in the soil environment. Biochar should not be stored over a long period of time prior to use in management of root rot diseases. Prolonged storage leads to reduction of its effectiveness against pathogen development.

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