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Antagonistic Activity of *Pseudomonas* Species Isolated from the Rhizosphere of Cowpea Against Some Fungal Pathogens

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Abstract

Soil samples collected from Crop, Soil and Pest departmental farm were subjected to chemical and microbiological analysis. The soil samples were then placed in polyethylene bags, inoculated with 0.2g of mancozeb, Benomyl and both Mancozeb and Benomyl respectively. Cowpea seeds (*Vigna unguiculata*) were planted in each of the experimental setup. Bacteria were isolated from the rhizosphere of cowpea treated with Mancozeb, Benomyl and combination of both of them. *Pseudomonas* species isolated from the rhizosphere of cowpea treated with Benomyl or / and Mancozeb was used to inhibit some fungal pathogen of plant origin. The chemical analysis revealed the soil to be acidic and high in Calcium, Phosphorus and Magnesium. The bacterial count of the soil before the planting of cowpea was 4.0 X 10^8, 10.33±3.09^a in Mancozeb treated soil and 6.03±0.888^b in Mancozeb and Benomyl treated soil. The bacterial isolated from the soil before planting of cowpea were *Actinomyces viscosus*, *Pasteurella species*, *Corynebacteria renale*, *Pseudomonas capacia*, *Bacillus pumilus*, *Pseudomonas stutzeri*, *Bacillus subtilis* while *Bacillus pumilus*, *Corynebacterium renale*, *Bacillus macerans*, *Actinomyces viscosus*, *Pseudomonas stutzeri*, *Bacillus subtilis*, *Corynebacterium ovis*, *Bacillus licheniformis*, *Bacillus firmus*, *Pasteurella multocida*, *Bacillus coagulans*, *Pseudomonas pseudomallei* were isolated from the rhizosphere of soil treated with Benomyl only. The bacterial isolated from the rhizosphere of soil treated with mancozeb only were *Clostridium botulinum*, *Staphylococci aureus*, *Plesiomonas shigelloides*, *Chromobacterium volaceum*, *Actinomyces viscosus*, *Pseudomonas stutzeri*, *Corynebacterium ovis*, *Bacillus licheniformis*, *Bacillus pantothenticus*, and *Bacillus firmus* while *Bacillus licheniformis*, *Pasteurella multicida*, *Bacillus subtilis*, *Pseudomonas florescens*, *Pseudomonas capacia* and *Pasteurella mutocida* from the rhizosphere of soil treated with both mancozeb and benomyl. *Pseudomonas pseudomallei* isolated from the rhizosphere of soil treated with Benomyl gives 28.5-25.0% inhibition at 24 to 96 hours against *Rhizotonia solani* while it inhibited *Sclerotium rolfsii* at 60 – 60.8% between 24 to 48 hours. *Pseudomonas strutzeri* gives 33.0-5.0% inhibition against *Rhizotonia solani* at 24 to 96 hours.

Keywords

Antifungal, Benomyl, Mancozeb, Pathogens

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1. Introduction

The Cowpea (*Vigna unguiculata*) is one of several species of the widely cultivated genus *Vigna*. Four subspecies are recognised, of which three are cultivated (more exist, including *V. textilis*, *V. pubescens*, and *V. sinensis*) [1]. It is one of the most important and native grain legume crops in Sub-Sahara Africa (SSA) which account for 64% of the world production. Cowpea is a member of the fabaceae family which is a primary source of protein in SSA where it is grown for fresh and dry grains, foliage and forage. It is also an important crop in part of Asia, South America and United State of America [2].

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Cowpeas are one of the most important food legumes. It is predominantly cultivated by resources limited small holder farmer usually women with average farm size of 0.5 to 1 hectare (ha). Cowpea can derive up to 99% of it Nitrogen (N) nutrition from symbiotic fixation and fix substantial amount of symbiotic nitrogen to soil hence contributing to sustainability of the cropping system through fixation of atmospheric nitrogen and prevention of soil erosion. In fact, it has been shown to contribute about 240 kg N/ha with nitrogen benefit of 60 to 70kg/ha to succeeding crops in rotation in unfertile soil [2]. It has the useful ability to fix atmospheric nitrogen through its root nodules by the association they formed with nitrogen fixing bacteria that lives in its root region [1]. The rhizosphere, that is, the narrow zone surrounding and influenced by plant roots, is a hot spot for numerous organisms and is considered as one of the most complex ecosystems on Earth. NRC, [3] postulated that plants may modulate the rhizosphere microbiome to their benefit by selectively stimulating microorganisms with traits that are beneficial to plant growth and health. Pathogens of food crop ranges from bacteria, fungi, nematodes to viruses. Plant diseases are causing severe losses to humans and if we look into history we will come to know about the starvation and uprooting of families resulted from the irish famine caused by Potato late blight disease. There are hundreds of Plant diseases which are causing economic losses throughout the world and some diseases are reducing the nutritional values of food crops [4]. The damage caused by plant pathogens often constitutes a great impediment to food production and security especially in developing countries. It has been estimated that without plant diseases, the world food production will be one third greater than its present level [5]. Also with plant diseases, it has been reported that food produce contain toxins that are harmful to human. Aflatoxins are hepatocarcinogenic in humans, particularly in conjunction with chronic infection by hepatitis B virus. Fumonisins are associated with liver and kidney tumours in rodents, with studies implying a possible link with increased oesophageal cancer and neural tube defects in humans. Mycotoxin contamination has become one of the most pressing and challenging problems facing plant pathologists today [6]. With respect to reduction in the yield of plant produce, leaf spot disease of cowpea that is caused by *Cercospora cruneta* had been reported to reduce the yield of cowpea by 70% if left unchecked. Massive efforts are use to address the problem of Fusarium head blight in wheat in Northern America and Western Europe but aflatoxins and fumonisins are contaminating a large fraction of the world's food, including maize, cereals, groundnuts, and tree nuts [6]. The resultant effect is that it negatively affects the economy of the country by reducing the gross domestic product and export per capital of the country. Different approaches may be used to prevent or control plant diseases, but prevention is generally cheaper than cure [5]. The use of synthetic pesticides has been most widely accepted as control for plant diseases but the danger pose by majority of these chemicals (*i.e* fungicides) include undesirable environmental side effect, accumulation of pesticide residues in the biosphere and the development of resistance among pathogens against conventional antibiotics has led scientists toward the development of alternative strategies for plant disease suppression [5]. This works thereby aims to isolate, characterize and use bacteria that are associated with the rhizosphere of cowpea planted with Benomyl and Mancozeb as antagonist against some fungal pathogen of plant.

2. Material and Methods

The experiment was carried out as a 2x2 factorial experiment in a complete randomized design with three replications. The factors considered are as follows;

1. Addition of mancozeb (with and without mancozeb)
2. Addition of benomyl (with and without benomyl).

2.1. Collection of Samples

The cowpea, soil samples and the pathogens (*Rhizotonia solani*, *Collectotricum capsici* (isolated from infected cowpea), *Fusarium oxysporium* (isolated from vegetable *i.e Amaranthus hybridus*) and *Sclerotium rolfsii* (isolated from infected tomato)) were collected from Crop Soil and pest department of the Federal University of Technology, Akure. The Mancozeb and Benomyl used were collected from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

2.2. Sterilization Techniques

All glass-wares and prepared media were all sterilized in the autoclave at 121°C for 15 minutes. The work bench was sterilized with cotton wool soaked in 96% ethanol.

2.3. Inoculation of Soil Samples

Desired amount of mancozeb and benomyl (0.2 g) was weighed each and added to the soil sample in the pots where necessary and then mixed with the soil.

2.4. Planting of Cowpea

Two seeds of the cowpea (*Vigna unguiculata*) were planted per polyethylene pots at a depth of 5 cm. The plants were grown under green-house condition at 25°C between May and June, 2010. The pots were watered regularly to maintain a good moisture condition. After days of growth, all seedlings were thinned to one seedling in each of the 12 polyethylene pots of the experimental set up [5]. The seedlings were harvested after 30 days of planting by uprooting with hand trowel.

2.5. Chemical Analysis of Soil Sample Used

Chemical analysis of the soil sample used for planting of cowpea was done according to A.O.A.C. [7].

2.6. Isolation and Identification of Bacterial from Soil Sample Before and After Planting of Cowpea

Bacteria isolation was done for the soil sample before and after planting of cowpea. The pour plate technique was used for the isolation of bacteria from the soil samples as described by Olutiola [8]. 8-fold serial dilution was plated and incubated at 37°C for 24 hours. Distinct colonies were then subcultured to obtained pure colonies on which Gram staining and other biochemical tests such as Sugar fermentation (glucose, inositol, starch, maltose, fructose, sucrose, lactose, mannitol) tests, Indole test, Citrate test, Urease test, Catalase test, Casein and Starch hydrolysis test were carried out. The methods described by Willey *et al*. [9] were adopted for characterization of isolated bacteria.

2.7. Determination of Antifungal Activity

The antifungal effect of isolated the *Pseudomonas* species was tested against the growing edge of 5 day old test fungal culture. The fungal culture was aseptically cut and placed at the centre of the solidified potato dextrose agar (PDA). Thereafter, 40 mm streak was made from the isolated bacterial culture 23 mm away from the centre of the Petri plate using a 10 mm diameter cork borer. The plates were incubated at 25°C in an inverted position and monitored for 4 days [10]. Measurement of zone of inhibition and intercolony distance were taken daily.

3. Results and Discussion

The percentage composition of soil sample is as shown in Table 1. The soil sample used for planting of Cowpea (*V. unguiculata*) was sand clay loam and the soil was acidic. The soil sample has high amount of Calcium, Phosphorus and Magnesium. Table 2 shows the characteristics of bacteria isolated from the soil before the planting of cowpea. Seven bacteria that were isolated from the soil before planting of cowpea which were *Actinomyces viscosus, Pasteurella species, Corynebacterium renale, Pseudomonas capacia, Pseudomonas stutzeri*, *Bacillus subtilis* and *Bacillus pumilus* of which four of the bacteria were Gram positive while three were Gram negative bacteria and this result agrees with the work of Wang *et al*., [11] which show that *Actinomyces*, *Bacillus* and *Pseudomonas* species are commonly encountered bacteria in soil samples.

Table 1. pH and mineral content of the soil before experimental trials.

pH and mineral content	values obtained
pH	5.6 ± 0.127^{d}
%OC	1.2 ± 0.129^{e}
%OM	2.1 ± 0.375^{f}
%N	0.1 ± 0.021^{c}
P (mg/kg)	6.2 ± 0.438^{f}
K (mg/kg)	0.5 ± 0.034^{g}
Na (Cmol/kg)	0.5 ± 0.014^{b}
Ca (Cmol/kg)	19 ± 0.141^{d}
Mg (Cmol/kg)	11 ± 0.141^{d}

Keys: Mean ± standard deviation of sample in duplicate. Values followed different alphabet mean there is significant difference. OC = Organic Carbon, OM = Organic Matter, N =Nitrogen, P = Phosphorus, K = Potassium, Na= Sodium, Ca = Calcium, Mg = Magnesium

Table 2. Characteristics of Bacteria isolated from soil before planting.

1	Nut.Agar	Glu	Fru	Suc	Man	Mal	Lac	St	Ino	Cat	Cit/Ur	St/Cas
A.V	+ve/rod White/R	+-	+-	++	+-	+-	+-	++	+-	+ve	+ve/-ve	+ve/+ve
P.S	-ve/rod Cream/F	++	++	+-	++	++	+-	++	+-	+ve	+ve/-ve	+ve/+ve
C.R	-ve/rod Pink/R	++	++	+-	+-	++	+-	+-	+-	+ve	+ve/-ve	-ve/+ve
P.C	+ve/rod Cream/F	++	--	++	+-	+-	+-	+-	+-	+ve	+ve/+ve	-ve/+ve
B.P	+ve/rod Cream/R	++	++	++	++	++	--	++	+-	+ve	+ve/+ve	-ve/+ve
P.S	-ve/rod White/F	+-	++	++	+-	++	--	++	+-	+ve	+ve/-ve	+ve/+ve
B.S	+ve/rod Cream/F	+-	++	++	+-	+-	--	+-	+-	+ve	+ve/-ve	+ve/+ve

Keys: 1-Isolates, A.V- Actinomyces viscosus, P.S- Pasteurella species, C.R- Corynebacteria renale, P.C- Pseudomonas capacia, B.P- Bacillus pumilus, P.S- Pseudomonas stutzeri, B.S- Bacillus subtilis, R-Raised, F-Flat, Glu-Glucose, Fru-Fructose, Suc-Sucrose, Man-Mannitol, Mal-Maltose, Lac-Lactose, St-Starch, Ino-Inositol, Cat-Catalase, Cit-Citrate, Ur-Urease, St-Starch hydrolysis, Cas-Casein hydrolysis, Nut.Agar-Nutrient Agar, ++ = Acid and gas, +- = Acid only, -- = No acid or gas

Table 3. Growth characteristics of cowpea under various condition of soil treatment.

Soil sample	Leaf length (cm)	Plant height (cm)	No of leaves
Z_oB_1	3.7	22.5	7
Z_1B_o	7.8	75	20
Z_1B_1	3.5	17.9	8

Keys: Z_0 = Soil without mancozeb, B_0 = Soil without Benomyl, Z_1 = Soil with mancozeb, B_1 = Soil with benomyl

The growth characteristics of cowpea under various condition of treatment are as shown in Table 3. The Highest height of cowpea seedling grew in soil treated with mancozeb only.

Table 4 shows the bacteria count of the soil from the rhizosphere of the cowpea after planting. The highest bacterial count was in soil treated with either mancozeb only or benomyl only. The bacterial load decreases from around

10 cfu (in mancozeb or benomyl treated soil) to 6×10^8 per gram in mancozeb and benomyl treated soil.

Table 4. Bacterial count of soil after planting of cowpea.

Soil Sample	Colony Count (Cfux10^8)
Z_oB_1	10.00±1.53[a]
Z_1B_o	10.33±3.099[a]
Z_1B_1	6.33±0.888[b]

Keys: Z_0 = Soil without mancozeb, B_0 = Soil without Benomyl, Z_1 = Soil with mancozeb, B_1 = Soil with benomyl.

Table 5 shows the characteristics of bacteria isolated from the rhizosphere of soil treated with Benomyl. The isolated bacteria are as follows *Bacillus pumilus, Corynebacterium renale, Bacillus macerans, Actinomyces viscosus, Pseudomonas stutzeri, Bacillus subtilis, Corynebacterium ovis, Bacillus licheniformis, Bacillus firmus, Pasturella multocida, Bacillus coagulans* and *Pseudomonas pseudomallei* in which ten isolates were Gram positive and two were Gram negative.

Table 5. Characteristics of bacteria isolated from soil treated with benomyl.

1	Gram stain/Nut.Agar	Glu	Fru	Suc	Man	Mal	Lac	St	Ino	Cat	Cit/Ur	St/Cas
B.P	+ve/rod Cream/R	++	+-	+-	+-	+-	--	+-	+-	+ve	+ve/-ve	+ve/-ve
C.R	+ve/rod Cream/F	++	++	+-	+-	++	+-	++	+-	+ve	-ve/+ve	+ve/-ve
B.M	+ve/rod Cream/R	++	+-	++	+	+-	++	+-	++	-ve	-ve/+ve	+ve/+ve
A.V	+ve/rod Cream/R	++	++	++	++	++	++	+-	+-	+ve	-ve/+ve	+ve/+ve
P.St	-ve/rod CreamY/R	++	+-	++	++	++	++	--	++	+ve	-ve/+ve	+ve/+ve
B.S	+ve/rod Cream/R	++	--	++	++	+-	++	+-	++	+ve	+ve/+ve	+ve/+ve
C.O	+ve/rod White/F	++	++	++	+-	++	++	+-	++	+ve	+ve/+ve	-ve/+ve
B.L	+ve/rod Cream/R	++	++	++	++	++	++	+-	+-	+ve	+ve/+ve	+ve/+ve
B.F	+ve/rod Cream/R	++	++	++	++	++	++	+-	++	-ve	-ve/+ve	+ve/-ve
P.M	+ve/rod Yellow/R	++	++	++	++	++	+-	+-	+-	-ve	-ve/+ve	+ve/-ve
B.C	+ve/rod Cream/R	--	+-	++	+	++	++	+-	++	+ve	+ve/+ve	+ve/-ve
P.P	-ve/rod Cream/R	++	++	+-	+-	++	++	+-	++	+ve	+ve/-ve	+ve/-ve

Keys: 1- Isolates, B.P- Bacillus pumilus, C.R- Corynebacterium renale, B.M- Bacillus macerans, A.V- Actinomyces viscosus, P.St- Pseudomonas stutzeri, B.S- Bacillus subtilis, C.O- Corynebacterium ovis, B.L- Bacillus licheniformis, B.F- Bacillus firmus, P.M- Pasteurella multocida, B.C- Bacillus coagulans, P.P- Pseudomonas pseudomallei R-Raised, F-Flat, Glu-Glucose, Fru-Fructose, Suc-Sucrose, Man-Mannitol, Mal-Maltose, Lac-Lactose, St-Starch, Ino-Inositol, Cat-Catalase, Cit-Citrate, Ur-Urease, St-Starch, Cas-Casein hydrolysis, Nut.Agar-Nutrient Agar, ++ = Acid and gas, +- = Acid only, -- = No acid or gas

Bacteria isolated from the rhizosphere of soil treated with mancozeb only are as shown in Table 6. *Clostridium botulinum*, *Staphylococci aureus*, *Plesiomonas shigelloides*, *Chromobacterium volaceum*, *Actinomyces viscosus*, *Pseudomonas stutzeri*, *Corynebacterium ovis*, *Baci*llus *licheniformis*, *Bacillus pantothenticus*, and *Bacillus firmus* out of which seven were Gram positive while three are Gram negative bacteria.

Table 6. Characteristics of bacterial isolate from soil treated with mancozeb.

1	Gramstain/Nut.Agar	Glu	Fru	Suc	Man	Mal	Lac	St	Ino	Cat	Cit/Ur	St/Cas
C.B	+ve/rod Cream/R	++	++	+-	+-	+-	++	+-	+-	+ve	+ve/-ve	-ve/+ve
S.A	+ve/cocci Cream/R	++	++	++	++	++	++	++	++	+ve	+ve/-ve	+ve/+ve
P.S	-ve/rod Cream/R	++	++	++	++	++	++	++	++	+ve	-ve/+ve	-ve/+ve
C.V	-ve/rod Cream/R	+-	++	++	++	++	++	+-	++	+ve	+ve/+ve	-ve/+ve
A.V	+ve/rod Cream/R	++	++	++	+-	++	++	+-	++	+ve	-ve/+ve	+ve/+ve
P.St	-ve/rod Cream/F	++	+-	++	++	++	++	--	++	+ve	+ve/+ve	-ve/+ve
C.O	+ve/rod Cream/R	++	++	++	+-	++	++	+-	++	+ve	+ve/+ve	-ve/+ve
B.L	+ve/rod Cream/R	++	++	++	++	++	++	+-	+-	+ve	+ve/+ve	+ve/+ve
B.P	+ve/rod White/F	--	++	++	++	+-	++	++	++	+ve	-ve/+ve	-ve/+ve
B.F	+ve/rod Cream/R	++	++	++	++	++	++	++	++	-ve	-ve/+ve	+ve/-ve

Keys: 1-Isolates C.B- Clostridium botulinum, S.A- Staphylococci aureus, P.S- Plesiomonas shigelloides, C.V- Chromobacterium volaceum, A.V- Actinomyces viscosus, P.St- Pseudomonas stutzeri, C.O- Corynebacterium ovis, B.L- Bacillus licheniformis, B.P- Bacillus pantothenticus, B.F- Bacillus firmus R-Raised, F-Flat, Glu-Glucose, Fru-Fructose, Suc-Sucrose, Man-Mannitol, Mal-Maltose, Lac-Lactose, St-Starch, Ino-Inositol, Cat-Catalase, Cit-Citrate, Ur-Urease, St-Starch, Cas-Casein hydrolysis, Nut.Agar-Nutrient Agar, ++ = Acid and gas, +- = Acid only, -- = No acid or gas.

The bacteria isolated from the rhizosphere of soil treated with both mancozeb and benomyl are as shown in Table 7. *Bacillus licheniformis*, *Pasteurella multicida*, *Bacillus subtilis*, *Pseudomonas florescens*, *Pseudomonas capacia* and *Pasteurella mutocida* out of which four are Gram positive while two are Gram negative and this agree with the work of Susilowath and Wiyono, [12] bacteria present in the rhizosphere of leguminous plant.

Table 7. Characteristics of bacterial isolated from soil treated with mancozeb and benomyl.

1	Gram Stain/Nut.Agar	Glu	Fru	Suc	Man	Mal	Lac	St	Ino	Cat	Cit/Ur	St/Cas
B.L	+ve/rod Cream/R	++	++	++	++	++	+-	+-	+-	+ve	+ve/+ve	+ve/+ve
P.M	+ve/rod Yellow/R	++	+	++	++	++	+-	+-	+-	-ve	-ve/+ve	+ve/-ve
B.S	+ve/rod Cream/R	++	--	++	++	+-	++	+-	++	+ve	+ve/+ve	+ve/+ve
P.C	-ve/rod Cream/F	--	+	++	++	++	++	++	++	+ve	+ve/+ve	-ve/+ve
P.F	-ve/rod Cream/R	+-	++	+-	++	+-	+-	+-	+-	-ve	-ve/+ve	+ve/+ve
B.Ce	+ve/rod Cream/R	++	+	++	++	++	+-	+-	+-	-ve	+ve/+ve	+ve/+ve

Keys: B.L-*Bacillus licheniformis*, P.M- *Pasteurella multocida*, B.S-*Bacillus subtilis*, P.C-*Pseudomonas capacia*, P.F-*Pseudomonas florescens*, B.Ce-*Bacillus cereus*, R-Raised, F-Flat, Glu-Glucose, Fru-Fructose, Suc-Sucrose, Man-Mannitol, Mal-Maltose, Lac-Lactose, St-Starch, Ino-Inositol, Cat-Catalase, Cit-Citrate, Ur-Urease, St-Starch, Cas-Casein hydrolysis, Nut.Agar-Nutrient Agar, ++ = Acid and gas, +- = Acid only, -- = No acid or gas.

Table 8 shows the percentage inhibition of *Pseudomonas pseudomallei* isolated from the rhizosphere of benomyl treated soil. *P. pseudomallei* inhibited *Sclerotium rolfsii* at 60 – 60.8% between 24 to 48 hours while it inhibited *Rhizotonia solani* at 28.5% at 24 hours, 25.0% at 48 hours, 30.0% at 72 hours and 28.5% at 96 hours which is in line with the work of Swati and Tiwani, [13] which state that some *Pseudomonas* species are capable of inhibiting *Rhizotonia solani*. It also inhibited *Fusarium oxysporium* at 48 - 40.4% at 24 to 48 hours while inhibiting *C. capsii* a 35.8-28.5% at 24-72 hours.

Table 8. Percentage inhibition of fungal species by *Pseudomonas pseudomallei.*

H	*R. solani*		*F. oxysporium*		*S. rolfsii*		*C. capsii*	
	ID	PI	ID	PI	ID	PI	ID	PI
24	18	28.5%	10	48.0%	13	60.0%	8	35.8%
48	17	25.0%	3	47.5%	5	60.8%	1	47.6%
72	16	30.0%	0	40.4%	0	0.0%	0	28.5%
96	15	28.5%	0	0.0%	0	0.0%	0	0.0%

Keys: H-Hours, ID= Inter colony distance (mm), PI= Percentage inhibition

Pseudomonas stutzeri isolated from the rhizosphere of soil treated with benomyl gives from 33.0% to 5% inhibition from 24 to 96 hours against *Rhizotonia solanii* although the percentage inhibition was reducing as the days were increasing while it gives between 23.5%-4.5% inhibitions against *Sclerotium rolfsii* from 24 to 72 hours which in agreement with the research work of Paramageetham and Babu, [14] which shows that *Pseudomonas* species were able to inhibit *Sclerotium rolfsii*.

Table 9. Percentage inhibition of fungal species by *Pseudomonas stutzeri.*

H	*R. solani*		*F. oxysporium*		*S. rolfsii*		*C. capsici*	
	ID	PI	ID	PI	ID	PI	ID	PI
24	17	33.0%	0	10.7%	10	23.5%	8	25.0%
48	13	23.0%	0	0.0%	4	9.5%	0	25.0%
72	6	10.5%	0	0.0%	0	4.5%	0	0.0%
96	4	5.0%	0	0.0%	0	0.0%	0	0.0%

Keys: H-Hours, ID= Inter colony distance (mm), PI= Percentage inhibition

Pseudomonas capacia isolated from the rhizosphere of soil treated with both mancozeb and benomyl gives 25.0% inhibition against *Sclerotium rolfsii* at 24 hours, 5.0 at 48 hours and 5.2% inhibition at 72 hours. *P. capacia* inhibited *Collectotricum capsici* by 80% on 24 hours of incubation. The results of the antagonistic activities shown by the *Pseudomonas* species were in agreement with the work of Susilowath and Wiyono, [12].

Table 10. Percentage inhibition of fungal species by *Pseudomonas capacia.*

H	*R. solani*		*F. oxysporium*		*S. rolfsii*		*C. capsici*	
	ID	PI	ID	PI	ID	PI	ID	PI
24	13	28.5%	8	48.3%	17	25.0%	18	80%
48	0	0.0%	0	0.0%	4	5.0%	0	0.0%
72	0	0.0%	0	0.0%	4	5.2%	0	0.0%
96	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Keys: H-Hours, ID= Inter colony distance (mm), PI= Percentage inhibition

4. Conclusion

Conclusively, the increased awareness of the constraints associated with the application of agrochemicals has stimulated active research to explore and prospect for alternative sources of pesticidal agent which are cheaper, safer and ecological friendly within the frame work of integrated pest management approaches. The findings of this study have been able to identified some bacteria that are possible degrader of Benomyl, Mancozeb and the combination of both. Also this study has been able to establish that *Pseudomonas capacia*, *Pseudomonas stutzeri* and *Pseudomonas pseudomallei* can serve as a good biocontrol agent for *Rhizotonia solanii*.

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