

## Antifungal Efficacy of Camazeb and Rhizosphere Bacteria on Damping Off Disease of Tomato Caused by *Rhizoctonia solani*

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**Abstract:** The antifungal efficacy of camazeb (fungicide) was determined on damping off disease of tomato caused by *Rhizoctonia solani*. Tomato seeds were planted in the soil samples (1.5 kg each) in which six different concentrations of camazeb (0.00, 0.25, 0.50, 1.00, 2.00 and 4.00 g/100ml) was applied and inoculated with or without *Rhizoctonia solani*. Plants were observed on the 30th day. Rhizosphere bacteria were isolated after 30 days of germination and harvesting. *In vitro* inhibitory effects of the isolated bacteria were compared with camazeb against *Rhizoctonia solani*. Damping off disease led to the death of tomato seedlings where *R. solani* was inoculated. It was observed that plants were healthy at 0.25g/100ml of camazeb in soil inoculated with *R. solani*. However, higher concentrations of camazeb (2.00 and 400g/100ml) were detrimental to tomato seedlings. Colony count of bacteria reduced from  $14 \pm 4.0 \times 10^3$  cfu/g at 0.00g/100ml to  $5 \pm 1.0 \times 10^3$  cfu/g at 4.00g/100ml of camazeb. The bacteria isolated from rhizosphere soil were *Pseudomonas aeruginosa*, *Bacillus thuringiensis*, *B. lentimorbus* and *B. subtilis*. *Pseudomonas aeruginosa* had higher zone of inhibition (38.80mm) against *R. solani* than camazeb (23.83mm) at 4.00g/100ml. Therefore, *Pseudomonas aeruginosa* may be exploited as biocontrol agent of *Rhizoctonia solani*.

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**Keywords:** camazeb, rhizosphere bacteria, damping off disease

### 1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the important sources among all the vegetables throughout the world (Jiskani *et al.*, 2007). It originated in tropical America and cultivated for thousand of years in Mexico and Peru before invasion of the Europeans. It is a good source of vitamins A, B and C (Khosro, 1994).

Plant pathogenic microorganisms are among biotic stresses that cause serious threat to crop production and ecosystem stability (Sabuquillo *et al.*, 2006). *Rhizoctonia solani* is one of the most important soilborne fungal pathogens which develop both in cultured and non-cultured soils, causing diseases in different crops such as rice, tomato, bean among others (Sneh *et al.*, 1991; Gull, 2008). It is the major fungus responsible for damping-off, black spot and root rot diseases (Neha and Dawande, 2010).

Camazeb is a fungicide which contains 120g of carbendazim and 630g mancozeb per kilo wettable powder. It is used as a protectant foliar spray to crops (including tomato) to control a wide range of fungal diseases such as damping off disease caused by *Rhizoctonia solani*. Camazeb is available as dusts, liquids, water dispersible granules, or wettable powders, and as ready-to-use (R-T-U) formulations (Meister, 1992).

The high cost of pesticides, development of fungicides resistance pathogen isolates, governmental restriction on the use of chemicals raises the need to find alternative control methods (Amel *et al.*, 2010). Biological control is a natural and specific way to control pathogens and enhance crop yield by growth promoting attributes of environment friendly microorganisms (Kiewnick and Sikora, 2006).

Studies have shown that the microbial population at a short distance from the root is little affected by the plant while soil immediately adjacent to the root contains an abundance of bacteria (Curl and Truelove, 1986). The rhizosphere is the soil surrounding the root that is subject to the influence of root exudates. Intense microbial activity and greater populations occurs in this micro-environment because of the release of large amount of organic matter from roots. The rhizosphere is also the site for interactions between plants pathogenic microorganisms and antagonist rhizobacteria and fungi (Sturz and Christie, 2003). The present research work was carried out to isolate and characterize bacteria that are associated with the rhizosphere of tomato plant, test the inhibitory effect of the isolated bacteria on *Rhizoctonia solani* and determine the influence of camazeb on *Rhizoctonia solani* in pots and plates (*in vitro*) experiment.

## 2.0 Collection of samples

Soil was also collected from the Federal University of Technology, Akure research farm, Obaekere. Tomato seeds and camazeb were purchased from Akure. *Rhizoctonia solani* was collected from the Department of Crop Soil and Pests Management (CSP), Federal University of Technology, Akure. Soil sample used for planting were sterilized in the oven (Gallenkamp BS 250) at 180°C for three hours while the media used were sterilized by autoclaving at 121°C for 15 min using Amsco 2022 Isothermal autoclave – Eagle series.

## 2.0 Materials and methods

### 2.1 Isolation and characterization of bacteria from rhizosphere and non-rhizosphere soil of tomato plant

Three fold serial dilution was performed on the rhizosphere and non-rhizosphere soil sample, 0.1ml of each of the diluents were then aseptically dispensed into the sterile petri plates and 20ml of molten Nutrient Agar was poured into the plates. They were allowed to set and incubated at a temperature of 37°C for 24 hours. The colonies that appeared after incubation were then counted. The isolated bacteria were subcultured until pure colonies were obtained. Characterization of the isolates was done according to the methods of Olutiola *et al.* (1991) and Holt (1989).

### 2.2 Planting and harvesting of tomato plant

The experimental design for the planting of tomato was a 2×2×6 factorial experiment in randomized design with three replicates. The factors considered were inoculation of soil with *Rhizoctonia solani* and application of camazeb at six different concentrations (0.00, 0.25, 0.50, 1.00, 2.00, and 4.00g/100ml). The average weight of soil in each plastic pot was 1.50kg.

The viable tomato seeds were planted in each of pots after inoculation of some of them with *Rhizoctonia solani*. After growth, all seedlings were thinned to one. The different concentrations (0.00, 0.25, 0.50, 2.00, and 4.00g/100ml) of camazeb were added to the soil samples after 5 days of planting. The pots were drenched with sterile distilled water regularly to maintain a good moisture condition. Various observations such as plant height, root development were made on the plant. The experiment was terminated after 30 days.

### 2.3 *In vitro* determination of the effect of camazeb on *Rhizoctonia solani*

Pure *Rhizoctonia solani* suspension (0.1ml) was discharged into Petri dishes after which molten

potato dextrose agar was poured into them. Thereafter, six wells were bored on the plates using a 10mm cork borer and camazeb concentrations (0.00, 0.25, 0.50, 1.00, 2.00, 4.00g/100ml) were dispensed into each well. Plates were incubated and zones of inhibition were measured after 72 hours at 28°C.

### 2.4 Inhibitory effects of the isolated bacteria on *Rhizoctonia solani*

Rhizosphere bacteria isolated from tomato were tested for antagonistic activities against *Rhizoctonia solani* on a nutrient agar prepared plate using the dual culture procedure. The inhibitory effect was observed as a clear zone around the fungi.

### 2.5 Physicochemical properties of soil

The soil sample used before and after planting was analyzed to determine its physicochemical properties using the Association of Officials Analytical chemist (A.O.A.C. 1980) method. Ten milliliters of sodium was added to 50g of soil sample in a beaker. Thereafter, 90ml of distilled water was added to the soil sample and left overnight. This was made up to the 100ml of the cylinder. A hydrometer was used to take the reading and value of the sand, clay and silt value were calculated.

#### 2.5.1 pH determination

Ten gram of 2mm sieved air dried soil sample was weighed into 100ml beaker in duplicate. Twenty milliliters of distilled water was added while 20ml of 1M potassium chloride was added to the soil sample in the second beaker. These mixture were stirred several times over a 30 minutes interval, the pH of the soil in the beaker containing water was measured by immersing the glass electrode into the partly settled suspension beaker.

#### 2.5.2 Determination of total nitrogen

The trimetric method of A.O.A.C (1980) was used. Two gram of the soil was weighed. Ten milliliters of concentrated sulphuric acid was introduced into the sample with one table spoon of catalyst copper sulphate. Heat was applied on digestion rack and the sample left to settle for 3 hours until a clear solution was obtained. This indicates completes digestion. The solution was left to cool. It was made to the mark with distilled water in a 100ml volumetric flask. The solution is titrated against 0.1M HCl until end point was reached i.e. color changes from blue to black.

#### 2.5.3 Determination of organic carbon

The walkey-black wet oxidation method of A.O.A.C (1980) was used. Ten milliliters of

potassium dichromate ( $K_2Cr_2O_7$ ) was added to pre-glue 50g well ground soil sample. Two hundred milliliters of concentrated sulphuric acid was added shaken gently and left to cool. The percentage of carbon was determined from reduction in the value of  $K_2Cr_2O_7$ .

#### 2.5.4 Determination of calcium, magnesium, potassium and sodium

Atomic absorption spectrophotometer method of A.O.A.C (1980) was employed to determine the composition of calcium, potassium, magnesium and sodium. One gram each of soil was transferred into 100ml conical flask and shake vigorously for 30 minutes. This was followed by the addition of 2ml aqua regia. The conical flask was left to stay for 3 days before they were made up to the 50ml mark with distilled water.

#### 2.5.5 Determination of phosphorus

Five grams of air-dried soil sample were transferred into 250ml flasks. Baryons solution was added and was left to stand for one minute before being filtered. Thereafter, 8ml of sample of standard solution or blank was pipette into a set of well-numbered glass vied with 5 drops of B. reagent (Ammoniummolydate solution) and 5 drops of  $FeSO_4$  solution were added and carefully mixed. This was allowed to stand for 15minutes. The samples were read in calorimeter using a green filter (600millmicrons weight) against a blank. The standard curve was then used to convert to appropriate values. The calorimeter read for standard and phosphorus was determined from graph.

#### 2.5.6 Determination of moisture content of the soil

A 50g of the soil sample were placed in the oven for 24h at a temperature of  $100^\circ C$ . The weight after drying (final weight) was determined. The difference between the final weight and the initial weight was calculated to obtain the moisture content (A.O.A.C., 1980).

#### 2.6 Statistical analysis

All data obtained were subjected to statistical analysis of variance (ANOVA) to determine the significance of the sources of variation.

#### 3.0 Bacteria isolated from rhizosphere soil of tomato

The average colony forming unit of non-rhizosphere bacteria isolates on plates was 4 cfu/ml while the average colony forming unit of bacterial isolates of rhizosphere soil at different concentration of camazeb is presented in Table 1. There was reduction in the count with increase concentration of

camazeb. Four Gram positive and a Gram negative bacteria were isolated from the rhizosphere of tomato and were identified as *Bacillus subtilis*, *B. lentimorbus*, *B. thuringiensis*, *Bacillus* species and *Pseudomonas aeruginosa*.

#### 3.1 Influence of camazeb and *Rhizoctonia solani* on tomato plant

The influence of camazeb at different concentration, *Rhizoctonia solani* and plant assessment based on damping off, root development and plant mortality is presented in Table 2. It was observed that *Rhizoctonia solani* caused damping off and plant mortality in  $RC_0$  while healthy growth was observed in  $R_0C_0$ . Slight disease was observed in  $RC_1$  with very good root development and slight disease and good root development in  $RC_2$  while  $R_0C_1$  shows healthy plant and  $R_0C_2$  exhibited slight disease and good root development. Moderate disease occurred in  $RC_3$  with moderate root development while  $R_0C_3$  shows slight disease good root development. At higher concentrations of camazeb, there was no root development and death of the plant occurred.

#### 3.2 *In vitro* inhibitory effect of camazeb on *Rhizoctonia solani*

The inhibitory influence of camazeb at different concentrations on *Rhizoctonia solani* is presented in Tables 4. It was observed that the inhibition of *Rhizoctonia solani* increased from 13.33 to 23.83mm as the concentration increased from 0.25g/100ml to 4.00g/100ml. There was an equal inhibitory effect at concentration 1.00g/100ml and 2.00g/100ml.

#### 3.3 Inhibitory effects of the isolated bacteria on *Rhizoctonia solani*

The zones of inhibition of rhizosphere bacteria against *Rhizoctonia solani* are presented in Table 5. It was observed that *Pseudomonas aeruginosa* showed the strongest antagonistic activity followed by *Bacillus subtilis* with zones of inhibition of 39.80 and 28.10mm respectively.

#### 3.4 Physicochemical analyses of soil sample

Physicochemical analysis of soil sample used show that the textural class is clayey loam and the soil was made up of 35% sand, 27% clay, and 38% silt. The results of the physicochemical characteristics of non-rhizosphere and rhizosphere soil sample are presented in Table 6. It was observed that phosphorous, potassium, and nitrogen content, organic matter reduced at 0.00g/100ml and intense reduction at 4.00g/100ml camazeb concentration compared to non rhizosphere soil. However, there was increase in calcium, magnesium, percentage

organic carbon, and soil pH content at 0.00g/100ml compared to the rhizosphere soil at 4.00/100ml and non rhizosphere soil.

### Disease assessment

**Damping off in plant:** 0: healthy plant, 1: slight disease, 2: moderate disease, 3: severe disease, 4: dead plant. **Root development in plant,** 0: no development/ dead plant, 1:poor development/weak plant, 2: moderate development/stable plant, 3: good development/healthy plant, 4: very good development/healthy plant.

**Table 1: Influence of camazeb on damping off disease caused by *R. solani***

Plant treatments	Damping off	Root development
RC <sub>0</sub>	4	0
R <sub>0</sub> C <sub>0</sub>	0	5
RC <sub>1</sub>	1	4
R <sub>0</sub> C <sub>1</sub>	0	4
RC <sub>2</sub>	1	3
R <sub>0</sub> C <sub>2</sub>	1	4
RC <sub>3</sub>	2	2
R <sub>0</sub> C <sub>3</sub>	1	3
RC <sub>4</sub>	4	0
R <sub>0</sub> C <sub>4</sub>	4	0
RC <sub>5</sub>	4	0
R <sub>0</sub> C <sub>5</sub>	4	0

**Table 2 Effect of camazeb on growth parameters of tomato plant**

Treatment	Leaf surface area	Shoot length	Root length	Leaf number
RC <sub>0</sub>	1.04 <sup>d</sup>	8.61 <sup>c</sup>	0.57±0.21 <sup>c</sup>	2.92b <sup>c</sup>
R <sub>0</sub> C <sub>0</sub>	2.78 <sup>a</sup>	11.6 <sup>a</sup>	2.6±0.1 <sup>a</sup>	3.67 <sup>a</sup>
RC <sub>1</sub>	0.80 <sup>f</sup>	8.79 <sup>d</sup>	0.53±0.058 <sup>eg</sup>	2.92b <sup>c</sup>
R <sub>0</sub> C <sub>1</sub>	1.72 <sup>b</sup>	11.15 <sup>b</sup>	2.4±0.1 <sup>b</sup>	3.08 <sup>b</sup>
RC <sub>2</sub>	1.2 <sup>c</sup>	8.11 <sup>f</sup>	0.53±0.058 <sup>ef</sup>	2.75b <sup>g</sup>
R <sub>0</sub> C <sub>2</sub>	0.96 <sup>e</sup>	9.63 <sup>c</sup>	2.17±0.58 <sup>c</sup>	3.0b <sup>e</sup>
RC <sub>3</sub>	0.4 <sup>g</sup>	5.45 <sup>h</sup>	0.43±0.15 <sup>eh</sup>	2.25 <sup>h</sup>
R <sub>0</sub> C <sub>3</sub>	0.25 <sup>h</sup>	5.89 <sup>g</sup>	2.07±0.12 <sup>cd</sup>	2.92 <sup>bf</sup>
RC <sub>4</sub>	0.19 <sup>hi</sup>	1.66 <sup>kl</sup>	0.00±0.00 <sup>ij</sup>	1.08 <sup>il</sup>
R <sub>0</sub> C <sub>4</sub>	0.13 <sup>ij</sup>	2.04 <sup>i</sup>	0.00±0.00 <sup>ik</sup>	1.33 <sup>l</sup>
RC <sub>5</sub>	0.092 <sup>jk</sup>	1.66 <sup>kl</sup>	0.00±0.00 <sup>i</sup>	1.25 <sup>il</sup>
R <sub>0</sub> C <sub>5</sub>	0.092 <sup>jl</sup>	2.02 <sup>ij</sup>	0.00±0.00 <sup>ik</sup>	1.33 <sup>jl</sup>

**Note:** Values are means of three replicates. Mean with similar letters are not significant at  $P < 0.05$ .

Keys:

RC<sub>0</sub>: Soil treated with *Rhizoctonia solani* and without camazeb (0.00g/100ml),

R<sub>0</sub>C<sub>0</sub>: Soil treated without *Rhizoctonia solani* and camazeb,

RC<sub>1</sub>: Soil treated with *Rhizoctonia solani* and 0.25g/100ml of camazeb,

R<sub>0</sub>C<sub>1</sub>: Soil treated without *Rhizoctonia solani* and 0.25g/100ml of camazeb,

RC<sub>2</sub>: Soil treated with *Rhizoctonia solani* and 0.50g/100ml of camazeb,

R<sub>0</sub>C<sub>2</sub>: Soil treated without *Rhizoctonia solani* and 0.50g/100ml of camazeb,

RC<sub>3</sub>: Soil treated with *Rhizoctonia solani* and 1.00g/100ml of camazeb,

R<sub>0</sub>C<sub>3</sub>: Soil treated without *Rhizoctonia solani* and 1.00g/100ml of camazeb,

RC<sub>4</sub>: Soil treated with *Rhizoctonia solani* and 2.00g/100ml of camazeb,

R<sub>0</sub>C<sub>4</sub>: Soil treated without *Rhizoctonia solani* and 2.00g/100ml of camazeb,

RC<sub>5</sub>: Soil treated with *Rhizoctonia solani* and 4.00g/100ml of camazeb,

R<sub>0</sub>C<sub>5</sub>: Soil treated without *Rhizoctonia solani* and 4.00g/100ml of camazeb.

**Table 3: Colony forming unit of bacterial isolates of rhizosphere soil at different concentration of camazeb**

Concentrations of camazeb (g/ml)	Colony forming unit/g (cfu/g)
0.00/100	14±4
0.25/100	10±3
0.50/100	9±3
1.00/100	7±2
2.00/100	6±1
4.00/100	5±1

**Table 4: Inhibitory effect of camazeb on *Rhizoctonia solani***

Camazeb at different Concentration (g/100ml)	Zone of inhibitions (mm)
0.00	0.00 ± 0.00a
0.25	13.33 ± 0.33b
0.50	15.00 ± 0.00c
1.00	21.00 ± 0.50d
2.00	21.00 ± 0.00d
4.00	23.83 ± 0.17e

**Note:** Values are means of three replicates. Mean with similar letters are not significant at  $P < 0.05$ .

**Table 5: Inhibitory effect of isolated bacteria on *Rhizoctonia solani***

Bacterial isolates	Zone of inhibition (mm)
<i>Bacillus thuringiensis</i>	17.80
<i>Pseudomonas aeruginosa</i>	39.80
<i>Bacillus subtilis</i>	28.10
<i>Bacillus</i> sp	9.80
<i>Bacillus lentimorbus</i>	12.40

**Table 6: Physico-chemical characteristics of soil sample used before and after experimental trials**

	Non rhizosphere soil	Rhizosphere soil treated with camazeb (0.00g/100ml)	Rhizosphere soil treated with camazeb (4.00g/100ml)
Phosphorus (mg/kg)	8.42	6.45	6.30
Sodium (Cmol/kg)	2.65	0.20	0.15
Potassium (Cmol/kg)	2.67	0.64	0.45
Calcium (Cmol/kg)	1.0	3.90	3.80
Magnesium (Cmol/kg)	1.0	2.90	2.60
Nitrogen (%)	0.923	0.30	0.29
% Organic matter	Not determined	4.13	3.99
% Organic carbon	1.78	2.39	2.31
Soil pH	5.05	6.90	6.73

#### 4.0 Discussion

Application of camazeb led to a reduction of the organic matter of the rhizosphere soil. Pereira *et al.* (2008) also noted that repeated applications of glyphosate in transgenic soybean can cause a reduction in root number and organic matter content. High pesticide concentrations, decreased organic matter amount and soil moisture contribute to a decline in the number and activity of soil fungi (Kjoller and Rosendahl, 2000), impacting also the plant nutrition itself and change in soil structure and fertility (Bethlenfalvai and Shuepp, 1994). There was reduction in the population of the rhizosphere soil with increase in concentration of camazeb. This result is in agreement with Barry and Davies (2004) who observed that populations of soil microorganisms were very sensitive to the environment's alterations mainly those caused by toxic substances. There was a slight reduction in the organic carbon at 4.00g/100ml of camazeb compared to control. However, Jakelaitis *et al.* (2006) study on the impact of herbicides atrazine and nicosulfuron, did not observe any alteration of soil microbial biomass carbon. There was increase in the growth parameters of tomato at lower concentrations of camazeb (0.25g/100ml). The results of the present investigation correspond with that of other workers Satija and Hooda (1987), Taha *et al.* (1988), Jiskani *et al.*, (2007). However at higher concentration, camazeb was detrimental to tomato plant. All the bacteria isolated from the rhizosphere of tomato had inhibitory effects on *R. solani* in the *in vitro* studies. However, *Pseudomonas aeruginosa* and *Bacillus subtilis* were the most effective with pathogen inhibition of 37.80 and 28.10 mm respectively which was higher than that of the pesticide camazeb even at

higher concentration. *Pseudomonas* sp. is ubiquitous in agricultural soils, well adapted to growing in the rhizosphere. *Pseudomonas* possesses many traits that make them well suited as biocontrol and growth-promoting agents (Weller, 1988; David and Weller, 2007). Since, intensified use of fungicides has resulted in the accumulation of toxic compounds potentially hazardous to humans and environment also in the buildup of resistance of pathogens (Amel *et al.*, 2010), ecofriendly antagonistic bacteria like *Bacillus subtilis* and *Pseudomonas aeruginosa* could be harnessed in the control of *Rhizoctonia solani*.

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