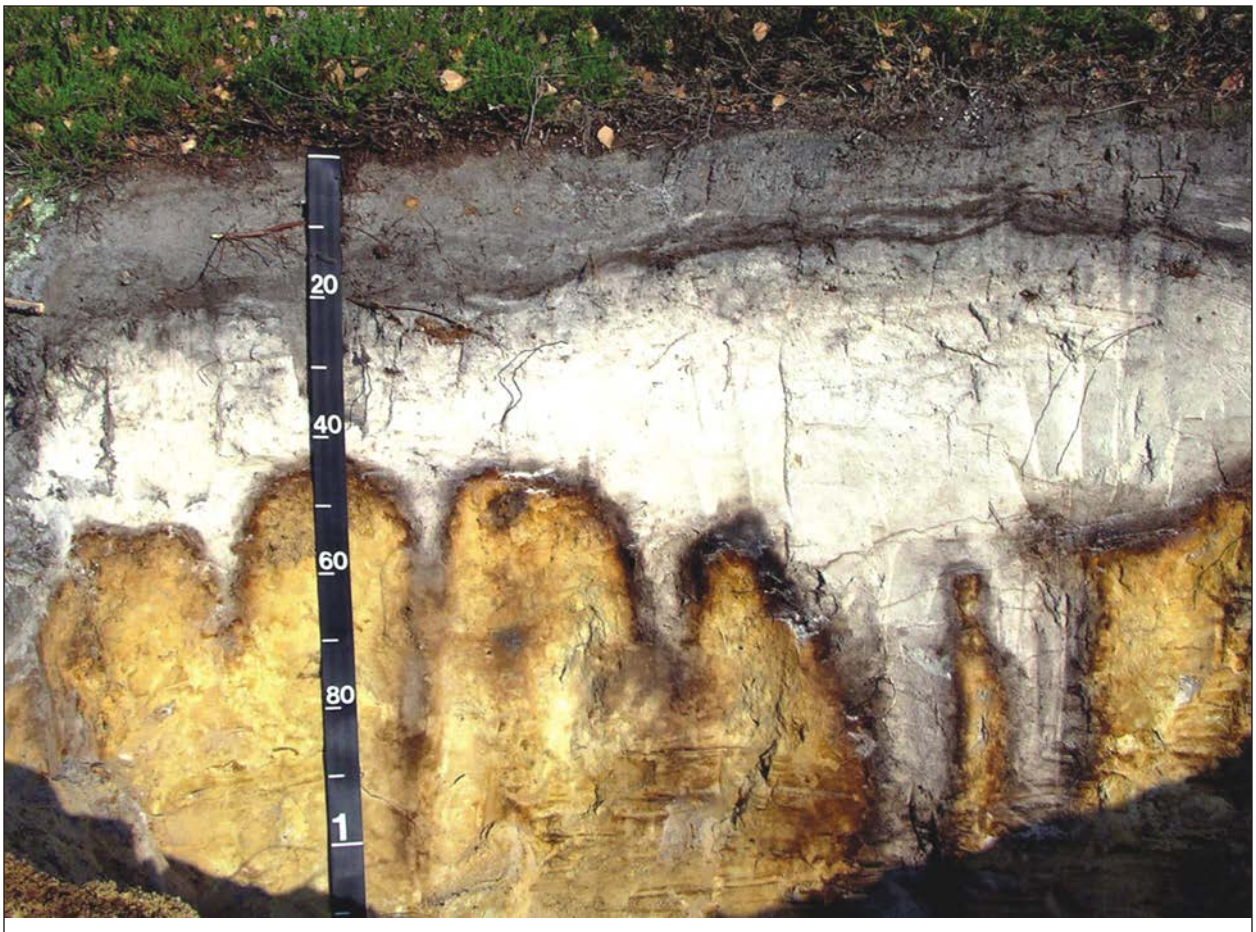


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# Influence of Benomyl on Ability of *Fusarium oxysporum* and *Fusarium solani* to Produce Beauvericin and Rhizosphere Organisms of Cow Pea

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**Abstract:** The ability of two *Fusarium* species to produce beauvericin in the presence or absence of benomyl was investigated. Production of beauveracin was measured by determining its antagonistic activity on some selected pathogens using agar cup plate and agar plug plate techniques. The two methods were found to be reliable for the susceptibility test of *Pseudomonas aeruginosa* to the metabolite produced by *Fusarium* sp. The results of this research work showed that only in the absence of benomyl in most cases allowed *Fusarium* sp. to exact antagonistic potency on some pathogenic bacteria like *P. aeruginosa, Staphylococcus aureus*. Nevertheless, the benomyl concentration of 0.002 g $mL^{-1}$could probably cause genetic modification to occur in *F. solani* that would stimulate the ability of the fungus to synthesize the antibiotic beauvericin that is capable of inhibiting *Klebsiella pneumoniae*. The presence of benomyl in the soil at high concentration of 0.8 g $mL^{-1}$ was found to have adverse effects on the microbial population and interaction of the rhizosphere microorganisms of cowpea (*Vigna unguiculata*).

**Key words:** Benomyl, beauvericin, rhizosphere, antagonistic activity

## INTRODUCTION

A large number of chemical compounds have the ability to inhibit the growth and metabolism of microorganisms or to kill these organisms (Michael *et al*., 2002). Fungicides are antibiological agents specifically designed to control one or several types of agricultural pests. These chemical control agents are collectively known as pesticides. Other Pesticides apart from fungicides are bactericides, herbicides, acarcides, insecticides, nematicides, algicides, plant growth stimulators/retarders etc. They have proved useful in meeting the food production needs of first world nations and will be just as important as the third world moves from food inadequacy to surplus production. Among pesticides, herbicides are generally least toxic (Howard, 1991).

Although fungicides are designed specifically for the control of soil/plant pathogenic fungi which reduce crop yields but the susceptibility of pathogenic fungi to fungicides varies and depends on many factors such as the concentrations of the fungicides under application, the strain type of the fungal organisms being applied to, the genetic constitution of the organism, the environmental factors and so on (Funke *et al*., 2005). Also, the fungi may develop a resistance to such chemical agent (Prescott *et al*., 2005), which may be due to competition between essential metabolites and a metabolic analog (the agent), development of an alternate metabolic pathway which by passes some reaction that would normally be inhibited or killed by the fungicide. It may also result from production of an enzyme altered in such a way that the function on behalf of the cell but is not affected by the fungicide or synthesis of excess enzyme over the amount that can be inactivated by the chemical agent under application, inability of the fungicide to penetrate the cell due to some alteration of the cell membrane and alternation of ribosomal proteins structure (Michael *et al*., 2002). Fungicide at low or slightly high

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concentration may be mutagenic to microbial cells which may affect their genetic make up. It may also induce resistance property (Madigan *et al*., 2005). Some essential metabolites may be over stimulated or under stimulated by organisms under such conditions which may make them change from their original state to another state called mutants. Nevertheless, fungicides kill fungi at high concentration instead of being fungistatic (Michael *et al*., 2002).

Effective fungicide disorganizes the ultra cellular structures of microbial cells and the microbial metabolism. Although, this action depends on the strain type of the organism, the concentration and the rate of application and other factors as stated before. So, much greater disruption of soil biota is caused by fungicides. The Rhizosphere population of nonpathogenic and asymbiotic organisms may be either increased or decreased, depending on the pesticide employed and its rate of application (Funke *et al*., 2005).

Benomyl is, since 1970 registered as a systemic fungicide in a great number of countries, including the United States of America (Prescott *et al*., 2005). It was first introduced into the UK market in 1971 by Dupont Agricultural products, an American company. It is marketed mostly as a 50% wettable powder (Viviana *et al*., 2000). Benomyl is a systemic benzimidazole fungicide that is selectively toxic to microorganisms. It is used against a wide range of fungal diseases of field crops, fruits, nuts and ornamentals. Similar to other benzimidazole fungicides, it is active against broad spectrum of fungi among which are ascomycetes, basidiomycetes and some deuteromycetes while it found completely inactive against the phycomycetes fungi (Madigan *et al*., 2005). Among the well controlled fungal diseases are powdery mildew, apple scab (*V. inaequalis*) and the green mould fungus *B. cinarea*. The commercial names for products containing benomyl include Agrocit, benex, fundazol, benosan, fungicide 1991 and Tersan 1991 but its principal trade name is Benlate. Benomyl is easily hydrolyzed to methyl 2-benzimidazole carbamate MBC (Carbendazim) in very dilute aqueous and in acidified methanolic solutions. MBC in turn hydrolyzes under basic conditions to give 2-amino benzimidazole (2-AB). Benomyl rapidly breaks down on contact with water (Acidic/neutral) to give MBC). Benomyl residues are quite stable; with 48 to 97% remaining as the parent compound 21 to 23 days after application. The makers of benomyl have recently experienced legal battles as farmers claim that the fungicide caused crop damage. The most recent proposed causes of damage is N,N-dibutylurea (DBU), a phytotoxic compound which can form during the manufacturing and storage of benlate ®, DBU formation experiments were conducted by applying the precursor n-butyl isocyanate (BIC) to soil under conditions similar to degradation studies.

The major metabolite produced by Fusarium species are been known to be beavercin (Liach *et al*., 2002). This have been reported to possess antimicrobial microbial activity against some plant pathogens (Machia *et al*., 1995). Benomyl is also a known pesticide and affects germination of fungal spores and the mode of action is similar to that of beavercin (Vivian *et al*., 2000). Also, the influence of pesticides on soil micro floral has been studied extensively (Ocanpo, 1993; Fotso and Smith, 2003). However, the effects of beavercin on human pathogens have not been reported as well as the influence of benomyl on its production by *F. solini* and *F. oxysporum* and other rhizosphere organisms of *Vigna unguiculata*. The aims and objectives of this project are to know effects of Benomyl on the ability of *Fusarium oxysporum* and *F. solani* to produce beauvericin and investigate the influence of Benomyl on the rhizosphere microorganisms of Cowpea (*Vigna unguiculata)* and ordinary soil microorganisms. The antagonistic activities against known human pathogens will also be investigated in order to establish the possibility of using the toxin in chemotherapy.

## MATERIALS AND METHODS

### Source of Organisms

Eight Pure cultures of *Fusarium solani* was collected from International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State, Nigeria. *Fusarium oxysporum* (pure culture) was collected from

Department of Microbiology, Federal University of Tech. Akure. Pathogenic microorganisms: *Klebsiella pneumonia*, *Escherichia coli*, *Salmonella paratyphi*, *Staphylococcus aureus*, *Shigella* sp., *Bacillus cereus* and *Pseudomonas aeruginosa* were collected from University Teaching Hospital (UCH), Ibadan and Microbiology Department, FUTA.

**Fungicide**

Benzimidazole fungicide benomyl (Benlate) was used at four different concentration Co, C1, C2, C3 containing 0.000, 0.002, 0.004 and 0.008 g $mL^{-1}$ or 0.00, 0.05, 0.1 and 0.2 g/25 mL, respectively. *Fusarium solani* (Fs) and *F. oxysporum* (Fo) were grown on sterile prepared sabouraud Dextrose agar in several plates for multiplicity. Some were also grown on agar slope for culture maintenance purpose. They were allowed to grow up to 7 to 10 days before use.

**Cultures of *Fusarium* sp. in Broth Containing Concentrations of Benomyl**

Twenty five milliliters of Sabouraud Dextrose Broth was prepared in 8 sterile bottles (30 mL capacity) each. To the first four Dextrose broths in bottles were added 0.00, 0.005, 0.1 and 0.2 g of weighed benomyl (benlate). They were stirred or mixed together to improve solubility. The second set of Sabouraud dextrose broths in bottles were done in the same way. Well sporulated *Fusarium oxysporum* (7-10 days old) were immersed into the first four Sabouraud Dextrose broths (25 mL) and *F. solani* (well sporulated 7-10 days old) was also added to the second 4 Broth. They were incubated at 25°C for 7-10 days so that enough metabolites would have been synthesized.

**Filtration**

The broth of the *Fusarium* culture in each bottle was filtered through sterile filter paper (Whatman No. 1) to separate the fungal mycelia from the broth. The filtrate from each subsequent filtration containing different concentration of benomyl was obtained after centrifugation at 10000 revolutions per minute.

**Antimicrobial Potency Tests**

The antimicrobial potency was carried out using the *Fusarium* sp. supernatants obtained against eight different pathogenic bacteria. The two methods used are agar cup plate technique (Prescott *et al*., 2005) and agar plug plate technique (Laich *et al*., 2002).

**The Pots Experiment**

Soil was obtained from the teaching and research farm of the Federal University of Yechnology, Akure, Nigeria and sterilized at 180°C for 3 h. Exactly 144 pots were used containing 1.5 kg of sterilized soil. About 10 g of Mycorrhiza (*Glomus mossae*) was inoculated into the pots where applicable at a depth of 5 cm. *Fusarium solani* suspension (5 mL) containing about $2.06\times10^{6}$ macro conidia spore $mL^{-1}$ was inoculated into pots where applicable at a depth of 5 cm. The suspension was prepared by scraping off top portion of the fungus inside the Petri dishes into 100 mL of sterile normal saline.

**Planting Procedure**

Seventy two hours after inoculation of the sample, a different concentration of benomyl was added to soil samples in all the polythene bags and mixed properly with the soil. Seeds of *Vigna unguiculata* (cowpea) were surface sterilized for 2 min in 70% alcohol, rinsed twice in water and planted 96 h after the addition of benomyl with two seeds per pot and planted at a depth of 3 cm. The plants were placed under green house conditions (25°C). The pots were watered every 7th day to field capacity to maintain soil moisture condition. Plants were harvested after 30 days of planting. The planting was done between 28th June, 2006 and 28th July, 2006.

**Isolation of Microorganisms from Rhizosphere of Cowpea (*Vigna unguiculata*)**

The organism in soil sample from the rhizosphere of cowpea was isolated, characterized and isolated using standard techniques (Prescott *et al*., 2005; Funke *et al*., 2005).

## RESULTS AND DISCUSSION

In the absence of benomyl *F. oxysporum* exacted antagonistic effects on *Pseudomonas aeruginosa* and *Staphylococcus aureus* by agar cup plate technique (Table 1), but the susceptibility of *S. aureus* is more than *P. aeruginosa*. The remaining pathogenic bacteria were not inhibited. This may be due to the fact that, these pathogens posses resistant genes in their plasmid which might have conferred resistance on them to the metabolite beauvericin (antibiotic) produced. Also, they may be capable of producing enzymes that could detoxify the antibiotic. In the presence of benomyl, *F. oxysporum* did not inhibit any of the pathogenic bacteria (Table 2).This may be due to the fact that the benomyl at various concentrations has killed the fungus or altered its genetic constitution (Prescott *et al*., 2005).

In the agar plug plate technique, *P. aeruginosa* was inhibited by *F. oxyporum* in the absence of benomyl (Table 2) while the remaining bacteria did not show zones of growth-inhibition. Both agar cup plate technique and agar plug plate technique are reliable methods for antimicrobial potency test (bioassay) for *P. aeruginosa* unlike, *S. aureus* that failed to be susceptible to *F. oxysporum* under Plug plate technique, but showed zone of inhibition of growth by agar cup plate technique. All the pathogenic bacteria used as test organisms showed zones of growth inhibition by *F. solani* in the absence of benomyl by agar plug plate technique (Table 4). Although, the degree of susceptibility of these pathogens varied, *S. paratyphi* has highest level of susceptibility while least susceptibility is found in both *Klebsiella pneumoniae* and *S. aureus*. In the presence of benomyl at various concentrations no zone of inhibition of growth was produced against the test pathogenic bacteria. This may be due to the fact that *F. solani* has been killed by the concentrations of benomyl (C1 to C3).None

Table 1: Antimicrobial effects of *Fusarium oxysporum* on pathogenic bacteria by Agar Cup Technique

| Supernatant of *F. oxysporum* at different concentrations of Benomyl | *Klebsiella Pneumoniae* | *Escherichia coli* | *Salmonella typhi* | *Staphylococcus aureus* | *Shigella* sp. | *Salmonella paratyphyi* | *Bacillus cereus* | *Pseudomonas aeruginosa* |
|---|---|---|---|---|---|---|---|---|
| | Average zones of growth–inhibition of pathogenic bacteria by *Fusarium oxysporum* measured in millimeters | | | | | | | |
| FoC0 | 0.00 | 0.00 | 0.00 | 2.67±3.57 | 0.00 | 0.00 | 0.00 | 9.67±3.67 |
| FoC1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| FoC2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| FoC3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Keys: F0- *Fusarium oxysporum*, C0- Concentration without Benomyl, C1- 0.05 g/25 mL Concentration of Benomyl equivalent to 0.002 g $mL^{-1}$, C2- 0.1 g/25 $mL^{-1}$ Concentration of Benomyl equivalent to 0.004 g $mL^{-}$ ,$^{1}$ C3- 0.2 g/25 mL Concentration of Benomyl equivalent to 0.008 g $mL^{-1}$

Table 2: Antimicrobial effects of *Fusarium oxysporum* on pathogenic bacteria by Agar plug plate technique

| Plugs of *F. oxysporum* at different concentrations of Benomyl | *Klebsiella Pneumoniae* | *Escherichia coli* | *Salmonella typhi* | *Staphylococcus aureus* | *Shigella* sp. | *Salmonella paratyphyi* | *Bacillus cereus* | *Pseudomonas aeruginosa* |
|---|---|---|---|---|---|---|---|---|
| | Average zones of growth-inhibition of pathogenic bacteria by *Fusarium oxysporum* measured in millimeters | | | | | | | |
| FoC0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 14.00±0.94 |
| FoC1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| FoC2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| FoC3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Keys: F0- *Fusarium Oxysporum*, C0- Concentration without Benomyl, C1- 0.05 g/25 mL Concentration of Benomyl equivalent to 0.002 g $mL^{-1}$, C2- 0.1 g/25 mL Concentration of Benomyl equivalent to 0.004 g $mL^{-1}$, C3- 0.2 g/25 mL Concentration of Benomyl equivalent to 0.008 g $mL^{-1}$

Table 3: Antimicrobial effects of *Fusarium solani* on pathogenic bacteria by Agar Cup plate Technique

| Supernatant of *F. solani* at different concentrations of Benomyl | *Klebsiella Pneumoniae* | *Escherichia coli* | *Salmonella typhi* | *Staphylococcus aureus* | *Shigella* sp. | *Salmonella paratyphyi* | *Bacillus cereus* | *Pseudomonas aeruginosa* |
|---|---|---|---|---|---|---|---|---|
| | Average zones of growth-inhibition of pathogenic bacteria by Fusarium solani measured in millimeters | | | | | | | |
| FsC0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| FsC1 | 11.00±2.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| FsC2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| FsC3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Keys: Fs- *Fusarium solani*, C0- Concentration without Benomyl, C1- 0.05 g/25 mL Concentration of Benomyl equivalent to 0.002 g $mL^{-1}$, C2- 0.1 g/25 mL Concentration of Benomyl equivalent to 0.004 g $mL^{-1}$, C3- 0.2 g/25 mL Concentration of Benomyl equivalent to 0.008 g $mL^{-1}$

Table 4: Antimicrobial effects of *Fusarium solani* on pathogenic bacteria by Agar plug plate Technique

| Plugs of *F. solani* at different concentrations of Benomyl | *Klebsiella Pneumoniae* | *Escherichia coli* | *Salmonella typhi* | *Staphylococcus aureus* | *Shigella* sp. | *Salmonella paratyphyi* | *Bacillus cereus* | *Pseudomonas aeruginosa* |
|---|---|---|---|---|---|---|---|---|
| | Average zones of growth-inhibition of pathogenic bacteria by *Fusarium solani* measured in millimeters | | | | | | | |
| FsC0 | 6.00±0.71 | 8.00±2.79 | 11.33±2.14 | 6.00±0.47 | 7.67±2.06 | 14.50±1.06 | 7.33±1.26 | 13.67±0.82 |
| FsC1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| FsC2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| FsC3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Keys: Fs- Fusarium *solani*, C0- Concentration without Benomyl, C1- 0.05 g/25 mL Concentration of Benomyl equivalent to 0.002 g $mL^{-}1$, C2- 0.1 g/25 mL Concentration of Benomyl equivalent to 0.004 g $mL^{-1}$, C3 0.2 g/25 mL Concentration of Benomyl equivalent to 0.008 g $mL^{-1}$

of the pathogenic microorganisms used as test organisms were susceptible to the metabolite beauvericin produced by *F. solani* in agar cup plate technique (Table 3), this may be due also to the possession of resistant plasmid or ability to synthesize antibiotic-detoxifying enzymes (Funke *et al*., 2005).

In the presence of benomyl at concentration of 0.002 g $mL^{-1}$, *F. solani* inhibited *Klebsiella pneumoniae* (Table 3). This may be due to the fact that genetic modification or mutation has occurred in the presence of the chemical agent (benomyl) on the fungus. Further increase in the benomyl concentration beyond 0.002 g $mL^{-1}$ was found to be detrimental to the fungus. This is probably due to the killing of the fungus by benomyl (Edward *et al*., 1991).

The soil microbes found in the rhizosphere of cowpea on the 30 days of planting are more in population in the pot tagged M0F0C0 containing 0.0 g $mL^{-1}$ than M0F0C5 containing 0.8 g $mL^{-1}$ of benomyl (Table 5).This showed that benomyl has adverse effects on the microbial population in the rhizosphere and thereby affecting various rhizopheric interactions that do normally occur in the soil under natural conditions like in the pot MoFoC0 (Howard *et al*., 1991) The ability of *Bacillus* sp. to exist only in non-benomyl inoculated soil M0F0C0 and benomyl inoculated soil M0F0C5 may be due to modified characteristics associated with the bacterium like, resistance property to heat and chemical due to possession of resistant spores and other changes in genetic constitution. This showed that *Bacillus* sp. was able to withstand the heat from the sterilization of soil used in pot experiment and also chemical effects of benomyl inoculated soil containing 0.8 g $mL^{-1}$.

The ability of *Variscosporum elodea* and *Artculosporium inflata* to exist in non-benomyl (0.0 g $mL^{-1}$) and benomyl (0.8 g $mL^{-1}$) inoculated soil may also be due to change in appropriate adaptive features that may be conferred to the organism. But, this was not so for the yeast (*Sachcaromyces cerevisiae*) that was only found in M0F0C0 predominantly. This probably indicated that *S. cerevisiae* was not able to withstand the fungitoxic effects of the chemical agent in M0F0C5.

Table 5: Characterization and Identification of Bacteria isolates of Rhizosphere soil of Cowpea

| Isolates | Cultural and morphological characteristics | Biochemical characteristics 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Probable identity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| X | Creamy, smooth, dull flat and long rods with central spore | + | + | + | - | + | - | - | - - | + - | - - | + - | *Bacillus* sp. |
| Y | Creamy, smooth, dull flat and long rods with central spore | + | + | + | - | + | - | - | - - | + - | - - | + - | *Bacillus* sp. |

1 = Gram staining, 2 = Spore staining, 3 = Motility test, 4 = Indole test, 5 = Catalase test, 6 = Capsule test, 7 = Coagulase test, 8 = Maltose, 9 = Glucose, 10 = Lactose, 11 = Sucrose, X: Bacterium isolate associated with the rhizosphere of cowpea tagged M0F0C0, Y: Bacterium isolate associated with the rhizosphere of cowpea tagged M0F0C5 ++ -acid and gas production; + - acid with no gas production; - - no acid and gas production, + Present; - Absent

Table 6: Characterization and Identification of Bacteria isolates from soil

| Isolates | Cultural and morphological characteristics | Biochemical characteristics 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Probable identity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Golden yellow, circular, entire, shiny, smooth, raised and long rods with central spore | + | + | + | - | + | - | - | ++ | ++ | + - | ++ | *Bacillus* sp |
| B | Creamy, circular, entire, dull, smooth, raised and short rods with central spore | - | + | - | - | + | - | - | + - | + - | - - | + - | *Artthrobacter globiformis* |
| C | White, irregular, rhizoid, dull, rough flat and short rods with central spore | + | + | + | - | + | - | - | + - | + - | - - | ++ | *Bacillus cereus* var. mycoides |
| D | Light yellow, circular, round, shiny, smooth flat and short rods with central spore | + | + | - | - | + | - | - | + - | + - | - - | + - | *Aureobacterium liquefaciens* |
| E | Light yellow, circular, round, shiny, smooth raised and clustered cocci with central spore | + | + | - | - | + | - | + | ++ | ++ | + - | + - | *Staphylococcus aureus* |

1 = Gram staining, 2 = Spore staining, 3 = Motility test, 4 = Indole test, 5 = Catalase test, 6 = Capsule test, 7 = Coagulase test, 8 = Maltose, 9 = Glucose, 10 = Lactose, 11 = Sucrose, ++ denotes acid and gas production; + - denotes acid with no gas production; - - denotes no acid and gas production, X: Bacterium isolate associated with the rhizosphere of cowpea tagged M0F0C0, Y: Bacterium isolate associated with the rhizosphere of cowpea tagged M0F0C5, ++: acid and gas production; +: acid with no gas production; - : no acid and gas production

*Diplosporium flavum* could not be found in non-benomyl inoculated soil (M0F0C0) but found to be associated with M0F0C5 soil only. This may be due to its ability to withstand the fungitoxic effects of the systemic benzimidazole chemical fungicide (benomyl). The fungi and bacteria associated with non-sterilized soil (ordinary soil) were numerous in number. This may be due to the available nutritional factors, micro environment factors and other microbial interactions in the soil (Chanway *et al*., 1991) Thus, the microbial population of the ordinary soil (non-sterilized) is more than rhizosphere soil of cowpea, (Table 6).

## RECOMMENDATION AND CONCLUSION

Benomyl in most cases has adverse effects on *Fusarium oxysporum* and *F. solani* and this may prevent them from synthesizing the metabolite beuvericin which has antimicrobial potency on pathogenic bacteria. It has also been found out that benomyl at low concentration (0.002 g $mL^{-1}$) may stimulate the production of antimicrobial agent like beuvericin due to inhibition of *Klebsiella pneumoniae by Fusarium solani*.

Since, *Fusarium* sp. is very effective against *P. aeruginosa,* it is therefore recommended that more toxicological works and pharmacokinetics researches should be done on beuvericin produced from *Fusarium* sp. probably it can be used as chemotherapeutic drug to treat infection caused by

*P. aeruginosa.* Also, the typhoid fever caused by *Salmonella typhi* may be treated with beauvericin if found to be pharmacologically proven for oral administration. Ojocins *et al*. (1998) reported on the possibility of beauvericin inducing apoptosis in mammalian cells and that the extent of human, animal and plant exposure to this toxin has not been established, therefore, it is very mandatory that more toxicological and mutagenic tests should be studied. Fotso and Smith (2003) have reported on toxicity of beuvericin from *Fusarium* spp. (which is still a subject of debate) can be suppressed by combined therapy or by the use of active toxin -suppressants. This would definitely make beauvericin to be generally acceptable as good antibiotic just like its world wide acceptability as insecticide.

It's therefore recommend that 0.002 g $mL^{-1}$ of benomyl be used to genetically modify *F. solani* in order to stimulate the fungus to produce antibiotic that can be used to control infections. It is not advisable to apply 0.8 g $mL^{-1}$ of the systemic fungicide to soil because it will reduce microbial population of the rhizosphere and various rhizopheric interactions that would have improved soil fertility.

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