JOURNAL OF BIOPESTICIDES AND AGRICULTURE
2465-7115 (Online); 2465-7123 (Print)
http://www.biopjournal.com

BIOCONTROL OF TWO PATHOGENS OF COWPEA *Colletotrichum lindemuthianum* and *Fusarium oxysporum* USING *Bacillus cereus*

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ABSTRACT

An *in vitro* experiment was carried out using *Bacillus cereus* as a biological agent to control *Colletotrichum lindemuthianum*, and *F. oxysporum* provides the use of potential antagonist capable of controlling the pathogenicity of *Colletotrichum lindemuthiam* and *Fusarium oxypsorum* in crops for sustainable agriculture. The experiment shows that for *C. lindeemuthian,* the percentage inhibition was low and the range was 22% to 38.09%. this indicates a slight inhibition.For *F. oxysporum,* the percentage inhibition was high and ranged from 62.50% to 66.90%. This percentage of inhibition was significant for the two fungi pathogen at 95% probability level. The bacterium *Bacillus cereus* can be used as a biopesticide for sustainable Agriculture.

Keywords: *Bacillus cereus*, Potato Dextrose Agar, Dual culture, *C. lindemuthianum*, Nutrient agar.

1. Introduction

Pests and microorganisms attack plants leading to low yields. Plant diseases cause losses in the field and storage. As a result of these, there is a heavy dependence on chemical pesticides to prevent or control these diseases. The high dependence on chemicals, result in environmental pollution and the presence of pesticide residues on food. Plant diseases are responsible for the loss of at least 10% of global food production, representing a threat to food security (Strange & Scott, 2005). Agrios, (2004) estimated that annual losses caused by diseases cost US$220 billion. All over the world, plant diseases were responsible for severe famines in the past (Agrios, 2004).

Cowpea (*Vigna unguiculata* L. Walp) is an annual legume that is very popular in tropical areas especially Africa. It is high in protein and also used as fodders for livestock (*Wong et al* 2006). Disease, especially those caused by fungi are the major constraints to cowpea production in Nigeria (Emechebe and Shoyinka, 1985).

Bacterial species such as *Bacillus* sp. have been used in controlling fungal diseases (Joseph *et al*., 2007), Micro organisms capable of lysing chitin, which is a major constituents of the fungal cell wall, play an important role in biological control of fungal pathogens (Abdullah *et al*., 2008). The genus *Bacillus* has such chitinolytic activity (Huang *et al.,* 2005) *Bacillus* species were found to colonize the root surface, increase the plant growth and cause the lysis of fungal mycelia. They also facilitate interaction between the host plant and other beneficial organisms (Antoun & Prevost, 2006). The United States Food and Drug Administration (USFDA) has granted the "Generally Regarded As Safe" (GRAS) status to species of *Bacillus* such as *Bacillus subtilis* which is regarded as non pathogenic (Harwood and Wipat, 1996). Bacillus species have the capacity to produce spores (Piggot and Hilbert, 2004), during adverse environmental conditions and this helps the bacteria to survive in the phytosphere (Monteiro *et al*., 2005).

Despite the great effectiveness and ease of utilization of pesticides, their use or misuse have caused many problems including significant pollution of soils and ground water reservoirs, accumulation of undesirable chemical residues on the food chain, emergence of fungicide-resistant strains of pathogens, as well as health concerns for growers. In recent time, there is less dependence on chemicals. So there is an increasing demand from consumers and authorities for more safe, rational, sustainable and eco-friendly strategies (Osunlaja and Alamutu 1999). (Osunlaja and Alamutu 1999).

Biopesticides are living organisms or natural substances that are derived from such natural materials as animals, plants, bacteria, and fungi. Biopesticides have an advantage over conventional pesticides.They decompose more quickly in the environment and are generally less toxic towards non target species (Thakore, 2006). Additionally, their modes of actions are usually distinct from those of conventional pesticides. Biopesticides-based products represents about 30% of total sales and have a variety of applications (*Amadioha 1998)*. These microorganisms are obtained from aerial or underground parts of plants that are naturally less or not all affected by a pathogen that devastates a nearby group of the same plant species.

The spores produced by Bacillus can be converted to suspension which can be converted to powder formulations for easy use (Lolloo *et al*., 2010). Shelf life of biopesticides formulated from sporulated bacteria is usually longer and require less storage precaution compared to other products containing living organisms Lolloo *et al* further observe(2010).

2. Materials and methods

Bacillus cereus were isolated from the soil of the experimental farm of Agronomy Department, Delta State University, Asaba Campus by the soil dilution method. The isolates obtained were screened for chitinase production based on the halo produced on plates with minimal salts medium amended with Chitin (1% chitin) (Cook, 1985). The *Bacillus cereus* thus obtained were maintained on Nutrient Agar (NA) amended with chitin. The strain obtained was characterized morphologically and biochemically by following the methods in Bergey's manual of systemic bacteriology (Sneath, 1986).

The fungi *Colletotrichum lindemuthianum* and *Fusarium oxysporum* were isolated from diseased parts of the cowpea plants, using Potato Dextrose Agar (PDA). The cultures were identified sub-cultured repeatedly until pure cultures were obtained. Identification was done using Barnett and Hunter (1987). Confirmation was done at the Pathology department of the Rubber Research Institute Iyanomo, Benin-City. Pathogenicity tests were done for the two fungi.

2.1 Dual Plate Assay

The fungal growth inhibition capacity of *Bacillius* was determined and using the method devised by Huang and Hoes, 1976. One 5mm-disc of a pure culture of the *Colletotrichum lindemuthianum* was centered in a petri dish containing PDA (Potato Dextrose Agar). The *Bacillus cereus* was inoculated at two opposing corners. Plates were incubated for 72 hours at 28°C and growth diameter of the pathogen was measured and compared to the growth in the control experiment where the bacterial suspension was replaced by sterile distilled water. Experiment was run in triplicates Results were expressed as the means of the percentage of inhibition of growth

$$\% \text{ Inhibition} = \frac{\text{Fungal growth}}{\text{Growth in control medium}}$$

$$\% \text{ inhibition} = \frac{DC - DT}{DC} \times \frac{100}{1}$$

DC = Average diameter of the fungus in the control

DT = Average diameter in treatment

Wokocha and Okereke (2005).

3. Result and discussion

The result as shown in Tables 1 and 2 indicated that for *C. lindemuthianum* the percentage of inhibition ranged from 22.00% to 38.09% for *F. oxysporum* the percentage inhibition ranged from 62.50% to 66.90%. T- tests ran on the two show that there is significant difference in the rate of inhibition as they had t-calculated values of 8.889 and 11.049 respectively with df=3 in both cases and significance = 0.003 and 0.002 respectively. They both show high levels of significant difference though that for *F. oxysporum* is higher. This work is in line with that of Chet *et al.* (1990) who used Bacillus sp. as a bio-control agent against some soil borne pathogens. Huang *et al.* (2012) reported on the

antifungal ability of *Bacillus pumilus* against *Rhizoctonia solani*. Gong *et al.* (2006) showed the antagonistic ability of *Bacillus subtilis* against phytopathogenic fungi. The *Bacillus cereus* act by competition for nutrients especially carbon which results in fungistasis and this inhibits the fungal spore germination in soil.

Stein (2005) showed the production of antimicrobial compounds such as antibiotics. Each family of *Bacillus* produces specific antibiotics.

Results as shown in Tables 1 and 2 indicated that there was no definite pattern or trend for the percentage of inhibition. It was high 2nd day, dropped on the 3rd day of assessment, there was a slight decrease on the sixth day but the Bacterium was able to suppress the radial growth of the fungi.

4. Conclusion

This *in vitro* studies reveal the success of *Bacillus cereus* as a bio-control agent against *C. lindemuthianum* and *F. oxysporum* pathogens of cowpea and the *Bacillus cereus* can be used as a potential biological control agent instead of the use of pesticides.

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Table 1: Effects of *B. cereus* on the mycelia growth of *C. lindemuthianum* on PDA at 25°C

Treatments	Days after inoculation/Radial mycelia growth (cm)				
	2	3	4	5	6
Growth of the fungus with *Bacillus*	1.40	1.63	2.15	2.73	2.75
Growth with sterile water (control)	2.10	2.54	3.30	3.50	4.21
% Inhibition	38.09	35.8	22.7	22.0	3.46

t=8.889; Significance= 0.003 at 95% probability

Table 2: Effects of *B. cereus* on the radial mycelia growth of *F. oxysporum* on PDA at 25°C

Treatments	Days after inoculation/Radial mycelia growth (cm)				
	2	3	4	5	6
Growth of the fungus with *Bacillus*	1.17	1.30	1.64	1.95	2.13
Growth with sterile water (control)	3.54	3.67	4.86	5.23	5.68
% Inhibition	66.9	64.57	66.25	62.71	62.50

t=11.049; significance= 0.002 at 95 % probability.

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