

**INTEGRATED MANAGEMENT OF BACTERIAL BLIGHT DISEASE OF COWPEA CAUSED BY
Xanthomonas axonopodis pv. *vignicola* (BURKHOLDER) VAUTERIN.**

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ABSTRACT

Cowpea bacterial blight (CoBB) disease interferes with the photosynthetic ability of the crop, thereby causing significant reduction in crop yield. A two-season experimental trial was conducted between March -May with a repeat in September -November in 2018 to evaluate the efficacy of fourteen treatment combinations on the management of CoBB using a susceptible cowpea variety ITIOK-305 and a pathogenic isolate of *Xanthomonas axonopodis* pv. *vignicola*. The experiment was laid out in a completely randomized design with three replicates. Data were collected on incidence and severity, growth and yield parameters following standard procedures. Cowpea plants that were inoculated but treated with combined application of cow dung and *T. harzianum* had significantly lower incidence of CoBB. Pod yield per hectare was significantly ($p < 0.05$) enhanced by the various treatment combinations and was highest in plants that were inoculated with the pathogen but treated with cow dung and *T. harzianum* with an average of 604-637 kg/ha⁻¹ in both seasons. Grain yield per hectare varied between 222.8-637.2 and 266.2-422.5 kg/ha in the two season trial and was significantly ($p < 0.05$) higher in inoculated and treated plants than the negative control. The combination of cow dung, neem and *T. harzianum* significantly reduced incidence and severity of CoBB and increased cowpea yield better than single application of the bactericide, bacteriomycin.

Keywords: Blight disease, Bacteriomycin, Cow dung, Grain yield, Susceptible

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) grain is an important source of food for man and animal, as well as source of income for farmers who sell the grains and fodder. It forms a major component of tropical farming system due to its ability to improve the soil with poor nutrient level through nitrogen fixation. As a cover crop, it improves the soil structure; controls weed infestation and also serves as live mulch which protects the soil from direct impact of the sun. The dry haulms serve as a source of organic matter when incorporated into the soil after a cropping season. Due to its ability to fix nitrogen in the soil, cowpea provides a cheap source of nitrogen for farmers who grow crops such as maize, wheat, barley and oat as rotational crops after cowpea to maximize yield.

Cowpea production in tropical Africa is constrained by biotic and abiotic factors and its output has been less than the potential grain yield of over 2,000kg/ha. Biotic factors such as pests, diseases, insects, and weeds account for significant yield reduction in the crop. Bacterial blight disease caused by *Xanthomonas axonopodis* pv. *vignicola* (Xav) has been identified as the most important biotic constraint to cowpea, causing huge losses in production (Ganiyu *et al.*, 2017). According to Okechukwu and Ekpo (2008) about 55% yield loss in cowpea has been attributed to cowpea bacterial blight (CoBB). Planting of infected seeds which serve as the main source of primary inoculum can result in pre or post-emergence seedling infection and subsequent mortality. The major impact of Xav infection is on the leaves and depending on the susceptibility of the genotype, it can cause complete defoliation (Claudius-Cole *et al.*, 2014). In severe cases, pods, seeds, and stems are also affected. CoBB infects all stages of cowpea plant including seedling, vegetative, flowering and podding.

The use of chemicals to control cowpea diseases has proved to be effective but may be too expensive for smallholder farmers and not readily available to them. The continuous and indiscriminate use of synthetic pesticides is accompanied by the development of resistant pathogen strains, increased cost of production and toxicity to man and the environment. Therefore, there is the need to explore organic and sustainable approach to plant disease control within the context of integrated management.

Cow dung is an important source of bio-fertilizer, which enhances soil fertility and stimulates the activities of beneficial soil microbes. Application of *Azadirachta indica* (neem) extract has been shown to reduce necrotic lesions in infected leaves, protect flowers and capsules from *Cercospora* spp. infection, thereby reducing incidence of leaf spot disease in sesame (Enikuomohin, 2005). *Trichoderma* spp. have been widely used in agriculture as biopesticides, and biofertilizers on a wide variety of plants against soil-borne, foliar and postharvest pathogens (Harman *et al.*, 2004). Therefore, this study evaluated the efficacy of various combinations of these organic control methods in the integrated management of CoBB disease in cowpea

MATERIALS AND METHODS

Experimental design and materials

The experiment was conducted in the screenhouse of the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria. The experiment was a two-season trial. The early season experiment was conducted between March 15 and May 31 and repeated with a late season planting between September 15 and November 15, 2018 to validate the results. The experiment was laid out in a completely randomized design and replicated three times.

Seeds of a susceptible cowpea variety ITI0K-305 and a pathogenic isolate of *Xanthomonas axonopodis* pv. *vignicola* (Xav) were obtained from the Genetic Resource Center and Germplasm Health Unit of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria respectively. *Trichoderma harzianum* was isolated from cowpea rhizosphere, while a synthetic bacteriomyacin bacteriocide, which served as positive control was purchased from a pharmaceutical store at Ibadan. Neem leaves were harvested from neem trees in the Faculty of Agriculture premises, while cow dung was collected from Teaching and Research Farm of the University of Ibadan.

Isolation of *Trichoderma harzianum* in the rhizosphere

Soil samples were collected from three different locations in a cowpea farm for the isolation of *Trichoderma* species and bulked together as composite. A three-fold serial dilution, 10^{-3} , 10^{-5} and 10^{-6} was prepared from each sample by dissolving one gramme of soil in sterile distilled water in sterile test tubes and mixed thoroughly. Isolation was enhanced by using a selective *Trichoderma harzianum* agar base medium, was prepared by dissolving 25.5 g of dehydrated powder in 960 mL distilled water (Kumar *et al.*, 2012). The agar was sterilized in an autoclave at 121°C and 1.05 kg/cm² pressure for 15 minutes. The medium was allowed to cool to 45°C before it was dispensed into sterile Petri dishes. An aliquot of 1 mL each of the three-fold dilution was dispensed into the Petri dishes and incubated at 28±2°C for three days. *Trichoderma* colonies were purified on potato dextrose agar medium and identified following standard procedures (Samuels, 1996; Barnett and Hunter, 1998). Sterilized wheat seeds were inoculated with a pure culture of *Trichoderma harzianum* and used as substrate carrier. The antagonist was applied at the rate of 5g per 5 kg pot.

Inoculation of cowpea plants

The pure culture of a pathogenic isolate of Xav obtained as described above was mass-produced by inoculating a loopful of the culture in nutrient broth medium in Erlenmeyer's flask and incubated at 28-30°C for three days. Inoculum suspension was prepared by adjusting bacterial cell concentration to 5×10^6 cfu/ml using a spectrophotometer (Nandini,

2012). Five grammes of starch were added as adhesive to allow the inoculum stick to leaves during inoculation. Young cowpea leaves were inoculated at four weeks after sowing (4WAS) by spraying Xav suspension on the surface of leaves and stems with low pressure sprayer. High relative humidity (70-90%) was maintained by spraying sterile distilled water in the screenhouse for seven consecutive days. The control consisted of inoculated plants that were sprayed with sterile distilled water. Inoculated plants observed for typical symptoms of CoBB beginning from two weeks after inoculation.

Preparation of neem extract and cow dung

Fresh leaves of *Azadirachta indica* were obtained as previously described and air-dried at room temperature (20-30°C) for 7-10 days. The dried leaves were ground to smooth powder using a rotary blender model MJ-BL 40G1 JA, USA. Two hundred grammes of the powder were weighed and dissolved in 1000 mL of sterile distilled water to obtain 20% w/v extract concentration, which had earlier proved to be effective in an *in vitro* trial (Dania and Okoye, 2017). The suspension was mixed thoroughly and allowed to stand for six hours before being filtered through a Whatman filter paper No. 4. The neem extract was applied a day after inoculation with Xav. Fresh cow dung was collected from the cattle ranch of the University of Ibadan Teaching and Research Farm and applied at the rate 5.0 t/ha^{-1} which resulted to 7.5g per 5kg pot (Waniyo *et al.*, 2013).

Effect of integrated management of CoBB on cowpea growth and yield

Experimental pots were filled with 5 kg of soil that was sterilized at 120 °C for four hours using an electrical sterilizer H1F 1650 USA. The cow dung was thoroughly mixed with the soil and watered to field capacity for ten days and applied two weeks before sowing of cowpea seeds. The experiment consisted of fourteen treatment combinations:

T1= Cowpea plants inoculated with Xav which served as negative control, T2= Uninoculated cowpea plants., T3= Inoculated cowpea plants treated with cow dung, T4= Inoculated cowpea treated with neem extract, T5= Inoculated cowpea plants treated with *T.harzianum*, T6= Inoculated cowpea plants treated with cow dung and bacteriomyacin, T7=Inoculated cowpea plants treated with neem extract and bacteriomyacin, T8= Inoculated cowpea plants treated with *T.harzianum* and and bacteriomyacin, T9= Inoculated cowpea plants treated with cow dung and *T.harzianum*, T10 = Inoculated cowpea plants treated with cow dung and neem extract, T11 = Inoculated cowpea plants treated with *T.harzianum* and neem extract, T12 = Inoculated cowpea plants treated with cow dung + neem extract + bacteriomyacin, T13 = Inoculated cowpea plants treated with cow dung + neem extract + *T.harzianum* and T14 = Inoculated cowpea plants treated with bacteriomyacin, which served as positive control.

Cowpea plants were inoculated with an inoculum concentration of 5×10^6 cfu/ml, while *Trichoderma harzianum* was applied at the rate of 5 g colonized thoroughly with soil and applied at 5 t/ha⁻¹ **two weeks** before planting to enhance quick release of nutrients for cowpea growth, while neem extract was sprayed on the leaves at 20% w/v concentration using hand sprayer. Plants that were inoculated and sprayed with sterile distilled water served as negative control while positive check consisted of plants that were treated with 0.5% w/v bacteriomyacin. Data were collected on weekly basis, beginning from two weeks after inoculation up till eight weeks at the onset of senescence. Data were collected on growth parameters such as number of leaves, plant height and stem girth, and yield indices including number of pods, number of seeds per pod, pod yield (kg/ha⁻¹) and grain yield (kg/ha⁻¹). Grain and pod yield per hectare were calculated according to AVSD (2013):

Number of pods per square meter = A

Average number of grains per pod = B

Number of grains per square meter = A x B = C.

Yield per square meter = C/100 x 3.4 g = D

Yield in t/ha = D/100

Disease incidence was determined by expressing the number of plants showing CoBB symptoms as a percentage of the total number of plants.

Disease severity was evaluated on a 6-point rating scale according to Okechukwu and Ekpo (2008)

1= No symptom, 2=Very mild symptoms (blight lesions on less than 10% of the leaf area), 3= Mild symptoms (blight lesions on 10-30% of the leaf area), 4= Moderate symptoms (blight lesions on 31-50% of the leaf area), 5= Severe symptoms (blight lesions on 51-75% of the leaf area), 6 = Very severe symptoms (blight lesions on 76% and above of the leaf area).

Data were analyzed using one-way analysis of variance (ANOVA), and mean differences between treatments were separated using Tukey's Honest Significant Difference (HSD) test to evaluate the

wheat substrate per 5kg experimental pot after inoculation with Xav. Cow dung was mixed

level of effects of the pair-wise comparisons at $p \leq 0.05$ using Statistical Analysis System (SAS) Institute (2008) Ver. 9.2.

RESULTS

Effect of treatments on incidence and severity of bacterial blight disease of cowpea

Cowpea plants that were inoculated with Xav but without the application of any treatment had the highest incidences of 78.7% and 83.35% in the early and late season planting at the end of the experiment and were significantly ($p < 0.05$) higher than other treatments (Table 1). Similarly, inoculated plants without treatment had the highest disease severity values of 66.6% and 82% in the early and late season trials respectively, and were also significantly higher ($p < 0.05$) than the other treatments. Plants that were inoculated with the test pathogen and treated with organic materials in various combinations had significantly lower incidences and severity of CoBB which did not differ significantly ($p > 0.05$). Plants that were uninoculated but sprayed with sterile distilled water had no incidence of the disease and differed significantly ($p < 0.05$) from those that were inoculated without treatment (negative control.). Cowpea plants that were inoculated but treated with a combined application of cow dung and *Trichoderma harzianum* had the lowest incidences of 11.8% and 14.7% in the early and late season experiments respectively and were not significantly ($p > 0.05$) different from the positive check. Incidence and severity of CoBB were higher in plants treated with combination of neem extract and bacteriomyacin. There was **significant** variation in the severity rate of symptoms of CoBB disease among inoculated plants, while control plants were asymptomatic (Figure 1)

Table 1. Percent incidence and severity of bacterial blight disease on cowpea plants inoculated with *Xanthomonas axonopodis* pv. *vignicola*

Treatment	Early planting		Late Planting	
	Incidence	Severity	Incidence	Severity
Inoculated with Xav	78.7±3.8a	66.6±3.1a	83.3±5.3a	82.0±4.5a
Uninoculated with Xav	0.0ab	0.0ab	0.0ab	0.0ab
Xav + CD	20.7±1.5ab	12.7±0.3b	21.3±1.1ab	10.4±1.2b
Xav + Neem extract	17.3±0.7ab	34.2±0.5ab	15.6±0.2ab	35.3±1.6ab
Xav + <i>T. harzianum</i>	17.3±0.5ab	18.8±1.1b	18.8±0.6ab	16.2±0.8b
Xav + CD + bacteriomicin	13.3±0.2ab	11.7±0.3b	20.8±0.2ab	13.7±0.3b
Xav + Neem extract + bacteriomicin	26.7±1.3ab	30.2±2.8ab	29.1±1.6ab	33.4±3.3ab
Xav + <i>T. harzianum</i> + bacteriomicin	18.6±0.4ab	12.3±1.0b	15.4±0.4ab	17.7±1.2b
Xav + CD + <i>T. harzianum</i>	11.8±0.3ab	14.5±1.3b	14.7±0.2ab	10.1±1.0b
Xav + CD + Neem extract	13.2±0.5ab	10.1±0.6b	15.3±0.3ab	9.3±0.4b
Xav + <i>T. harzianum</i> and Neem extract	15.5±0.7ab	11.4±0.5b	20.2±0.3ab	10.2±0.4b
Xav + CD + Neem extract + bacteriomicin	14.3±0.3ab	15.7±0.5b	17±0.4ab	12.2±1.1b
Xav + CD + Neem extract + <i>T. harzianum</i>	16.7±0.5ab	13.6±1.2b	15.7±1.2ab	12.6±0.8b
Xav + bacteriomicin	17.8±1.2ab	13.3±1.1b	19.5±1.1ab	10.4±0.5b

Means with same superscript along a column are not significantly different using Tukey's Honest Significant Difference (HSD) Test at P<0.05). Xav = *Xanthomonas axonopodis* pv. *vignicola*, CD = Cow dung. Each value represents mean ± standard error

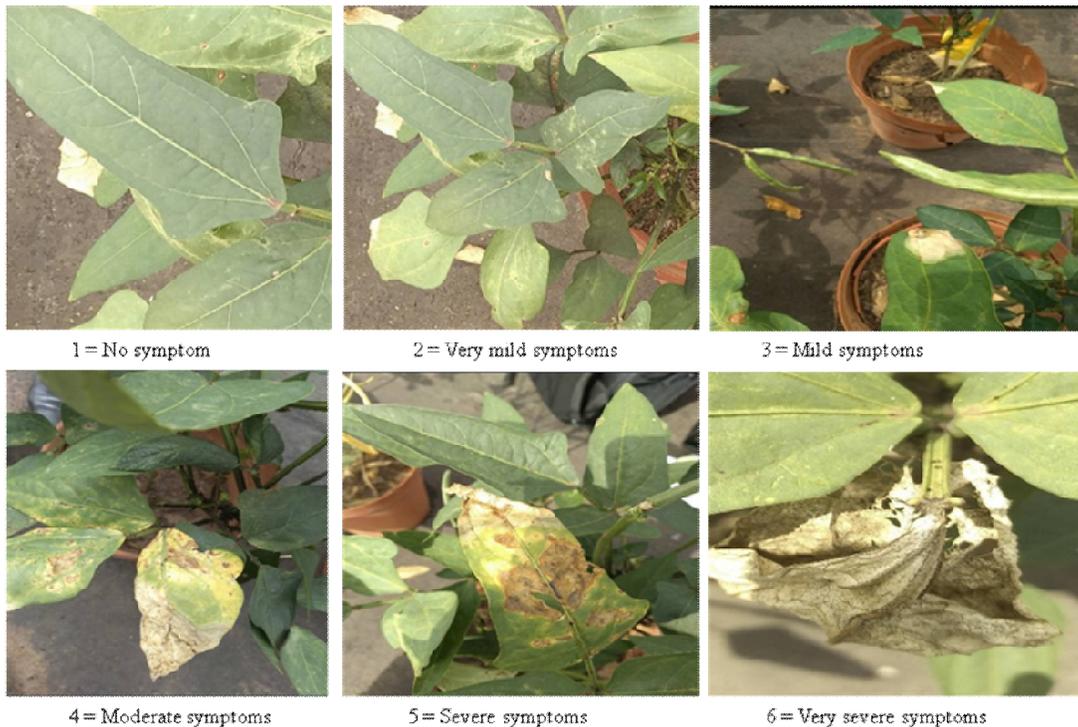


Figure 1. Disease severity rating in cowpea plants inoculated with *Xanthomonas axonopodis* pv. *vignicola*

Effect of treatments on number of leaves, plant height and stem girth

The number of leaves ranged between 20.7- 40.5 and 23.2-38.3 in the early and late season trial

respectively (Table 2). However, the leaf number in cowpea plants that were inoculated without treatment application, uninoculated or inoculated with the application of treatments did not differ significantly (p>0.05). Plant height varied between 22.7-35.3 cm

in early planting and 26.2%-36.5% in the late season trial. Similarly, plant height was not significantly ($p>0.05$) affected by the treatments. The trend of observation was similar in terms of stem girth, which did not differ significantly ($p>0.05$) among the various treatment combinations and control.

Effect of integrated disease management of CoBB on cowpea yield

The number of pods per plant varied between 31.7-65.6 and 36.1-68 among the treatments in the early and late season planting respectively (Table 3). Cowpea plants that were inoculated but treated with cow dung and *T. harzianum* had the highest number of 65.6-68.7 pods per treatment in both trials, which was significantly ($p<0.05$) higher than the yield obtained in both the negative control and positive check. The highest number of seeds of 13.3-14 was obtained in plants that were treated with cow dung and *T. harzianum*. Pod yield per hectare was significantly ($p<0.05$) different among the various treatment combinations and was highest in plants that were inoculated with the pathogen but treated with cow dung and *T. harzianum* with an average yield of 604-637 kg/ha⁻¹ in the two-season trial. Grain yield per hectare varied between 222.8-637.2 and 266.2-422.5 kg/ha in both seasons and was significantly ($p<0.05$) higher in inoculated and treated plants than the negative control.

Table 2. Effect of treatments on number of leaves, plant height and stem girth

Treatment	Early planting			Late planting		
	No.of leaves	Plant height (cm)	Stem girth (cm)	No. of leaves	Plant height (cm)	Stem girth (cm)
Inoculated with Xav	30.2±1.5b	27.2±1.8bc	0.44±0.03bc	25.2±1.2bc	31.2±2.1ab	0.46±0.01b
Uninoculated with Xav	26.2±1.6bc	25.1±1.1bc	0.53±0.01ab	26.9±2.2bc	32.7±1.2ab	0.51±0.01ab
Xav + CD	22.6±2.1c	30.9±0.2b	0.41±0.05bc	23.2±1.5c	31.8±1.8ab	0.53±0.01ab
Xav + Neem extract	32.2±3.2b	25.3±0.9bc	0.55±0.01ab	27.4±1.5bc	26.2±2.1b	0.55±0.03ab
Xav + <i>T. harzianum</i>	20.7±1.4c	30.1±2.8b	0.54±0.01ab	25.9±2.0bc	28.4±1.7b	0.64±0.05a
Xav + CD +Carbendazim	33.2±1.4b	26.2±3.1bc	0.49±0.04b	31.4±1.8b	32.7±1.8ab	0.63±0.05a
Xav + Neem extract +bacteriomyacin	27.4±2.1bc	32.3±1.7b	0.60±0.01a	30.6±3.2b	34.6±3.4ab	0.51±0.01ab
Xav + <i>T. harzianum</i> + bacteriomyacin	31.6±2.1b	32.5±2.7b	0.55±0.07ab	30.2±2.5b	32.2±3.0ab	0.53±0.01ab
Xav + CD + <i>T. harzianum</i>	39.4±0.3a	28.9±2.8bc	0.50±0.05b	33.3±2.5ab	30.0±1.6b	0.65±0.02a
Xav + CD + Neem extract	40.5±1.5a	35.3±2.1ab	0.53±0.05ab	40.1±3.3a	36.5±3.5a	0.45±0.05b
Xav+ <i>T. harzianum</i> + Neem extract	41.1±2.3a	28.8±1.9bc	0.47±0.01b	33.2±1.7ab	37.7±3.4a	0.58±0.01a
Xav + CD + Neem extract + bacteriomyacin	36.3±2.2ab	22.7±2.1c	0.49±0.01b	38.3±2.8a	34.4±1.6ab	0.48±0.01b
Xav+CD + Neem extract + <i>T. harzianum</i>	29.2±2.3b	38.6±3.3a	0.62±0.02a	35.2±3.3ab	33.7±2.1ab	0.54±0.03ab
Xav + bacteriomyacin	35.1±2.8ab	34.4±3.1ab	0.55±0.05ab	26.8±2.1	30.4±1.4b	0.6±0.01

Means with same superscript along a column are not significantly different using Tukey's Honest Significant Difference (HSD) Test at P<0.05). Xav = *Xanthomonas axonopodis* pv. *vignicola*, CD = Cow dung
Each value represents mean ± standard Error

Table 3. Effect of integrated disease management of bacterial blight disease on cowpea yield

Number of leaves Treatment	Number of pods per plant		Number of seeds per pod		Pod yield (kg/ha)		Grain yield (kg/ha)	
	Early Planting	Late Planting	Early Planting	Late Planting	Early Planting	Late Planting	Early Planting	Late Planting
Inoculated with Xav	31.4±0.3ab	36.1±0.1bc	8.5±0.1ab	10.2±0.2	316.8±5.8gh	344.1±6.0e	222.8±4.2de	266.2±3.5ef
Uninoculated with Xav	47.2±0.5ab	51.3±0.5a	10.1±0.1ab	12.4±0.5a	557.3±5ab	468.8±2.4ab	254.2±4.5e	292.3±3.7cd
Xav + CD	40.3±0.5a	59.4±0.1ab	11.9±0.4ab	11.7±0.1a	504.7±3.4bc	580.7±3.8d	277.4±3.5cd	280.8±3.6bc
Xav + Neem extract	48.1±0.2ab	52.2±0.2a	13.3±0.4a	13.1±0.5a	551.4±3.4b	482.3±3.0bc	286.5±3.8ab	350.1±3.2b
Xav + <i>T. harzianum</i>	46.2±0.6a	56.3±0.1a	9.3±0.5ab	11.8±0.1a	485.1±5.7de	551.6±5.3cd	290.8±4.4bc	301.5±4.5cd
Xav + CD +bacteriomycin	59.3±0.4a	60.2±0.3a	11.4±0.2a	10.8±0.1a	498.6±6.6c	557.7±5.2cd	295.6±5.5c	288.2±4.5c
Xav + Neem extract +bacteriomycin	35.7±0.2ab	55.1±0.6ab	12.2±0.2a	12.5±0.3a	408.3±7.3ef	402.3±4.3de	305.5±3.7ef	310.3±4.3bc
Xav + <i>T. harzianum</i> + bacteriomycin	48.3±0.2ab	62.2±0.7a	10.7±0.2ab	11.1±0.6a	502.3±4.8bc	581.5±2.7bc	310.3±7.2b	302.2±3.5c
Xav + CD + <i>T. harzianum</i>	65.6±0.3a	68.7±0.5bc	14.1±0.3a	13.3±0.5a	604.5±8.2a	637.2±1.8a	338.7±3.9a	422.5±2.2a
Xav + CD + Neem extract	49.0±0.1ab	47.2±0.5bc	11.7±0.5a	11.0±0.2a	478.2±5.4ab	577.1±3.4b	255.9±5.4de	300.7±1.7ab
Xav + <i>T. harzianum</i> and Neem extract	56.5±0.1ab	50.3±0.8ab	12.6±0.5a	10.1±0.2ab	403.6±3.8f	583.6±7.8	241.4±4.3d	271.1±3.4e
Xav+CD+ Neem extract + bacteriomycin	62.1±0.1ab	58.5±0.5bc	13.2±0.5a	11.8±0.2a	554.9±5.4ab	601.3±3.4b	323.4±5.4de	388.9±1.7ab
Xav+CD + Neem extract + <i>T. harzianum</i>	50.8±0.5a	57.9±0.1bc	11.1±0.1a	10.3±0.5ab	494.4±6.7bc	556.2±6.2b	225.5±3.3d	287.2±5.1cd
Xav + bacteriomycin	61.3±0.5ab	58.9±0.2	13.2±0.3a	12.5±0.2a	5018±2.9cd	555.7±4.1c	280.2±3.1d	291.8±3.2cd

Means with same superscript along a column are not significantly different using Tukey's Honest Significant Difference (HSD) Test at P<0.05)

Xav = *Xanthomonas axonopodis* pv. *vignicola*, CD = Cow dung

Each value represents mean ± standard Error

DISCUSSION

The amount of soil moisture in the soil, prevailing relative humidity (RH) and ambient temperature are critical indices that influence pathogenesis in plants. Thus, these environmental factors must be favourable to interact with a susceptible host plant in the presence of a virulent pathogen for infection and subsequent disease development. Results from this study showed that CoBB was less prevalent in the early season than during the late season. This may be attributed to the early planting that was done at the beginning of the rains in mid-March when environmental conditions were quite unfavourable to the pathogen development. Shenge *et al.* (2017) also reported higher incidence of bacterial spot and speck during periods of favourable weather conditions to disease development in tomato plants.

Regardless of the treatment combinations, the incidence and severity of CoBB was significantly reduced in both the early and late season experiments. Cowpea plants that were inoculated but treated with a combined application of cow dung and *Trichoderma harzianum* had the lowest incidences in the early and late season experiments respectively. This result is consistent with the previous findings of Basak and Lee (2002) that reported cow dung as an effective manure in the suppression of mycelial growth of *Sclerotinia sclerotiorum*. Previous reports by Akhtar *et al.* (2006) had shown that organic manure reduced disease incidence caused by a wide range of plant pathogens. Rhizosphere-competent species of *Trichoderma* have direct effects on seed germination, fertilizer use efficiency, nutrient uptake, and enhancement of plant defences against biotic and abiotic stress (Shoresh *et al.* 2010). The application of combination of *T. harzianum* and neem extract also significantly reduced CoBB incidence to between 13.2 and 15.3% in the early and late season trials. This result agrees with the findings of Dania and Okoye (2017) that reported increased tomato yield following treatment with neem extract than in control plants that were infected with *Alternaria solani*. Seed oil produced from neem has also been found to significantly reduce the incidence and severity of bacterial blight of cotton (Khan *et al.*, 2000)

Cowpea plants that were inoculated with Xav but treated with a combination of cow dung and *T. harzianum* produced the highest pod and grain yield in the two trial experiments. This result agrees with the previous report of Mandal *et al.* (2013) that the integration of cow dung as biofertilizers in the soil increases crop yield. In addition to supplying major plant nutrients such as nitrogen, phosphorus and potassium, organic manure improves soil structure, aeration, and water holding capacity and stimulates the activities of beneficial soil microorganisms such as *Rhizobium leguminosarum* which helps in nitrogen fixation in cowpea. The carbon-nitrogen ratio in cow dung manure shows that it is a rich

source of protein for microbes that help in the degradation of organic matter, and the release of valuable nutrients in available forms for plant growth (Adegunloye *et al.*, 2007). Cowpea pod and grain yield were found to be higher in the late season than during the early season. This may be due to the prevailing environmental conditions in the early season which were less favourable to crop growth and ultimate yield.

This study has shown that application of cow dung, *T. harzianum* and neem extract in various combinations is more effective in the management of CoBB than treatment with single application of synthetic bacteriomycin. Therefore, the judicious and sustainable application of these treatments in cowpea production will significantly reduce the incidence and severity of the disease, and ultimately increase growth and yield of the crop.

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