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(Research Article)

Synergistic effects of intercropping green beans with okra on the bacteria blight disease

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Abstract

Green bean is a widely consumed as vegetable mostly for its nutritional, health and economic values. In Cameroon, the production is hindered by bacterial blight disease cause by *Xanthomonas campestris*. The aim of this study is to evaluate the synergistic effects of intercropping green bean with okra on bacterial blight disease of green beans. Beans varieties (local green bean, jackpot green bean and dolly green bean) were planted in a field in a Randomized Complete Block Design with six treatments and three replicates in Department of Crop Production Technology of the University of Bamenda research farms. The first, second and third treatments consisted of inter-cropping of dolly green bean, jackpot green beans and local green with okra and fourth, fifth and sixth consisted of mono-cropped of dolly green bean, jackpot green beans and local green beans varieties which serves as the control experiments. Data was collected for disease incidence, disease severity, and number of pods and weight of pods were recorded in all the fields. Pathogenicity assessment was conducted in the green house by inoculating healthy green bean plants with bacterial blight isolate and lesion area measured. Significant variations (P≤0.05) were observed in disease incidence, disease severity and yield. The highest mean disease incident (100 %) severity (64 %)and low number of pods (4.3) were recorded in monocropped fields and the intercropped fields had lowest mean disease incidence(40 %), severity (23 %)and high number of pods (27.3).Inter-cropping green beans with okra can be recommended to control bacterial blight disease of green beans varieties.

Keywords: Green beans; Okra; Bacterial blight; Intercropping; Synergistic; Bambili

1. Introduction

Green beans *(Phaseolus vulgaris L.*) are an annual vegetable crop belonging to the family Fabaceae (1). Green bean originated from Central and East Asia and spread to South and Central America (2, 3). Green bean grow best when the temperature ranges from 24 to 25 °C, and best cultivated on soils that drain well such as Sandy loam soils (1). Green bean is consumed by many and it is a good source of protein, carbohydrates, fibre, vitamin A and C, zinc and iron (4). Green bean is used to prevent some human diseases such as diabetes, cancer and cardiovascular diseases (5, 6). It also helps in improving the soil through symbiotic nitrogen fixation (7).

In 2020, the world production of green beans was 23 million tones with china producing 77 % of the total production (8).Green beans production in Cameroon is done by small scale farmers which serves as source of income for their

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family (9, 10). The Western highlands Agro ecological Region of Cameroon are the highest producers of green bean in Cameroon producing more than 90 % of national production (9).

The production of green beans is hindered by pest and diseases which are fungi, viruses and bacteria. Among the diseases, bacterial blight caused by *Xanthomonas campestris* is one of the most devastating and widely distributed bacteria diseases of green bean plants (11). This disease reduces yields up to 40 % in most localities where green bean is cultivated (12). Bacteria blight affects the foliage, pods of beans and causes significant losses in both yield and seed quality (13). Disease occurrence, epidemic development and severity dare influenced by cropping systems and production practices, topographical features, crop genotypes, altitudinal ranges, cropping season and field management practices under a given environment (14).

There is high inoculums rate of *Xanthomonas campestris* in the field which has reduced green bean production of 40 %and those farmers who depend on it for income has also reduce their house hold income. In the last years, chemical has been used to control the disease. Spraying foliage with a copper-based bactericide once the disease appears reduces disease development (15). Applying contact bactericides early in the seasons every 7 to 10 days intervals during cool, moist weather can decrease establishment of bacteria pathogens (16). However, these methods are not very friendly because they caused harm to the environment and the organisms in the environment (17).Intercropping has many advantages, such as increased diversity, which facilitates better biological control of pests and reduced soil erosion, resulting in reduced pest and disease incidence (18). There's no documented information on the synergistic effects of intercropping combination of green beans with okra on bacterial blight of green beans in Bambili. Okra belongs to the family malvaceae and it is not a host of bacterial blight so it can be used to intercrop with green beans to control the spread of bacteria blight. Therefore, the aim of this study is to assess the synergistic effects of intercropping green beans with okra on the bacteria blight disease.

2. Materials and methods

2.1. Location of experimental sites

The study was carried out in the research plot and green house of the Department of Crop Production Technology of the University of Bamenda, located in Bambili, Tubah Sub-division in the North West region of Cameroon. It is located at latitude 5^{o} 60' 33" North and longitude 10^{o} 15' 21" East. This area is found 1444 m, 4737.53 ft., 56850.42 above sea level. Laboratory analysis was carried out in Catholic University, Bamenda, Cameroon (19).

2.2. Climate dynamics of Bambili

Bambili falls under the western highlands plateau agro ecological region of Cameroon. The climate is tropical, characterized by two seasons; a long rainy season which starts from mid- match and ends in mid-November and a dry season which begin from mid-November to mid-March and has an average temperature of 23 °C, ranging between 15-32 °C, characterized by annual rainfall of 2400 mm (20)

2.3. Effect green beans inter-cropped with okra on bacteria blight disease and yield of green beans

Three varieties of green beans (dolly green bean, jackpot green bean and local green bean), and one variety of okra (Cafeier variety) were used for this study. The layout was done in a randomized complete block design (RCBD) with 6 treatments and 3 replications. Treatments one, two and three consisted of dolly green bean, jackpot green bean and local green bean intercropped with okra at inter-row spacing of 30 cm for okra and 25 cm for green bean. Treatment four, five and six consisted of mono-cropping of dolly green bean, jackpot green bean and local green bean which serves as the control methods. The field was planted on the 8 April 2023. Two seeds were planted per hole for all the treatments. Weeding and molding was done manually with the use of a hoe and hands at two weeks' interval till maturity. Disease incidence and severity were recorded at two weeks' intervals starting from the first appearance of the symptoms in the field. Disease incidence was assessed by counting the number of infected plants of each variety times a hundred percent.

$$\text{Disease incidence} = \frac{\text{Number of infected green bean plants}}{\text{Total number of green bean plants}} \text{ x } 100$$

Disease severity symptoms of each green bean variety was scored using the scale of 0 to 4 (21)

0 = no symptoms.
1= Presence of lesions 1-25 % of leaf area
2= Presence of lesions 26-50 % of leaf area
3= Presence of lesions 51-75 % of leaf area
4= Presence of lesions 76-100 % of leaf area.

2.4. Yield assessment of green beans varieties

The yields of all the green bean varieties were assessed 70 days after planting. Pods from fifty plants in each plot were counted and recorded. The weights of these pods were taken by weighing pods of plants in each plot using a weighing balance.

2.5. Collection, isolation, and identification of *Xanthomonas campestris*

Infected leaves of three green beans varieties were collected from diseased green bean plants showing symptoms of common bacterial blight disease in the field in Bambili, preserved in separate fuel papers and transported to the Catholic University of Cameroon (CATUC) laboratory, Bamenda town. Leaves were cut into small fragments of 2 mm from the portion having common bacterial blight symptoms and sterilized for 2 minutes in 75 % ethanol, and was rinsed in 2 changes of distilled water. The plants materials were transferred with a sterile pair of forceps into a mortar and crushed. Ten mills of distilled water was added in the mortar and the leaf extract transferred into Petri dishes and allowed for 5 minutes (22).Bacterial suspension were streaked over the surface of Nutrient agar dishes and allowed for 48 hours in a sterile inoculating chamber to obtain single bacterial colonies. Single bacteria colonies were inoculated by drawing four perpendicular sets of three streaks each at the edge of each agar dishes and incubated at 18 °C, Bacterial cultures were purified 3 times by single colony transfer on fresh nutrient agar dishes until a pure culture was obtained (figure 1). Spores were observed with the use of a microscope and counted with a haemocytometer. The number of spores/ml on the haemocytomer was estimated and calculated using the formula adopted from (23).

$$S=NV/v$$

Where
S= Number of spores per milliliter
N= Mean number of spores in 10 large squares counted

$$V=1\ ml=1000\ mm^3$$

v=volume of spore suspension under glass cover = 2 $x10^4 mm^3$

Figure 1 Pure culture of *Xanthomonas campestris*

2.6. Preparation of inoculums

Four days- old culture of bacterial isolates was used to prepare spore suspension. 2.5 ml of sterile distilled water was poured into a beaker. The spores in each Petri dish were brushed into three separate beakers. The spores were adjusted using haemocytometer to a spore density of $2x10^4$ spores/ml of distilled water. The spores were transferred into three separate syringes and then inoculated on the leaves of different green bean varieties in the green house (24).

2.7. Green house test

The three green bean varieties (dolly green bean, jackpot green bean and local green bean) were planted in plastic pots filled with steam sterilized soils in the green house. These plants were arranged in completely Randomized Design with four replications of three plants. Green beans were inoculated 14 days after planting with four days old spore suspension of common bacteria blight. Syringe was used to inject the spore suspension on a punctured spot on the leaf. The leaves were observed and the area of the lesions of inoculated leaves was recorded at 3, 6, 9, 12, 15, 18 days after inoculation by multiplying the width and length of infected area.

2.8. Statistical analysis

Data collection for disease incidence, severity, yield parameters, and area of lesion of *Xanthomonas campestris* for the three varieties of green beans were subjected to analysis of variance (ANOVA) using statistical software (Originlab, 2021). Their treatment means were separated using Tukey HSD and least significant difference (LSD) t statistical significance of 95% confident interval (P≤0.05). Mean data were used to plot graphs for appropriate representation of the results.

3. Results

3.1. Effect of green beans and okra inter-cropped and mono-cropped green beans on bacteria blight disease incidence

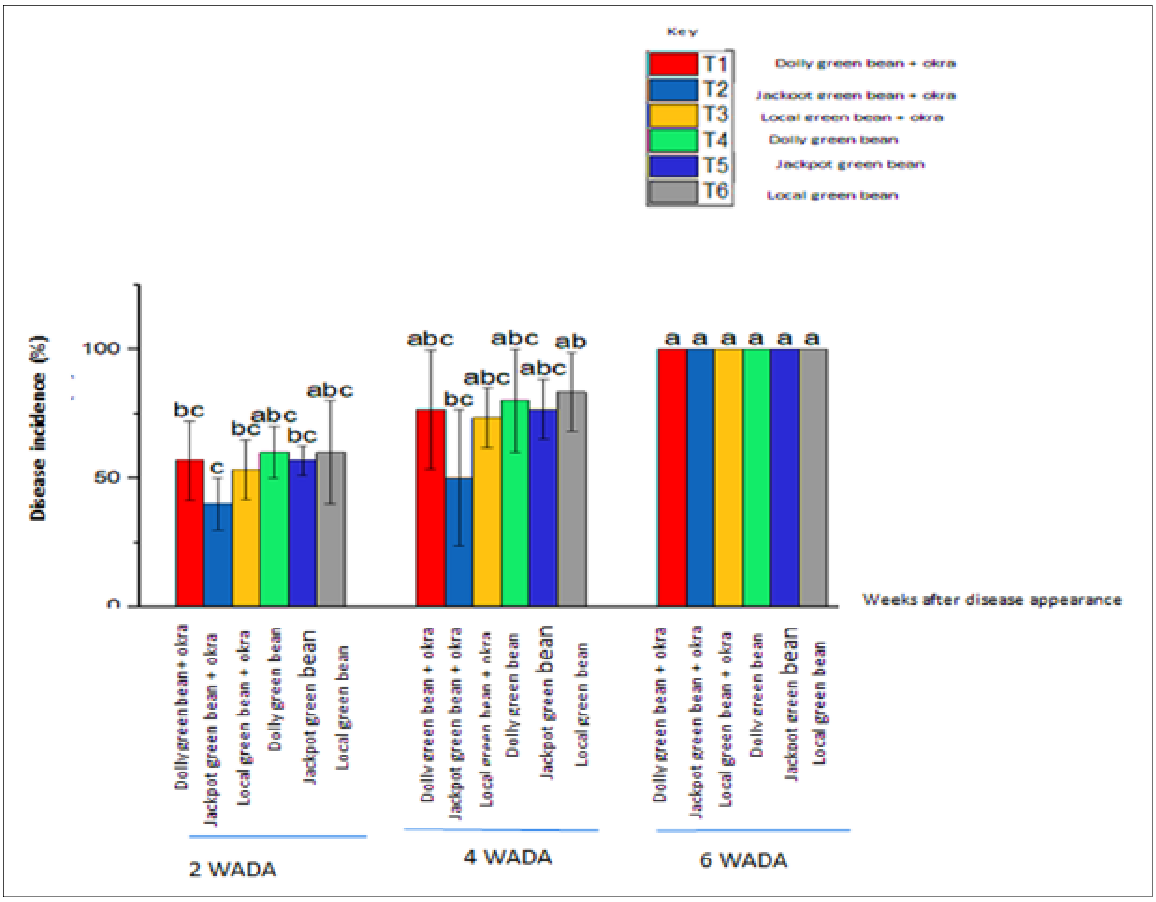


Figure 2 Incidence of bacteria blight disease at different intervals on green beans in green bean – okra intercrop and monocrop. Mean bars with the same letter within an interval are not significantly different (Tukey HSD, α = 0.05)

Bacterial blight disease symptoms appeared in the field 20 days after planting with black lesions on the leaves. The rate of infection increased in both inter-cropped and mono-cropped plots. At six weeks of disease incidence, all green bean plants were affected with bacteria blight disease. The highest mean disease incidence of 100 % was recorded in all the treatments at six weeks of disease appearance and the lowest mean disease incidence of 40 % was recorded in inter-cropped plots of jackpot green bean with okra. Mono- cropped and inter-cropped plots showed no significant difference ($p > 0.05$) in the mean disease incidence at 6 weeks after disease appeared in the field (figure 2).

3.2. Effect of green beans and okra inter-cropped and mono-cropped green beans on bacteria blight disease severity

Disease severities for the different treatments were different from two to six weeks after disease appeared in the field. The leaves of all the green bean plants were highly infected for the mono-cropped treatments as compared to those of the inter-cropped treatments. Inter-cropped treatments, jackpot green bean and local green bean showed less infection rate. There was decrease in disease severity for local green bean inter-cropped with okra at 4 weeks after disease appeared in the field. The highest mean disease severity of 51, 57.3 and 64 were recorded in jackpot green bean, dolly green bean and local green bean variety for mono-cropped plots as compared to low mean disease severity of 32.6, 36.3 and 43.6 on local green beans, jackpot green beans and dolly green beans inter-cropped with okra at week six respectively. Generally, there was a significant difference ($p \leq 0.05$) in mean disease severity on green bean varieties for both mono-cropped and inter-cropped plots in the field (figure 3).

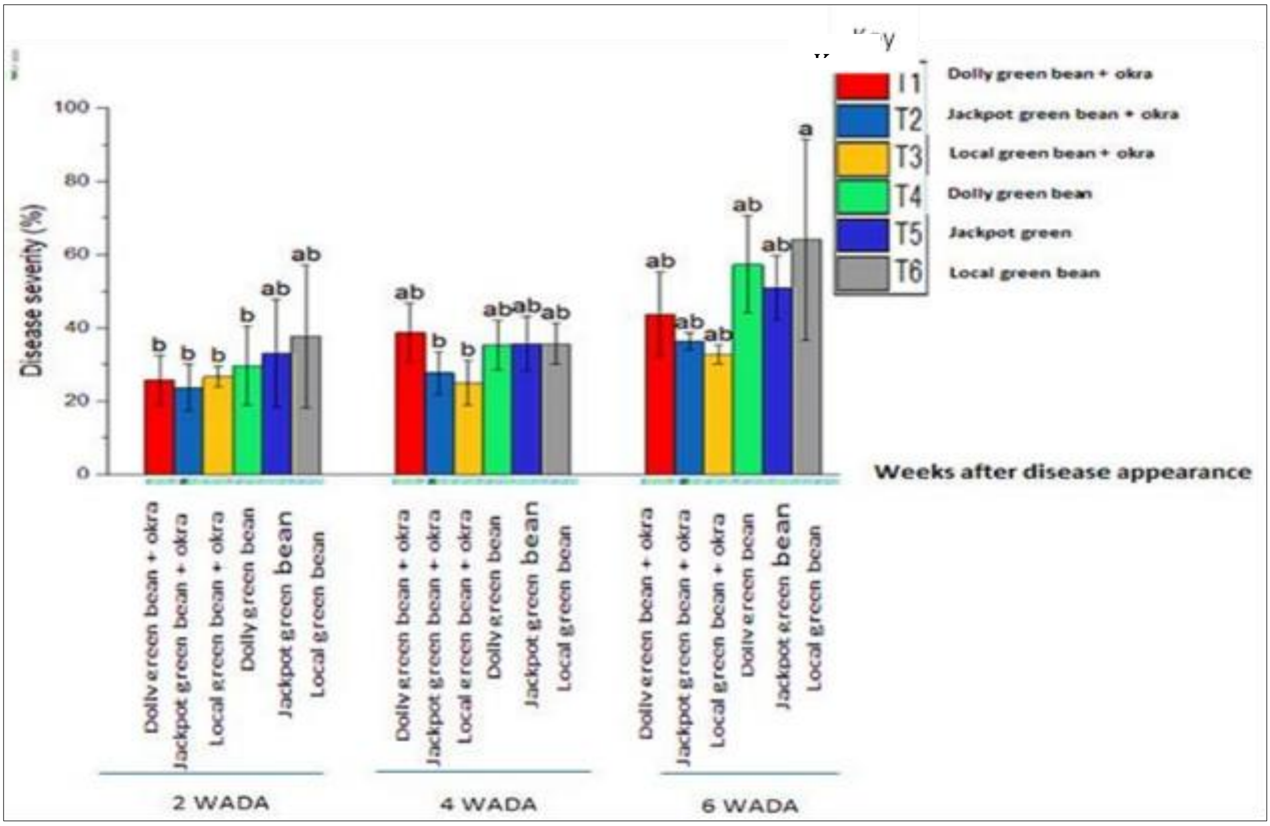
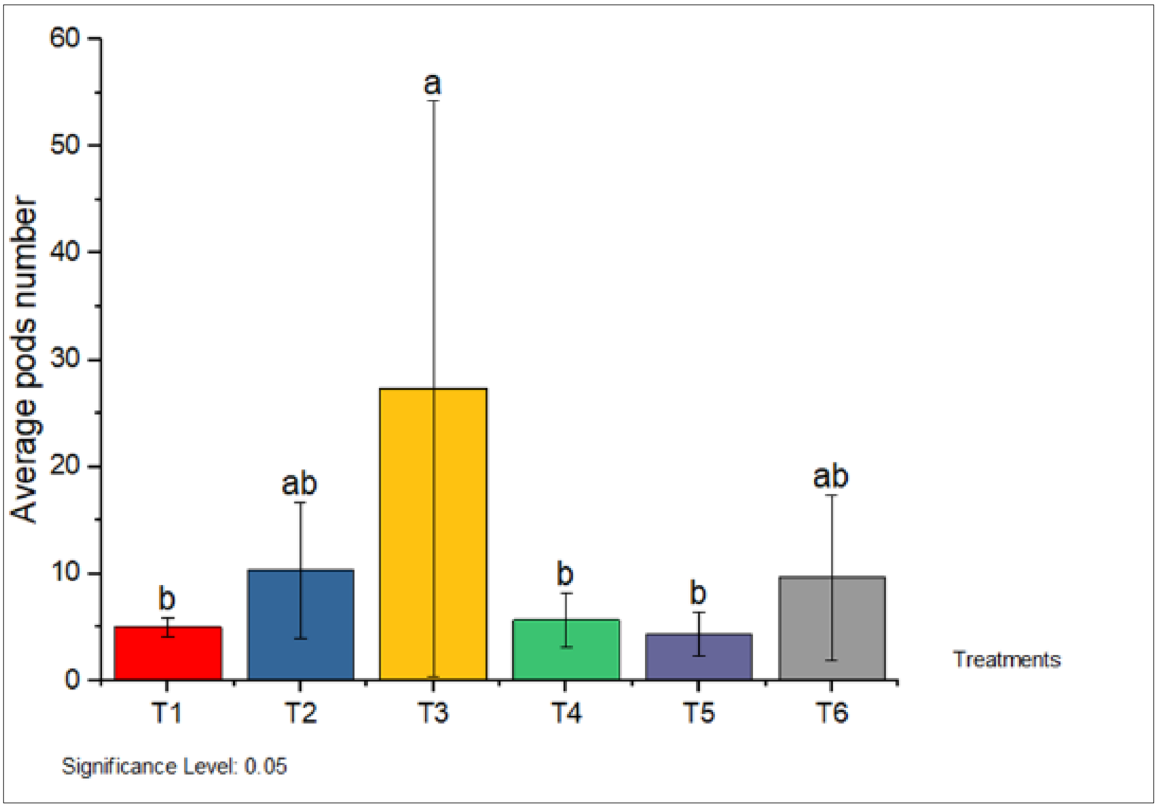


Figure 3 Severity of bacteria blight disease at different intervals on green beans in green bean – okra intercrop and monocrop. Mean bars with the same letter within an interval are not significantly different (Tukey HSD, α = 0.05)

3.3. Assessment green beans intercropped with okra and mono-cropped green beans on number of pods per plot

Number of pods was recorded 70 days after planting. The number of pods was different for the three green bean varieties (jackpot green bean, local green bean and dolly green bean) both in the mono-cropped and inter-cropped patterns. The highest mean number of pods of 27.3as recorded on local green bean variety in the inter-cropped treatment while the lowest mean number of pods of 4 was recorded on jackpot green bean in the mono-crop treatment. This study showed that there was significant difference ($P \leq 0.05$) in the mean number of pods amongst the treatments (figure 4)

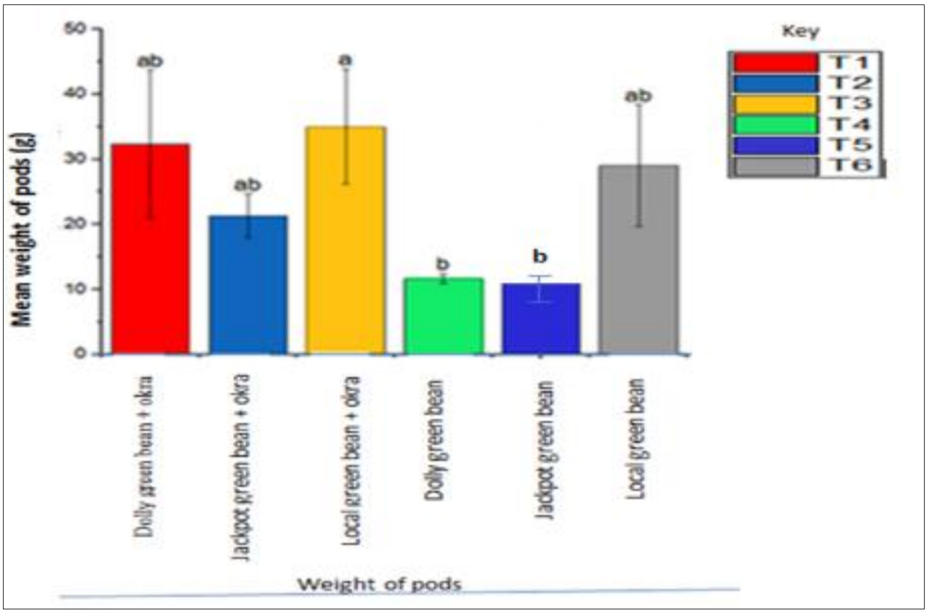


T1= dolly green bean intercropped with okra;T2= jackpot green bean intercropped with okra;T3= local green bean intercropped with okra ;T4= mono-cropping of dolly green bean;T5= mono-cropping of jackpot green bean ;T6= mono-cropping of local green bean

Figure 4 Effect of inter-cropping and mono-cropping on mean number of pods of different green bean varieties

Mean bars with the same letter are not significantly different (Tukey HSD, α = 0.05).

3.4. Assessment green beans intercropped with okra and mono-cropped green beans on weights of pods per plot



Mean bars with the same letter are not significantly different (Tukey HSD, α = 0.05).

Figure 5 Effects of inter-cropping and mono-cropping on the weight of pods of different green bean varieties

The highest mean weight of 35g was recorded on the pods of local green bean variety in the inter-cropped treatment while the lowest mean weight of 11.6g was recorded on the pods of jackpot green bean in the mono-cropped treatment. Dolly green bean had the largest pod weight and jackpot green bean had the smallest pod size. There was no significant variation in the mean weight of green bean pods for both mono-cropped and inter-cropped treatments (figure 5)

3.5. Pathogenicity assessment of *Xanthomonas campestris* from infected plant materials

The results obtained in the laboratory showed that spores were visible four days after it was sub cultured on nutrient agar. There was a significant difference (p≤0.05) in the mean number of spores observed among the green bean varieties (jackpot green bean, local green bean and dolly green bean) isolates. Local green beans had the highest number of spores of 7 while the lowest number of spores of 4 was observed in dolly green bean at four days of culture (figure 6)

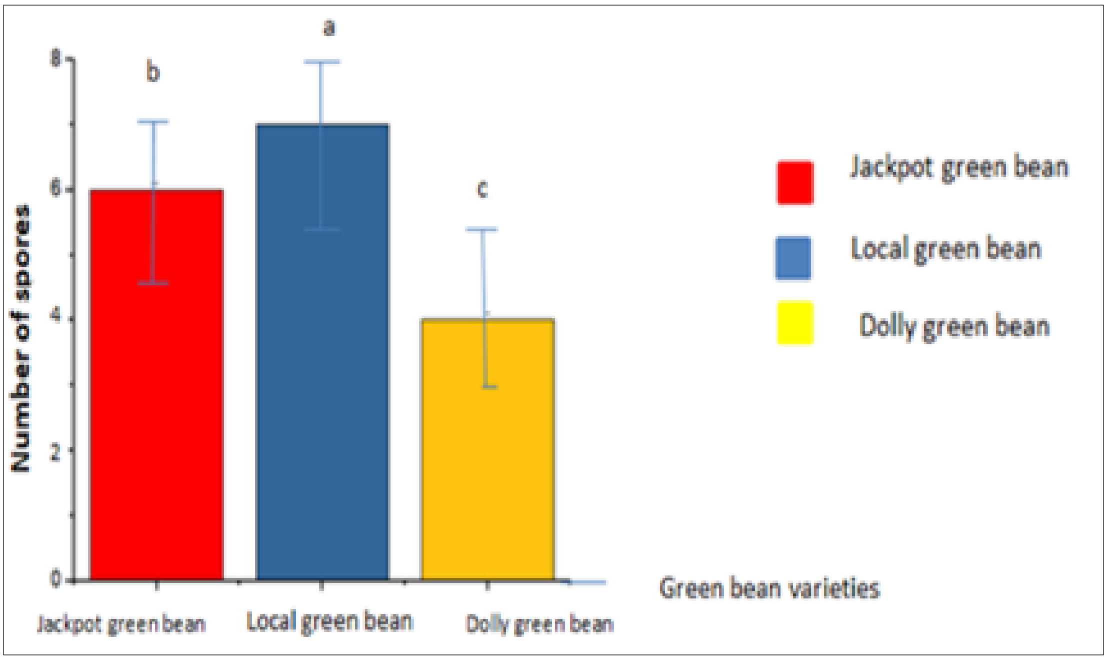


Figure 6 Number of spores of three green bean varieties

Mean bars with the same letter are not significantly different (Tukey HSD, α = 0.05).

3.6. Pathogenicity test of Xanthomonas campestris

Three days after disease inoculation in the green house, disease appeared on the leaves of three green bean varieties (jackpot green bean, local green bean and dolly green bean). Black spots were observed on the leaves with higher rate of infection in jackpot green bean. Lesion area increased from day three to eighteen day and became stable. There was a significant difference (p≤0.05) in the lesion area of the green bean varieties (jackpot green bean, local green bean and dolly green bean). The highest lesion area of 1 mm^2 was observed in all the green bean varieties while the least lesion area of 0.1 mm^2 was observed in dolly green bean and local green bean varieties. All the varieties were susceptible to the disease because all measured the same area from day 18 after inoculation (figure 7).

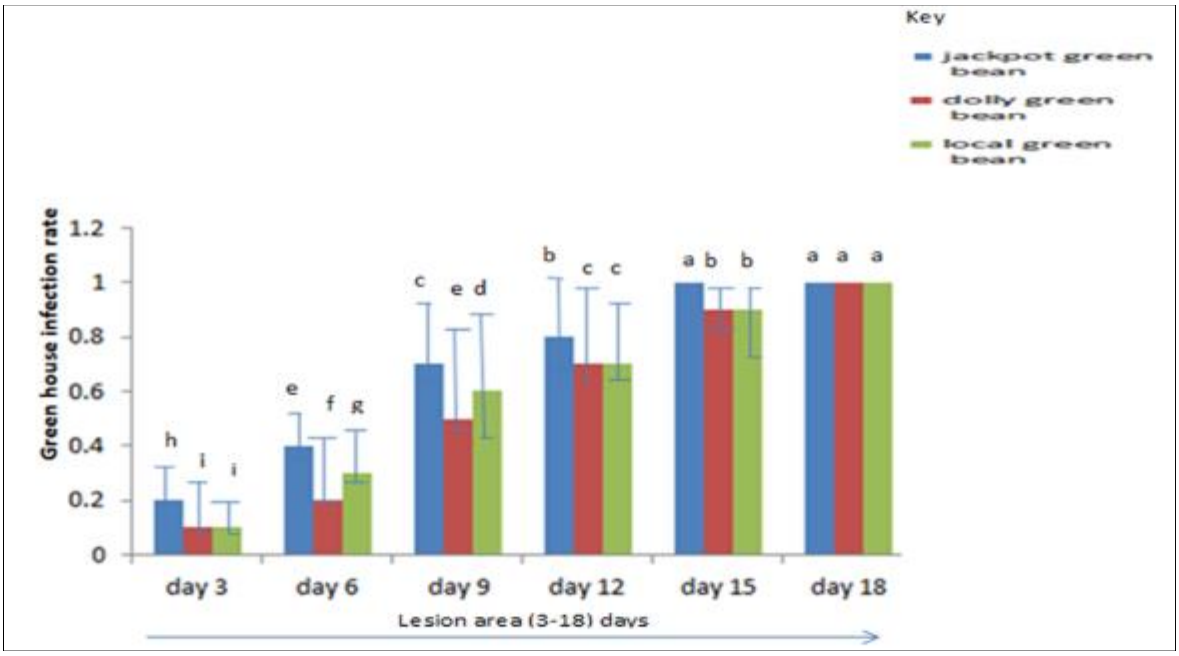


Figure 7 Effect of spores' inoculation on the leaves of jackpot green bean, local green bean and dolly green bean plants in the green house

Mean bars with the same letter are not significantly different (Tukey HSD, $\alpha = 0.05$).

4. Discussion

Mono- cropping and inter-cropping showed no significant difference in the mean disease incidence at six weeks of disease appearance in the field. This may be due to primary inoculum on crop residues which are likely to have more consistent impact on disease risk under conducive environmental conditions (25, 26).

This study revealed that disease severity was higher for the mono-cropped treatments as compared to those of the inter-cropped treatments. This might be associated to the use of same plant variety which is a host to the same pathogen that permit rapid spread of pathogen from neighboring plants during favorable environmental conditions for disease development (27). The beans plants in the intercropped field had less disease damage this could be due to the okra plant which intercepts disease spread from the okra to beans plant since okra is not a host of *Xanthomonas campestris.* Furthermore, the distance between the plants could reduce free dispersal of pathogens (28). There was a decrease in disease severity at four weeks of disease appearance because of no rainfall and decrease in humidity. This result is confirmed by (29) who reported that low humidity and rain decreases disease development in the field**.** Some of the green beans varieties were highly susceptible (local green beans and dolly green beans) while the other was moderately susceptible (Jack pod green beans) this could be attributed to the difference in the genetic constitution amongst the green beans varieties (30).

The traits which are directly responsible for crop yield such as number and weights of pods showed that there was a significant difference ($P \leq 0.05$) in the mean number of pods and weight of pods for all the treatments. The number and weight of pods in all the green bean varieties was higher for inter-cropped treatments as compared to mono-cropped treatments. This variation in yield could be attributed to the type of cropping system that may have influence on the overall crop output (31).

Laboratory results showed that spores were visible four days after it was sub cultured on nutrient agar and there was a significant difference ($p \leq 0.05$) in the mean number of spores observed among the green bean varieties (jackpot green bean, local green bean and dolly green bean) isolates. This might be due to the different concentration spores in the nutrient agar in different Petri dishes. Variability in number of spores might be attributed to different types of isolate in nutrient agar medium which produces colonies of different kinds and characteristics (32). Furthermore variability in number of spores can be compared to the study of (33) which reported that genetic variability in plant species influences the diversity of fungal spores associated with these plants

Black lesions were observed on inoculated green bean leaves three days after inoculation and bacteria blight infection rate on local green beans variety was higher than those of jackpot green bean and dolly green bean varieties. This may be because of the genetic variation of the green bean varieties. This result is confirmed by (34) who reported that large pathogenic variation could have been associated with mutation, recombination and migration. Also, Goodwin(35), Agrios (36) reported that physiological mechanisms in *phaseolus vulgaris* such as the release of chemical compounds (phenols, tannins and avenalin) into its environment that inhibits the movement of bacteria in plant tissues and thereby reduce the accumulation of bacteria attacking the plant or internal tissues in seeds. Susceptible varieties accumulate larger bacterial populations in the leaves that translocate rapidly through vascular tissues than resistant and partially resistant genotypes (35).

5. Conclusion

This study indicates that inter-cropping green beans varieties with okra reduces common bacterial blight disease in the field and increases yield. The intercropped of local green beans with okra was more resistant than intercropped of jackpot green bean and intercropped of dolly green bean with okra. Pathogenicity test of bacterial blight on the different green bean varieties revealed that all the three varieties were infected by spores of 2 x 10^4 spores/ml of distilled water. Lesions observed on inoculated leaves showed that jackpot green beans was more susceptible to common bacterial blight disease than local green bean and dolly green bean. Thus, inter-cropping can be used to control common bacterial blight disease of all the green bean varieties used in this study.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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