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Evaluation of rhizobium and nitrogen fertilizer for the control of bacterial blight in green beans (*Phaseolus vulgaris* L.) varieties

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Bacterial blight is a serious disease affecting green beans (*Phaseolus vulgaris L.*), impacting all growth and yield parameters and potentially causing a yield loss of about 40%. Inadequate information exists on the evaluation of nitrogen fertilizer potential on bacterial blight diseases in green bean cultivars. The aim of this study was to assess the potential of nitrogen fertilizer in managing bacterial blight disease in green bean varieties to improve growth and yield. Three bean varieties (Cora, Dolly, and Local Black Green Beans) were planted in the field using a completely randomized block design with twelve treatments and three replicates. Fields were treated with nitrogen fertilizer at different concentrations of 30 and 60 g, and subsequently treated with rhizobium inoculants, except in the control field where no treatment was applied. Data for disease incidence, disease severity and yield parameters were collected. Pathogenicity assessment was conducted in the greenhouse by inoculating healthy plants with a bacterial isolate (2×10^4 spores/ml) and measuring the lesion diameter. A significant difference ($p \leq 0.05$) was observed in disease incidence, severity, and yield parameters across the different treatments. The highest average number pods per plant of 21 and lowest mean disease severity of 32.6% was recorded in local black green bean variety treated with rhizobium (bacteria) inoculant. This indicates that the application of rhizobium inoculant can be the best management option for controlling bacterial blight disease in all three bean varieties studied.

Key words: Bacteria blight disease, nitrogen fertilizer, green beans varieties.

INTRODUCTION

Green beans (*Phaseolus vulgaris L.*) are an annual herbaceous plant in the Fabaceae family (Kaplan et al., 1999). They originated from South Mexico and Central America (Bitocchi et al., 2012). Green beans are cultivated mainly in areas of high rainfall and grow best when the air temperature is 29°C (Branka et al., 2021). Green beans are an important leguminous crop consumed worldwide and have high nutritional value, including protein, zinc, vitamin K, iodine, calcium, and iron (Petret et al., 2015; Savita, 2020). Green beans also

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have medicinal properties; they contain calcium, which is important for strong, healthy bones and reducing the risk of fractures. They decrease the risk of obesity, diabetes, and heart diseases (Savita and Rimsi, 2014). Green bean cultivation is a good source of income for local producers, with some subsistence farmers generating enough revenue from green bean production to meet basic family needs (Akibode et al., 2011). In Cameroon, the Western Highlands are the biggest producers of green beans, accounting for more than 90% of the total production (Anonymous, 2010). The Food and Agricultural Organization Statistical Database (FAOSTAT, 2017) showed that the world's production of green beans in 2017 was 24,221,252 tons, with Cameroon ranked 57th in the world with a production of 4,631 tons. Despite the high nutritional and economic value of green beans, their production faces major constraints such as diseases and pests (Thibaud et al., 2017; Mieke et al., 2013; Jaffee and Henson, 2004). The crop is susceptible to many diseases, including fungal, bacterial, and viral infections (Gupta et al., 2021).

Among these diseases, bacterial blight caused by *Xanthomonas axonopodis pv. phaseoli* is a major economically important disease of green beans, causing crop yield losses of up to 40% (Ferreira et al., 2003; Miklas et al., 2011). This disease is usually severe in warm, humid climates with high levels of rainfall, leading to losses in both yield and seed quality (Jadon et al., 2020).

Bacterial blight disease causes lesions on the leaves, pods, branches and petioles that result in severe premature defoliation (Belete and Bastas, 2017). The margin of the lesion is marked by brown spots with lemon yellow narrows (Harveson and Schwartz, 2007). The problems associated with bacterial blight are exacerbated by the difficulty in controlling it due to the seed-borne nature of the bacteria and its capacity to produce large amounts of secondary inoculum (Belete and Bastas, 2017). Despite all research that has been carried out on the bacterial blight disease management (biological, chemical and cultural) of green beans, its production is still a major problem (Takudzwa et al., 2017). To address this issue, effective management strategies for green bean production in Cameroon are needed. Nitrogen fertilizers are commonly used to increase crop yields by enriching the soil with nutrients, which promotes healthy plant growth and enhances disease resistance. Thus, the aim of this study is to evaluate the potential of nitrogen fertilizers in managing bacterial blight disease in green bean varieties.

MATERIALS AND METHODS

Experimental sites

This research was carried out on school research farms and in a screen house at the University of Bamenda, located at a latitude of 6º north of the Equator and a longitude of 10º east of the Greenwich Meridian, at an altitude of 1600 m above sea level. Additionally, part of the research was conducted at the Phytopathology Laboratory in the Catholic University of Bamenda.

Field evaluation of nitrogen fertilizer on incidence and severity of bacterial blight disease

Green bean seeds of three different varieties (Dolly Green Bean, Cora Green Bean, and Local Black Green Beans) were inoculated with a powdered rhizobium inoculant (Rhizobium TMG) at a rate of 3.5 g per 100 g of seeds. Each 100 g of seeds from the varieties was spread separately in clean plastic bowls and mixed with a solution of 200 ml of water containing 3.5 g of rhizobium inoculant and 20 g of sugar; the sugar helped the inoculant adhere to the seeds. The mixture was stirred for 30 s with a wooden spoon. The inoculated seeds were then dried in a cool, shaded place to protect the rhizobium from sunlight (Deaker et al., 2004). These bean varieties were planted on the 8th of April, 2023.

Seeds were planted by placing three seeds per hole at a depth of 2.5 cm into the soil with 15 cm spacing between plants, and later thinned to two seeds per hole after two weeks of planting. Planting of these bean varieties was done using a completely randomized block design with 12 treatments and 3 replicates. These treatments consist of the following:

T1 consist of seeds treated with rhizobium bacteria inoculant (V1-dollygreen beans)
T2 consist of seeds treated with rhizobium bacteria inoculant (V2-coragreen beans)
T3 consist of seeds treated with rhizobium bacteria inoculant (V3 - local black green beans)
T4 consist of uninoculated dolly green seeds applied with 30 g of nitrogen fertilizer (NPK) in the field (V1)
T5 consist of uninoculated cora green beans seeds applied with 30 g of NPK fertilizer in the field (V2)
T6 consist of uninoculated local black green beans seeds applied with 30 g of NPK fertilizer in the field (V3)
T7 consist of uninoculated dolly green beans seeds applied with 60 g of NPK fertilizer in the field (V1)
T8 consist of uninoculated cora green beans seeds applied with 60 g of NPK fertilizer in the field (V2)
T9 consist of uninoculated local black green beans seeds applied with 60 g of NPK fertilizer in the field (V3)
T10 consist of the Control field, dolly green beans seeds not inoculated nor fertilized (V1)
T11 consist of the Control field, cora green beans seeds not inoculated nor fertilized (V2)
T12 consist of the Control field, local black green beans seeds not inoculated nor fertilized (V3)

The uninoculated seeds were planted first to avoid contamination of the inoculated seeds. The nitrogen fertilizer (NPK) was applied by burying it in 5 cm deep trenches beside the plants. It was applied twice: once during planting and again two weeks after planting. Disease incidence was assessed immediately after the disease appeared by counting the number of infected plants in the middle bed at two-week intervals. Percentage incidence was calculated using the standards adopted from Groth et al. (1999).

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

The disease severity symptoms of each plant of the various green beans varieties were scored using the scale from 0 to 4 of (Asad et al., 2018).

O= no symptoms, 1= 1-25 % leaf areas infected with bacteria blight disease, 2=26-50% leaf areas infected with bacteria blight disease, 3= 51-75% leaf areas infected with bacteria blight disease, 4=76-100% leaf areas infected with bacteria blight disease.

$$\text{Disease severity} = \frac{Area\ of\ infected\ leaves}{Total\ number\ of\ leaves} X\ 100$$

Yield assessment of green beans

All the green bean varieties were assessed 65 days after planting for yield by recording the number and weight of the pods. The number of pods per plant was counted, and the weight of pods per plant was measured using an electronic scale.

Test for pathogenicity *Xanthomonas axonopodis pv. phaseoli*

Preparation of Nutrient Agar

The nutrient agar used for this experiment consists of 5 g of peptone, 5 g of NaCl, 1.5 g of beef extract, and 15 g of agar. Twenty-eight grams of this agar mixture was measured and placed in a conical flask containing 500 ml of distilled water. The solution was mixed thoroughly and boiled for 10 min. The dissolved mixture was then sterilized at 121°C for 15 min. After allowing it to cool, the nutrient agar was poured into different petri dishes in a sterile laminar flow chamber to solidify. The petri dishes were covered and stored in a refrigerator.

Collection, isolation, and identification of *X. axonopodis pv. phaseoli* from infected plant materials

Infected leaves of green beans displaying symptoms of bacterial blight disease were collected from the field in Bambili, preserved in separate plastic bags, and transported to the Catholic University of Bamenda laboratory for isolation of *X. axonopodis pv.* phaseoli. The edges of the infected portions of the green bean leaves were cut into smaller pieces measuring 2 mm each. These plant materials underwent sterilization for 2 min in 75% ethanol and were then rinsed in three changes of sterile distilled water. A pair of sterilized forceps was used to transfer the treated plant materials to a few drops of sterile distilled water in a mortar, where they were pounded and allowed to stand for up to 5 min. The resulting bacterial suspension was streaked over the surface of Nutrient agar dishes and incubated for 48 h in a sterilized inoculating chamber to obtain single bacterial colonies. These single bacterial colonies were inoculated by drawing perpendicular sets of three streaks each at the edge of each agar Petri dish and then incubated at 18°C.

Bacterial cultures were purified three times by single colony transfer on fresh nutrient agar Petri dishes until an axenic culture was obtained (Fokunang, 2000). Spores were observed using a microscope and counted using a haemocytometer. The number of spores/ml was estimated and calculated using the formula adopted from Duncan and Torrance (1992).

$S = NV/v$

where S = number of spores per milliliter; under glass cover = 0.0002 mm^3 (2 x 10^4 mm^3).

Preparation of inoculum

The pure culture obtained previously was used to prepare a spore suspension. In a beaker, 2.5 ml of sterile distilled water was poured. The spores from each Petri dish were brushed and placed in three separate beakers. The spores were adjusted using a haemocytometer to a density of 2×10^4 spores/ml of distilled water. Subsequently, the spores were transferred into three separate syringes and then inoculated onto the leaves of various green bean varieties in the greenhouse.

Green house test

The three green bean varieties (Dolly, Cora, and local black green beans) were planted in plastic pots filled with steam-sterilized soil in a greenhouse. These plants were arranged in a completely randomized design with three replicates, each containing four plants. Green beans were inoculated with a spore suspension of *X. axonopodis pv. phaseoli* 14 days after planting. Inoculation was performed by using a syringe to inject the spore suspension onto one spot on each leaf. Observations were carried out, and the lesion diameter was measured using a ruler. Data for the average lesion diameter were recorded at 3-day intervals for 18 days by multiplying the length and width of the infected area (Manju et al., 2020).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using statistical software (Originlab, 2021). Mean data were used to graphically represent the results appropriately.

RESULTS

Impact of nitrogen fertilizer application on disease incidence

Bacterial blight disease symptoms appeared at 2 weeks after planting (WAP), black spots were observed on the leaves. Disease incidence of 100% was recorded at 6 weeks after planting for all the twelve treatments of the three green bean varieties.

For all the twelve treatments of the three green bean varieties there was a significant difference ($p \leq 0.05$) on disease incidence from 2 to 4 weeks after disease appeared in the field (Figure 1).

Impact of nitrogen fertilizer application on disease severity

Disease severity was significantly different ($p \leq 0.05$) among all twelve treatments across the different varieties of green beans. The Cora green bean variety exhibited the highest infection levels, except in the treatment involving 30 g of NPK fertilizer. Conversely, the local black green bean variety consistently displayed the lowest infection rates across all twelve treatments.

The highest mean disease severity of 68.6% was recorded in the Cora bean variety, which served as the control group in the field, while the lowest mean severity of 32.6% was observed in the local black green beans treated with rhizobium bacteria inoculant at the sixth week, respectively (Figure 2).

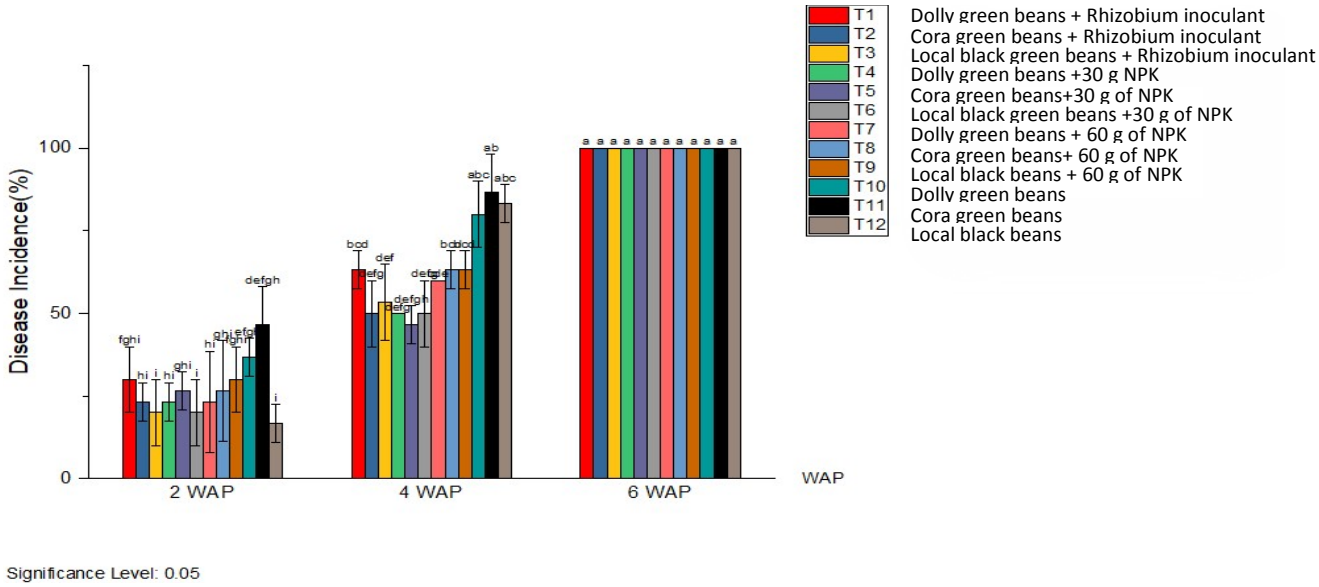

Figure 1. Disease incidence on green beans varieties at 2 to 6 weeks after planting. Bars represent mean incidence and standard errors, WAP= weeks after planting.

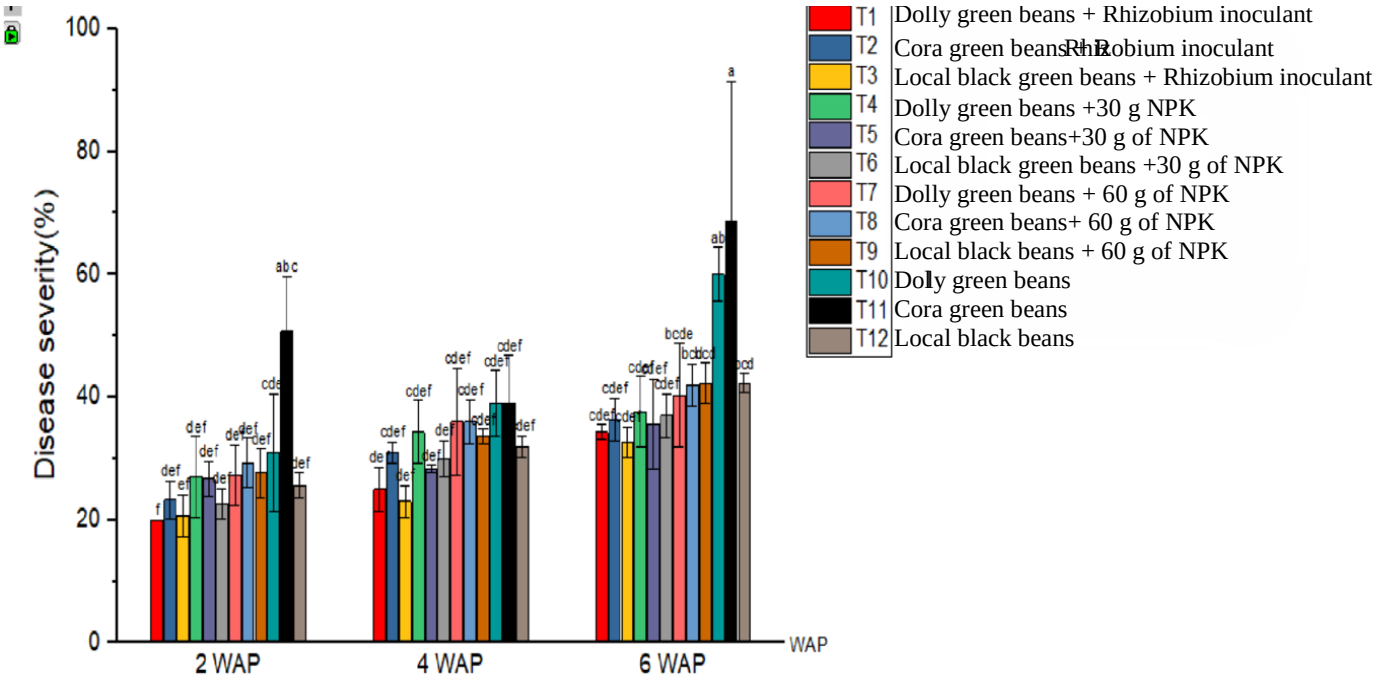

Figure 2. Disease severity on green beans varieties at 2 to 6 weeks after planting. Bars represent mean incidence and standard errors. WAP= weeks after planting.

Assessing the effects of Nitrogen Fertilizer on number of Pods of green beans

Crops matured at 65 days after planting. This study revealed significant differences ($p \leq 0.05$) in the number of pods among all twelve treatments across the three green bean varieties. The highest average number of pods per plant, totaling 21, was recorded in the local black green beans variety treated with rhizobium bacteria inoculant, whereas the lowest number of pods per plant, only 2, was observed in the control field of the Cora green beans variety (Figure 3).

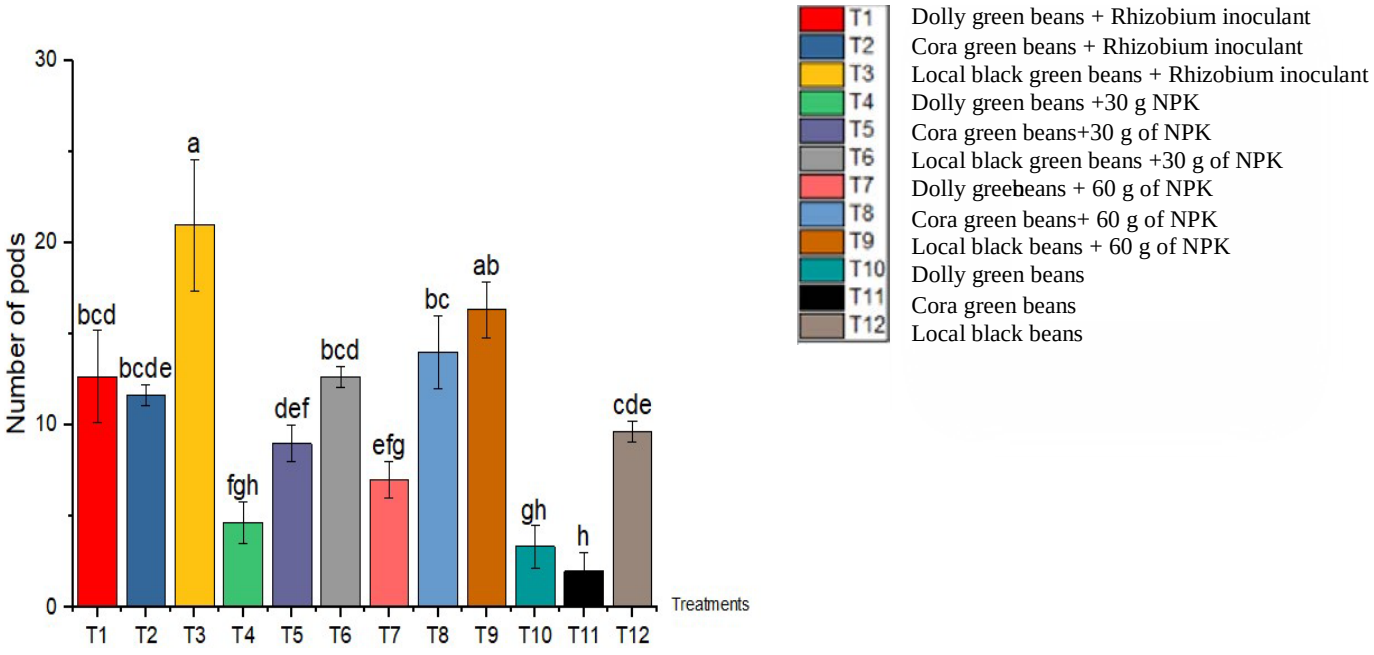

Figure 3. Number of Pods showing different treatments of green beans varieties. Bars represent mean number of pods and standard errors.

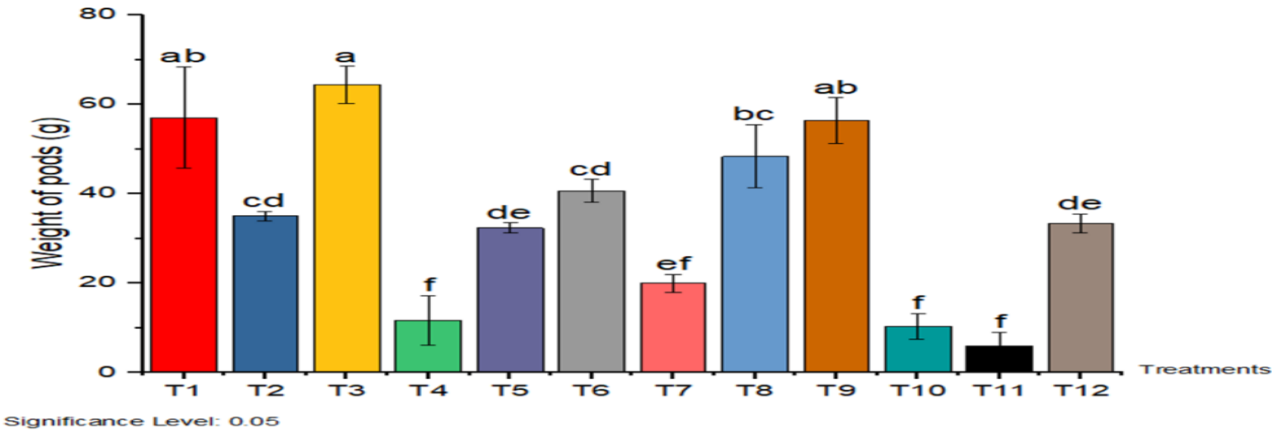

Figure 4. Weight of pods. Bars represent mean n umber of pods and standard errors. T1 = Dolly green beans + Rhizobium inoculant, T2 = Cora green beans + Rhizobium inoculant, T3= Local black green beans + Rhizobium inoculant, T4= Dolly green beans +30 g NPK, T5= Cora green beans+30 g of NPK, T6= Local black green beans +30 g of NPK, T7= Dolly green beans + 60 g of NPK, T8= Cora green beans+ 60 g of NPK, T9= Local black beans + 60 g of NPK, T10= Dolly green beans, T11= Cora green beans, T12= Local black green beans.

Weight of pods

Significant differences ($p \leq 0.05$) were observed among all twelve treatments across the three green bean varieties. The average mean pod weights indicated that the local black green beans variety treated with rhizobium inoculant exhibited the highest pod weight of 64.3 g, whereas the control field of the Cora green beans variety displayed the lowest pod weight of 6 g (Figure 4).

Pathogenicity assessment of bacterial blight of green beans

Numbers of spores observed in culture media in the laboratory

Results obtained in the laboratory indicated that creamy white spores were visible 4 days after it was sub cultured on nutrient agar to obtain a pure culture (Figure 5). There

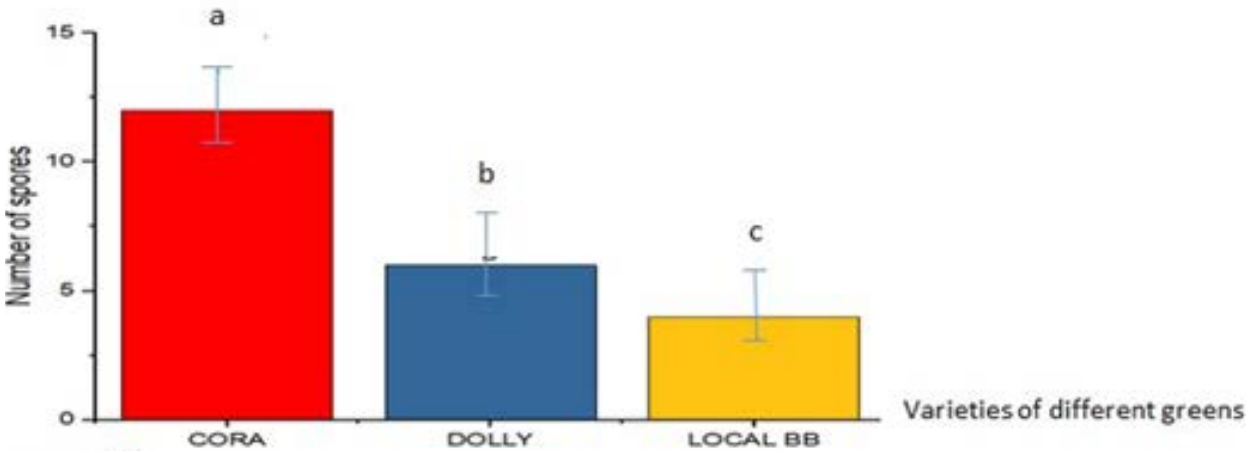

Figure 5. Growth of spores in culture media.

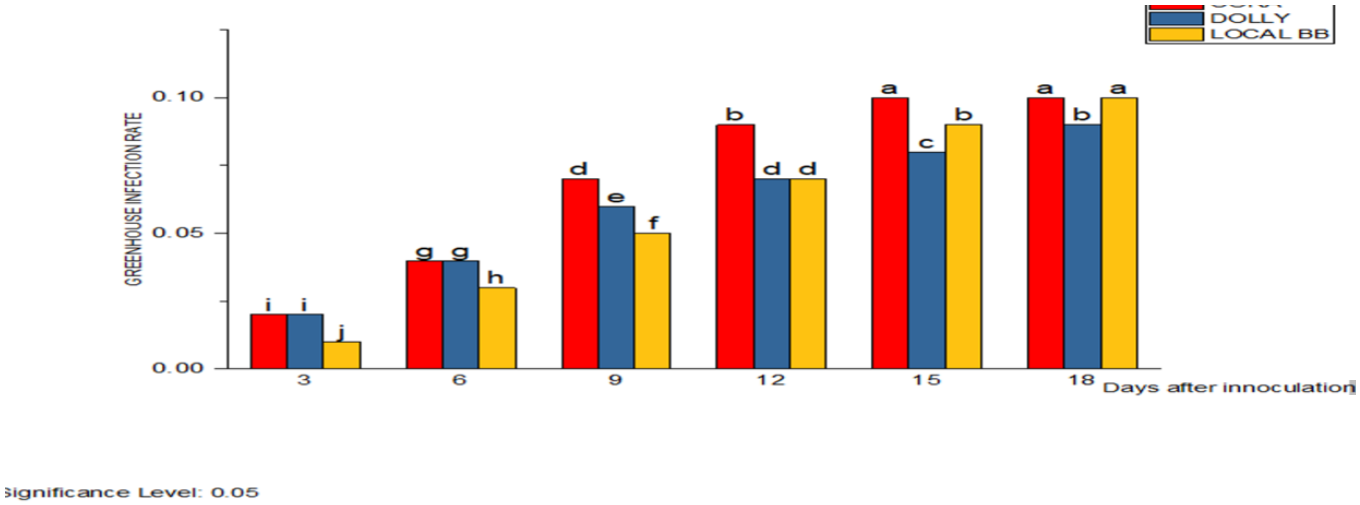

Figure 6. Effect of spores' inoculation on the leaves of cora, dolly and local black green beans in the green house. Bars represent mean lesions and standard errors.

was a significant difference (P≤0.05) in the number of spores of all the varieties of green beans isolates. Highest mean number of spores of 12 was recorded for Cora green beans variety and lowest mean number of spores of 4 was recorded for local black green beans variety at 4 days of culture.

Effect of inoculation on green beans leaves in the greenhouse

The disease manifested three days after inoculation in the greenhouse. Cora and Dolly green bean varieties exhibited a higher infection rate compared to the local black green bean variety (Figure 6). The lesion area increased from day 3 to day 15 and remained stable up to day 18 for all three bean varieties tested. At 3 days post-inoculation, the highest lesion area recorded was 0.1 mm^2 in the Cora and Dolly green bean varieties, while the lowest lesion area recorded was 0.01 mm^2 in the local black green bean variety.

DISCUSSION

This study revealed a significant difference in disease incidence among all twelve treatments of the three green bean varieties at 2 to 4 weeks, while no significant difference was observed at 6 weeks after disease appearance in the field.

This observation could be attributed to environmental factors such as temperature, humidity, and soil conditions, which can influence disease incidence and severity in the

three green bean varieties. This finding is supported by Wang et al. (2018), who reported that high humidity levels can contribute to the development of diseases such as powdery mildew and downy mildew.

The results obtained for disease severity indicated a significant difference ($p \leq 0.05$) among all treatments for the three green bean varieties. The highest mean disease severity was recorded in the Cora green bean variety in the control treatment, while the lowest mean severity was recorded in the local black green beans treated with rhizobium bacteria inoculant. The effects of rhizobium bacteria inoculant on disease severity may be attributed to the bacteria's ability to convert atmospheric nitrogen, thereby enhancing resistance in the three green bean varieties. Additionally, genetic variation may render some green bean varieties more susceptible or moderately resistant to bacterial blight (Wang et al., 2018).

All seeds treated with 60 g of nitrogen exhibited greater severity than those treated with 30 g of nitrogen fertilizer for Cora, Dolly, and local black green beans. This study demonstrated that higher rates of nitrogen fertilizer resulted in increased severity of bacterial blight, consistent with the findings of Davis et al. (2015), who observed increased severity of bacterial symptoms with higher rates of nitrogen fertilizer, likely due to enhanced plant growth contributing to disease severity. Furthermore, the effects of nitrogen fertilizer and rhizobium inoculation on disease severity may be attributed to nitrogen fertilizer's role in enabling plants to resist biotic stress such as common bacterial blight disease. Anderson (2002) suggested that nutritional factors alter the chemical composition of cells by reinforcing their pecto-cellulosic membranes, thus impeding pathogen penetration.

The study also indicated that disease severity of bacterial blight in the control fields was higher in all green bean varieties compared to other treatments, likely due to the absence of nitrogen fertilizer. Nitrogen fertilizer aids in the development of a cuticle, which serves as a significant physiological barrier against infections (Sharma et al., 2005).

Results obtained for the number of pods and weight of pods per plant indicated a higher number of pods and pod weight in the inoculated seeds of Cora and local black green beans compared to their corresponding uninoculated seeds, except for Dolly, which exhibited higher values in the treatment with 60 g of nitrogen fertilizer. This finding aligns with the study of Ahmad and Mohammad (2007), which reported that rhizobium inoculation led to improved growth, although this growth can be significantly influenced by environmental and genetic factors such as soil temperature and moisture content. Gunarto (2000) also suggested that the increase in crop establishment could be attributed to enhanced soil productivity resulting from the activities of bacteria and the availability of nutrients. Results from Dahmardeh et al. (2010) and Morad et al. (2013) support the findings of this study, indicating that inoculation with rhizobium inoculant increases the number of pods per plant, seeds per plant, and seed weight compared to different levels of nitrogen fertilizers and control fields. Rhizobium is known to be a source of nitrogen, phosphorus, potassium, and other micronutrients, thus enhancing nutrient uptake in the soil (Tousi-Hammami et al., 2016).

The highest number of spores was recorded for the Cora green beans variety, while the lowest number of spores was recorded for the local green beans variety in the laboratory. Variability in the number of spores may be attributed to different types of isolates in the nutrient agar medium, which produce colonies with varying characteristics (Carlucci and Pramer, 1957). Furthermore, variability in the number of spores can be compared to the findings of Smith et al. (2018), who reported that genetic variability in plant species influences the diversity of fungal spores associated with these plants. The spores observed were dull white in color on the agar medium, consistent with the report of Phondekar et al. (2020), which stated that virulent colonies of the test bacterium developed on TZC (Thiosulfate zone colonies) agar medium appeared as dull white or creamy.

Pathogenicity assessment of common bacterial blight disease in the greenhouse after inoculation of *X. axonopodis spores* on green bean leaves showed that the Cora green bean variety had the highest lesion area, while the lowest lesion area was recorded in the local black green beans variety. This variability among genotypes of the three green bean varieties may contribute to differences in disease severity (Eyuel et al., 2022).

Conclusion

This study demonstrated that treating green bean seeds with rhizobia inoculants significantly reduced the severity of bacterial blight and increased both the number and weight of pods, compared to treatments using 30 g and 60 g of nitrogen fertilizer. These results highlight the potential of rhizobia inoculants as an effective and sustainable alternative to traditional nitrogen fertilizers for controlling this disease. Furthermore, uniform infection of all tested varieties with *Xanthomonas axonopodis* spores (2 × 10^4 spores/. ml) highlights the widespread vulnerability of green beans to bacterial blight, regardless of the treatment applied. Future investigations should explore the mechanism by which rhizobia inoculants confer resistance to plants and test the effectiveness of these agents in different environmental conditions and against other pathogenic strains. The incorporation of integrated management practices that combine the use of rhizobia, selection of resistant varieties and environmental control can offer a more resilient and

productive strategy for green bean agriculture.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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