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Scrutinizing the efficacy of plant extracts and bactericidal agents applied against *Xanthomonas axonopodis*, the causative agent of bean blight

Estudio de la eficacia de los extractos de plantas y agentes bactericidas aplicados contra *Xanthomonas axonopodis*, el agente causante del tizón del frijol

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Abstract

Common bean (a major staple seed crop and legume) is susceptible to bean blight (*Xanthomonas axonopodis*). The study controlled bean blight in the laboratory and screen-house using botanicals and bactericides. Completely randomized and replicated design was used and recorded percentage germination, number of leaves, shoot length, seed weight, shoot fresh weight, disease incidence, and severity. *In vitro*, control of the pathogen depended on the application of antibiotics: tetracycline, cephalosporin, lincomycin, and erythromycin in order of efficacy, giving 52.2–100% inhibition of the pathogen. *In vitro*, aqueous extracts of *Eucalyptus globulus*, *Aframomum melegueta*, *Ricinus communis*, and *Acmella oleracea* effectively inhibited 25.0–62.5% of the bacterial growth. In screen-house, effects of chemical bactericides on *Xanthomonas* species revealed a significant difference in the number of leaves at 49 days after inoculation (DAI). Also, percentage inhibition of *Xanthomonas* species by the bactericides ranged from 46.2-97.5% from 6-56 DAI. Shoot lengths were significantly different under the influence of plant extracts at 35 DAI and 49 DAI. Plant extracts caused 36.4-90.9% percentage inhibition of the pathogen from 6-56 DAI. Formulation of agricultural applications using these control agents is required.

Keywords: Bactericides, common bean blight, plant protein, *Xanthomonas campestris*, *Xanthomonas phaseoli*.

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Resumen

El frijol común (un cultivo básico de semilla y una leguminosa importante) es susceptible al tizón del frijol (*Xanthomonas axonopodis*). El estudio controló el tizón del frijol en el laboratorio y en el invernadero utilizando productos botánicos y bactericidas. Se utilizó un diseño completamente aleatorizado y replicado y se registró el porcentaje de germinación, el número de hojas, la longitud del brote, el peso de la semilla, el peso fresco del brote, la incidencia de la enfermedad y la severidad. Por medio del *in vitro*, el control del patógeno dependió de la aplicación de antibióticos: tetraciclina, cefalosporina, lincomicina y eritromicina en orden de eficacia, dando un 52,2–100 % de inhibición del patógeno. Los extractos acuosos *in vitro* de *Eucalyptus globulus*, *Aframomum melegueta*, *Ricinus communis* y *Acmella oleracea* inhibieron eficazmente el 25,0–62,5 % del crecimiento bacteriano. En el invernadero, los efectos de los bactericidas químicos en las especies de *Xanthomonas* revelaron una diferencia significativa en el número de hojas a los 49 días después de la inoculación (DDI). También la inhibición porcentual de las especies de *Xanthomonas* por los bactericidas osciló entre el 46,2 % y el 97,5 % entre los 6 y los 56 DDI. La longitud de los brotes fue significativamente diferente bajo la influencia de los extractos vegetales a los 35 y 49 DDI. Los extractos vegetales causaron una inhibición porcentual del patógeno del 36,4 % al 90,9 % entre los 6 y los 56 DDI. Se requiere la formulación de aplicaciones agrícolas utilizando estos agentes de control.

Palabras clave: Bactericidas, tizón común del frijol, proteína vegetal, *Xanthomonas campestris*, *Xanthomonas phaseoli*.

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1 Introduction

Common bean (*Phaseolus vulgaris* L. in the plant family Fabaceae) is a principal grain legume globally. Its varieties include French bean, haricot bean, salad bean, snap bean, string bean, and kidney bean. Around 2019-2022, approximately 28 million metric tonnes of dry common beans were produced globally (Kadege et al., 2022; FAOSTAT, 2024). FAO (1999) and Porch et al. (2013) acknowledged that the market value of these beans exceeds that of all the other legumes combined. While its yield data is unreliable in Africa, the continent accounted for 7.8 million hectares (circa 25%) of the total global area under common beans.

Beans are a staple food in millions of households in Africa. They are a major source of income and food security in sub-Saharan Africa, especially in eastern Africa (Ethiopia, Kenya, Burundi, Tanzania, Uganda), and West Africa - especially in Nigeria (Howard et al., 2005; CABI, 2022; FAO, 1999; Kadege et al., 2022). Beans are cultivated worldwide for their edible seeds/pods and occasionally for their edible leaves and straw fodder.

Beans are rich in dietary fibre, protein, vitamins (like vitamin A and vitamin C), and minerals (i.e., iron, zinc, copper, potassium, calcium, and magnesium). Common beans account for 8–10% protein per 100 g daily intake and they are rich in all the essential amino acids, especially lysine and tryptophan, but deficient in methionine. Beans have very low fat and unhealthy cholesterol content (CABI, 2022; Câmara et al., 2013; Chen et al., 2021).

Câmara et al. (2013) and Kadege et al. (2022) pointed out that this bean is a functional food because it helps improve our health. This is tied to its high levels of phenols, starch, vitamins, and fructo-oligosaccharides that combat distresses like heart disease, sugar-related diseases, and various oncological conditions. Beans could be preserved by drying, cooking, canning, or processed as gluten-free wheat flour.

The production constraints related to beans include diseases, insect pests, low soil fertility (mainly phosphorus deficiency), abiotic stresses (especially drought), inadequate adaptation of introduced varieties, low access to inputs, and inappropriate management of production processes (Kimani et al., 2005; Akibode and Maredia, 2011; Porch et al., 2013; Beebe et al., 2014; OECD, 2016; Mondo et al., 2019; Kadege et al., 2022).

Bean blight or common bean blight (caused by *Xanthomonas axonopodis* pv. *phaseoli* in the bacteria family Lysobacteraceae (i.e. Xanthomonadaceae)) is one of the five major bacterial diseases of common beans. It attacks the foliage, pods, seeds, and stems (ISTA, 2007; Muedi and Fourie, 2014; Chen et al., 2021). Karavina et al. (2011) and Manju et al. (2024) agreed that bean blight is more severe at temperatures between 25-35 °C, especially when coupled with heavy rainfall, and high relative humidity. Both authors estimated that this infection can result in 40% yield loss.

Due to this significant bean yield loss, researchers have been trying to develop sustainable management options for bean blight. Chen et al. (2021) pointed out that insufficient options (either chemical or biological) exist against bacterial diseases. Thus, the options available to producers are limited to cultural practices. They confirmed that copper-based fungicides (cum bactericides), streptomycin, kasugamycin, and manganese-based foliar fertilizers are effective against plant-infecting bacteria.

Karavina et al. (2011) and Muedi and Fourie (2014) affirmed that copper-based bactericides (like copper oxychloride, copper oxide, copper sulphate, copper hydroxide), and potassium di-ethyl-dithiocarbamate can effectively control bacterial foliar infections. Some plant-based agents like essential oils from various plants may be effective against bean blight. Synthetic antibiotics (i.e. streptomycin and kasugamycin) successfully control external bacterial agents even though no seed treatment agent has been developed to completely eradicate *X. axonopodis* situated inside seeds.

Câmara et al. (2013) and Porch et al. (2013) stated that the common bean is under-researched especially in Africa. This study presents effective solutions to address the urgent problem of *X. axonopodis* infections in common beans. The aim is to assess the effectiveness of different plant extracts and bactericidal agents against this pathogen, and show their potential as viable management strategies.

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2 Materials and Methods

2.1 Site of the study

This study was performed in Nigeria at the Alex Ekwueme Federal University Ndufu-Alike, Abakaliki (6.069°N by 8.199°E). Legumes like cowpea, common bean, pigeon pea, Bambara groundnut, soya bean, mung bean, sword bean, and so on are widely cultivated in all the agroecological zones of West Africa including Nigeria.

However, the yields of beans are mostly below the global average due to various production constraints, among which are pests and diseases. The ecological requirements for the production of beans are similar to those required for *Xanthomonas axonopodis* (Figure 1). This is a conundrum that leaves farmers and researchers feeling impotent against this pathogen.

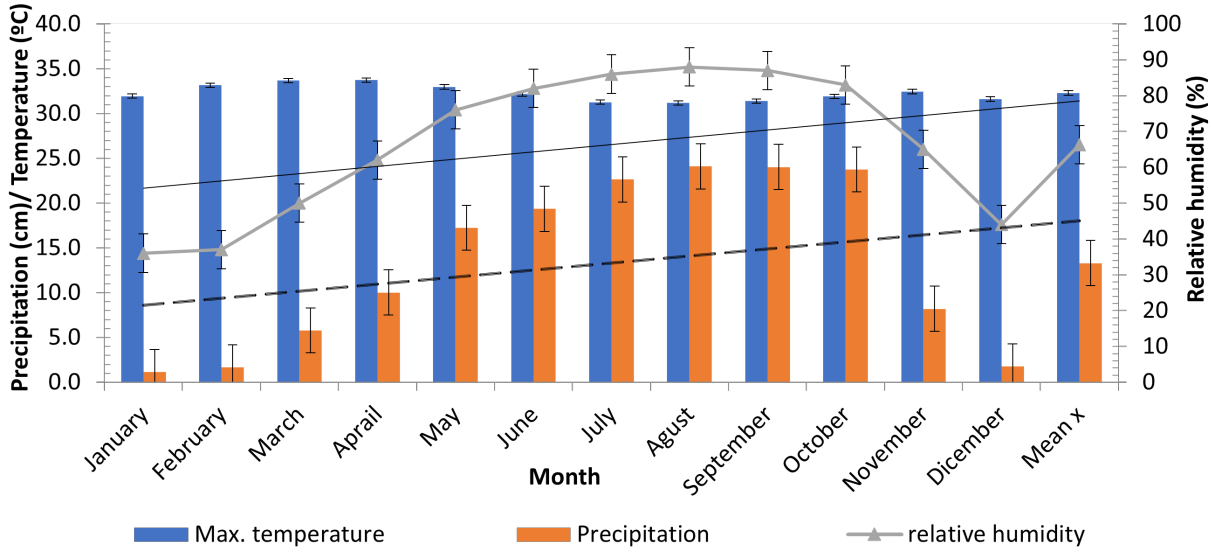

Figure 1. The typical temperature, relative humidity, and rainfall patterns for the study sites at Abakaliki, Ebonyi State. Figure modified from Ndifon (2022).

2.2 Preparation of the plant extracts

The plant organs (like *Ricinus communis* seed soap, flowers of *Acmella oleracea*, *E. globulus* resin, and *A. melegueta* seeds) utilized for the control of common bean bacterial blight were sourced from Abakaliki and its surroundings. They were washed with tap water, and surface sterilized with 1% sodium hypochlorite for 5 minutes. These plant materials were macerated into paste/powder using a mortar and pestle. Each plant material contained 165 g plant tissue per liter of sterile distilled water and it was extracted for 24 hours. For clarity, the plants used included castor bean which is *Ricinus communis*, toothache plant which is *Acmella oleracea*, blue gum which is *Eucalyptus globulus*, and alligator pepper which is *Aframomum melegueta*.

2.3 Isolation of the bacteria pathogen

The seeds and shoots of kidney beans utilized for the trial were initially obtained from Jos (in Plateau State), Nigeria. The plant organs were surface sterilized using 1% sodium hypochlorite for two minutes and rinsed with sterile distilled water. Autoclaved nutrient agar enriched with glucose-containing fluconazole (1 g per L) was used for the isolation of the pathogen (Kado and Heskett, 1970). Three seeds per Petri dish were placed on the agar aseptically.

The plates were subsequently incubated at 28±2°C for 24 hours. The bacteria growths that were noticed were individually sub-cultured and observed for colony similarity. Luckily, only one type of bacteria colony was isolated from the bean tissues. Sub-culturing was continued to purify the iso-

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late. The pathogen was stored at 4°C and used subsequently for morphological and biochemical characterization of the pathogen (Sinclair and Dhingra, 1995; Grimault et al., 2024).

2.4 Total microbial count

A serial dilution to 1×10^6 of the homogenate was made in sterile test tubes using the direct suspension method (Ordóñez et al., 2023). One mL of the serially diluted bean sample was pipetted into each serially marked Petri dish.

Nutrient agar was used for the total count. The streak plate method was utilized to culture the bacteria. Autoclaving was carried out at 120 °C, 15 psi for 15 minutes. At the end of incubation, the colonies were counted and CFU per mL of the suspension were calculated, giving a good estimate of the viable count of the bacterial CFU.

2.5 Characterization and identification of the bacteria isolates

Phenotypic and biochemical tests were relied on for the characterization and identification of the pathogen (ISTA, 2007; Rajyalakshmi et al., 2016; Ordóñez et al., 2023; Grimault et al., 2024). A combination of tests is preferable for in-depth identification. We utilized the phenotypic and biochemical test options for this pathogen.

2.5.1 Gram staining of the bacteria isolates

The bacteria colony was smeared on a clean glass slide and flamed briefly over a Benson burner. Aqueous crystal violet solution (0.5%) was added to the smeared section for 30 seconds and washed with water for one minute. It was replaced with Gram's iodine solution for one minute and rinsed using a wash bottle. Rapid de-colorization with 95% ethanol was carried out.

The smear was counterstained with safranin for 10 seconds, washed using a wash bottle, dried, and observed under the microscope for the presence of staining of the bacteria (Rajyalakshmi et al., 2016; Ordóñez et al., 2023).

2.5.2 Motility test for the bacteria isolates

The test was carried out using nutrient + glucose broth, in a semi-solid medium. For this motility test, this medium was prepared according to the manufacturer's instructions. The broth was poured into the test tubes and inoculated with the bacteria growth using the stab inoculation technique. The stab inoculation was done using a young growth (cultured for 24 hours). The incubation was carried out at 28±2°C. The tubes were examined for growth and signs of motility. If the bacteria are motile and they are inoculated using stab technique, the bacteria growth spreads laterally in the medium with passage of time.

2.5.3 Morphological characterization of the bacteria isolates

The bacteria were streaked on the respective medium and incubated at 28±2°C for 24 hours. At the end of the incubation, the colonies were observed for morphological and cultural characteristics including the form of the margin, nature of the surface, texture, elevation, shape, color, and transparency/translucency of the colony (Wogu and Ofuase, 2014; Rajyalakshmi et al., 2016).

2.5.4 Biochemical characterization of the bacteria isolates

The bacteria isolate was identified using the following biochemical tests: carbohydrate utilization (carried out using glucose for carbohydrate utilization test), catalase, urease, aesculin, oxygen usage, hydrogen sulphide, starch hydrolysis, nitrate reduction, oxidase, KOH, and urease tests (Saddler and Bradbury, 2005; Porch et al., 2013; Wogu and Ofuase, 2014; Rajyalakshmi et al., 2016; Grimault et al., 2024).

The biochemical and morphological characteristics of the isolate revealed that the pathogen is *X. axonopodis* (strongly tied to pathovar. *phaseoli* based on literature/manuals, pathogenicity, and contrasting with likely bacteria agents of bean) (Table 1). Pathogenicity tests were carried out *in vivo* using these isolates, and the kidney bean was highly susceptible (Grimault et al., 2024).

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2.6 Preparation of the bacteria strain used for both *in vitro* and *in vivo* trials

Bean blight or common bean blight is caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Vauterin et al. (syn. *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye; or *Xanthomonas phaseoli* pv. *fuscans* (Burkholder) Starr & Burkholder).

The plates were incubated on nutrient agar at 28±2°C for 24 hours. Serial dilution to 1×10^6 of the homogenate culture was made in sterile test tubes. A 0.5 McFarland standard was used to create inoculum densities of 1.0×10^6 CFU mL^{-1} using the direct suspension method in saline water (containing NaCl 8.5 g L^{-1}) (Wogu and Ofuase, 2014; Ordóñez et al., 2023; Grimault et al., 2024).

2.7 *In vitro* test

2.7.1 Evaluation of bactericides against *X. axonopodis*

Antibiotic resistance patterns and antimicrobial activity of bacterial isolates were studied *in vitro* using chemical bactericides. The antibiotics included erythromycin, tetracycline, cephalosporin, and lincomycin (all at 500 mg of the commercial preparations) per liter (Wogu and Ofuase, 2014; Rajyalakshmi et al., 2016; Ordóñez et al., 2023). A negative control was included using only sterile distilled water as placebo.

Nutrient agar enriched with dextrose was utilized for this test. The entire agar surface was covered with the antibiotic agent using the spread plate technique. The rates were 0%, 50%, or 100% concentrations. A cork-borer (diameter of 6 mm) was utilized to aseptically make a hole in the center of the agar. *X. axonopodis* suspension (50 μL) was applied to the well.

The antibiotic diffused into the agar and inhibited the growth of the pathogen. Incubation of the bacteria was carried out at 28±2°C for 24 hours. Observation of the plates for zones of inhibition was carried out; the diameter (mm) was measured and recorded using a pair of calipers (Saddler and Bradbury, 2005; Mounyr et al., 2016).

2.7.2 Evaluation of plant extracts against *X. axonopodis*

Antibiotic resistance patterns and antimicrobial activity of bacterial isolates were studied *in vitro* using plant extracts. The plant extracts included *E. globulus*, *A. oleracea*, *A. melegueta* and *R. communis*. A negative control was included among the treatments. A negative control is a treatment that is inoculated with the pathogen and is not controlled/inhibited at all. A negative control shows or suffers the full effects of the pathogen.

A nutrient agar medium with fluconazole was utilized for this test. The test was replicated three times. The entire agar surface was inoculated using a spread plate technique with 50 μL of *X. axonopodis* suspension prepared above. A cork-borer (diameter of 6 mm) was utilized to aseptically make a hole in the center of the agar and 100 μL of the plant extract was applied to the well.

The antibiotic diffused into the agar and inhibited the growth of the pathogen. Incubation of the plates was carried out at 28±2°C for 24 hours. Observation of the plates for zones of inhibition was carried out, and the diameter of growth (mm) was measured using a pair of calipers and recorded accordingly.

2.8 *In vivo* trials

Trial 1: Effects of plant extracts on common bean blight infecting kidney bean: consequences on growth and yield of bean plants in the screen-house

The plant extracts (prepared above) were utilized at the dose rates of 0.0, 50, and 100% prepared through arithmetic progression dilutions. The *in vitro* CRD trial was carried out using nine treatments, which included a control, *A. oleracea* (50% and 100%), *E. globulus* (50% and 100%), and *R. communis* (50% and 100%). The plants were cultivated for 90 days before the termination of the trial.

Trial 2: Effects of bactericides on *X. axonopodis* infecting kidney bean: consequences on growth and yield of this blight on bean plants in the screen-house

The CRD pot experiment (replicated three times) had the following treatments: a negative control, te-

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tracycline (0, 50, and 100 %), lincomycin (0, 50, and 100 %), and cephalosporin (0, 50, and 100 %). The plants were cultivated for 90 days before the termination of the trial. Each fungicide was prepared at the rate of 500 mg L^{-1} from commercial formulations.

2.9 Data collection and analysis

Percentage germination was calculated by dividing the total number of seeds that germinated over the total number of seeds sown per plot times 100 %. Seedling vigor was obtained using a scale (Ndifon, 2023). Disease severity was obtained using the scale presented in Table 1.

The percentage inhibition of the pathogen was calculated using the equation 1.

$$PI = \left(\frac{C-T}{C}\right) * 100\,\% \quad (1)$$

Where, PI = Percentage of inhibition of the growth of the pathogen
C = Radius covered by the pathogen in the negative control
T = Radius covered by the pathogen in the treated plate

The data collected included: percentage germination, seedling vigor, number of leaves, shoot length, stem diameter, number of branches, seed weight, shoot fresh weight, number of pods, incidence, and severity of blighting. The incidence of blighting was recorded after counting the infected plants divided by the total number of plants in the accessed treatment times 100 %. The data were subjected to an analysis of variance test and the means were separated using Duncan's multiple range test (DMRT) ($p \leq 0.05$).

Table 1. Disease severity scale.

Rating	Severity %	Description of severity on shoot/leaves
0	0-0.99 %	No lesions, good color of leaves and vigorous plants
1	1-3.0 %	Hard to discern visible symptoms
2	3.1-10 %	A few blighted lesions on plant
3	10.1-20 %	Slightly more blighted lesions than in 1
4	20.1-30 %	Small blighted lesions with limited sporulation
5	30.1-40 %	Plants having few large blighted surfaces and sporulation
6	40.1-50 %	Abundant and generally large sporulation blighted surfaces
7	50.1-60 %	Plants having large lesions, chlorotic, and necrotic tissue
8	60.1-70 %	Highly discernable sporulation and coalescing lesions
9	70.1-80 %	Large sporulation, coalescing lesions, and few fallen leaves
10	80.1-90 %	Leaf fall very rampant, large sporulation, and coalescing lesions
11	90.1-100 %	Highest leaf fall, drooping leaves, death plants

3 Results and Discussion

The results of the phenotypic characterization and biochemical tests carried out on the bacteria are presented in Table 2. These tests together with literature (Schaad et al., 2001; Lacy and Lukezic, 2004; Saddler and Bradbury, 2005; ISTA, 2007; Wogu and Ofuase, 2014; Rajyalakshmi et al., 2016; Ordóñez et al., 2023; Grimault et al., 2024), were used to confirm the identity of the bacterium *Xanthomonas axonopodis*.

The attempt to control the bacterium *in vitro* using bactericides was successfully carried out and presented in Figure 2. The efficacy of tetracycline (at 50 % and 100 % concentrations) was significantly superior to other treatments (Table 3). The other treatments (erythromycin 50 %, lincomycin, and cephalosporin (each at 50 % and 100 % concentrations) were superior to the control. The bactericides achieved excellent inhibition of the bacteria to the tone of 52.2-100 % inhibition based on the amount of growth inhibition zone.

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Table 2. Characterization of the bacterial strain used.

Characterization for the bacterium specimen												
Morphological characteristics	Gram staining	Motility	Margin of the colony	Surface	Texture	Elevation	Colony shape	Color	Transparency			
traits	-	+	Entire	Shiny	Smooth	Raised	Circular, convex, irregular	Orange-yellow	-			
Biochemical characteristics	Gluscose/ Carbohydrate utilization -glucose	Catalase	Hydrogen sulphide production (H_2S)	Starch hydrolysis	Oxidase	KOH test	Fermentation in Oxi-Fem	Hydrolysis of Aesculin	Aerobic / oxygen	Nitrate reduction	Urease	
traits*	+	+	+	+	-	+	-	+	+	-	-	

*Traits could be positive (+) or negative (-). The + or – indicates the type of response to the agent used to affect the organism. Expected responses are tagged + and lack of expected responses are tagged -.

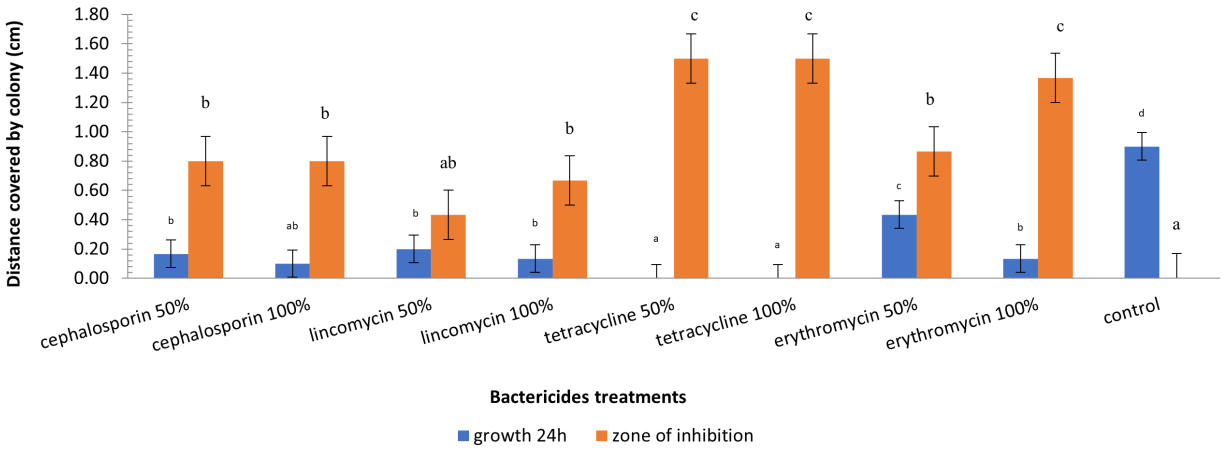

Figure 2. Effect of synthetic bactericides on *X. axonopodis in vitro*.
*Means overshadowed by the same letter(s) are statistically similar using DMRT ($p \leq 0,05$).

The botanicals effectively controlled *X. axonopodis in vitro*, leading to a reduction of the radial growth (Table 3). The plant extracts caused between 28.8-62.5% inhibition of the pathogen. *E. globulus* 100%, *A. melegueta* 100% and *R. communis* 100%, *R. communis* 50%, then *A. oleracea* 100% were the best treatments applied against *X. axonopodis in vitro*.

Meanwhile, controlling the bacterium *in vitro* using botanicals was equally successful and is presented in Figure 3. The zone of inhibition was recorded 48 hours after inception. It shows that *E. globulus* (at 100% concentration) produced the largest zone of inhibition, followed by the *A. oleracea* (at 100% concentration), then *A. melegueta* (at 100% concentration), and *Eucalyptus* sp. 50% well before *A. melegueta* (at 50% concentration).

The effects of chemical bactericides on *Xanthomonas* species revealed a significant difference in the number of leaves between the treatments at 49 DAI (Figure 4). No significant differences existed

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between the shoot lengths. The percentage of inhibition of *Xanthomonas* species by the bactericides ranged from 46.2-97.5% over time. The inhibition was highest at the beginning of the trial (20 DAI) for all the chemicals, but this reduced with time.

Plant extracts caused no significant difference between the treatments based on the number of leaves produced in the screen house (Figure 5). The shoot lengths were significantly different under the influence of plant extracts at 35 DAI, and 49 DAI. Nevertheless, these differences were inconsistent with time. The percentage of inhibition of the pathogen varied from 36.4-90.9% with time. It can be mentioned that the 50% and 100% rates of *E. globulus* are the best treatments, followed by *R. communis* 100% rate, and *A. oleracea* 50%.

Table 3. Inhibition of the growth of *X. axonopodis* by selected plant and chemical bactericides *in vitro*.

Chemical bactericides	**% inhibition**
Cefalosporina 50%	81.1
Cefalosporina 100%	88.9
Lincomicina 50%	77.8
Lincomicina 100%	85.6
Tetraciclina 50%	100.0
Tetraciclina 100%	100.0
Eritromicina 50%	52.2
Eritromicina 100%	85.6
Plant extracts	**% inhibition**
Aframomum melegueta 100%	53.8
Aframomum melegueta 50%	28.8
Eucalyptus globulus 100%	62.5
Eucalyptus globulus 50%	33.8
Acmella oleracea 100%	46.3
Acmella oleracea 50%	33.6
Ricinus communis 100%	53.8
Ricinus communis 50%	50.1

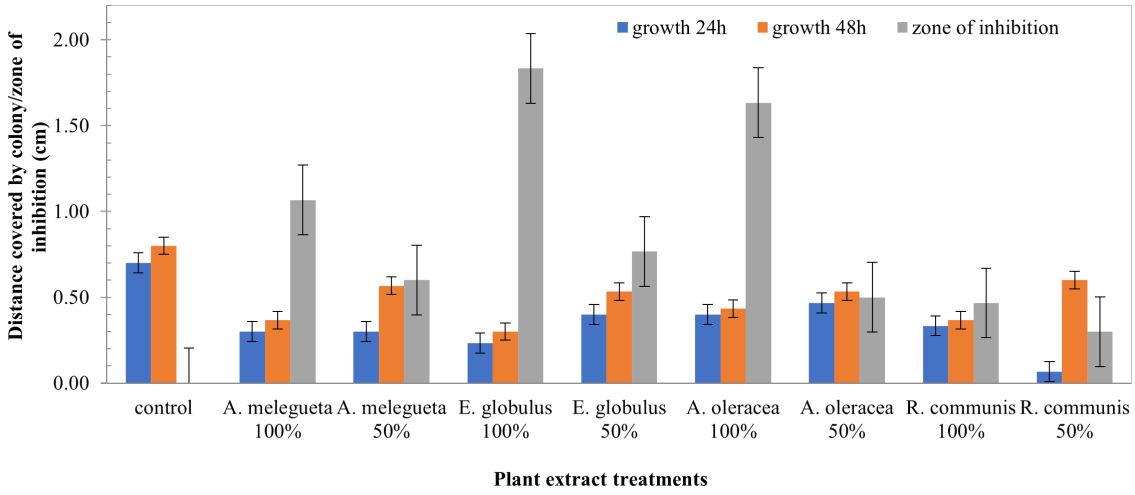

Figure 3. Effect of botanicals on *X. axonopodis in vitro*.

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Fresh pod weight and seed weight were not significantly different between the treatments when chemical bactericides were applied against the bacterium (Figure 6). There were differences between treatment means when the fresh pod and seed weights were compared in a non-statistical manner. It is likely that for these beans with indeterminate growth patterns, the yields could be significantly different later. The fresh shoot weight varied significantly from the control.

Likewise in Figure 7, the fresh pod weight and seed weight were generally lower than those in the trial where chemical bactericides were applied. The time to first flower produced was longer for plant extract plots compared to the time for chemical plots. The reason for this was not clear. It is probable that the chemical agents boost time to flowering or pod production. Maybe some factor was present in plant tissue that favors continuation of vegetative growth. These are basically untested conjectures. There were visible differences between the seed, shoot, and pod weights which turned out not to be significant.

Finally, Figure 8 is attached to help readers appreciate the magnitude of the damage bacteria diseases can pose to plants including beans. It shows the dead seedlings planted in plot trials and damaged tissues of the beans during the experiment. The quality of *in vitro* pictures was low so they are not included. The trial will be conducted in the field when safer preparations of chemicals are available.

Bean blight is indeed a devourer of plants, which leaves the farmer without leaves or fruits. It devours the fodder, hay, and seeds seamlessly and mercilessly. Unfortunately, perfect control may be unattainable once the disease is in the field. This means no export or import to many countries where the producers could get premium prices. Researchers are up and doing in the struggle to ensure healthy production. This can enable producers to make a profit, value their crops more, and thus adopt modern production paraphernalia.

Howard et al. (2005) contended that bean blight agents could be controlled using chemical fungicides (cum bactericides). Buruchara et al. (2010) agreed that seed treatment using Copper compounds and Streptomycin, as well as restriction of field operation during rains has proven effective. Muedi and Fourie (2014) affirmed this opinion but reiterated that these chemicals cannot eliminate established bacteria from crops/fields. They believed that such chemicals could only reduce the spread of bacterial disease. These views were corroborated in this present study *in vitro* and *in vivo*.

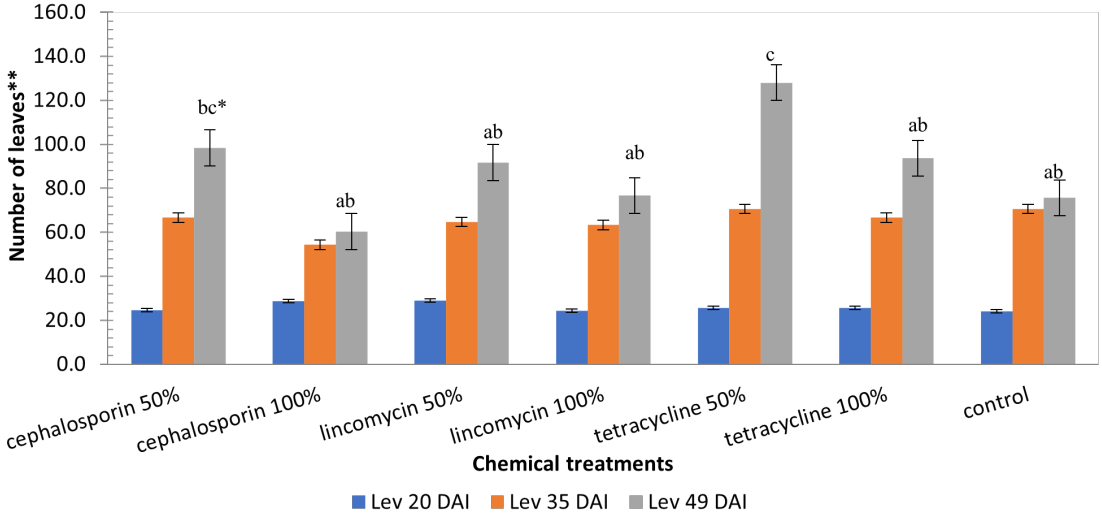

Figure 4. Effects of synthetic bactericides on crop growth and *X. axonopodis* in the screen-house. Note: ** Lev = leaves, Sht = shoot, %INH = percentage of inhibition. *Means overshadowed by the same letter(s) are statistically similar using DMRT ($p \leq 0{,}05$).

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But Karavina et al. (2011) pointed out that the use of these measures by researchers is only possible in the short run. They stated that control of bean blight is possible by using disease-free seeds and chemicals. Thus, we embarked on this research and successfully proved the usefulness of bactericides in the production of common beans.

Research on control of bacterial blight of beans using plant extracts is rare. Control of fungi has been severally proven with the aid of plant materials. Wavare et al. (2017) reported that aqueous extracts of marigold (*Tagetes erecta*) flowers exhibited potential antifungal activity against *Sclerotium rolfsii* under greenhouse conditions.

Sanasam et al. (2018) proved that plant extracts of garlic and turmeric inhibited (67.7%) *S. rolfsii*. Ndifon et al. (2022) revealed that aqueous extracts made from ginger and garlic for seed and soil dressing significantly controlled *Fusarium* wilt during the cultivation of *Solanum aethiopicum*.

Hussain et al. (2009) revealed that six plant extracts, including *Eucalyptus camaldulensis*, inhibited fungi species by suppressing the mycelia growth. Ndifon and Lum (2021) reported that all the plant extracts (including *Eucalyptus globulus*) assayed inhibited the growth of *Aspergillus niger* significantly compared to the control. The plant species applied in this study have all proven very effective in our laboratory against fungi. They showed much promise in this study but they could not surpass synthetic bactericides. Chemical bactericides still have a role in bean blight management.

Since bacterial diseases of plants are often treated using fungicides for instance copper oxides and copper oxychlorides, much research on use of plant extracts against fungi has been carried out. This study was carried out with the hope that some plant extracts that have been effective against fungi will also be effective against bacteria. This could be a plus for pathogen control if the control materials are dual-purpose pesticides.

Many researchers have raised alarm about the safety of chemical pesticides to man, animals, and the environment. Attempts to offer farmers different control agents are rare as far as bean blight is concerned. Cultural control and the use of resistant materials may be feasible for small plots or isolated fields. Breeding for resistance is made difficult because of the high rate of self-pollination in beans.

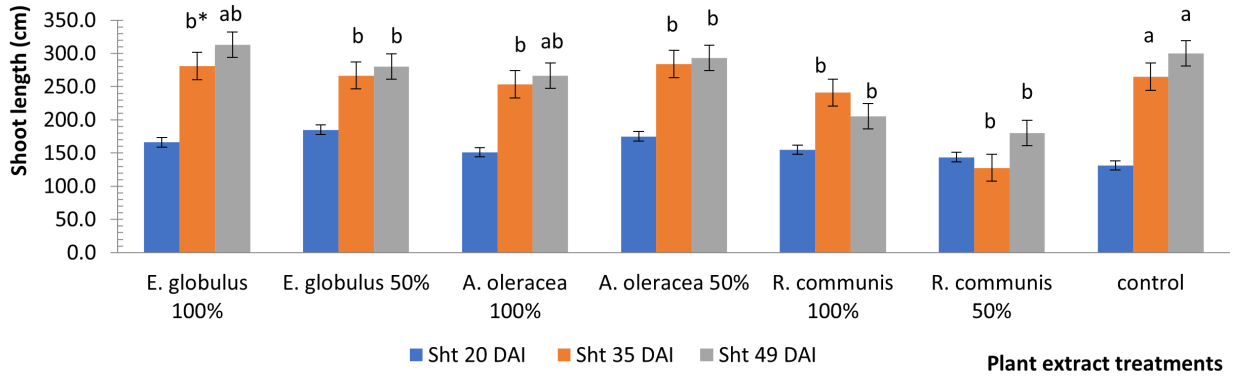

Figure 5. Effects of plant extracts on crop growth and *X. axonopodis* in the screen-house.
*Means overshadowed by the same letter(s) are statistically similar using DMRT ($p \leq 0{,}05$).

However, resistance against this disease agent is complex and many variants of the disease agent may co-exist. Resistance to common bean blight is particularly complex as 26 quantitative resistance loci to common bean blight have been described so far. To date, transcriptomic studies after common bean blight infection have been very scarce and the molecular mechanisms underlying susceptibility or resistance are largely unknown (Foucher et al., 2020).

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Increased resistance can be developed by selecting for horizontal rather than vertical resistance (Garcia-Espinosa, 1997; Muimui et al., 2011). These results provide a basis to further understand the complex inheritance of common bean blight resistance in Mesoamerican common beans (Ambachew et al., 2021). These are just a few direct quotations to encourage researchers to breed for resistance to this disease.

In disease-complex situations, the tolerance/resistance to bean blight may be negligible. ISTA (2007), Karavina et al. (2011) and Chen et al. (2021) recommended an integrated disease management approach, which should include quarantine, cultural practices, and resistant varieties.

Trutmann et al. (1993) pointed out the efficacy of manipulating the microclimate in small fields as a viable measure to control this blight. Integrated management of this pathogen will likely be the trump card in the arsenal of farmers. This research is ongoing in the field to include elements of integrated management of common bean blight.

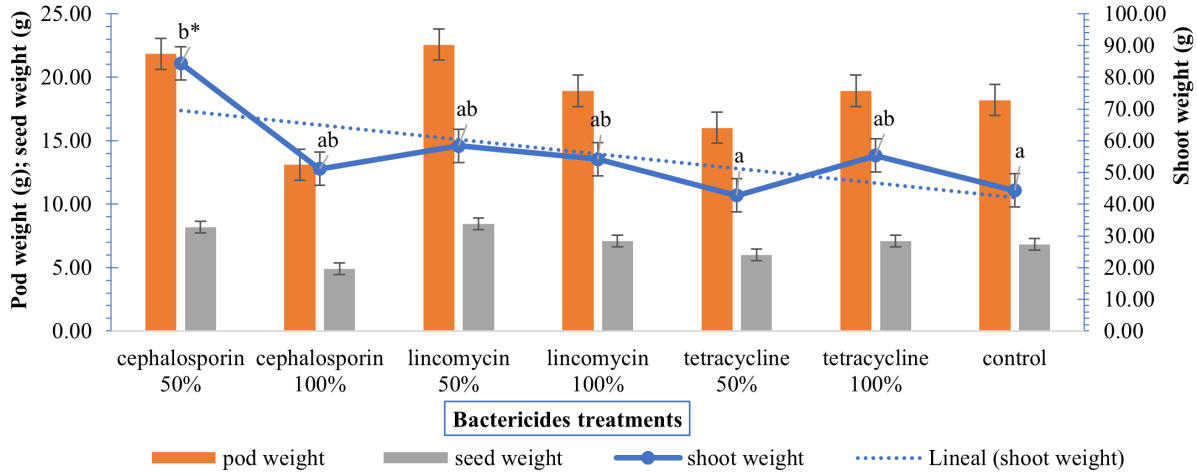

Figure 6. Effects of *Xanthomonas sp.* on shoot weight per plant, pod weight per plant, and seed weight per plant under the influence of bactericides in the screen-house. *Means overshadowed by the same letter(s) are statistically similar using DMRT ($p \leq 0{,}05$).

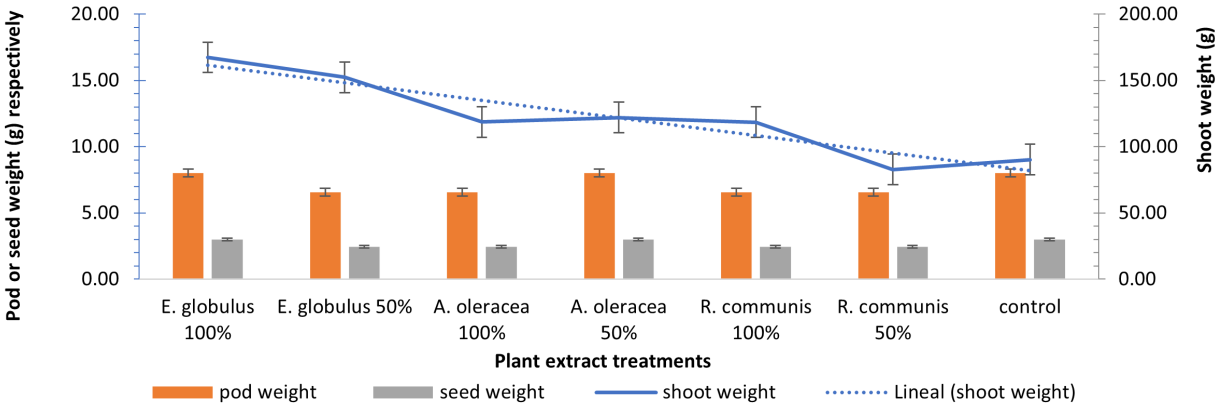

Figure 7. Effects of *Xanthomonas sp.* on shoot weight per plant, pod weight per plant, and seed weight per plant under the influence of plant extracts in the screen-house.

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Figure 8. Symptoms of bean blight during the trial. **Top**: left = infected leaf in screen house. Middle = very early symptoms in screen house. Right = dying seedling in field. **Bottom**: left and middle = symptomatic and healthy plants. Right = highly infected or death seedlings.

4 Conclusions

Delving into the power of plant extracts and bactericidal agents applied against *Xanthomonas axonopodis* (the plant pathogenic bacterium behind bean blight disease) revealed that the bacteria could be managed effectively using botanicals and bactericides. Lincomycin, erythromycin, cephalosporin, and tetracycline were very effective against the pathogen. Likewise, *E. globulus*, *A. oleracea*, *A. melegueta* and *R. communis* also prevailed against the pathogen.

The synthetic antibiotics were better than the plant extracts at all times. Healthy beans could be produced using these plant extracts and bactericides. Meanwhile, work will continue on the availability, formulation, safety, and integration of these bacteria management agents for sustainable bean production. This study was carried out without hassles in the laboratory, in the screen-house the work became much difficult to carry out without contamination. In the field the work will be most difficult, hence we will need to obtain standard facilities to continue the work.

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Authors' contribution

E.M.N.: Conceptualization, Data curation, Formal analysis, Funding acquisition, Research, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing– original draft, Writing– review and editing.

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