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Research Article

Effect of Plant Extracts on Angular Leaf Spot Disease and Yield Variables of Common Beans (*Phaseolus vulgaris L.*) Varieties in Bambili (North-West, Cameroon)

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is one of the essential leguminous vegetable crops grown in Cameroon mainly for its economic and nutritional values. Its productivity is highly limited by Angular leaf spot caused by the fungus *Phaeoisariopsis griseola* (Sacc.). This study evaluated the effects of two plant extracts (ginger and neem oil aqueous solution) in the control of angular leaf spot disease for four varieties of beans (GLP 190 C, PNN, ECAPAN 021, and GL22) in two seasons (July – November 2021 and March – July 2022). The trials were carried out at the teaching and research farm in the University of Bamenda campus, in the North-West region of Cameroon. The experimental field design was a randomized complete block design of four treatments replicated three times on four varieties of beans. The data on disease incidence, disease severity, pod weight, and the number of pods were collected for the four beans varieties. The results obtained for disease incidence, severity, and yield revealed differences at $p \leq 0.05$ amongst the different treatments in different fields. The highest mean disease incidence (95 %), severity (4.25), and low yield (4 pods weighing 0.3 grams) were observed in both seasons in untreated plots (T_0) in all varieties. The treated plots recorded the lowest average disease severity of (1), incidence (10 %), and high yields (16 pods weighing 6.8 grams). Disease incidence for treated plots with ginger aqueous extract (T_2) and neem oil aqueous solution (T_1) recorded a relatively lower mean disease incidence (20 %). Treatment with neem oil aqueous solution showed a low disease incidence (10 %) at week 3 for the PNN variety. Disease severity rate for plots treated with neem oil aqueous solution (T_1) had the lowest mean value of 1.2 for medino long (GLP 190C) and PNN varieties at week four, while ginger aqueous extract (T_2) showed an infection of 1.5. The pod weights revealed the highest mean weight of 6.8 grams for plots treated with neem oil aqueous solution for PNN (black bean), while GL22 and GLP 190C (medino long) had the lowest mean of 0.2 grams in season one. From this study, ginger aqueous extract could therefore be recommended as a biocontrol pesticide against angular leaf spot disease of bean in the dry season and neem oil aqueous solution in the rainy season.

Key words: Angular leaf spot disease, beans, biocontrol, plant extracts, yield.

RÉSUMÉ

Le haricot (*Phaseolus vulgaris* L.) est une légumineuse essentielle cultivée au Cameroun, principalement pour ses valeurs économiques et nutritionnelles. Sa productivité est fortement limitée par la tache angulaire des feuilles cause par le champignon *Phaeoisariopsis griseola* (Sacc.) *griseola* (Sacc.). Cette étude a évalué l'effet de deux extraits de plantes antimicrobiens (gingembre et huile de neem) dans la lutte contre la maladie des taches angulaires en utilisant quatre variétés de haricot (GLP 190 C, PNN, ECAPAN 021 et GL22) pendant deux saisons (juillet-novembre 2021 et mars-juillet 2022). Les essais ont été réalisés dans le champ d'enseignement et de recherche du campus de l'Université de Bamenda, region du Nord-Ouest Cameroun. Le dispositif expérimental en blocs randomisés complets de quatre traitements répétés trois fois sur quatre variétés de haricots était utilisé. Les données sur l'incidence et la gravité de la maladie, le poids et le nombre de gousses ont été collectées. Les résultats obtenus concernant l'incidence, la gravité et le rendement de la maladie ont révélé des différences significatives à $p \leq 0,05$ entre les différents traitements des différentes parcelles. L'incidence moyenne de la maladie la plus élevée (95 %), la gravité (4,25) et le faible rendement (4 gousses pesant 0,3 gramme) ont été observés au cours des deux saisons, dans les parcelles non traitées (T0) pour toutes les variétés. Les parcelles traitées ont enregistré la gravité moyenne de la maladie la plus faible (1), une faible incidence (10 %) et les rendements élevés (16 gousses pesant 6,8 grammes). L'incidence de la maladie pour les parcelles traitées avec de l'extrait de gingembre (T2) et de l'huile de neem (T1) a été relativement plus faible (20 %). Le traitement avec la solution acqueuse d'huile de neem a montré une faible incidence de la maladie (10 %) à la 3^{e} semaine pour la variété PNN. Le taux de gravité de la maladie pour les parcelles traitées avec la solution acqueuse d'huile de neem (T1) avait la valeur moyenne la plus basse de 1,2 pour les variétés medino long (GLP 190C) et PNN à la 4^{e} semaine, tandis que l'extrait de gingembre (T2) a montré une infection de 1,5. Les poids des gousses ont révélé la moyenne la plus élevée avec un poids moyen maximal de 6,8 grammes pour les parcelles traitées à l'huile de neem pour le haricot noir (PNN), tandis que GL22 et GLP C 190 (haricots longs) présentaient le poids moyen le plus faible de 0,2 gramme la première saison. De cette étude, l'extrait aqueux de gingembre pourrait donc être recommandé comme pesticide de biocontrôle contre la maladie des taches angulaires du haricot en saison sèche et l'huile de neem en saison des pluies.

Mots clés : Maladie des taches angulaires, haricots, bio contrôle, extraits de plantes, rendement.

1. INTRODUCTION

Beans (*Phaseolus vulgaris* L.) are one of the most important leguminous vegetable crops, originating from Mexico and Central America and spreading to other countries in Africa, including Cameroon (Bitocchi *et al*. 2012, Broughton, 2003). Cameroon produces beans mostly in the Western Highlands (Broughton, 2003). Bean is mainly cultivated and consumed worldwide because its pods contain nutrients such as protein, carbohydrates, fiber, vitamins, minerals (iron and zinc), and a low glycemic index (Lajolo *et al*. 2002, Singh *et al*. 2017, ISAR 2011). Bean sales exceed US$500 million annually (FAO, 2011). According to FAO (2014), half of the world's standard bean production is being done mainly by developing countries and serves as a staple crop. Tropical Africa produces 75,000 tons of beans annually and Northern Africa 312,000 tons (FAOSTAT 2018). In Cameroon, bean is mainly produced and marketed in the Western Highlands of Cameroon (Siri *et al*. 2017) and contributes more than 90 % of the national bean production (Anonymous, 2010).

Bean production faces many constraints that account for low national yields (Sanyang *et al*. 2019). Among these constraints are diseases, insects, and low soil fertility. Bean diseases such as angular leaf spots caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris is one of the most devastating diseases, causing yield losses of 80 % (Adikshita *et al*. 2016, Buruchara *et al*. 2010, Mahuku *et al*. 2004). Angular Leaf Spot develops rapidly under favorable weather conditions, causing premature defoliation, pods, and crop losses (Mwang' Ombe *et al*. 2007). Most of the bean cultivars are susceptible to angular leaf spot disease; hence, the incidence and severity of the disease increase rapidly in the cultivated areas (Stenglein *et al*. 2003, Robert *et al*. 2016).

Angular leaf spot disease causes 50 % yield loss of beans in Cameroon and 80 % yield loss of beans in other parts of the world has been recorded (Sartorato *et al*. 2000, Stenglein *et al*. 2003).

Farmers spray different fungicides on the crops, leading to problems like eliminating natural enemies, environmental hazards like air, soil, and water pollution, and the development of resistance to diseases (Mahuku *et al*. 2003, Celleti 2006). Numerous studies have shown that ginger aqueous extract and neem aqueous solution have a wide range of antimicrobial activities and act as botanical fungicides by inhibiting spore germination and growth of plant pathogens. Ginger rhizome extract has been reported to possess compounds that have antifungal properties against *Fusarium solanum* (Ke-Yong *et al*. 2022), while neem aqueous oil solution was also found to be effective in the control of soilborne pathogens (Chava 2020). Sanyang *et al*. (2019) reported the use of difenoconazole fungicide in the control of angular leaf spot infection in the Western region of Cameroon. Most synthetic chemicals are not readily available and are expensive to buy; hence, the need for alternative pest control strategies that will help reduce attacks on farms (Adeola *et al*. 2014). Nowadays, many research studies have shown the use of botanicals in the control of *Phaeoisariopsis griseola* in the field because they are biodegradable, have low mammalian toxicity, are safe to natural enemies, cheap, and can be easily produced by skilled and unskilled farmers (Adeola *et al*. 2014). According to Damalas *et al*. (2014), most bio-pesticides are slow-acting and give better results when used in an integrated pest management (IPM) program. Limited information exists on extracts from botanicals and some synthetic insecticides (difenoconazole). Therefore, the objective of this study was to assess the influence of ginger and neem oil aqueous solution in the control of angular leaf spot disease in beans.

2. MATERIALS AND METHODS

2.1. Location of experimental site

The trial site for the research was Bambili, located in the North-West region of Cameroon, at the University of Bamenda campus, and the *in vitro* experiment was carried out at the Catholic University (CATUC) Laboratory

Bamenda, from August 2021 to July 2022. The University of Bamenda lies at latitude 6°C North of the equator, longitudes 10 °C East, and an altitude of between 1600 and 2000 m above sea level.

2.2. Assessment of effect of the biological activity of botanicals on angular leaf spot during cropping seasons

This experiment was carried out in two cropping seasons: the first season from August to November 2021 (wet season/ season 1) and the second from April to July 2022 **(**dry season/season 2). For both seasons, seeds were sown one per hole at a distance of 25 cm apart on 10 m ridges with a total seed density of 720 seeds in a total surface area of 264m^2 for each season. This trial was carried out using a Randomized Complete Block Design (RCBD), made up of three treatments (T_1 (treatment with neem oil aqueous solution), T_2 (treatment with ginger), and T_0 (control) replicated 3 times.

2.3. Processing of ginger (*Zingiber officinale)* and neem oil aqueous solution *(Azadirachta indica)*

Rhizomes of ginger were bought from a local market in Nkwen and thoroughly washed. Five hundred grams (500 g) were weighed on a scale, sliced, and crushed to obtain a paste. The paste obtained was mixed in 2 liters of distilled water and mixed after, which it was left for 24 hours. The aqueous solution was filtered using a sieve and stored in a refrigerator. Ginger aqueous extract was prepared by mixing ginger juice of 0.5 liters in a liter of water in the ratio 1:2 before spraying. Neem oil aqueous solution was prepared by mixing five milliliters of the neem oil aqueous solution in 1 liter of tap water in the ratio 1:200, and 10 grams of detergent (emulsifier) was added to the mixture. Ginger aqueous extract was prepared by mixing ginger juice of 0.5 liters in a liter of water. These plant extracts were used to spray the crop once every week using a Knapsack sprayer for six weeks, except for the control experimental units that were not sprayed with plant extract.

Disease incidence was assessed by counting the number of infected plants in the middle beds twice a week and calculated using the formula:

$$\text{Disease incidence} = \frac{\text{Total number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

Disease severity was evaluated based on the degree of infection caused by the fungus and graded on a scale of 1 to 5 according to Bensaci *et al.* (2021), where:

1=No symptoms

2= Low percentage (0-30 %) leaf infection

3= Moderate percentage (30-50 %) of plant leaves affected with ALS

4= High percentage (50-70 %) of plant leaves affected with ALS

5= high percentage (70-100 %) leading to complete damage of leaves

2.4. Assessment of yield

Total bean pods per treatment on each plot were counted at 9 weeks after planting when pods were fully mature. This was done by weighing (using a scale balance) and recording the weight of all pods in each treatment to give the yield.

2.5. Pathogenicity test of angular leaf spot (*Phaeoisariopsis griseola*) in the screen house

Young bean leaves with lesions of angular leaf spot disease were collected from the field. These leaves were cut into small fragments of 2mm from the edges of the lesion and surface-sterilized in 5% dilute solution of sodium hypochlorite for 20 seconds and rinsed four times in sterilized distilled water for 2 minutes in each Petri dish. The leaf fragments were dried on sterilized filter paper, and three fragments were placed on solidified cool V8 juice culture medium in a Petri dish. The petri dishes were labelled and placed in a laminar flow chamber at room temperature of 22-26^{0}C for four days, after which subculturing was done to obtain pure cultures (Fig.1). Fungi spores of *Phaeoisariopsis griseola* were identified using a light microscope. Spores' suspension was prepared by flooding the surface of the growing mycelia on the Petri dish with 5ml of sterile distilled water, and the spores were dislodged with a small brush. The suspension was centrifuged for 5 minutes and used to inoculate healthy plants in a screen house. The inoculation was done by using a syringe to inject the spore solution into two spots on the leaves. Data for average lesion area were collected 3 days after inoculation, and the data were recorded for 7 days by measuring the length and width of lesions for each plant using the adopted procedure of Manju *et al*. (2020).

Figure 1: Axenic Culture of *Phaeoisariopsis griseola*

2.6. Statistical analysis

Data on disease incidence, severity, and yield were subjected to analysis of variance (ANOVA) using statistical software (JUMP11). The treatment means were separated using Turkey HSD and least significant difference (LSD) at the statistical significance of 95% confidence interval ($p \leq 0.05$). Mean data were used to plot graphs of results.

3. RESULTS

3.1. Effect of ginger aqueous extracts and neem oil aqueous solution on disease incidence of *Phaeoisariopsis griseola*

Symptoms of Angular leaf spot were seen in the field 21 days (3 weeks) after planting for seasons one and two. They appeared as whitish spots centered on the tissues and central veins, presenting an angular shape. The result for disease incidence showed an increase during the trial period from 21, 28, and 35 (weeks 3, 4, and 5, respectively) for season one (rainy season) (Fig. 2). Disease incidence in plants progressed based on the age of the plants in the treated and untreated plots. From observations, the control plots (T_0) per variety of beans (GLP 190C, PNN, ECAPAN 021 and GL22) recorded the highest mean disease incidence at day 35 of 95 % for GLP 190C and ECAPAN 021 and the treated plots with ginger aqueous extract (T_2) and neem oil aqueous solution (T_1) recorded a relatively lower mean disease incidence of 20 % (Fig. 2). Treatment with neem oil aqueous solution showed a low disease incidence of 10 % at week 3 for the PNN variety. The analysis of variance revealed a mean significant difference of value ($p \leq 0.05$) among all bean varieties treated with plant extract at the 4th and 5th week (Fig. 2). Furthermore, season two (dry season) experienced disease progression from 21 days to 35 days and the treated plots with ginger aqueous extract (T_2) and neem oil aqueous solution (T_1) recorded a low mean disease incidence of 20 % at week 3 (Fig. 2). PNN recorded the lowest mean of 0 % with ginger aqueous extract at week 3. There was a significant difference of $p \leq 0.05$ between varieties in weeks 4 and 5.

3.2. Effect of ginger aqueous extracts and neem oil aqueous solution on disease severity of *Phaeoisariopsis griseola*

Disease severity appeared as an extension of lesions on bean leaves in the field at 28 days after planting. The degree of infection by the disease increases as the plants age in the treated and untreated plots from days 28, 35, 42, 49, and 56 (weeks 4, 5, 6, 7, and 8, respectively) in season one (rainy season). From observations, the control plots (T_0) for the GLP 190C variety recorded the highest infection rate of 4.3 at week 8, while the treated plots with neem oil aqueous solution (T_1) and ginger aqueous extract (T_2) showed a low disease infection of 1.5 for season one (Fig. 3). Neem oil aqueous solution had the lowest mean value of 1.2 for GLP 190C and PNN varieties at week 4. There was a significant difference at $p \leq 0.05$ between bean varieties in weeks 4 and 5.

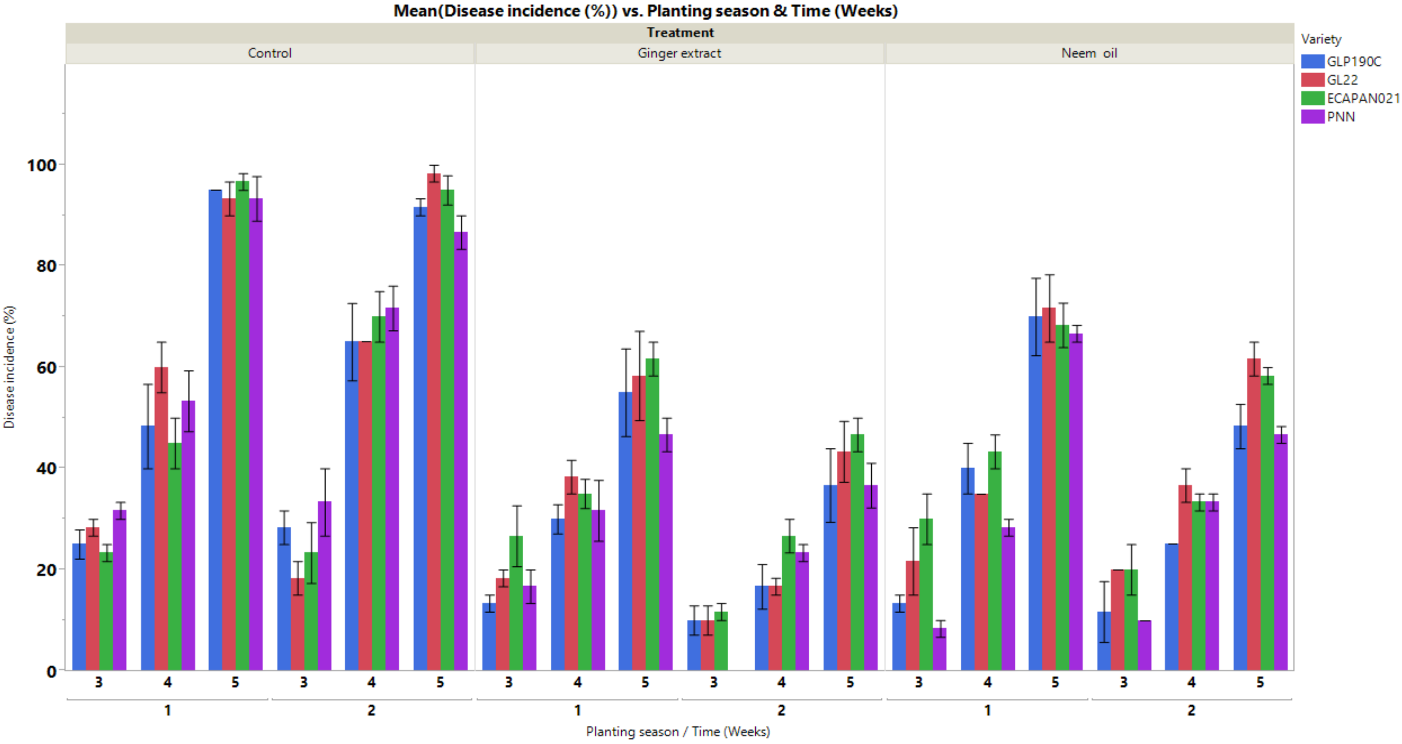

Figure 2: Effect of plant extract on disease incidence for seasons 1 and 2

Error bars represent the mean standard deviation at p≤0.05

The rate of disease severity on bean plants also increased with the age of the plants in the treated and untreated plots from days 28, 35, 42, 49, and 56 (weeks 4, 5, 6, 7, and 8, respectively) in season two (dry season). From observations, the control plots (T_0) GLP 190C variety recorded the highest mean infection of 3.25 and week 8, while the treated plots with neem oil aqueous solution (T_1) and ginger aqueous extract (T_2) showed a lower disease infection of 1.5 for season two. Also, the neem oil aqueous solution had the lowest mean value of 1.2 for the GLP 190C in week 4. There was a significant difference at p≤0.05 between bean varieties and treatments in weeks 4 and 5.

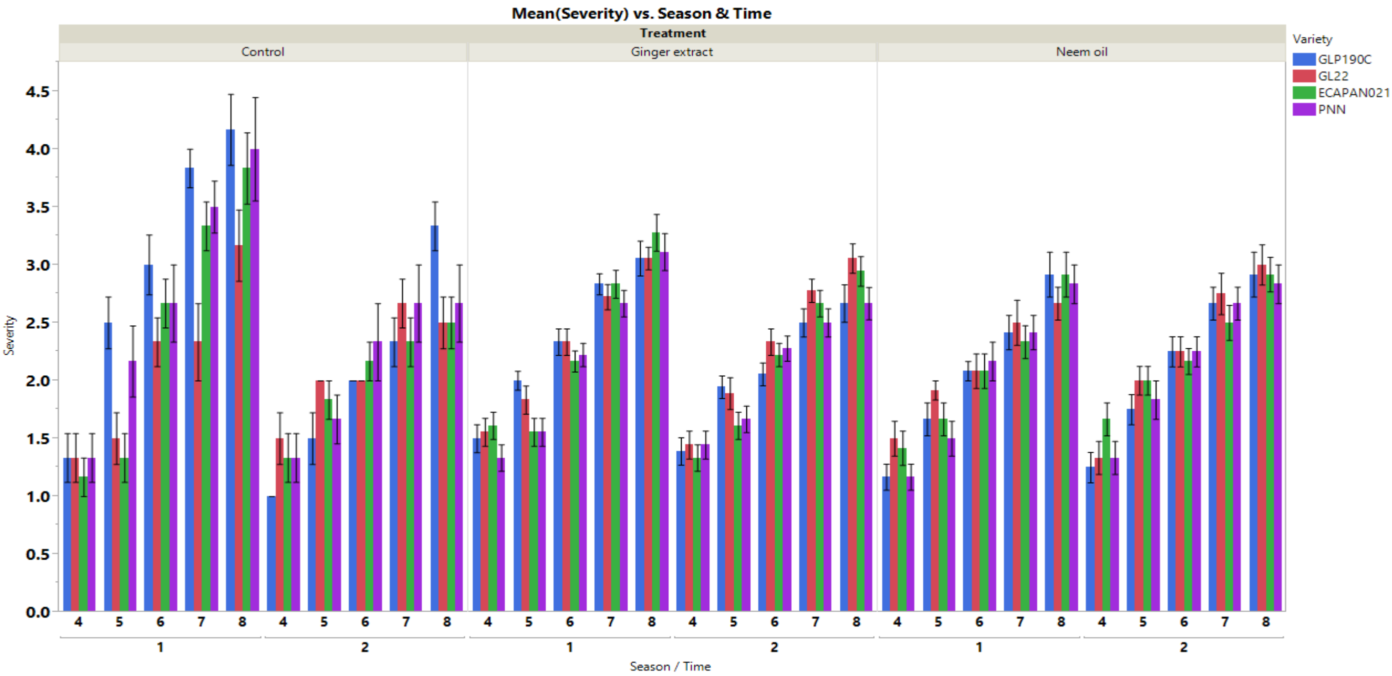

Figure 3: Effect of plant extract on disease severity for seasons 1 and 2

Error bars represent the mean standard deviation at p≤0.05

3.3. Assessment of plant extracts on the average number of bean pods

At 74 days after planting, mature bean pods were harvested per variety. The mean numbers of pods per plant (6 plants used) were recorded for each variety with, PNN (black beans) having the highest mean of 15 pods for the plots treated with, ginger and the lowest mean of 4 pods for medino long (GLP 190C) for the control plots in season one (Fig. 4). There were variations in the other varieties for both ginger and neem oil aqueous solution treatment, with ginger having higher yields than neem. There was a significant difference in the mean number of pods between varieties at $p \leq 0.05$.

For season two, the PNN variety (black beans) had the highest mean of 16 pods for the plots treated with ginger and the lowest mean of 5 pods for medino long (GLP 190C) for the control plots in season one (Fig. 4). There were variations in the other varieties for both ginger and neem oil aqueous solution treatment, with ginger having higher yields than neem. There was a significant difference in the mean number of pods between varieties at $p \leq 0.05$.

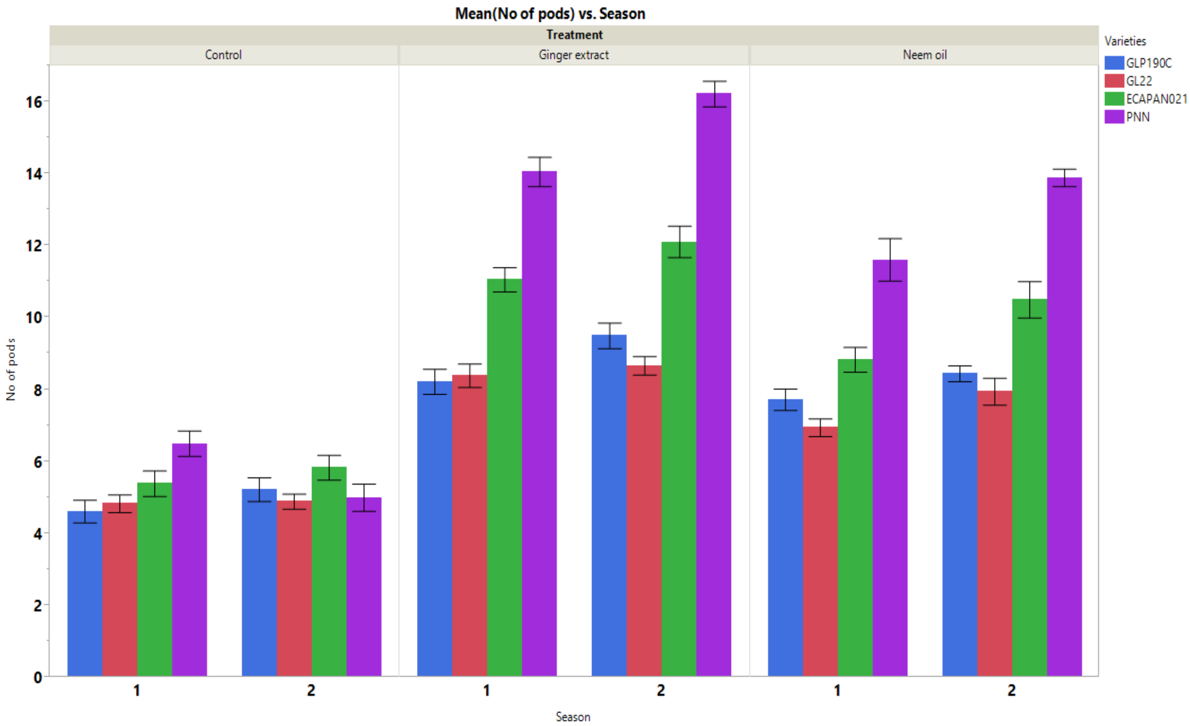

Figure 4: Effect of ginger aqueous extract and neem oil aqueous solution on mean number of pods per plant for seasons 1 and 2. Error bars represent the mean standard deviation at $p \leq 0.05$

3.4. Effect of ginger aqueous extract and neem oil aqueous solution on the mean weight of bean

Figure 4 shows the pod weight recorded after harvesting. The highest mean weight (4 grams) was recorded for plots treated with neem oil aqueous solution for the PNN (black bean) variety, while GL22 and GLP 190C had the lowest mean of 0.2 grams in season one. There was a significant difference in the mean number of weights between the varieties at $p \leq 0.05$. The highest mean weight of 6.8 grams was recorded for plots treated with ginger aqueous extract for the PNN (black bean) bean variety, while GL22 and GLP 190C had the lowest mean of 0.2 grams in season two (Fig. 5). There was a significant difference in the mean number of weights between varieties at $p \leq 0.05$.

3.5. Effect of inoculum on plant leaves in the screen house

The result of fungi growth on plants in the screen house shows a progression from day one to seven. Two days after disease inoculation in the screen house, disease appeared on the leaves. The infection rate in the screen house was highest at 0.8 for the GLL 22 variety at day 7 and lowest of 0.0 for GLP 190 C variety at day 1 (Fig. 6). There was a variation in all varieties from day one to seven at $p \leq 0.05$.

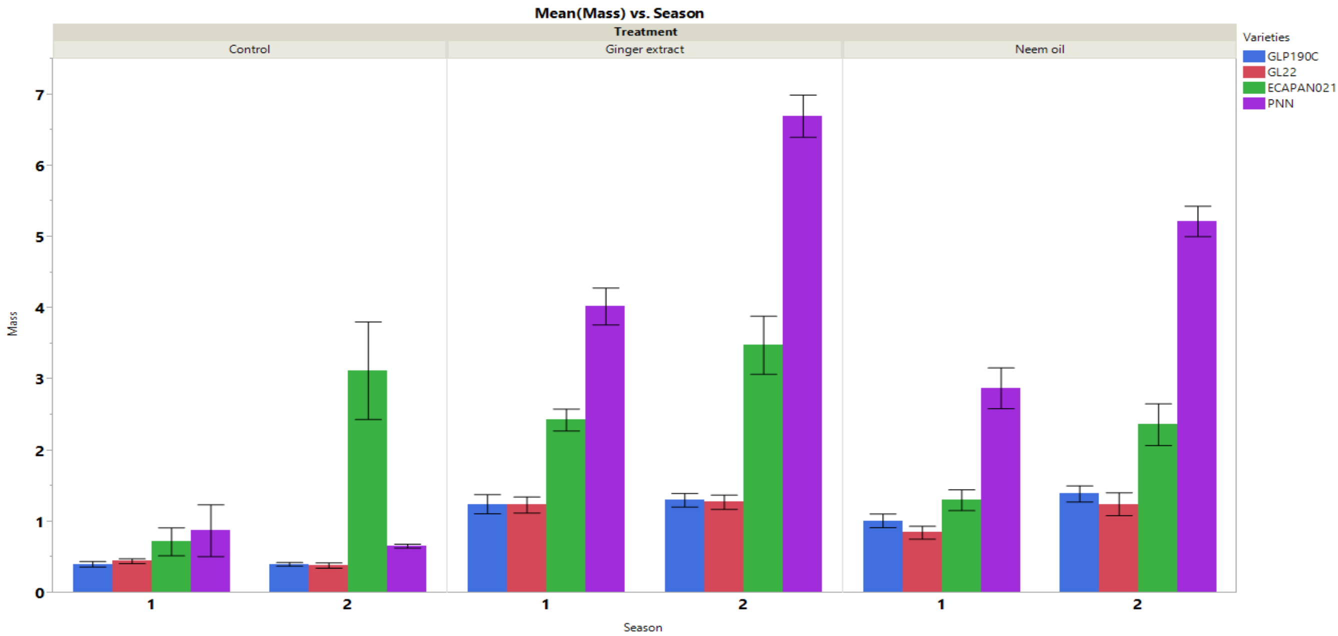

Figure 5: Effect of ginger aqueous extract and neem oil aqueous solution on mean weight of pods per plant for seasons 1 and 2. Error bars represent the mean standard deviation at p≤0.05.

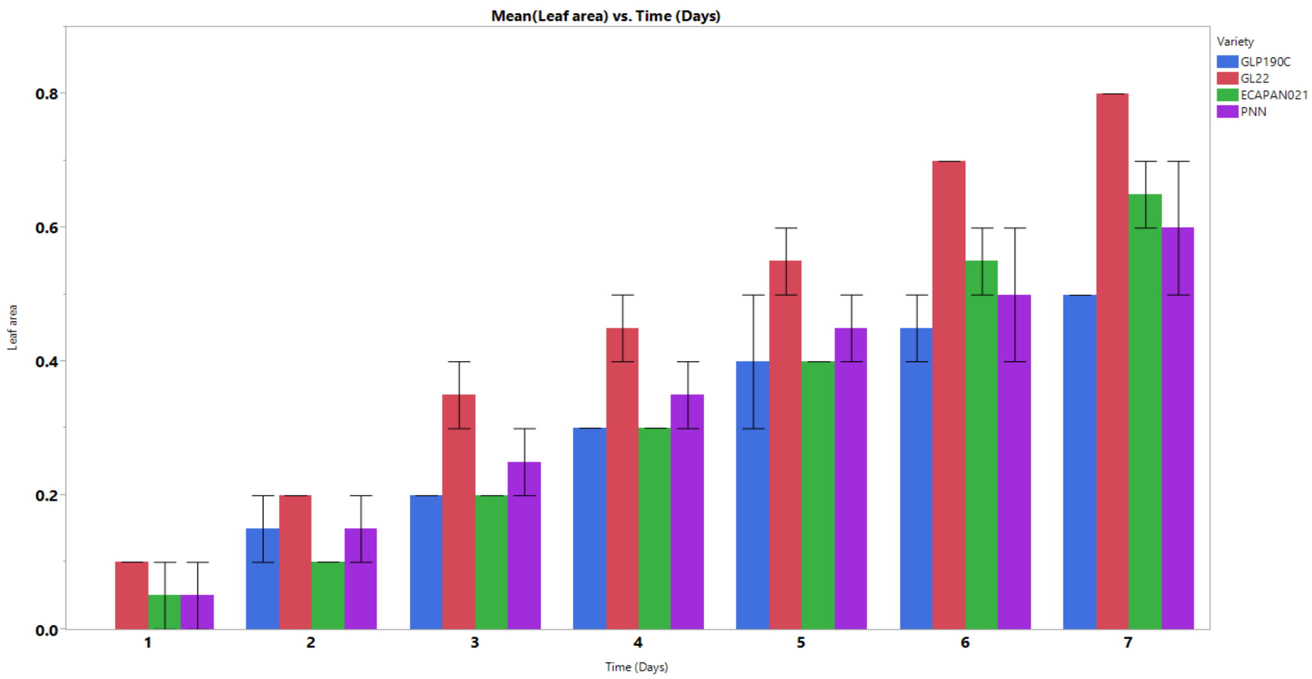

Figure 6: Effect of inoculated spores on bean plant leaves in the screen house.

Error bars represent the mean standard deviation at p≤0.05.

4. DISCUSSION

Angular leaf spots uniformly appeared in the field for all the different varieties of beans before the application of plant extracts. These extracts revealed a considerable reduction in infections on the bean leaves. This aligns with previous studies carried out by Ke-Yong (2022) and Chava (2020), who demonstrated fungicidal effects of ginger and neem oil against fungi and other soilborne pathogens. Disease incidence spread more rapidly in the untreated plots than in the treated plots, which may be a result of crop residues from previous farming, susceptibility of seeds to the disease, and favorable environmental conditions like high humidity, temperature, and rainfall. This result is in line with the work of Tesfay *et al*. (2024), who reported that poor farming practices by smallholder farmers in the area, like the use of poor-quality planting materials, lack of crop rotation due to land scarcity, and poor management practices, and favorable environmental conditions, enhance disease development. There was a significant difference in disease incidence between season one and season two, which can be due to climatic influences on the environment like temperature and humidity. Kijana *et al.* (2017) in their study reported that high rainfall and relative humidity, common in high altitudes, favour ALS infection and disease development. Pastor-Corrales (2016) and Santos (2019) reported that altitude affects temperature and rate of evaporation, and, in so doing, also influences the incidence and severity of certain diseases.

Angular leaf spot was more severe in untreated plots than in the treated plots with plant extracts for both seasons. The disease severity in season one was higher compared to season two. This could be because of favorable environmental conditions for disease development, such as high temperature and high humidity. Steinglein *et al.* (2003) reported that moderate temperatures and high relative humidity were the most favourable conditions for the fungal disease epidemic development. Also, high disease infection could be the result of the presence of debris, creating a survival medium for the disease. This result agrees with the work of Celletti *et al.* (2005), indicating that pathogens can survive on infested crop debris over two winters and the stroma that forms in lesions, allowing the pathogen to remain dormant until environmental conditions are favorable for sporulation. Inoculum from nearby bean farms acted as a means of disease spread and severity in both seasons. Gomez (2018) and Schwartz (2017) reported that a high spread of ALS occurred in areas where beans were planted in the previous one or two seasons. Severity was lower in plots treated with neem and ginger, leading to a reduction in the rate of defoliation after applying plant extracts due to their antifungal activity.

Yield data was obtained from the number of pods and weight per plant and showed that the mean yield was generally higher in the dry cropping season than the wet season, with the black beans (PNN) generally having a high mean yield for both seasons. The treated plots showed a significant difference in yield from the untreated plots for all bean varieties. This trial aligns with the results of Mahuku *et al.* (2002), suggesting that fungicide application results in significant yield increments compared to non-treated plots. This trial agrees with an earlier study in snap beans, where the use of Orius® fungicide to control ALS and rust resulted in significant yield increments (Paparu *et al.* 2014). Results obtained after inoculation in the screen house show that there was the presence of *P. griseola* spores that were cultured from the infected plant in the laboratory.

The spores cause lesions on the inoculating plant. Angular Leave infection rate on the white bean variety (GL22) was higher than the medino long variety (GLP 190 C) after inoculation in the screen house. This may be due to genetic variations of the individual bean variety. This result is confirmed by (Leung *et al.* 2003) who reported that large pathogenic variation could have been associated with mutation, recombination, and migration. Infection of leaves in the screen house after spore injection of the fungus caused stress to the plants, which resulted in disease development depending on the bean variety. This result is confirmed by (Chavarria *et al.* 2007), who reported that the pathogen has mostly colonized the damaged tissue at the early stage to cause disease development.

5. CONCLUSION

The study has revealed that ginger and neem oil aqueous solution both have fungicidal properties against angular leaf spot diseases. However, the effectiveness of each botanical depends on the season of application. Ginger (*Zingiber officinale*) was most effective during the dry season (season one), while neem oil aqueous solution showed the highest potential for reducing disease infection during the rainy season (season two). PNN bean variety was more resistant to the disease when treated with ginger in season two than other varieties. PNN bean variety had a higher number of pods and weight for both seasons than other varieties. The weight of the pods was higher for season two than season one, indicating that the dry season can be used to obtain more yield in the area. Pathogenicity assessment of *P. griseola* on the different bean varieties revealed that all bean varieties were infected by *P. griseola* spores on inoculation in the screen house. Moreover, these eco-friendly disease control approaches with minimum disturbance to natural beneficial insects and non-target organisms should be highly encouraged. Ginger and neem oil are more eco-friendly, readily available, cheap, easily biodegradable, and less toxic to mammals.

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CONFLICT OF INTERESTS: The authors have not declared any conflict of interests

AUTHOR CONTRIBUTIONS: All authors collaborated in carrying out this research. Author MEB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ANT, CTA, and NTN managed the study design, statistics, and the literature searches. All authors read and approved the final manuscript.

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