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Impact of Azadirachta Indica Leaf-Infused Soil Amendment Options and Okra Types on Root-Gall Nematode (M. Incognita) Infection of Okra (Abelmoschus Esculentus) in Owerri Ultisol

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Abstract: This study investigated the impact of Azadirachtaindica leaf-infused soil amendment options and okra types on root-gall nematode infection. It utilized a Randomized Complete Block Design with four replications, organized in a 2 × 3 factorial layout. The treatments included two combinations of organic soil amendments: Azadirachtaindica leaves and Carica papaya leaves, Azadirachtaindica leaves and Chromolaenaodorata applied at 8.33 tons/ha, along with a control. Clemson spineless and Yeleen were the two okra types studied. Juvenile root-gall nematode populations were assessed using the modified Baermann technique, and a galling index ranging from 0 to 4 was employed. Statistical analysis was performed using analysis of variance at a significance level of 5%. Yeleen exhibited superior performance over Clemson spineless, demonstrating higher leaf production and yielding greater fresh pod weight, shoot weight, seed number, and pod number. The findings indicated that soil amendments, particularly, Azadirachtaindica leaves + Carica papaya leaves, Azadirachtaindica leaves + Chromolaenaodorata effectively controlled root-gall nematode compared to the control plots. Application of these amendments enhanced growth and yield parameters compared to untreated plots, suggesting their potential as effective control measures in fields heavily infested by M. incognita, especially the Azadirachtaindica + Carica papaya leaves mixture.

Keywords: Root-gall nematode, soil amendments, okra types, ultisol, Azadirachtaindica leaves, M. incognita.

1. Introduction:

Across tropical, subtropical, and warm temperate regions of the world, okra (Abelmoschusesculentus (L) Moench) is a widely grown annual vegetable crop (Swamy, 2023). Okra was first domesticated in Western and Central Africa, but it is now widely farmed for local food across the tropics (Abelmoschusesculentus (Okra), 2022).

In Nigeria, okra is the third most widely consumed and produced crop, following tomatoes and peppers (Tijani and Kehinde, 2022). In 2021, global okra production amounted to 10.8 million tons, with India leading at 60% of the total output and Nigeria and Mali being notable secondary producers (Okra, 2024). Nigeria alone accounts for 200,000 to 320,000 tons of the 500,000 to 600,000 tons of okra produced annually in West Africa, indicating its substantial contribution to the region's okra output (Nkongho et al., 2022).

Okra is rich in essential nutrients such as calcium, magnesium, phosphorus, potassium, iron, sodium, zinc, and some vitamins, along with carbohydrates, fiber, sugar, and lipids (Hussain et al., 2023). Its mucilaginous texture enhances its economic importance by making it easier to consume staple foods like yam puree, garri, and potatoes.

Several living components contribute to the decline in the production of okra, a crucial crop. Nematodes that produce root-knots are noteworthy among these; they are ranked in the top 10 genera of plant-parasitic nematodes worldwide and in the top five plant diseases (Rusinque et al., 2023). Phytopathogenic nematodes cause substantial harvest losses in various agricultural crops, including vegetables. Symptoms of nematode infestation in okra include galls, abnormal root growth, chlorosis, early senescence, wilting, and growth retardation. Additionally, nematode-infested plants show reduced mucilaginous and nutritional qualities in okra (Agu et al., 2009).

Extreme root-knot infection caused by Meloidogyne spp. in okra can lead to yield losses of up to 27% (Sikora and Fernandez, 2005). In severe cases, nematode infections can cause losses of up to 90% in vegetable crops (Pandey and Kalra, 2003). Despite their effectiveness in eliminating nematodes, synthetic nematicides are becoming less common due to their high toxicity and unintended effects on collateral species and the ecosystem (Anastasiadis et al., 2008). Farmers face significant crop failures due to current nematode infestations, necessitating the development of natural nematicides that are less harmful to humans, animals, and the environment yet equally effective against root-gall nematodes.

Several studies have examined the effectiveness of soil amendment sources in combating nematodes (Cookeyet al., 2015; Ogwudire et al., 2022). This research aims to evaluate the impact of Azadirachtaindica leaf-infused soil amendment options and okra types in an ultisol infested with root-gall nematodes.

2. Research methods:

2.1.Study site and land preparation

The field experiment was carried out at the Federal University of Technology, Owerri (FUTO), in Nigeria, at the Demonstrative Farms of the Department of Crop Science and Technology. The site is 91 meters elevated from sea level, between latitudes 050 and 270 N and longitudes 020 and 070 E. With an annual rainfall of 130 mm, the mean lowest and highest temperatures are 29.4°C and 31.7°C, respectively. Meloidogyne spp., a kind of root-gall nematode, was naturally present in the loamy sand soil (Agu, 2008). Using a machete and shovel, an area measuring 11.5 m by 19.5 m (224.25 m) was physically cleared of existing bush, and the rubbish was burned. Ranging poles, ropes, pegs, and a 50 m measuring tape were used for marking out.

2.2. Experimental Design, Plant Materials and Planting

A 2 x 3 factorial in a Randomized Complete Block Design (RCBD) with four (4) replications made up the experimental setup. This resulted in twenty-four experimental plots overall, with six plots in each block. Treatments included two okra cultivars (yeleen and Clemson spineless) and two blends of organic soil amendments (Carica papaya (pawpaw) and Azadirachtaindica (neem) leaves, Chromolaenaodorata (siam weed), and Azadirachtaindica leaves). Six therapy combinations were obtained as a result of this. Plots were spaced 300 cm by 200 cm (6 m^2), and there were 100 cm and 50 cm between each plot and block, respectively. One of the six treatment combinations was assigned at random to each plot.

Around the base of the plants, soil amendments were applied at a rate of 8.33 tons/ha (5 kg/plot). This was carried out twice (at planting and 4 weeks after planting). Prior to planting, the okra seeds were immersed in water for a whole day, with two seeds placed in each hole, at a planting depth of two centimeters, and a planting distance of 50 x 50 cm.

Throughout the trial, manual weeding was used to maintain the experimental site free of weeds, particularly at 4 and 8 weeks after planting. The site was also watched until it reached maturity and was harvested at 12 weeks after planting.

2.3. Collection of data.

To obtain information on the number of leaves per plant, each leaflet of a trifoliate leaf was directly counted and recorded, or counted as a single leaf. The leaf area (cm2) was obtained by measuring the midrib length and broadest breadth, and it was then calculated as follows: An okra leaf with lobed surfaces has an area of L × B × 0.56. According to Asoegwu (1988), the formula for calculating the leaf area of an unlobed okra is Leaf Area = L x B x 0.89, where L stands for leaf length, B for leaf width, and 0.89 for the correction factor. The number of pods produced by each plant was counted to calculate the plant height, which was measured using a meter rule positioned from the base of the plant to the shoot apex. Similarly, the number of seeds per pod was ascertained by counting the number of seeds per pod, and the fresh pod weight was ascertained by weighing the total number of pods harvested/plot. Weighing the okra plants' above-ground portion allowed us to determine the weight of the fresh shoots, and weighing the okra plants' below-ground portion allowed us to determine the weight of the fresh roots.

2.4.Evaluation of plant root infection.

To evaluate the extent of root disease caused by root-gall nematodes in each plot, the roots were carefully extracted intact. This process involved gently removing the plant and washing away the soil from the roots using tap water. Each root system was then individually assessed according to the scoring system outlined by Agu and Ogbuji (1996), where: 0 = no infection (no galls present), 1 = rare infection (1-3 galls present), 2 = light infection (4-10 galls present), 3 = moderate infection (11-30 galls present), and 4 = severe infection (30 or more galls present).

2.5.Determination of preliminary and terminalnematode population in the soil.

Soil samples were collected from a designated study area to determine the preliminary and terminal nematode populations. The study area was divided into multiple sampling sites to obtain representative samples. Using a soil auger, soil samples were collected from a depth of 5-20 cm. A total of 20 subsamples were taken from each site and combined to form a composite sample for each site. The composite samples were then placed in labeled sampling bags for further analysis. Each composite soil sample was thoroughly mixed to ensure homogeneity. A 100-gram aliquot of soil was weighed for nematode extraction. The soil sample was placed on a tissue paper within a Baermann funnel. The funnel was filled with water until the soil was submerged. The setup was left undisturbed for 48 hours to allow nematodes to migrate out of the soil into the water. The nematode-containing water was then collected in a beaker. The extracted nematode suspension was transferred to a counting dish. Using a light microscope, nematodes were counted and identified to the genus level. Counts were recorded for each sample. At the conclusion of the study, final soil samples were collected from the same sites following the same procedure as the initial sampling. The extraction and counting methods described above were repeated to determine the final nematode population.

The experiment's data were put through an analysis of variance (ANOVA), following Steel and Torrie's (1981) instructions, and the means were separated using the least significant difference (L.S.D.) at the 5% probability level.

3.Results and Discussion:

3.1. Impact of Soil amendment sources and variety on preliminary and terminalnematode population / 100 g soil

The effects of different okra cultivars and types of soil amendments on the initial and terminal populations of soil nematodes are depicted in Figure 1. The data showed that the interactions between okra types and soil amendment sources had no effect on the preliminary nematode population ($P > 0.05$). Nonetheless, a noteworthy influence ($P<0.05$) was detected for the terminal nematode population. The highest reduction in post-harvest nematode count (50/100 g of soil) was achieved by combining Azadirachtaindica and Chromolaenaodorata with the Yeleen okra variety. This was followed by soils treated with a combination of Azadirachtaindica and Carica papaya leaves (125/100 g of soil). In control plots, the Clemson Spineless Okra variety had the highest nematode count (875/100 g of soil) following harvest. The highest nematode count after harvest (875/100 g of soil) was recorded in the Clemson Spineless okra variety in control plots. The juveniles' direct contact with the active components in the metabolites of biodegraded soil amendment materials could be one reason for the decrease in the number of root-gall nematodes. This is consistent with research by Chitwood (2002), who found that nematodes' direct interaction with the active components in moringa leaf extracts reduced both the final population of nematodes and root-galling in sweet pepper roots. Nematicidal chemicals in the soil amendment sources may have contributed to the ultimate nematode population reduction, corroborated by findings published in Alasmary et al. (2020) and Aji (2024).

3.2.Influence of soil amendments sources and okra varieties on number of leaves, leaf area (cm)^2 and plant height (cm).

Figure 2 illustrates how different okra types and soil amendment sources affect the number of leaves, leaf area (cm^2), and plant height (cm). Across all treatments, the Yeleen type continuously displayed more leaves than the Clemson spineless variant.

When treated with Azadirachtaindica leaves along with either Chromolaenaodorata or Carica papaya, both varieties displayed more leaves than the control. The highest number of leaves were produced by combining the leaves of Azadirachtaindica and Carica papaya; Yeleen reached 21.35 leaves, while Clemson spineless reached 16.06 leaves. The treatments involving Azadirachtaindica leaves significantly improved the number of leaves in both plant varieties, with the combination of Azadirachtaindica leaves + Carica papaya being the most effective.

Clemson spineless demonstrated a consistent increase in leaf area (cm^2) with each treatment, peaked in 283 cm^2 with the combination of Azadirachtaindica leaves and Carica papaya treatment. With the Azadirachtaindica leaves and Chromolaenaodorata treatment, Yeleen had the largest leaf area at 213.7 cm^2, but with the Azadirachtaindica leaves and Carica papaya treatment, the leaf area decreased somewhat to 196.4 cm^2. Overall, the combination of Azadirachtaindica leaves and Carica papaya proved to be the most successful in expanding the leaf area of Clemson spineless plants. For Yeleen, however, the combination of Azadirachtaindica leaves and Chromolaenaodorata plants yielded marginally better results. In comparison to the control plots, which generated the fewest leaf areas in the two types under investigation (Clemson spineless: 143, Yeleen: 132.4), all of these showed a significant difference.

Our findings on the plant height show that the application of soil amendments considerably ($P<0.05$) increased the height of the okra plants relative to the control plot. With each treatment, the Clemon spineless okra variety showed a noticeable rise in height; the highest height was attained with the application of Carica papaya and Azadirachtaindica leaves. With both Azadirachtaindica leaf treatments, the Yeleen okra variety showed a notable increase in plant height; the Azadirachtaindica leaves + Carica papaya treatment resulted in the maximum height of 23.63 cm. The control plots, which showed the fewest plants in both kinds (19.05 cm and 15.40 cm), were noticeably different from these.

The presented findings showed that soil amendments significantly affect the growth characteristics of okra, particularly the number of leaves, leaf area, and height of the plant. This may be explained by the high nutrient content of Azadirachtaindica leaves, which may have supplied vital nutrients that encourage leaf growth. Additionally, a synergistic impact between several organic amendments may have increased the availability of nutrients. Allelopathic qualities of Azadirachtaindica may inhibit soil root knot nematodes, promoting healthier plant growth. This aligns with the conclusions drawn by other writers (Cookey et al., 2019; d'Errico et al., 2023). The development of the leaf area in each variety may have been impacted differently by the various amendments' effects on the soil microbiome.

It is possible that the higher okra plants observed under the treated plots were caused by the improved soil structure brought about by the organic amendments, which in turn improved root development and taller plants. They may also contain precursors or natural growth hormones that encourage the development of height. According to Kwayep et al. (2017), applying several modifications enhanced the development and production of okra.

3.3. Effects of soil amendment sources and varieties on number of pods per plant, number of seeds per pod and fresh pod weight of okra

Fig. 3 shows the impact of soil amendment sources and cultivars on okra's fresh pod weight, number of seeds per pod, and number of pods per plant. Both varieties' control plots produced the fewest pods per plant (Clemson spineless: 7.25, Yeleen: 11). These were followed by plots treated with a combination of Azadirachtaindica leaves and Chromolaenaodorata (Clemson spineless: 16.5, Yeleen: 23.75), which produced the most pods per plant (22 for the Clemson spineless variety and 23.75 for the Yeleen variety). In a similar vein, the control plots yielded the fewest seeds overall (Clemson spineless: 34.8, Yeleen: 35.5 seeds per pod). The combination of Azadirachtaindica leaves and Carica papaya showed the greatest quantity of pods per plant and seeds per pod, especially in the Yeleen variety (63.8), and these differed considerably ($P < 0.05$) from the other treatments.

The okra types planted in the control plots, Clemson spineless and Yeleen, yielded the lowest fresh pod weights, weighing 58 g and 172 g, respectively. The fresh pod weight of both varieties was significantly increased by both treatments involving Azadirachtaindica leaves (Azadirachtaindica leaves + Chromolaenaodorata: Clemson spineless, 138 g; Yeleen, 335 g; Azadirachtaindica leaves + Carica papaya: Clemson spineless, 137 g; Yeleen, 330 g); however, the combination of Azadirachtaindicaleaves + Chromolaenaodorata was slightly more effective for both varieties.

The study's lower okra production parameter could be the result of severe galling and a greater nematode count in the control plots without soil amendments, which could have hindered the plant's ability to absorb nutrients. This result aligns with the findings of Etim et al. (2023), who stated that okra cultivated in contaminated soil had a lower growth parameter than okra grown in improved soil.

Rich in minerals and organic matter, soil amendments—particularly organic ones like Carica papaya and Azadirachtaindica (neem)—improve the fertility and structure of the soil. According to research byIrin and Hasanuzzaman (2024), organic amendments improved the microbial activity and soil nutrient content, which in turn greatly raised crop productivity.

According to Sharma et al. (2023), the improved nutrient availability and soil health that come from mixing several organic amendments led to higher crop yields than single amendments. Amendments to the soil can enhance the general health of plants, resulting in improved flowering and development of seeds. According to a study by Ranjan et al. (2023), increased vigor of plants and decreased pest pressure were the reasons why organic amendments like neem leaves promoted blooming and seed set in a variety of crops. Organic amendments improve the physical characteristics of soil, including as aeration and water-holding capacity, which are essential for pod growth. Organic additions enhanced soil structure and boosted okra pod weight, according to a study by Shampazuraini et al. (2023)

Plant biomass can be increased overall by enriching the soil with organic materials, which can result in heavier pods. According to research by Ghani et al. (2022) adding organic amendments significantly enhanced the amount of biomass produced and fruit yield of okra.

Enhancing the nutritional condition of the soil through organic amendments has a direct impact on seed growth. Better seed production and more seeds per pod are the results of improved nutrient uptake from organic amendments in African yam bean, according to Agu (2008). Additionally, a number of authors have observed that a higher crop output results from the absence of root galls (Cookey et al., 2021; Ogwudire et al., 2022).

3.4. Effects of soil amendment sources and varieties on fresh shoot weight (g), fresh root weight (g) and root-gall index of okra plant

Figure 4 illustrates how different soil amendment sources and varieties affect the okra plant's fresh shoot and fresh root weights (g) and root-gall index. According to our findings, the Yeleen okra variety produced the greatest fresh shoot weight of 76.8 g on plots treated with a combination of Azadirachtaindica and Chromolaenaodorata leaves. This was followed by Azadirachtaindica leaves and Carica papaya leaves (68 g), and the control plot produced the lowest shoot weight (50.4 g). In contrast, the combination of Azadirachtaindica leaves and Carica papaya had the highest fresh shoot weight (50.7 g) in the Clemson okra variety, followed by Azadirachtaindica leaves and Chromolaenaodorata (48 g), with the control plots having the lowest fresh shoot weight (37 g).

As contrasted with the control plots, the application of soil amendments had a substantial ($P<0.05$) impact on the fresh root weights of both kinds. Plots altered with Azadirachtaindica leaves and Carica papaya yielded the lowest fresh root weights (7.7 g and 12.8 g) for both the Clemson spineless and Yeleen okra types. These plots were followed by Azadirachtaindica leaves and Chromolaenaodorata (12.6 g and 13 g). The control plots' okra types yielded roots that weighed considerably more (30.1 g and 18 g).

Based on the root gall index results (Fig. 4), it was found that the okra varieties utilized in this study that were planted on the control plots had the highest root gall index of 4, which indicates that they were severely galled, significantly ($P < 0.05$).

The root gall indexes of Clemson spineless and Yeleen okra varieties planted on plots amended with a combination of Azadirachtaindica leaves and Carica papaya were 0.75 and 0.53, respectively; on plots amended with a combination of Azadirachtaindica leaves and Chromolaenaodorata, the root gall indexes of Clemson spineless and Yeleen okra varieties were 0.24 and 0.31, respectively. The root galling was significantly lower in these than in the control plots.

Comparing the fresh shoot weights with organic amendments to the control plots with nematode infection, there was a substantial increase, suggesting improved nutrient availability and uptake. Neem, or Azadirachtaindica, along with other organic amendments, are well-known for their abundant nutritional profiles, capacity to enhance soil fertility, and therapeutic qualities against soil microorganisms, all of which promote plant development and productivity. According to Emeghara (2023), organic amendments increase plant development and soil fertility, which raises crop yields for a variety of crops, including okra. Additionally, Ogwudire et al. (2022) observed that applying Jatrohacurcas tannins enhanced the nutritional composition and mucilaginous quality of okra while reducing the detrimental effects of root-knot nematode disease. Higher fresh shoot counts and healthier roots are signs of robust, healthy plants, which are likely to yield more. This is especially crucial for crops like okra, where more pods and higher-quality yield are directly correlated with plant vigor.

In contrast to the greater root weights and root-gall index seen on the severely galled untreated control plots, the lower root weights and root-gall index in the plots treated with organic amendments showed a reduced infestation of root-gall nematodes. The nematicidal qualities of neem (Azadirachtaindica) are especially well-known because they aid in controlling worm populations and reducing root damage. These outcomes were consistent with studies byWylie and Merrell (2022) and Adhikari, Bhandari, Niraula, and Shrestha (2020) that demonstrated the beneficial effects of neem amendments on nematode infestation reduction and plant health. Better nutrient and water uptake by healthier root systems with reduced root-gall indices promotes overall plant development and yield (Habteweld et al., 2024)

4. Conclusions:

The findings showed that soil amendments, particularly those that combined Azadirachtaindica leaves with either Carica papaya or Chromolaenaodorata, greatly enhanced okra yield and growth while also lowering nematode populations and reducing root galling in the okra varieties that were utilized. The yeleen variety, on the other hand, performed better in terms of growth and yield characteristics as well as the inhibition of the root-gall nematode on the contaminated soil in plots supplemented with a combination of Azadirachtaindica and Carica papaya leaves. Thus, applying a blend of Carica papaya and Azadirachtaindica leaves can be a sustainable addition for managing M. incognita on an ultisol that is naturally infested.

Conflict of Interest: The Authors declare that there is no conflict of interest.

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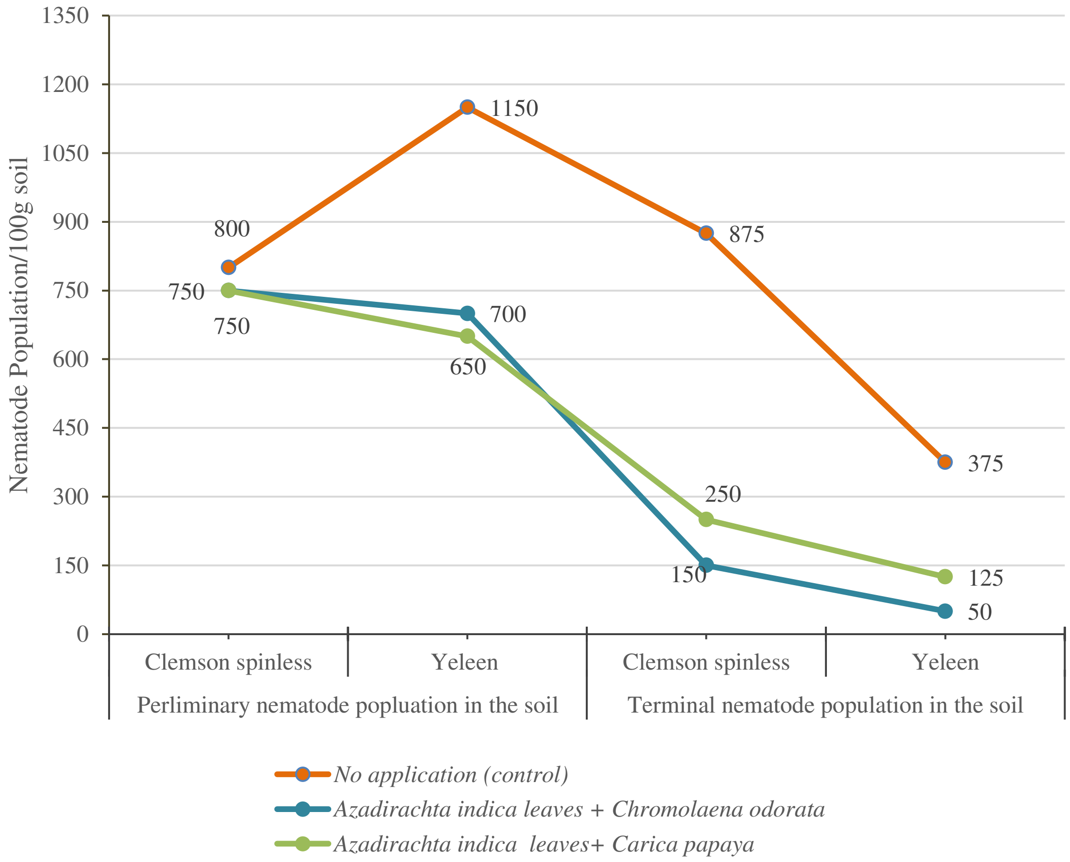
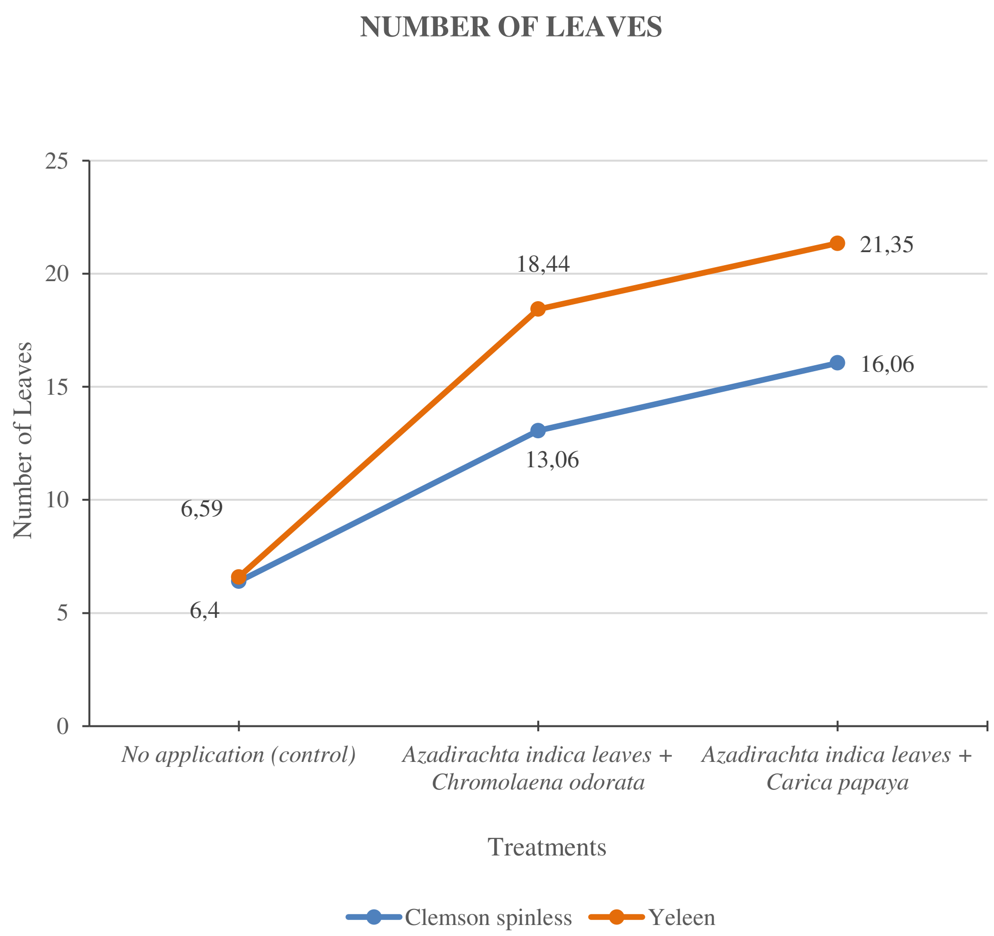


Fig 1: Impact of Soil amendment sources and variety on preliminary and terminalnematode population / 100 g soil.


NUMBER OF LEAVES
Number of Leaves
25
20
15
10
5
0
18,44
21,35
16,06
13,06
6,59
6,4
No application (control)
Azadirachta indica leaves + Chromolaena odorata
Azadirachta indica leaves + Carica papaya
Treatments
Clemson spinless
Yeleen

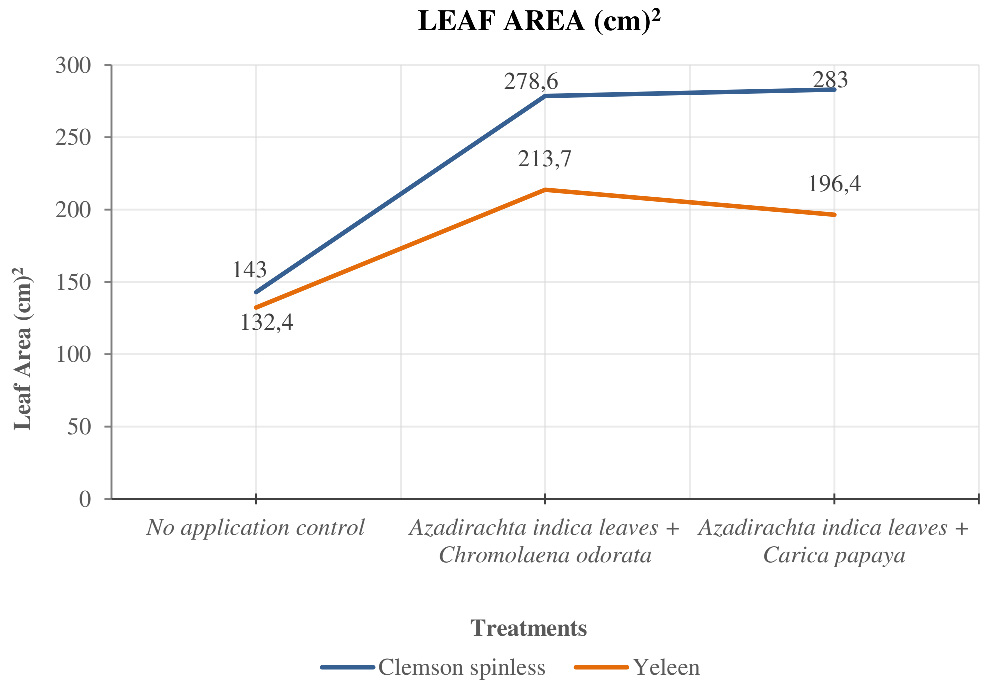
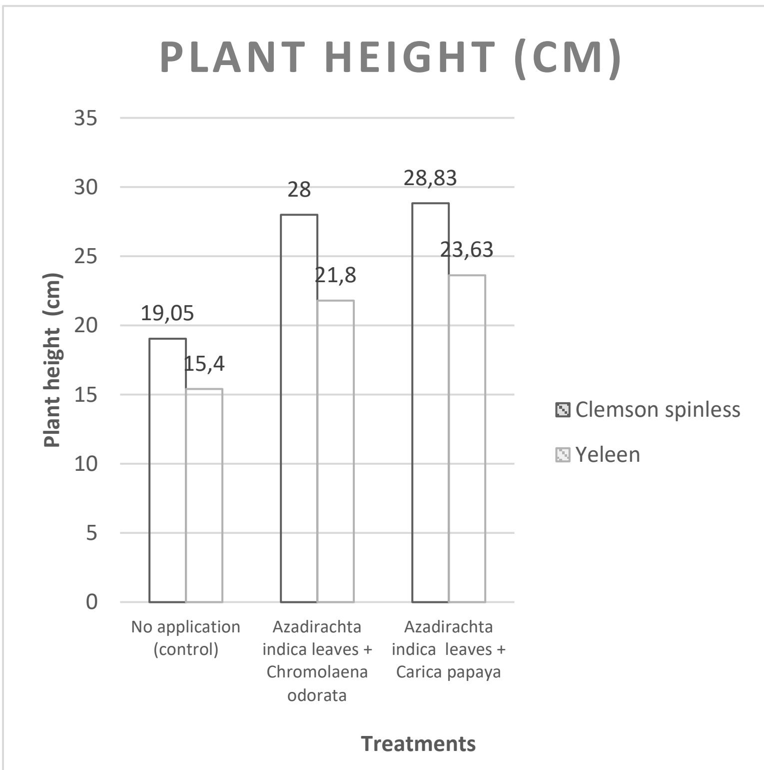


Fig. 2. Influence of soil amendments sources and okra varieties on number of leaves, leaf area $(cm)^2$ and plant height (cm).



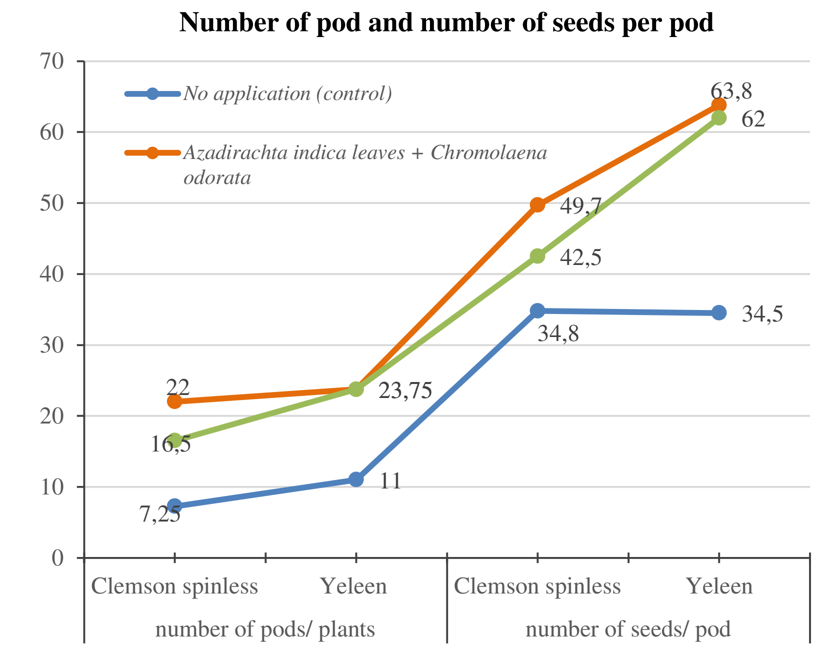
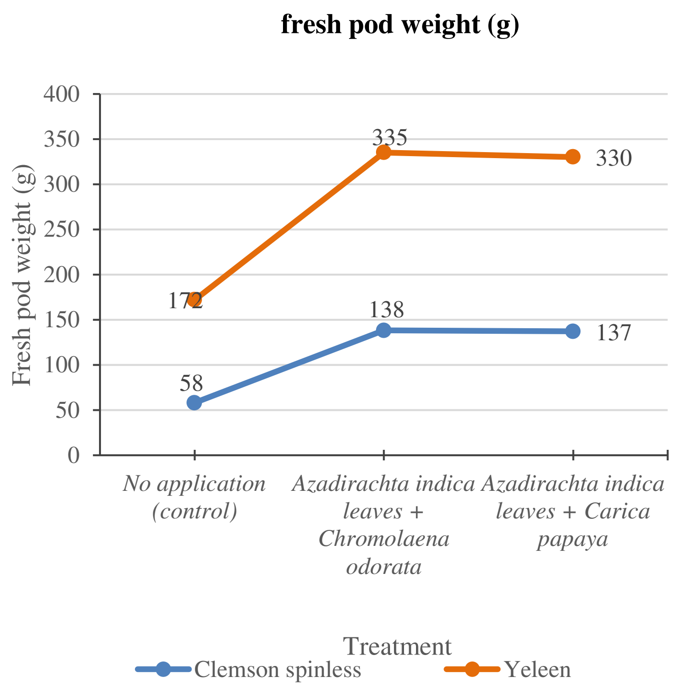


Fig.3. Effects of soil amendment sources and varieties on number of pods per plant, number of seeds per pod and fresh pod weight of okra



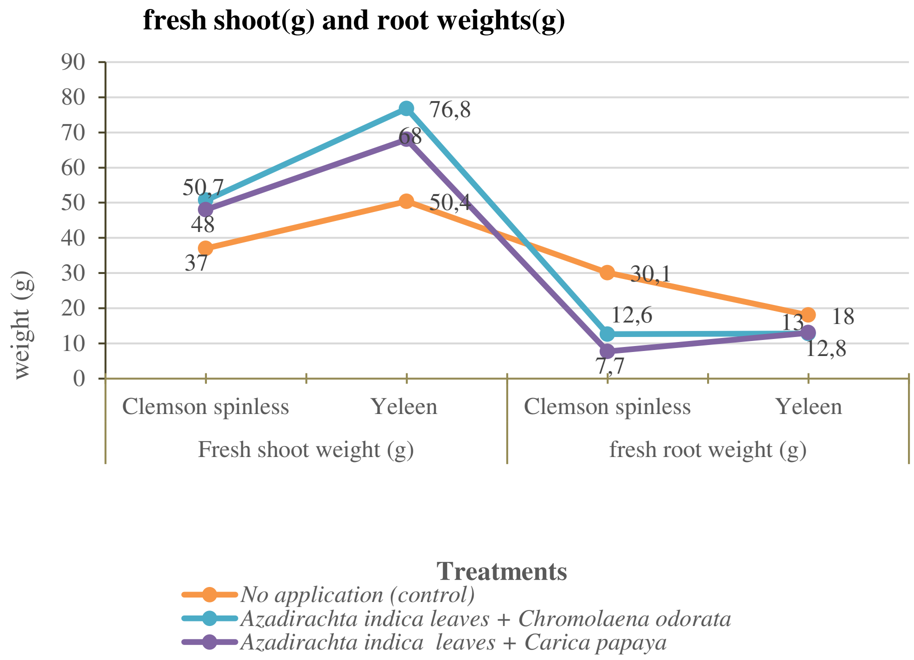


Fig. 4.Effects of soil amendment sources and varieties on fresh shoot and fresh root weights (g) and root-gall index (0 - 4) of okra plant.