Hindawi
International Journal of Agronomy
Volume 2024, Article ID 4025844, 11 pages
https://doi.org/10.1155/2024/4025844

Research Article

Evaluation of Preplant Seed Protectants for the Management of Root-Knot Nematode of Okra in Ghana

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Received 10 January 2024; Revised 9 February 2024; Accepted 29 February 2024; Published 7 March 2024

Academic Editor: Amri Ismail

Root-knot nematode (*Meloidogyne incognita*) poses a significant threat to okra production, resulting in substantial yield losses. The objectives of this study were to assess the impact of biological seed protectants on the growth and establishment of okra plants and nematode population reduction in soil. Okra seeds were coated with 40% sesame oil, 50% neem oil, 100% citrus oil, velum at 3.8 ml/7l of water, and a control (sterilized distilled water) at different time intervals of 30 min, 60 min, 90 min, and 120 min to determine the germination percentages and vigor. The experimental setup was laid out in a completely randomized design (CRD), with three replications, utilizing a Jacobson table for the germination test. The laboratory results demonstrated significant differences ($P < 0.05$) in germination percentage and vigor index across the different time intervals. Neem oil, citrus oil, and velum exhibited higher germination percentages and vigor indices at all time intervals. Notably, 30-minute time interval proved to be efficient with 100% citrus oil producing 80.33% germination and 965 vigor index and 50% neem oil producing 75% germination and 994 vigor index. Field evaluations revealed citrus at 100% concentrations as seed coating (T5) and neem at 50% concentrations as seed coating (T3), with the highest nematode reductions (90.1% and 90.4%) and least reproductive factors (RFs) of 0.05 and 0.04, respectively, at the Atomic farms. The study has revealed that treating okra seeds with 100% citrus oil and 50% neem oil has the efficacy of reducing nematode reproduction in soil, while enhancing germination and seedling vigor, together with an improvement in growth and yield. Sesame oil has a negative influence on seed germination and vigor and is therefore not recommended as a preplant protectant.

1. Introduction

The production of quality okra seeds and maximum yield largely depend on the healthy root system and growth parameters of the okra plant. Damage caused by plant-parasitic nematodes (PPNs) is one of the greatest factors that reduce the optimum yield and quality of seeds in Ghana. Plant-parasitic nematodes normally produce symptoms on aboveground plant parts which are presumed to be a nutrient or water deficiency or a disease. Nematode infestations on plants therefore go unnoticed by farmers, which results in crop losses with negative impacts on the economy. Nematode parasitism on plants results in stunted growth and chlorosis and this is heightened in instances of increased moisture stress and temperature [1]. However, these symptoms can be confused with symptoms from abiotic infections. Most farmers involved in okra cultivation are into the monocropping system [2], which enhances the rapid buildup of root-knot nematode population densities in these soils.

Effective management of plant-parasitic nematodes will involve the use of integrated management approaches. Methods currently utilized in nematode management include cultural, nematicide application, and physical methods; however, there are some limitations to these approaches [3].

The need for the use of environmentally sustainable control methods such as seed treatments will enhance

effective nematode management. These seed treatment methods can easily be used by farmers. The active ingredients (AI) in these seed protectants limit the buildup of plant-parasitic nematode populations. These protectants provide room for multiple treatment applications (i.e., fungicides, nematicides, and insecticides) to the same seed [4]. Kesba et al. [5] compared the effects of aqueous and ethanolic extracts from solidago and periwinkle on *Meloidogyne incognita* J2. The aqueous extracts caused a higher mortality rate, and the periwinkle extracts showed a greater inhibition of egg hatching relative to solidago extracts. In another study involving plant seed oils (canola, cotton, flax, olive, soybean, and sesame), against root-knot nematode in tomatoes, sesame oil had the least efficacy potential in reducing nematode populations in tomatoes and tomato shoot lengths were also reduced [6]. Other studies conducted have revealed some variations in relation to the effectiveness of the seed treatments on nematode population reduction, as a consequence of prevailing soil and environmental conditions, as well as chemical concentrations [7–9]. Further studies are needed to understand the relationships between nematode population densities and the effectiveness of nematode-protectant seed treatments [10]. Therefore, there is an urgent need to investigate the efficacy of new biological and plant extracts for root-knot nematode management in okra. This study assessed the efficacy of biological seed protectants on the growth and establishment of okra plants and root-knot nematode population reduction in soils planted to okra.

2. Materials and Methods

2.1. Study Sites. The study was conducted between January 2020 and August 2021. Laboratory experiments took place at the Ghana Seed Inspection Division's (GSID) Seed Testing Laboratory (latitude 5°42′ 0″ north and longitude 0° 18′ 7.2″) and the Plant Pathology Laboratory at the University of Ghana, located in Accra (latitude 5° 39′ 1.79″ north and longitude 0° 11′ 7.80″ east). Fieldwork was carried out at the University of Ghana farm and the Ghana Atomic Energy vegetable growers' farm, both situated in the Greater Accra Region.

The materials used in this study were obtained from various sources: the okra variety (Legon Finger) was acquired from the Crop Physiology Laboratory at the University of Ghana, the nematicide Velum (fluopyram SC 400 G) was obtained from Bayer West and Central Africa Limited, and the sesame seed extract, neem seed extract, and orange peel oil were acquired from Brimkata Enterprise (B.K Naturals) (Table 1). Petri dishes for the seed germination test were obtained from GSID.

2.2. Methodology. The study involved two experimental setups. Experiment one was conducted in a laboratory to evaluate the effect of biological seed protectants on the germination and vigor of okra seeds. Experiment two (fieldwork at two separate locations) was conducted from August to November 2020 at the University of Ghana farm and from March to June 2021 at the Ghana Atomic Energy vegetable growers' farm.

2.3. Laboratory Experiment. Okra seeds were coated with 40% sesame oil, 50% neem oil, 100% citrus oil, velum at 3.8 ml/7L of water, and a control (seeds were soaked in sterile distilled water) at different time intervals of 30 min, 60 min, 90 min, and 120 min to determine the germination percentage and vigor. The coating was done by immersing the seeds in the various oils and nematicide for the defined time intervals, and then, the seeds were removed and dried before sowing. The experimental setup was laid out in a completely randomized design (CRD) with three replications using a Jacobson table for the germination test. In a laboratory experiment, the following data were collected. The percentage of germination was calculated by dividing the number of seeds that sprouted by the total number of seeds planted and then multiplying the result by 100:

$$\text{percentage germination (\%)} = \left(\frac{\text{NSG}}{\text{TNSP}}\right) \times 100, \quad (1)$$

where NSG is the number of seeds germinated and TNSP is the total number of seeds planted [11, 12].

$$\text{Vigor index} = (\text{MRL} + \text{MSL}) \times \text{PSG}, \quad (2)$$

where MRL is the mean root length, MSL is the mean shoot length, and PSG is the percentage of seed germination [13]. Root and shoot lengths were measured using a meter rule.

2.4. Field Experiment. The farms were ploughed to loosen the soil, and the field layouts were performed with pegs and lines as well as a tape measure. The total field size was 34.1 m × 51.5 m. Each plot size was 3.2 m × 2.5 m with a 1 m alley left between plots.

There were nine treatments in total (4 treatments for seed coating prior to sowing for 30 min and 4 treatments for drenching 2 weeks after sowing and one control). The drenching was performed by making a hole (drench) around the base of the plant and pouring the chemical in and covering it. Treatments were laid out in a randomized complete blocked design (RCBD) with four replications. The various treatments used are as follows:

T1 = sesame oil at 40% as seed coating for 30 min prior to sowing

T2 = sesame oil at 40% as drenching two weeks after germination

T3 = neem oil at 50% as seed coating for 30 min prior to sowing

T4 = neem oil at 50% as drenching two weeks after germination

T5 = citrus oil at 100% as seed coating for 30 min prior to sowing

T6 = citrus oil at 100% as drenching two weeks after germination

Table 1: Treatments and their active ingredients, formulation type, and mode of action.

Treatment	Active ingredients	Formulation type	Mode of action	Reference
Sesame oil (seed extract)	Sesamin	Liquid	Systemic	Moazzami et al. [11]
Neem oil (seed extract)	Azadirachtin	Liquid	Antifeedant	Isman et al. [12]
Citrus oil (peel extract)	Limonene	Liquid	Systemic	Anwar et al. [13]
Velum	Fluopyram	Liquid	Systemic	Faske and Hurd [14]

T7 = velum (check) at 3.8 ml/1L as seed coating for 30 min prior to sowing

T8 = velum (check) at 9.8 ml/15L of water as drenching two weeks after germination.

T9 = control (no application)

2.5. Sowing and Treatment Application for Both Farms. Okra seeds for T1, T3, T5, and T7 were coated with 40% sesame oil, 50% neem oil, 100% citrus oil, and velum at 3.8 ml/1L for 30 minutes and then dried for 5 minutes. Seeds for T2, T4, T6, T8, and T9 were sown without coating. Seeds were picked and sown with a pair of forceps after drying. Drenching was performed two weeks after sowing seeds for T2, T4, T6, and T8 at 100 ml/plant each of 40% sesame oil, 50% neem oil, 100% citrus oil, and velum at 9.8 ml/15L.

The planting distance was 80 cm × 50 cm within and between rows, with each plot containing 20 plants and a total population of 720 plants.

2.6. Cultural Practices for Both Farms. Handpicking and occasional use of a hoe were the main methods for controlling less dense weeds, with no weedicides applied. Daily watering was performed using watering cans. At 4 weeks after planting, the entire field received 0.5 t/ha of fertilizer N : P : K 15 : 15 : 15. Insects on the field were managed by spraying Orizon.

2.7. Nematode Extraction and Counting for Both Farms. Nematode extraction and count were performed in the Plant Pathology Laboratory of the Department of Crop Science, University of Ghana, Legon. Nematode populations were determined before sowing and after harvesting at 13 weeks. Nematode extraction was undertaken using the sieving and sucrose-centrifugation method [15].

Sucrose solution was prepared by adding deionized water to 454 g of sugar to bring the volume to 1 liter and stirred until completely dissolved.

200 cc soil sample after rocks and roots were removed was packed lightly into a beaker for uniformity. Soil samples were placed in a bucket and covered with water twice its volume, vigorously mixed for complete dispersion, and allowed to settle for 5 minutes. Clayey soil samples were left to settle for 7 minutes. Three laboratory test sieves with different apertures were used: 90 μm, 71 μm, and 36 μm. The sieves were nested onto each other in the order listed and the supernatant was carefully poured through the sieves. The 90 and 71 μm sieves trapped the soil particles while the 36 μm trapped both nematodes and nematode eggs. Using the fine spray wash bottle, samples trapped on the 36 μm mesh sieve were washed into centrifuge tubes at equal volumes (where necessary, water was added to equalize volumes in centrifuge tubes). Tubes were placed in balanced pairs in the centrifuge and spun at 1700 rpm for 5 minutes and then allowed to settle for 5 minutes. Supernatants were aspirated to about 1 cm above the pellets. The tubes were filled with sucrose solution to cover the pellets after which a spatula was used to stir to break the pellets until complete dispersion. Samples were spun at 1000 rpm for 1 minute. Nematodes and clay were suspended in the sucrose supernatant. The supernatant was then sieved out through the 36 μm sieve to trap the nematodes and then transferred into the labeled vials using a fine spray water bottle. A Hand tally counter was used to keep track of each nematode counted within the liquid under the compound microscope.

2.8. Nematode Reproductive Factors. The reproductive factor (an indicator of the suitability of a host plant for nematodes) of the nematodes was determined using Oostenbrink's reproduction factor (RF) [16], which was calculated as follows: RF = (Pf)/(Pi), where Pf is the final nematode population and Pi is the initial nematode population.

2.9. Nematode Identification. Nematodes were identified only at the genera level. They were identified based on their morphological features (length of stylet, head region, stylet knob, intestine thickness, and tail shape of adult nematodes) as described by Luc et al. [17] and Siddiqi and Alam [18] and the website of the University of Nebraska–Lincoln for nematode identification (https://nematode.uml.edu/konzlistbutt.htm). Nematode contents in plastic vials were separately emptied gently into 50 mm round plastic counting Petri dishes internally calibrated with 2 mm × 2 mm blue grid lines. These lines served as a guide for systematic analysis of nematodes across the liquid. Nematodes were sucked using a small syringe and transferred to another counting dish where selected nematodes for identification were mounted on a drop of water on a microscopic slide and placed on a 60°C hot plate briskly to allow nematode to straighten for identification [19]. The compound light microscope with a magnification of 400x, manufactured by Optec Optical Technology, was used for nematode identification.

2.10. Data Collected on Plant Parameters for Both Farms. Data were collected on six record plants on each plot and the mean number of each plot was determined for each treatment (Table 2).

Table 2: Data collected on plant growth parameters in field experiments.

Plant growth parameter	Mode and duration of assessment
Plant height (cm)	Measured with the meter rule for shorter plants and tape measure for taller plants at 5, 8, and 11 weeks of treatment application
Number of leaves	Leaves of 6 record plants were counted after 5, 8, and 11 weeks after emergence on each plot and the mean number of leaves per plot was determined for each treatment
Plant girth (cm)	Plant girth was measured with the digital micrometer screw gauge at 5 and 8 weeks after application of treatments
Chlorophyll content	Chlorophyll content index (CCI) was measured with SPAD-502Plus electronic chlorophyll meter (Konica Minolta Optics, Japan) at weeks 5 and 8
Number and weight of fruits	Number of fruits for each plot was counted individually. The weight of the fruit for each plot was weighed on a scale
Shoot fresh weight (g)	After week 13, the plants were uprooted, shoots were separated from the roots, attached soil was washed off, and then, it was allowed to air dry for five minutes and then weighed on the weighing balance
Shoot dry weight (g)	Shoot fresh weights taken after week 13 were dried in the oven at 70°C for 72 hours and weighed
Root fresh weight (g)	After 13 weeks, the roots were abscised from the shoots and washed thoroughly then allowed to dry for five minutes and weighed on a balance
Root dry weight (g)	Root fresh weights taken after week 13 were dried in an oven at 70°C for 72 hours and weighed

2.11. Data Analysis. Data collected were subjected to analysis of variance (ANOVA) using GenStat Statistical Package Edition 12, and means were separated using LSD at 5% where significant differences existed.

3. Results

3.1. Laboratory Experiment

3.1.1. Effect of Duration of Coating of Biological Seed Protectants on Seed Germination and Vigor. Okra seeds were treated with different biological seed protectant's (citrus oil, sesame oil, neem oil, velum, and control), and their germination percentages and vigor indices were evaluated at 30 min, 60 min, 90 min, and 120 min intervals. There were significant differences ($P < 0.05$) across all four different time intervals that were assessed for each of the five treatments (Figures 1 and 2). There was a general decrease in germination percentages for all treatments as the time for seed treatment increased from 30 min to 60 min, with the exception of the control (Figure 1). Sesame oil had the least germination rates for the four time points and showed a sharp decrease (64% to 3%), as the soaking time increased from 30 min to 120 min. The germination percentage of the control however increased gradually over the time points (64% to 80%). At 30 min, velum and citrus oil had the highest germination percentage (90% and 80%), respectively. There was a general decrease in seedling vigor for neem (994 to 343) and sesame (610 to 7) oils, for the time points 30 min and 120 min, respectively (Figure 2). Sesame oil therefore had the least seedling vigor among the four treatments. The control had a gradual increase in seedling vigor (899 to 1036) for the time points 30 min and 120 min, respectively.

3.2. Biological Seed Protectants on Okra Growth and Establishment (University of Ghana Farm)

3.2.1. Effect of Treatments on the Height of Okra Plants. Plant height increased as the plants aged over 5–11 weeks. Treatment 9 (control) had the tallest plants of 19 cm, 69 cm, and 71.4 cm for weeks 5, 8, and 11, respectively (Figure 3). Treatment 8 (velum at 9.8 ml/15L of water as drenching two weeks after germination) had the shortest plants. The treatments did not show any significant differences in plant height for the specific weeks.

3.2.2. Mean Effect of the Treatments on the Number of Leaves on Okra. Over the course of weeks 5 to 11, there was a noticeable increase in the number of leaves as the plants matured. Specifically, treatments 6, 5, and 2 exhibited the most robust leaf growth at weeks 5 (47 leaves), 8 (110 leaves), and 11 (127 leaves), respectively. Conversely, treatment 8 displayed the lowest leaf count at week 8, while treatment 1 consistently had the least number of leaves at both weeks 5 and 11. The difference between the treatments with a higher number of leaves and those with a lower number of leaves was only significant ($P < 0.05$) at week 11.

3.2.3. Effect of Treatments on the Chlorophyll Content of Okra Plants. Treatment 3 had the highest chlorophyll content of 53.1 CCI, while treatment 8 (velum) at 9.8 ml/15L of water as drenching two weeks after germination had the lowest chlorophyll content of 44.2 CCI at week 5, and difference in chlorophyll content between these two treatments was not significant ($P < 0.05$). At week 8, the highest chlorophyll content of 61.8 CCI was observed on treatment 2 (sesame at 40% as drenching two weeks after germination) and the

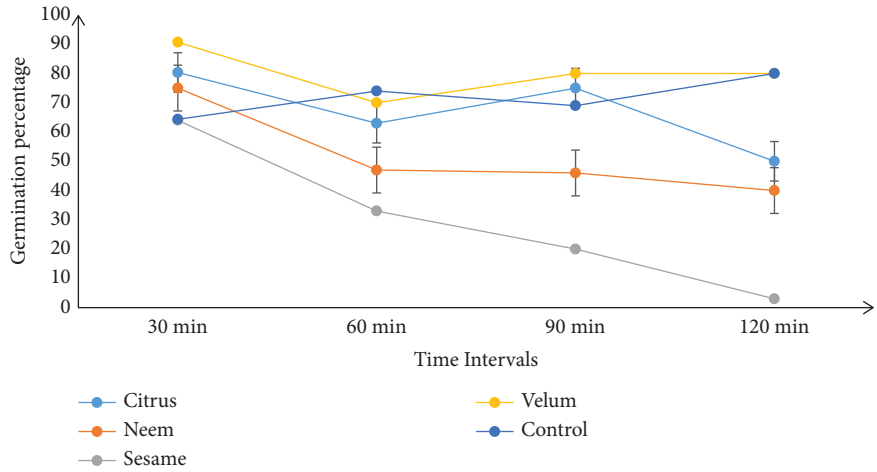

Figure 1: Germination percentage (%) of treatments at four time intervals. T1 = sesame at 40% as seed coating for 30 min prior to sowing, T2 = sesame at 40% as drenching two weeks after germination, T3 = neem at 50% as seed coating for 30 min prior to sowing, T4 = neem at 50% as drenching two weeks after germination, T5 = citrus at 100% as seed coating for 30 min prior to sowing, T6 = citrus at 100% as drenching two weeks after germination, T7 = velum (check) at 3.8 ml/1L as seed coating for 30 min prior to sowing, T8 = velum (check) at 9.8 ml/15L of water as drenching two weeks after germination, and T9 = control (no application).

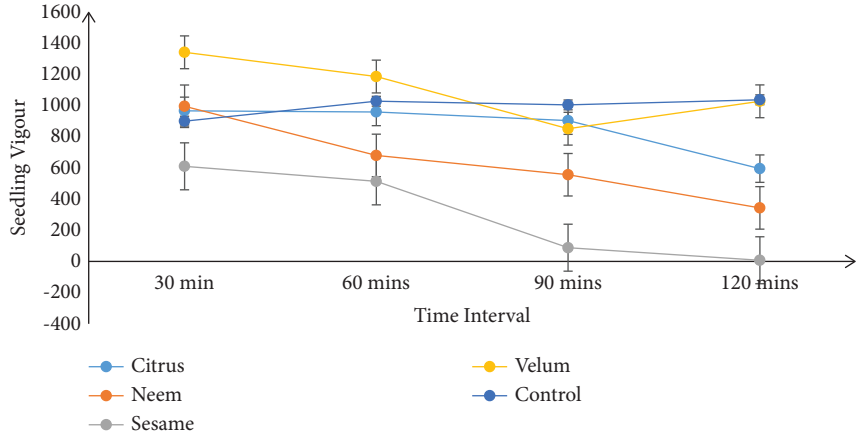

Figure 2: Seedling vigor index for treatments at four time intervals. T1 = sesame at 40% as seed coating for 30 min prior to sowing, T2 = sesame at 40% as drenching two weeks after germination, T3 = neem at 50% as seed coating for 30 min prior to sowing, T4 = neem at 50% as drenching two weeks after germination, T5 = citrus at 100% as seed coating for 30 min prior to sowing, T6 = citrus at 100% as drenching two weeks after germination, T7 = velum (check) at 3.8 ml/1L as seed coating for 30 min prior to sowing, T8 = velum (check) at 9.8 ml/15L of water as drenching two weeks after germination, and T9 = control (no application).

lowest chlorophyll content of 48.8 CCI was observed on treatment 4 (neem at 50% as drenching two weeks after germination). There were significant differences ($P < 0.05$) between these two treatments in their effect on the chlorophyll content of the leaves.

3.2.4. Mean Effect of the Treatments on Plant Girth at Weeks 5 and 8. Plant girth increased as the plants aged from weeks 5 to 8. Treatment 6 (citrus at 100% as drenching two weeks after germination) had the highest girth of 11.54 cm, with treatment 8 (velum at 9.8 ml/15L of water as drenching two weeks after germination) having the lowest girth of 8.84 cm at week 5. At week 8, treatment 1 (sesame at 40% as seed coating for 30 min prior to sowing) had the highest girth of 4.33 cm, while treatment 3 (neem at 50% as seed coating for 30 min prior to sowing) obtained the lowest girth of 3.96 cm. There were no significant differences ($P > 0.05$) between any of the treatments either at week 5 or week 8.

3.2.5. Mean Effect of Treatments on the Number of Fruits and Weight of Fruits at Two Weekly Intervals. Treatment 9 had the highest fruit count (20) during week 1, which is significantly different from treatments 6 and 7 with the lowest counts (12) ($P < 0.05$) (Table 3). However, within week 2, treatment 6 had the most fruits (35), followed by treatment 1 (34), and treatment 8 had the least (27); however, there were no significant differences among the treatments ($P > 0.05$).

Regarding fruit weight, treatment 9 had the highest weight (0.7 kg/ha) during the harvest in week 1, which is significantly different from treatment 6 with the lowest

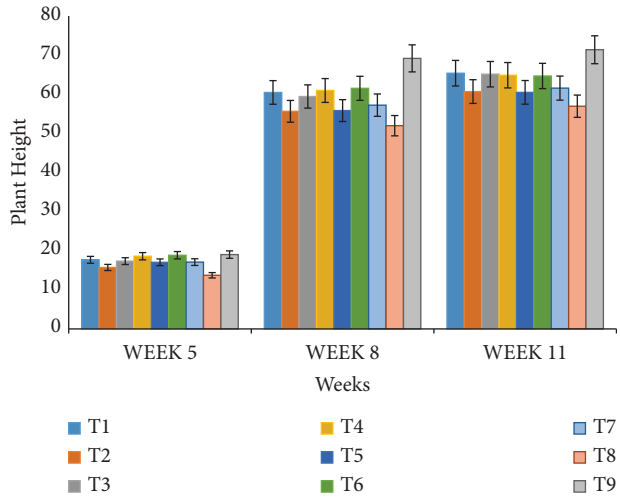

Figure 3: Plant height from weeks 5 to 11 after treatment application (University of Ghana farm). T1 = sesame at 40% as seed coating for 30 min prior to sowing, T2 = sesame at 40% as drenching two weeks after germination, T3 = neem at 50% as seed coating for 30 min prior to sowing, T4 = neem at 50% as drenching two weeks after germination, T5 = citrus at 100% as seed coating for 30 min prior to sowing, T6 = citrus at 100% as drenching two weeks after germination, T7 = velum (check) at 3.8 ml/1L as seed coating for 30 min prior to sowing, T8 = velum (check) at 9.8 ml/15L of water as drenching two weeks after germination, and T9 = control (no application).

Table 3: Effect of treatments on the number and weight of fruits, at two-week intervals (University of Ghana farm).

Treatment	Number of fruits WK 1	Number of fruits WK 2	Weight of fruits (kg/ha) WK 1	Weight of fruits (kg/ha) WK 2
T1	15.0ab	34.0a	0.6a	1.2a
T2	14.0ab	28.0a	0.5a	0.9a
T3	13.0ab	33.0a	0.5a	1.0a
T4	17.0ab	33.0a	0.7a	1.1a
T5	13.0ab	28.0a	0.5a	0.9a
T6	12.0a	35.0a	0.4a	1.1a
T7	12.0a	30.0a	0.5a	1.0a
T8	17.0ab	27.0a	0.6a	0.9a
T9	20.0b	33.0a	0.7a	1.2a
LSD	6.0	12.0	0.3	0.5
Grand mean	14.0	31.0	0.6	1.0

Means followed by the same letters in a column are not significantly different at LSD ($P < 0.05$). T1 = sesame at 40% as seed coating for 30 min prior to sowing; T2 = sesame at 40% as drenching two weeks after germination; T3 = neem at 50% as seed coating for 30 min prior to sowing; T4 = neem at 50% as drenching two weeks after germination; T5 = citrus at 100% as seed coating for 30 min prior to sowing; T6 = citrus at 100% as drenching two weeks after germination; T7 = velum (check) at 3.8 ml/1L as seed coating for 30 min prior to sowing; T8 = velum (check) at 9.8 ml/15L of water as drenching two weeks after germination; T9 = control (no application).

weight (0.4 kg/ha) ($P < 0.07$) (Table 3). Treatments 1 and 9 had the highest fruit weights (1.2 kg/ha), while treatment 8 had the lowest fruit weight (0.9 kg/ha) in week 2 of the harvest. However, there were no significant differences observed among the treatments ($P > 0.05$).

3.2.6. Mean Effect of Treatments on Fresh and Dry Shoot Weights and Fresh and Dry Root Weights. There were no significant differences ($P > 0.05$) among the treatments for fresh and dry shoots and root weights (Table 4).

3.2.7. Mean Effect of Biological Seed Protectants on Nematode Populations. Significant differences ($P < 0.05$) existed among the treatments in relation to nematode population reduction percentages (Table 5). The treatment with the least nematode reduction percentage was T9. This is also reflected in the high RF value of 0.9. However, there were no significant differences ($P < 0.05$) among the other treatments. Nematode RF values ranged from 0.1 to 0.4 for treatments (T1 and T3), respectively.

3.3. Results from the Ghana Atomic Energy Vegetable Growers' Farms

3.3.1. Effect of the Treatments on the Height of the Okra Plants. Treatment 4 (neem at 50% after 2 weeks of germination) had the highest plant height of 18.41 cm at week 5 (Figure 4). Treatment 9 (control) showed a change in trend, reaching the highest plant height of 65.8 cm and 71.4 cm at weeks 8 and 11, respectively. Treatment 8 (check) had the lowest plant height of 14.74 cm, 47.1 cm, and 57.2 cm for

Table 4: Effect of treatments on fresh and dry shoot and root weights (University of Ghana farm).

Treatment	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)
T1	45.8a	22.67a	30.36a	17.87a
T2	47.5a	24.75a	31.61a	18.13a
T3	45.6a	24.16a	32.43a	19.59a
T4	46.6a	23.76a	30.28a	18.91a
T5	44.6a	22.89a	28.41a	17.84a
T6	49.6a	25.39a	34.19a	21.15a
T7	39.0a	19.23a	27.66a	17.61a
T8	45.0a	22.77a	30.93a	18.32a
T9	33.4a	15.92a	21.81a	14.43a
LSD	11.71	7.81	7.31	4.02

Means followed by the same letters in a column are not significantly different at LSD ($P < 0.05$). T1 = sesame at 40% as seed coating for 30 min prior to sowing; T2 = sesame at 40% as drenching two weeks after germination; T3 = neem at 50% as seed coating for 30 min prior to sowing; T4 = neem at 50% as drenching two weeks after germination; T5 = citrus at 100% as seed coating for 30 min prior to sowing; T6 = citrus at 100% as drenching two weeks after germination; T7 = velum (check) at 3.8 ml/1L as seed coating for 30 min prior to sowing; T8 = velum (check) at 9.8 ml/15L of water as drenching two weeks after germination; T9 = control (no application).

Table 5: Percent reduction in nematode populations (University of Ghana farm).

Treatment	Initial nematode count	Final nematode count	Reproductive factor (RF)	Percentage reduction in nematode population (%)
T1	310.0	41.0	0.1	80.0b
T2	184.0	40.0	0.2	70.8b
T3	128.0	46.0	0.4	67.0b
T4	319.0	80.0	0.3	76.6b
T5	203.0	63.0	0.3	68.8b
T6	153.0	25.0	0.2	77.4b
T7	605.0	104.0	0.2	75.8b
T8	67.0	19.0	0.3	70.9b
T9	370.0	327.0	0.9	21.0a

T1 = sesame at 40% as seed coating for 30 min prior to sowing; T2 = sesame at 40% as drenching two weeks after germination; T3 = neem at 50% as seed coating for 30 min prior to sowing; T4 = neem at 50% as drenching two weeks after germination; T5 = citrus at 100% as seed coating for 30 min prior to sowing; T6 = citrus at 100% as drenching two weeks after germination; T7 = velum (check) at 3.8 ml/1L as seed coating for 30 min prior to sowing; T8 = velum (check) at 9.8 ml/15L of water as drenching two weeks after germination; T9 = control (no application).

weeks 5, 8, and 11, respectively, which was also the case at the University of Ghana farm. A significant difference was observed between treatments 9 and 8 as okra plants aged from weeks 8 to 11.

3.3.2. Effect of Treatments on the Number of Leaves on Okra Plants. At week 5, treatment 7 had the highest number of leaves (47), followed by treatments 5 (46) and 6 (46), while treatment 1 had the least number of leaves (41). At week 8, treatments 2 and 8 obtained the highest (107) and lowest (81) number of leaves, respectively, and the difference between these two treatments was significant ($P < 0.05$). Treatment 5 had a significantly ($P < 0.05$) higher number of leaves (128) than treatment 1, which had the lowest number of leaves (106) at week 11.

3.3.3. Mean Effect of the Treatments on Plant Girth at Weeks 5 and 8. Treatments 6 (citrus at 100% as drenching two weeks after germination) and 9 (control, no application) had the highest (12.15 cm) and lowest (9.19 cm) girths at week 5, while at week 8, treatments 1 and 9 had the highest (19.18 cm) and lowest (15.41 cm) girths. Treatments 6 and 1 consistently had the largest girths in both farms and at weeks 5 and 8, respectively. Both at week 5 and week 8, treatments with the highest and lowest plant girths were significantly different ($P < 0.05$).

3.3.4. Mean Effect of Treatments on Chlorophyll Content at Weeks 5 and 8. There were significant differences ($P < 0.05$) among the treatments for chlorophyll content for weeks 5 and 8 (Table 6). Treatment 1 (sesame at 40% as seed coating for 30 minutes prior to sowing) had the highest chlorophyll content of 51.83 CCI at week 5, with treatment 2 having the highest chlorophyll content at week 8 (60.0 CCI). Treatment 9 had the lowest chlorophyll content, measuring 36.2 CCI and 37.2 CCI at weeks 5 and 8, respectively.

3.3.5. Mean Effect of Treatments on the Number of Fruits and Weight of Fruits at Two Weekly Intervals. There were no significant differences ($P > 0.05$) among the different treatments for the number of fruits and fruits harvested. Treatment 2 (sesame at 40%, applied as a drench two weeks after germination) had the most fruits (17) during the

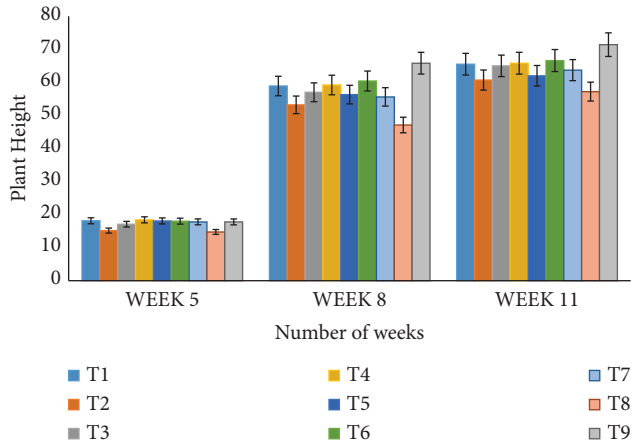

Figure 4: Plant height from weeks 5 to 11 after treatment application (Ghana Atomic Energy vegetable growers' farm). T1 = sesame at 40% as seed coating for 30 min prior to sowing, T2 = sesame at 40% as drenching two weeks after germination, T3 = neem at 50% as seed coating for 30 min prior to sowing, T4 = neem at 50% as drenching two weeks after germination, T5 = citrus at 100% as seed coating for 30 min prior to sowing, T6 = citrus at 100% as drenching two weeks after germination, T7 = velum (check) at 3.8 ml/1L as seed coating for 30 min prior to sowing, T8 = velum (check) at 9.8 ml/15L of water as drenching two weeks after germination, and T9 = control (no application).

Table 6: Effect of treatments on chlorophyll content at week 5 and week 8.

Treatment	Chlorophyll (week 5)	Chlorophyll (week 8)
T1	51.83b	54.90bc
T2	50.98b	60.00c
T3	47.64b	58.20bc
T4	46.15b	56.80bc
T5	49.77b	58.50bc
T6	50.82b	50.00b
T7	46.15b	54.40bc
T8	46.07b	54.40bc
T9	36.20a	37.20a
LSD	8.36	9.48
Grand mean	47.29	53.8

Means followed by the same letters in a column are not significantly different at LSD ($P < 0.05$). T1 = sesame at 40% as seed coating for 30 min prior to sowing; T2 = sesame at 40% as drenching two weeks after germination; T3 = neem at 50% as seed coating for 30 min prior to sowing; T4 = neem at 50% as drenching two weeks after germination; T5 = citrus at 100% as seed coating for 30 min prior to sowing; T6 = citrus at 100% as drenching two weeks after germination; T7 = velum (check) at 3.8 ml/1L as seed coating for 30 min prior to sowing; T8 = velum (check) at 9.8 ml/15L of water as drenching two weeks after germination; T9 = control (no application).

harvest in week 1 (Table 7). On the other hand, treatments 6, 7, and 9 had the least number of fruits (12 each).

The harvest in week 2 showed treatment 6 (citrus at 100%, applied as a drench two weeks after germination) with the most fruits (35), while treatment 9 had the least fruits (28).

3.3.6. Mean Effect of Treatments on Fresh and Dry Shoot Weights and Fresh and Dry Root Weights. There were no significant differences ($P > 0.05$) among the treatments for fresh and dry shoots and root weights (Table 8).

3.3.7. Mean Effect of Biological Seed Protectants on Nematode Populations. There were significant differences ($P < 0.05$) among the various treatments for nematode reduction percentages (Table 9). The treatment with the least nematode reduction percentage (19.5%) was T9 and the two treatments T5 and T3 had the highest nematode reduction percentages (90.1% and 90.4%), respectively. However, there were no significant differences ($P < 0.05$) among the treatments (T7, T2, and T1).

4. Discussion

In a previous study by Oyekale et al. [20], natural botanicals such as sesame, neem, and citrus extracts were utilized as short- and medium-term storage treatments for seeds and these gave the highest germination and vigor percentages. The germination % and vigor index of all treatments varied significantly; however, the 30-minute seed treatment was the most effective. Other investigators, such as Hoque et al. [21], Ashrafi et al. [22], and Lawan et al. [23], have indicated that there are specific weeds and plant species which contain secondary plant products with allelopathic potentials, influencing a decrease in seed germination and growth.

Seed treatment with biocontrol agents offers an effective approach for introducing beneficial organisms into the soil-root environment. This method protects the seed from both seed and soilborne pathogens, such as plant-parasitic nematodes, enabling successful germination and healthy seedling establishment [24].

The current study demonstrates that the use of biological protectants leads to improved plant growth, e.g., plant height, compared to synthetic protectants used as controls. In a previous study, biological protectants provided superior protection against root-knot nematodes and improvement in plant establishment [20].

Kumar and Khanna [25] have also observed the suppressive effects of neem extract, a natural protectant, on nematode multiplication and root galling severity, leading to enhanced plant growth in tomatoes. Investigations reveal that soil amendments with aqueous and powdered citrus peel extracts not only show the absence of phytotoxicity to cowpea plants but also exhibit nematicidal properties, thus improving the growth and yield of cowpea plants. Other reports have also confirmed growth improvements in plants [26, 27]. Seed treatment with biocontrol agents, such as neem extract and citrus-based compounds, proves to be a promising approach for enhancing plant growth by reducing nematode populations and providing protection against harmful pathogens [24]. These natural protectants offer significant advantages over synthetic alternatives and contribute to the establishment of healthy and thriving crops [20, 25–27].

Table 7: Effect of treatments on the number and weight of fruits, at two-week intervals (Ghana Atomic farm).

Treatment	Number of fruits WK 1	Number of fruits WK 2	Weight of fruits (kg/ha) WK 1	Weight of fruits (kg/ha) WK 2
T1	16.0a	32.0a	0.7a	1.3a
T2	17.0a	32.0a	0.7a	1.2a
T3	13.0a	30.0a	0.5a	1.3a
T4	16.0a	32.0a	0.6a	1.5a
T5	16.0a	29.0a	0.6a	1.3a
T6	12.0a	35.0a	0.4a	1.5a
T7	12.0a	29.0a	0.5a	1.1a
T8	17.0a	29.0a	0.7a	1.3a
T9	12.0a	28.0a	0.5a	1.1a
LSD	5.0	11.0	0.3	0.5

Means followed by the same letters in a column are not significantly different at LSD ($P < 0.05$). T1 = sesame at 40% as seed coating for 30 min prior to sowing; T2 = sesame at 40% as drenching two weeks after germination; T3 = neem at 50% as seed coating for 30 min prior to sowing; T4 = neem at 50% as drenching two weeks after germination; T5 = citrus at 100% as seed coating for 30 min prior to sowing; T6 = citrus at 100% as drenching two weeks after germination; T7 = velum (check) at 3.8 ml/1L as seed coating for 30 min prior to sowing; T8 = velum (check) at 9.8 ml/15L of water as drenching two weeks after germination; T9 = control (no application).

Table 8: Effect of treatments on fresh and dry shoot and root weights (Ghana Atomic farm).

Treatment	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)
T1	41.0a	22.8a	29.5a	11.9a
T2	47.9a	28.5a	27.0a	15.0a
T3	41.7a	24.5a	32.9a	16.0a
T4	40.2a	21.5a	27.7a	14.3a
T5	47.0a	25.5a	24.8a	15.6a
T6	51.5a	26.4a	30.2a	14.8a
T7	38.0a	18.7a	29.4a	16.6a
T8	40.4a	21.3a	25.2a	14.9a
T9	39.6a	20.8a	26.0a	15.6a
LSD	14.5	12.6	9.1	3.6

Means followed by the same letters in a column are not significantly different at LSD ($P < 0.05$). T1 = sesame at 40% as seed coating for 30 min prior to sowing; T2 = sesame at 40% as drenching two weeks after germination; T3 = neem at 50% as seed coating for 30 minutes prior to sowing; T4 = neem at 50% as drenching two weeks after germination; T5 = citrus at 100% as seed coating for 30 min prior to sowing; T6 = citrus at 100% as drenching two weeks after germination; T7 = velum (check) at 3.8 ml/1L as seed coating for 30 min prior to sowing; T8 = velum (check) at 9.8 ml/15L of water as drenching two weeks after germination; T9 = control (no application).

Table 9: Percent reduction in nematode populations (Ghana Atomic farm).

Treatment	Initial nematode count	Final nematode count	Reproductive factor (RF)	Percentage reduction in nematode nos. (%)
T1	609.0	66.0	0.10	87.6bcd
T2	336.0	32.0	0.09	78.9bcd
T3	595.0	23.0	0.04	90.4d
T4	109.0	33.0	0.30	69.0bc
T5	219.0	13.0	0.05	90.1d
T6	261.0	7.0	0.02	89.1cd
T7	135.0	30.0	0.22	74.9bcd
T8	60.0	23.0	0.38	68.5b
T9	79.0	63.0	0.79	19.6a

Means followed by the same letters in a column are not significantly different at LSD ($P < 0.05$) T1 = sesame at 40% as seed coating for 30 min prior to sowing; T2 = sesame at 40% as drenching two weeks after germination; T3 = neem at 50% as seed coating for 30 min prior to sowing; T4 = neem at 50% as drenching two weeks after germination; T5 = citrus at 100% as seed coating for 30 min prior to sowing; T6 = citrus at 100% as drenching two weeks after germination; T7 = velum (check) at 3.8 ml/1L as seed coating for 30 min prior to sowing; T8 = velum (check) at 9.8 ml/15L of water as drenching two weeks after germination; T9 = control (no application).

Other investigators have indicated the usefulness of neem applied as a biocontrol formulation against plant-parasitic nematodes [28, 29]. In the current study, neem applied at a concentration of 50% as seed coating for 30 min prior to sowing (T3) had a high efficacy of percent nematode reduction. Neem releases phenols, amino acids, aldehydes, and fatty acids that are hostile to root-knot nematodes [30]. Metabolites produced by neem's chemical ingredients

(azadirachtin, salannin, limonoids, triterpenoids, phenolic compounds, carotenoids, steroids, and ketones) drive plant cells to produce aberrant metabolites that deter nematodes from infecting plant roots [31]. Studies have shown bioactive extracts including saponins, flavonoids, sterols, and alkaloids are poisonous to pathogenic nematodes and inhibit their activities [27]. In addition, citrus oil contains limonene viz. limonene tetrabromide, carvone, and monoterpenes such as a-terpineol, linalyl acetate, linalool, and limonene [32, 33]. These oils pose the potential to limit fungal growth [31] and nematicidal activity against *Meloidogyne incognita* [33]. In a previous study, maximum root-knot nematode egg hatch inhibition and juvenile mortality were by carvone at 1500 μg ml^{-1} after 96 hours of treatment application [33]. In our study, field evaluations showed citrus at 100% concentrations as seed coating (T5) with the highest nematode reductions (90.1%) and least reproductive factors (RFs) of 0.05 at the atomic farms. The potential of this oil in limiting nematode production can be attributed to limonene and its derivatives.

Nematodes, in particular *Meloidogyne incognita*, which has the ability to alter the normal physiology of plants and cause poor nutrient uptake, lower growth, and consequently low quality and quantity of the yield, are implicated in the improved performance of the treated plants. Sesame oil in the current study had the least efficacy in reducing nematode reproduction in soil and compared to another study which involved plant seed oils such as cotton, flax canola, olive, soybean, and sesame applied against root-knot nematode in tomato; sesame oil was inefficient in reducing nematode populations in tomato [6].

5. Conclusion

Okra seeds treated with 100% citrus oil and 50% neem oil were efficacious in reducing nematode reproduction in soil and also enhanced seed germination and seedling vigor, with an improvement in growth and yield. Sesame oil was the least effective in enhancing seed germination and vigor and is therefore not recommended as a preplant protectant.

Data Availability

The datasets used and/or analyzed in this study have been provided as tables or figures and incorporated into the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Special thanks go to Mr. Emmanual Armah for assisting with nematode isolation and quantification from the various farms. The authors thank the World Bank for providing a bursary to support the research for PB.

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