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Biofumigation Efficacy of Spider Plant (*Cleome gynandra* L.) Accessions on Nematode Control in Tuberose (*Polianthes tuberosa* L.)

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ARTICLE INFO

Article history

Received: 7 September 2020

Accepted: 25 September 2020

Published Online: 30 September 2020

Keywords:

Nematode management

Biofumigation

Flower growth

Flower quality

ABSTRACT

Tuberose (*Polianthes tuberosa* L.) is a perennial summer flower grown by smallholders in Kenya for export. However, its production and export volumes have declined drastically due to nematodes infestation. This study evaluated the effect of *Cleome gynandra* accessions on nematode management on tuberose. Experiment was carried out at Egerton University, Kenya using a randomized complete block design with four replications. The treatments were *C. gynandra* namely “Simlaw”, “Egerton”, “Taastrup”, “PS” and “IP8”, applied at 6 kg/m² and compared with *Brassica napus*, solarization and untreated control. Data was collected on growth and yield parameters, nematode infestation and quality of tuberose. Data collected was subjected to analysis of variance at $p \leq 0.05$ and means separated using Tukey’s test. Biofumigation with *Cleome gynandra* accessions helped to reduce nematode population by 34%, gall numbers by 83% and galling index by 96% when compared with the control. Use of biofumigation helped to improve plant height and leaf number of tuberose by 16% and 87%, respectively, when compared with the control. Use of biofumigation helped to improve spike length by 32%, marketable spikes by 80%, and flower yield by 90% and reduced nonmarketable spikes by 95% when compared with the control. Based on the above results, use of *Cleome gynandra* accessions and other biofumigants such as rape seed can be used to manage nematodes and improve growth, yield and quality of tuberose.

1. Introduction

Floriculture is one of the fastest growing sub-sectors of agriculture in Kenya. The sub-sector is one of the Kenya’s main foreign exchange earners^[13]. However, the quantity of cut-flower production has been on the decline since 2012^[17]. This has been mainly attributed to pest attacks and high costs involved due to compliance to standards such as Global Good Agricultural Practices

(Gobal GAP), hence being a challenge to smallholder farmers. Use of pesticides to control pests impact heavily on flower industry since they affect global market access due to interceptions during exportations caused by non-compliance to phyto-sanitary requirements such as pesticide residues^[13].

Tuberose (*Polianthes tuberosa* L.) is a cut-flower which originated from Mexico, belonging to the family Amaryllis and Genus *Polianthes*^[31]. It is grown in the

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tropical and sub-tropical areas as a summer flower^[14]. In Kenya, summer flowers comprise of 2.9% of the total production of cut-flowers and are mainly done by smallholder's growers with tuberose contributing to 0.09% of the 2.9%^[13].

In Kenya, Kiambu County has the highest hectares under tuberose (MOALD, 2002)^[24] which is grown primarily for the export market. The main export destination is the Netherlands with negligible amounts to other countries^[13]. Tuberose is graded according to stem length, namely: Grade I (≥ 70 cm), Grade II (69-60 cm), Grade III (59-50 cm) and Grade IV (49-40 cm)^[3]. The flowers that do not meet the export standard are sold to the local market at lower prices. Tuberose export volumes and values have been declining since 1997 (HCD, 2017)^[13]. This has been mainly due to pest attack including nematodes^[9].

Nematode (*Meloidogyne spp*) belong to the order Tylenchida and family Heteroderidae. They are parasitic on a wide range of flowers causing up to 5% yield losses^[8,26]. The most common nematodes in tuberose is root-knot nematode (RKN)^[9]. The RKN form disease complex with plant pathogenic bacteria and fungi, thus their management is important^[9]. Although nematicides are available for the control of nematodes in tuberose, most of the synthetic nematicides are too expensive for small-scale farmers who account for the majority of tuberose growers to afford, besides they are unfriendly to the environment^[26]. For instance, most nematicides belong to toxicity class 1 or 2 which are either prohibited or restricted for use in horticultural crops in Kenya. However, concerns about the environment and the general public health has led to re-evaluation of these products^[2,12,32]. Environmentally sound alternative methods including organic amendments such as biofumigation have been suggested for use in the management of nematodes^[1,11,26,33,34].

Biofumigation is the suppression of soil-borne pests and pathogens by biocidal compounds released when plant materials especially brassicaceous residues are crushed and incorporated into the soil^[1,21]. The use of biofumigants is a safer alternative method of soil-borne pest management which can be applied on plants containing significant quantities of thioglucoside compounds known as glucosinolates (GSLs). Brassicaceae plant suppress nematodes through volatile toxic compound, such as isothiocyanates generated from glucosinolates which are also known to possess broad activity against weeds, bacteria and fungi^[22]. Synthetic commercial nematicides, such as dazomet and metham sodium also contain isothiocyanates especially methyl isothiocyanates (MITIC) which is the active ingredient to manage nematodes^[10,23].

Spider plant (*Cleome gynandra L. syn. Gynandropsis*

L.) belongs to the family Cleomoideae, formerly Capparidaceae, which is a phylogenetic relative of Brassicas family^[7]. According to^[28], homogenized leaves of spider plant emit significant quantities of biologically active isothiocyanates including methyl isothiocyanate and their levels of methyl isothiocyanate vary among *C. gynandra* accessions. However, its use in nematode management has not been extensively studied. This study therefore evaluated the efficacy of biofumigation with different *Cleome gynandra* accessions on nematode infestation, growth, yield and quality of tuberose.

2. Materials and Methods

2.1 Experimental Site

The study was done at the experimental field at Egerton University, Kenya. The field is within the latitude of 0.23° South and longitudes 35.35° East in the Lower Highland III Agro Ecological (LH3) at 2,238 meters above sea level. Average maximum and minimum temperature ranges between 19 °C to 22 °C and 5 °C to 8 °C respectively, with a mean annual rainfall of 1,000 mm. The soil are predominately sandy-vitric mollic andosols^[15].

2.2 Planting Materials

Tuberose bulbs was bought from PJ Dave Flora Limited and thoroughly cleaned and treated with Bavistin (0.2%) for 30 minutes and dipped in thiourea solution to break dormancy. *Brassica napus* (rape seed) and *Cleome gynandra* accession "Simlaw" seeds was obtained from Kenya Seed Company, Nakuru. Seeds of other *C. gynandra* accessions were obtained from previous collections: "Egerton", from Egerton University; "Taastrup" from Denmark; "PS" and "IP8" from AVRDC, Arusha, Tanzania.

Brassica napus and *C. gynandra* seeds was planted and all agronomic and maintenance practices was done following the technical recommendation for the respective crops. The leafy twigs at flowering were harvested. The two are known to contain glucosinolates which has nematicidal properties^[18]. At flowering stage, the *C. gynandra* plants were uprooted, chopped into small pieces of equal or less than three centimeters (≤ 3 cm) and applied immediately to the experimental plots.

2.3 Nematode Collection, Augmentation and Inoculation

Nematodes were extracted from infested tomato plants and augmented in two weeks old tomato seedlings established outdoor^[30]. Specifically, galls were extracted from

the roots of infested tomato seedlings, chopped and mixed with native soil. To augment the nematode inoculum, the mixture was added to the soil with 2 weeks old tomato seedlings. The nematode inoculum was allowed to infest, develop and multiply on the tomato plants for 8 weeks. After augmentation, nematode egg masses were extracted from the galled tomato roots to prepare inoculum. Galled root tissues were chopped to a length of 0.5 cm and macerated to release the egg masses. This was placed in 15 cm diameter sieves of 1 mm pore size, lined with cross-layered tissues paper and placed for hatching in a glass petri-dishes containing distilled water and incubated at 27 °C. After hatching, it was transferred into a 2 L conical flask. Quantification of the juveniles was done under light microscope with gridded petri dishes. Ten 1 ml replicate samples were drawn from the well mixed suspension to establish the average number of juveniles per ml. Nematodes suspension sample was adjusted to contain approximately 596 juveniles in 1000 ml of distilled water.

2.4 Experimental Design and Treatment Application

The experiment was laid in a randomized complete block design with 4 replications. There were 9 treatments consisting of chopped *C. gynandra* accessions: “Simlaw”, “Egerton”, “Taastrup”, “PS” and “IP8”; *Brassica napus*; fumigant, solarization and; untreated control. Plots of 1.2 m × 1.2 m was dug to a depth of 20 cm, lined with polyethylene sheet and filled with the top soil. The second instar juvenile stage of *Meloidogyne spp.* inoculum suspension was added to each experimental unit ensuring uniform distribution. The chopped plant materials (*C. gynandra*) were incorporated into the soil up to 0.3 m depth and plots covered with 0.14 mm thick clear polyethylene sheet. At the same time, plots treated with Basamid® were also re-dug and fumigated at the rate of 0.029 kg m⁻² and covered. The edges of the polyethylene sheet were buried 0.15 m into the soil to ensure air tight conditions for four weeks. The untreated plots (negative control) were re-dug and left without incorporating *Cleome gynandra* or Basamid® application. After four weeks; the treated plots were uncovered and left to aerate for 14 days.

2.5 Tuberose Establishment

A plot measuring 12.4 m × 6.3 m was marked, cleared, ploughed, harrowed and demarcated into 36 plots each measuring 1.2 m × 1.2 m. Spacing of the tuberose was carried out at 20 cm by 20 cm. There was a spacing of 0.5 m between blocks and 0.2 m between plots. Each plot had 5 rows each having 5 plants each. Diammonium phos-

phate (DAP; 18% N, 46% P₂O₅) fertilizer was applied at the rate of 240 kg ha⁻¹ (approximately 10 g per hole) (HCD, 2017)^[13] and a thorough mixed with soil prior to planting the bulbs. Soil was sampled before application of the nematode suspension and incorporation of amendments to check moisture content and plant parasitic nematode population.

2.6 Data Collection

Data was collected and recorded on the following parameters:

(a) Nematode population

To determine the nematode population in the soil, soil was extracted from 100 cm³ soil from each experimental unit, using the method described by ^[17]. At 80 days after planting, the soil was sampled by taking 100 cm³ of sample. The sample was placed in a plastic mesh with pore diameter of 1 mm lined with double layered tissue paper. The sieves were half immersed in plastic beakers containing 250 ml of distilled water to allow nematode migration into the water underneath for 24 hours. Nematode counts were determined in 3 replicate sample of 1 ml for each.

(b) Gall numbers

For gall assessment, 6 plants were gently uprooted, at the end of the experiment and their roots washed under tap water to remove excess adhering soil. The plant material containing nematode was chopped to small pieces (≤ 1 cm length), placed in a folded muslin cloth to enclose the material and gently submerged into the water in the funnel. Nematode emerged from the tissues and sunk to the bottom of the funnel stem. After 24 to 48 hours, the clamp was fully opened and 5 to 10 ml of water containing nematodes was rapidly withdrawn and transferred in to a shallow viewing dish for examination.

(c) Galling index

Galling was determined by counting the number of galls of 1 mm diameter and above. The index score was in a scale 1 to 10. Where 0:no gall, 1:10-50 galls, 2:51-100 galls, 3:101-150 galls, 4:151-200 galls, 5:201-250 galls, 6:251-300 galls, 7:301-350 galls, 8:351-400 galls, 9:401-450 galls, 10:451 and above ^[17]. The scores were converted into numerical entries and their mean determined.

(d) Number of leaves

Leaf count data was collected from the 6 plants in each plot. Leaf count data collection commenced 21 days after planting and continued at an interval of 14 days up to 100 days after planting. At each instance of the data collection the mean number per leaves per plant from each replicate was computed. The mean number of leaves per plant was determined by computing the means of the replicate mean.

(e) Plant height

Plant height data was collected from 6 plants in each plot. This started at 21 days after planting and continued for 14 days interval up to 100 days. The plant height was measured using a tape measure. At each instance of data collection, the mean height per plant from each replicate was computed.

(f) Yield and quality variables

Weekly harvesting at bud-burst stage from the 6 tagged plants per treatment was done. At each harvest, stems were sorted into marketable and non-marketable categories. Tuberose is graded according to stem length, namely: Grade I (≥ 70 cm), Grade II (69 - 60 cm), Grade III (59 - 50 cm) and Grade IV (49 - 40 cm)^[3]. The height was measured and stem length of 50 cm and above was considered marketable stems. Spike length and flower yield was also determined.

2.7 Data Analysis

Data was analysed using SAS statistical package (version 9.1). The proc univariate procedure was used to check for normality of the data before analysis, and data transformation was done. All numerical data was subjected to analysis of variance (ANOVA) at $P \leq 0.05$ and significant means were compared using Tukey's test at $P \leq 0.05$.

3. Results

3.1 Efficacy of Biofumigation with Different *Cleome gynandra* Accessions on Nematode Infestation during Production of Tuberose

Cleome gynandra accessions had significant effect on nematode population. Untreated (control) soil had the highest nematode population followed by solarization when compared with the rest of the treatments which were not significantly different from each other (Figure 1).

Similar to nematode population, gall number was significantly affected by the treatments. Gall numbers was the highest in the untreated tuberose roots, followed by the solarization, when compared with the rest of the treatments that were not significantly different (Figure 2).

Galling index was significantly affected by the treatments. Galling index was the highest in the untreated tuberose roots followed by solarization when compared with the rest of the treatments that were not significantly different (Figure 3). Untreated control was significantly different from solarization for the galling index, nematode population and gall numbers.

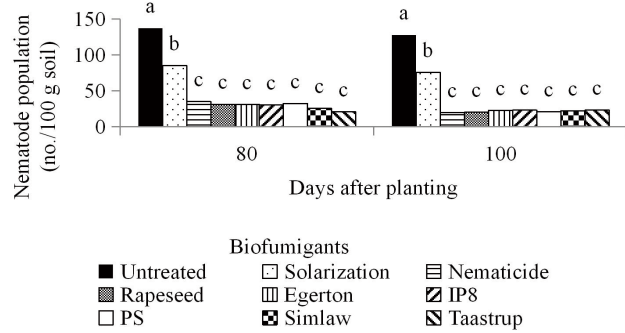


Figure 1. Effect of biofumigation with different *Cleome gynandra* accessions on nematode population during tuberose production. Means followed by the same letter within an evaluation period is not significantly different according to Tukey's test at 5% level of significance

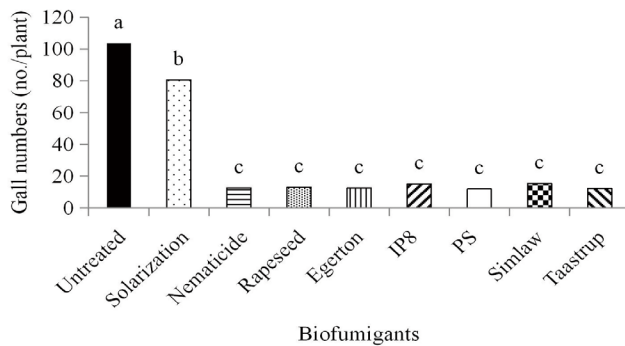


Figure 2. Effect of biofumigation with different *Cleome gynandra* accessions on gall numbers on tuberose roots during production. Means followed by the same letter is not significantly different according to Tukey's test at 5% level of significance

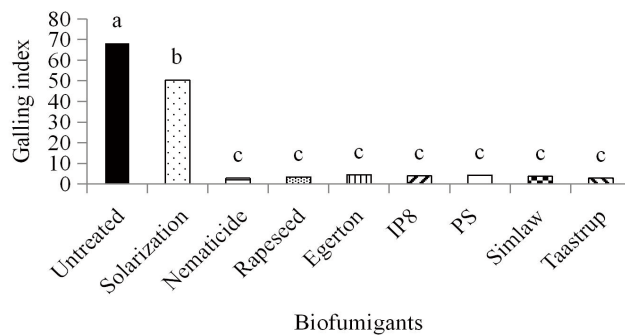


Figure 3. Effect of biofumigation with different *Cleome gynandra* accessions on galling index during tuberose production. Means followed by the same letter is not significantly different according to Tukey's test at 5% level of significance

3.2 Effect of Biofumigation with Different *Cleome gynandra* Accessions on Growth of Tuberose

Use of *Cleome gynandra* accessions had significant influence on the height of tuberose plants. Taastrup and

nematicide had the tallest tuberose plants throughout the study when compared with the untreated, solarisation and rapeseed. In most of the evaluation days all the *Cleome gynandra* accessions had similar effect on plant height compared with use of nematicide and solarization (Table 1).

Biofumigation with different *Cleome gynandra* accessions had effect on leaf number of tuberose plants. Taastrup and nematicide had the highest number of leaves when compared with the untreated which had the lowest number of leaves, followed by rapeseed and then solarization (Table 2). *Cleome gynandra* accessions were not different with the highest treatments. However, in the remaining evaluation period, untreated tuberose had the lowest number of leaves compared with the rest of the treatments which were not significantly different from each other.

Table 1. Effect of biofumigation with different *Cleome gynandra* accessions on plant height (cm) of tuberose during production

Treatments	49 days	63 days	77 days	91 days
Untreated	30.1 ^d	35.9 ^d	40.2 ^d	53.5 ^c
Solarization	34.8 ^c	38.8 ^c	42.1 ^c	62.3 ^b
Nematicide	41.2 ^a	50.5 ^a	63.4 ^a	84.5 ^a
Rapeseed	39.2 ^b	42.1 ^b	50.5 ^b	71.7 ^{ab}
Egerton	38.7 ^{ab}	48.2 ^{ab}	60.9 ^{ab}	84.5 ^a
IP8	37.0 ^{ab}	46.9 ^{ab}	60.0 ^{ab}	82.3 ^a
PS	36.6 ^{ab}	45.2 ^{ab}	58.6 ^{ab}	81.1 ^a
Simlaw	36.4 ^{ab}	46.4 ^{ab}	59.6 ^{ab}	79.7 ^a
Taastrup	41.3 ^a	51.2 ^a	65.5 ^a	86.0 ^a

Means followed by the same letter within a column is not significantly different according to Tukey’s test at 5% level of significance

Table 2. Effect of biofumigation with different *Cleome gynandra* accessions on leaf number (no./plant) of tuberose during production

Treatments	49 days	63 days	77 days	91 days
Untreated	12.7 ^d	14.8 ^c	18.0 ^c	19.8 ^c
Solarization	20.2 ^c	23.5 ^b	25.0 ^b	28.1 ^b
Nematicide	30.1 ^a	30.8 ^a	34.0 ^a	36.8 ^a
Rapeseed	22.9 ^b	24.2 ^b	26.1 ^b	30.1 ^b
Egerton	27.6 ^{ab}	30.9 ^a	34.5 ^a	39.7 ^a
IP8	27.4 ^{ab}	31.1 ^a	33.3 ^a	36.5 ^a
PS	26.6 ^{ab}	31.6 ^a	34.2 ^a	37.5 ^a
Simlaw	24.1 ^{ab}	27.1 ^a	29.7 ^a	32.2 ^a
Taastrup	32.1 ^a	34.4 ^a	38.3 ^a	41.2 ^a

Means followed by the same letter within a column is not significantly different according to Tukey’s test at 5% level of significance

3.3 Effect of Biofumigation with Different *Cleome gynandra* Accessions on Yield and Quality of Tuberose

Spike length was significantly affected by the treatments. All the biofumigants used produced the longest spike (best quality) when compared with the untreated (control) but was not different with the solarization (Figure 4).

Similar to the trend on spike length, *Cleome gynandra* accessions had significant effect on marketable spikes (>50 cm). At the treatments had the highest number of marketable spikes compared with the untreated and solarization (Figure 5).

Cleome gynandra accessions had significant effect on nonmarketable spikes (<50 cm). Untreated tuberose had the highest number of nonmarketable spikes followed by solarisation when compared with the rest of the treatments (Figure 6).

Cleome gynandra accessions had significant effect on yield of tuberose flowers. *Cleome gynandra* accessions namely. All the treatments had the highest tuberose yield when compared with the untreated which had the lowest followed by solarization (Figure 7).

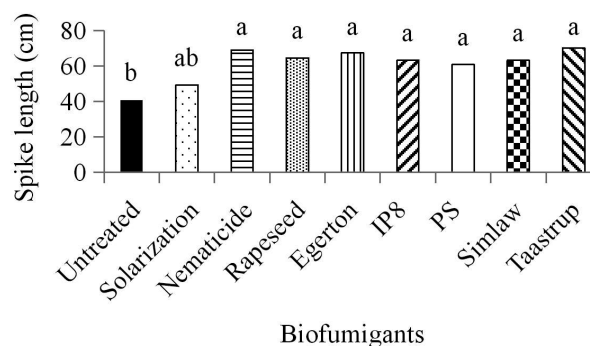


Figure 4. Effect of biofumigation with different *Cleome gynandra* accessions on spike length of tuberose production. Means followed by the same letter is not significantly different according to Tukey’s test at 5% level of significance

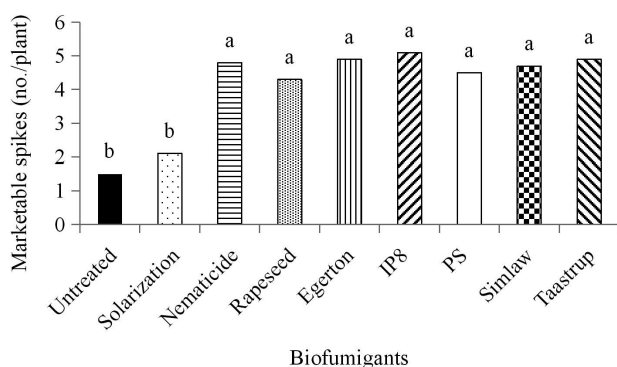


Figure 5. Effect of biofumigation with different *Cleome gynandra* accessions on marketable spikes of tuberose during production. Means followed by the same letter is not significantly different according to Tukey’s test at 5% level of significance

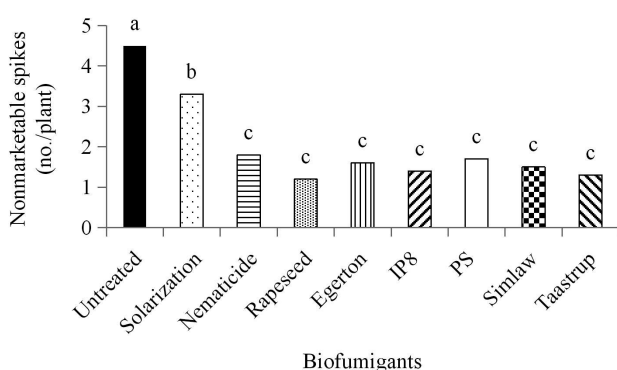


Figure 6. Effect of biofumigation with different *Cleome gynandra* accessions on nonmarketable spikes of tuberose during production. Means followed by the same letter is not significantly different according to Tukey’s test at 5% level of significance

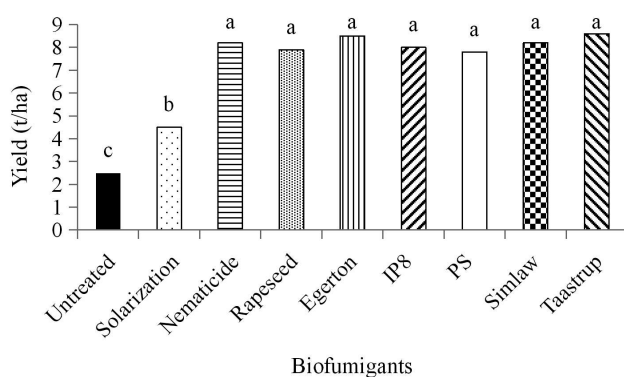


Figure 7. Effect of biofumigation with different *Cleome gynandra* accessions on flower yield of tuberose during production. Means followed by the same letter is not significantly different according to Tukey’s test at 5% level of significance

4. Discussion

Biofumigation helped to reduce nematode population by 34%, gall numbers by 83% and galling index by 96% when compared with the control, with the best effect observed when *Cleome gynandra* accessions, mainly Taastrup was used. This was comparable with the use of nematicide. The results of the study is corroborated by the findings of Argento *et al.* [4] Who observed that application of different concentrations of *Brassica macrocarpa* helped to reduce nematode population and galling index in root-knot nematodes (*Meloidogyne* spp.) on greenhouse tomato plants. Use of biofumigants has been observed to result in enhanced soil properties and nematicidal activities [4]. Biofumigants incorporated into the soil decompose thereby releasing various mineral elements and also improve soil organic matter content. This helps in enhancing soil biological, physical and chemical properties. This therefore helps in better crop development as observed in the study. Similarly, biofumigants release volatile compounds with nematicidal properties such as isothiocyanates. The promotion of plant yield parameters may be as a result of increased availability of nutrients in the root zone and inducing root growth that led to nutrient uptake. Biofumigation was also observed to reduce pest population, disease incidences, and increased crop yield by 30% compared with untreated controls [30]. The enhanced yield may also due to the suppression of nematodes in the soil by the volatile compounds released during decomposition of the studied biofumigants

In another study, Lwande *et al.* [20] were unable to test the efficacy of methyl isothiocyanate, one of the biochemical compounds found in *C. gynandra* on ticks because of the practical difficulties caused by its toxicity, but suggested that essential oil of *C. gynandra* could be contributing to the tick repellency effect. Lwande *et al.* [20] reported that terpenes and their derivatives as the main constituents of the essential oil of *C. gynandra* extracted by hydro distillation, and two of them, β -cyclocitral and β -ionone. *Cleome gynandra* has been observed also to have traces of methyl isothiocyanate that possess miticidal properties as a result of the volatile emissions from entire plants and their detached leaves [27]. It has also been found that hydrolysates of *Cleome spinosa*, a related species to *C. gynandra* contain methyl isothiocyanate and have similar active ingredient of pesticides such as metam sodium, metam potassium and dazomet which are effective biofumigants against fungi and nematodes [23,29].

Barros *et al.* [6] also observed that sulfur-containing compounds, mostly isothiocyanates, were found in mustard, a close relative of rape seed used in the present

study. Barros *et al.* [6] further demonstrated that irrigation applied directly after biofumigation may trap the volatile organic compounds in soil water thus keeping nematode toxicity longer. *Brassica juncea* eco-types namely Nemfix, Fumus, and ISCI99 were observed to contain high contents of 2-propenyl glucosinolate and resulted in more than 95% mortality of encysted eggs of *G. pallida* in soils covered with polyethylene [19]. Aydınlı & Mennan [5] observed that galling index and nematode eggs were on tomatoes biofumigated with *E. sativa* and *R. sativus*. Therefore, it is possible that the effect of the studied biofumigants on nematode population and galling index could be associated with the repellence and toxicity effects of the biochemicals that they produce.

Use of biofumigation helped to improve growth of tuberose, such as plant height and leaf number of tuberose by 16% and 87%, respectively, when compared with the control. The results were comparable with the use of nematicide. Similar results were observed by Argento *et al.* [4] when *Brassica macrocarpa* was used as a biofumigant against root-knot nematodes (*Meloidogyne* spp.) on greenhouse tomato plants. According to Argento *et al.* [4] most crop residues incorporated into the soil tend to release nutrients to crops, that can also improve the physical, chemical and biological properties of the soil. This results in better physiological development of tuberose plants. In addition, biofumigant also release volatile compounds against various insect pests, including nematodes [1]. Argento *et al.* [4] observed that biofumigation with *Brassica macrocarpa* in the open field was better than in the greenhouse since it allows for natural decomposition process and better chemical changes to reactive volatile molecules which are released in a more controlled manner, resulting in a longer residual nematicidal activity. Improved growth of tuberose plants may be attributed to improved soil physico-chemical and nematicidal properties of the biofumigants.

Use of biofumigation helped to improve spike length by 32%, marketable spikes by 80%, and flower yield by 90% and reduced nonmarketable spikes by 95% when compared with the control. The results were similar to the use of nematicide. Argento *et al.* [4] observed increase in marketable yield and dry matter on greenhouse tomatoes when *Brassica macrocarpa* was used as a biofumigant against root-knot nematodes (*Meloidogyne* spp.). Hassan *et al.* [12] also observed that biofumigation with radish crushed leaves or seed meal induced high inhibition activity against and *Meloidogyne* spp resulting in higher eggplant yield. Use of biofumigants have been observed to result in enhanced soil properties and nematicidal activities [4]. Biofumigants incorporated into the soil

decompose releasing various mineral elements and also improve soil organic matter content. This helps in enhancing soil biological, physical and chemical properties which result in better leaf development as observed in the study. Similarly, biofumigants release volatile compounds with nematicidal properties. The enhanced yield may also be due to the suppression of nematodes in the soil by the volatile compounds released during decomposition of the studied biofumigants.

5. Conclusions and Recommendations

Biofumigation helped to reduce nematode population by 34% and galling index by 96% when compared with the control, with the best effect observed when *Cleome gynandra* accessions. Use of biofumigation helped to improve growth of tuberose, such as plant height and leaf number of tuberose by 16% and 87%, respectively, when compared with the control. Use of biofumigation helped to improve spike length by 32%, marketable spikes by 80%, and flower yield by 90% and reduced nonmarketable spikes by 95% when compared with the control. This was comparable with the use of nematicide. Based on the above results, use of *Cleome gynandra* accessions and other biofumigants such as rape seed can be used to manage nematodes and improve growth, yield and quality of tuberose. However, further studies on the optimization of the rates of the studied biofumigants against nematode management and improving growth and yield of tuberose is necessary.

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