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Inhibition of *Fusarium oxysporum* wilt of scarlet eggplant (*Solanum aethiopicum*) using plant extracts

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Abstract

Solanum aethiopicum cultivation is highly constrained by *Fusarium* wilt. A trial was conducted to control soil-borne and seed-borne *F. oxysporum* f.sp. *melongenae* using botanicals (ginger and garlic extracts). Seeds of Bello variety of eggplant were used for the trial. The trial was set up using completely randomized design with nine treatments and each treatment replicated thrice. Data were collected on percentage germination, shoot height, number of leaves, plant vigour, shoot weight, disease incidence and disease severity. The results shows that treatment T9 has the lowest percentage germination (18.3%) at 7 DAS which is lower than T1 (44.2%) or mean (48.1%). T7 permits 72.5% germination which is at par with T6 (83.3%) at 15 DAS. Based on germination, T8, T5, T6 and T7 tops the chart (≥ 72.3% germination) compared to T9 (50.8%) at 15 DAS. The incidence of wilt is highest (33.4%) in T9 compared to all treated plots. T4, and T2 are the best treatments (0.0% incidence) at 45 DAS. Percentage severity of wilt follows the same trend as incidence. Based on shoot weight, T8 (8.3 g) and T7 (7.0 g) are the best treatments. The highest vigour index (45 DAS) is from T3 (823) followed by T2 (769), T5 (692), T7 (658) then T1 (604) in descending order of vigour index and mean vigour index is 582. It is concluded that the infection of eggplant by *Fusarium* sp. affects germination of seeds and subsequent growth in the absence of control measures. However, control of *Fusarium* sp. is possible with the use of botanicals.

Keywords: African garden egg; garlic; ginger; management; organic farming; seed treatments; seed dressing

Abbreviations: T1 = Positive control (uninfected seeds); T2 = Ginger and Garlic extracts applied to seeds and to soil; T3 = Ginger extract applied to seeds; T4 = Garlic extract applied to seeds; T5 = Garlic extract applied to soil; T6 = Ginger extract applied to soil; T7 = Garlic extract applied to seeds and to soil; T8 = Ginger extract applied to seeds and to soil; T9= Negative control (infected seeds and no plant extract)

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Introduction

The sale of *Solanum aethiopicum* L. (in subgenus *Leptostemonum,* genus *Solanum*) brings in a lot of income to farmers and traders. The fruits of *S. aethiopicum* (scarlet eggplant) are usually consumed raw, boiled, or roasted as a snack or sauce at home, social and religious gatherings. It is easy to cultivate African garden egg although it suffers from many abiotic and biotic stresses including soil-borne diseases like wilts induced by *Fusarium oxysporum* Schelecht ex Fries (Syn. and Hans) f. sp. *melongenae* Mauto and Ishigami (Chinedu *et al*., 2011; Ndifon, 2019).

The need to reduce primary pathogen inoculants and ensure vigorous seedlings during the early phase of cropping is felt most by organic farmers and vegetable gardeners. However, the use of organic seed treatment agents is still in its infancy. Besides seeds constitute very effective means of transporting plant pathogens over long distances. It has often been lamented that getting enough certified seeds proves impossible especially for vegetables in the tropics. Thus, scarlet eggplant seed requirement is often met through local uncertified seed supply and by importing some seeds from other climes (Habib *et al*., 2007).

In vegetable gardening, the over-use of agrochemicals results in negative health and environmental impacts. The vegetables produced with excess pesticides and agrochemicals constitute environmental and health hazards to mankind and animals. Generally, seed treatment is more economical and effective when it is carried out with respect to the nature of pathogen and the level of infection. The degree of control using seed treatment agents may depend on the active ingredients of the fungicide, its rate, the seed-borne, and soil-borne pathogens present, environmental conditions and application of coverage materials on the soil (Syngenta Canada Ltd., 2012). Habib *et al.* (2007) carried out component analysis of African garden egg seeds and found that most fungi associated with the seeds were located in the seed coat and tegmin but not in the embryo. *F. oxysporum* and *Fusarium solani* were found in the embryo of *S. aethiopicum* seeds.

Studies on the application of garlic and ginger extracts against microflora are limited even though they have antimicrobial properties (Haciseferogullari *et al*., 2005; Perelló *et al*., 2013). Perelló *et al.* (2013) reported that allicin in garlic juice corrected the poor germination of wheat seeds caused by natural mycoflora of grain leading to improved vigour. Treating seed ensures that the crop gets off to a good start, although this treatment will not cure a poor seed lot that has high proportions of dead, damaged, or infected seed.

Kebede *et al.* (2013) reported that ginger extract completely controlled (100%) *Xanthomonas campestris* pv. *vesicatoria* in tomato seeds (soaked in the bacterium inoculum for 24 hours). This control enabled 81-97% germination of tomato seeds *in vitro*.

Olaniran *et al.* (2 013) reported that the combination of garlic and ginger increased the total phenolic and lycopene contents of tomato paste. Their study showed that the use of garlic and ginger either separately or in combination increased fats, lycopene and total antioxidant potential of tomato paste during storage.

These plant materials are readily available in the tropics. Thus this trial was set up with the objective of assessing the efficacy of these plant extracts against *Fusarium oxysporium* f.sp *melongenae* wilt on eggplant in the screen-house. Hopefully, the findings of this research will help in accepting the premises that ginger and/or garlic extracts boost percentage germination, seedling vigour, number of leaves, shoot weight and shoot height but reduce wilt incidence and disease severity.

Materials and Methods

Preparation of plant extracts

The ginger rhizomes (*Zingiber officinale*) and garlic (*Allium sativum* L.) cloves were bought from the market in Makurdi. The ginger rhizome and garlic clove extracts were prepared by macerating 250 g of each plant material in a mortar. The macerated plant material was soaked in sterile distilled water (300 ml) and strained after four hours through double-layer muslin clothe. This crude extract constituted the 100% concentration. The seeds that needed treatment were soaked in 5 ml of the extract for one hour before draining away the extract and for soil that required plant extract, another 5 ml of the plant extract (from the conical flask) was sprayed on the soil for treatments that required it.

Sterilization of soil

Soil to be sterilized was obtained from the top-most 30 cm of the soil (in order to remove organic matter that may have pesticide effects, top-most 5 cm of soil was discarded first before digging). The soil was mixed with sand collected at the Teaching and Research Farm of the Federal University of Agriculture, Makurdi. Loamy soil and sand were thoroughly mixed at the ratio of 3:1 soil to sand. This mixture was sieved with a coarse metal sieve (1 cm mesh).

The soil-sand mixture was steam sterilized with heat from fuelwood for one hour using a big metal container (Netscher and Sikora, 1990). The soil was turned and stirred every 20 minutes to ensure uniform sterilization. Each plastic pot (25 cm diameter, 25 cm high) was filled with steam-sterilized soil.

Preparation of Fusarium inoculum and infection of seeds

The *Fusarium* isolate utilized in this experiment were obtained from infected plants grown in farmers' fields in Zaria. The fungus was isolated and cultured in the laboratory using acetate differential agar (Difco dehydrated medium) enriched with dextrose. The medium was autoclaved at 121 °C, at 15 psi, for 15 minutes as recommended by the manufacturer. The pure culture of the wilt pathogen was identified using literature and microscopy.

The conidia inoculum concentration of *Fusarium oxysporum* f.sp. *melongenae* was calculated based on haemocytometry counts. The seeds were surface sterilized with sodium hypochlorite (1%). Then, they were artificially inoculated by spraying them with a 1 ml suspension of *Fusarium* species (1.0×10^4 spores). These seeds were air-dried for a week in a cool dry aseptic environment. Negative control was composed from uninfected seeds obtained from the same seed and it was prepared by surface sterilization with sodium hypochlorite (1%) for 15 minutes at sowing time.

Experimental design

To layout the trial, each plastic pot was filled with steam-sterilized soil. Twenty infected seeds were sown in each pot. The treatments were applied using plant extracts from ginger and garlic. This pot experiment was setup using a completely randomized design (CRD) layout with 9 treatments and each treatment was replicated three times. Only the main effects were of interest in this trial.

The treatment set utilized included:

T1 = Positive control (uninfected seeds);
T2 = Ginger and garlic extracts applied to seeds and to soil;
T3 = Ginger extract applied to seeds;
T4 = Garlic extract applied to seeds;
T5 = Garlic extract applied to soil;
T6 = Ginger extract applied to soil;
T7 = Garlic extract applied to seeds and to soil;
T8 = Ginger extract applied to seeds and to soil;
T9= Negative control (infected seeds and no plant extract)

Data collection

Collected data included percentage germination, seedling vigour, wilt incidence, disease severity, number of leaves, shoot weight and shoot height.

Disease incidence: This was calculated using equation 1 (Momma, 2008 (as modified)).

Equation 1: $D.I = \{(\sum I_p)/\sum A_p\} \times 100\%$

Where:

D.I = disease incidence

Ip = number of infected plants (wilted, stunted, drooped leaves, epinastic leaves, chlorosis, dead plants)

Ap = number of assessed plants

Disease severity: Disease severity was obtained using equation 2 (Momma, 2008 (as modified)).

Equation 2: $\mathrm{D.S} = \{(\sum \mathrm{I_s})/3 \times \sum \mathrm{P_a}\} \times 100\%$

Where:

D.S = disease severity

3 = highest severity score (Three (3) was highest score)

P_a = number of plants assessed

I_s =Individual disease severity scores (obtained using the modified individual disease severity scale) (Momma, 2008 (as modified))

Modified individual disease severity scores (as in equation 2 (Momma, 2008 (as modified))):

0 = No wilting at all, healthy plants

1 = Less than 1/3 of leaves wilted/dropped off

2 = More than 1/3 of leaves wilted/dropped off

3 = More than 2/3 of leaves wilted/dropped off or dead plants

Plant vigour index was calculated using equation 3 (Kebede *et al.*, 2013 (as modified))

Equation 3 V.I. = L x G

Where:

V.I = vigour index

L= mean shoot length (cm)

G = percentage germination of seeds (%)

Data analysis

The collected data were presented using descriptive statistics. The data collected as percentages were arcsine transformed. Then the transformed data were subjected to analysis of variance (ANOVA) and the means were separated using FLSD ($P \leq 0.05$).

Results and Discussion

The results of different plant extracts' potential on the germination of African garden egg infected with *F. oxysporum* are presented in Figure 1. It is observed that negative control has the lowest percentage germination (18.3%) at 7 DAS which is significantly (based on ANOVA and SNK test $P \leq 0.05$) lower than the positive control (44.2%) and below mean percentage germination (48.1%) at 7 DAS. When garlic extract is applied to seeds and soil the percentage germination (33.3%) is lower than that of positive control (44.2% germination) at 7 DAS ($P \leq 0.05$). However, the same garlic extract applied to seeds and soil enables above average germination (72.5%) which is at par with one of the best treatments (i.e., Ginger extract applied to soil (83.3%)) at 15 DAS($P \leq 0.05$).

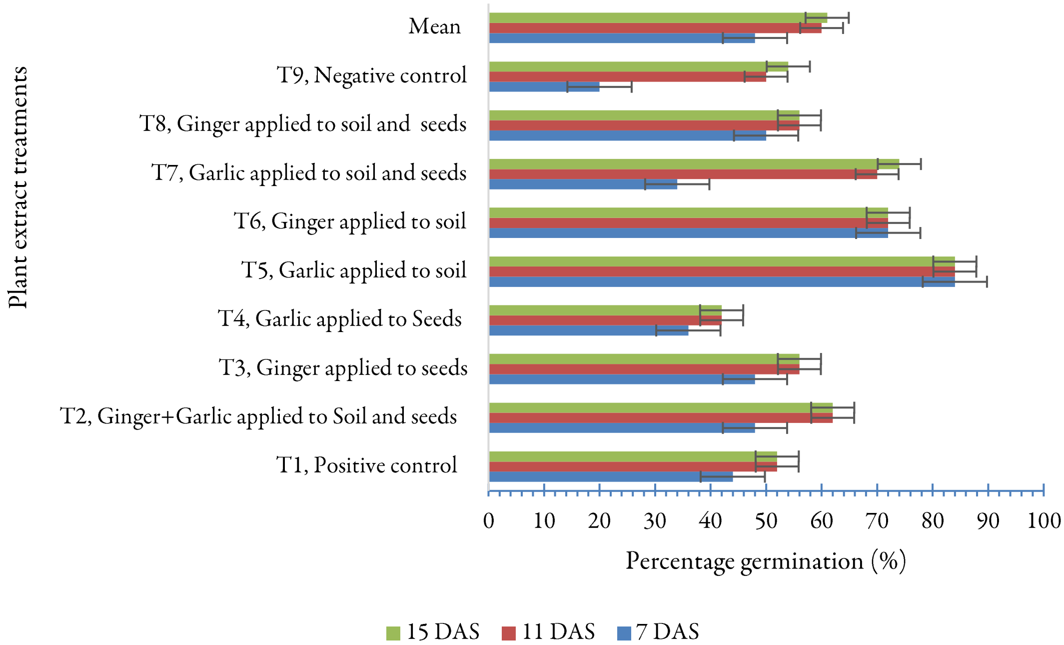

Figure 1. The germination of African garden egg infected with *F. oxysporum* f.sp *melongenae* as affected by applied plant extracts
Error bars were based on standard error

In terms of percentage germination, ginger extracts performed better than the controls but garlic extracts are the best treatments irrespective of the mode of application. Combining both garlic and ginger extracts gives lower percentage germination (47.5-60.8%) at levels that are below that of best garlic extract (T5) (i.e., 83.3%)) germination) and performance of the best ginger extract (T6) (i.e., 72.5%)).

It is observed that infection of seeds by *F. oxysporum* has not stopped the seeds from germinating, in fact, at 15 DAS half of the seeds (50.8%) had germinated in T9 which is comparable with the T1 (52.5%). Thus, in terms of percentage germination, T2, T5, T6 and T7 are the treatments that top the chart with a percentage germination greater than 72.3%.

All the plots have some germinated seeds notwithstanding the treatments they received. This corroborates the findings of Habib *et al.* (2007) who states that even if the seed lot has a high level of *Fusarium* spp. infection, it may still have high germination levels. This is likely an indication that the pathogen has only infected the seed coat or endosperm and not killed the embryo, thus the severity of damage to the seed may be less.

The results of different plant extracts' potential on the number of leaves of African garden egg infected with *F. oxysporum* are presented in Table 1. The number of leaves was not highly affected by the plant extracts at the early seedling stage. All the plant extracts are comparable to the positive control (4 and 9 leaves respectively at 30 and 45 DAS) but better than the negative control (1.8 and 2.3 leaves respectively at 30 and 45 DAS). A similar trend is observed with the shoot heights (Table 2).

Table 1. The number of leaves of African garden egg infected with *Fusarium oxysporum* f. sp *melongenae* as affected by applied plant extracts

	Treatments	Number of leaves	
		30 DAS	45 DAS
TI	Positive control	4.0	9.0
T2	Ginger and garlic extracts applied to seeds +soil	4.9	10.5
T3	Ginger extract applied to seeds	3.7	9.7
T4	Garlic extract applied to seeds	4.9	7.3
T5	Garlic extract applied to soil	3.9	8.3
T6	Ginger extract applied to soil	4.1	7.8
T7	Garlic extract applied to seeds +soil	3.8	8.3
T8	Ginger extract applied to seeds +soil	4.2	9.5
T9	Negative control	1.8	2.3
	SED	1.0	1.6
	LSD	2.0	3.4

Table 2. The shoot height of African garden egg infected with *Fusarium oxysporum* as affected by the application of plant extracts

	Treatments	Shoot heights	
		30 DAS	45 DAS
T1	Positive control	5.9	11.1
T2	Ginger and garlic extracts applied to seeds +soil	7.4	12.3
T3	Ginger extract applied to seeds	7.2	13.7
T4	Garlic extract applied to seeds	4.9	8.2
T5	Garlic extract applied to soil	7.1	11.3
T6	Ginger extract applied to soil	6.9	11.3
T7	Garlic extract applied to seeds +soil	7.3	10.3
T8	Ginger extract applied to seeds +soil	5.2	9.9
T9	Negative control	1.8	1.9
	SED	2.1	2.0
	LSD	4.3	4.3

The results of different plant extracts' potential on the incidence and severity of wilt of scarlet eggplant infected with *F. oxysporum,* at 45 DAS, are presented in Table 3. The percentage incidence of wilt on the seedlings was highest (33.4%) in the negative control. The treated plots show a below-average percentage (12.4%) incidence of wilts which are lower than the negative control.

T4 and T2 are the best treatments with (0.0%) percentage incidence of wilt, which is the same results in T1 at 45 DAS. The percentage severity of wilt followed the same trend as incidence of wilt and the best treatments are the same ones for percentage incidence at 45 DAS.

The percentage incidence of wilt on the seedlings is highest (33.4%) in the infected control while the treated plots show a below-average percentage incidence (12.4%) of wilts. Tagoe *et al.* (2011) reported that all organic plant extracts utilised inhibited fungi growth resulting in a significant difference ($P \le 0.01$) in the growth diameter of fungi on extract treated plots compared with control *in vitro* which corroborates these findings. Perelló *et al.* (2013) reported that garlic extract reduced the severity of disease caused by *Bipolaris sorokiniana* and *Drechslera tritici-repentis* on wheat seeds which confirms the findings of this research.

Garlic extract applied to seeds, and combined Ginger + Garlic extracts applied to seeds+soil were the best treatments with a 0.0% percentage incidence of wilt, which was similar to the healthy control at 45 DAS. Siva (2008) reported that crude medicinal plant extracts of 20 plant species (at 50% concentration) were effective in reducing the mycelial growth of *F. o.* f.sp. melongenae thus corroborating the findings of this experiment.

Table 3. The incidence and severity of wilt of African garden egg infected with *Fusarium oxysporum* after 45 DAS as affected by the application of plant extracts

	Treatments	Incidence of wilt (%)	Severity of wilt (%)
T1	Positive control	0.0	0.0
T2	Ginger and garlic extracts applied to seeds+soil	0.0	0.0
T3	Ginger extract applied to seeds	11.2	12.5
T4	Garlic extract applied to seeds	0.0	0.0
T5	Garlic extract applied to soil	11.2	4.2
T6	Ginger extract applied to soil	22.3	4.2
T7	Garlic extract applied to seeds+soil	11.2	8.3
T8	Ginger extract applied to seeds+soil	22.3	8.3
T9	Negative control	33.4	20.8
	Mean x	12.4	7.3

The results of different plant extracts' potential on shoot weight of African garden egg seedlings infected with *F. oxysporum,* at 45 DAS, are presented in Figure 2. The results shows that all the treatments that received plant extracts perform slightly better than the positive control (5.2 g) in terms of shoot weight. The best treatment is ginger extract applied to seeds + soil (8.3 g) which is better than all the other treatments and the controls.

This good performance is followed by that of ginger and garlic extracts applied to seeds + soil (7.0 g). All the treated plots with plant extracts are at par with Ginger extract applied to seeds+soil plots (8.3 g). The negative control has very slender seedlings that weighs less than half of the weight of positive control plants.

It is observed that plant extracts do not cause significant changes in growth parameters early in the trial. This may be due to the fact that *Fusarium* species mostly affect the water and nutrient uptake which may not be limiting at early growth until the vascular vessels are completely blocked as the fungus grew. However, at 45 DAS, growth parameters of plant extract treated plots are higher than those of the negative control.

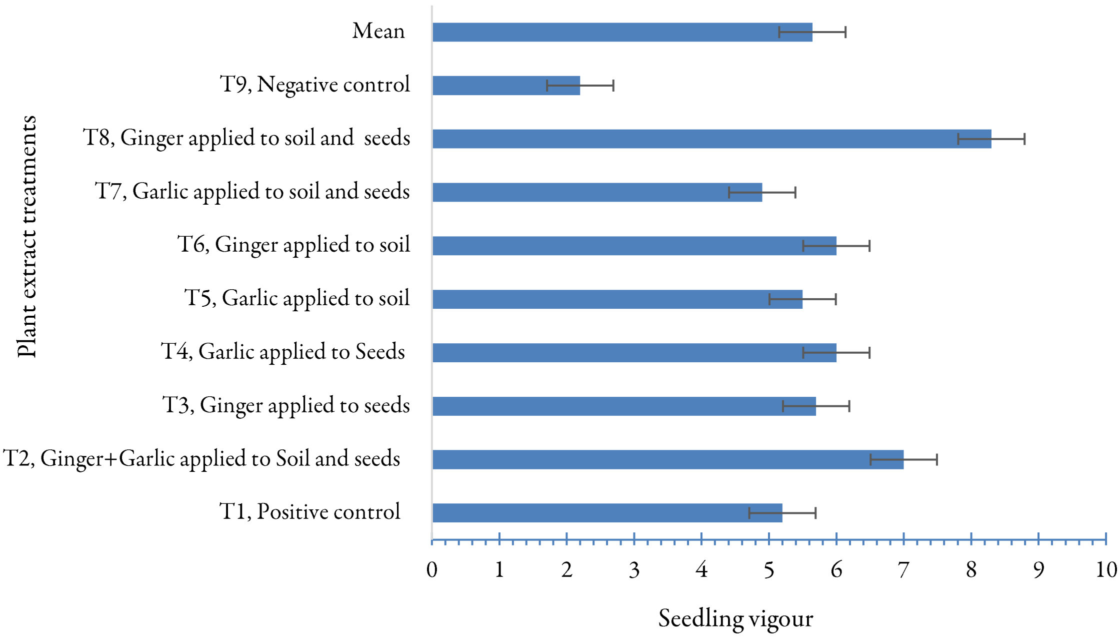

Figure 2. The shoot weight of African garden egg seedlings infected with *Fusarium oxysporum* after 45 DAS as affected by the application of plant extracts
Error bars were based on standard error

The obtained results of different plant extracts' potential on vigour index of African garden egg seedlings infected with *F. oxysporum* are presented in Figure 3. The results shows that the highest vigour index at 45 DAS is from Ginger extract applied to seeds (823) followed by ginger and garlic extracts applied to seeds + soil (769) then Garlic extract applied to soil (692) and Garlic extract applied to seeds and soil (658) respectively. These high vigour indices are also above the vigour index of healthy control (604) and the mean vigour index (582). The same pattern of vigour indices is observed for vigour indices calculated at 30 DAS.

The correlation of results shows that germination is negatively correlated to the incidence and severity of wilt throughout the 45 days of cultivation (Table 4). Incidence and severity of wilt are positively correlated at 30 and 45 DAS.

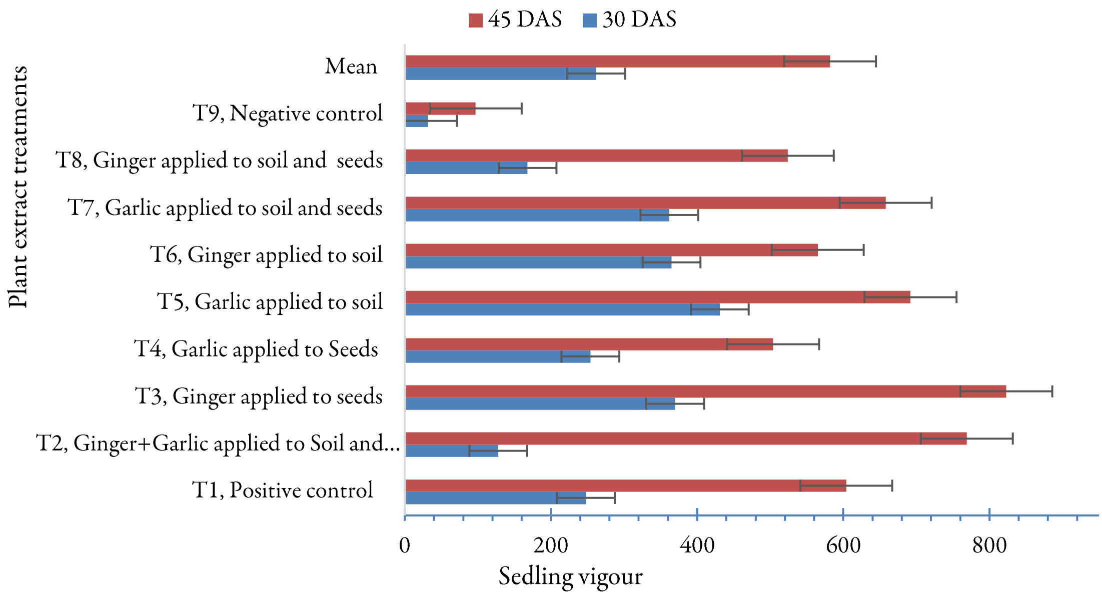

Figure 3. The seedling vigour index of African garden egg infected with *Fusarium oxysporum* as affected by the application of plant extracts
Error bars were based on standard error

Table 4. Correlation of percentage germination and disease parameters on the use of plant extracts to treat seeds of *S. aethiopicum* against *Fusarium* sp. wilt after 30 and 45 DAS

	Germination 7 DAS	Germination 11 DAS	Germination 15 DAS	Incidence 30 DAS	Severity 30 DAS	Incidence 45 DAS	Severity 45 DAS
Germination 7 DAS	1						
Germination 11 DAS	0.06	1					
Germination 15 DAS	0.42*	0.78**	1				
Incidence 30 DAS	-0.29	-0.28	-0.14	1			
Severity 30 DAS	-0.43*	-0.24	-0.14	0.72**	1		
Incidence 45 DAS	-0.38	0.09	0.17	0.17	0.21	1	
Severity 45 DAS	-0.33	-0.45*	-0.54**	0.23	0.20	0.06	1

Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).

The high vigour indices from garlic and ginger treated plots are above the vigour index of healthy control (604) and the mean vigour index (582) at 45 DAS. The findings of this trial shows that seed treatment using organic materials lasts longer. Thus, the results do not corroborate the statement that in most cases, seed treatments are no longer effective after the seedling emergence stage (Syngenta 2010). Kebede *et al.* (2013) reported that ginger extract seed dressing resulted in 2303-2270 plant vigour indexes of tomato seed. This corroborates the findings of this research.

Conclusions

This trial was set up using ginger and garlic plant extracts as a seed treatment to control the *Fusarium* wilt disease of scarlet eggplant/African garden egg. The performance of the botanicals (ginger and garlic) against *Fusarium* wilt showed that they were very effective as seed and soil treatment materials. The most prominent among these botanicals was garlic irrespective of mode of application. The incidence and the severity of this pathogen were lowest when combined treatment was applied on soil and seed using garlic, which was comparable to the positive control. It was concluded that ginger and/or garlic extracts boosted percentage germination, seedling vigour, number of leaves and shoot height, plant weight but reduced wilt incidence and disease severity up to 45 DAS in the screen house. The use of garlic and ginger as antimycotic seed and soil treatment materials is strongly recommended.

Authors' Contributions

Conceptualization; Visualization; Writing - original draft: MN; Investigation; Project administration; Resources: MN and MC; Data curation; Formal analysis: MN, PI, EA; Validation: MN, CPOE and IC; Writing - review and editing: MN, MC, PI, EA, CPOE and IC.
All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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