Seed Dressing with Aqueous *Allium sativum* L. Extracts Enhanced the Tolerance of Maize Plant to Stalk and Ear Rot Disease Caused by *Fusarium verticillioides* (Sacc.) Nirenberg

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ABSTRACT

Stalk and ear rot is one of the most common fungal disease of maize. Significant crop failure and yield loss, arising from the disease, are reported annually. Maize grains infected with the pathogen pose serious threat to human and animal health, owing to mycotoxins contamination. The present study evaluated aqueous extracts from *Allium sativum* in the management of the disease. Four concentrations of the extracts were evaluated against the pathogen *in vitro*. The most promising concentrations, were applied as seed dressing agents *in vivo* at 5, 10 and 15-minutes duration for each concentration. Garlic extracts-dressed seeds were then transferred to *F. verticilliodes* infested medium and allowed to germinate and grow for 9 days. Seedlings were transplanted into sterile soils in plastic buckets thereafter. Data collected were subjected to analysis of variance and mean separation. The results obtained showed that peak inhibition of mycelial growth was achieved by 80% garlic extract concentration (76.29% at three days after inoculation). The *in vivo* study showed that the concentration of garlic extracts as well as the duration of treatment had significant effect on seed germination. Maize seeds dressed with 80% aqueous garlic extract had the least percentage germination, 43.33%, but recorded the least disease incidence and severity at 2 weeks after transplanting. It also had the best yield, fresh weight of cobs, 303.86 g, and the least percentage of kernel infection with *F. verticillioides*, 15.00%. Garlic extract has potential, as a bio-fungicide, in the management of stalk and ear rot disease of maize.

Keywords: *Maize, Stalk and ear rot disease, Fusarium verticillioides Allium sativum, Seed dressing.*

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Highlights of this paper

- This study evaluated aqueous garlic extract as seed dressing agent in the management of stalk and ear rot disease of maize.

1. INTRODUCTION

Maize, (*Zea mays* L.) has been described as the most important cereal with economic significance in Africa [1]. The crop is of immense importance to man nutritionally and medicinally. Maize grains are important component in the formulation of feed for farm animals and are also essential in the production line of some industries as raw material [2, 3].

Pests and diseases are major limitations to the cultivation of maize worldwide. One of the most common disease is stalk and ear rot (SER) caused by *Fusarium verticillioides* (Sacc.) Nirenberg [4]. It is a systemic disease favoured by a warm temperature and a dry field condition, especially in the early life of the crop. Infected seed is one major means of dissemination, although residue of the infected maize plants from the previous harvest also play a major role in disease development, serving as a reservoir of inoculum for succeeding planting season. Such residues are also known to be a major source of soil contamination with *F. verticillioides* [5]. Insect vectors, *Ostrinia nubilalis* (Humber) as an example, spread the disease through the creation of wounds that serves as entry point for the pathogen. The disease cycle usually starts with the infection of the radicle. The pathogen then grows within the stem of standing maize plant and finally get to the ear and kernels, where it causes ear and kernel rot respectively [6, 7]. In certain cases, infection with the pathogen produce no symptoms and infected plants are said to be asymptomatic. In most cases, however, the leaves of infected plants appear dull-green, while the lower portion of stalk turn yellow. Stalk rot, resulting from the disintegration of the tissues making up the pith, can result in the death and lodging of the infected plants [8] before ear and kernel formation. Infected kernels are characterized by white streaking of the hull, a phenomenon referred to as "starburst". Over time, the entire ear and kernels may rot with conspicuous white mycelium of the infecting pathogen [9]. Poor growth and yield loss, as much as 10 – 50%, are some of the other symptoms associated with *F. verticillioides* infection of maize [10]. Nutritionally, infected maize grains pose a serious threat to human health and that of farm animals. This is because *F. verticillioides* is known to produce numerous mycotoxins, such as; fumonisins, furasic acid and fusarins [11]. Fumonisins are the most common of these mycotoxins and have been implicated in the development of oesophageal cancer in humans, while aiding cancer-promoting activities in experimental animals like rat [12]. Leukoencephalomalacia disease in horse and pulmonary edema infection in pig are some of the other diseases associated with fumonisins mycotoxins [13].

Current management strategies for stalk and ear rot disease (SERD) have remained largely ineffective, owing to the variability in *F. verticillioides* strains [14]. The use of resistant cultivars seems to be the most promising option, but that again is faced with several challenges, one of which is that not many of such cultivars are available to the rural African farmer [15]. There is a need to look inward and develop easy to use and environment-friendly management options for the disease in rural Africa.

Aqueous extracts from several plants found in Africa are known to possess antifungal properties against several plant pathogenic fungi. Results from the *in vitro* evaluation of extracts from such plants on their antifungal properties have been reported [16-18]. Reports on the efficacy of garlic extracts in the management of some common plant pathogenic organisms, *in vitro*, abound in literature [19, 20]. The active ingredient responsible for its antimicrobial properties has been identified to be Allicin. The mode of action of Allicin has been studied and explained elaborately [21-23].

There is a need to go beyond the *in vitro* studies and evaluate these extracts for practical application in plant disease management under field condition. This study was designed as a step in this direction. The first objective was to evaluate *A. sativum* (garlic) extracts for antifungal properties against *F. verticillioides in vitro*, while the second was to evaluate its effectiveness in the management of SERD as a seed dressing agent.

2. MATERIALS AND METHODS

2.1. Collection of the Test Crop

The yellow maize cultivar evaluated in this study was obtained from The International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

2.2. Preparation of Artificial Growth Medium For Isolation of the Test Organism

Potato Dextrose Agar (PDA) was the artificial growth medium employed in the isolation of the test organism (*F. verticillioides*). Powdered PDA was purchased and prepared based on the recommendation of the manufacturer. Sterilization of prepared PDA, all glass and metal wares for the isolation process was done in an autoclave at 121°c for 15 minutes. Amendment of PDA with streptomycin antibiotic, 0.2 g/100 ml of agar, after sterilization, was done to inhibit bacterial growth. Pour plating in Petri-dishes, at 15 ml PDA/Petri-dish, was carried out when sterilized and amended PDA cooled to 45°c. Pour plated PDA were then allowed to gel.

2.3. Isolation/Identification of the Test Organism And Preparation of Its Pure Culture

Fusarium verticilliodes, the test organism, was isolated from infected maize seeds, showing the characteristic white streaking of hull, from a farmers field in Akure. Infected seeds were surface sterilized in 70% alcohol, rinsed in three changes of sterile distilled water and inoculated on prepared PDA in Petri-dishes. Three seeds were inoculated/Petri-dish, equidistant to one another, after drying in sterile absorbent paper Figure 1a. Six of such plates were prepared and incubated at 28°c ± 2°c. At day 3 after inoculation, *Fusarium* spp. was seen growing out from some of the inoculated seeds Figure 1b. Sub-culturing of the isolated *Fusarium* spp. was done to obtain a pure culture of the organism. Confirmation of the isolated organism as *F. verticillioides* was based on visual observation of the morphological characteristics in culture plate. Microscopic examination was also done with a CX40 Trinocular Olympus microscope, with reference to the description given by Deepta and Sreenivasa [24]. Pathogenicity test was also carried out for further confirmation.

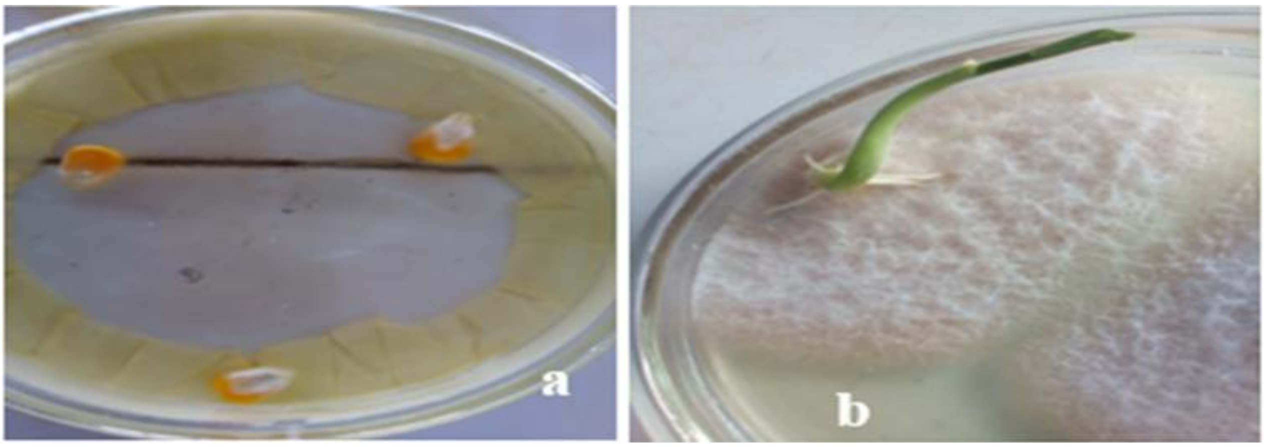

Figure-1 (a). *F. verticillioides* infected maize seeds inoculated on PDA. (b). *Fusarium* spp. growing from inoculated maize seeds.

2.4. Collection and Preparation of A. Sativum Aqueous Extract

Allium sativum, garlic, was purchased from a fruit and vegetable store at the main market in Akure, Ondo State, Nigeria. Preparation of the aqueous extract was at the pathology laboratory of the Department of Crop, Soil and Pest Management (CSP) the Federal University of Technology, Akure (FUTA). Ondo State, Nigeria. Debris, flaky scale leaves and unhealthy cloves were removed, after which the healthy cloves were washed in sterile distilled water and air-dried. Hot (70^{o}c) aqueous extract was prepared following the method described by Ajayi [25]. Four different concentrations of 20, 40, 60, and 80% were prepared from the stock solution (100%) using appropriate dilutions with sterile distilled water. Ten (10) ml of each concentration was prepared in sterile specimen bottles.

2.5. Evaluation of A. Sativum for Antifungal Properties Against F. Verticillioides

Potato dextrose agar (PDA) growth medium was prepared as described previously but with a little modification. This was to make up for the expected decrease in the PDA content of the growth medium after the addition of garlic extracts. Instead of the recommended 3.9 g of powdered PDA in 100 ml of distilled water by the manufacturer, 4.4 g was used. Pour plating was done at the rate of 13 ml of PDA/Petri-dish when sterilized PDA was 50^{o}c. Two (2) ml of garlic extracts was added to the pour plated agar in each Petri-dish, using a sterile syringe, followed by gentle swerving to ensure proper mixing of the PDA and garlic extracts. The same procedure was adopted in the preparation of the standard check and the control. The standard check was a synthetic fungicide "Dress Force 42 WS" (Imidacloprid 20%, Metalaxyl-M 20% and Tebuconazole 2%), while the control was sterile distilled water. Each Petri-dish, therefore, contained 15 ml of PDA/Garlic extracts. The standard check had PDA/Dress Force, while the control had PDA/Distilled water complex. The garlic extracts were at four different concentrations, 20, 40, 60 and 80%. Powdered Dress Force was diluted to a concentration of 12.5 mg/ml, based on an estimation of the recommendation for use by the manufacturer. The width of each Petri-dish was 8.50 cm. A 5 mm agar disc, from a 5-day old culture of *F. verticillioides* was inoculated at the center of the PDA complex in each Petri-dish. Each treatment was replicated four times, such that a total of 24 experimental units were evaluated in all. The treatments were laid out in a completely randomized design. Incubation was done at 28^{o}c ± 2^{o}c, while mycelial growth was measured at three-day interval.

2.6. Preparation of Garlic Extract/Starch Slurry and Seed Dressing of Maize

Twenty (20) % aqueous garlic extracts was the least effective form the *in vitro* study. It was consequently excluded in the preparation of garlic extract/starch slurry for seed dressing. Three (3) concentrations, 40, 60 and 80% of the extracts were prepared as described previously. Dry granules of commercially produced instant cold-water starch, to mimic adjuvant and adhesive agent in conventional fungicides, was added to each concentration of garlic extracts at the rate of 25 mg/ml, after which stirring was done to allow for complete dissolution of the granules and the formation of garlic extract/starch slurry. Twenty (20) ml of the slurry was obtained from each concentration and 60 healthy maize seed were placed in each. The seeds were allowed to remain in the respective garlic slurry concentrations for 5, 10 and 15 minutes. A separate garlic slurry concentration was prepared for each treatment duration. The standard check, "Dress Force" was prepared as a slurry, based on manufacturer's recommendations. Starch granules was not added. The control had 20 ml of distilled water/starch slurry.

2.7. Preparation of F. Verticillioides Infested Medium and Sowing of Dressed Maize Seeds

The sowing medium was 6 layers of sterile absorbent paper in each Petri-dish. Each medium was moistened with 30 ml of spore suspension, at 10^6 spores/ml, of *F. verticillioides*. The spores were harvested from 6-day old cultures of the pathogen, while the required spore concentration was obtained with the aid of a haemocytometer slide. Dressed maize seeds for all treatments (garlic extract concentrations at three-time interval, the standard check and control) were placed in each Petri-dish at the rate of 20 seeds/Petri-dish. Fifteen (15) treatments were evaluated and each was replicated thrice, making a total of 45 experimental units. The treatments were laid out on the side bench in the pest laboratory of CSP Department. Wetting was done as required with equal volume of sterile distilled water for all treatments. Sowed seeds were examined daily and seedlings were allowed to grow in the *F. verticillioides* infested medium for 9 days.

2.8. Collection/Sterilization of Soil and Transplanting of Seedlings From F. Verticillioides Infested Medium

Sandy loam soil was collected from FUTA Teaching and Research Farm and was sterilized using the steam heat method as described by Bollen [26]. The sterilized soil was transferred into 5-liter capacity plastic buckets at the rate of 4 kg of soil/bucket. Each bucket had 6 small holes, 3 mm diameter each, at the base. Two 9-day old seedlings from 5 minutes treatment, being the best in terms of germination percentages, in each garlic extracts complexes were selected randomly and were transferred to sterile soil in plastic buckets. Each bucket had a corresponding treatment label with the *F. verticillioides* infested medium (FvIM) from where the maize seedlings were transplanted. The experiment was laid out in a completely randomized design in the screen house of CSP Department. Irrigation was done as required with sterile distilled water, while the fertilizer applied was NPK 15:15:15 at the recommended rate of 200 kg/ha. (3.8 g/stand).

2.9. Data Collection and Statistical Analysis

Data were collected on the following parameters.

2.9.1. Mycelial Growth of F. Verticillioides.

The measurement of mycelial growth was done at 3-day interval and was terminated on the 9th day when complete coverage of the growth medium in the control plate with the mycelium of *F. verticillioides* was achieved. The measurement of mycelial growth was with a meter rule. The values obtained for the treatments were converted to percentage mycelial growth inhibition using Equation 1.

$$im = \frac{mgc - mgt}{mgc} x \frac{100}{1} \quad (1)$$

Where;

im = inhibition of mycelial growth.

mgc = mycelial growth in control plate.

mgt = mycelial growth in treated plate.

2.9.2. Germination of Maize Seeds in F. Verticillioides Infested Medium (%)

Maize seeds sown in FvIM in Petri-dishes were examined daily. The number of germinated seeds, those with emerged radicle, were counted. The values obtained were converted to percentage seed germination with Equation 2.

$$psg = \frac{ngs}{nss} x \frac{100}{1} \quad (2)$$

Where;

psg = percentage seed germination.

ngs = number of germinated seeds.

nss = number of seeds sown.

2.9.3. Shoot Length

On the 9th day, after sowing in FvIM, maize seedlings were carefully removed, to prevent the roots from breaking off. The shoot of each seedling was measured one after the other with a meter rule. The mean of the values obtained in each replicate was calculated and recorded.

2.9.4. Root Length

After 9 days of growth in the pathogen infested medium (PIM), the length of the primary root of each seedling was measured. The mean values obtained for each experimental unit was also recorded.

2.9.5. The Number of Roots Produced by Seedlings

At the end of the 9-day growth period in FvIM, the number of roots produced by each seedling in all the treatments was counted. The mean value was calculated and recorded.

2.9.6. Incidence of Stalk and Ear Rot Disease (%)

The transplanted maize in plastic buckets were examined weekly for expression of symptoms of SERD from the 2nd week after transplanting (WAT). The incidence of the disease was determined and recorded in percentage for each treatment with Equation 3.

$$di = \frac{ndl}{tnl} \; x \; \frac{100}{1} \qquad (3).$$

Where;

di = disease incidence.

ndi = number of diseased leaves showing symptoms of SERD.

tnl = total number of leaves on the plant.

2.9.7. Severity of Stalk and Ear Rot Disease

Symptoms indicative of SER infection on maize stem was observed visually during the growing period of the plant and at harvest. The modified severity rating scale cited by Afolabi, et al. [15] was adopted. It consisted of a 0 – 5 rating where;

0 = No symptom of infection.

1= 1– 25% of the leaf shows a symptom of infection/1-2 cm diameter lesion of rot on the stem.

2 = 26 – 50% of the leaf shows a symptom of infection/2.1-3 cm diameter lesion of rot on the stem.

3 = 51 - 75% of the leaf shows a symptom of infection/3.1- 4 cm diameter lesion of rot on the stem.

4 = 76 – 100% of the leaf shows a symptom of infection/4.1- 5 cm diameter lesion of rot on the stem.

5 = Death and lodging of the maize plant.

2.9.8. Cob Yield of Maize Plants (g)

Maize cobs were allowed to dry before harvest. Weighing of cobs was done immediately after harvest and the value obtained for each treatment was recorded as cob fresh weight.

2.9.9. Infection of Kernels with F. Verticillioides

Harvested cobs were shelled and sun-dried for one week. Thereafter, 20 seeds were selected randomly from each treatment. The selected seeds were surface sterilized in 70% alcohol, rinsed in several changes of sterile distilled water and pat dried with sterile absorbent paper. Sterilized maize seeds were inoculated on PDA medium in Petri-dishes at the rate of 5 seeds/Petri-dish. The number of *F. verticillioides* infected seeds were counted and expressed in percentage infection of kernels with Equation 4.

$$pis = \frac{tis}{tin} \, x \, \frac{100}{1} \qquad (4)$$

Where;

pis = percentage infection of seed.

tis = number of infected seeds.

tin = number of inoculated seeds.

2.10. Treatments Evaluated in the in Vivo Study

The treatments were evaluated in the in vivo study were

i. Garlic extracts at 40%concentration = G40 at 5, 10- and 15-minutes seed treatment duration.

ii. Garlic extracts at 60% concentration = G60 at 5, 10- and 15-minutes seed treatment duration.

iii. Garlic extracts at 80% concentration = G80 at 5, 10- and 15-minutes seed treatment duration.

iv. Synthetic fungicide as standard check = SDC at 5, 10- and 15-minutes seed treatment duration.

v. Control = CNT at 5, 10- and 15-minutes seed treatment duration.

All data collected were subjected to analysis of variance (ANOVA) using Minitab software, Version 17. Mean separation was achieved with Tukey's test.

3. RESULTS

3.1. Effect of Aqueous A. Sativum Extract on the Mycelial Growth of F. Verticillioides

The inhibition of mycelial growth (IMG) of *F. verticillioides*, by aqueous garlic extracts, increased with the increasing concentration of the extracts. Significant differences were observed among treatments, especially at the 3rd and 6th day after inoculation Figure 2a, Table 1. A decreased efficacy in the inhibitory activity of the extracts was however observed with the increasing number of days after inoculation Table 1. On the 3rd day after inoculation, 80% garlic concentration inhibited the mycelial growth of *F. verticillioides* by 76.29%. This was the highest value among the extract concentrations. Next was 60% garlic extract concentration with 51.33% IMG, while 40% concentration had 40.95% IMG. The least value of mycelial growth inhibition, 20.74%, was exhibited by 20% gallic extract concentration Table 1. The same pattern of IMG was observed on the 6th and 9th day after inoculation. It must be pointed out however that 40 and 60% garlic extract concentrations were not significantly different in their IMG of *F. verticillioides* on the 9th day after inoculation Table 1.

3.2. Effect of Seed Dressing of Maize with Garlic Extracts on Germination (%)

A surprising trend of decreasing germination percentage with an increasing concentration of garlic extracts was observed Table 2. On the 2nd day after sowing, the control (CNT) had the highest percentage germination, 58.33%, among seeds that were treated for 5 minutes in garlic extracts slurry (GES). This value was however not statistically ($p \leq 0.05$) different from garlic extract slurry at 40% concentration (G40) and the standard check (SDC).

In seeds treated for 10 minutes in GES, SDC had the highest germination percentage, 60.00%. G40, CNT and SDC were statistically similar.

Table-1. Inhibition of mycelial growth of *F. verticillioides* by *A. sativum* extract (%).

Treatments	Inhibition of mycelial growth/ days after inoculation		
	3	**6**	**9**
G20	20.74e	10.18e	8.68d
G40	40.95d	36.34d	32.22c
G60	51.33c	42.87c	33.72c
G80	76.29b	65.64b	63.04b
SDC	83.09a	71.80a	68.87a

Note: Means in the same column followed by the same alphabets are not significantly different ($p \leq 0.05$) according to Tukey's test.
Key. G20 = Garlic extract at 20% concentration, G40 = Garlic extract at 40% concentration, G60= Garlic extract at 60% concertation, G80 = Garlic extract at 80% concentration, SDC= Standard check.

A similar trend was observed among seeds treated for 15 minutes, but CNT had the highest germination percentage, 60.00%. On a general note, maize seeds treated with 80% GES had significantly lowest germination percentages on the 2nd day after sowing, 18.33%, 18.33% and 15.00% at 5, 10- and 15-minutes treatment time Table 2. On the 3rd day after sowing in PIM, germination percentages were higher in all treatments than the previous day. Among seeds treated for 5 minutes before sowing in PIM, SDC had the highest germination percentage of 75.00%, while G80 had the lowest, 40.00%,. The two values were significantly ($p \leq 0.05$) different. SDC, CNT and G40 were not statistically similar Table 2. When seeds were treated for 10 minutes GES before sowing in the PIM, the highest germination percentage of 76.00% was obtained from SDC, while the least, 36.66% was obtained from G80. Germination percentage in G80 further declined to 26.66% when seeds were treated in slurry for 15 minutes before sowing. SDC had the highest germination percentage at this period, 73.33%, Table 2. Peak germination was attained on the 4th day after sowing.

Table-2. Effect of seed treatment with different concentrations of garlic extracts on the germination of maize seeds (%).

Treatments	Germination (%)/Duration of treatment in garlic extract (Minutes)		
	5	**10**	**15**
	2 Days after sowing in *F. verticillioides* infested medium		
G40	56.66a	53.33a	55.00a
G60	40.00b	31.66b	40.00b
G80	18.33c	18.33c	15.00c
CNT	58.33a	56.66a	60.00a
SDC	56.66a	60.00a	58.33a
	3 Days after sowing in *F. verticillioides* infested medium		
G40	70.00ab	71.66a	66.66ab
G60	63.33b	58.33b	58.33b
G80	40.00c	36.66c	26.66e
CNT	71.66ab	70.00a	70.00ab
SDC	75.00a	76.00a	73.33a
	4 Days after sowing in *F. verticillioides* infested medium		
G40	73.33a	75.00a	68.33b
G60	65.00b	61.66b	61.66c
G80	43.33c	38.33c	26.66d
CNT	76.66a	75.00a	73.33ab
SDC	75.00a	78.33a	76.66a

Note: Means in the same column followed by the same alphabets for each day after sowing are not significantly different ($p \leq 0.05$) according to Tukey's test.
Key. G40 = Garlic extract at 40% concentration, G60= Garlic extract at 60% concertation, G80 = Garlic extract at 80% concentration, CNT = Control, SDC= Standard check.

Among seeds treated with slurry for 5 and 10 minutes before sowing in PIM G40, CNT and SDC both produced germination percentages above 70.00%. The values of germination percentages produced by the three treatments were all similar statistically. G80 produced significantly lowest germination percentages of 43.33%, 38.33% and 26.66% when seeds were treated in garlic extracts for 5, 10 and 15 minutes respectively Table 2. Finally, the duration of treatment before sowing had no noticeable effect on seeds from SDC, and CNT as no definite germination pattern was observed across the 3-time treatment durations for both treatments.

3.3. Effect of Seed Dressing of Maize With Garlic Extracts on the Shoot Length of Seedlings (Cm)

The least value of shoot length, 3.77 cm, was obtained from maize seeds treated with 80% garlic extract concentration for 15 minutes (T9) before transferring to the PIM Figure 2b, Table 3. The highest value, 6.69 cm was obtained from the standard check (T11) at 5 minutes treatment before transferring to the PIM Table 2. An interesting observation, however, was that shoot length was statistically very similar among most of the treatments Table 3.

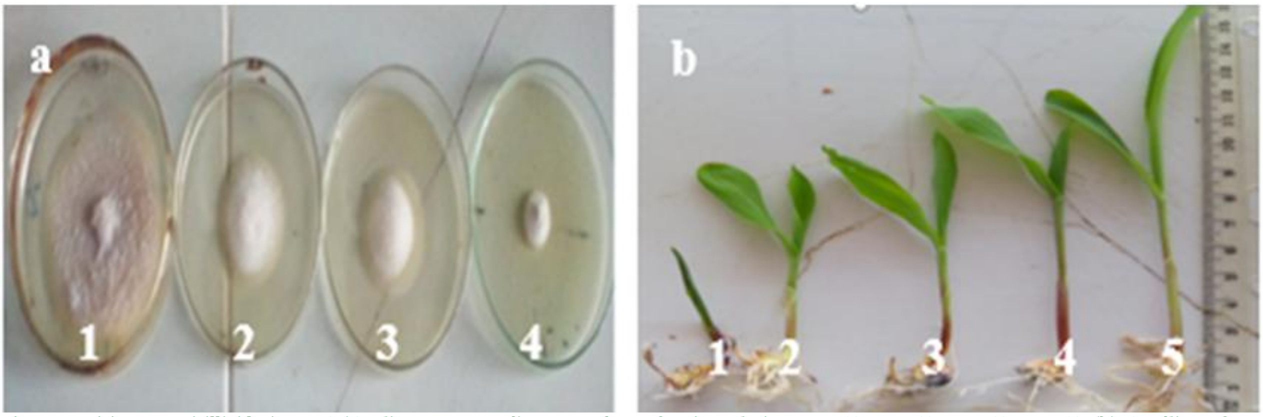

Figure-2. (a). *F. verticillioides* in PDA/Garlic extract medium at 9 days after inoculation 1=20%, 2=40%, 3=60%, 4=80%. (b). Seedlings from maize seeds dressed with different concentrations of garlic extract and grown in *F. verticillioides* infested medium for 9 days. 1=80%, 2=60%, 3=40%, 4=SDC, 5=CNT.

3.4. Effect of Seed Dressing of Maize with Garlic Extracts on the Root Length of Seedlings (cm)

In a similar fashion to the result obtained for shoot length, the shortest root length from seedlings was also obtained from maize seeds treated with 80% garlic extract for 15-minutes, T9. Root length was 2.26 cm. The highest root length value of 7.06 cm was obtained from the standard check, T11, Table 3. Root length was, however, very similar statistically among most of the treatments Table 3.

Table-3. Effect of treatment with different concentrations of garlic slurry on the shoot length, root length and the number of roots of maize seedlings.

Treatments	Parameters evaluated		
	Shoot length(cm)	Root length (cm)	Number of roots
T1	4.91bc	5.78ab	7.34abc
T2	5.47ab	5.89ab	9.14a
T3	5.90ab	6.48a	7.61abc
T4	5.70ab	6.46a	5.91cd
T5	4.91bc	4.81ab	5.74cde
T6	4.80bc	4.86ab	6.55bcd
T7	4.87bc	5.84ab	4.99de
T8	4.53bc	3.85bc	5.77cd
T9	3.77c	2.26c	3.66e
T10	6.69a	6.89a	8.61ab
T11	6.81a	7.06a	8.91a

Note: Means in the same column followed by the same alphabets are not significantly different ($p \leq 0.05$) according to Tukey's test.

Key; T1= 40% garlic slurry concentration, 5 minutes duration of maize seeds treatment, T2= 40% garlic slurry concentration, 10 minutes duration of maize seeds treatment, T3= 40% garlic slurry concentration, 15 minutes duration of maize seeds treatment, T4= 60% garlic slurry concentration, 5 minutes duration of maize seeds treatment, T5=60% garlic slurry concentration, 10 minutes duration of maize seeds treatment, T6= 60% garlic slurry concentration, 15 minutes duration of maize seeds treatment, T7= 80% garlic slurry concentration, 5 minutes duration of maize seeds treatment, T8=80% garlic slurry concentration, 10 minutes duration of maize seeds treatment, T9= 80% garlic slurry concentration, 15 minutes duration of maize seeds treatment, T10= Sterile water slurry, 5 minutes duration of maize seeds treatment (Control), T11= Synthetic chemical slurry, 5 minutes duration of maize seeds treatment (Standard check).

3.5. Effect of Seed Dressing of Maize with Garlic Extracts on the Number of Roots Produced by Seedlings

The highest number of roots, 9.14, was produced by maize seeds treated with 40% garlic extract concentration for 10 minutes before sowing in PIM (T4) while the least, 3.66, was produced by seeds treated with 80% garlic extract concentration for 15 minutes before sowing in FvIM, T9. Most of the treatments were not significantly different with regard to root production Table 3.

3.6. Effect of Seed Dressing of Maize With Garlic Extract on the Incidence of Stalk and Ear Rot Disease (%)

On the 2nd WAT, 54.66% disease incidence was recorded from G40. This was significantly higher than all other treatments. G80 and SDC both had 0.00% disease incidence and were significantly the lowest Table 4. By the 4th WAT, G40 had 86.83% disease incidence, while CNT had 89.33%. The two values were not significantly ($p \leq 0.05$) different, but differed from 17.23% obtained from G80. G80 had significantly lowest disease incidence at this period. On the 6th WAT, disease incidence declined in G40, G60 and CNT, while G80 and SDC increased moderately. Two treatments, G60 and G80 were statistically similar at this period and were significantly lower than the remaining three treatments Table 4.

3.7. Effect of Seed Dressing of Maize with Garlic Extract on the Severity of Stalk and Ear Rot Disease

The result obtained on disease severity showed that on the 2nd WAT, G40 had the highest value, 1.50. This was however statistically similar to 1.00 and 1.33 obtained from G60 and CNT respectively. By the 4th WAT, a different picture emerged. The severity of SERD in CNT had increased to 3.66. This was the highest value. It differed significantly from all other treatments. G40 had the second highest disease severity value of 2.33 Table 4. G60, G80 and SDC had 1.66, 1.00 and 1.00 respectively. The three values were not significantly different. Result for disease severity on the 6th WAT showed that G60, G80 and SDC all had 1.0, while G40 had 1.66. The four values were not significantly different. Significantly highest value of 3.90 was obtained from CNT Table 4.

Table-4. Effect of seed dressing of maize with garlic extracts on the incidence and severity of stalk and ear rot disease.

Treatments	Disease incidence (%)/WAT			Disease severity/WAT		
	2	**4**	**6**	**2**	**4**	**6**
G40	54.66a	86.83a	32.77a	1.50a	2.33b	1.66ab
G60	21.66c	27.23b	23.46b	1.00a	1.66bc	1.00b
G80	0.00d	17.23c	21.36b	0.00b	1.00c	1.00b
CNT	45.00b	89.33a	39.77a	1.33a	3.66a	3.90a
SDC	0.00d	23.61b	31.94a	0.00b	1.00c	1.00b

Note: Means in the same column followed by the same alphabets are not significantly different ($p \leq 0.05$) according to Tukey's test.

Key: WAT= Weeks after transplanting, G40 = Garlic extract at 40% concentration, G60= Garlic extract at 60% concertation, G80 = Garlic extract at 80% concentration, CNT = Control, SDC= Standard check.

3.8. Effect of Seed Dressing of Maize with Garlic Extracts on Cob Fresh Weight (G)

Maize plants from seeds treated with 80% garlic extract, G80, produced the biggest cobs with significantly ($p \leq 0.05$) heaviest weight of 303.86 g. Figure 3. Cobs from G60 and SDC weighed 270.71 and 270.54 g respectively. The two values were statistically similar. The least weight of cobs, 110.44 g was obtained from CNT Figure 3.

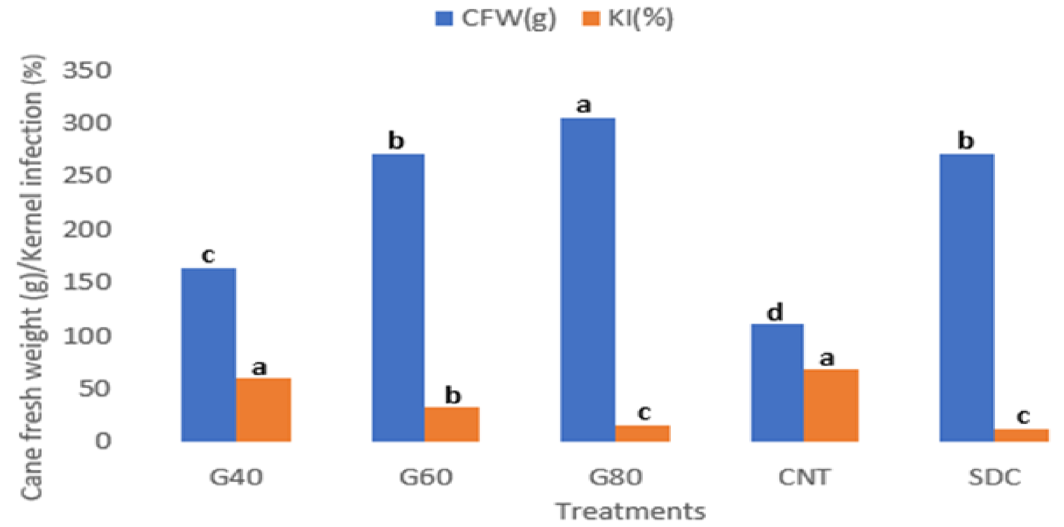

Figure-3. Effect of seed treatment with garlic extracts on cob fresh weight and kernel infection of maize.
Key: CFW = Cob fresh weight, KI= Kernel infection, G40 = Garlic extract at 40% concentration, G60= Garlic extract at 60% concertation, G80 = Garlic extract at 80% concentration, CNT = Control, SDC= Standard check.

3.9. Effect of Seed Dressing of Maize With Garlic Extracts on Kernel Infection with F. Verticillioides

The lowest percentage of kernel infection with *F. verticillioides*, 15.00%, was obtained from G80 Figure 3. This value was however statistically similar to 11.66% obtained from SDC. The highest percentage infection, 68.33% was obtained from CNT. It was statistically not different from 60.00% obtained from G40 Figure 3.

4. DISCUSSION

Result from the *in vitro* studies showed that garlic extract possesses antifungal properties against *F. verticillioides*. The observed trend was an increasing percentage inhibition of mycelial growth of the pathogen as the concentrations of garlic extracts increased. The inhibitory effect of the extract however declined with increasing number of days after inoculation of the pathogen across all the concentrations evaluated. Similar findings on the antifungal properties of garlic extract against other common fungal pathogens of crops have been reported by scholars [20, 25]. The decrease in fungistatic property with increasing days after inoculation may be due to the degradation of the bioactive components in the garlic extract.

The germination of treated maize seeds in FvIM started from the second day after sowing and increased progressively until the fourth day when no new germinations were recorded. A surprising observation was that overall percentage germination decreased with increasing concentration of garlic extracts, such that the lowest overall percentage germination was obtained from maize seeds treated with 80% garlic extract concentration. This was a direct opposite of the impact of garlic extract on *F. verticillioides* in the *in vitro* study and was unexpected. Report from literature however, confirmed the observed result of decreasing germination percentage of maize seeds with increasing concentration of garlic extracts. Cheng, et al. [27] reported that certain allelochemicals are present in garlic. They pointed out that such chemicals exert allelopathic effect on seed germination, root growth and seedling development when garlic extracts are used as seed treatment agent. The most prominent of these chemicals was reported to be Dailly Disulfide (DDS). At low concentrations of garlic extracts, the impact of DDS is minimal or non-existent. Consequently, rapid and high overall percentage germination, root development and good seedling vigour are enhanced. This observation was reported earlier by Perello, et al. [23]. At high concentrations however, DDS concentration increases, so also does it allelopathic effect, bringing about poor seed germination, poor root development and seedlings with less vigour. This explanation must have accounted for the poor overall percentage germination in maize seeds treated with 80% garlic extract concentration, as well as the few number and poorly developed roots exhibited by them within the PIM. It also explains the decreased percentage germination with increased length of time maize seeds were allowed to remain in garlic extracts concentrations before sowing. Ali, et al. [28] reported a similar finding on the effect of garlic extracts on the germination of *Solanum melongena*.

Disease incidence and severity were high in maize plants whose seeds were treated with 40% garlic extract and the control. Seeds from the two treatments germinated quickly and grew rapidly within the *F. verticillioides* infested medium, but succumbed to infection by the pathogen easily after transplanting. This may be due to the fact that the low concentration/non-availability of garlic extract allowed unhindered growth, infection and colonization of the root system by *F. verticillioides*. Pathogenesis continued unhindered after transplanting, resulting in the manifestations of symptoms of SERD.

Few weeks after transplanting, growth activities in maize plants whose seeds were subjected to 80% garlic extract treatment suddenly picked up. By the 8th weeks after transplanting, the treatment produced plants with the best growth parameters. The most reasonable explanation is that few numbers of roots produced, due to the effect of DDS, brought about less contact with *F. verticillioides* while growing within the PIM. The high concentration of garlic extracts around the seeds may have equally inhibited the growth of *F. verticillioides*. These facts may have prevented infection and the onset of pathogenesis. It is also possible that a defense response may have been initiated in the maize seedlings as a result of some priming effects that may have been conferred by garlic extracts at 80% concentration. Hayat, et al. [29] had reported a similar finding. The fact that disease incidence and severity were very low in the treatments may be a confirmation of this view.

The control had the least fresh weight of cob and an equally high percentage of kernel infection with *F. verticillioides*. This is understandable, considering the high incidence and severity of SERD. Poor yield, in terms of quality and quantity is often associated with diseased crops, especially in susceptible varieties [10, 30]. The high percentage of infected kernels, especially in 40% garlic slurry and the control is a confirmation of report from literature on the seeds as the most important source of transmission of *F. verticilliodes* [6].

5. CONCLUSION AND RECOMMENDATION

Results from this study showed that garlic extracts possess antifungal properties against *F. verticillioides*. It may also have conferred protection on maize plant against SERD at 80% concentration, leading to improved growth performance and yield. Further work needs to be done on the DDS component of the extract before outright recommendation of its use in the management of SERD of maize. There is also a need for the evaluation of garlic extracts as foliar spray against SERD of maize.

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