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COMBINATION OF BIOLOGICAL CONTROL AGENTS AND GARLIC (*ALLIUM SATIVUM*) EXTRACT IN REDUCING DAMPING-OFF DISEASE OF TOMATO

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Abstract

Damping-off *(Pythium aphanidermatum)* is a soil-borne disease which accounts for seedling mortality and significant yield losses in tomato production. Laboratory and screenhouse experiments were conducted in 2017, with a repeated field trial in 2018 to evaluate the efficacy of combining three biological control agents (BCAs), *Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* with *Allium sativum* extract for the integrated management of the disease in tomato crop. Treatments were laid out in a completely randomized design and randomized complete block design in the screenhouse and field experiments, respectively with eighteen treatments and three replications. The BCAs and extract were formulated and applied using seed treatment and soil sprinkling methods. Treatment combinations of BCAs with *A. sativum* were more effective in the reduction of mycelial growth of the pathogen with inhibitory values that ranged between 77.6-91.2% than single inoculation. Seed treatment before planting was more effective than soil sprinkle method, reducing pre-emergence and damping-off incidence to between 6.8-18.3% and 9.7-26.3% under screenhouse and field conditions, respectively than the sprinkling method. Soil sprinkle with *T. harzianum* in combination with *A. sativum* extract had the highest cumulative tomato fruit yield of 902 kg/ha^{-1} under field conditions. This study showed that combined application of BCAs and *A. sativum* extract reduced damping-off disease and thereby improved the fruit yield of tomato.

Keywords: *Bacillus subtilis*, damping-off, integrated management, Seed treatment, Soil sprinkle, Tomato

Introduction

Tomato (*Solanum lycopersicum* L.) is the most commonly grown and consumed vegetable worldwide (Kurze *et al*., 2018), because of its rich source of vitamin A and it plays an important role in maintaining the human health being a high source of lycopene which acts as an antioxidants and free radical scavenger.

Nigeria is ranked second largest producer of tomato in Africa, with an output of 1.81 million metric tonnes of tomato annually under rainfed production (Ibitoye *et al*., 2015). It is a food and cash crop which contributes significantly to the economy of the local population (Kator *et al*., 2015).

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The crop is propagated mainly through seeds by farmers, especially in Nigeria, that rely on their own saved seeds from previous season for planting. These helpless farmers often suffer from seed and seedling losses resulting in significant yield reduction arising from cultivation of unhealthy seeds. Among the main constraints to tomato production is the damage caused by soil-borne pathogens, mainly bacteria and fungi, which result in severe losses in production (Barone and Frusciante, 2007). Tomato plants are susceptible to many soil-borne fungal genera such as *Fusarium*, *Rhizoctonia*, *Pythium* and *Phytophthora*, causing serious diseases such as root rot, wilt and damping-off (Srinon *et al.,* 2006).

Pre and post emergence damping-off disease of tomato is caused by *Pythium aphanidermatum*, a soil-borne pathogen which results in seed rot or death of young seedlings (Ashwathi *et al.,* 2017). The pathogen can survive in the soil through the production of perennating structures such as oospores or sporangia (Agrios, 2005).

Currently, damping-off disease *(Pythium aphanidermatum)* of tomato is being managed in Nigeria through the use of synthetic fungicides such as Benlate. Although this approach has produced some desired results, the indiscriminate use has contributed to adverse environmental effects including reduction in beneficial soil-borne microbes and evolvement of new virulent races of pathogens (Thakur and Tripathi, 2015). The use of biocontrol agents (BCAs) in combination with other organic materials is a suitable alternative to chemical applications which ensures sustainable disease management within the concept of an integrated approach. The biocontrol agents are highly effective, inexpensive with excellent shelf life and suitable method of delivery (Someshwar *et al.,* 2013). The antimicrobial activities of garlic, a common Nigerian spice, has been reported (Obagwu and Korsten, 2003). Botanicals are cheap, biodegradable and eco-friendly. However neither of these strategies could achieve a complete control.

Therefore, this study was designed to evaluate the effect of different formulation and combination of control options for the integrated management of damping-off disease of tomato under screenhouse and field conditions

Materials and Methods

Experimental site

The *in vitro* experiment was conducted in the Plant Pathology laboratory of the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria. The *in-vivo* experiments were carried out in the screen house during rainy seasons in 2017 with a repeated field trial in 2018 to validate the screenhouse results. Ibadan is located on latitude 7°33´N 3°56´E with an elevation of 213 m above sea level and annual rainfall ranging between 1250 mm and 1500 mm in the Derived savanna agroecological zone of Nigeria.

Isolation and preparation of fungal inoculum

Pythium aphanidermatum was isolated from roots of tomato seedlings that showed damping-off symptoms such as water-soaked and shriveled stem base leading to rotting of the collar immediately above soil level. Soil debris was washed off the roots, surface-sterilized in 10 % sodium hypochlorite for one minute and rinsed in three changes of sterile distilled water. Infected tissues were then cut into small sizes measuring 2mm× 2mm washed in sterile distilled water for 1 minute and dried on sterile filter paper. Tissue samples were plated on water agar medium in 9 cm Petridishes and incubated at 28±2°C for 10 days under alternating 12 h photoperiod of light and darkness. The fungus was then transferred to 1.7 % corn meal agar (CMA) medium amended with 100µl pimaricin (Davet and Rouxel, 2000). Pathogen cultures were maintained on corn meal agar slants at 8°C. A 3-mm diameter agar plug of mycelia was sub-cultured on a freshly prepared water agar in Petridishes and incubated in the dark at 28-30°C (Zamanizadeh *et al*., 2011). The culture plates were observed for 5 to 10 days for the development of reproductive structures such as sporangia and oospores. Identification of species was carried out using a standard monograph guide for the *Pythium* species (Plaats-Niterink, 1981) and following confirmation at the Mycological Herbarium at the International Institute of Tropical Agriculture, Ibadan, Nigeria.

Substrate for the growth of *P. aphanidermatum* was prepared using sorghum grains as carrier according to modified method of Khiyami *et al*. (2014). Grain weights of 50 g sorghum were poured into a 500-ml Erlenmeyer flasks, each containing 100 ml of water. Contents of bottles were autoclaved at 121°C and 1.05 kg/cm^2 pressure for 30 min. Excess water in the sterilized sorghum grains was drained off using sterile cheese cloth. Fungal inoculum was taken from the edge of a 7-day old culture on Potato Dextrose Agar and introduced into bottles and allowed to colonize sorghum grains for 14 days. At full mycelial ramification, the substrate in each bottle was emptied on sterile filter paper, air-dried in a Laminar flow hood for two days and then ground to fine powder using a rotary blender and sieved. Sandy loam soil was sterilized at using an Sussman Electrical Sterilizer Model SG 12-04-220, USA at 71°C for 1h prior to inoculum application at 10 g/kg of sterilized soil (Adandonon *et al*., 2006).

Isolation and formulation of biological control agents

Trichoderma species were isolated from tomato rhizosphere from four different locations using serial dilution method. Ten grammes of soil adhering to roots of each sample were dissolved in 100 ml of sterile distilled water, shaken for 20 minutes on a rotary shaker, and diluted serially in sterile distilled water. One ml from each of 10^{-4} to 10^{-6} dilutions was dispensed on acidified potato dextrose agar with ten replicates and incubated at 28±2°C for 3 days. Selected cultures

with typical green *Trichoderma* colonies were purified and identified using standard taxonomical keys for *Trichoderma* (Rifai, 1969, Bissett, 1991). Mass production of *Trichoderma* species was carried out using modified Richards medium (Harman *et al*., 1991) which contained 10 g KNO_3, 5 g KH_2PO_4, 1.3 g anhydrous $MgSO_4$, 8 g sucrose, 20 mg $FeCl_3$ and 100 ml of tomato juice in 1 litre of sterile distilled water. An aliquot of 100 ml of the medium was dispensed in 250- ml conical flask and inoculated with a 5-mm agar plug from a 7-day-old culture on acidified potato agar medium and incubated at 28±2°C. After 21 days incubation period, the mycelia were harvested and air-dried in plastic plates under sterile conditions at room temperature (28-30°C) for two days. They were thereafter ground to a fine powder in a blender and stored at 4°C in polyethylene bags until it was required for the study. Colony forming units in formulation of *Trichoderma* spp. mixtures was adjusted to 3 x 10^7 cfu/g (Nashwal *et al.,* 2008). A powder formulation of *Bacillus subtilis* containing 10^8 colony forming units per gram (cfu/g^{-1}) used in this experiment was collected from the Microbiology unit of the International Institute of Tropical Agriculture, Ibadan, Nigeria.

Inhibitory effect of biological control agents and *Allium sativum* extract on radial mycelial growth of *Pythium aphanidermatum in vitro*

Fresh healthy bulbs of *A. sativum* were washed with sterile distilled water (SDW) and ground to a fine pulp using a rotary blender MJ-BL 40G1 JA. Four samples of 50 g, 100 g, 200 g and 250 g were weighed separately. The weights were ground to a pulp of each sample weight and dissolved in 1litre of SDW to produce final extract concentrations of 5%, 10%, 20% and 25% w/v, respectively. The extract suspensions were agitated intermittently for 10 minutes and left in the Laminar flow hood overnight to enable the active constituents dissolve in the medium. The suspension was then filtered through sterile cheese cloth into Erlenmeyer flask. An aliquot of 1ml was dispensed into 15 ml acidified PDA in 9 cm diameter Petridishes and swirled gently to allow proper mixing of extract and medium. After solidification of the PDA, a 3-mm cork borer was used to obtain mycelial disc from the edge of 7-day old colonies of *P. aphanidermatum* and placed at the centre of each Petridish. Biological control agents, *T. viride, T. harzianum* and *B. subtilis* were inoculated at four equidistant points relative to the pathogen at the center of each Petridish. The control consisted of the pathogen grown on unamended PDA. While a synthetic mancozeb fungicide applied at the rate of 0.5 g/l served as positive check. Each concentration consisted of four replicates in a completely randomized design with 19 treatments. Petridishes were incubated at 28±2°C and the radial growth of the colony treatment was measured at 5 days of incubation and expressed as a percentage of the control.

Screenhouse and field evaluation of effect of single and combined application of *Allium sativum* extract and biological control agents on incidence of damping-off disease

Healthy seeds of a susceptible tomato variety UC82B used in this experiment were obtained from a certified agricultural commercial seed center in Ibadan, Nigeria. Treatments were applied using two methods i.e. seed treatment and soil sprinkle (Harman *et al*., 1991). The seeds were slightly moistened with sterile distilled water and then treated with the powdered formulation of *Trichoderma* species and *B. subtilis* with spore concentration of 3×10^7 cfu/g^{-1} and 10^8 cfu/ g^{-1} respectively as previously described, at the rate of 10 g/kg seeds. Sterilized starch was added to the powdered seed mixture at 5 g/kg as adhesive and mixed thoroughly in aseptic plastic bowls before being planted in soil artificially inoculated with the pathogen at 10 g/kg of soil 2 days before planting. In the seed treatment with the extract, a 25% w/v *A. sativum* concentration which proved to be most effective in the *in vitro* trial was selected and used in the screenhouse and field experiments. It involved soaking the tomato seeds in the extract solution for 10 minutes. The seeds were then air-dried at room temperature (28±2°C) for 1 h before planting. Soil inoculation with *P. aphanidermatum* was done at 10 g/kg of sterilized soil as previously described. The soil sprinkle method involved mixing of 0.5 g powdered formulation of BCAs with 5 g of sterilized soil and sprinkling of the mixture in each planting hole. Experimental pots measuring 25 cm in diameter were each filled at 90% capacity with 5 kg sandy loam soil sterilized using a Sussman Electrical Sterilizer Model SG 12-04-220, USA at 71°C for 2 hours. Four seeds were planted per pot which were later thinned to two and the experimental layout was a randomized complete design with three replications. Screen house environmental conditions varied between 74±2% to 90±2% relative humidity with a temperature range of 28±2°C. The experiment consisted of 18 treatment combinations: T_1=*Trichoderma viride* seed treatment, T_2=*Trichoderma viride* soil sprinkle, T_3=*Trichoderma harzianum* seed treatment, T_4=*Trichoderma harzianum* soil sprinkle, T_5=*Bacillus subtilis s*eed treatment, T_6=*Bacillus subtilis* soil sprinkle, T_7=*Allium sativum* seed treatment, T_8=*Allium sativum* soil sprinkle, T_9=*Trichoderma viride* seed treatment + 25% *A. sativum*, T_{10}=*Trichoderma viride* soil sprinkle + 25% *A. sativum*, T_{11}=*T. harzianum* seed treatment + 25% *A. sativum*, T_{12}=*T. harzianum* soil sprinkle + 25% *A. sativum*, T_{13}=*Bacillus subtilis s*eed treatment + 25% *A. sativum*, T_{14}=*Bacillus subtilis* soil sprinkle + 25% *A. sativum*, T_{15}=Inoculated and untreated, T_{16}=Uninoculated and untreated, T_{17}=Mancozeb seed treatment and T_{18}= Mancozeb soil sprinkle.

The percentage of pre-emergence and post emergence damping-off were recorded weekly and calculated:

$$\text{Pre-emergence damping-off} = \frac{\text{No. of ungerminated seeds}}{\text{Total number of planted seeds}} \times \frac{100}{1}$$

$$\text{Post-emergence damping-off} = \frac{\text{No. of infected seedlings}}{\text{Total number of germinated seedlings}} \times \frac{100}{1}$$

The effect of treatment combinations on growth and yield parameters were determined according to Khiyami *et al*. (2014). Disease incidence was calculated by expressing the number of symptomatic plants as a percentage of the total plants evaluated. Disease control was calculated and expressed as a percentage:

Disease control = [1-DT/DC]× 100

Where DR = Disease reduction, DT = Disease incidence on treatment

DC = Disease incidence on control.

Disease severity rating was evaluated on a scale of 1-5 using the modified method of Adandonon *et al*. (2006):

1=No symptoms, 2=No wilting of leaves, seedlings fell on the ground after fourth day, 3=Wilting of leaves on 3rd day with plants falling to the ground on fourth day, 4=Wilting of leaves on third day with plants falling on ground same day, 5=Wilting of leaves on second day with seedlings falling to the ground same day or next day. 6=Wilting of leaves on first day with seedlings falling over on second day.

Disease severity values obtained were converted to percentages using the method os Assad *et al.* (2010):

$$\text{Disease severity} = \frac{\text{Sum of disease ratings}}{\text{Total number of ratings}} \times \frac{100}{\text{Maximum disease grade}}$$

The field experiment was conducted in 2018 to validate results obtained in the screenhouse in 2017 at the University Teaching and Research Farm, Ibadan, Nigeria. Therefore, the same treatments that were used in the screenhouse were also evaluated in the field trials. However, the experiment was conducted under natural conditions in a planting site with history of damping -off disease. Each plot was 3x3 m in area with three rows of 3m in length and 50 cm in width. The trial consisted of plots (treatments) in a randomized complete block design with three replications. Four tomato seeds were planted per hole at a spacing of 60 cm × 50 cm which were later thinned to two. All parameters that were assessed in the screenhouse experiment were also evaluated in the field. Seed-to-plant transmission of damping off disease was evaluated by planting seeds harvested from control plants in the previous screenhouse experiment that were inoculated with *P. aphanidermatum* but untreated. Seeds that were harvested from uninoculated plants served as control. Data collected included percentage pre- and, post-emergence damping-off, and seedling mortality as described above.

Plant growth parameters such as plant height, number of leaves per plant, stem diameter, fresh weight of plant, and yield were determined according to Abd-El-Khair *et al.* (2010).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of the Statistical Analysis System Version 9.1, Institute (SAS institute Inc., 2002). ANOVA was made by F variance test and the pair comparisons were done by Duncan's Multiple Range Test (DMRT) at P=0.05.

Results and Discussion

The inoculation of individual biological control agent reduced radial mycelial growth of *P. aphanidermatum* to between 64.8-67.2% (Table 1). Similarly, the four extract concentrations of *A. sativum* evaluated also had inhibitory effect on growth of the test pathogen varying from 54.2-67.3% relative to the control which was neither amended with BCA nor extract with 100% growth and no inhibition. However, the efficacy of the extract increased with concentration, being most effective at 25% w/v. Generally, treatment combinations of BCAs with *A. sativum* proved to be more effective in the reduction of mycelial growth of the pathogen with inhibitory values that ranged between 77.6-91.2%. Their effects were significantly higher than those of single inoculation of individual BCA or extract against the pathogen.

Table 1. Inhibitory effect of *Allium sativum* extract combinations with BCAs on mycelia growth of *P. aphanidermatum in-vitro*

Treatment	Mycelial growth (mm)	Reduction (%)
Pathogen alone	100a	0.0c
Pathogen + *T. viride*	34.8bc	65.2bc
Pathogen + *T. harzianum*	32.8bc	67.2b
Pathogen + *B. subtilis*	39.2b	64.8bc
Pathogen + 5% *A. sativum*	45.8ab	54.2c
Pathogen + 10 % *A. sativum*	41.2b	58.8c
Pathogen + 20 % *A. sativum*	34.8bc	65.2bc
Pathogen + 25 % *A. sativum*	32.7bc	67.3b
Pathogen + *T. viride* + 5% *A. sativum*	22.4cd	77.6ab
Pathogen + *T. viride* + 10% *A. sativum*	13.5d	86.5a
Pathogen + *T. viride* + 20% *A. sativum*	11.7d	88.3a
Pathogen + *T. viride* + 25% *A. sativum*	12.3d	87.7a
Pathogen + *T. harzianum* + 5% *A. sativum*	19.1cd	80.9ab
Pathogen + *T. harzianum*+ 10% *A. sativum*	10.3de	89.7a
Pathogen + *T. harzianum*+ 20% *A. sativum*	13.2d	86.8a
Pathogen + *T. harzianum*+ 25% *A. sativum*	8.8de	91.2a
Pathogen + *B. subtilis* + 5% *A. sativum*	32.2bc	67.8b
Pathogen + *B. subtilis* +10% *A. sativum*	27.1c	72.9b
Pathogen + *B. subtilis* + 20% *A. sativum*	20.4cd	79.6ab
Pathogen + *B. subtilis s* + 25% *A. sativum*	18.1cd	81.9ab
Pathogen + Mancozeb	9.6dde	90.1a

Means with same letters along a column are not significantly different using DMRT test at P=0.05.

However, inoculation of *T. harzianum* in combination with 25% w/v *A. sativum* concentration had significantly higher inhibition of the the test pathogen than other treatments and control. In contrast, inoculation of *B. subtilis* in the dual culture was least effective among the BCAs. All the BCAs significantly ($P<0.05$) inhibited radial mycelial growth of *P. aphanidermatum* when applied either singly or in combination with varying concentrations of *A. sativum* in the *in vitro* trial compared to the control. This result was consistent with the findings of Idowu *et al.* (2016) who reported the ability of *Trichoderma* species to inhibit *Pythium* spp, causing damping-off disease in seedlings of sweet pepper variety. A zone of inhibition was observed between *B. subtilis* and the pathogen with an apparent lysis of the pathogen mycelia, which may have been due to the production of metabolites or antibiotics by the BCA (Demoz and Korsten, 2006); while *Trichoderma* spp. grew on the mycelia of the test pathogen, under microscopic examination suggesting that the mode of control by the BCAs could be hyper parasitism (Howell, 2003; Harman *et al*,. 2004). *Allium sativum* extract also reduced mycelial growth of the test pathogen, but was more effective when applied in combination with the BCAs. Its inhibitory action may be due to its active ingredient 'allicin', which enhances its potency against plant pathogens (Obagwu and Korsten, 2003).

Screenhouse and Field evaluation of single and combined application of *A. sativum* leaf extract and Biological control agents on incidence of damping-off disease

Tomato seeds that were treated with inoculum powder of either the BCA or extract alone had better inhibitory effect on the test pathogen than the control in the screenhouse experiment of 2017 and the repeated field experiment in 2018 (Table 2). Seed treatment before planting was more effective than soil sprinkle method, reducing pre-emergence damping off incidence to between 6.8-18.3% and 9.7-26.3% under screenhouse and field conditions respectively. Treatment of tomato seeds with *T. harzianum* in combination with *A. sativum* extract proved to be most effective in the single application of the BCAs with a pre and post emergence disease reduction. In contrast, the application of *B. subtilis* either as seed treatment or soil sprinkle was least effective among the BCAs in overall disease incidence, severity and control. Although single dosages of the BCAs and *A. sativum* extract significantly ($P<0.05$) reduced damping-off disease, better results were obtained with the treatment combinations. Seeds that were treated with a mixture of BCA and *A. sativum* extract significantly ($P<0.05$) reduced disease incidence relative to single treatments and compared well to mancozeb fungicide.

Table 2. Effect of *Allium sativum* extract combinations with BCAs on pre and post emergence damping- off in the screenhouse and field conditions

Treatment	Pre-emergence						Post emergence			
	Disease Incidence %		Disease control (%)		Disease incidence %		Disease severity (%)		Disease control (%)	
	Screenhouse	Field	Screenhouse	Field	Screenhouse	Field	Screenhouse	Field	Screenhouse	Field
Trichoderma viride seed treatment	9.0b	14.8bc	67.9bc	53.8bc	22.2ab	23.8ab	48.0bc	24.0bc	72.8b	76.1ab
Trichoderma viride Soil sprinkle	7.3bc	1.5bc	55.2bc	65.8b	26.1b	33.4b	42.0bc	32.0bc	71.6b	65.5bc
Trichoderma harzianum seed treatment	12.1b	11.5bc	77.1b	74.4ab	25.7b	31.7cd	42.0bc	54.0b	81.7ab	75.2ab
Trichoderma harzianum soil sprinkle	11.1b	15.0bc	73.6b	58.2bc	26.1b	20.7cd	34.0c	46.0b	80.2ab	73.1b
B. subtilis seed treatment	16.7ab	22.2b	54.8bc	54.1bc	33.8b	49.4ab	74.0ab	74.0ab	63.1bc	62.5bc
B. subtilis soil sprinkle	18.3ab	18.8b	51.1bc	58.1bc	21.5ab	47.1bc	76.0ab	81.0a	54.7c	56.4c
A. sativum seed treatment	15.3ab	22.3b	51.7bc	47.6bc	34.3ab	47.2b	68.0ab	62.0ab	49.6c	50.2c
A. sativum soil sprinkle	15.1ab	26.3ab	53.6bc	50.7bc	28.3ab	31.3bc	62.0b	50.0b	53.1c	51.8c
T. viride seed treatment + 25% *A. sativum*	10.2b	9.7c	82.8ab	72.1ab	28.2cd	17.6c	46.0bc	44.0b	88.8a	80.6ab
T. viride soil sprinkle treatment + 25% *A. sativum*	9.2b	11.8bc	75.7b	78.5a	22.4b	21.5c	32.0c	46.0b	90.1a	81.2ab
T. harzianum seed treatment + 25% *A. sativum*	6.8bc	19.3c	88.3a	80.0a	19.7b	20.4cd	26.0c	28.0bc	93.7a	81.6ab
T. harzianum Soil sprinkle + 25% *A. sativum*	4.3bc	7.7c	64.8bc	71.1ab	21.2b	23.7cd	32.0c	54.0b	80.3ab	77.1ab
B. subtilis seed treatment + 25% *A. sativum*	14.8ab	25.3ab	63.4bc	33.2c	42.8ab	46.1b	62.0b	72.0ab	59.7c	54.2c
B. subtilis Soil sprinkle + 25% *A. sativum*	13.1ab	18.4b	67.7bc	24.8cd	43.3ab	49.2ab	74.0ab	70.0ab	53.4	66.3bc
Inoculated and untreated	72.7a	78.3a	0.0c	0.0d	78.8a	81.7a	84.0a	92.0a	0.0cd	0.0cd
Uninoculated and untreated	0.0c	10.6c	0.0c	0.0d	0.0d	22.1bc	20.0cd	30.0bc	0.0cd	0.0cd
Mancozeb seed treatment	5.1bc	9.2cd	70.4b	72.8ab	17.5b	11.2cd	44.0bc	26.0bc	86.7a	83.2ab
Mancozeb soil sprinkle	4.1bc	8.2c	87.4a	78.3a	9.5b	14.2c	58.0b	28.0bc	88.4.7a	87.2a

Means with same letters along a column are not significantly different using DMRT test at P=0.05.

These results agree with the earlier report of Adanonon *et al*. (2006) that found seed treatment with BCAs to be more effective than using the soil drench method in the control of soil-bone *Sclerotium rolfsii*. Also, *Trichoderma* spp. used as seed treatment was reported to significantly reduce the incidence of damping-off disease than other formulation methods (Idowu *et al*., 2016). Disease incidence was apparently higher under field conditions than in the screenhouse. The better disease control achieved in the screenhouse may be due to the asepetic condition that was maintained in the screenhouse which included sterilization of soil that was used in planting and the controlled environment. On the contrary, the environmental conditions could not be regulated in the field, where planting was also done without soil sterilization under natural conditions. However, under field conditions, it was observed that some of the uninoculated and untreated plants were symptomatic, although with a comparatively lower disease incidence. Disease incidence and severity were significantly ($P<0.05$) lower in case of treatment combinations than single treatment dosage and untreated control. The application of the treatment combination as seed treatment was generally more effective than the sprinkle method in reducing both incidence and of disease severity. Seed treatment with *T. harzianum* in combination with *A. sativum* extract had significantly ($P<0.05$ lower incidence when compared to other treatments and the untreated control.

Trichoderma harzianum when applied in combination with 25% w/v concentration of *A. sativum* extract was most effective in reducing damping-off incidence, severity and control. Several reports had corroborated the efficacy of *T. harzianum* in the control of soil-borne pathogens (Howell 2003; Manjula *et al.*, 2005; Kumari *et al.*, 2015). In contrast, *Bacillus subtilis* was least effective among the BCAs in disease control both in the screenhouse and field. While *Trichoderma* spp. are fortified with several methods of pathogen control such as rapid competition and colonization of substrate, hyperparasitism and production of lytic enzymes, *B. subtilis* rely mainly on the mechanism of antibiosis (Dania *et al.*, 2016).

All treatments and their combinations significantly ($P<0.05$) influenced plant height and stem diameter both in the screenhouse and field trials (Table 3). However, tomato plants that were planted under field conditions had significantly higher shoot weight, number of leaves and fruit yield than those grown in the screenhouse. These might be due to the inability of the tomato plant roots in the screenhouse to expand fully due to the reduced space in each experimental pot, hence limited access to nutrients which ultimately influenced the overall yield compared to those that were planted in the open and unrestricted field (Muriungi *et al*., 2014).

Table 3. Effect of *Allium sativum* extract combinations with BCAs on growth and yield parameters of tomato in screenhouse and field conditions

Treatment	Plant height (cm)		Number of leaves		Stem diameter (cm)		Fresh shoot wt (g)		Yield (kg ha^{-1})	
	Screenhouse	Field	Screenhouse	Field	Screen house	Field	Screenhouse	Field	Screenhouse	Field
T. viride seed treatment	63.2bc	67.2ab	98.2b	106.4bc	0.60a	0.61a	94.4d	111.7d	722.5f	731.1e
T. viride Soil sprinkle	71.1ab	68.2ab	107.3a	101.3cd	0.63a	0.72a	105.2bc	101.2de	692.2g	852.5b
T. harzianum seed treatment	64.3bc	68.6ab	85.1c	117.2ab	0.65a	0.64a	98.4cd	128.4cd	801.7c	790.6d
T. harzianum soil sprinkle	64.8bc	72.8ac	98.0b	114.5b	0.61a	0.58ab	103.5c	153.5b	775.4de	802.1cd
B. subtilis seed treatment	68.4b	64.0bc	96.1b	104.3c	0.38b	0.56ab	112.3ab	182.3a	784.2d	807.5c
B. subtilis soil sprinkle	73.0ab	75.1a	104.2ab	100.2cd	0.49ab	0.54ab	101.5c	151.5bc	774.5de	781.2de
A. sativum seed treatment	63.2bc	60.8b	98.1b	109.4bc	0.72a	0.69a	123.9a	143.9bc	770.6e	806.7c
A. sativum soil sprinkle	66.1b	64.6b	103.2ab	101.5cd	0.55ab	0.61ab	120.8a	180.8a	801.2c	867.4ab
T. viride seed treatment + 25% *A. sativum*	63.7bc	68.9sb	90.8bc	107.4bc	0.52ab	0.54ab	107.1b	142.1bc	831.2b	777.7de
T. viride soil sprinkle treatment + 25% *A. sativum*	77.1a	75.2a	92.6bc	111.2b	0.61a	0.68a	114.3ab	164.1b	820.7bc	851.3b
T. harzianum seed treatment + 25% *A. sativum*	64.5bc	66.8b	103.9ab	118.4ab	0.64a	0.67a	109.7b	177.3ab	886.1a	688.5ef
T. harzianum soil sprinkle + 25% *A. sativum*	68.2b	70.6ab	96.6b	108.8bc	0.60a	0.58ab	101.7c	126.4cd	882.3ab	902.2a
B. subtilis seed treatment + 25% *A. sativum*	63.3bc	67.1ab	94.3bc	117.6ab	0.52ab	0.57ab	108.3b	103.8de	770.3e	707.5ef
B. subtilis Soil sprinkle + 25% *A. sativum*	62.7bc	65.5b	102.4ab	122.2a	0.58ab	0.65a	107.8b	114.2d	788.8d	835.6bc
Inoculated and untreated	66.2b	69.9ab	103.2ab	100.1cd	0.63a	0.78a	115.2ab	132.2c	603.3g	634.1ef
Uninoculated and untreated	66.1b	65.7b	97.0b	113.3b	0.43b	0.56ab	100.7c	114.7d	732.2ef	808.4 c
Mancozeb seed treatment	66.3b	65.2b	105.5ab	112.7b	0.57ab	0.55ab	120.9a	150.9b	702.5fg	865.4ab
Mancozeb soil sprinkle	74.3ab	69.3ab	109.5a	117.7ab	0.63ab	0.58ab	122.9a	148.9bc	794.6d	728.4e

Means with same letters along a column are not significantly different using DMRT test at P=0.05.

Table 4. Effect of treatments on percent incidence of seed-to-plant transmission of damping-off disease in seedlings grown from infected tomato seeds

Treatment	Screen house trial in 2017					Field trial in 2018				
	7WAS	14DAS	21DAS	28DAS	Mortality (%)	7WAS	14DAS	21DAS	28DAS	Mortality (%)
Trichoderma viride seed treatment	3.8b	7.0ab	8.9b	13.1ab	7.3b	4.1bc	9.2b	10.7	11.3bc	9.2b
Trichoderma viride soil sprinkle	5.1b	10.1ab	10.2b	12.3ab	7.1b	3.5bc	8.1b	12.1b	12.8bc	7.4bc
Trichoderma harzianum seed treatment	3.2b	4.9b	9.3b	8.8b	6.7b	6.1b	8.4b	7.7bc	10.4c	7.7bc
Trichoderma harzianum soil sprinkle	3.4b	6.3ab	8.4b	9.7b	5.4b	10.8ab	7.8b	9.5b	11.8bc	8.6b
Bacillus subtilis seed treatment	7.5ab	9.5ab	12.5ab	14.2ab	8.2ab	4.8b	10.5ab	11.2b	13.4bc	13.6ab
Bacillus subtilis soil sprinkle	6.1b	12.3ab	14.6ab	15.8ab	10.1ab	7.7b	10.2ab	14.2ab	16.1b	14.3ab
Allium sativum seed treatment	4.1b	9.4ab	10.8b	15.0ab	9.5ab	6.8b	8.4b	10.1b	14.8b	11.3ab
Allium sativum soil sprinkle	6.2b	8.2ab	12.1ab	17.3ab	13.3ab	5.8b	11.2ab	14.4ab	17.4b	11.8ab
T. viride seed treatment + 25% *A. sativum*	2.5b	3.5b	8.1b	9.9b	7.1b	3.7bc	7.3b	10.5b	12.4bc	8.1b
T. viride soil sprinkle +25% *A. sativum*	1.7bc	4.0b	7.7b	13.3ab	6.3b	3.4bc	5.9bc	9.9b	10.7c	7.4bc
T. harzianum seed treatment + 25% *A. sativum*	2.9b	3.8b	6.5b	7.9b	3.7b	5.7b	8.8b	11.8b	14.0b	9.3b
T. harzianum soil sprinkle + 25% *A. sativum*	6.6b	7.8ab	9.2b	10.6b	10.4ab	6.3b	6.0bc	8.7bc	12.6bc	9.5b
B. subtilis seed treatment + 25% *A. sativum*	6.0b	10.4ab	15.3ab	16.9ab	9.2ab	8.8ab	12.6ab	12.0b	20.2ab	10.8b
B. subtilis soil sprinkle + 25% *A. sativum*	7.9ab	11.2ab	18.2ab	18.8ab	11.6ab	8.1ab	13.9ab	16.6ab	17.5b	12.3ab
Infected and untreated	12.7a	26.1a	47.6a	77.4a	36.8a	15.1a	38.6a	53.5a	83.3a	68.7a
Healthy and untreated	0.0b	0.0b	0.0c	0.0bc	0.0bc	3.7bc	4.8bc	5.7bc	10.2c	10.8b
Mancozeb seed treatment	2.3b	3.5b	4.1bc	7.7b	3.0b	4.3bc	5.4bc	5.9bc	5.9cd	8.4b
Mancozeb soil sprinkle	6.2b	4.1b	7.4b	5.9b	4.0b	6.3b	7.0b	8.5 bc	12.2bc	10.1ab

DAS= Days after sowing. Means with same letters along a column are not significantly different using DMRT test at P=0.05.

Soil sprinkle with *T. harzianum* in combination with *A. sativum* extract had the highest cumulative tomato yield of 902 kg/ha^{-1} under field conditions. Fruit yield was significantly higher in plots that were treated with either BCAs or extract alone and their combinations than those of inoculated but untreated that served as control. The inherent ability of *Trichoderma harzianum* to reduce disease incidence of several pathogens by stimulating vegetative growth and enhancing root development and overall yield of the treated plants were also reported (Harman *et al*., 2004; Singh and Singh, 2004).

The application of treatments reduced the incidence of seed-to-plant transmission of damping-off disease among seeds that were inoculated before planting (Table 4). Seed-to-plant transmission was reduced to between 7.9-18.8% and 10.2-20.2% in screenhouse and field trials respectively relative to control. Similarly, the treatment combinations reduced seedling mortality better than individual application at 28 days after inoculation.

Disease incidence was significantly lower among plants that were inoculated and treated with combinations of BCAs and *A. sativum* extract than control plants in both trials. Also, seedling mortality was significantly reduced ($P<0.05$) among the treatments compared to control. However, seedling mortality was comparatively higher under field conditions than the screenhouse. Healthy seeds that were uninoculated and untreated were asymptomatic in the screenhouse; however, some of the emerging seedlings became symptomatic under field condition.The combination of biological control agents with *Allium sativum* extract significantly reduced the incidence, severity and seedling mortality caused by damping-off disease. Therefore, the treatment combination as shown in this study could be explored to reduce annual seedlings and yield losses due to the damping-off disease among tomato farmers in Nigeria.

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