FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 3 No. 1, March, 2019, pp 152 - 159

GROWTH RESPONSES OF NANOPARTICLES-TREATED TOMATO (*Solanum lycopersicum* L.) CULTIVARS TO FUNGAL DISEASE STRESS

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ABSTRACT

A study was carried out to evaluate the effect of nanoparticles treatment on the response of different tomato cultivars to fungal disease stress in Lafia, Nasarawa State, Nigeria. Two grams each of seeds belonging to the Roma Savana and UC 82B tomato cultivars were exposed to Alphaspin Nanoparticles for 15, 30, 45 and 60 minutes respectively, and challenged with 21 days old culture filtrates of *Sclerotinia sclerotiorum* and *Alternaria triglochinicola*, using the poisoned food technique. Germination and growth responses of Nanoparticles-treated seeds plated on sterile solidified water agar impregnated with culture filtrates of fungal pathogens were assessed for a duration of 5 days. Seeds of the Roma Savanna tomato variety challenged with fungal disease stress after treatment with Alphaspin nanoparticles had the highest mean total percentage germination of 13.32, root length 0.68cm and shoot length 36.67cm, compared to UC 82B with 13.24% germination, 0.61cm root length, and 5.87cm mean total shoot length. UC 82B had the highest and significantly different ($P \leq 0.05$) stem girth of 62.41cm from that of Roma Savanna, 36.67cm. Differences in mean total disease effect of the test fungi on growth parameters of the studied tomato varieties were significant ($P \leq 0.05$). Alphaspin nanoparticles promoted germination of treated tomato seeds under conditions of biotic stress induced by toxic metabolites of fungal pathogens. The findings of this research further emphasizes the usefulness of nanotechnology in enhancing crop productivity, and could be harnessed in the improvement of tomato yield and productivity in the study area.

Keywords: Culture Filtrates, Disease Stress, Fungal Pathogens, Nanoparticles, Tomato Cultivars

INTRODUCTION

The cultivated tomato, *Solanum lycopersicum* L. belongs to the family *Solanaceae*, which includes more than 3000 species, occupying a wide variety of habitats (Knapp, 2002). It is ranked above most fruits and vegetables as an excellent source of vitamins and minerals (Cobley and Steele, 1976). Although the yield potential of tomato has been reported to range from 60 to 100 tons per hectare (Varela *et al*., 2003; Bok *et al*., 2006), the productivity of tomatoes among small scale farmers is generally far below the potential of the crop, mostly due to the lack of tomato breeding efforts to develop tomato cultivars that are adapted to the local environment, and the devastating effect of pests, diseases and other environmental stresses (Monamodi *et al.*, 2013).

Nanoparticles (Nanoscale particles - NSPs) are atomic or molecular aggregates with at least one dimension between 1 and 100nm (Ball, 2002; Roco, 2003), that can drastically modify their physio-chemical properties compared to the bulk material (Nel *et al.*, 2006). The ability of nanoparticles to penetrate plant cells and deliver energy at different molecular levels has been exploited to enhance yield and performance of different crop plants. Foliar spray of cucumber with microscale CeO_2 and CuO has been reported to increase fruit fresh weight and enhance nutritional properties of cucumber (*Cucumber sativus*) (Hong *et al.*, 2016). Mahajan *et al.* (2011) also reported improvement of seedling growth in mung (*Vigna radiata*) and gram (*Cicer arietinum*) by suspensions of nano-ZnO particles.

Screening of different tomato cultivars treated with Alpha-spin nanoparticles for disease stress tolerance will further pave way for enhanced yield and productivity of tomatoes in the study area and other tomato growing communities in Africa and beyond.

MATERIALS AND METHODS

Source and Maintenance of Test Organisms

Isolates of two tomato wilt fungi namely; *Sclerotinia sclerotiorum* and *Alternaria triglochinicola* were obtained from the culture bank of the Plant Pathology Unit of the Department of Botany, Federal University Lafia, and maintained on Potato Dextrose Agar (PDA) slants at ambient conditions of

temperature (28°C±2°C) prior to pathogenicity bioassays (Elliot, 2017).

Source of Tomato Varieties

Certified seeds of two tomato varieties namely; Roma Savana and UC 82B were purchased from Agritropic Seed Company, Lafia.

Seed Treatment with Alphaspin Nanoparticles

Two (2) grams each of seeds of the different tomato varieties were dispensed in sterile transparent polyethylene bags and separately exposed to Alphaspin Nanoparticles for 15, 30, 45 and 60 minutes respectively, on an Alphaspin disc (Plate 1) (Oshinowo, 2018).

Plate 1: Alphaspin Nanoparticles Exposure Disc (Oshinowo, 2018)

Culture of Fungal Pathogens for Production of Pathogenicity-Related (PR) Compounds

The method reported by Zheng *et al*. (2010) was used as follows: Ten 6cm diameter mycelia plugs were obtained from actively growing regions of 7 days old cultures of the pathogenic fungi *Alternaria triglochinicola* and *Sclerotinia sclorotiorum* on Potato Dextrose Agar (PDA) and transferred into 900ml flasks containing 500 ml of Potato Sucrose Broth (PSB) (Potato infusion – 200g; Sucrose – 20g; Water – 1L) and incubated at ambient temperature (28°C±2°C) for 21 days. Cell-free culture filtrates were obtained by passing the liquid through Whatman no. 1 filter paper.

Seed Germination and Seedling Growth Bioassay of Crude Toxin Filtrates

The method reported by Terna *et al.* (2018) was adopted as follows; five milliliters (5ml) of fungal culture filtrates were gently mixed with 20 ml of 1.5% (Wt/Vol) molten Agar Agar (LOBA Chemie) in 9cm diameter Petri dishes using the pour plate method, and allowed to solidify. Ten tomato seeds were surface sterilized by vigorously shaking in 3.5% Sodium Hypochlorite solution for 1 minute, rinsed in 4-5 changes of sterile water, inserted half way in solidified agar-filtrate gel using a pair of sterile forceps, and incubated for 5 days. Surface sterilized seeds plated on agar mixed with sterile water served as control. Seeds were monitored daily for the onset of germination. Photographs of germinated seedlings taken 5 days after germination were used for the measurement of seedling height and girth with the aid of ToupView Camera Software Version 3.7., caliberated using the pixel:length relationship (ToupTek Photonics, 2013). Percentage germination inhibition was determined using the formula:

$$\%GI = \frac{No.\ GSC - No.\ GSTP}{No.GSC} X\ 100$$

Where; % GI = Percentage germination inhibition

No. GSC = No. of germinated seeds in control

No. GSTP = No. of germinated seeds in treated plates

Experimental Design and Statistical Analysis

Experimental treatments consisting of twenty replicates were laid out in Completely Randomized Design (CRD). All data collected were subjected to Analysis of Variance (ANOVA) at 5% level of significance. Means were separated by Duncan Multiple Range Test (DRMT).

RESULTS

Seeds of the Roma Savanna tomato variety treated with different exposures of Alphaspin nanoparticles showed variations in percentage germination, when challenged with culture filtrates of different fungal pathogens (Table 1). Percentage germination increased with increase in exposure time from 15 minutes to 60 minutes. Roma Savanna seeds challenged with culture filtrates of *A. triglochinicola* and *S. sclerotiorum* after exposure to Alphaspin nanoparticles for 15 minutes, showed the least percentage germination of 33.33 and 22.22 respectively. Germination was highest in seeds challenged with culture filtrates of *A. triglochinicola* and *S. sclerotiorum* exposed to nanoparticles for 60 minutes (40.74% and 48.15% respectively). Percentage germination of nanoparticles-treated seeds challenged with *A. triglochinicola* was significantly less ($P \leq 0.05$) than control (62.96%). Seeds exposed to Alphaspin

nanoparticles for 60 minutes did not differ in percentage germination (48.15) from control, following contact with culture filtrates of *S. sclerotiorum* (Table 1).

Root length decreased with increase in nanoparticles exposure time, and was highest in the controls (1.74cm in roots challenged with *A. trighlichinicola* and 1.75cm in roots challenged with *S. sclerotiorum*). Differences in root length were significant between nanoparticles-treated and untreated seedling roots challenged with culture filtrates of the tested fungal pathogens (P≤0.05) (Table 2).

Variations in shoot length were observed in seedlings of the Roma Savanna tomato variety treated with different levels of Alphaspin nanoparticles and challenged with culture filtrates of different fungal pathogens (Table 3). Mean shoot length was highest in the controls challenged with culture filtrates of *A. triglochinicola* (65.63cm) and *S. sclerotiorum* (33.87cm), and differed significantly from seedlings treated with different levels of nanoparticles exposure (P≤0.05).

Table 1: Effect of Different Alphaspin Nanoparticles Treatment Durations on Seed Germination of Roma Savana Tomato Variety Challenged with Different Fungal Pathogens

	% Germination	
Nanoparticles Treatment Time	*Alternaria triglochinicola*	*Sclerotinia sclerotiorum*
15 minutes	33.33^{abc}	22.22^{a}
30 minutes	33.33^{abc}	29.63^{ab}
45 minutes	25.92^{ab}	22.22^{a}
60 minutes	40.74^{bc}	48.15^{cd}
Untreated (Control)	62.96^{d}	48.15^{cd}

Means followed by same superscripts within same column are not significantly different (P≤0.05)

Table 2: Effect of Different Alphaspin Nanoparticles Treatment Durations on Root Length of Roma Savana Tomato Variety Challenged with Different Fungal Pathogens

	Root Length (cm)	
Nanoparticles Treatment Time	*Alternaria triglochinicola*	*Sclerotinia sclerotiorum*
15 minutes	0.69^{b}	0.37^{ab}
30 minutes	0.52^{ab}	0.33^{a}
45 minutes	0.43^{ab}	0.26^{a}
60 minutes	0.43^{ab}	0.29^{a}
Untreated (Control)	1.74^{c}	1.75^{c}

Means followed by same superscripts within same column are not significantly different (P≤0.05)

Table 3. Effect of Different Alphaspin Nanoparticles Treatment Durations on Shoot Length of Roma Savana Tomato Variety Challenged with Different Fungal Pathogens

	Root Length (cm)	
Nanoparticles Treatment Time	*Alternaria triglochinicola*	*Sclerotinia sclerotiorum*
15 minutes	4.93^{a}	3.95^{a}
30 minutes	2.56^{a}	3.06^{a}
45 minutes	6.29^{a}	2.25^{a}
60 minutes	7.23^{a}	3.40^{a}
Untreated (Control)	65.63^{b}	33.87^{b}

Means followed by same superscripts within same column are not significantly different (P≤0.05)

Seedlings of the Roma Savanna tomato variety raised from seeds previously treated with different levels of Alphaspin nanoparticles showed variations in stem girth, following contact with culture filtrates of the tested fungal pathogens (Table 4). Mean stem girth was highest in controls (48.49cm) after contact with *A. triglochinicola* filtrates, and after contact with filtrates of *S. sclerotiorum* (10.23cm) respectively, compared to treated seedlings. Differences in stem girth were significant between nanoparticles-treated and untreated seeds (P≤0.05).

Seeds treated with nanoparticles for 30 and 60 minutes had the highest percentage germination in the presence of culture filtrates of *A. triglochinicola* (81.48% respectively), and differed significantly at P≤0.05 from controls (50.00). Highest germination percentage of UC 82B seeds (55.56) was observed in seeds treated with Alphaspin nanoparticles for 45 minutes before exposure to culture filtrates of *Sclerotinia sclerotiorum,* but did not differ significantly from controls (P≤0.05) (Table 5).

Seeds of the UC82B tomato variety treated with Alphaspin nanoparticles differed significantly in root length (P≤0.05) from the controls, when challenged with disease stress by culture filtrates of the test fungi (Table 6). Seeds treated with nanoparticles for 45 minutes had the least root lengths (0.29cm respectively), compared with controls with the highest root lengths when exposed to *A. triglochinicola* (1.91cm) and *S. sclerotiorum* (1.56cm).

Table 4: Effect of Different Alphaspin Nanoparticles Treatment Durations on Stem Girth of Roma Savana Tomato Variety Challenged with Different Fungal Pathogens

	Stem Girth (cm)	
Nanoparticles Treatment Time	*Alternaria triglochinicola*	*Sclerotinia sclerotiorum*
15 minutes	1.27[a]	1.07[a]
30 minutes	1.27[a]	0.86[a]
45 minutes	1.77[a]	0.88[a]
60 minutes	1.51[a]	0.94[a]
Untreated (Control)	48.49[b]	10.23[b]

Means followed by same superscripts within same column are not significantly different (P≤0.05)

Table 5: Effect of Different Alphaspin Nanoparticles Treatment Durations on Seed Germination of UC 82B Tomato Variety Challenged with Different Fungal Pathogens

	% Germination	
Nanoparticles Treatment Time	*Alternaria triglochinicola*	*Sclerotinia sclerotiorum*
15 minutes	70.37[bc]	51.85[a]
30 minutes	81.48[c]	55.55[ab]
45 minutes	77.78[c]	55.56[ab]
60 minutes	81.48[c]	48.15[a]
Untreated (Control)	50.00[a]	51.85[a]

Means followed by same superscripts within same column are not significantly different (P≤0.05)

Table 6: Effect of Different Alphaspin Nanoparticles Treatment Durations on Root Length of UC 82B Tomato Variety Challenged with Different Fungal Pathogens

	Root Length (cm)	
Nanoparticles Treatment Time	*Alternaria triglochinicola*	*Sclerotinia sclerotiorum*
15 minutes	0.33[a]	0.32[a]
30 minutes	0.38[a]	0.31[a]
45 minutes	0.31[a]	0.35[a]
60 minutes	0.29[a]	0.29[a]
Untreated (Control)	1.91[b]	1.56[b]

Means followed by same superscripts within same column are not significantly different (P≤0.05)

Results of the effect of different Alphaspin nanoparticles treatment durations on shoot length of the UC 82B tomato variety is presented in Table 7. Mean shoot length was highest in controls challenged with culture filtrates of *A. triglochinicola* (50.85cm) and *S. sclerotiorum* (24.25cm), compared to seedlings exposed to Alphaspin nanoparticles for durations ranging from 15 to 60 minutes. Differences in shoot lengths between nanoparticles-treated and untreated seedlings were significant (P≤0.05).

Stem girth was highest in controls subjected to disease stress by culture filtrates of *A. triglochinicola* (37.88cm) and *S. sclerotiorum* (9.03cm), and differed significantly from Alphaspin nanoparticles-treated seedlings (Table 8).

All seeds not previously treated with Alphaspin nanoparticles had the highest and significantly different (P≤0.05) mean percentage germination (43.65), root length (1.74cm), and stem girth (26.41cm), compared to seeds previously treated with nanoparticles. Mean total shoot length of tomato seeds treated with Alphaspin nanoparticles for 60 minutes had the highest mean shoot length (54.63cm), but did not differ significantly from control, when challenged with disease stress by culture filtrates of the test fungal pathogens of tomato (Table 9).

Table 7: Effect of Different Alphaspin Nanoparticles Treatment Durations on Shoot Length of UC 82B Tomato Variety Challenged with Different Fungal Pathogens

	Shoot Length (cm)	
Nanoparticles Treatment Time	*Alternaria triglochinicola*	*Sclerotinia sclerotiorum*
15 minutes	8.27[a]	6.00[a]
30 minutes	8.85[a]	6.44[a]
45 minutes	7.11[a]	6.97[a]
60 minutes	8.26[a]	5.36[a]
Untreated (Control)	50.85[c]	24.25[b]

Means followed by same superscripts within same column are not significantly different (P≤0.05)

Table 8: Effect of Different Alphaspin Nanoparticles Treatment Durations on Stem Girth of UC 82B Tomato Variety Challenged with Different Fungal Pathogens

	Stem Girth (cm)	
Nanoparticles Treatment Time	*Alternaria triglochinicola*	*Sclerotinia sclerotiorum*
15 minutes	1.49[a]	1.19[a]
30 minutes	1.96[a]	1.49[a]
45 minutes	1.45[a]	1.31[a]
60 minutes	1.48[a]	1.43[a]
Untreated (Control)	37.88[b]	9.03[b]

Means followed by same superscripts within same column are not significantly different (P≤0.05)

Table 9: Mean Total Effect of Different Nanoparticle Treatments on Growth Parameters of Tomato Seedlings Challenged with Fungal Disease Stress

	Growth Parameters			
Nanoparticles Treatment Time	% Germination	Root Length (cm)	Shoot Length (cm)	Stem Girth (cm)
15 minutes	5.79[a]	0.43[a]	44.44[a]	1.26[a]
30 minutes	5.23[a]	0.38[a]	50.00[abc]	1.39[a]
45 minutes	5.66[a]	0.34[a]	45.37[ab]	1.36[a]
60 minutes	6.06[a]	0.33[a]	54.63[c]	1.34[a]
Untreated (Control)	43.65[b]	1.74[b]	53.24[bc]	26.41[b]

Means followed by same superscripts within same column are not significantly different (P≤0.05)

The Roma Savanna tomato variety challenged with fungal disease stress after treatment with Alphaspin nanoparticles, had the highest mean total percentage germination (13.32%), root length (0.68cm) and shoot length (36.67cm), compared to UC 82B with 13.24 percentage germination, 0.61cm root length, and 5.87cm mean total shoot length, respectively. UC 82B had the highest and significantly different (P≤0.05) stem girth (62.41cm) from Roma Savanna (36.67cm) (Table 10).

Tomato seeds challenged with culture filtrates of *Sclerotinia sclerotiorum* following exposure to different levels of Alphaspin nanoparticles had the least mean total percentage germination (9.56), root length (0.58cm), shoot length (2.84cm) and stem girth (43.33cm), compared to *Alternaria triglochinicola* with 17.00, 0.70cm, 9.86cm, and 55.74cm in percentage germination, root length, shoot length and stem girth respectively. Differences in mean total disease effect of the test fungi on growth parameters of the studied tomato varieties were significant (P≤0.05) (Table 11 and Plate 2).

Table 10: Mean Total Growth Responses of Nanoparticles-Treated Tomato Varieties to Disease Stress by Fungal Pathogens

Tomato Variety	Growth Parameters			
	% Germination	Root Length (cm)	Shoot Length (cm)	Stem Girth (cm)
Roma Savanna	13.32[a]	0.68[a]	6.83[a]	36.67[a]
UC 82B	13.24[a]	0.61[a]	5.87[a]	62.41[b]

Means followed by same superscripts within same column are not significantly different (P≤0.05)

Table 11: Mean Total Effect of the Test Fungal Pathogens on Growth Parameters of Nanoparticles-Treated Tomato Seeds

Pathogen	% Germination	Root Length (cm)	Shoot Length (cm)	Stem Girth (cm)
Alternaria triglochinicola	17.00[b]	0.70[a]	9.86[b]	55.74[b]
Sclerotinia sclerotiorum	9.56[a]	0.58[a]	2.84[a]	43.33[a]

Means followed by same superscripts within same column are not significantly different (P≤0.05)

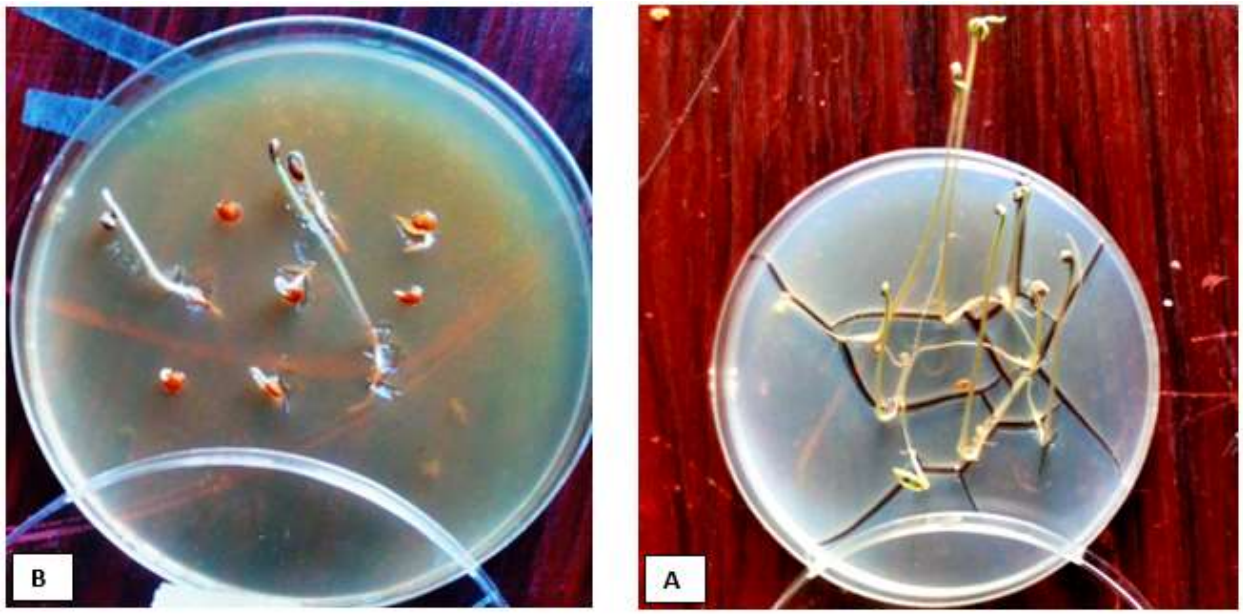

Plate 2. UC82B Tomato seedlings growing on water agar incorporated with culture filtrates of: A) *Sclerotinia sclerotiorum*; B) *Alternaria triglochinicola*.

DISCUSSION

Tomato varieties treated with different levels of Alphaspin nanoparticles responded differently to disease initiation by culture filtrates of the tested fungal pathogens. Seed germination was highest in nanoparticles-treated seeds compared to the controls. Enhancement of seed germination in different plants has been previously reported by several authors. Siddiqui and Al-Whanbi (2014) reported that lower concentrations of nano-SiO_2 improved seed germination of tomato. Suriyaprabha *et al.* (2012) also reported that nano-SiO_2 increased seed germination by providing better nutrients availability to maize seeds, and pH and conductivity to the growing medium. The use of nano-anatase on parsley seeds by Dehkourdi *et al*. (2013) resulted in increased percentage of germination, germination rate index, root and shoot length, fresh weight, vigor index, and cholorphyll content of the seedlings. Krishnaraj *et al.* (2012) studied the effect of biologically synthesized AgNPs on hydroponically grown Bacopa monnieri growth metabolism, and found that biosynthesized AgNPs showed a significant effect on seed germination and induced the synthesis of protein and carbohydrate and decreased the total phenol contents and catalase and peroxidase activities. The germination enhancing property of nanoparticles can be attributed to their ability to penetrate cell walls and membranes and also provide suitable delivery systems of growth enhancing energy and chemicals to cells.

Root length, shoot length and stem girth were least in Alphaspin nanoparticles-treated tomato varieties challenged with fungal pathogens, compared to the controls. In similar studies by Shaw *et al.* (2013) on the effect of nano-CuO on rice, it was reported that seed germination was significantly reduced, and there was damage to the root cells, an increase in H_2O_2, accumulation of proline, and a decrease in the level of carotenoids. In addition to the possibility of increased abiotic stress on the treated plants by the nanoparticles, the reduction in growth parameters of treated tomato plants in this concluded study could be as a result of the inadequacy of the Alphaspin exposure levels used in the study to sufficiently counter the biotic stress induced by the tested fungal pathogens. This is however subject to further investigation.

S. sclerotiorum was most pathogenic on the tested tomato varieties. Reports by several workers (Maxwell and Lumsden, 1970; Marciano *et al*., 1983; Dutton and Evans, 1996; Zhou and Boland, 1999; Cessna *et al.*, 2000) have attributed the remarkable success of *S. sclerotiorum* in the production of plant disease symptom complexes, to its ability to produce large quantities of oxalic acid which acts to achieve maximum toxicity and tissue damage, leading to the eventual death of infected plants.

CONCLUSION

Alphaspin nanoparticles promoted germination of treated tomato seeds under conditions of biotic stress induced by toxic metabolites of fungal pathogens. The findings of this research further emphasizes the usefulness of nanotechnology in enhancing crop productivity, and could be harnessed in the improvement of tomato yield and productivity in the study area.

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