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Effect of Alphaspin® Nanoparticles on the Susceptibility of Tomatoes (*Solanum lycopersicum* L.) to Southern Blight Caused by *Sclerotium Rolfsii* in Lafia

S. Abaine, T. P. Terna✉ & J. I. Okogbaa
Department of Plant Science & Biotechnology, Federal University of Lafia, PMB 146, Nasarawa State, Nigeria
✉ternapaul@yahoo.com

Abstract: A study was carried out to investigate the disease responses of Alphaspin nanoparticles-treated tomato (*Solanum lycopersicum* L.) to Southern blight caused by *Sclerotium rolfsii* in Lafia, Nasarawa State, Nigeria. Two grams each of seeds belonging to the Syria, Roma Savana, and UC82B tomato varieties were exposed to Alphaspin nanoparticles for 10, 20, 30, 40, and 50 min respectively, and challenged with sclerotia obtained from isolates of *S. rolfsii.* Growth and disease responses of Alphaspin nanoparticles treated tomato plants to *S. rolfsii* were assessed 4 weeks after inoculation. UC82B tomato exposed to Alphaspin nanoparticles for 40 min proved to be the most susceptible variety to *S. rolfsii*, with the mean total leaf yellowing 87.50%, leaf necrosis 81.25%, flower wilt 82.50%, stem necrosis 40.00%, stem rot 35.00%, stem wilt 42.50%, plant wilt 45.00%, plant height 37.40 cm, number of leaves 88.00, stem girth 2.25 cm, number of branches 0.75, and number of flowers 7.75. The differences in growth and disease responses were significant among the different tomato varieties ($P<0.05$). The study revealed that plants exposed to Alphaspin nanoparticles were more vulnerable to southern blight than the untreated control; hence, studies involving the use of other nano materials such as green synthesized nanoparticles are required to further our understanding of the effect of nanoparticles on tomato resistance to southern blight caused by *S. rolfsii.*

Keywords: Alphaspin nanoparticles, tomato varieties, *Sclerotium rolfsii*, Southern blight, disease responses

Introduction

Tomatoes (*Solanum lycopersicum* L.) are a widely grown and significant crop. It is the second most popular vegetable in terms of consumption and production [1]. With a global production of around 170 million tonnes in 2018 on a cultivated area of nearly 5.2 million hectares, it ranks seventh in terms of production worldwide, behind wheat, rice, maize, potatoes, soybeans, and cassava [2]. The discovery of lycopene in tomatoes, a highly medicinal compound with anti-oxidative and anticancer capabilities, has further increased the popularity of the crop worldwide [3, 4].

Despite the economic importance of tomato, the tomato crop is often attacked by fungal pathogens, resulting in huge annual yield loses. *Sclerotium rolfsii*is known to be one of the major causal organisms of tomato diseases accounting for huge yield losses annually [5]. The common management strategy for the disease often involves the treatment of plants and seeds with chemical fungicides which are harmful to humans, soil health, non-target organisms, and often leads to the development of resistance in the target organisms [6, 7]. Hence, there is need for the development of more effective and environmentally friendly alternatives in the management of southern blight disease of tomato caused by *S. rolfsii* in the study area.

Nanoparticle materials have distinctive physicochemical characteristics that are not found in their bulk counterparts, which improve their ability to interact with microbes and perform a variety of antimicrobial actions [8, 9]. In the present study, tomato seeds treated with Alphaspin nanoparticles were screened for their tolerance to southern blight disease caused by *S. rolfsii.* The findings of this study will contribute to efforts aimed at providing environmentally friendly and sustainable alternatives in the control of southern blight disease of tomato in the study area.

Materials and Methods

Source of tomato seeds

Seeds of the local variety, Syria were purchased from a local farmer in Araho village of Lafia Local Government Area, Nasarawa State, Nigeria, while Roma and UC 82B tomato seeds were purchased from Royal Seeds Company Jos, Plateau State, Nigeria.

Source of *S. rolfsii*

Sclerotia of the tomato pathogen *S. rolfsii* obtained from a previous study were collected from the Department of Plant Science and Biotechnology, Federal University of Lafia.

Seed treatment with alpha spin nanoparticles

The method of Terna and Oshinowo [10] was adopted. Two grams each of seeds of the different tomato varieties were dispensed in sterile transparent polyethylene bags and separately exposed to Alphaspin nanoparticles for 10, 20, 30, 40 and 50 minutes respectively on an Alphaspin disc (Plate 1).

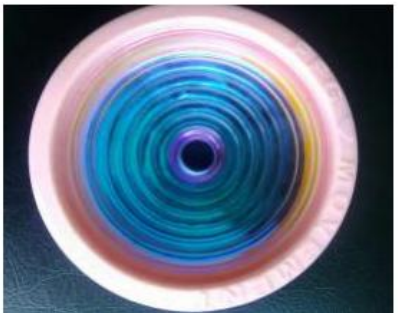

Plate 1: The alphaspin nano disc [11]

Pathogenicity of *S. rolfsii*on tomato plants treated with alphaspin nanoparticles

Four weeks old seedlings of nanoparticles-treated seeds raised and on sterile Sandy-loam garden soil were inoculated with sclerotia using the method of Terna *et al.* [7]. Inoculated seedlings were monitored and assessed for the incidence and severity of disease symptoms such as leaf yellowing (LY), leaf necrosis (LN), stem rot (SR), stem wilt (SW), plant wilt (PW), stem necrosis (SN) and flower wilt (FW), for a total duration of four weeks.

Experimental design and data analysis

Experimental treatments consisting four replicates were laid out in Randomized Complete Blocked Design (RCBD) for all field experiments. Data obtained from the experiment was subjected to Analysis of Variance (ANOVA) at 5% level of probability using the Minitab Statistical Software version 29. Means were separated using the Tukey's Honestly Significant Difference Test.

Results and Discussion

The growth responses of Syrian variety infected with *S. rolfsii*, after four weeks of seed exposure to different levels of Alphaspin nanoparticles are presented in Table 1. Among the nanoparticles treated plants infected with *S. rolfsii*, plants exposed for 50 min had the highest plant height (62.93 cm), number of leaves (187.80), number of branches (4.00), and number of flowers (21.00), while stem girth was higher in plants exposed for 10 min and 20 min (2.95 cm each), compared to the other treatments. However, growth responses observed in the Alphaspin nanoparticles-treated tomato plants infected with *S. rolfsii* were significantly lower than those observed in treated but uninfected plants ($P<0.05$). Table 2 presents the disease responses of the Syrian variety infected with *S. rolfsii*, after four weeks of seed exposure to Alphaspin nanoparticles. Among the infected plants, those previously exposed to Alphaspin nanoparticles for 50 min showed the least leaf yellowing (57.50 %), while leaf necrosis (51.25%), flower wilt (71.25%), stem necrosis (0.00%), stem rot (0.00%), stem wilt (0.00%), and plant wilt (0.00%) were least observed in infected tomato plants previously exposed for 30 min. Leaf yellowing, leaf necrosis, and flower wilt observed in the infected plants were significantly higher than what was observed respectively in control experiments exposed for 10, 30 and 40 min ($P<0.05$).

Table 1: Growth responses of alphaspin-treated syrian tomato variety after four weeks of post-inoculation with *S. rolfsii*

Treatment	PH (cm)	NL	SG (cm)	NB	NF
10 min + pathogen	49.33^{cdef}	170.80^{bcd}	2.95^{a}	2.50^{ab}	17.25^{a}
20 min + pathogen	41.15^{f}	139.30^{de}	2.95^{a}	1.50^{b}	15.50^{a}
30 min + pathogen	43.83^{ef}	133.00^{de}	2.80^{abc}	3.00^{ab}	17.50^{a}
40 min + pathogen	48.10^{def}	132.00^{de}	2.53^{bc}	2.50^{ab}	15.50^{a}
50 min + pathogen	62.93^{b}	187.80^{bcd}	2.80^{abc}	4.00^{ab}	21.00^{a}
Control 1 (10 min – pathogen)	62.22^{b}	198.80^{bc}	3.03^{a}	3.25^{ab}	24.50^{a}
Control 2 (20 min – pathogen)	56.90^{bcd}	329.50^{a}	2.98^{a}	6.00^{a}	22.25^{a}
Control 3 (30 min – pathogen)	52.40^{bcde}	188.00^{bcd}	2.88^{ab}	3.25^{ab}	19.00^{a}
Control 4 (40 min – pathogen)	60.15^{bc}	231.30^{b}	2.65^{abc}	4.00^{ab}	22.75^{a}
Control 5 (50 min – pathogen)	74.42^{a}	227.00^{bc}	2.85^{abc}	4.75^{ab}	24.25^{a}

Means followed by different superscripts within the same column are significantly different ($P<0.05$)
PH = Plant Height, NL = Number of Leaves, SG = Stem Girth, NB = Number of Branches, NF = Number of Flowers

Table 2: Disease responses of alphaspin-treated syrian tomato variety after four weeks of post-inoculation with *S. rolfsii*

Treatment	LY (%)	LN (%)	FW (%)	SN (%)	SR (%)	SW (%)	PW (%)
10 min + pathogen	71.25^{a}	63.75^{a}	77.50^{a}	36.30^{a}	27.50^{a}	40.00^{a}	43.80^{a}
20 min + pathogen	66.30^{ab}	60.00^{ab}	88.75^{a}	17.50^{a}	12.50^{a}	20.00^{a}	25.00^{a}
30 min + pathogen	58.80^{abc}	51.25^{abc}	71.25^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
40 min + pathogen	68.75^{a}	55.00^{abc}	87.50^{a}	20.00^{a}	13.80^{a}	17.50^{a}	20.00^{a}
50 min + pathogen	57.50^{abc}	55.00^{abc}	72.50^{a}	25.00^{a}	25.00^{a}	25.00^{a}	25.00^{a}
Control 1 (10 min – pathogen)	17.50^{c}	15.00^{c}	26.25^{c}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control 2 (20 min – pathogen)	20.00^{c}	17.50^{bc}	28.75^{c}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control 3 (30 min – pathogen)	23.75^{bc}	15.00^{c}	21.25^{c}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control 4 (40 min – pathogen)	20.00^{c}	15.00^{c}	36.25^{bc}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control 5 (50 min – pathogen)	23.75^{bc}	18.75^{bc}	28.75^{c}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}

Means followed by different superscripts within the same column are significantly different ($P<0.05$)
LN = Leaf Necrosis, LY = Leaf Yellowing, FW = Flower Wilt, SN = Stem Necrosis, SR = Stem Rot, SW = Stem Wilt, PW = Plant Wilt

Table 3: Disease responses of alphaspin-treated Roma Savana tomato variety after four weeks of post-inoculation with *S. rolfsii*

Treatment	PH (cm)	NL	SG (cm)	NB	NF
10 min + pathogen	43.27^{de}	157.75^{bc}	2.65^{abc}	2.00^{abc}	15.75^{a}
20 min + pathogen	51.45^{bcd}	142.00^{bc}	2.78^{ab}	2.50^{abc}	16.00^{a}
30 min + pathogen	43.48^{de}	134.00^{c}	2.65^{abc}	1.50^{abc}	13.75^{a}
40 min + pathogen	41.83^{e}	124.25^{c}	2.43^{bc}	1.25^{bc}	12.75^{a}
50 min + pathogen	49.60^{bcde}	167.67^{bc}	2.78^{ab}	2.67^{abc}	16.00^{a}
Control 1 (10 min – pathogen)	55.00^{ab}	229.30^{a}	3.00^{a}	3.50^{a}	23.75^{a}
Control 2 (20 min – pathogen)	61.40^{a}	149.75^{bc}	2.85^{a}	3.25^{ab}	20.50^{a}
Control 3 (30 min – pathogen)	54.90^{abc}	152.30^{bc}	2.78^{ab}	2.00^{abc}	21.75^{a}
Control 4 (40 min – pathogen)	53.47^{abc}	137.75^{bc}	2.43^{bc}	1.75^{abc}	19.50^{a}
Control 5 (50 min – pathogen)	58.75^{ab}	181.50^{b}	2.75^{ab}	2.00^{abc}	19.50^{a}

Means followed by different superscripts within the same column are significantly different (P<0.05)
PH = Plant Height, NL = Number of Leaves, SG = Stem Girth, NB = Number of Branches, NF = Number of Flowers

Results showing the growth responses of Roma Savana variety infected with *S. rolfsii*after four weeks of seed exposure to different levels of Alphaspin nanoparticles are presented in Table 3. Among the nanoparticles treated plants infected with *S. rolfsii*, plants exposed for 20 min had the highest plant height (51.42 cm), number of leaves (167.67), number of branches (2.67), and number of flowers (16.00) were higher in plants exposed for 50 min, while stem girth was higher in plants exposed for 20 and 50 min (2.78 cm each), compared to the other treatments. However, growth responses observed in the Alphaspin nanoparticles treated tomato plants infected with *S. rolfsii* were significantly lower than those observed in treated but uninfected plants (P<0.05).

Table 4 presents the disease responses of Roma Savana variety infected with *S. rolfsii*, after four weeks of seed exposure to Alphaspin nanoparticles. Among the infected plants, those previously exposed to Alphaspin nanoparticles for 30, and 50 min showed the least leaf yellowing (60.00% each), leaf necrosis (43.33%) was least observed in plants exposed for 50 min, flower wilt (65.00%) was least observed in plants exposed for 40 min, while stem necrosis (0.00%), stem rot (0.00%), stem wilt (0.00%), and plant wilt (0.00%) were least observed in infected tomato plants previously exposed for 30, 40, and 50 min. Leaf yellowing, leaf necrosis, and flower wilt observed in the infected plants were significantly higher than what was observed respectively in control experiments exposed for 30, 40, and 50 min (P<0.05). Results showing the growth responses of the UC82B variety infected with *S. rolfsii*, after four weeks of seed exposure to different levels of Alphaspin nanoparticles are presented in Table 5. Among the nanoparticles treated plants infected with *S. rolfsii*, plants exposed for 10 min had the highest plant height (52.83 cm), number of leaves (125.50), number of branches (1.25), and number of flowers (10.25), while stem girth was higher in plants exposed for 40 and 50 min (2.25 cm each), compared to other treatments. However, growth responses observed in Alphaspin nanoparticles treated tomato plants infected with *S. rolfsii* were significantly lower than those observed in treated but uninfected plants (P<0.05).

Table 4: Disease responses of alphaspin-treated Roma Savana tomato variety after four weeks of post-inoculation with *S. rolfsii*

Treatment	LY (%)	LN (%)	FW (%)	SN (%)	SR (%)	SW (%)	PW (%)
10 min + pathogen	82.50^{a}	77.50^{a}	88.75^{ab}	12.50^{a}	7.50^{ab}	15.00^{ab}	20.00^{ab}
20 min + pathogen	78.75^{a}	63.75^{ab}	91.25^{a}	27.50^{a}	18.75^{a}	36.30^{a}	46.30^{a}
30 min + pathogen	60.00^{a}	55.00^{ab}	70.00^{ab}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
40 min + pathogen	65.00^{a}	53.75^{ab}	65.00^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
50 min + pathogen	60.00^{ab}	43.33^{bc}	66.67^{ab}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control 1 (10 min – pathogen)	26.25^{c}	21.25^{cd}	27.50^{c}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control 2 (20 min – pathogen)	31.25^{bc}	21.25^{cd}	31.25^{c}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control 3 (30 min – pathogen)	22.50^{c}	15.00^{d}	23.75^{c}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control 4 (40 min – pathogen)	27.50^{c}	25.00^{cd}	22.50^{c}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control 5 (50 min – pathogen)	25.00^{c}	22.50^{cd}	20.00^{c}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}

Means followed by different superscripts within the same column are significantly different (P<0.05)
LN = Leaf Necrosis, LY = Leaf Yellowing, FW = Flower Wilt, SN = Stem Necrosis, SR = Stem Rot, SW = Stem Wilt, PW = Plant Wilt

Table 5: Growth responses of alphaspin-treated UC82B tomato variety after four weeks of post-inoculation with *S. rolfsii*

Treatment	PH (cm)	NL	SG (cm)	NB	NF
10 min + pathogen	52.83^{b}	125.50^{ab}	2.35^{a}	1.25^{a}	10.25^{cd}
20 min + pathogen	39.40^{c}	85.50^{c}	2.20^{a}	0.50^{a}	7.00^{d}
30 min + pathogen	42.27^{c}	93.75^{bc}	2.33^{a}	0.75^{a}	7.50^{d}
40 min + pathogen	37.40^{c}	88.00^{c}	2.25^{a}	0.75^{a}	7.75^{d}
50 min + pathogen	43.07^{c}	96.25^{abc}	2.25^{a}	0.50^{a}	9.25^{cd}
Control 1 (10 min – pathogen)	63.42^{a}	128.30^{a}	2.40^{a}	1.25^{a}	23.75^{ab}
Control 2 (20 min – pathogen)	53.75^{b}	102.00^{abc}	2.28^{a}	0.75^{a}	15.25^{bcd}
Control 3 (30 min – pathogen)	55.60^{ab}	107.75^{abc}	2.18^{a}	1.00^{a}	19.50^{abc}
Control 4 (40 min – pathogen)	52.27^{b}	97.75^{abc}	2.28^{a}	0.75^{a}	16.75^{abcd}
Control 5 (50 min – pathogen)	53.38^{b}	104.50^{abc}	2.43^{a}	0.50^{a}	27.25^{a}

Means followed by different superscripts within the same column are significantly different (P<0.05)
PH = Plant Height, NL = Number of Leaves, SG = Stem Girth, NB = Number of Branches, NF = Number of Flowers

Table 6: Disease responses of alphaspin-treated UC82B tomato variety after four weeks of post-inoculation with *S. rolfsii*

Treatment	LY (%)	LN (%)	FW (%)	SN (%)	SR (%)	SW (%)	PW (%)
10 min + pathogen	71.25^{abcd}	65.00^{abc}	77.50^{abc}	17.50^{a}	10.00^{a}	18.80^{a}	22.50^{a}
20 min + pathogen	70.00^{abcde}	65.00^{abc}	67.50^{abcd}	25.00^{a}	25.00^{a}	25.00^{a}	25.00^{a}
30 min + pathogen	77.50^{ab}	63.80^{abc}	85.00^{a}	22.50^{a}	15.00^{a}	25.00^{a}	31.30^{a}
40 min + pathogen	87.50^{a}	81.25^{a}	82.50^{a}	40.00^{a}	35.00^{a}	42.50^{a}	45.00^{a}
50 min + pathogen	75.00^{abc}	70.00^{ab}	80.00^{ab}	22.50^{a}	17.50^{a}	28.80^{a}	32.50^{a}
Control 1 (10 min – pathogen)	33.75^{def}	23.75^{bc}	26.25^{de}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control 2 (20 min – pathogen)	37.50^{cdef}	30.00^{bc}	28.75^{de}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control 3 (30 min – pathogen)	27.50^{f}	25.00^{bc}	32.50^{bcde}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control 4 (40 min – pathogen)	40.00^{bcdef}	33.75^{abc}	25.00^{de}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control 5 (50 min – pathogen)	31.30^{ef}	26.25^{bc}	30.00^{cde}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}

Means followed by different superscripts within the same column are significantly different (P<0.05)
LN = Leaf Necrosis, LY = Leaf Yellowing, FW = Flower Wilt, SN = Stem Necrosis, SR = Stem Rot, SW = Stem Wilt, PW = Plant Wilt

Table 6 and Plate 2 presents the disease responses of UC82B variety infected with *S. rolfsii*, after four weeks of seed exposure to different levels of Alphaspin nanoparticles. Among the infected plants, those previously exposed to Alphaspin nanoparticles for 20 min showed the least leaf yellowing (70.00%), and flower wilt (67.50%), leaf necrosis (63.80%) was least observed in plants exposed for 30 min, while stem necrosis (17.50%), stem rot (10.00%), stem wilt (8.80%), and plant wilt (22.50%) were least observed in infected tomato plants previously exposed for 10 min. Leaf yellowing, leaf necrosis, flower wilt, stem necrosis, stem rot, stem wilt, and plant wilt observed in the infected plants were significantly higher than what was observed respectively in control experiments exposed for 10, 20, and 30 min (P<0.05).

Plate 2: UC82B tomato variety exposed to alphaspin nanoparticles for 40 min. (A) Leaves of *S. rolfsii*-infected plant showing chloroses; (B) Necrosis on leaf of an infected plant; (C) Stem necrosis; (D) Healthy leaves of uninfected plant (Control)

Tomato varieties treated with Alphaspin nanoparticles at different levels prior to inoculation with sclerotia responded differently to disease initiation and symptoms development by *Sclerotium rolfsii*. Inoculated tomato plants had higher disease incidence, resulting in the significant decrease in growth parameters such as plant height, plant girth, number of leaves, and number of flowers, compared to the uninoculated tomato plants. Similar findings had been reported by Liamngee *et al*. [12] that soil infestation with *S. rolfsii* showed reduction in germination parameters such as number of leaves, branches, and height of the tomato cultivars. In a related study, Terna and Oshinowo [10] also reported that root length, shoot length, and stem girth were least in Alphaspin nanoparticles-treated tomato varieties challenged with fungal pathogens, compared to the controls. The authors attributed the higher growth suppression of alphaspin nanoparticles-treated seedlings to the inadequacy of the alphaspin nanomaterials to successfully counter the biotic stress initiated in the plant tissues by the pathogen. It is also likely that the exposure to alphaspin nanoparticles may have resulted in genetic alterations that were disadvantageous to the plant with respect to growth and disease resistance.

Conclusion

The study revealed that plants exposed to Alphaspin nanoparticles were more vulnerable to southern blight than the untreated control; hence, studies involving the use of other nano materials such as green synthesized nanoparticles are required to further our understanding of the effect of nanoparticles on tomato resistance to southern blight caused by *S. rolfsii.*

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