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Annals of Biological Research, 2015, 6 (9):7-15 (http://scholarsresearchlibrary.com/archive.html)



Evaluation of the disease causing potential of *Sclerotium rolfsii* on tomato cultivars in bioassay (pathogenicity test)

¹Liamngee Kator, ²Okoro James Kalu and ³Akomaye Maria Umgbeb

¹Department of Biological Sciences, Benue State University, Makurdi, Nigeria ²College of Agronomy, Federal University of Agriculture, Makurdi, Nigeria ³Department of Biology, Federal College of Education, Obudu, Cross River State, Nigeria

ABSTRACT

An evaluation of the disease causing potential of Sclerotium rolfsii on some tomato cultivars in bioassay was conducted. The cultivars showed disease symptoms such as chlorosis, wilting, damping off, blighting and necrosis. For Shase, Hoozua and UTC cultivars, chlorosis wilting and damping off ranged from 50 –100% in week three while blighting and necrosis was 0%. In week four, chlorosis, wilting and damping off was 100% while blighting and necrosis was 0%. In week four, chlorosis, wilting and damping off was 100% while blighting and necrosis was 0%. In week five and six, disease symptoms were 100% on all cultivars. Analysis of Variance (ANOVA) revealed significant differences (P < 0.05) in the response of the different cultivars to Sclerotium rolfsii infection with respect to their controls. Severity of Sclerotium rolfsii infection on the tomato cultivars ranged from 1-5 which indicated 1-100% of plant tissue damage. There was no significant difference (P > 0.05) in the severity of Sclerotium rolfsii proved to be highly pathogenic on the evaluated cultivars of tomato in pot experiments.

Keywords: Evaluation, Sclerotium rolfsii, Disease, Potential, Bioassay, Tomato.

INTRODUCTION

Sclerotium rolfsii is a soil-borne plant pathogen that causes damping off of seedlings, stem canker, crown blight, root, crown, bulb, tuber and fruit rots [1] (Farr *et al.*, 1989). Sclerotia diseases caused by *Sclerotium rolfsii* occur primarily in the tropics, sub tropics and other warm temperate regions of the world, especially at high moistures and high temperatures [2] (Aycock, 1996). The pathogen frequently affects more than 500 species of plants, including most vegetables, flowers, legumes, weeds and forage plants [3] (Agrios, 1998). This kind of disease is often called Sclerotia rot in general. An estimated loss of up to 20-30 million US dollars caused by *Sclerotium rolfsii* has been reported in southern USA on peanut with yield depletion ranging from 1-60% in different fields [2] (Aycock, 1996). Experiments were therefore carried out to evaluate the disease causing potential of *Sclerotium rolfsii* on some tomato cultivars in bioassay.

MATERIALS AND METHODS

1. Preparation of *Sclerotium rolfsii* Inoculum for Artificial Inoculation

Potato Dextrose Agar (PDA) was dispensed in 9cm diameter Petri dishes which were then inoculated with 5mm agar plugs of 7 day old PDA cultures of *Sclerotium rolfsii* isolated from tomato plants. The plates were then incubated at

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 $25-27^{\circ}$ C for three weeks. The Sclerotia were collected from the plates and dried for 3days in an incubator at $25-30^{\circ}$ C.

2. **Pathogenicity Test:** Seeds of three cultivars of tomato namely Shase, Hoozua and UTC were sown in pots (2 seeds per pot) each containing 0.5kg of sterilized sandy loam soil. Two weeks after germination (5 leaf stage), these were inoculated with 12 dried Sclerotia of *Sclerotium rolfsii*. Sclerotia were placed beneath the soil surface contacting the stem of the plant. A non inoculated pot served as control. There were 4 pots per cultivar and 2 plants per pot laid out in complete randomized design. Data collected include weekly incidence and severity of *Sclerotium rolfsii* disease.

Incidence = <u>Number of plants infected</u> X 100 Total number of plants

Severity Scale - 0 = No Infection

1 = 1 - 20% of plant tissue damage 2 = 21-40% of plant tissue damage 3 = 41-60% of plant tissue damage 4 = 61-80% of plant tissue damage

5 = 81-100% of plant tissue damage

3. **Data Analysis:** Data generated from the study was analyzed using Analysis of Variance (ANOVA) and the Fishers Least Significant Difference (FLSD) was used to separate the means at 5% level of significance.

RESULTS

Pathogenicity Test

Incidence of *Sclerotium rolfsii* disease on the three cultivars of tomato in bioassay showed disease symptoms such as chlorosis, wilting, damping off, blighting and necrosis.

For Shase cultivar, chlorosis, wilting and damping off ranged from 50-100% in week three, while blighting and necrosis was 0%. In week four, incidence of chlorosis, wilting and damping off was 100% while blighting and necrosis was 0%. In week five and six, incidence of all disease symptoms was 100% as shown in Table I.

Variety/Replications	Chlorosis (%)	Wilting (%)	Damping off (%)	Blighting (%)	Necrosis	(%)
V ₁ A	0	0	0	0	0	
V_1B	50	50	50	0	0	week 3
V ₁ C	100	100	100	0	0	
V ₁ D	100	100	100	0	0	
V ₁ A	0	0	0	0	0	
V_1B	100	100	100	0	0	week 4
V ₁ C	100	100	100	0	0	
V ₁ D	100	100	100	0	0	
V ₁ A	0	0	0	0	0	
V_1B	100	100	100	100	100	week 5 & 6
V ₁ C	100	100	100	100	100	
V_1D	100	100	100	100	100	

Table I: Incidence of Sclerotium rolfsii Infection on Shase cultivar of Tomato in Bioassay from week 3-6

Key hase Cultiv

V₁.Shase Cultivar V₁A - Shase Cultivar Control V₁B - Replicate 1 V₁C - Replicate 2

 V_1D - Replicate 3

Analysis of Variance revealed a significant difference (P < 0.05) in the response of Shase cultivar to *Sclerotium rolfsii* infection with respect to the control as shown in Table II.

Variety	Chlorosis	Wilting	Damping off	Blighting	Necrosis	
Shase	83.33a	83.33a	83.33a	0.00a	0.00a	
Control	0.00b	0.00b	0.00b	0.00a	0.00a	week 3
LSD (0.05)	(46.3)	(46.3)	(46.3)	(0.00)	(0.00)	
Shase	100a	100a	100a	0.00a	0.00a	
Control	0.00b	0.00b	0.00b	0.00a	0.00a	week 4
LSD (0.05)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	
Shase	100a	100a	100a	100a	100a	
Control	0.00b	0.00b	0.00b	0.00b	0.00b	week 5 & 6
LSD (0.05)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	

Table II: Analysis of Variance in the Response of Shase Cultivar of Tomato to S. rolfsii infection in week 3-6

Footnote: Means having different alphabets in each week are significant at P=0.05, otherwise, they are the same. For Hoozua cultivar, chlorosis, wilting and damping off ranged from 50 -100% in week three while blighting and necrosis was 0%. In week four, incidence of chlorosis, wilting and damping off was 100% while blighting and necrosis was 0%. In week five and six, incidence of all disease symptoms was 100% as shown in Table III.

Table III: Incidence of Sclerotium rolfsii Infection on Hoozua cultivar of Tomato in bioassay from week 3-6

Variety/Replications	Chlorosis (%)	Wilting (%)	Damning off (%)	Blighting (%)	Necros	sis (%)
V A	0	0		0	0	15 (70)
V ₂ A	0	0	0	0	0	
V_2B	50	50	50	0	0	week 3
V_2C	100	100	100	0	0	
V_2D	100	100	100	0	0	
V_2A	0	0	0	0	0	
V_2B	100	100	100	0	0	week 4
V_2C	100	100	100	0	0	
V_2D	100	100	100	0	0	
V_2A	0	0	0	0	0	
V_2B	100	100	100	100	100	week 5 & 6
V_2C	100	100	100	100	100	
V_2D	100	100	100	100	100	

Key V2. Hoozua Cultivar V2A - Hoozua Cultivar Control V2B - Replicate 1 V2C - Replicate 2 V2D - Replicate 3

Analysis of Variance revealed significant difference (P < 0.05) in the response of Hoozua cultivar to *Sclerotium rolfsii* infection with respect to the control as shown in Table IV.

Table IV: Analysis of Variance in the Response of Hoozua Cultivar of Tomato to S. rolfsii infection in week 3-6

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Variety	Chlorosis	Wilting	Damping Off	Blighting	Necrosis	
Hoozua	66.67a	66.67a	66.67a	0.00a	0.00a	
Control	0.00b	0.00b	0.00b	0.00a	0.00a	week 3
LSD (0.05)	(46.3)	(46.3)	(46.3)	(0.00)	(0.00)	
Hoozua	100a	100a	100a	0.00a	0.00a	
Control	0.00b	0.00b	0.00b	0.00a	0.00a	week 4
LSD (0.05)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	
Hoozua	100a	100a	100a	100a	100a	
Control	0.00b	0.00b	0.00b	0.00b	0.00b	week 5 & 6
LSD (0.05)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	

Footnote: Means having different alphabets in each week are significant at P=0.05, otherwise, they are the same. For UTC cultivar, incidence of chlorosis, wilting and damping off ranged from 50-100% in week three, while blighting and necrosis was 0%. In week four, chlorosis, wilting and damping off was 100% while blighting and necrosis was 0%. In week five and six, incidence of all disease symptoms was 100% as shown in Table V.

Variety/Replications	Chlorosis (%)	Wilting (%)	Damping off(%)	Blighting (%)	Necrosis (%	(0)
V ₃ A	0	0	0	0	0	
V_3B	50	50	50	0	0	week 3
V ₃ C	50	50	50	0	0	
V_3D	100	100	100	0	0	
V ₃ A	0	0	0	0	0	
V_3B	100	100	100	0	0	week 4
V ₃ C	100	100	100	0	0	
V_3D	100	100	100	0	0	
V ₃ A	0	0	0	0	0	
V_3B	100	100	100	100	100 v	veek 5 & 6
V ₃ C	100	100	100	100	100	
V_3D	100	100	100	100	100	

Table V: Incidence of Sclerotium rolfsii Infection on UTC cultivar of tomato in Bioassay from week 3-6

Key V3. UTC cultivar V3A - UTC cultivar Control V3B - Replicate 1 V3C - Replicate 2 V3D - Replicate 3

Analysis of Variance showed a significant difference (P < 0.05) in the response of UTC cultivar to *Sclerotium rolfsii infection* with respect to the control as shown in Table VI.

Table VI: Analysis of Variance in the Response of UTC Cultivar of Tomato to S. rolfsii infection in week 3-6

Variety	Chlorosis	Wilting	Damping Off	Blighting	Necrosis	
UTC	83.33a	83.33a	83.33a	0.00a	0.00a	
Control	0.00b	0.00b	0.00b	0.00a	0.00a	week 3
LSD (0.05)	(46.3)	(46.3)	(46.3)	(0.00)	(0.00)	
UTC	100a	100a	100a	0.00a	0.00a	
Control	0.00b	0.00b	0.00b	0.00a	0.00a	week 4
LSD (0.05)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	
UTC	100a	100a	100a	100a	100a	
Control	0.00b	0.00b	0.00b	0.00b	0.00b	week 5 & 6
LSD (0.05)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	

Footnote: Means having different alphabets in each week are significant at P=0.05, otherwise, they are the same.

Severity of *Sclerotium rolfsii* on all tomato cultivars ranged from one to five as shown in Table VII which indicated 1-100% of plant tissue damage.

Table VII: Severity of <i>Sclerotium rolfsii</i> Infection on Tomato cu	ltivars in	ı Bioassay
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Variety/Week	3	4	5	6	
V ₁ A	0	0	0	0	
V_1B	1	2	4	5	Shase cultivar
V ₁ C	1	2	4	5	
V_1D	1	2	4	5	
V_2A	0	0	0	0	
V_2B	1	2	4	5	Hoozua cultivar
V_2C	1	2	4	5	
V_2D	1	2	4	5	
V ₃ A	0	0	0	0	
V_3B	1	2	4	5	UTC cultivar
V ₃ C	1	2	4	5	
V ₃ D	1	2	4	5	

Severity Scale

O – No infection

1 - 1 - 20% of plant tissue damage

2-21-40% of plant tissue damage

3-41-60% of plant tissue damage

4-61-80% of plant tissue damage

5 - 81 - 100% of plant tissue damage

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There was no significant difference (P > 0.05) in the severity of *Sclerotium rolfsii* on the tomato cultivars in each week as shown in Table VIII.

Table VIII: Analysis of Variance in the Severity of Sclerotium rolfsii infection on Tomato cultivars in Bioassay

Variety/Week	3	4	5	6
Shase	1.00a	2.00a	4.00a	5.00a
Hoozua UTC	1.00a 1.00a	2.00a 2.00a	4.00a 4.00a	5.00a 5.00a

	LSD (0.05)	(NS)	(NS)	(NS)	(NS)	
Footnote:	Means having same	letters	in each w	eek are i	the same	at P=0.05.
	NS - Nc	signifi	ant diffe	rence		



Shase cultivar of Tomato



Hoozua cultivar of tomato



UTC cultivar of tomato

Table I: Incidence of Sclerotium rolfsii Infection on Shase cultivar of Tomato in Bioassay from week 3-6

Variety/Replications	Chlorosis (%)	Wilting (%)	Damping off (%)	Blighting (%)	Necrosis	s (%)
V ₁ A	0	0	0	0	0	
V_1B	50	50	50	0	0	week 3
V ₁ C	100	100	100	0	0	
V ₁ D	100	100	100	0	0	
V ₁ A	0	0	0	0	0	
V_1B	100	100	100	0	0	week 4
V ₁ C	100	100	100	0	0	
V ₁ D	100	100	100	0	0	
V ₁ A	0	0	0	0	0	
V_1B	100	100	100	100	100	week 5 & 6
V ₁ C	100	100	100	100	100	
V_1D	100	100	100	100	100	

*Key V*₁.Shase Cultivar *V*₁A - Shase Cultivar Control *V*₁B - Replicate 1 *V*₁C - Replicate 2 *V*₁D - Replicate 3

Table II: Analysis of Variance in the Response of Shase Cultivar of Tomato to S. rolfsii infection in week 3-6.

Variety	Chlorosis	Wilting	Damping off	Blighting	Necrosis	
Shase	83.33a	83.33a	83.33a	0.00a	0.00a	
Control	0.00b	0.00b	0.00b	0.00a	0.00a	week 3
LSD (0.05)	(46.3)	(46.3)	(46.3)	(0.00)	(0.00)	
Shase	100a	100a	100a	0.00a	0.00a	
Control	0.00b	0.00b	0.00b	0.00a	0.00a	week 4
LSD (0.05)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	
Shase	100a	100a	100a	100a	100a	
Control	0.00b	0.00b	0.00b	0.00b	0.00b	week 5 & 6
LSD (0.05)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	

Footnote: Means having different alphabets in each week are significant at P=0.05, otherwise, they are the same.

Variety/Replications	Chlorosis (%)	Wilting (%)	Damping off (%)	Blighting (%)	Necros	is (%)
V_2A	0	0	0	0	0	
V_2B	50	50	50	0	0	week 3
V_2C	100	100	100	0	0	
V_2D	100	100	100	0	0	
V_2A	0	0	0	0	0	
V_2B	100	100	100	0	0	week 4
V_2C	100	100	100	0	0	
V_2D	100	100	100	0	0	
V_2A	0	0	0	0	0	
V_2B	100	100	100	100	100	week 5 & 6
V_2C	100	100	100	100	100	
V_2D	100	100	100	100	100	

Table III: Incidence of Sclerotium rolfsii Infection on Hoozua cultivar of Tomato in bioassay from week 3-6

Key

V₂ .Hoozua Cultivar V₂A - Hoozua Cultivar Control V₂B - Replicate 1 V₂C - Replicate 2 V₂D - Replicate 3

Table IV: Analysis of Variance in the Response of Hoozua Cultivar of Tomato to S. rolfsii infection in week 3-6.

Variety	Chlorosis	Wilting	Damping Off	Blighting	Necrosis	
Hoozua	66.67a	66.67a	66.67a	0.00a	0.00a	
Control	0.00b	0.00b	0.00b	0.00a	0.00a	week 3
LSD (0.05)	(46.3)	(46.3)	(46.3)	(0.00)	(0.00)	
Hoozua	100a	100a	100a	0.00a	0.00a	
Control	0.00b	0.00b	0.00b	0.00a	0.00a	week 4
LSD (0.05)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	
Hoozua	100a	100a	100a	100a	100a	
Control	0.00b	0.00b	0.00b	0.00b	0.00b	week 5 & 6
LSD (0.05)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	

Footnote: Means having different alphabets in each week are significant at P=0.05, otherwise, they are the same.

 Table V: Incidence of Sclerotium rolfsii Infection on UTC cultivar of tomato in Bioassay from week 3-6.

Variety/Replications	Chlorosis (%)	Wilting (%)	Damping off (%)	Blighting (%)	Necrosis (%	(0)
V ₃ A	0	0	0	0	0	
V_3B	50	50	50	0	0	week 3
V ₃ C	50	50	50	0	0	
V_3D	100	100	100	0	0	
V ₃ A	0	0	0	0	0	
V_3B	100	100	100	0	0	week 4
V ₃ C	100	100	100	0	0	
V_3D	100	100	100	0	0	
V ₃ A	0	0	0	0	0	
V_3B	100	100	100	100	100 v	week 5 & 6
V ₃ C	100	100	100	100	100	
V_3D	100	100	100	100	100	

Key V₃. UTC cultivar V₃A - UTC cultivar Control V₃B - Replicate 1 V₃C - Replicate 2 V₃D - Replicate 3

Variety	Chlorosis	Wilting	Damping Off	Blighting	Necrosis	
UTC	83.33a	83.33a	83.33a	0.00a	0.00a	
Control	0.00b	0.00b	0.00b	0.00a	0.00a	week 3
LSD (0.05)	(46.3)	(46.3)	(46.3)	(0.00)	(0.00)	
UTC	100a	100a	100a	0.00a	0.00a	
Control	0.00b	0.00b	0.00b	0.00a	0.00a	week 4
LSD (0.05)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	
UTC	100a	100a	100a	100a	100a	
Control	0.00b	0.00b	0.00b	0.00b	0.00b	week 5 & 6
LSD (0.05)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	

Table VI: Analysis of Variance in the Response of UTC Cultivar of Tomato to S. rolfsii infection in week 3-6

Footnote: Means having different alphabets in each week are significant at P=0.05, otherwise, they are the same.

Table VII	: Severity of	of Sclerotium	rolfsii Infection	n on Tomato	cultivars in	Bioassay

Variety/Week	3	4	5	6	
V ₁ A	0	0	0	0	
V_1B	1	2	4	5	Shase cultivar
V ₁ C	1	2	4	5	
V ₁ D	1	2	4	5	
V_2A	0	0	0	0	
V_2B	1	2	4	5	Hoozua cultivar
V_2C	1	2	4	5	
V_2D	1	2	4	5	
V ₃ A	0	0	0	0	
V_3B	1	2	4	5	UTC cultivar
V ₃ C	1	2	4	5	
V ₃ D	1	2	4	5	

Severity Scale

O - No infection

1 - 1 - 20% of plant tissue damage

 $2 - 21 \neg - 40\%$ of plant tissue damage

3 - 41 - 60% of plant tissue damage

4 – 61-80% of plant tissue damage

5 - 81 - 100% of plant tissue damage

Footnot

Table VIII: Analysis of Variance in the Severity of Sclerotium rolfsii infection on Tomato cultivars in Bioassay.

Variety/Week	3	4	5	6
Shase	1.00a	2.00a	4.00a	5.00a
Hoozua	1.00a	2.00a	4.00a	5.00a
UTC	1.00a	2.00a	4.00a	5.00a
LSD (0.05)	(NS)	(NS)	(NS)	(NS)

DISCUSSION

Soil infestation with *Sclerotium rolfsii* showed a reduction in germination parameters such as number of leaves, branches and height of the tomato cultivars as compared to the control. The inoculated tomato plants developed symptoms that were identical to those observed on naturally infested plant. Initially, their leaves became yellow and gradually, the entire plant turned brown to black and became blighted. This decrease in growth parameters showed disease condition of the plants. Similar findings had been reported by [4] (Sherf and MacNab, 1986) that decrease in growth parameters of tomato is associated with disease conditions.

Further observations showed that Shase, Hoozua and UTC cultivars treated with *Sclerotium rolfsii* recorded total necrosis of 100% in week five and six. This indicates that cultivars to resist Southern blight disease of tomato are not yet found. Similar results were observed by [5] (Shokes and Gorbert, 1998) who reported that *Sclerotium rolfsii* produced stem and pod rot in groundnut with potential death and estimated field losses of 89% or more. Similarly, [6] (Blum and Rodriguez, 2004) also reported reduction in seed germination and plant growth in soybean. Likewise,

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[7] (Khalequzzaman, 2003) recorded a reduction in length of shoot and root, fresh weight of shoot and root with nodules, number of pods, number of nodules and yield in soybean plants inoculated with *Sclerotium rolfsii* as compared to non inoculated plants. Similar results on the Pathogenicity of *Sclerotium rolfsii* have also been reported on *Edgeworthia papyrifera* from Taiwan [8] (Chang, 1994), maize and apple from Pakistan [9] (Ahmed *et al.*, 1984), [10] (Jahangir *et al.*, 1995), *Phaius flavas and Paphiopedidilum venustum* from India [11] (Bag, 2004), Chilli from Malaysia [12] (Jomduang, 1995) and apple from USA [13] (Conway and Tomasino, 1985).

The Pathogenicity of *Sclerotium rolfsii* on the tomato cultivars as reported in this study can be attributed to the ability of the pathogen to produce a mass of mycelium on the plant surface after which it produces an enzyme which deteriorates the host's outer cell layer for penetration of the host tissues [14] (Sadana *et al.*, 1983). *Sclerotium rolfsii* produces extracellular enzymes including pectinmethylesterases [15] (Bateman and Beer, 1965), cutinases [16] (Baker and Bateman, 1978), phosphatidase [17] (Kaveriappa, 1979), oxalic acid and polygalacturonases [18] (Bateman, 1972).

CONCLUSION

Results obtained from this study show that *Sclerotium rolfsii* proved to be highly pathogenic on the evaluated cultivars of tomato in pot experiments. Analysis of Variance revealed significant differences (P < 0.05) in the response of the cultivars to *Sclerotium rolfsii* infection with respect to their controls.

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