THE EFFECT OF *FUSARIUM OXYSPORUM f.sp. LYCOPERSICI* METABOLITES ON DROUGHT STRESS TOMATO PLANTS.

BY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY

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DECLARATION

I hereby declare that this project report written under the supervision of Dr. M.A. Abiala and is a product of my own research work. Information gotten from various sources has been duly referenced. This project report has not been previously presented anywhere for the award of any degree or certificate.

AKPATA E. JOY

Date

CERTIFICATION.

This is to certify that this research project **THE EFFECT OF** *Fusarium oxysporum* **METABOLITES ON DROUGHT STRESS TOMATO** was done by AKPATA, Joy Eloho, with matriculation number 17010101001. This project meets the requirement guiding the award of Bachelor of Science (B.Sc.) Degree in Microbiology Department of Biological Sciences of the Mountain Top University, Ogun State, Nigeria.

DR. M.A. ABIALA (Project Supervisor) Date

DR O.T. KAYODE (Head of Department) Date

DEDICATION

This work is dedicated to my creator and all-time support and strength giver God Almighty, he saw me through from the beginning to the end.

ACKNOWLEDGEMENT

All adorations goes to God Almighty who made the completion of this program a success. He has been my rock and fortress my all-time supporter and I'm forever grateful.

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I want to use this medium to say a big thank you to my project partner Oluwalaanumi Oshodi Abike for the love, moral and physical support and also her motivations, I really appreciate it.

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ABSTRACT

Tomatoes are one of the most widely grown and consumed vegetables on the planet. The effect of *Fusarium oxysporum f.sp. lycopersici* (FOL) metabolite on tomato, on the other hand, is a major challenge, especially during drought. Though, much research has been done on tomato plant growth, however, the impact of FOL metabolite on tomato seed germination has been overlooked, particularly when tomato seeds encounter dry condition. The effect of FOL metabolite on tomato seed germination under drought stress is investigated in this study using blotter techniques method. FOL metabolite and PEG-induced drought stress were evaluated individually and in combination on germination of tomato seeds. The results revealed that PEGinduced drought stress enhanced the effect of FOL metabolite on sensitive tomato cultivars more, in comparison to drought stress tolerant tomato cultivars that were slightly affected by FOL metabolites. Finally, the FOL metabolite had a high harmful effect on tomato seeds germination, especially when subjected to drought stress.

Keywords: *Fusarium oxysporum f.sp. Lycopersici* metabolite, PEG-induced drought stress, tomato.

ABBREVIATIONS

- FO Fusarium oxysporum
- F. Sp. Fusarium specie
- FA Fusaric acid
- FOM Fusarium oxysporum metabolites
- FOL Fusarium oxysporum f.sp. Lycopersici
- FOLM Fusarium oxysporum f.sp. Lycopersici metabolites
- PDA Potato dextrose agar
- PEG Polyethylene Glycol
- TC Tomato cultivar

CHAPTER ONE

1.0 INTRODUCTION

The *Fusarium* genus is one of the most complex and adaptable in the Eumycota, and the *Fusarium oxysporum* (FO) species complex comprises pathogens for plants, animals, and humans, as well as a varied spectrum of non-pathogens.

In nature, water is usually the most limiting factor for plant growth, according to (Khan et al., 2015). Drought stress, which occurs when plants do not receive enough rain or irrigation, can stunt growth more than all other environmental stresses combined. To conserve water, a plant slows its growth and reduces photosynthesis and other plant processes. Some species' leaves may appear to change color, usually to a blue-green, as water loss progresses. Foliage starts to wilt, and if the plant is not irrigated, leaves fall off and the plant dies. Aside from soil moisture content, high light intensity, high temperature, low relative humidity, and high wind speed all contribute to increased plant water loss. Drought stress can also be influenced by a plant's previous environment (Khan et al., 2015).

Tomato (*Solanum lycopersicum L.*) is one of the world's most important crops. It is consumed as a fresh or processed fruit because of its excellent nutritional properties, including vitamins, folate, and phytochemicals (Aldrich HT, et al. 2010). Various fungi, such as *Alternaria, Aspergillus, Fusarium, Rhizopus, Penicillium,* and *Trichoderma* species, can infect fresh vegetable fruits such as tomatos. A proteomic study was carried out by (Brackett, 1988) of a drought-stressed tomato plant which identified several differentially accumulated proteins in its root, with the majority in the down-regulated fraction in both genotypes. These belonged to cellular metabolic activities and protein translation categories. The accumulation of secondary metabolites involved in water stress protection was highlighted in metabolomic analyses (Guoting et al., 2020).

Fusarium oxysporum is a fungus that causes a variety of plant and human diseases as well as toxigenic microorganisms (Nelson et al., 1981; Laurence et al., 2014). The mycotoxin Fumonisins is produced by *oxysporum* (Desjardins, 2006). According to Proctor et al., (2008); Ramana et al., (2011), F. The presence of *oxysporum* on plants and other commodities, as well as the wide variety and frequent presence of

metabolites, has created a major constraint in major food crops. Some of *F. oxytoca's* metabolites Tomato wilt has been linked to *oxysporum f. sp. radicis-lycopersici* (Giovannucci 1999).

1.1 JUSTIFICATION

Tomato is one of the important vegetable to exemplify the challenges of food insecurity in Nigeria. The threat of *Fusarium oxysporum f.sp. lycopersici* metabolites on tomato cultivars from Nigeria has been established in Nigeria, However not in the presence of drought most especially during seed germination.

1.2 OBJECTIVES

This main objectives of this research work are;

- \checkmark To identify the effect of F. oxysporum metabolites on tomato cultivars
- ✓ To enumerate possible effect of F. oxysporum metabolites on morphological structure of drought stress tolerant tomato

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fungi secondary metabolites

Fungi's ability to manufacture a vast range of secondary metabolites, which are tiny chemicals that aren't required for regular growth or development, is one of its most remarkable characteristics (Fox and Howlett 2008). The evolutionary factors that led in their formation may not be the same as the functions that are observed or suggested today (Davies 1990). These metabolites, according to Demain and Fang (2000), serve as a competitive weapons against other bacteria, fungi, amoebae, plants, insects, and large animals; (ii) metal transporting agents; (iii) agents of symbiosis between microbes and plants, nematodes, insects, and higher animals; or (iv) agents of symbiosis between microbes and plants, nematodes Secondary metabolites, which allow organisms, particularly microbes, to carve out an ecological niche, are now widely accepted (Gloer 2007; Keller et al. 2005). The creation of secondary metabolites by fungi, bacteria, plants, and other organisms as a stress reaction to other fungi, insects, or animals demonstrates their ecological significance. Fungi will occupy an ecological niche if nutrients are available; however, other species will compete for these resources. Before these resources can be used, some type of interference competition, either behavioural or pharmacological, is thought to occur (Wicklow 1981). The ability of a rival to acquire a resource would be directly affected by the development of poisonous secondary metabolites by an organism, boosting their own chances of survival. Fungi can colonise a resource and develop secondary compounds that make the nutrient substrate undesirable to animals and microbes. Fungal endophytes, which live in plant vascular or leaf tissue without causing damage, are an example of this.

2.2 FOL of tomato plant

It's a soil-borne pathogen that produces metabolites like fusaric acid, which causes tomato wilt, making it one of the most common and dangerous diseases of tomato plants. Tomato wilt caused by *Fusarium oxysporum f.sp.*, according to pirsa, (2020).

Lycopersici is a devastating disease that has been reported in at least 32 countries. It is found in major tomato-growing regions around the world. *Fusarium oxysporum* f.sp. has been divided into three races. It has been reported that *lycopersici* exists. Their virulence towards tomato cultivars with single resistance genes distinguishes them. Race 1 was first documented in 1886, and Race 2 in 1945. In Australia in 1978, Race 3 was observed (pirsa 2020). Djordjevi et al. (2011) found that while race 1 of Fusarium wilt is not a limiting factor for successful tomato production, race 3 of *Fusarium oxysporum* f. sp. In Serbia, *lycopersici* is found and can pose a serious threat to tomato production (Djordjevic et al., 2011b).

2.3 F. oxysporum metabolites

Metabolites production is induced by a variety of conditions that are still being investigated. Some secondary metabolites, such as polyols, microsporines, and pigments, are thought to be created as a result of fungal adaptation to stressful situations (Kimura 2007). Some of the metabolites produced by *F. oxysporum* spp. are listed below:

a) Fusaric acid

Apart from fusarins, fusaric acid (FA) (figure 2.1) is well known for it's phytotoxicity and is one of the earliest identified phytotoxin in the tomato wilt symptoms induced by *F. oxysporum f. sp. lycopersici*. Although the toxin does not play any role in the early infection stage, it strongly helps to the pathogenesis process during the next stage (Selim 2015). Recently, the ion chelating activity of FA has been found in contaminated tomato (López-Díaz 2018). The severe phytotoxicity demonstrated by FA is mitigated by the exogenous input of copper, iron, and zinc. Also, the toxin can increase reactive oxygen species (ROS) level, inhibit the activity of antioxidant enzymes like catalase and ascorbate peroxidase, and promote cell death in detached tomato leaves (Singh 2014). External application of FA lowers chlorophyll pigments and raises total proteolytic enzyme levels in tomato, lowering photosynthetic rate, cellular metabolism, and inducing cell structure disruption, resulting to wilt (Singh 2017).



Fig 2.1 Chemical structure of fusaric acid (Singh 2017).

b) Fusarins

Polyketide compounds having a substituted 2-pyrrolidone on a polyenic chromophore are called fusarins (Gelderblom 1984). *Fusarium avenaceum, Fusarium culmorum, Fusarium fujikuroi, Fusarium graminearum, Fusarium oxysporum, Fusarium poae, Fusarium sporotrichioides, Fusarium venenatum, Fusarium sporotrichioides, Fusarium venenatum* (Niehaus 2014). Wiebe and Bjeldanes in Berkeley (California, USA) discovered fusarins A, B, C, and D (Figure 2.2) in 1981. (Wiebe 1981). Fusarin C, a mutagen that can convert auxotrophic Salmonella strains to prototrophic in the Ames *Salmonella typhimurium* test, was identified and partially characterised among the different types (Cheng 1985). The existence of the C13-14 epoxide ring is responsible for fusarin C's mutagenicity, whereas fusarin A and D lack this ring and are hence not mutagenic (Desjardins 2007). They may also play a function in esophageal cancer in humans (Li 1992).



Fig 2.2. Chemical structures of fusarins (Desjardins 2007).

c) Moniliformin

According to Vesonder, (1992) study, moniliformin (Figure 2.3) is less poisonous than T-2 toxin, fumonisins, butenolide, and dihydrofusaric acid and has the ability to impede plant growth by lowering the efficiency of photosynthentic pigment. *Fusarium avenaceum, Fusarium proliferatum, Fusarium subglutinans, Fusarium oxysporum, Fusarium chlamydosporum, and Fusarium anthophilum all produce moniliformin,* which is a prominent contaminant in cereals (Bullerman 2003). The toxin can prevent wheat seedlings from growing leaves and lower their bulk (Wakuli 1989).



Fig 2.3 Chemical structure of moniliformin (Bullerman 2003).

2.4 Drought stress

Drought is by far the most serious environmental stress in agriculture, and many efforts have been made to increase crop output in drought-stricken areas. Drought stress has a number of negative effects, including negative leaf water balance, turgor loss, chlorophyll (Chl) degradation, and photosynthetic down-regulation via affecting stomatal function and limiting carbon dioxide supply (Zhou et al. 2017). Drought stress affects cell enlargement, leaf growth, root and shoot development, dry matter partitioning, and, as a result, yield (Farooq et al. 2009). The preceding habitat of a plant can also influence drought stress. A plant that has been drought stressed in the past and recovered may become drought resistant in the future. Furthermore, a well-watered plant that has previously been drought-stressed will normally be able to resist the drought better than one that has been drought-stressed on a regular basis.

2.5 FOL metabolites and it's effect on drought stress tolerance tomato

Many metabolites are produced by *Lycopersici*, according to singh VK et al., (2017), however only a few have been identified. The mycotoxin fusaric acid causes Fusarium wilt in the tomato (*Lycopersicon esculentum*). By causing lipid peroxidation,

a rise in reactive oxygen species, and finally the death of host cells, it causes "Fusarium wilt." Fusaric acid produced by *Fusarium oxysporum* causes tomato wilt, which is one of the most frequent tomato diseases. *Fusarium oxysporum sp. Lycopersici* produces metabiolites, which is a key component in the development of wilt disease in tomato plants. It may cause disease symptoms in tomato plants depending on the dosage and exposure time (Singh and Upadhyay, 2014).the metabolites causes wilting of tomato stems and petioles, as well as necrotic areas on leaves, shrivelling and drying of leaves, and wilting of leaves. They also cause a transient membrane hyperpolarization, which limits the growth of roots and root hairs (Bouizgarne et al., 2006). Tomato plants develop wilt symptoms as a result of these metabolites causes variety of symptoms (leaf withering and necrosis), showing that thier metabolites is a factor in disease progression.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

To study "The effect of *Fusarium oxysporum f.sp. lycopersici* on drought stress tolerant tomato", an experiment was conducted at Mountain Top University during May- June 2021. Seeds of tomato cultivars namely NGB00725, NGB00714, NGB00740, NGB00715 were collected from National Center for Genetic Resources and Biotechnology Apata, Ibadan (NACGRAB). The seeds were grown in transparent disposable culture plate of 47mm in diameter, 15mm in height and 166ml in volume weighing 3.20 ounces and each culture plate was filled with 0.4g of cotton wool and then covered with filter paper

3.2 METHODS

3.2.1 Collection of Tomato and Fusarium oxysporum

The tomato cultivars, TC25, TC14, TC40, and TC15 were collected from National Center for Genetic Resources and Biotechnology Apata, Ibadan (NACGRAB), while the *Fusarium oxysporum f.sp. lycopersici* cultured plate was collected from The National Horticultural Research Institute and Training (NIHORT).

3.2.2 Screening of the tomato seeds

Invitro germination of the seeds using petri dish, cotton wool, and moisting filter paper was done. A quantifiable amount of 0.4g of cotton wool was put in a petri dish of 47mm in diameter, 15mm in height, 166ml in volume and 3.20 ounces in weight and was covered with filter paper to create a suitable seed bed for the seeds to grow and 15ml of water was added to damping the cotton wool in absence of soil to maintain adequate water capacity for the tomato cultivars. Therefore, 12 seeds from each seed samples were placed accordingly in each laelled petri dish for each tomato

sample. Each tomato cultivars were replicated 5 times and the seeds were then kept in a dark room for 3 days before exposure to light for four days.

3.2.3 Screening of the tomato seeds for drought stress (wilting)

PEG (polyethylene glycol) solution was prepared. 45g of PEG was weighed, 150ml of sterile distilled water was heated in a sterile beaker covered with aluminum foil on a magnetic bead stirrer. The PEG was then added bit by bit into the water and distilled water was then added to it till it reached 300ml. the solution was set aside to cool down. 15ml of the PEG solution was pippetted on the tomato seed bed and 10 seeds placed on the seed bed. It was replicated 5 times for each tomato cultivars and it kept in the dark for 3 days and exposed to light for 4 days

3.2.4 Purification of F. oxysporum

Exactly 11.7g of PDA was put in a borosilicate glass bottle, 300ml of distilled water was added to it and was autoclaved at 121°C for 15mins. The PDA was allowed to cool to bearable temperature and then 250mg of chlorephenicol mixed with sterile water and was then added to the agar before pouring. The agar was poured into 9 sterile petri dishes under aseptic conditions and allowed to solidify. It was incubated at room temperature for 2day after which the agar was checked for any contamination. Sub-culturing was done under sterile conditions. Cultured plate was cork bored using a sterile cork borer in creating smaller sections of the *F. oxysporum* colony. Colonies was transfer with sterile forcep and placed inversely onto the newly prepared agar and incubated for 5 days.

3.2.5 Extraction of F. oxysporum f.sp. lycopersici metabolites

An average of 200ml of potato dextrose broth was prepared in a conical flask. Smaller colonies of FOL culture initially bored was transfered with sterile forcep into the broth .Cultured broth was incubated in a shaking incubator for 7 days. After which the culture was filter with a muslin cheese cloth folded in 4 layers and filtered again with memberane milipore filter ($0.22\mu m$) in a buchner set up. Metabolites were collected in a sterile conical flask.

3.2.6 Inducing of tomato cultivars with FOL metabolites

A total of 10 seeds from each seed samples was put in 4 seperate seed beds. 10 seeds from each variety was soaked in 10mls of FOL metabolites for 30 second. The seeds were transferred onto the seed bed containing 15ml of water (FOLM+H2O). Each tomato cultivars were replicated 5 times and kept in a dark room for 3 days before exposure to light for four days.

3.2.7 Assessment of drought stress tolerance tomato and FOL metabolites interaction (toxicity and wilting)

A total of 10 seeds from each seed samples was put in 4 separate seed beds. 10 seeds from each variety was soaked in 10mls of FOL metabolites for 30 second. The seeds were transferred onto seed bed containing 15ml of PEG (FOLM+PEG). Each tomato cultivars were replicated 5 times and kept in a dark room for 3 days before exposure to light for four days.

CHAPTER FOUR

4.0 RESULTS

4.1 Screening of tomato seeds

After 4 days of cultivation in a dark room, growth as shown in Table 4.1 was observed. Tomato cultivar TC25 has the highest % germination followed by TC40, TC14 and TC15. After 7 days the results were recorded again and TC25 still has the highest %germination followed by TC40, TC14, TC15 this is shown in Table 4.2 To calculate the % of seed germination for each replicate:

% of seed germination = <u>number of seed germinated in each replicate x</u> 100 Total number of seed planted in each replicate 1 For example: To calculate the % germination for TC14 replicate 1

% of seed germination = 9×100 12 1 = 75%

To calculate for the average mean of % germination:

Average mean of % germination = <u>Addition of all replicate</u> Total number of replicate

For example: To calculate the average mean of % germination for TC14

Average mean of % germination = $\frac{R1+R2+R3+R4}{4}$ = $\frac{75+75+91.6+66.6}{4}$ = 77.05

4.2 Effect of drought stress (PEG) on the germinating tomato cultivars (wilting)

The result showed in Table 4.3 reviewed that TC15 has zero tolerance for drought stress i.e in the presence of PEG solution TC15 will not germinate. Meanwhile, TC25 showed high level of tolerance towards PEG solution with 38.36% of growth proving

it to be a drought stress tolerant plant and it was the first variety to achieve the dicot stage.Next is TC14 (36.66%) and the least TC40 (26.68%). The result was tabulated and the average mean of the five replicates calculated.

4.3 Effect of FOL metabolites on the germinating tomato cultivars (toxicity test)

Shown in table 4.4 is a result of the toxicity of FOL metabolites on tomato cultivars. FOL metabolites inhibited the growth of TC15 leading to seedling death of some of the seeds which is denoted by blackish appearance of the seed and no growth at all of others. FOL metabolites had effect on TC25 but did not inhibit the growth of TC25 in any form. Visible effect seen on TC25 include brown lesion girdling the hypocotyl, yellow and wilting of the leaves, root rot, and seedling death denoted by black coloration of the seeds. But the growth rate of the seeds were not affected in anyway. In growth rate, TC25 had the most growth and was the least affected by FOL metabolites followed by TC40 and the TC14 in decreasing level of growth and resistance.

4.4 Effect of FOL metabolites on the germination of drought stress tomato cultivars

Based on this experiment, FOL metabolites had effect on TC15. The only effect observed was seedling death and also due to drought stress (PEG solution) no growth was observed. FOL metabolites had an effect on the morphology of TC25 such as brown lesion girdling the hypocotyl, yellow and wilting of the leaves, root rot, and seedling death denoted by black coloration of the seeds but not the growth as the growth was not stunted. The PEG solution had no effect on the TC25 as it is drought stress tolerant. This mean that TC25 is resistant to drought with 96% average mean germination followed by TC40 with 68% and TC14 with 56%.

TC	No o	f seed	s plar	nted	No germ	of inated		seeds	% of s	seed ge	Average mean of germination (%)		
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	
TC14	12	12	12	12	10	10	12	9	83.3	83.3	100	75	85.4
TC15	12	12	12	12	2	1	6	1	16.6	8.3	50	8.3	20.8
TC25	12	12	12	12	10	12	12	12	83.3	100	100	100	95.8
TC40	12	12	12	12	12	11	11	12	100	91.6	91.6	100	93.8

Table 4.1: % Mean germination of the tomato cultivars at 7th day

Table 4.2:	% Mean germination of PEG induced drought stress tomato cultivars
at 7 th day	

TC	No	of see	eds cu	ıltiva	ted	No	of see	ed gei	mina	tion	% of	seed g	ermina	Average mean		
														of %germination		
	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	
TC14	10	12	12	12	10	4	6	0	0	2	22.2	50	0	75	25	26.66
1014	12	12	12	12	12	4	0	0	9	3	33.3	50	0	13	23	30.00
TC15	12	12	12	12	12	0	0	0	0	0	0	0	0	0	0	0
TC25	10	12	12	10	10	5	6	2	5	5	41.7	50	167	41.7	41.7	28.26
1025	12	12			12	3	0		3	3	41./	30	10.7	41./	41./	38.30
TC40	12	12	12	12	12	1	2	8	3	2	8.3	16.7	66.7	25	16.7	26.68

TC	No	of see	eds cu	ıltiva	ted	No	of see	ed gei	mina	tion	% of	seed	germ	Average mean		
														of %germination		
	D 1	DO	DO	DA	DC							DO	DO	1		
	KI	K2	K3	K4	КЭ	KI	K2	R3	K4	КЭ	KI	K2	K3	K4	КS	
TC14	10	10	10	10	10	7	6	4	4	8	70	60	40	40	80	58
TC15	10	10	10	10	10	0	0	0	0	0	0	0	0	0	0	0
TC25	10	10	10	10	10	10	8	8	10	8	100	80	80	100	80	88
TC40	10	10	10	10	10	7	5	4	9	6	70	50	40	90	60	62

Table 4.3: % Mean germination of FOL metabolites on germination of tomatocultivars at 7th day

Table 4.4: % Mean germination of Drought stress tomatos + FOL metabolites +PEG at 7th day

TC	No	of see	eds cu	ıltiva	ted	No	of see	ed gei	rmina	tion	% of	seed	germi	Average mean		
														of %germination		
	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	
TC14	10	10	10	10	10	6	5	6	5	6	60	50	60	50	60	56
TC15	10	10	10	10	10	0	0	0	0	0	0	0	0	0	0	0
TC25	10	10	10	10	10	10	10	10	8	10	100	100	100	80	100	96
TC40	10	10	10	10	10	7	7	6	7	7	70	70	60	70	70	68



Comparison between TC15 and TC25 on the effect of fol metabolite on peg induced drought stress tomato cultivar.

4.2 DISCUSSION

The tomato seeds were screened in 5 replicates to rate thier % germination. After 7 days of growth some variety of seed grew best than others. TC25 proved to grow best with a germination rate of 95.8% and TC15 had a very poor germination rate of 20.8%.

The actuality of FOL metabolites having an effect on drought stress tolerance tomato is good, as evidenced by this study. PEG, a chemical, was used to increase drought stress tolerance in tomato cultivars. PEG (polyethelene glycol) is a stress inducer that causes drought stress by depleting the plant's water supply. PEG was used on the tomato cultivars which reflected in thier %germination as shown in table 4.3. This was concorded to by Jokanović and Zdravković (2015), where according to thier researchthey stated that polyethylene glycol (PEG) treatments are commonly employed to cause drought stress in plants, especially in the early phases of their life cycle. The varying % germination reflect the drought stress tolerance of the tomato cultivar ranging from 38.36% of TC25 to 0% of TC15. TC25 is drought stress tolerant and TC15 having 0% growth is highly drought stress sensitive this is showed by van and zeng (2006) research work.

Metabolites produced by *Fusarium oxysporum* are part of FOL metabolites. *Fusarium oxysporum fungus. Schlect f. sp. lycopersici* (FOL) generates metabolites that are responsible for the most damaging soil-borne tomato disease. Although TC25 had the highest % germination rate of 88%, next is TC40 (62%) and then TC14 (58%) and the absolute least is TC15 with 0% growth this is shown in (table 4.4). TC25 still showed the effects of the metabolites also observed in (Vavrina, 1993) research which reflect the same results observed which showed symptoms like stunted growth, yellowing and wilting of the leaves, reddish discolorations of the xylem vessels (visible inside the stem as lines or spots in cross section), and root or stem degradation are all indications of Fusarium wilt (McGovern, et al., 1998).

Fusarium oxysporum is a sophisticated adaptable fungus that adapts quickly to changes in the environment. Because it has evolved to the drought stress state in the environment, *Fusarium oxysporum* can attack a drought stressed plant. Metabolites are primarily created when the plant has been attacked. Due to a lack of water and a weakened defense system, FOL metabolites have a greater impact on drought-stressed plants. The tomato cultivars were cultivated in PEG solution and induced with FOL

metabolites. After 7 days of cultivation TC25 had the best % germination of 96% and TC15 having 0% with seedling death due to the metabolites. TC25 had effects like root rot, brown lesion girding the hypocotyl, leaf wilting, yellowing of leaves followed by necrosis, seedling death which is in line with (McGovern, et al., 1993) where he noted that early symptoms of FOL in tomato seedlings include stunting, yellowing, and premature abscission of cotyledons and lower leaves, as well as a noticeable brown lesion that girdles the hypocotyls, root rot, wilting, and seedling mortality (McGovern, et al., 1998).

CHAPTER FIVE

5.1 CONCLUSION

This study found that a infected tomato cultivar with Fusarium oxysporum f.sp. lycopersici metabolites had negative effect on the tomato cultivar, regardless of whether the tomato cultivar is drought stress tolerant or not. It was also discovered that the effect of the FOL metabolite on PEG induced drought stress tolerant tomato cultivars is more than that of a tomato cultivar reared in normal conditions. This had an impact on their growth rate and morphology, causing brown lesions around the hypocotyls, yellowing and withering of the leaves, fusarium crown and root rot, seedling death, and even plant death.

REFRENCES

- Aldrich HT, Salandanan K, Kendall P, Bunning M, Stonaker F, Kulen O, Stushnoff C. Cultivar choice provides options for local production of organic and conventionally produced tomatoes with higher quality and antioxidant content. J Sci Food Agric. 2010;90:2548–55.
- Bouizgarne, B., El-Maarouf-Bouteau, H., Frankart, C., Reboutier, D., Madiona, K., Pennarun, A.M., et al., 2006. Early physiological responses of Arabidopsis thaliana cells to fusaric acid: toxic and signaling effects. New Phytol. 169, 209e218.
- Brackett, R.E.: Changes in the microflora of packaged fresh tomatoes. Journal of Food Quality, 2: 89-105, 1988.
- Bullerman, L.B. Mycometabolites Classififications. In *Encyclopedia of Food Sciences and Nutrition*, 2nd ed.; Caballero, B., Ed.; Academic Press: Cambridge, UK, 2003; pp. 4080–4089.
- Cheng, S.J.; Jiang, Y.Z.; Li, M.H.; Lo, H.Z. A mutagenic metabolite produced by *Fusarium moniliforme* isolated from Linxian County, China. *Carcinogenesis* 1985, 6, 903–905.
- Davies J (1990). What are antibiotics? Archaic functions for modern activities. Molecular microbiology, 4: 1227-1232.
- Demain A and Fang A (2000) The natural functions of secondary metabolites. History of Modern Biotechnology I. Springer Berlin Heidelberg, 1-39.
- Desjardins, A.E., 2006. Fusarium Mycometabolites Chemistry, Genetics and Biology. APS Press, St. Paul.
- Desjardins, A.E.; Proctor, R.H. Molecular biology of Fusarium mycometabolites. *Int.* J. Food Microbiol. 2007, 119, 47–50.
- Djordjević, M. Zečević, B., Djordjević, R., Zdravković, J. i Mijatović, M.: Uticaj rase 3 fuzarioznog uvenuća na pojedine sorte i hibride paradajza. Zbornik abstrakata IV simpozijuma Sekcije za oplemenjivanje organizama Društva genetičara Srbije, Kladovo, 2011b, str. 69.
- Djordjević, M., Zdravković, J., Zecević, B., Ugrinović, M. and Ivanović, M.: Reaction of different tomato cultivars to race 1 of Fusarium oxysporum f. sp.

lycopersici. Program and Abstracts ISHS Symposium on High Tunnel Horticultural Crop Production, State College, PA, USA, 2011, (no pages).

- Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: efects, mechanisms and management. In: Lichtfouse E, Navarrete M, Debaeke P, Véronique S, Alberola C (eds) Sustainable agriculture. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-2666-8_12
- Fox E and Howlett B (2008). Secondary metabolism: regulation and role in fungal biology. Current opinions in microbiology, 11: 481-487.
- Gelderblom, W.C.; Marasas, W.F.; Steyn, P.S.; Thiel, P.G.; van der Merwe, K.J.; van Rooyen, P.H.; Vleggaar, R.; Wessels, P.L. Structure elucidation of fusarin C, a mutagen produced by *Fusarium moniliforme. J. Chem. Soc. Chem. Commun.* 1984, 2, 122–124
- Giovannucci E (1999) tomatos,tomato-based products, lycopene and cancer: Review of the epidemiologic literature. J Natl Cancer Inst 91: 317-331.
- Gloer J (2007) Applications of fungal ecology in the search for new bioactive natural products. Environmental and Microbial Relationships, 2nd Edition, 257-283.
- Gordon TR, Okamoto D, Jacobson DJ (1989). "Colonization of muskmelon and nonsusceptible crops by Fusarium oxysporum f. sp. melonis and other species of Fusarium"(https://www.apsnet.org/publications/phytopathology/backissues/ documents/1989Abstracts/Phyto79_1095.htm) .Phytopathology.(10): 1095– 1100. doi:10.1094/Phyto-79-1095 (https://doi.org/10.1094%2FPhyto-79-1095).
- Guoting Liang, Junhui Liu, and Jingmin Zhang effects of drought stress on photosynthetic and physiological parameters of tomato145(1):12–17. 2020. https://doi.org/10.21273/JASHS04725-19
- Jokanović M. B. & Zdravković J. (2015). Germination of tomatoes under PEGinduced drought stress. *Ratar. Povrt.*, 52(3), 108-113. http://dx.doi.org/10.5937/ratpov52-8324
- Keller NP, Turner G, Bennett J. (2005) Fungal secondary metabolism from biochemistry to genomics. Nature Reviews in Microbiology, 3: 937-947.
- Khan SH, Khan A, Litaf U, Shah AS, Khan MA, et al. (2015) Effect of Drought Stress on Tomato cv. Bombino. J Food Process Technol 6: 465.

- Kimura, M.; Tokai, T.; Takahashi-Ando, N.; Ohsato, S.; Fujimura, M. Molecular and genetic studies of *Fusarium* trichothecene biosynthesis: Pathways, genes, and evolution. *Biosci. Biotechnol. Biochem.* 2007, *71*, 2105–2123.
- Laurence MH, Summerell BA, Burgess LW, Liew ECY (2014) Genealogical concordance phylogenetic species recognition in the *Fusarium oxysporum* species complex. Fungal Biol 118(4):374–384
- Li, M.X. Fusarin C induced esophageal and forestomach carcinoma in mice and rats. *Chin. J. Oncol.* 1992, *14*, 27–29.
- López-Díaz, C.; Rahjoo, V.; Sulyok, M.; Ghionna, V.; Martín-Vicente, A.; Capilla, J.;
 Di Pietro, A.; López-Berges, M.S. Fusaric acid contributes to virulence of *Fusarium oxysporum* on plant and mammalian hosts. *Mol. Plant Pathol.* 2018, 19, 440–453.
- Marre, M.T., Vergani, P., Albergoni, F.G., 1993. Relationship between fusaric acid uptake and its binding to cell structures in leaves of Egeria densa and its toxic effects on membrane permeability and respiration. Physiol. Mol. Plant Pathol. 42, 141e157.
- McGovern, R.J., Vavrina, C.S., MacKay, L.A., 1993. The effect of transplant tray type and tomato cultivar on the incidence of Fusarium crown and root rot in tomato transplants. Proc. Fla. State Hortic. Soc. 106, 173e175.
- McGovern, R.J., Vavrina, C.S., Noling, J.S., Datnoff, L.E., Yonce, H.D., 1998. Evaluation of application methods of metam sodium for management of Fusarium Crown and Root Rot in tomato in southwest Florida. Plant Dis. 82, 919e923.
- Nelson P.E., Toussoun T.A., Cook R.J., In: *Fusarium*: Disease, Biology and Taxonomy Pensylvania University Press, University Park, 1981, pp. 391-399.
- Niehaus, E.M.; Díaz-Sánchez, V.; von Bargen, K.W.; Kleigrewe, K.; Humpf, H.U.; Limón, M.C.; Tudzynski, B. Fusarins and fusaric acid in fusaria. In *Biosynthesis and Molecular Genetics of Fungal Secondary Metabolites*; Springer: New York, NY, USA, 2014; pp. 239–262.
- Pirsa (2020) Fusarium oxysporum as a multihost model for the genetic dissection of fungal virulence in plants and mammals. Infect Immun 72:1760–1766
- Proctor, R.H., Busman, M., Jeong-Ah, S., Lee, Y.W., Plattner, R.D., 2008. A fumonisin biosynthetic gene cluster in Fusarium oxysporum strain O-1890 and

the genetic basis for B versus C fumonisin production. Fungal Gene. Biol. 45, 1016–1026.

- Ramana, M.V., Balakrishna, K., Murali, H.S., Batra, H.V., 2011. Multiplex PCRbased strategy to detect contamination with mycotoxigenic Fusarium species in rice and finger millet collected from southern India. J. Sci. Food Agri. 91, 1666–1673.
- Selim, M.E.; El-Gammal, N.A. Role of fusaric acid mycotoxin in pathogensis process of tomato wilt disease caused by *Fusarium oxysporum*. J. Bioprocess Biotech. 2015, 5, 1
- Singh, V.K., Singh, H.B., Upadhyay, R.S., 2017. Role of fusaric acid in the development of 'Fusarium wilt' symptoms in tomato: physiological, biochemical and proteomic perspectives. Plant Physiol. Biochem. 118, 320– 332.
- Singh, V.K., Upadhyay, R.S., 2014. Fusaric acid induced cell death and changes in oxidative metabolism of Solanum lycopersicum L. Bot. Stud. 55, 66. http:// dx.doi.org/10.1186/s40529-014-0066-2.
- Van den Berg, L., & Zeng, Y. J. (2006). Response of South African indigenous grass species to drought stress induced by polyethylene glycol (PEG) 6000. *Afr. J. Bot.*, 72: 284-286.
- Vavrina, C.S. (Ed.), 1993. Fusarium crown and root rot of tomato: reevaluation of management strategies. In: Fla. Tom. Instit. Proc., Vegetable Crops Special Series, SS HOS1UniversityofFlorida-IFAS,pp.75e82.In: <u>http://swfrec.ifas.uflfl.edu/docs/pdf/veg-hort/tomatoinstitute/</u> proceedings/ti92_proceedings.pdf.
- Vesonder, R.F.; Labeda, D.P.; Peterson, R.E. Phytotoxic activity of selected watersoluble metabolites of *Fusarium* against *Lemna minor* L. (duckweed). *Mycopathologia* 1992, *118*, 185–189.
- Wakuli 'nski, W. Phytotoxicity of the secondary metabolites of fungi causing wheat head fusariosis (head blight). *Acta Physiol. Plant.* 1989, *11*, 301–306.
- Wicklow D (1981) Interference competition and the organization of fungal communities. In: Wicklow D, Carroll, G. The fungal community, its organization and role in the ecosystem. Marcel Dekker Inc.
- Wiebe, L.A.; Bjeldanes, L.F. Fusarin C, a mutagen from *Fusarium moniliforme* grown on corn. J. Food Sci. 1981, 46, 1424–1426.

Zhou R, Yu X, Ottosen CO, Rosenqvist E, Zhao L, Wang Y, Yu W, Zhao T, Wu Z (2017) Drought stress had a predominant effect over heat stress on three tomato cultivars subjected to combined stress. BMC Plant Biol 17:24. https://doi.org/10.1186/s1287 0-017-0974-x-017-0974-x