

EFFECT OF *FUSARIUM OXYSPORUM* f.sp. *LYCOPERSICI* ON DROUGHT STRESS
TOLERANT TOMATO

BY

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SCIENCES (B.Sc.) Hons DEGREE IN MICROBIOLOGY

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DECLARATION

I hereby declare that this project work was written under the supervision of DR. M.A. ABIALA.

This is a product of my own research work. Information derived from various sources have been duly acknowledged in the text and a list of references provided.

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Date

CERTIFICATION

This is to certify that this project report titled “**EFFECT OF *FUSARIUM OXYSPORUM f.sp. LYCOPERSICI ON DROUGHT STRESS TOLERANT TOMATO***” was carried out by OSHODI, Oluwalaanumi Abike with matriculation number 17010101011 of Microbiology in the department of biological sciences, in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) degree in microbiology.

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DEDICATION

I dedicate this work to God almighty for his divine strength, wisdom and for his guidance.

ACKNOWLEDGEMENT

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ACRONYMS

FO – *Fusarium oxysporum*

PEG- Polyethylene Glycol

PDA- Potato Dextrose Agar

FOS1- *Fusarium oxysporum* spore sample 1

FOS2- *Fusarium oxysporum* spore sample 2

FOS3- *Fusarium oxysporum* spore sample 3

TC- Tomato cultivars

R1- Replicate 1

R2- Replicate 2

R3- Replicate 3

R4- Replicate 4

R5- Replicate 5

FOL- *Fusarium oxysporum* f.sp. *Lycopersici*

ABSTRACT

Tomatoes are one of the most extensively produced vegetables globally and mostly consumed worldwide. However, the effect of *Fusarium oxysporum f.sp. lycopersici* (FOL) on tomato is a serious challenge most especially under drought condition. Though, lots of work have been done on tomato plant growth while the effect of *Fusarium oxysporum f.sp. lycopersici* (FOL) on tomato seed germination have been relegated to the background most especially under drought condition. This study hereby investigates the effect of FOL on tomato seed germination under drought stress condition. Blotter techniques method was employed to check the effect of *Fusarium oxysporum f.sp. lycopersici* (FOL) on tomato seed germination under PEG induced drought stress condition. Observation revealed that FOL inhibited germination of both drought sensitive and stress tolerant tomato cultivars. In addition, the effect of FOL was more on drought sensitive tomato in comparison to tomato that is not resistant to PEG induced drought stress. In conclusion, FOL exhibited high pathogenic effects on the tomato cultivars most especially in the absence of water.

Keyword: Tomatoes, *Fusarium oxysporum f.sp. lycopersici*, PEG-induced drought stress.

CHAPTER ONE

1.0 INTRODUCTION

Fusarium comes from a Latin word 'fusus' meaning a spindle. Fusarium is a vast genus of filamentous fungi that belongs to the hyphomycetes family of fungus. It is found in soil and is commonly connected with plants. *Fusarium oxysporum* (FO) is widely distributed in soilborne fungus communities in all types of soils around the world (Burgess, 1981). This species is also regarded as a natural component of plant rhizosphere fungal communities. All strains of *Fusarium oxysporum* are saprophytic, meaning they can grow and thrive on organic matter in soil and in the rhizosphere of many plant species for lengthy periods of time (Garrett, 1970). Pathogenic and non-pathogenic *Fusarium oxysporum* can coexist and cannot be separated morphologically unless pathogenic tests are conducted. Some strains of *F. oxysporum* are pathogenic to various plant species, penetrating the roots and causing root rots or tracheomycosis when they infiltrate the vascular system. Many other strains are capable of penetrating roots but do not infiltrate the vascular system or cause disease (Olivain and Alabouvette, 1997). As a result, nonpathogenic strains are being developed as biocontrol agents. Many economically important plant species have been severely harmed by wilt-inducing strains of *F. oxysporum*. Fusarium wilt infections have a high level of host specificity, and they are classified into more than 120 formae speciales and races depending on the plant species and cultivars they can infect (Armstrong and Armstrong, 1981).

Tomatoes (*Solanum lycopersicum*) are one of the most widely grown vegetable crops in the world, and Fusarium wilt, caused by *F. oxysporum* f.sp. *Lycopersici* is a dangerous disease that affects tomatoes and causes significant economic losses (Snyder and Hansen, 1940). It happens in the middle of the summer when air and soil temperatures are high, resulting in drought in most

cases. The infected plants' older leaves (those closest to the ground) turn yellow. The yellowing is usually limited to one side of the plant or to leaflets on one side of the petiole. Plants infected with *F. oxysporum* in the nursery stage may wilt and die soon after symptoms appear. Chemical soil fumigation and resistant cultivars are the mainstays of Fusarium wilt management. Broad-spectrum biocides, such as methyl bromide, are used to fumigate soil before planting and are harmful to the environment. If resistant cultivars are available, they are the most cost-effective and environmentally friendly method of control.

1.2. Justification

Tomato is one of the most important vegetables to exemplify the challenge of food insecurity in Nigeria. The threat of *Fusarium oxysporum* on tomato cultivars in Nigeria has been established. However, not in the presence of drought most especially during seed germination.

1.3. Objectives

This present project work was conducted in order to achieve the following objectives.

- To identify the effect of *Fusarium oxysporum* spores on tomato cultivars.
- To enumerate possible effect of *Fusarium oxysporum* spores on morphological structure of drought stress tolerant tomato.

CHAPTER TWO

2.0 Literature Review

This literature review shows various types of experiments that have been conducted exhibiting the response of tomato to drought stress condition. Some examples are given below

2.1. Drought

Drought is an event of protracted shortages in the water supply, whether atmospheric, surface water or ground water. Drought stress tolerance is when a plant maintains its biomass output during arid or drought circumstances. In most regions of the world, it is a periodic aspect of the climate. Some plants are naturally adapted to dry environments, surviving thanks to defense mechanisms like desiccation tolerance, detoxification, or repair of xylem embolism. Because of the decreased P N, leaf area, shoot fresh and dry weight, drought stress may cause a greater drop in tomato output than heat stress. However, further research is needed into the effects of single and combined stress on tomato flowering, fruit set, and yield. Arid biomes such as deserts and grasslands result from semi-permanent drought (Keddy, 2007). Drought symptoms are similar to salt stress because excessive salt concentrations in the root zone cause osmotic water loss from roots. Aside from the soil moisture content, high light intensity, high temperature, low relative humidity, and high wind speed will all contribute to increased plant water loss. A plant's previous environment can also have an impact in the development of drought stress. A plant that has previously been drought stressed and recovered may become drought resistant. Also, a plant that was well-watered before the drought would usually do better than one that has been drought-stressed for a long time. Drought is by far the most serious environmental stress in agriculture, and various efforts have been undertaken to increase crop yield while reducing conditions While

natural selection has prioritized adaptability and survival mechanisms, breeding activity has focused selection on enhancing the economic yield of cultivated species.

Leperen et al. (2003), looked at how water stress affected the distribution of xylem hydraulic resistance in tomato fruiting trusses. The xylem hydraulic resistances of peduncles (truss stalks), pedicels (fruit stalks), and the future abscission zone (AZ) halfway along the pedicel of tomato (*Solanum lycopersicum*) plants were measured in this study at successive levels of fruit development in plants grown under two levels of water availability in the root environment. They discovered that the highest resistances were found in the AZ, where the majority of individual vessels terminated. Plants growing in a root environment with low water availability had xylem with increased hydraulic resistances in the peduncle and pedicel segments on both sides of the AZ, with the AZ having the biggest increase in hydraulic resistance. Hydraulic resistances in peduncle and pedicel segments reduced on both sides of the AZ throughout fruit growth, but tended to keep rising in the AZ. Because of the dominant role of hydraulic resistance in the AZ, the overall xylem hydraulic resistance between the shoot and fruit tended to grow with fruit development.

Gabriella et al. (2004), investigated the histone-like protein H1-S and tomato leaf water deprivation response. They researched the tomato H1-S linker histone, which is activated by drought. For their failure to accumulate H1-S in response to water stress, they chose three separate H1- antisense tomato mutants. The physiological and morphological characteristics of these mutants have been studied. They discovered that histone H1-S antisense transgenic plants developed normally, showing that H1-S does not play a role in tomato development's fundamental activities. There were no alterations in chromatin structure, indicating that H1-S does not have a structural role in chromatin organization.

With a study on variations in proline content, Hatem et al. (2006), looked at the physiological reactions. In tomato plants, the effects of various levels of water stress on leaf water potential, stomatal resistance, protein and chlorophyll content, and particular anti-oxidative enzymes were examined.

2.2. Drought Stress in Germination Stage

Germination is one of the most crucial stages of plant development, and it is frequently influenced by environmental conditions such as dehydration (Leila, 2007). Both live and dead seeds absorb water and swell during the first stage of germination. The amount of water absorbed is determined by the seed's chemical composition. Protein's mucilage and pectin are more hydrophilic colloid and absorbs more water than starch. Grains such as corn, soybeans, almost a third of a second seed weight to absorb water. Soil moisture that is lower than the field capacity is usually advantageous for germination. The germination rate is reduced by using a wilting point moisture farm. Water absorption and germination stop if the water level isn't high enough. (Leila, 2007) Water is the most important factor in germination, because osmotic and matric potential decrease availability to water. The pace of water absorption and, as a result, the germination plant is affected by the water potential environment. Drought stress can reduce germination rates, but it's important to pay attention to drought stress at different stages of germination to figure out what's different. (Zareian, 2004).

2.3. Drought Stress in Vegetative Stage

Drought stress in the vegetative state has a lower impact on yield and yield components than it does in the reproductive stage, but because the stress at this stage of development of leaf, stem development, photosynthesis, leaf, and accumulation is so important, it has a significant impact

on plants (Kabiri, 2010). The primary reasons of dry weight in the vegetative stage of stress can include genuine photosynthesis, leaf area index, and stimulating the reproductive phases of the plant (Diallo et al. 2001). Drought has two main effects: reduced germination and poor stand establishment (Harris et al. 2002). Drought stress has been shown to significantly affect seedling germination and stand (Kaya et al. 2006).

2.4. Spores of *Fusarium Oxysporum*

Because a teleomorph has never been detected in nature or created in the laboratory, *Fusarium oxysporum* is thought to reproduce asexually. The possibility of a cryptic sexual cycle, on the other hand, cannot be fully ruled out. Both mating-type genes have been discovered in FOS members (Yun et al. 2000). Although some studies (Bentley et al. 1998; Koenig et al. 1997) supported the clonality of FO based on allele association, reanalysis of the data (Taylor et al. 1999) revealed that the possibility of recombination could not be ruled out. Asexual spores come in three varieties. Microconidia are one or two cells that are oval or elliptical in shape. They're made in a variety of environments, including liquid and solid culture media, the rhizosphere, and the vascular system of infected plants. Macroconidia are three to five-celled organisms with gradually pointed and curved ends. Macroconidia are commonly found on the surface of FO-killed plants as well as in sporodochia (Katan et al. 1997). FO's long-term survival is ensured by thick-walled chlamydospores. Chlamydospores are produced either terminally or intercalarily on older mycelium or in macroconidia, and they can survive in the soil for many years, posing a long-term crop production constraint in previously infested fields. The molecular mechanism that underpins the formation of these spores in FO is unknown. However, a combination of genome-enabled approaches can quickly address this deficiency (e.g., profiling of expressed genes and proteins, systematic mutagenesis of candidate genes). New insights into asexual reproduction

may offer novel ways to manage FO diseases, given the importance of these spores for FO pathology.

CHAPTER THREE

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- July 2021. Seeds of tomato cultivars namely NGB00725, NGB00714, NGB00740, NGB00715 were collected from National Center for Genetic Resources and Biotechnology Apata, Ibadan (NACGRAB) and plated *Fusarium oxysporum* culture was also collected from. 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

The tomato culture namely NGB00725, NGB00714, NGB00740, NGB00715 were collected from National Center for Genetic Resources and Biotechnology Apata, Ibadan (NACGRAB), while the *Fusarium oxysporum* were collected from The National Horticultural Research Institute and Training (NIHORT).

3.2.2 Screening of The Tomato Seeds

A quantifiable amount of 0.4g of cotton wool was put in a medium sized transparent disposable 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 substrate for the seeds to grow in absence of soil. Therefore, 12 seeds were placed accordingly in each cup for each tomato sample. Watering was done to maintain adequate water capacity for the tomato cultivars till each tomato grow leaves and each tomato cultivars were replicated 5 times. 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

Forty-five gram (45g) of PEG (polyethylene glycol) 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 then set aside to cool down and 15ml of the PEG solution was used to plant the tomato seed samples which was repeated 5 times for each tomato cultivars.

3.2.4 Purification of *Fusarium oxysporum*

A total of 11.7g of PDA (PDA is a general-purpose medium for the cultivation of yeast and mold that can be supplemented with antibiotics to inhibit bacteria growth (Sadar, 2019)) was put in a borosilicate glass bottle, 300ml of distilled water was added to it and was autoclaved at 121c for 15mins. The PDA was allowed to cool 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 before putting it into the incubator at room temperature for 2day after which the agar was checked for any contamination. Then sub culturing was done under sterile conditions by cutting part of the fusarium from the initial agar which it was growing from to the newly prepared agar for growth.

3.2.5 Preparation of *Fusarium Oxysporum* inoculum size using single cell techniques

Six (6) plates of uncontaminated *Fusarium Oxysporum* was used. The *Fusarium Oxysporum* samples were washed with sterile water into a sterile beaker covered with a sterile muslin cloth, to allow only the spores to drop into the beaker and not the mycelium. After which 100ml of water was added to the 1st spore sample (FOS1) and from the first sample 100ml was poured into a 2nd sterile beaker (FOS2) and 100ml of sterile was added to the 2nd beaker making it 200ml. From the 200l in the second beaker 100ml was poured into a 3rd beaker (FOS3) and 100ml of sterile water was added to it. The 3 beakers named FOS1, FOS2, and FOS3 where then kept in the fridge. Then spectrophotometer reading was done for all 3 samples using wavelength 540nm, 600nm, 700nm and culturing was done to check for viable and non-viable spores.

3.2.6 *Fusarium Oxysporum* Spores interaction with Drought Stress Tomato (pathogenicity and wilting)

After seed sterilization, the Tomato cultivars was planted in 5 replicates as follows: Tomato alone planted with distilled water, Tomato + FOS1 planted with distilled water, Tomato + PEG, Tomato + FOS1 + PEG. For the Tomato + FOS1, the tomato seeds were soaked into 5ml of the FOS1 for 30secs to maximum of 1 minutes and the same procedure was done for Tomato + PEG + FOS1. But for Tomato alone and Tomato + PEG, the seed was not soaked in the FOS1 and all these were done in sterile condition and it was done to check the for the interaction of the FOS1 with drought stress tomato and to see the growth of all samples under the same condition.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1. Screening of the tomato seeds

It was observed that after 5 days of planting it was noticed that tomato cultivar NGB00725 has the highest % germination followed by TC40, TC14 and TC15. Then after 7 days the results were recorded again and TC25 still has the highest % germination followed by TC40, TC14 and TC15 has the lowest germination rate as shown in Table 4.1.

To calculate the % of seed germination for each replicate:

$$\% \text{ of seed germination} = \frac{\text{number of seed germinated in each replicate}}{\text{Total number of seed planted in each replicate}} \times 100$$

For example: To calculate the % germination for TC14 replicate 1

$$\begin{aligned} \% \text{ of seed germination} &= \frac{9}{12} \times 100 \\ &= 75\% \end{aligned}$$

To calculate for the average mean of % germination:

$$\text{Average mean of \% germination} = \frac{\text{Addition of all replicate}}{\text{Total number of replicate}}$$

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

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

4.1.2 Effect of drought stress on the tomato cultivars (wilting)

Planting the tomato cultivars with PEG (polyethylene glycol solution) for 7 days it was observed that TC25 has the highest germination rate (38.36) even in the presence of drought as shown in Table 4.2 which means TC25 is a drought stress tolerant tomato followed by TC14(36.6) and TC40. It was also observed that TC15 has no sign of growth as shown in Table 4.2 meaning it is not resistant to drought. The results were noted down and the mean of the 5 replicates of the %germination was done.

4.1.3 Results of Spectrophotometer Reading

After sub culturing to get pure culture samples of *Fusarium oxysporum*, using single cell techniques has done in 3.2.5, Spectrophotometer reading was done for all 3 samples (FOS1, FOS2 and FOS3) using wavelength 540nm, 600nm, and 700nm. The result gotten from 600nm wavelength of the FOS1 sample has the best result according to (Schitz, 2020) which stated that 600nm wavelength of results 1×10^7 spores/ml between 2.015 ± 0.083 is suitable to be used and has shown in Table 4.3

4.1.4 Effect of *Fusarium Oxysporum* Spores with Tomato cultivars TC14, TC15, TC25 and TC 40 (Pathogenicity and wilting)

Based on this experiment, In the presence of FOS1, *Fusarium oxysporum* inhibited the seeds of TC15. However, TC14, TC40 and TC25 exhibited slight tolerance to FOS1. Quantitatively, TC25 had the highest germination% in the presence of FOS1, followed by TC40 and TC14 as shown in Table 4.4 while TC15 has no sign of germination or seedling death in the presence of FOS1. In the presence on FOS1 + PEG, FOS1 + PEG inhibited the growth of TC15 as shown in table 4.5. Also, TC25 exhibited tolerance to both the FOS1 and PEG and TC25 still had the highest germination % even in the presence of FOS1 and PEG as shown in table 4.5 and also exhibited symptoms of FO.

Table 4.1 Mean germination of tomato seeds at 7 days of planting

TC	No of seeds planted				No of seeds germinated				% of seed germination				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	95.82
TC40	12	12	12	12	12	11	11	12	100	91.6	91.6	100	93.8

Table 4.2 Screening of tomato cultivars for drought stress using PEG.

TC	No of seeds planted					No of seeds germinated					% of seed germination					Average mean of % germination
	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	
TC14	12	12	12	12	12	4	6	0	9	3	33.3	50	0	75	25	36.6
TC15	12	12	12	12	12	0	0	0	0	0	0	0	0	0	0	0
TC25	12	12	12	12	12	5	6	2	5	5	41.7	50	16.7	41.7	41.7	38.36
TC40	12	12	12	12	12	1	2	8	3	2	8.3	16.7	66.7	25	16.7	26.68

Table 4.3 Spectrophotometer reading to determine the inoculum size

Wavelength	Blank	FOS1			FOS2			FOS3		
		R1	R2	R3	R1	R2	R3	R1	R2	R3
540nm	0.00	0.653	0.790	0.498	1.201	0.697	0.511	0.685	0.601	0.729
600nm	0.00	1.368	1.472	0.775	0.932	1.098	1.134	0.808	0.779	0.272
700nm	0.00	0.648	1.532	1.531	0.963	0.929	0.863	0.195	0.211	0.210

Table 4.4 Effect of FOL on germination of tomato seeds.

TC	No of seeds planted					No of seeds germinated					% of seed germination					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	9	3	8	6	9	90	30	80	60	90	70
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	9	10	10	100	100	90	100	100	98
TC40	10	10	10	10	10	10	9	10	8	10	100	90	100	80	100	94

Table 4.5 Effect of FOL on germination of tomato seeds under PEG- induced drought stress.

TC	No of seeds planted					No of seeds germinated					% of seed germination					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	9	10	8	9	5	90	100	80	90	50	82
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	9	8	100	100	100	90	80	94
TC40	10	10	10	10	10	7	8	9	10	10	70	80	90	100	70	82

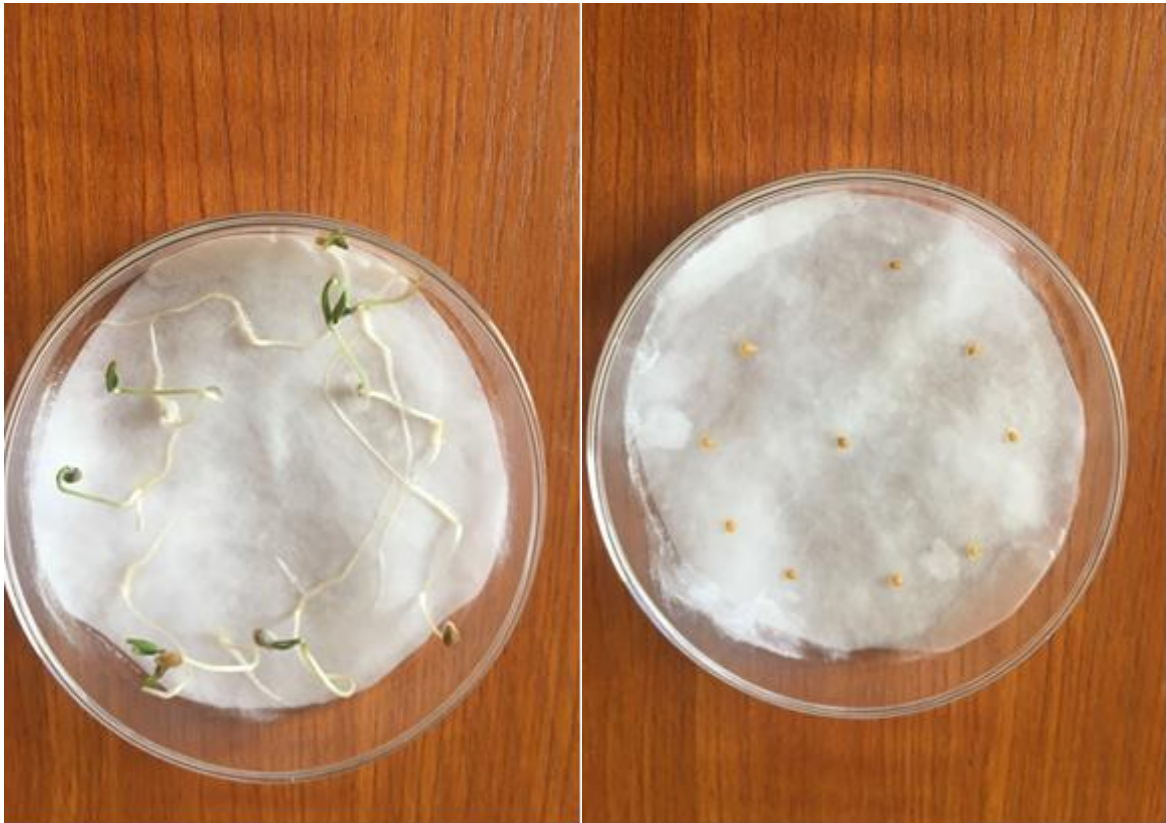


Figure 4.1: Comparison of TC25 and TC15 on the effect of FOL and PEG-induced drought stress.

4.2 Discussion

PEG is a stress inducer that causes drought stress by depleting the plant's water supply. Polyethylene glycol (PEG) treatments are commonly employed to cause drought stress in plants, according to Rageb et al. (2007), especially in the early phases of their life cycle. However, the PEG solution concentrations that have been described as useful in discriminating between tolerant and sensitive genotypes differ. This is most likely related to the features of the material evaluated in such research (number of genotypes, divergence, origin). The stage of plant growth during which the treatment was administered, as well as the length and kind of the experiment (in vitro, hydroponic, pot). From this research work, it was observed that after 7 days of planting the Tomato cultivars with PEG, TC25 has the highest germination % followed by TC14 and TC40 which means they exhibited drought resistant as shown by George et al. (2013). But the PEG inhibited the growth of TC15 which means TC15 is not resistant to drought. In the presence of FOS without inducing the tomato cultivars with PEG TC25 also had the highest germination followed by TC40 and TC14 has it was in concordance to Ghebremariam et al. (2013). Also, after the addition of FOS to the tomato seeds induced with PEG, the FOS and PEG inhibited the seeds of TC15 with 0% germination because there was no sign of germination of the seeds or seedling death. But TC14, TC25 and TC40 exhibited slight tolerance to the FOS1 and PEG especially TC25 which had the highest % germination and exhibited symptoms of *F. oxysporum* f.sp. *Lycopersici*.

CHAPTER FIVE

5.0 CONCLUSION

5.1. Conclusion

Fusarium oxysporum inhibited the germination of TC15 with and without PEG induced drought stress and the symptom include no sign of germination of the seeds. In comparison to TC15, *Fusarium oxysporum* only had slight effect on TC25 with or without PEG induced drought stress and symptoms include seedling death, root rot, wilting of the leaves, brown lesion hypocotyls etc.

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