American Journal of Biomedical Science & Research

www.biomedgrid.com

ISSN: 2642-1747

Research Article

Impacts of Inorganic Nitrogen Compounds on Fusarium - Potential Control Measures for Sustainable Agriculture

H Akram[1], S Hussain[2] and TE Butt[3]*

[1]*Institute of Biological Sciences, Faculty of science, University of Malaya, Malaysia*

[2]*College of Earth and Environmental Sciences, University of the Punjab, Pakistan*

[3]*Faculty of Engineering & Environment, Northumbria University, UK*

***Corresponding author:** TE Butt, 3Faculty of Engineering & Environment, Northumbria University, City Campus, Newcastle upon Tyne. Postcode: NE1 8ST. England, UK.

To Cite This Article: *H Akram, S Hussain and TE Butt*. Impacts of Inorganic Nitrogen Compounds on Fusarium - Potential Control Measures for Sustainable Agriculture. Am J Biomed Sci & Res. 2023 20(3) AJBSR.MS.ID.002701,* DOI: 10.34297/AJBSR.2023.20.002701

Received: September 25, 2023; **Published:** October 16, 2023

Abstract

Fusarium Oxysporum F.Sp. *Lycopersici* (FOL) is one of the most common soil-dwelling pathogens which causes wilt on tomato crop. On the contrary, tomato is one of the most important economic crops of the world, which is susceptible to invasion of FOL. Although different growth media have been used for invitro studies of FOL growth, FOL has still not been much studied in varying concentrations and combinations of these three inorganic nitrogen compounds: KNO_3, $(NH_4)2SO_4$ and Urea, and these three media: Malt Extract Agar, Potato Dextrose Agar and V8. As opposed to organic compounds, this study innovatively regards the aforesaid three nitrogen compounds to observe the FOL growth in the three aforesaid different culture media. The isolate of FOL is derived from soil sample of Kasur region of district Lahore, Pakistan. Then the isolate is cultured under different nutritional conditions by providing aforesaid media and nitrogen compounds. Varying combinations of these media and compounds are studied, in which the impacts of the nitrogen compounds in the different culture media, have been observed for 7 days to establish the growth behavior of FOL. Concentrations of each of the three nitrogen compounds are varied by preparing solutions of 0.5g/L, 1.0g/L and 1.5g/L of each compound. Radial growth is measured in mm while mass is determined in mg. Results are presented in tables and graphs are drawn for analyses. It is found that the growth pattern varies with combination of media and nitrogen compounds. The MEA is observed to be the best medium for the growth inhibition as opposed to the other two media. Additionally, the best nitrogen source for the growth control is noticed to be Urea. These findings pave a path for further research where invitro FOL growth control is required in morphological, anatomical, physiological and biochemical studies of pathogen.

Keywords: Sustainable agriculture, Nitrogen compounds, Inorganic compounds, *Fusarium oxysporum* f.sp. *Lycopersici* (FOL), Culture media (Table 1)

Introduction

Background

Table 1

Abbreviation	Full name	Abbreviation	Full name
MEA	Malt Extract Agar	V8	V8 juice
PDA	Potato Dextrose Agar	Temp	Temperature
f.sp	Fusarium species	Comp	Compound
g	gram	F	*Fusarium*
L	Liter	FOL	*Fusarium oxysporum* f.sp. *lycopersici*
mm	millimeter	mg	milligram

The development and advancement in biotechnology has been playing a vital role in the field of agriculture [1]. Even though this agricultural revolution has multiplied food production, still the gap between the food demand and the food production is increasing, for which one crucial reason is that the human population has been growing faster than ever [2]. Despite the advancement in agricultural technologies, the pathogens community of microbes is still posing greatest risk to crops because of the diversity and cosmo politan nature of microbes [3]. One of the ways to overcome the gap between the food demand and the food supply is to safe food crops from pathogenic invasion particularly those of tender and sensitive for pathogens. Of such crops, tomato is one of the most susceptible crops in demand being a second largest crop of the world in terms of productivity, and can easily be exploited by pathogens. Because of its status as food, tomato has been propagated to increase values like, productivity, fruit value, and resistance to biotic and abiotic pressures. It has been extensively used not only as food, but also as investigation material. On a worldwide scale, the yearly production of fresh tomatoes has been estimated approximately 159 million tons. But, more than one fourth of those 159 million tons are grown aimed at the processing industry, which marks tomatoes the world's principal vegetable for processing. The nine chief producing countries contribute 74.2% of the world's annual production are china, India, USA, Turkey, Egypt, Iran, Italy, brazil and Spain. Hence this crop is to be saved to ensure its contribution in the fulfillment of the food demand of accelerating human population.

Among a number of diseases in tomato, *Fusarium* wilt is, so far, the most common wilt disease in the world [4]. Since the establishment of tomato as prime crop in food industry, *Fusarium* wilt has always been persistent problem in tomato crop. Even though, no clear-cut information on the damages caused by the disease is existing, but the yield loss may fluctuate from 10 to 100% subject to the environmental conditions. Estimated yearly loss of millions of dollars has been reported owing to wilt disease. At least 80% of all cultivated plants are associated with at least one disease caused by a *Fusarium* species [5]. Thus, they are responsible for huge economic losses due to reductions in harvest yields and/or the quality of staple foods.

The genus *Fusarium* is related with class Hyphomycetes from the group of Mitosporic fungi (formerly Deuteromycotina) [6]. This is well adapted to diverse habitats and is supposed to be the earliest fungal colonists on earth. It occurs in various environments such as the deserts and arctic [7]. A large number of species within this genus are extensively distributed soil borne species that cause diseases to many economically important crops. Various members of this genus are important plant pathogens. It was investigated that they have the greatest pathogenic domain based on the number of pathogenic taxa, the host range they attack, and the diversity of habitats in which they cause disease in plant [8]. Among them, *Fusarium oxysporum* f.sp. *lycopersici* is well signified in the world. *Fusarium oxysporum* f.sp. lycopersici is saprophytic. It can endure for a considerable time on soil organic matter and in the rhizosphere of many kinds of plants [9]. Physical and chemical environments have a significant impact on diagnostic traits of fungi. Therefore, it is essential to use different media in order to identify a fungus in culture as mycelial growth and sporulation on artificial media are chief biological features.

State of the Existing Knowledge

Different media and nutrient variables methods have been developed to reduce the occurrence and harshness of *Fusarium* crown rot affected by *Fusarium oxysporum*. The two isolates of *F. oxysporum* f.sp. *elaeidis* were used to investigate the nitrogen requirements. Out of the 10 tested nitrogen compounds, conspicous growth and sporulation was observed on potassium nitrates, ammonium and peptone. But no substantial increase in growth was noticed when the nitrogen (KNO_3) of the medium was increased [8].

The outcome of nitrogen concentration in the tissues of tomato was explored as a result of nitrogen supply frequency, on the vulnerability of tomato plants to the selected pathogens. They varied tissue nitrogen concentration by providing nitrogen at regular intervals by increasing quantities to the growth medium on which tomatoes were grown. Experiments were carried out to check the susceptibility of tomato plants to various pathogens including the wilt agent *Fusarium oxysporum* f.sp. *lycopersici*. The effect of nitrogen concentration in the tissues seemed to be greatly pathogen-dependent, there was no influence on proneness to *F. oxysporum* [10]. While in this study nitrogen compounds are used instead of nitrogen only, in different media with varying combinations to observe the growth of the pathogen.

The *Fusarium oxysprum* f,sp. *lycopersici* as the causal agent of tomato wilt was studied. The specimen was tested for its pathogenicity. The PDA has been the most suitable medium for the growth as compared to the other selected media. It was also observed that the calcium and potassium nitrate were the best sources of nitrogen [11]. While in the current study, on the top of the Ibrahim's remit, two additional nitrogen compounds with two other media have been studied to diversify and observe the FOL growth on a wider scale (details in the Research Methodology section).

The effects of several nitrogen compounds on mycelial growth of *Fusarium* soil sp. selected from two fields of Murshidabad district in West Bengal, India have been reported. Eight nitrogen compounds were selected to observe the growth of the pathogen. The organic nitrogen compounds were observed to be more favorable for mycelial growth of the isolates as compared to the inorganic nitrogen compounds. All the *Fusarium* sp. could consume glycine well. Out of the inorganic nitrogen compounds, sodium nitrate was observed to be most suitable for growth of *Fusarium* isolate [12]. However, in this research, experiments are designed by using inorganic nitrogen compounds.

In summary, it is established that there is a number of publications which covers the influence of nitrogen compounds on different physiological and morphological aspects of *Fusarium oxysorum* f.sp. *lycopersici*. However, among all the publications reviewed in this study, none of them has applied these three nitrogen com-

pounds- KNO_3, $(NH_4)2SO_4$, and UREA- at the same time, in these three media- Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and V8- in varying combinations in the same study. This fact is further highlighted and objectively captured in Table 2 where the red color indicates the overall knowledge gap that this research focuses on (Table 2).

Table 2: Literature Review Matrix.

KEY LITERAURE / PUBLICATION			Fusarium Oxysporum f.sp. lycopersici	Tomato (specimen)	INORGANIC NITROGEN COMPOUNDS			MEDIA			All features in previous columns	BRIEF REMARK
Author	Year	Type			KNO3	(NH4)-2SO4	Urea	PDA	MEA	V8		
Garcia, et al. [13]	2019	Journal						ü ü	ü ü			Tomato is not focused. Impact of nitrogen compounds is not studied and V8 is not considered.
Mezzomo, et al. [14]	2018	Journal						ü ü	ü ü	ü ü		Tomato is not focused; nitrogen sources are missing.
Hoffland, et al. [10]	2018	Journal		ü ü				ü ü				MEA and V8 are not used, nitrogen compounds are not studied.
Friis [15]	2017	journal										Banana is focused but not tomato. These media and nitrogen sources are not considered.
Fovo, et al. [16]	2017	Journal	ü					ü ü	ü ü	ü ü		Tomato is not focused. Nitrogen sources are not considered.

Jie jia, et al. [17]	2017	Journal	ü							ü ü		Tomato and nitrogen compounds are not focused. PDA & MEA are not considered.
Porter, et al. [5]	2015	Journal	ü					ü ü				Tomato and nitrogen compounds are not part of the study and only PDA is considered.
Juber, et al. [18]	2014	Journal	ü					ü ü				FAO should pay attention towards tomato crop.
Nokano, et al. [8]	2013	Journal	ü	ü ü	ü ü							Tomato is not focused. These three media are not considered.
Prasanna, et al. [19]	2013	Journal	ü	ü ü								Media and nitrogen compounds are different.
Sundaramoorthy, et al. [20]	2013	Journal	ü	ü ü				ü ü				Impact of nitrogen compounds is not focused. PDA is considered but not MEA & V8.

Pradeep, et al. [21]	2013	Journal	ü					ü ü				Tomato and nitrogen compounds are not focused. Only PDA is considered but not MEA & V8.
Rajmane, et al. [22]	2012	Journal	ü			ü ü						Tomato is not taken into account; these media are not considered. Ammonium sulfate is considered but not urea and potassium nitrate.
Ignjatov, et al. [23]	2012	Journal	ü	ü ü				ü ü				Impact of nitrogen compounds is not observed. PDA is considered but not MEA & V8.
Sharma, et al. [24]	2011	Journal	ü	ü ü		ü ü		ü ü		ü ü		Among media MEA is not considered. Among nitrogen compounds only ammonium sulfate is used.

Sharma, et al. [12]	2010	Journal	ü					ü ü				Tomato is not focused. Impact of nitrogen compounds is not observed. PDA is considered but not MEA & V8.
Ibrahim, et al. [11]	2003	Journal	ü	ü ü				ü ü				nitrogen utilization is not observed. PDA is considered but not MEA & V8.
Mc Callum, et al. [25]	2004	Journal						ü ü		ü ü		Tomato is not focused. These nitrogen compounds are not used. MEA is missing.

Aim and Objectives

Fusarium oxysporum f.sp. *lycopersici* (FOL) is the most common causal agent of tomato wilt. The purpose of the study is to establish the potential of nitrogen compounds as a means of chemical control on FOL and also to identify the best medium for the growth control of FOL both, as an individual as well as in combination with the corresponding nitrogen compound. The study applies nitrogen compounds that are considered from the group of inorganic compounds. This is in order to contribute to the existing knowledge with the new aspect, where the studies to date are predominantly based on organic nitrogen compounds. Below are the key objectives to manage the aim of this research:

a. Select a suitable set of three inorganic nitrogen compounds (as sources of nitrogen) along with a set of three appropriate culture media to generate varying combinations with different concentrations.

b. Observe and compare the growth behavior of (FOL) in the aforesaid combinations of nitrogen compounds and culture media.

c. Identify the most effective combination of nitrogen compound, its concentration and the corresponding medium in which Fusarium growth is most inhibited.

Materials and Method

Materials

Chemicals: Calcium carbonate, Sodium carbonate, Ammonium sulfate, Urea, Potassium nitrate, Methylated, Spirit, Antibacterial agents (Amoxicillin).

Media: Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), V8 Agar Juice.

Instruments: Autoclave, Refrigerator, Laminar Flow Hood, Incubator, Analytical balance.

Apparatus: Petri plates, spirit lamp, inoculation needles, measuring flasks (250, 500,1000ml), measuring cylinder (1000 ml).

Other Materials: Measuring scale, Parafilm, Cotton, Masking tape, permanent marker, gloves, spray bottle, distilled water, rubber band, sterilized polythene bags, baskets, insulated gloves, digital camera.

Cleaning and Sterilization of Equipment

Sterilization of glass wares: Glass wares were cleaned with

chronic acid, followed by thorough washing in liquid detergent under running tap water, and rinsed with distilled water 2-3 times. These were air-dried and then kept in oven for sterilization at 180°C for at least 2 hrs. Plastic wares were autoclaved at 121°C, 15 psi for 15 minutes [26].

Sterilization of inoculating needles and working table: Clean inoculating needle was sterilized by dipping the loop of needle in spirit and heating over the flame until red- hot. The process was repeated 2-3 times. The working table of laminar air flow was disinfected by sweeping with cotton soaked in absolute alcohol and exposing it to UV light for 30 minutes [26].

Sterilization of media and distilled water: Sterilized glassware and plastic wares were used for dispensing media and distilled water. All media were autoclaved at 121°C, 15 psi pressure for 15-30 minutes [26].

Sterilization of laminar air flow: Prior to the day of inoculation of fungus sample, the laminar air flow was saturated with alcohol vapors. At the time of inoculation, the laminar air flow chamber was wiped with 70% alcohol or general spirit. Then only required instruments were kept in the chamber and exposed to UV rays for 15-20 min. All the operation viz., transfer, inoculation etc. were done over a gas burner flame [26].

Culture media: All the solid media were sterilized in an autoclave at 121^0C and 15 lbs. pressure (p.s.i) for 20 minutes [26].

Selection of Strain and Revival of Fungus

The strain was isolated from the soil sample collected from Kasur region of district Lahore, Punjab, Pakistan. The fungus was revived by inoculation of fungal culture on Malt Extract Medium by adding 2g Malt Extract and 1.5g Agar powder (weighing by digital balance) in 100ml of distilled water in a conical flask followed by autoclaving and pouring of media into Petri plates.

Preparation of Media with Nitrogen Sources

Three types of media namely PDA, MEA, and V8 Agar were used to assess the best medium for growth of the fungus.

a. **PDA*:*** Potato Dextrose Agar (PDA) medium was used. For preparation of PDA, 250g peeled potatoes were cut into slices and boiled in 500 ml of distilled water in conical flask. The extract was strained through a piece of muslin cloth and 20g dextrose was added in it. 20g Agar-Agar was melted in 500 ml of distilled water separately and was mixed in potato dextrose solution and the volume was made up to 1000 ml by adding distilled water [27].

b. **V8*:*** V8 Agar was prepared by mixing 2g $CaCO_3$ and 15g agar with 180 ml V8 juice. Finally, distilled water was added into solution to make volume up to 1000 ml [27].

c. **MEA*:*** For preparation of MEA 20g of Malt Extract and 15g of agar were added in 1000 ml distilled water [27].

After media preparation, three nitrogen compounds, which are Urea, KNO_3 and $(NH_4)2SO_4$, were added, each with three different concentrations in g/L viz, (0.5g/L, 1.0g/L and 1.5g/L of solution) in each selected media and autoclaved. After autoclave media with nitrogen compounds were poured in petri-plates and inoculated with pure culture of the Fusarium to establish the growth behavior of *Fusarium oxysporum* f.sp. *lycopersici*.

Preparation of Control Media*:* Three control plates of the three aforesaid media (with the same procedure as described above) were prepared without any additional nitrogen compound and inoculated with (FOL) to check the growth of this pathogen, see Figure 1.

Pouring Media in Plates

Pouring was done in laminar flow. Before pouring laminar chamber was fully saturated with spirit and wiped out with cotton to make it sterilized in order to avoid contaminations. Spirit lamp was flamed and kept in laminar chamber. Already sterilized petri plates were taken. Medium was poured carefully in each plate of 90 mm diameter and covered with lids. Four replicates of each treatment were made. Let them cooled down for 30 minutes. Then plates were inverted and kept at rest for 48 hours in laminar flow at room temperature.

Inoculation

Inoculation was performed with the help of sterilized Inoculation needle. Firstly, culture was Picked from collected isolate culture with help of needle and inoculated in the MEA plate. Same procedure was repeated for rest of two media namely, PDA and V8 plates.

Incubation

After inoculation the plates were covered with sterilized polyethene and kept in incubator at 28 ±1 ºC temp for growth of culture (Figure 1).

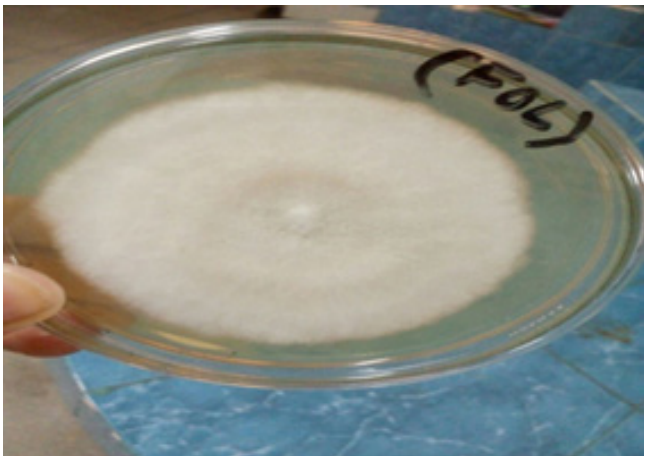

Figure 1: Pure Culture of Fusarium Oxysporum f.sp. Lycopersici – Isolated from Lahore soil sample.

Results Analysis and Discussion

As explained in Methodology section, there are three different media with three different nitrogen compounds, each with three different concentrations. Thus, there are 27 combinations of culture plates, each with different set of a medium, compound and its concentration. After seven days of inoculation, observations are recorded and the data is compiled in the form of a matrix. The values mention in table are the mean of the values of three replicates of each treatment, see Table 3. The results and their interpretation, particularly from the perspective of fungus growth behavior are described below (Table 3).

Table 3: Growth behavior of *Fusarium Oxysporum* f.sp. *Lycopersici* (Day 7. observations).

MEDIA	Nitrogen Compounds	Growth of *Fusarium oxysporum* f.sp. *lycopersici* with different nitrogen compounds' concentrations.					
		Radial growth (in mm)			Dry mass (in mg)		
		Conc. 0.5 g/L	Conc. 1.0 g/L	Conc. 1.5 g/L	Conc.0.5g/l	Conc. 1.0 g/L	Conc. 1.5 g/L
PDA	Potassium Nitrate	71.1	77	79.3	4.77	4.79	4.81
	Ammonium Sulfate	75.3	54.7	52.7	4	3.01	3.01
	Urea	80.3	76	49.3	5.1	4.5	2.05
	Control	85mm			5.5mg		
V8	Potassium Nitrate	81.3	77	70	6.01	5.83	5.83
	Ammonium Sulfate	71.7	65.7	76.3	4.74	4.45	5.11
	Urea	67.7	50	45.3	3.07	2.88	2.62
	Control	78mm			4.2mg		
MEA	Potassium Nitrate	56.7	65.7	69.3	2.16	2.19	2.21
	Ammonium Sulfate	63.7	72.3	67	2.03	2.31	2.22
	Urea	78	72.7	33.7	3.64	3.09	1.67
	Control	70mm			3.4mg		

Growth in Control Media

Figure 2 clearly reflects that growth of FOL is maximum in PDA followed by V8 and MEA, respectively, under control conditions i.e., media without nitrogen sources (Figure 2).

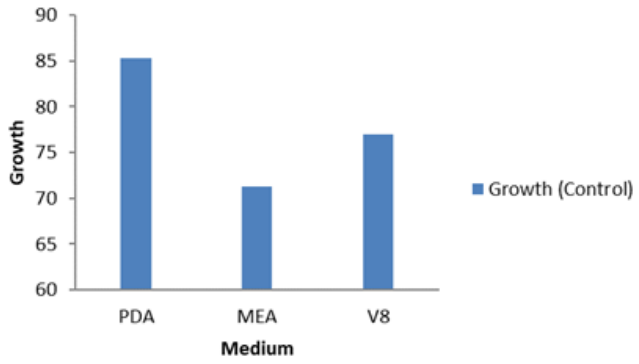

Figure 2: Growth (in mm) of *Fusarium Oxysporum* f.sp *Lycopersici* in Control Media.

PDA with Nitrogen Compounds

Referring to Table 3, in this scenario the growth behavior of FOL studied with three different compounds of nitrogen that are KNO_3, $(NH_4)2SO_4$ and Urea, each with a set of three concentrations (0.5g/L, 1.0g/L, 1.5g/L).

Table 4: Growth comparison of *Fusarium Oxysporum* f.sp. *Lycopersici* in PDA, MEA and V8 with different sources of nitrogen.

Factor	N	Mean	St.Dev
V8	9	77.56	0.49
PDA	9	75.61	0.67
MEA	9	66.33	0.82
P-Value		0.042	

Note*: N = NO of treatments.
P-Value = Significance value.

a. **KNO_3**-FOL shows minimum radial growth for the lowest concentration of this compound; similarly, maximum growth is seen for the highest concentration; and intermediate growth for the medium concentration. Therefore, it is established in this case that the *Fusarium* radial growth is directly proportional to the compound concentration in the range of 0.5 to 1.5g/L in KNO_3. However, almost the same quantity of mass with similar leathery texture of mycelium is observed in each case.

b. **$(NH_4)2SO_4$-** In comparison to KNO_3 *Fusarium* shows inverse growth behavior in $(NH_4)2SO_4$ i.e., maximum growth is observed for the lowest concentration while minimum growth is noticed in the highest concentration and shows intermediate growth for medium concentration. The texture of mycelial is matt like in all three cases but dry mass is inversely proportional to the concentration in the range of .5 to 1.0g/L, while the mass remains same in the range of 1.0 to 1.5 g/L.

c. **Urea**- In this compound, Fusarium growth behavior is similar to $(NH_4)2SO_4$ i.e., inversely proportional to compound concentration, but the magnitude of growth is greater in urea than $(NH_4)2SO_4$. The texture in the said compound is paper like and amount of dry mass is inversely proportional to the concentration in all cases.

V8 with Nitrogen Compounds

Referring to Table 3, in this scenario the growth behavior of Fusarium oxysporum is studied with three different compounds of nitrogen that are KNO_3, $(NH_4)2SO_4$ and Urea, each with a set of three concentrations (0.5g/L, 1.0g/L, 1.5g/L).

a. **KNO_3**- *Fusarium* shows maximum radial growth for the lowest concentration of this compound; similarly, minimum growth is seen for the highest concentration; and intermediate growth for the medium concentration. Therefore, it is established in this case that the *Fusarium* radial growth is inversely proportional to the compound concentration in the range of 0.5 to 1.5g/L in KNO_3. The mass also varies inversely with the change in concentration of compound. However, similar fluffy texture of mycelium is observed in each case.

b. **$(NH_4)2SO_4$-** In this compound, *Fusarium* displays inconsistent behavior for both the radial growth and the amount of mass i.e., maximum growth and greater mass are observed for the highest concentration while minimum growth and less mass are noticed in the medium concentration and shows intermediate growth and moderate mass for lowest concentration with similar fluffy texture of mycelium is in each case.

c. **Urea**- *Fusarium* growth response is maximum in lowest con-

centration of this compound; and minimum in highest concentration; whereas, intermediate growth response in medium concentration. It shows that the radial growth of fusarium is inversely proportional to concentration in the range of 0.5 to 1.5g/L in Urea. The texture of mycelial is fluffy in all three cases. Dry mass also varies inversely with concentration of the compound, i.e., more mass in the lowest concentration of compound and less dry mass in highest concentration.

MEA with Nitrogen Compounds

Referring to Table 3, in this scenario the growth behavior of Fusarium oxysporum is studied with three different compounds of nitrogen that are KNO_3, $(NH_4)2SO_4$ and Urea, each with a set of three concentrations (0.5 g/L, 1.0 g/L, 1.5 g/L).

a. **KNO_3**- *Fusarium* shows minimum radial growth for the lowest concentration of this compound; similarly, maximum growth is seen for the highest concentration; and intermediate growth for the medium concentration. Therefore, it is established in this case that the *Fusarium* radial growth is directly proportional to the compound concentration in the range of 0.5 to 1.5 g/L in KNO3. The same paper like texture of mycelium is to be observed in each case. However, the quantity of dry mass of fungus is directly proportional to its concentration in the range of 0.5 to 1.5g/L.

b. **$(NH_4)2SO_4$**- In this compound *Fusarium* manifests unusual behavior in both the growth and the quantity of mass i.e., maximum growth is observed in the medium concentration while minimum growth is noticed in the lowest concentration and shows intermediate growth for the highest concentration. The texture of mycelium is paper like in all three cases.

c. **Urea**- *Fusarium* growth response is maximum in lowest concentration of this compound; and minimum in highest concentration; whereas, intermediate growth response in medium concentration. It shows that radial growth of Fusarium is inversely proportional to concentration in the range of 0.5 to 1.5g/L in Urea. The texture of mycelial is paper like in all three cases. Dry mass varies inversely with concentration of the compound, i.e., lowest the concentration of compound more is the dry mass and vice a versa.

Comparison Between Media

In Table 4, ANOVA analysis shows that the growth behavior of Fusarium is dependent on the type of selected media which was also verified from the work of Hoffland [10]. In fact, the growth varies from medium to medium. In terms of radial growth, maximum growth of FOL was observed in V8 and minimum growth was observed in MEA. whereas, in PDA, intermediate growth of FOL was observed. As the p value (significance value) i.e., 0.042, is less than 0.05 which rejects the null hypothesis, also indicates that growth of fusarium is affected by the addition of nitrogen compounds as well as by the nature of growth media. Moreover, with the addition of nitrogen compounds in media the impact of media on growth also varies when compared with the control environment. Additionally, in Figure 3, Interval Plot is used to assess and compare confidence intervals of the means of three selected media i.e., PDA, MEA and V8. An interval plot shows a 95% confidence interval for the mean of each growth medium which also interprets the results obtained via ANOVA analysis.

Furthermore, referring to Figure 4, the effectiveness of nitrogen compounds is related to the nature of the media. In V8 all the three nitrogen compounds with selected concentrations seem to be in favor of the Fusarium growth. Whereas, MEA restrains the availability of the nitrogen to FOL. Among compounds, in Urea with the concentration of 1.5g/L, the least radial growth and minimum quantity of dry mass of Fusarium was observed in all above said media. The overall growth comparison among different media and different nitrogen compounds has been illustrated in Figure 4 (Table 4) (Figure 3).

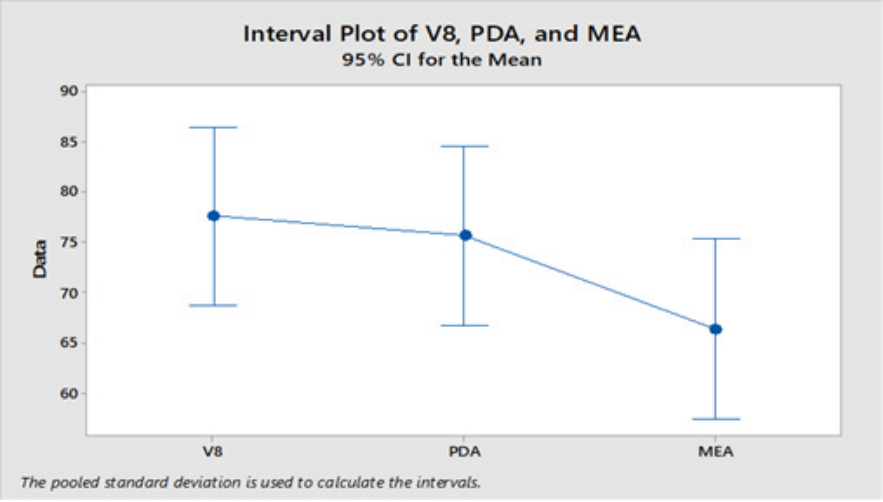

Figure 3: Interval Plot of Growth of *Fusarium Oxysporum* f. sp. *Lycopersici* n PDA, MEA and V8 with different sources of nitrogen.

Other Considerations

Sulfur being antifungal in nature may play its role as growth suppressor [28], which is why growth of FOL in the media containing ammonium sulfate is less than that of media containing potassium nitrate. Potassium is an important cation and may play role in context of 'availability efficiency' of nitrogen for the uptake by FOL [29].

However, the behavior of urea is unique in relation to growth of FOL, as both carbon and nitrogen are fundamental structural elements of fungus. Still the least growth has been observed in urea as compared to aforesaid other two nitrogen compounds (see Figure 4). This is the area that requires further research and is not in the scope of this study (Figure 4).

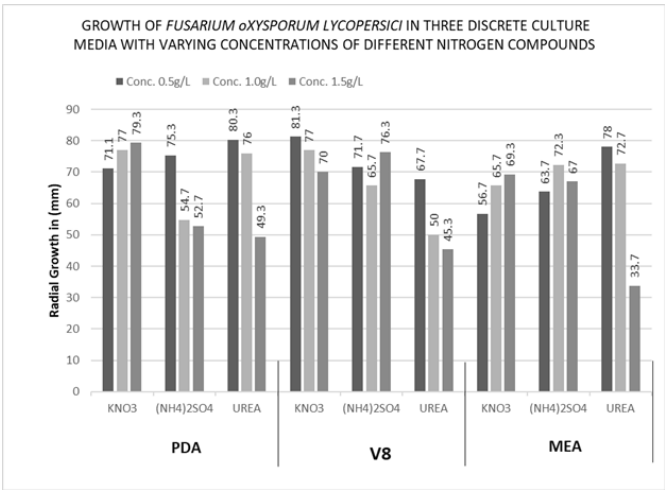

Figure 4: Radial growth of *Fusarium Oxysporum* f.sp. *Lycopersici* in three culture media with different nitrogen sources of varying concentrations.

Concluding Remarks

Fusarium Oxysporum f.sp. *Lycopersici* (FOL) has been and still is the most detrimental reason for mass destruction of tomato crop resulting in considerable decrease in the crop yields. This disease can not only huge economic but also the nutritional loss to the world. In view of this significant problem, in-vitro investigations have been conducted in this study in order to observe changes and development of growth behaviour of FOL.

In this study, a set of various inorganic nitrogen compounds is deployed with different media in varyingly specified proportions to observe the growth behaviour of the isolate (FOL). MEA medium is found to be the best medium as a growth inhibitor for FOL in comparison to the other tested media i.e., PDA and V8. Among nitrogen compounds, urea is found to be the most effective nitrogen source to control the FOL growth in comparison to the other two compounds that are potassium nitrate and ammonium sulfate. Additionally, the growth is not only minimum in MEA and urea individually, but also in the combination of these two.

The current study can be regarded as a precursor or a preliminary study before being applied to tomato directly, where media would be replaced instead of soil. This study drives results which can be used as benchmarks to compare against when the direct application of nitrogen compounds to the combination of fusarium and tomato in soil is studied, be it in a laboratory setting or eventually in the field, in the real-world. Finally, this study can also be extended to examine impacts of other *Fusarium* species on a wide range of other crops such as potato, onions, lentils, banana and the like, and then accordingly frame recommendations to devise wilt control measures to conserve such crucial crops for sustainable agriculture.

Acknowledgement

None.

Conflict of Interest

None.

References

1. Varshney RK, Bansal KC, Aggarwal PK, Datta SK, Craufurd PQ, et al. (2011) Trends in Plant Science 16(7): 363-371.
2. Smith P (2013) Delivering food security without increasing pressure on land. Global Food Security 2(1): 18-23.
3. Ranjard L, Dequiedt S, Jolivet C, Saby NP, Thioulouse J, et al. (2010) Biogeography of soil microbial communities: a review and a description of the ongoing french national initiative. Agronomy for Sustainable development 30(2): 359-365.
4. Bawa I (2016) Management strategies of Fusarium wilt disease of tomato incited by Fusarium oxysporum f. sp. lycopersici (Sacc.): A Review. Int J Adv Acad Res 2(5).
5. Porter LD, Pasche JS, Chen W, Harveson RM (2015) Isolation, identification, storage, pathogenicity tests, hosts, and geographic range of Fusarium solani f. Sp. pisi causing fusarium root rot of pea. Plant Health Progress 16(3): 136-145.

6. Shimizu K, Matsuda Y, Nonomura T, Ikeda H, Tamura N, et al. (2007) Dual protection of hydroponic tomatoes from rhizosphere pathogens Ralstonia solanacearum and Fusarium oxysporum f. sp. radicis-lycopersici and airborne conidia of Oidium neolycopersici with an ozone-generative electrostatic spore precipitator. Plant pathology 56(6): 987-997.

7. Nirenberg HI (1981) A simplified method for identifying Fusarium spp. occurring on wheat. Canadian Journal of Botany 59(9): 1599-1609.

8. Nakano A, Uehara Y, Yamauchi A (2003) Effect of organic and inorganic fertigation on yields, δ 15N values, and δ 13C values of tomato (Lycopersicon esculentum Mill. cv. Saturn). In Roots: The Dynamic Interface between Plants and the Earth, Springer, Dordrecht 343-349.

9. Fourie G, Steenkamp ET, Ploetz RC, Gordon TR, Viljoen A (2011) Current status of the taxonomic position of Fusarium oxysporum formae specialis cubense within the Fusarium oxysporum complex. Infect Genet Evol 11(3): 533-542.

10. Hoffland E, Jeger MJ, van Beusichem ML (2000) Effect of nitrogen supply rate on disease resistance in tomato depends on the pathogen. Plant and Soil 218(1-2): 239-247.

11. Ibrahim NF, Mohd MH, Nor NMIM, Zakaria L (2015) First report of Fusarium oxysporum and F. solani associated with pineapple rot in Peninsular Malaysia. Plant Disease 99(11): 1650-1650.

12. Sharma GPRR (2010) Influence of Culture Media on Growth, Colony Character and Sporulation of Fungi Isolated From Decaying Vegetable Wastes. Journal of yeast and fungal research 1(8): 157-164.

13. Garcia E, Paiva D, Costa J, Portugal A, Ares A (2019) First Report of Fusarium Wilt Caused by Fusarium oxysporum f. sp. passiflorae on Passion Fruit in Portugal. APS publication 10(103).

14. Mezzomo R, Rolim JM, Poletto T, Rosenthal VC, Savian LG, et al. (2018) Morphological and Molecular Characterization of Fusarium Spp. Pathogenic to Ilex Paraguariensis. Cerne 24(3): 209-218.

15. Friis C, Nielsen J (2017) Land-use change in a telecoupled world: the relevance and applicability of the telecoupling framework in the case of banana plantation expansion in Laos. Ecology and Society 22(4).

16. Fovo JD, Dostaler D, Bernier L (2017) Influence of culture media and temperature on growth and sporulation of Lasiodiplodia theobromae, Pestalotiopsis microspora and Fusarium oxysporum Isolated from Ricinodendron heudelotii in Cameroon. International Journal of Current Microbiology and Applied Sciences 6(6): 3098-3112.

17. Jia LJ, Wang WQ, Tang WH (2017) Wheat coleoptile inoculation by Fusarium graminearum for large-scale phenotypic analysis. Bio Protoc 7(15): e2439.

18. Juber KS, Al Juboory HH, Al Juboory SB (2014) Fusarium wilt disease of strawberry caused by Fusarium oxysporum f. sp. Fragariae in Iraq and its control. Journal of Experimental Biology and Agricultural Sciences 2(4): 419-427.

19. Prasanna R, Chaudhary V, Gupta V, Babu S, Kumar A, et al. (2013) Cyanobacteria mediated plant growth promotion and bioprotection against Fusarium wilt in tomato. European Journal of Plant Pathology 136(2): 337-353.

20. Sundaramoorthy S, Balabaskar P (2013) Biocontrol efficacy of Trichoderma spp. against wilt of tomato caused by Fusarium oxysporum f. sp. lycopersici. Journal of Applied Biology & Biotechnology 1(3): 36-40.

21. Pradeep FS, Begam MS, Palaniswamy M, Pradeep BV (2013) Influence of culture media on growth and pigment production by Fusarium moniliforme KUMBF1201 isolated from paddy field soil. World Applied Sciences Journal 22(1): 70-77.

22. Rajmane SD, Korekar SL (2012) Impact of carbon and nitrogen sources on pectinase production of post-harvest fungi. Current Botany 3(3).

23. Ignjatov M, Milosevic D, Nikolic Z, Gvozdanovic Varga J, Jovicic D, et al. (2015) Fusarium oxysporum as causal agent of tomato wilt and fruit rot.

24. Sharma BK, Singh RP, Saha S, Kumar A, Rai AB (2011) Effect of temperature, pH and media on the growth and sporulation of Fusarium oxysporum f. sp. lycopersici causing wilt of tomato. Progressive horticulture 43(2): 186-192.

25. McCallum BD, Tekauz A, Gilbert J (2004) Barrage zone formation between vegetatively incompatible Fusarium graminearum (Gibberella zeae) isolates. Phytopathology 94(5): 432-437.

26. Patra JK, Das G, Das SK, Thatoi H (2020) Isolation, Culture, and Biochemical Characterization of Microbes. In A Practical Guide to Environmental Biotechnology pp.83-133.

27. Teye MM (1994) Studies on the Mycoflora of Okra, Onion, Pepper and Tomato plant and their stored products.

28. Bhargava SN, Tandon RN (1963) Sulphur and Phosphorus Requirements of Three Fungi Causing Diseases in Storage. Mycopathologia et mycologia applicate 21(3-4): 169-178.

29. Jones EBG, Jennings DH (1965) The Effect of Cations on The Growth of Fungi. New Phytologist 64(1): 86-100.