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Article

Potential control measures for *Fusarium* wilt in tomato for sustainable agriculture–impacts of nitrogen compounds and culture media on Fusarium

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Abstract: *Fusarium oxysporum* is one of the most common soil-dwelling pathogens that causes wilt on tomato crops. There is a plethora of literature on Fusarium, but comparatively fewer studies exist when it comes down to *Fusarium* and tomato together. Different experimental models on tomato infestation with *Fusarium* were tried. Three nitrogen compounds: KNO_3, $(NH_4)_2 SO_4$ and Urea, and three media: Malt Extract Agar, Potato Dextrose Agar and V8 were used in this study. Concentrations of each of the three nitrogen compounds were varied by preparing solutions of 0.5 g/L, 1.0 g/L and 1.5 g/L of each compound. Radial growth is measured in mm while mass is determined in mg. V8 medium was found to be the best medium for the growth as compared to tested media. The best nitrogen source for the growth of the isolate was potassium nitrate. The optimum temperature for the growth of the fungus was found to be in the range of 25–30.

Keywords: *Fusarium oxysporum* f. sp. lycopersici (FOL); inorganic compounds; culture media; nitrogen compounds

1. Introduction

The development and advancement in biotechnology have 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 [2,3]. One of the ways to overcome the gap between the food demand and the food supply is to save food crops from pathogenic invasion, particularly those of tender and sensitive for pathogens [4]. Of such crops, tomatoes are one of the most susceptible crops in demand, being the second largest crop in the world in terms of productivity, and can easily be exploited by pathogens [5]. Because of its status as food, tomatoes have been propagated to increase values like productivity, fruit value, and resistance to biotic and abiotic pressures [6]. It has been extensively used not only as food but also as investigation material [7]. On a worldwide scale, the yearly production of fresh tomatoes has been estimated at approximately 159 million tons [8]. But more than one-fourth of those 159 million tons are grown for the processing industry, which marks tomatoes as the world's principal vegetable for processing [9]. The nine chief producing countries that contribute 74.2% of the world's annual production are China, India, the USA, Turkey, Egypt, Iran, Italy, Brazil, and Spain [10]. Hence, this crop is to be saved to ensure its contribution to the fulfillment of the food demand of the accelerating human population.

Among a number of diseases in tomatoes, *Fusarium* wilt is, so far, the most common wilt disease in the world [11]. Since the establishment of tomatoes as a prime crop in the food industry, *Fusarium* wilt has always been a persistent problem in tomato crops [9]. Even though no clear-cut information on the damages caused by the disease exists, the yield loss may fluctuate from 10 to 100% subject to the environmental conditions. An 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 [12]. 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*) [13]. This is well adapted to diverse habitats and is supposed to be the earliest fungal colonist on earth. It occurs in various environments such as the deserts and arctic [14,15]. A large number of species within this genus are extensively distributed soil-borne species that cause diseases in many economically important crops [16]. Various members of this genus are important plant pathogens [17]. It was investigated that they have the greatest pathogenic domain based on the number of pathogenic taxa [18], the host range they attack, and the diversity of habitats in which they cause disease in plants [19]. Among them, *Fusarium oxysporum* f. sp. *lycopersici* is well signified in the world [11]. *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 [11]. Physical and chemical environments have a significant impact on the diagnostic traits of fungi [19]. 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.

Different media and nutrient variable methods have been developed to reduce the occurrence and harshness of *Fusarium* crown rot affected by *Fusarium oxysporum* [20]. The two isolates of *F. oxysporum* f. sp. *elaeidis* were used to investigate the nitrogen requirements. Out of the 10 tested nitrogen compounds, conspicuous growth and sporulation were 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 [21].

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* [22–24]. The effect of nitrogen concentration in the tissues seemed to be greatly pathogen-dependent; there was no influence on proneness to *F. oxysporum* [25,26]. 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 [27]. It was also observed that the calcium and potassium nitrate were the best sources of nitrogen

[28–31]. While in the current study, on the top of 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 the 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 the 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 the growth of *the Fusarium* isolate [32]. However, in this research, experiments are designed by using inorganic nitrogen compounds.

In summary, it is established that there is a number of publications that cover 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 compounds–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.

Among a number of diseases in tomatoes, *Fusarium* wilt is, so far, the most common wilt disease in the world. Since the establishment of tomatoes as a prime crop in the food industry, *Fusarium* wilt has always been a persistent problem in tomato crops.

This paper focuses on *Fusarium* Wilt, caused by *Fusarium oxysporum* f. sp. *Lycopersici* (Sacc.) W.C. Snyder and H.N. Hans, a soil-borne plant pathogen in the class Hyphomycetes, cause *Fusarium* wilt specifically in tomato. It can endure indeterminately without a host. Most occurrences are linked with disease-ridden tomato debris left in the soil. The *Fusarium* in soil endures through resting spores called chlamydospores. As the fungus can live in the soil for many years, the control of disease through normal crop rotation is not possible. Even though a number of tomato lines have been testified as resistant to wilt from several countries throughout the world, they have a very narrow range of success because of area-specific races of the pathogen.

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 and 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:

1) 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.

2) Observe and compare the growth behavior of (FOL) in the aforesaid combinations of nitrogen compounds and culture media.
3) Identify the most effective combination of nitrogen compound, its concentration and the corresponding medium in which *Fusarium* growth is most inhibited.

2. Materials and method

2.1. Materials

Chemicals: Calcium carbonate $CaCO_3$, Sodium carbonate Na_2CO_3, Ammonium sulfate $(NH_4)_2SO_4$, Urea $(NH_2)_2CO$, Potassium nitrate K_2NO_3, Methylated spirit (C_2H_5OH), antibacterial agents [Amoxicillin ($C_{16}H_{19}N_3O_5S$)].

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, 1000 mL), 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.

2.2. Cleaning and sterilization of equipment

Sterilization of glassware: Glassware was 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 the oven for sterilization at 180 ℃ for at least 2 h. Plastic wares were autoclaved at 121 ℃, 15 psi for 15 min [33].

Sterilization of inoculating needles and working table: The clean inoculating needle was sterilized by dipping the loop of the needle in spirit and heating it 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 min [33].

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 ℃, 15 psi pressure for 15–30 min [33].

Sterilization of laminar air flow: Prior to the day of inoculation of the 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 the required instruments were kept in the chamber and exposed to UV rays for 15–20 min. All the operations, *viz.*, transfer, inoculation, etc., were done over a gas burner flame [33].

Culture media: All the solid media were sterilized in an autoclave at 121 ℃ and 15 lbs. pressure (p.s.i.) for 20 min [33].

2.3. Selection of strain and revival of fungus

Selection of strain was made by studying the already isolated and identified strains of *Fusarium oxysporum*. Isolate FCBP-SF-0060 seven-day-old culture was collected from the First Fungal Culture Bank of Pakistan (Institute of Agricultural Sciences Punjab University Lahore). The fungus was revived by inoculation of fungal culture on Malt Extract Medium by adding 2 g Malt Extract and 1.5 g of Agar powder (weighing by digital balance) in 100 mL of distilled water in a conical flask, followed by autoclaving and pouring of media into Petri plates.

2.4. Preparation of media with nitrogen sources

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

- PDA: Potato Dextrose Agar (PDA) medium was used. For the preparation of PDA, 250 g of peeled potatoes were cut into slices and boiled in 500 mL of distilled water in a conical flask. The extract was strained through a piece of muslin cloth, and 20 g of dextrose was added to it. 20 g of 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 [34].
- V8: V8 Agar was prepared by mixing 2 g $CaCO_3$ and 15 g Agar with 180 mL V8 juice. Finally, distilled water was added into the solution to make the volume up to 1000 mL [34].
- MEA: For preparation of MEA 20 g of Malt Extract and 15 g of agar were added in 1000 mL distilled water [34].

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., (0.5 g/L, 1.0 g/L and 1.5 g/L of solution) in each selected media and autoclaved. After autoclaved 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 aforementioned 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**.

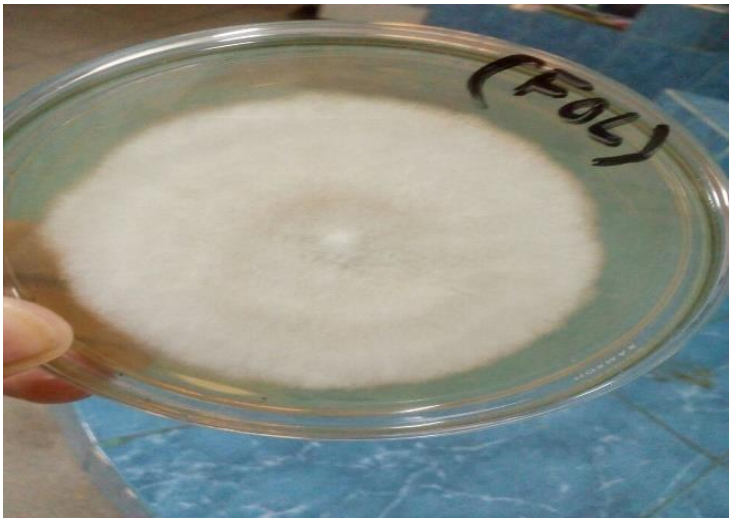

Figure 1. Pure culture of *Fusarium oxysporum* f. sp. *lycopersici*–isolated from Lahore soil sample.

2.5. Pouring media in plates

Pouring was done in laminar flow. Before pouring, the laminar chamber was fully saturated with spirit and wiped out with cotton to make it sterilized in order to avoid contaminations. The spirit lamp was flamed and kept in a 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 cool down for 30 min. Then plates were inverted and kept at rest for 48 h in laminar flow at room temperature.

2.6. Inoculation

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

2.7. Incubation

After inoculation, the plates were covered with sterilized polyethylene and kept in an incubator at 28 ℃ ± 1 ℃ temp for the growth of culture.

3. Results analysis and discussion

There are 27 combinations of culture plates, each with a 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. Data was analyzed by using the one-way analysis of variance (ANOVA).

3.1. Growth in control media

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

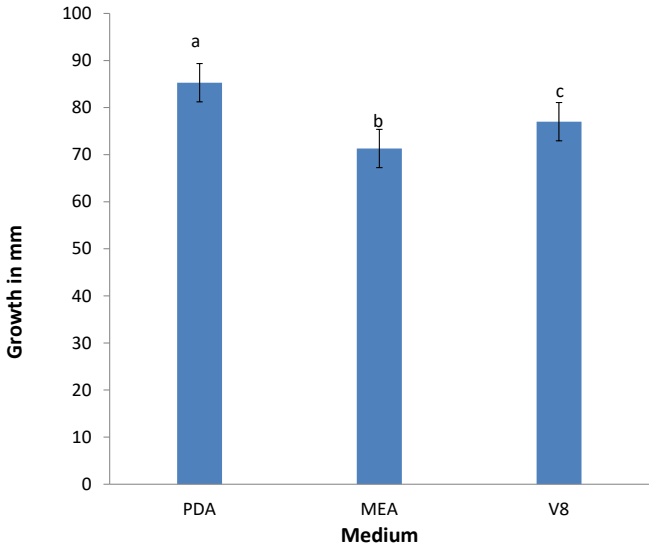

Figure 2. Growth (in mm) of *Fusarium oxysporum* f. sp. *lycopersici* in control media.

Note: The values are mean ± SD (n = 9). Different letters represent significant differences at ($p < 0.05$) using ANOVA and Tukey post-hoc tests.

3.2. PDA with nitrogen compounds

The growth behavior of FOL was 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).

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 1.5 g/L in KNO_3. However, almost the same quantity of mass with a similar leathery texture of mycelium is observed in each case.

$(NH_4)_2SO_4$–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 mycelium is matte like in all three cases, but dry mass is inversely proportional to the concentration in the range of 0.5 to 1.0 g/L, while the mass remains the same in the range of 1.0 to 1.5 g/L.

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 the amount of dry mass is inversely proportional to the concentration in all cases.

3.3. V8 with nitrogen compounds

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).

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 1.5 g/L in KNO_3. The mass also varies inversely with the change in concentration of compound. However, a similar fluffy texture of mycelium is observed in each case.

*$(NH_4)_2SO_4$–*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 show intermediate growth and moderate mass for the lowest concentration with a similar fluffy texture of mycelium in each case.

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

3.4. MEA with nitrogen compounds

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).

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 KNO_3. 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 1.5 g/L.

*$(NH_4)_2SO_4$–*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.

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

3.5. Comparison between media

The growth behavior of *Fusarium* is dependent on the type of selected media, which was also verified from the work of Hoffland [35,26]. In fact, the growth varies from medium to medium (**Figure 3**). 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, it also indicates that the growth of *Fusarium* is affected by the addition of nitrogen compounds as well as by the nature of growth media, which is in agreement with the study of Palmieri [36]. 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 4**, an 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 3**, the effectiveness of nitrogen compounds is related to the nature of the media. In V8, all 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 1.5 g/L, the least radial growth and minimum quantity of dry mass of *Fusarium* was observed in all the above-mentioned media.

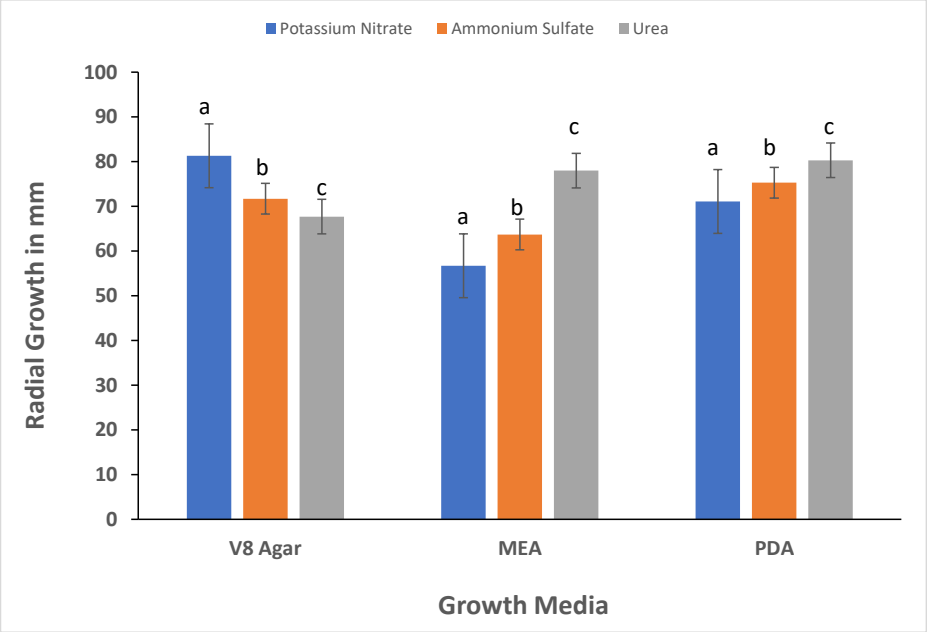

Figure 3. Growth behavior of *Fusarium oxysporum* f. sp. *lycopercici* in different growth media under different chemical environments.

Notes: The values are mean ± SD (n = 9). Different letters represent significant differences at ($p < 0.05$) using ANOVA and Tukey post-hoc tests.

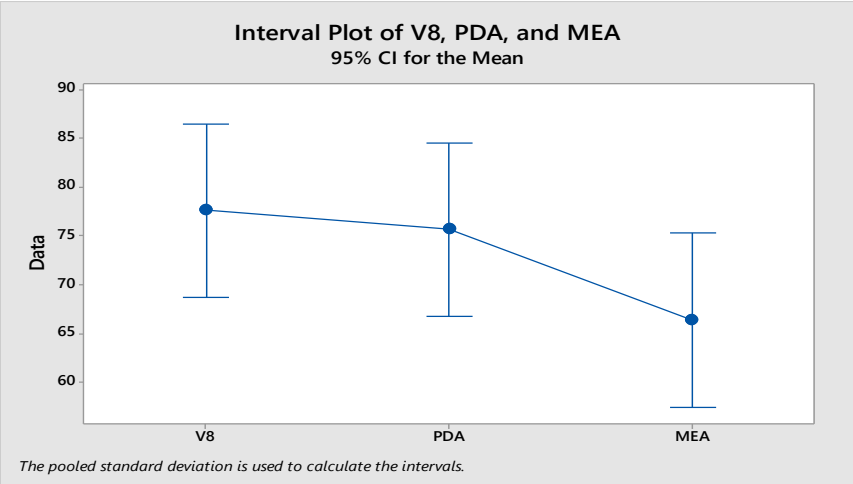

Figure 4. Interval plot of growth of *Fusarium oxysporum* f. sp. *lycopersici* in PDA, MEA and V8 with different sources of nitrogen.

3.6. Other considerations

Sulfur being antifungal in nature may play its role as a growth suppressor [37,38], which is why the 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 a role in the context of the 'availability efficiency' of nitrogen for uptake by FOL [39,40]. However, the behavior of urea is unique in relation to the growth of FOL, as both carbon and nitrogen are fundamental structural elements of fungus [41]. Still, the least growth has been observed in urea as compared to the aforementioned other two nitrogen compounds (**Figure 3**). This is the area that requires further research and is not in the scope of this study. A brief comparison of results with published literature has been presented in **Table 1**.

Table 1. Literature review matrix.

KEY LITERAURE/PUBLICATION	*Fusarium Oxysporum* f. sp. *lycopersici*	Tomato (specimen)	INORGANIC NITROGEN COMPOUNDS			MEDIA			BRIEF REMARK
Author			**KNO_3**	**$(NH_4)_2SO_4$**	**Urea**	**PDA**	**MEA**	**V8**	
Garcia et al. [42]	✓					✓	✓		Tomato is not focused. The impact of nitrogen compounds is not studied and V8 is not considered.
Mezzomo et al. [43]	✓					✓	✓	✓	Tomato is not focused; nitrogen sources are missing.
Hoffland et al. [26]	✓	✓				✓			MEA and V8 are not used, nitrogen compounds are not studied.
Friis [44]	✓								Banana is focused but not tomato. These media and nitrogen sources are not considered.
Fovo et al. [45]	✓					✓	✓	✓	Tomato is not focused. Nitrogen sources are not considered.
Jie et al. [46]	✓							✓	Tomato and nitrogen compounds are not focused. PDA & MEA are not considered.
Porter et al. [47]	✓					✓			Tomato and nitrogen compounds are not part of the study and only PDA is considered.
Juber et al. [48]	✓					✓			FAO should pay attention to tomato crop.
Nokano et al. [49]	✓	✓	✓						Tomato is not focused. These three media are not considered.
Prasanna et al. [50]	✓	✓							Media and nitrogen compounds are different.
Sundaramoorthy et al. [51]	✓	✓				✓			The impact of nitrogen compounds is not focused. PDA is considered but not MEA & V8.

Table 1. (*Continued*).

KEY LITERAURE/PUBLICATION	*Fusarium Oxysporum* f. sp. *lycopersici*	Tomato (specimen)	INORGANIC NITROGEN COMPOUNDS			MEDIA			BRIEF REMARK
Author			**KNO_3**	**$(NH_4)_2SO_4$**	**Urea**	**PDA**	**MEA**	**V8**	
Pradeep et al. [52]	✓					✓			Tomato and nitrogen compounds are not focused. Only PDA is considered but not MEA & V8.
Rajmane et al. [53]	✓			✓					Tomato is not taken into account; these media are not considered. Ammonium sulfate is considered but not urea and potassium nitrate.
Ignjatov et al. [54]	✓	✓				✓			The impact of nitrogen compounds is not observed. PDA is considered but not MEA & V8.
Sharma et al. [55]	✓	✓		✓		✓		✓	Among media, MEA is not considered. Among nitrogen compounds, only ammonium sulfate is used.
Sharma et al. [32]	✓					✓			Tomato is not focused. The impact of nitrogen compounds is not observed. PDA is considered but not MEA & V8.
Ibrahim et al. [28]	✓	✓				✓			Nitrogen utilization is not observed. PDA is considered but not MEA & V8.
McCallum et al. [56]	✓					✓		✓	Tomato is not focused. These nitrogen compounds are not used. MEA is missing.

4. Conclusions and recommendations

Many chemical methods have been proposed, and some are now established for use. Even though comparatively operative, such chemical methods undergo some in-built disadvantages. One such disadvantage relates to economies of attaining a satisfactory level of fungus control. Therefore, the long-term control measures must be devised because the pathogens survival structures (chlamydospores) are at such a depth in the soil that complete eradication is almost impossible.

This study has carried out research in vitro investigation in order to observe changes and development of growth behavior of *Fusarium oxysporum* f. sp. *lycopersici* as a pathogen that causes tomato wilt disease. This disease has been the reason for mass destruction in history, resulting in a considerable decrease in crop yields. Still, this disease can not only cause huge economic but also nutritional loss to the world.

Being a pathogen, FOL has always been a potential hazard to the tomato crop, but environmental changes are continuously encouraging this pathogen to display itself even more adversely. At present, acid rain is one of the greatest consequences of environmental changes that is playing a vital role in the augmentation of harmful chemicals in soil that pose a risk to plant crops on one hand, and on the other hand, it is providing a congenial environment for pathogens in soil to flourish. Therefore, this issue needs to be addressed.

This study establishes that generally organic (nitrogen) compounds have been deployed to investigate the FOL growth behavior, while on the other hand, the employment of inorganic nitrogen compounds in this regard is next to none. In this study, different media are deployed with a set of three different inorganic nitrogen compounds (as an innovative approach) in specified proportions to observe the efficacy of the media and the chemicals on the growth of the isolate (FOL). MEA medium is found to be the best medium as a growth inhibitor for *Fusarium* in comparison to the other tested media, viz. 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, which are potassium nitrate and ammonium sulfate. Furthermore, the growth is minimum in the combination of the MEA and Urea not just individually, as said above.

This study can be helpful in devising chemical control measures to combat FOL. Such measures can be operated by adjusting the chemical composition of the soil and providing nutrients in a proportion that could reduce the mycelial growth. Thereby reducing the likelihood of spore proliferation in the environment. In order to investigate the growth behavior of FOL in soil, further research is recommended to replace the media with soil. This can lead to a safer agricultural environment for tomato production, thereby resulting in greater yields. This study can also be extended to examine the impacts of *Fusarium* and accordingly devise control measures of wilt to conserve a wide range of other staple crops such as potatoes, onions, lentils, bananas, and the like. Such studies can contribute to one of the 17 SDGs of the United Nations, “No Poverty”, which includes the indicators of ending hunger, achieving food security, improving nutrition, and promoting the sustainable agricultural environment.

Furthermore, another SDG is "Climate Action", in which climate change adaptation is one of the key targets. The development of nitrogen-based fungicides as a control measure to combat the risk of *Fusarium* growth in tomatoes and other vital crops posed by accumulating nitrogen compounds in soil as a result of acid rain can play a role in contributing to these two SDGs.

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