

Original Research

**Impacts of Different Sources of Carbonates on Growth of *Fusarium oxysporum* f. sp. *lycopersici* in Different Growth Media**Hina Akram<sup>1,2</sup>, Shoaib Hussain<sup>3</sup>, Talib E. Butt<sup>4,\*</sup>

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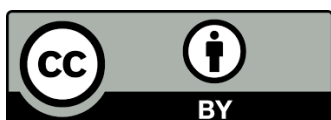
\* **Correspondence:** Talib E. Butt; E-Mail: [t.e.butt@northumbria.ac.uk](mailto:t.e.butt@northumbria.ac.uk)**Academic Editor:** Daniel M. Alongi**Special Issue:** [Environmental Management](#)*Adv Environ Eng Res*

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**Received:** November 18, 2022**Accepted:** November 07, 2023**Published:** November 13, 2023**Abstract**

*Fusarium* is the most common soil-borne pathogen that causes wilt in many plant crops, among which the tomato is one of the most susceptible crops. This experiment is conducted to analyse the impacts of inorganic carbon compounds i.e., calcium carbonate  $\text{CaCO}_3$ , sodium carbonate  $\text{Na}_2\text{CO}_3$  and potassium bicarbonate  $\text{KHCO}_3$  on the growth of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) causing wilt in tomato plant in different culture media including Malt Extract Agar (MEA), Potato Dextrose Agar (PDA) and V8. Three concentrations viz 0.5 g/L, 1 g/L and 1.5 g/L of each carbonate are applied. The study indicates that the efficiency of carbon compounds is related to the nature of the media. Among various carbon compounds tested in the present study, maximum growth is obtained with  $\text{KHCO}_3$  while FOL showed least growth with  $\text{Na}_2\text{CO}_3$  in each of the three media. MEA has more profound effect on limiting the growth



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and biomass of the fungus than compared to V8 and PDA. This shows *Fusarium* growth is restricted in the chemical environment containing  $\text{Na}_2\text{CO}_3$  and MEA. In addition, the study reflects that *Fusarium* thrives well in PDA containing  $\text{KHCO}_3$  in each of its three concentrations which may be involved in vegetative and reproductive growth. This study paves a path for further research on how the growth behavior of FOL can be controlled under the influence of inorganic carbon compounds in the soil and yet in the face of environmental changes.

### Keywords

Carbonates; Culture Media; *Fusarium oxysporum* f. sp. *lycopersici*; Fusarium wilt; Fusarium growth; inorganic carbon compounds

## 1. Introduction

Tomato is the second-largest crop of the world in terms of its demand and productivity [1]. Unfortunately, it is among one of the most vulnerable to disease causing pathogen crops as well [2]. Globally, the annual production of fresh tomatoes amounts to approximately 189.1 million tons [3], around one-fourth of which are grown for the processing industry, which renders it to be the world's principal vegetable for processing [4]. Furthermore, tomato is a widely used foodstuff, hence, this crop is to be safeguarded against various threats including pathogenic invasion to endorse its contribution in the fulfilment of the food demand of increasing human population. Among the wide range of diseases in tomato, Fusarium wilt is the most commonly occurring disease [5]. There are a number of pathogens that can cause wilt, but Fusarium wilt is, so far, the most common wilt disease in the world [6]. Since the establishment of tomato as a major crop in the food industry, the incidence of Fusarium wilt has always been a persistent problem in tomato crop [7]. Although no clear-cut statistics on the damages caused by the disease are existing, the yield loss may vary from 10 to 80% subject to the environmental conditions [8]. Approximately 80% of all cultivated plants are associated with at least one disease caused by a *Fusarium* species [9]. Thus, this group is accountable for huge economic losses due to declines in harvest yields and/or the quality of staple foods [10]. Because of the diversity and cosmopolitan distribution of *Fusarium*, they have gained considerable attention by the plant pathologists worldwide [11].

This paper focuses on *Fusarium oxysporum* f. sp. *lycopersici* (FOL) which is soil-borne plant pathogen in the class Hyphomycetes, and causes Fusarium wilt specifically in tomato [12]. It can survive for a long period without a host [13]. Most incidences are linked with disease-ridden tomato debris left in the soil [14]. The *Fusarium* species *lycopersici* in soil withstands through resting spores called as chlamydospores [15]. As the fungus can exist in the soil for many years [16], the control of disease through normal crop rotation is not promising [17]. Even though several tomato breeds have been appeared as resistant to wilt from many countries all over the world, but they have a very narrow range of success because of area-specific species of the pathogen [18]. The present investigation aims to assess the role of carbonates as a source to control the growth of the fungal pathogen FOL which is the biggest causal agent of tomato wilt.

## 2. Material and Method

In this study the inorganic carbon compounds specifically from the group of carbonates have been used as carbon source along with appropriate culture media, to generate varying combinations with different concentrations of carbon compounds to observe the growth behavior of the FOL in different combinations of inorganic carbon compounds and culture media. All the glassware were cleaned and sterilized according to Bhowmik method [19]. Sterile plastic or glass Petri dishes were used to culture the FOL. Standard size plates for fungal cultures with 9 cm in diameter and 1.3 cm or 2.0 cm in height were taken.

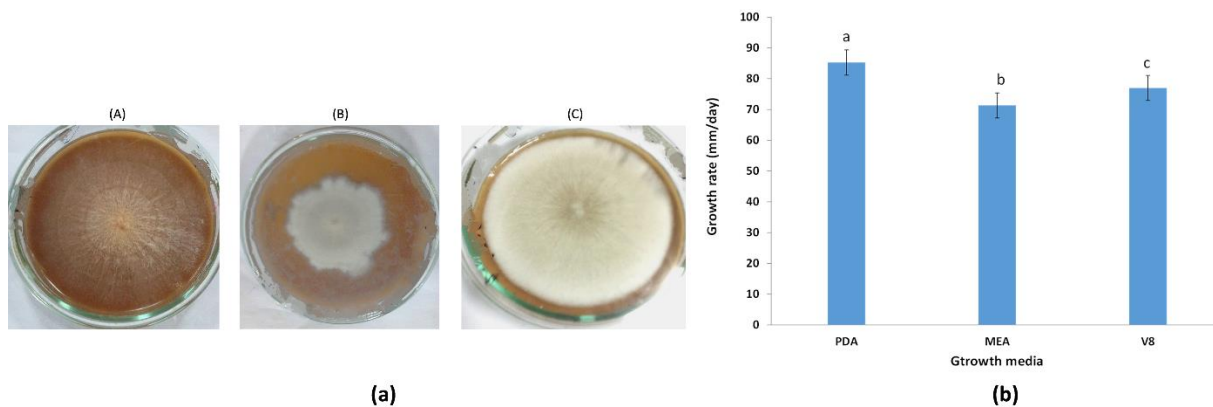
*Isolation of strain:* The strain was isolated from the soil sample collected from Kasur region of district Lahore, Punjab, Pakistan. The serial dilution plating method was used to dilute the soil sample as described by Waksman [20] with the purpose of minimizing the fungi in the soil in each dilution.

*Revival of fungus:* The fungus was revived by inoculation of fungal culture on Malt Extract Medium (MEA) with method used by Nevalainen [21]. Flask was filled up to 2/3 to leave room for the MEA to boil in the autoclave, and covered with foil or a lid not fully closed, and was sterilized at 121°C for 20 min. MEA media was cooled down after autoclaving to about 60-70°C for pouring of plates or making additions. Required amount of the antibiotic was added to the sterilized and well-cooled MEA plates, and were kept in incubator cabinets set at 28°C to grow the plate cultures for 7 to 10 days. All procedures were carried out at room temperature.

*Media with carbonates:* Three types of media namely Potato Dextrose Agar (PDA) prepared by using Razak A. [22] method, Malt Extract Agar (MEA) prepared by using Ottow J. [23] method, and V8 Agar was prepared using the Jaffers S. [24] protocol, were used to assess the growth of the fungus. Three carbon compounds namely:  $\text{CaCO}_3$ ,  $\text{Na}_2\text{CO}_3$  and  $\text{KHCO}_3$  were used with three different concentrations in g/L viz, 0.5 g/L, 1.0 g/L and 1.5 g/L in all selected media to observe the best combination for inhibition of the fungus and to establish the growth behavior of FOL.

*Inoculation on experimental media:* Plates can be inoculated from colonies growing on older plate cultures. Transfer is carried out by lightly touching the growing colony by a sterile rod or toothpick and making a 1-2 mm streak onto the new plates.

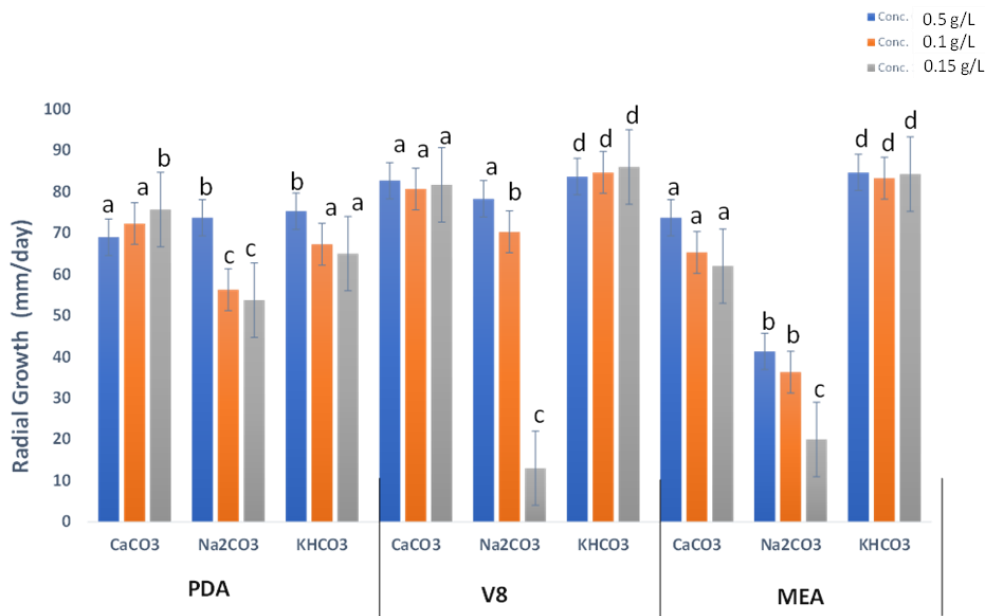
Three control plates of selected media were also prepared and inoculated to check the growth of pathogen with the method suggested by Waksman [20] (Figure 1a and b). The growth was observed and recorded on alternate days till day 10.



**Figure 1 (a)** Growth behaviour of *Fusarium oxysporum* f. sp. *lycopersici* in three culture media **(A)** Malt Extract Agar (MEA), **(B)** V8 Agar, and **(C)** Potato Dextrose Agar (PDA). **(b)** 10 days growth of *Fusarium oxysporum* f. sp. *lycopersici* in control media.

### 3. Results and Discussion

The growth behavior of FOL is not independent of the type of selected media. In fact, the growth varies from medium to medium (Figure 2). In terms of radial growth, V8 is found to be a favorable medium for the growth of FOL in vitro, followed by PDA and MEA, respectively. On the other hand, in respect to the negative impact on radial growth, MEA is observed to be most suitable medium to inhibit the growth of FOL, while PDA shows intermediate response in both the aforesaid situations in comparison to V8 and MEA.



**Figure 2** 10 days radial growth of *Fusarium oxysporum* f. sp. *lycopersici* in three culture media PDA (Potato Dextrose Agar), MEA (Malt Extract Aagar), and V8 Agar with different carbon sources of varying concentrations.

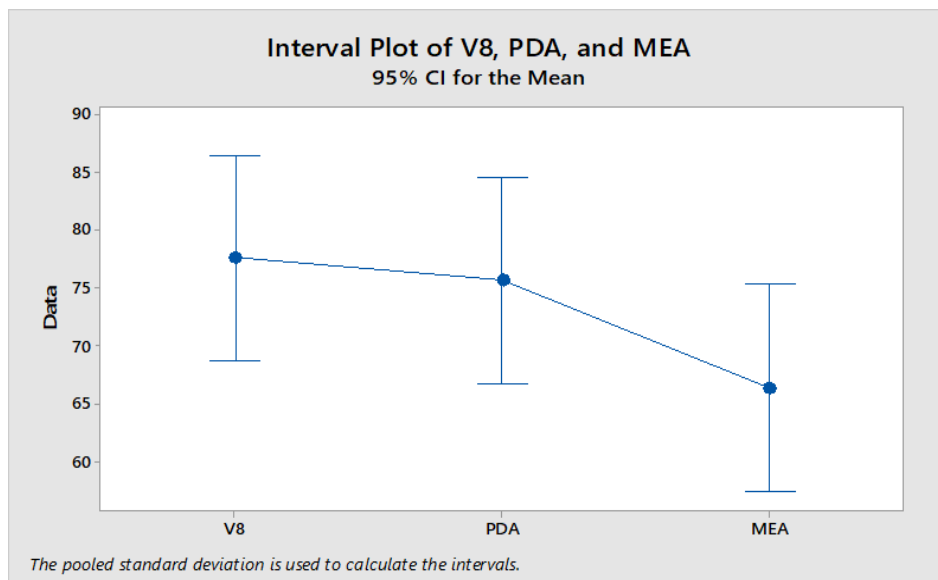
The efficiency of carbon compounds is also related to the nature of the media. Sodium carbonate is observed to be the least effective for growth of FOL in all three selected media. In PDA, all the

three carbon compounds with selected concentrations seem to be in favor of the FOL growth. Whereas, MEA restrains the availability of the carbon compounds to the FOL. Among compounds, Na<sub>2</sub>CO<sub>3</sub> with the concentration of 1.5 g/L is established as the least effective carbon compound for radial growth and quantity of dry mass of FOL in all above said media. MEA is observed to be most suitable medium to inhibit the growth of FOL, while V8 shows intermediate response in comparison to PDA and MEA (Table 1).

**Table 1** Growth Comparison of *Fusarium oxysporum* f. sp. *lycopersici* in PDA, MEA and V8 with different sources of Carbon.

Factor	N	Mean	St. Dev
V8	6	77.56	1.49
PDA	6	75.61	1.67
MEA	6	66.33	1.82
P-Value	0.021		

From the Figure 3, It is clear that FOL could grow at all tested media with 95% CI between the fungal growth. The V8 medium provided maximum growth over the other tested media. The MEA supported the least growth, respectively with P-Value 0.021.



**Figure 3** Interval Plot of growth of *Fusarium oxysporum* f. sp. *lycopersici* n PDA, MEA and V8 with different carbon sources.

Different chemicals have been reported to use against plant pathogens, either to stunt the growth of pathogens or kill the pathogens [25]. There are a number of compounds used as fungicides like, epoxiconazole, propioconazole, prochloraz, tebuconazole and azoxystrobin with in vitro activity against *Fusarium* wilt pathogen [26]. In this *in vitro* study, carbonates have been used as chemical control agent to control the growth of *Fusarium* causing tomato wilt. The efficacy of carbonates to control the growth of *Fusarium* varies with the concentration. Such effects have been observed in several in vitro studies with some compounds inhibiting the growth of *Fusarium* species at specific dose level [27]. There is a close relationship between presence of carbonate, soil organic

matter stability, and soil microbial communities, as microorganisms depend on substrate availability [28].

Carbonates are known to alleviate soil organic matter by enhancing soil aggregation and binding soil organic matter by calcium [29]. Carbonates also have impact on microbial communities because soil organic matter content is greater in carbonated soils [30].

The integration of different methods for sustainable management of Fusarium wilt in tomato is the need of the hour to significantly reduce the disease severity [31]. The nexus of the epidemiological characteristics of the pathogen, less genetic resistance of the host and significant environmental impacts on the disease development, make it essential to adopt some integrated measures to reduce disease damage [32]. In both laboratory studies with pure cultures of phytopathogens and field trials with crop plants, the overall evidence concerning the effectiveness of fungicide is contradictory [33]. But the application of fungicides is one of the efficient control measures when weather conditions are favorable to infection from flowering to harvest [34]. On commercial scale, the development of environmentally safe fungicides is encouraged because of rising concerns related to environmental risks. Therefore, synthesis of carbonate-based fungicides can be crucial step towards sustainable disease management.

#### 4. Conclusion

The research presented in this study is *in vitro* investigation to observe changes and development in mycelial growth behavior of FOL that causes tomato wilt disease. In this study, different media are deployed with a set of three different inorganic carbon compounds 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 FOL in comparison to the other tested media viz PDA and V8. Among carbon compounds,  $\text{Na}_2\text{CO}_3$  is found to be the most effective carbon source to control the FOL growth in comparison to the other two compounds that are  $\text{CaCO}_3$  and  $\text{KHCO}_3$ . Regardless of MEA and  $\text{Na}_2\text{CO}_3$  as individual entities, the growth is observed to be most minimum in the combination of these two specifically with the concentration of 1.5 g/L. The study establishes that carbonates have the potential to be employed as a controlling agent for Fusarium wilt management via devising chemical control measures in the form of fungicides derived from carbon compounds. Finally, this study can also be extended to examine impacts of other *Fusarium* species involve in pathogenic impact on wide range of other crops such as potato, onions, lentils, banana and the like.

#### Author Contributions

**Hina Akram** was responsible for conceptualization, methodology, investigation, software, validation, formal analysis, data curation, writing of original draft and proofreading. **Dr. Shoib Hussain** performed data analysis, formal analysis, proofreading. **Dr. Talib E. Butt** conducted formal analysis, review and editing. All authors have read and agreed to the published version of the manuscript.

## Competing Interests

The authors have declared that no competing interests exist.

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