Open Access

Advances in Environmental and Engineering Research

LIDSEN Publishing Inc.

Original Research

Impacts of Different Sources of Carbonates on Growth of *Fusarium oxysporum* f. sp. *lycopersici* in Different Growth Media

Hina Akram [1, 2], Shoaib Hussain [3], Talib E. Butt [4, *]

1. Institute of Biological Sciences, Faculty of science, University of Malaya, Kuala Lumpur, Malaysia; E-Mail: s2136490@siswa.um.edu.my
2. Minhaj University, Civic Centre, Hamdard Chowk, Lahore, Pakistan; E-Mail: h.a.akram@outlook.com
3. College of Earth and Environmental Sciences University of the Punjab Canal Bank Road, Lahore, Pakistan; E-Mail: Shoaibhussainsatti@yahoo.com
4. Faculty of Engineering & Environment, Northumbria University, City Campus, Newcastle upon Tyne, England, UK; E-Mail: t.e.butt@northumbria.ac.uk

* **Correspondence:** Talib E. Butt; E-Mail: t.e.butt@northumbria.ac.uk

Academic Editor: Daniel M. Alongi

Special Issue: Environmental Management

Adv Environ Eng Res
2023, volume 4, issue 4
doi:10.21926/aeer.2304049

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 $CaCO_3$, sodium carbonate Na_2CO_3 and potassium bicarbonate $KHCO_3$ 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 $KHCO_3$ while FOL showed least growth with Na_2CO_3 in each of the three media. MEA has more profound effect on limiting the growth

and biomass of the fungus than compared to V8 and PDA. This shows *Fusarium* growth is restricted in the chemical environment containing Na_2CO_3 and MEA. In addition, the study reflects that *Fusarium* thrives well in PDA containing $KHCO_3$ 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: $CaCO_3$, Na_2CO_3 and $KHCO_3$ 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.

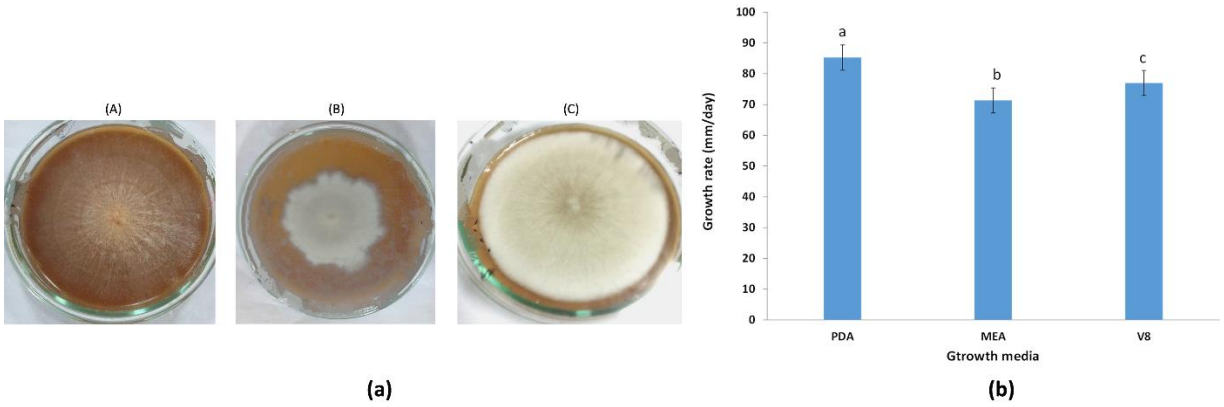

Figure 1 (a) Growth behaviour *of Fusarium oxysporum* f. sp. *lycopersici* in three culture media **(A)** Malt Extract Agar (MEA), **(B)** V8 Agar, and **(C)** Potato Dextrose Agar (PDA). **(b)** 10 days growth of *Fusarium oxysporum* f. sp. *lycopersici* in control media.

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.

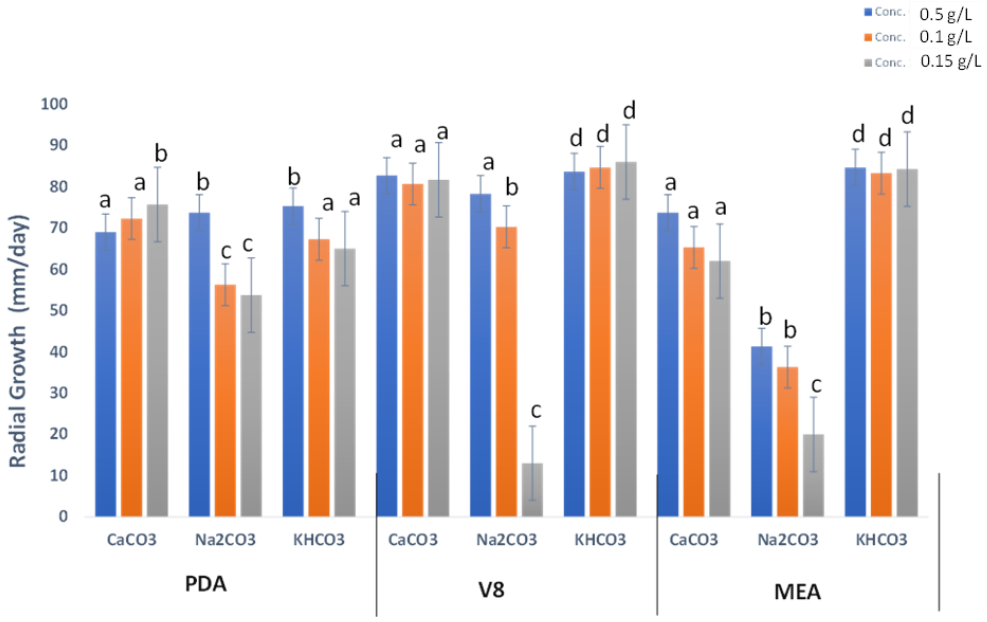

Figure 2 10 days radial growth of *Fusarium oxysporum* f. sp. *lycopersici* in three culture media PDA (Potato Dextrose Agar), MEA (Malt Extract Aagar), and V8 Agar with different carbon sources of varying concentrations.

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_2CO_3 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.

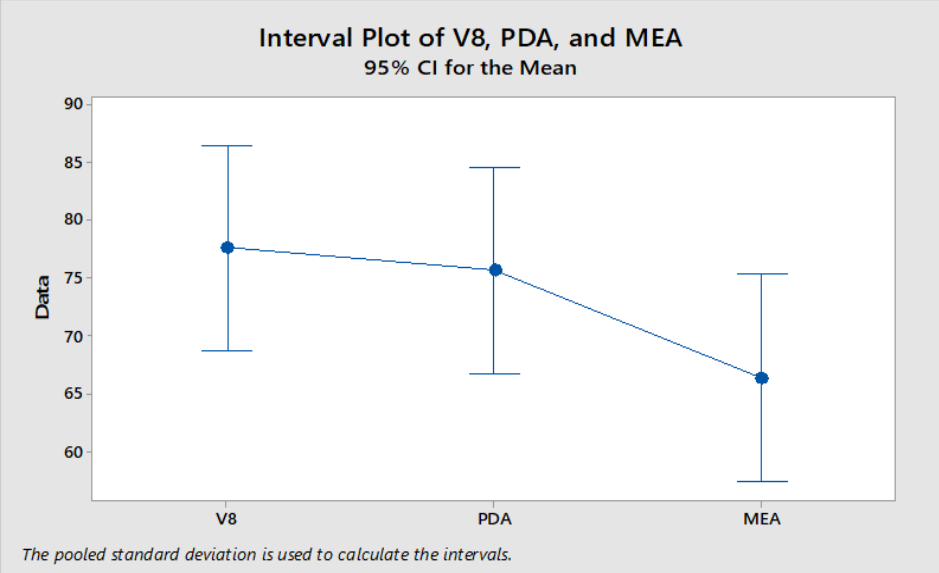

Figure 3 Interval Plot of growth of *Fusarium oxysporum* f. sp. *lycopersici* n PDA, MEA and V8 with different carbon sources.

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, Na_2CO_3 is found to be the most effective carbon source to control the FOL growth in comparison to the other two compounds that are $CaCO_3$ and $KHCO_3$. Regardless of MEA and Na_2CO_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. Shoaib 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.

References

1. Nimbrayan PK, Jaslam PM, Chandanshive A. Modelling and forecasting of area, production and productivity of tomatoes in Haryana and India. Indian J Ext Educ. 2022; 58: 205-208.
2. Ihuoma SO, Madramootoo CA. Sensitivity of spectral vegetation indices for monitoring water stress in tomato plants. Comput Electron Agric. 2019; 163: 104860.
3. Liu Z, Jiang J, Li J. GWAS characterization of the genetic regions associated with nine important agronomic traits in tomato (solanum lycopersicum L.). Agronomy. 2023; 13: 1191.
4. Samoggia A, Grillini G, Del Prete M. Price fairness of processed tomato agro-food chain: The Italian consumers' perception perspective. Foods. 2021; 10: 984.
5. Naqvi SF, Javaid A, Khan IH. Fungicidal activity of stem extract of chenopodium murale L. against the pathogen of fusarium wilt of tomato. Allelopath J. 2023; 59: 69-80.
6. Drenth A, Kema G. The vulnerability of bananas to globally emerging disease threats. Phytopathology®. 2021; 111: 2146-2161.
7. Swett CL, Del Castillo Múnera J, Hellman E, Helpio E, Gastelum M, Lopez Raymundo E, et al. Monitoring for a new I3 resistance gene-breaking race of F. oxysporum f. sp. lycopersici (fusarium wilt) in california processing tomatoes following recent widespread adoption of resistant (F3) cultivars: Challenges with race 3 and 4 differentiation methods. Front Plant Sci. 2023; 14: 1088044.
8. Jamil A, Musheer N, Kumar M. Evaluation of biocontrol agents for management of wilt disease of tomato incited by *Fusarium oxysporum f. sp. lycopersici*. Arch Phytopathol Plant Prot. 2021; 54: 1722-1737.
9. Pfordt A, Ramos Romero L, Schiwek S, Karlovsky P, von Tiedemann A. Impact of environmental conditions and agronomic practices on the prevalence of fusarium species associated with ear- and stalk rot in maize. Pathogens. 2020; 9: 236.
10. Akhtar R, Javaid A, Qureshi M. Bioactive constituents of shoot extracts of sisymbrium irio L. against fusarium oxysporum f. sp. cepae. Planta Daninha. 2020; 38: e020200961.
11. Abdel Azeem AM, Abdel Azeem MA, Darwish AG, Nafady NA, Ibrahim NA. Fusarium: Biodiversity, Ecological Significances, and Industrial Applications. Recent Advancement in White Biotechnology Through Fungi: Volume 1: Diversity and Enzymes Perspectives. Cham, Switzerland: Springer International Publishing; 2019. pp. 201-261.
12. Maurya S, Dubey S, Kumari R, Verma R. Management tactics for fusarium wilt of tomato caused by *Fusarium oxysporum f. sp. lycopersici* (Sacc.): A review. Management. 2019; 4: 1-7.
13. Bahadur A. Current Status of Fusarium and their management strategies. Fusarium-An Overview of the Genus. London, UK: IntechOpen; 2021.
14. Yetukuri NK, Maddali K, Jayapandian N, editors. Machine Learning based Plant Disease Identification by using Hybrid Naïve Bayes with Decision Tree Algorithm. 2023 5th International Conference on Smart Systems and Inventive Technology (ICSSIT); 2023; Tirunelveli, India: Piscateville, NJ, US: Institute of Electrical and Electronics Engineers (IEEE).

15. Arya M, Vikrant, Majumder S, Chaturvedi SK. Exploiting Host Resistance in Management of Vascular Wilt in Major Pulses of India. Plant Stress: Challenges and Management in the New Decade. Cham, Switzerland: Springer; 2022. pp. 73-87.
16. Alkhalifah DHM, Damra E, Melhem MB, Hozzein WN. Fungus under a changing climate: Modeling the current and future global distribution of fusarium oxysporum using geographical information system data. Microorganisms. 2023; 11: 468.
17. Sampaio AM, Araujo SdS, Rubiales D, Vaz Patto MC. Fusarium wilt management in legume crops. Agronomy. 2020; 10: 1073.
18. Chitwood Brown J, Vallad GE, Lee TG, Hutton SF. Breeding for resistance to fusarium wilt of tomato: A review. Genes. 2021; 12: 1673.
19. Bhowmik A. Phylogenetic analysis of drug resistant environmental Escherichia coli. Dhaka, Bangladesh: University of Dhaka; 2021.
20. Waksman SA. A method for counting the number of fungi in the soil. J Bacteriol. 1922; 7: 339-341.
21. Nevalainen H, Kautto L, Te'o J. Methods for isolation and cultivation of filamentous fungi. In: Environmental Microbiology: Methods and Protocols. Totowa, NJ: Humana Press; 2014. pp.3-16.
22. Razak A, Bachmann G, Ali TM, Farrag R. Activities of microflora in soils of upper and lower Egypt. Afr J Mycol Biotechnol. 1999; 7: 1-20.
23. Ottow J, Glathe H. Rose bengal-malt extract-agar, a simple medium for the simultaneous isolation and enumeration of fungi and actinomycetes from soil. Appl Microbiol. 1968; 16: 170.
24. Jeffers S. Protocol 07-11.1: V8 agar (V8A) or broth. Laboratory protocols for Phytophthora species. St. Paul, MN, US: American Phytopathological Society; 2015. pp. 1-2.
25. Collinge DB, Sarrocco S. Transgenic approaches for plant disease control: Status and prospects 2021. Plant Pathol. 2022; 71: 207-225.
26. Rad AK, Astaikina A, Streletskii R, Zarei M, Etesami H. Fungicide and pesticide fallout on aquatic fungi. Holland: Elsevier; 2022. pp. 171-191.
27. Shcherbakova L, Mikityuk O, Arslanova L, Stakheev A, Erokhin D, Zavriev S, et al. Studying the ability of thymol to improve fungicidal effects of tebuconazole and difenoconazole against some plant pathogenic fungi in seed or foliar treatments. Front Microbiol. 2021; 12: 629429.
28. Rasul M, Cho J, Shin HS, Hur J. Biochar-induced priming effects in soil via modifying the status of soil organic matter and microflora: A review. Sci Total Environ. 2022; 805: 150304.
29. Li T, Zhang Y, Bei S, Li X, Reinsch S, Zhang H, et al. Contrasting impacts of manure and inorganic fertilizer applications for nine years on soil organic carbon and its labile fractions in bulk soil and soil aggregates. Catena. 2020; 194: 104739.
30. Martí Roura M, Hagedorn F, Rovira P, Romanya J. Effect of land use and carbonates on organic matter stabilization and microbial communities in Mediterranean soils. Geoderma. 2019; 351: 103-115.
31. Chandhrapati A, Archana T, Kumar D. Integrated disease management of wilt of tomato caused by *Fusarium oxysporum f. sp. lycopersici*. Plant Dis Res. 2022; 37: 257-258.
32. Suman A, Govindasamy V, Ramakrishnan B, Aswini K, SaiPrasad J, Sharma P, et al. Microbial community and function-based synthetic bioinoculants: A perspective for sustainable agriculture. Front Microbiol. 2022; 12: 805498.

33. Corkley I, Fraaije B, Hawkins N. Fungicide resistance management: Maximizing the effective life of plant protection products. Plant Pathol. 2022; 71: 150-169.
34. Janssen E, Mourits M, Van Der Fels Klerx H, Lansink AO. Pre-harvest measures against fusarium spp. infection and related mycotoxins implemented by dutch wheat farmers. Crop Prot. 2019; 122: 9-18.