



Official publication of Pakistan Phytopathological Society

Pakistan Journal of Phytopathology

ISSN: 1019-763X (Print), 2305-0284 (Online)

http://www.pakps.com



DISEASE CAUSING PHYTOPATHOGENIC MICROMYCETES IN CITRUS IN UZBEKISTAN

^aBakhora I. Turaeva, ^bAzamjon B. Soliev, ^aHusniddin K. Karimov, ^aNodira S. Azimova, ^aGuzal J. Kutlieva, ^aKhurshida M. Khamidova, ^aNigora Y. Zuxritdinova

^a Institute of the Microbiology, Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan.

^b Republican Scientific and Practical Center of Sports Medicine, Tashkent, Uzbekistan.

ABSTRACT

This research work provides information on phytopathogenic micromycetes that cause major and emerging diseases posing a significant threat to the cultivation of citrus in Uzbekistan. The purpose of the study is to develop strategies for the management of diseases by biological methods and to improve the methods of eliminating the causative agents of diseases, as well as to determine the effectiveness of micromycetes with high antifungal properties in the cultivation of citrus. The issues affecting the citrus industry and scientific recommendations to overcome those issues are discussed. Micromycetes of phytopathogens that cause diseases in the roots, rhizosphere and leaves of lemon and tangerine in closed ground conditions, including *Fusarium vasinfectum*, *Verticillium dahliae*, *Alternaria alternata*, *F. semefectum*, *Aspergillus oryzae*, *A. flavus*, *Penicillium funiculosum*, *F. sporotrichiella*, *Aspergillus sp.*, *A. terreus*, *P. chrysogenum*, *P. digitatum*, *F. solani*, *P. sp.1*, *A. sp.2*, *A. sp.3*, *F. sp.1*, *P. sp.2*, *F. sp.2*, *A. sp.4*, *F. sp.3* were identified.

Keywords: *Trichoderma harzianum* 55, lemon, tangerine, micromycete, phytopathogen, antagonism.

INTRODUCTION

Citrus fruits are among the top ten and the most productive fruits in the world, grown in more than 100 countries and ranked first in international fruit trade by value (Khanchouch *et al.*, 2017). In Uzbekistan, lemons and tangerines are grown mainly in closed ground conditions (greenhouses), and are also imported from Turkey and Pakistan to meet the needs of the population. The constant use of chemical fungicides against disease-causing micromycetes in the cultivation of citrus has led to the emergence of resistant strains of phytopathogenic fungi (Sánchez-Torres, 2011). Every year, in total of 25% of fruit crops are infected with phytopathogenic microorganisms, resulting in up to 50% loss of its yield in the world (Barkai, 2001; Díaz, 2020). In optimal humidity and temperature, the

phytopathogenic microflora present in the soil develops very rapidly and damages the plants. Recently, the problem of decrease in the yield of perennial citrus plantations has become an urgent issue. Mostly the risk of the mass loss of the fruit yield is being increased due to pathogens in citrus products (Kulyan, 2017; Cronje, 2002). *Alternaria alternata* phytopathogenic fungus infects lemons and tangerines causing 7 different diseases considered as dangerous for citrus (Tsuge, 2013). Phytopathogen micromycetes such as, *A. alternata*, *A. brassicicola*, *A. solani*, *Stemphylium loti*, *S. calilstephi*, *S. trifolii*, *S. sarciniforme*, *S. lancipes*, *Stemphylium solani* were isolated from lemon plantations, and their mechanisms of propagation and pathogenicity features have been determined (Peever, 1999). Phytopathogenic microorganisms belonging to *Dothiorella viticola*, *Lasiodiplodia theobromae*, *Neoscytalidium hyalinum*, *Phaeoacremonium (P.) parasiticum*, *P. italicum*, *P. iranianum*, *P. rubrigenum*, *P. minimum*, *P. croatiense*, *P. fraxinopensylvanicum*, *Phaeoacremonium sp.*, *Cadophora luteo-olivacea*, *Biscogniauxia (B.) mediterranea*, *Colletotrichum*

Submitted: October 01, 2021

Revised: November 19, 2021

Accepted for Publication: December 10, 2021

* Corresponding Author:

Email: jaloliddinshavkiev1992@gmail.com

© 2017 Pak. J. Phytopathol. All rights reserved.

gloeosporioides, *C. boninense*, *Peyronellaea* (*Pa.*) *pinodella*, *Stilbocrea* (*S.*) *walteri*, *Pestalotiopsis* and *Fusarium* genera were identified in infected parts and tissue of citrus trees in Iran (Espargham, 2020) Diatrypaceae sp. micromycetes have been isolated from citrus leaves in Australia and Greece (Elena *et al.*, 2006; Trouillas, 2010). In Australia, Argentina, Brazil, the United States, and the Philippines, phytopathogenic microorganisms *C. paradisi*, *C. maxima* and *C. aurantium* belonging to the genus of *Eutypella* were found to induce diseases in lemons. Phytopathogenic micromycetes because dangerous diseases such as root rot, gray rot, dry root rot in plants that affect the yield. *D. limonicola* and *D. melitensis* micromycetes spread gray rot disease, which is the most common disease in citrus, leading to complete loss of yield. These phytopathogenic fungal strains develop very rapidly and maintain viability in plant stems, leaves, roots and soil even under extremal stress conditions (Guarnaccia, 2017). The major threat group of phytopathogens common in agriculture around the world is *Fusarium* species. A healthy and strong development of the root system of the seedling is required in growing seedlings in the establishment of citrus plantations. *Fusarium* phytopathogens infect lemon and tangerine seedlings with dry root rot, which has become a serious problem in some countries. *F. solani* micromycetes strain causes dry root rot disease and leads to yield loss due to its destructive effects on lemon cultivation. The varieties of orange Valencia Temprana, Valencia Tardía, Navel, Comondú and Sur are grown in Mexico and considered as main export product. Up to 40% of tangerine yields are lost each year due to damage caused by micromycetes of the genus *Aspergillus*, *Fusarium* and *Penicillium*. Particularly, *A. flavus*, *F. oxysporum*, *P. digitatum*, *P. italicum* and *P. variables* strains of phytopathogenic micromycetes were identified (Ochoa, 2007). In the lemon plantation, 30% of the fruits of 400 trees were found to be covered with white mycelium and Green conidia. Their infected parts became rotten and soft, and in the samples taken from the affected parts of trees; *P. italicum* micromycetes were detected and isolated for morphologic analysis. Investigations have shown that phytopathogens cause gray rot in fruits (Hernández-Montiel, 2007). Mold fungi can also be included in the group of phytopathogenic fungi that damage citrus. Mold fungi cause fruit spoilage. *A. niger* micromycetes isolated from lime were found to

develop actively at high temperatures (Sandoval-Contreras, 2010). High temperature and humidity in the closed groundsoils create favorable conditions for the development of the phytopathogenic fungus *A. niger*.

From various citrus fruits, 294 species of phytopathogenic micromycetes have been isolated and their feature of synthesizing secondary metabolites (toxins) have been studied. The fungi belonging to *Penicillium*, *Aspergillus*, *Alternaria* and *Fusarium* species actively synthesized the mycotoxins with strong toxic features. Death was observed in animals when the toxins synthesized by phytopathogenic micromycetes were tested in them (Albinas, 2002; Leila *et al.*, 2014). *P. expansum* and *P. crustosum* phytopathogenic micromycetes are widespread fungi that threaten the storage of citrus (lemon, tangerine), pear and apple fruits. They develop in plant tissues and infect fruits (Gonzalez-Candelas, 2010). A common post-harvest disease in citrus is a green mold disease spread by the phytopathogenic fungus *Penicillium digitatum* that can cause 90% loss of total yield in dry and subtropical climates (Eckert, 1989; Moraes Bazioli, 2019). *P. italicum* (blue mold) is one of the most dangerous pathogens of citrus and can cause damage to citrus fruits even at low temperatures (<10 °C) that are strictly controlled for transportation and storage (Palou, 2002; Tingfu, 2020). *A. fumigatus*, *Aspergillus niger*, *A. flavus* pathogenic fungi causing various putrefactive diseases were detected in sweet orange (*Citrus sinensis* L.) in Sokoto Metropolis city that may cause fruit spoilage by 22, 17, 25 and 36%, respectively. When healthy oranges were artificially infected with phytopathogenic fungi, *Rhizopus stolonifer*, *A. flavus* were found to form a decay zone of 45 mm and 35 mm, *A. niger* 25 mm (Tafinta, 2013).

When using fungicides against phytopathogenic micromycetes, it is important to determine the fungicide resistance of the isolates because a very large fine is paid for any fungicide applied against a phytopathogen with high fungicidal resistance (Dukare *et al.*, 2018). Global climate change and the need for sustainable agriculture require biological control of phytopathogenic diseases in citrus using antagonistic microorganisms, and acceptance of biocontrol as an alternative to the use of fungicides, and the production of ecologically pure products (Vega, 2014). Several mechanisms work in the tripartite plant-

phytopathogen-antagonist interaction system to stop disease and phytopathogen development (Spadaro, 2016). The main affecting mechanisms of the antagonist include competition in the nutrient medium and proliferation, antibiotic production according to the nature of the formation of substances of antibiotic nature, the formation of resistance induction through the synthesis of enzymes affecting mycoparasitism and the pathogenic cell wall (Dukare *et al.*, 2018). As a result of the use of chemicals, there is an accumulation of pathogenic pests in the soil, a decrease in soil microflora, in particular, antagonists, and an increase in resistance to phytopathogens. Diseases transmitted by phytopathogenic microorganisms develop very rapidly, damaging the plant, the yield and the soil. During the years of strong development of diseases, in some districts of Uzbekistan, crop damage was observed at 50-70% (Green, 1999; Howell, 2003). This situation demonstrates the need for the use of biological control agents in protecting crops from disease-causing microorganisms. The biological control method is based on the use of natural antagonists in the struggle against phytopathogens (Wells, 1988; Harman, 2000). Biological control is an important strategy to combat phytopathogenic fungi and the diseases they spread. This principle is based on the use of living cells of antagonists in the elimination of economic damage and the struggle against phytopathogenic microorganisms (Cuthbert, 2018; Hernandez, 2019; Safdarpour, 2019). Micromycetes belonging to the family *Trichoderma* synthesize a number of biologically active substances as primary metabolites (enzymes), secondary metabolites (phytohormones) and more than 100 antibiotics and are used worldwide as a biocontrol agent (Elad, 1983; Hammond-Kosack, 1995; Sivasithamparam, 1998). The fungal strain *T. harzianum* 55 was found to synthesize phytohormone activity, secondary metabolites gibberellin acid (GA) and indole acetic acid (IAA) and antagonistic features against phytopathogen fungi (*Fusarium*, *Alternaria*, *Verticillium*, *Aspergillus*, *Scopulariopsis*, *Rhizoctonia* species) that is, *A. flavus*, *F. vasenfectum*, *F. solani*, *S. brevicaulis*, *A. tenuis*, *R. solani*, *F. verticillioides*, *S. carbonaria*, *F. oxysporum*, *F. avenaceum*, *F. semitectum*, *F. gibbosum*, *F. sambucinum*, *F. javanicum*, *F. culmorum* (Harman, 2004; Turaeva *et al.*, 2020). IAA activates cell division in the growing part of the plant root and enhances root development, eliminates toxic

metabolites produced by phytopathogenic microorganisms and directly controls root pathogens (Turaeva *et al.*, 2019). Micromycetes protect plants from phytopathogens, increase seed germination capacity, enhance plant growth, increase metabolism of substances, expand leaf plate surface, improve soil structure, and increase porosity; they are considered as highly effective biological control agents with a mechanism of pleiotropic effect on soil and plant. *T. asperellum* MG/6 and *T. T-30* strains have high antagonistic activity against *F. sambucinum* and *F. sporotrichioides* strains (Inbar, 1994; Chet *et al.*, 1998). Antagonistic effect of *T. virens* 3X and *T. lignorum* M-10 strains was studied against phytopathogen fungi *F. oxysporum*, *F. solani*, *F. sporotrichiella* Bilai, *Fusarium* sp. that cause root rot and Fusarium diseases, white rot pathogens - *Sclerotinia sclerotiorum*, rhizoctonia pathogen - *Rhizoctonia solani*, it was also found that fungal strains belonging to the family *Trichoderma* had a complete dominance over pathogens (Lorito, 1998; Alizadeh *et al.*, 2020; Vafaie, 2018; Rosado, 2007). Rational use of biocontrol agents to protect crops from phytopathogens in closed ground conditions, to obtain high and qualitative yields gives effective results (Srinivasa *et al.*, 2014).

MATERIALS AND METHODS

Sample collections: Samples were brought from the local zonal varieties of lemon (F-1 Tashkent, F-2 Yubileyniy), tangerine (Tashkent), pampela (Zayniddin) grown in greenhouses (Fakhriddinov *et al.*, 2020). From the leaves, roots, fruits and root rhizosphere of the diseased lemon and tangerine plant the samples were taken for analysis. Samples from roots and leaves were purified with 3% hydrogen peroxide, alcohol, and distilled water. The samples were ruptured in the cell shell using crushed glass under laboratory conditions and inoculated in nutrient media in a sterile state. 1 g was taken by weighing from samples obtained from the root rhizosphere and diluted to 0.5 ml in 1–10 test tubes filled with 5 ml of sterile water. 3-4 and 5-6 diluted samples were inoculated in nutrient media. Samples prepared for microbiological analysis were grown in meat-peptone agar (MPA), potato-dextrose agar (PDA), Mandels, Oats agar (OA), agar Chapek nutrient media and placed in thermostats at temperatures from 20°C to 38°C. XSP-136 B and OLYMPUS BX 41 light magnifiers (capable of magnification up to 400 times) were used to identify

the types of microorganisms. In identifying species of microscopic fungi determiners of Pidoplichko N.M. (Pidoplichko, 1971), Litvinov M.A. (Litvinov, 1967), Bilay V.I., Aristovskaya T.V., (Bilay, 1982; Bilay, 1977; Aristovskaya, 1962; Tepper, 2004; Heng Mei Hsuan, 2011; Garibova, 2005) were used. Also, phytopathogenic fungi were detected by a mass spectrometry (MALDI TOF) in the sanitary-hygienic laboratory under the Ministry of Healthcare of the Republic of Uzbekistan (Kazakov, 2017). The antagonistic properties of *Trichoderma harzianum* 55 micromycete strain against phytopathogenic micromycetes isolated for the development of biological control measures against phytopathogenic fungi were determined by agar block method (Turaeva *et al.*, 2019; Karimov *et al.*, 2020; Turaeva *et al.*, 2016).

STATISTICAL ANALYSIS

The experiments were carried out in three independent replicates and the results were expressed as mean standard deviations (SD). The SD values (represented as deviation bars) were determined using Microsoft Excel 2016 (Microsoft Corp., Redmond, Washington, DC, USA). The data were subjected to one-way analysis of variance (ANOVA) followed by a Tukey's post hoc test where applicable, with the significance evaluated at $p < 0.05$.

Antagonistic property: The antagonistic property of microorganisms (micromycetes, bacteria, actinomycetes) is explained by the fact that they inhibit the growth of phytopathogenic micromycetes due to the synthesis of substances of antibiotic nature or form a growth ring against them (Bekmukhamedova, 2020).

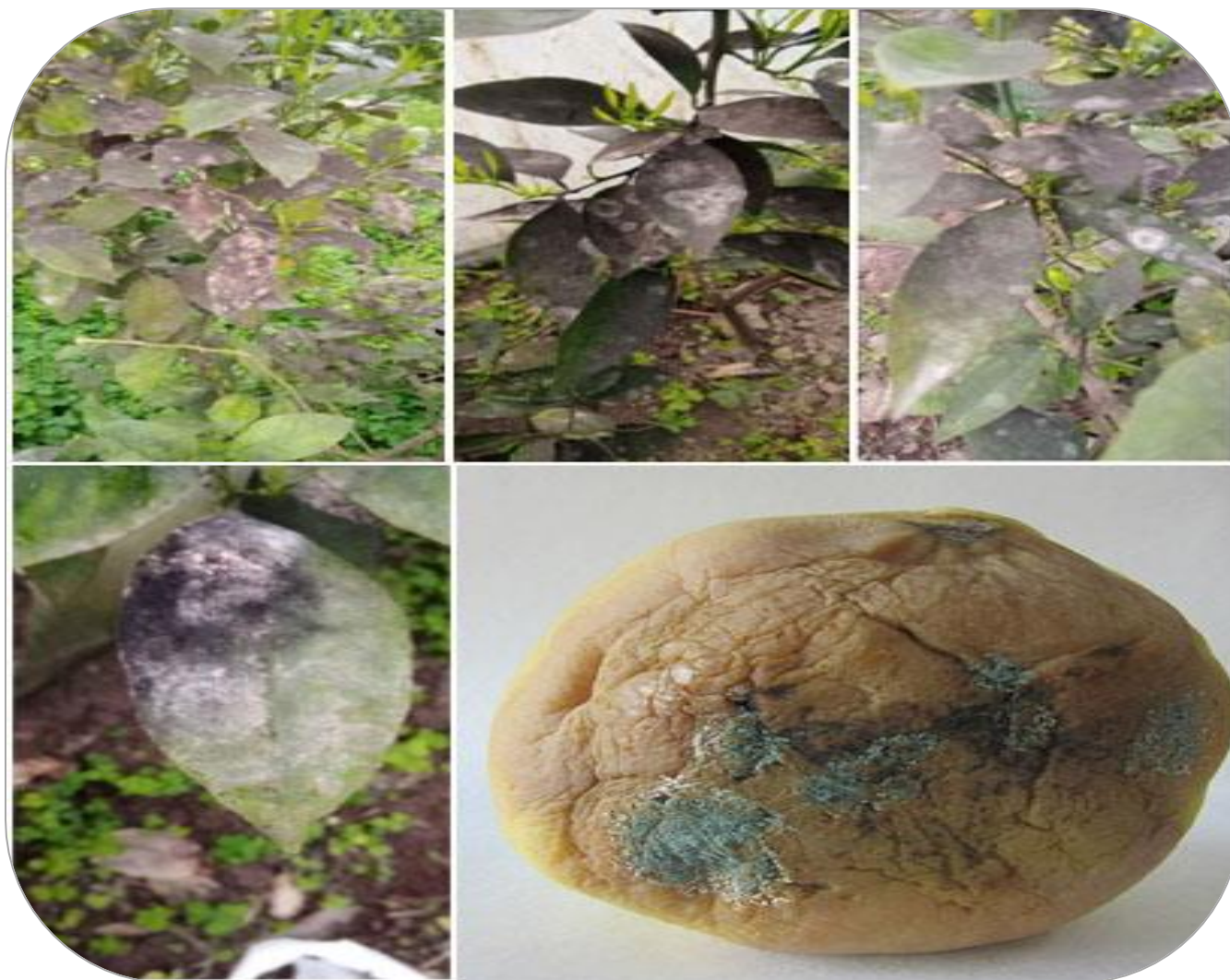


Figure-1. Citrus plants grown in green house and infected pamella (Zayniddin) fruit.

Antagonistic properties of the fungal strain of *T. harzianum*-55 in relation to phytopathogens isolated from citrus:

The antagonistic property of the *T. harzianum*-55 fungal strain was determined by the agar block method. In agar block method the followings were used: agar Mandels media with 2% saccharose(g/l: KH_2PO_4 - 2,0; $(\text{NH}_4)_2\text{HPO}_4$ - 1,4; MgSO_4 - 0,5; CaCl_2 - 0,3; saccharose - 2; agar - 16; microelements - 1 ml; (mixture of microelements: 500 mg FeSO_4 ; 156 mg $\text{MnSO}_4 \times 4\text{H}_2\text{O}$; 167 mg ZnCl_2 ; 200 mg CoCl_2 ; 1 mL 19% HCl in 100 mL of distilled water)) (Mandels, 1962). It was grown on a Petri dish for 6 days and 8 mm agar blocks were prepared. Phytopathogenic fungi isolated from citrus were inoculated in Chapek nutrient medium (g /l: KH_2PO_4 - 1,0; MgSO_4 - 0,5; NaNO_3 - 3,0; KCl - 0,5;

FeSO_4 -granules; saccharose -2; microelements - 1 mL; (mixture of microelements: 500 mg FeSO_4 ; 156 mg $\text{MnSO}_4 \times 4\text{H}_2\text{O}$; 167 mg ZnCl_2 ; 200 mg CoCl_2 ; 1 mL 19% HCl in 100 mL distilled water)) in 24 * 150 mm test tubes and grown in a thermostat at 28 °C for 6 days, and then poured 5 ml of sterilized water, 0.1 ml of the prepared suspension was taken and then planted in Petri dishes. 20 minutes after planting phytopathogenic fungi, 8 mm agar blocks of *T. harzianum*-55 fungal strain were placed. Grown and monitored for 5 days in a thermostat at 30 °C. The experiments were performed in three replications, the antifungal activity of the antagonist fungus was determined by the formation of a growth ring relative to the pathogen and the diameter of the ring.

Table 1. Antagonistic effect of *T. harzianum* 55 fungus on phytopathogens (4 days)

S.No.	Strains of phytopathogen micromycetes	<i>T.harzianum</i> -55 antagonist strain formed ring relative to phytopathogen micromycetes (mm)
1	<i>Fusarium vasinfectum</i>	Complete stop of pathogen growth
2	<i>Verticillium dahliae</i>	Complete stop of pathogen growth
3	<i>Alternaria alternata</i>	Complete stop of pathogen growth
4	<i>Fusarium semefectum</i>	Complete stop of pathogen growth
5	<i>Aspergillus oryzae</i>	60 ±1,1
6	<i>Aspergillus flavus</i>	55 ±1,2
7	<i>Penicillium funiculosum</i>	63 ±0,9
8	<i>Fusarium sporotrichiella</i>	70 ±0,3
9	<i>Aspergillus sp.</i>	80±0,9
10	<i>Aspergillus terreus</i>	70±0,7
11	<i>Penicillium chrysogenum</i>	Complete stop of pathogen growth
12	<i>Penicillium digitatum</i>	60±0,6
13	<i>Fusarium solani</i> .	Complete stop of pathogen growth
14	<i>Penicillium sp. 1.</i>	45±0,7
15	<i>Aspergillus sp.2.</i>	Complete stop of pathogen growth
16	<i>Aspergillus sp.3</i>	80±0,9
17	<i>Fusarium sp.1.</i>	65±1,1
18	<i>Penicillium sp.2</i>	45±0,9
19	<i>Fusarium sp.2.</i>	70±0,6
20	<i>Aspergillus sp.4.</i>	Complete stop of pathogen growth
21	<i>Fusarium sp.3.</i>	45±0,9

RESULTS AND DISCUSSION

Trichoderma harzianum-55 fungus manifested the highest level of antagonistic activity against phytopathogens *Fusarium vasinfectum*, *F.solani*, *Verticillium dahliae*, *Alternaria alternata*, *F.semefectum*, *Penicillium chrysogenum*, *Aspergillus sp.4* and it was found to stop growth of phytopathogen micromycetes (Figure 2). Pereira, (2014); Silva *et al.* (2016) noted in their research that *Trichoderma* fungi had high antifungal feature against micromycetes of

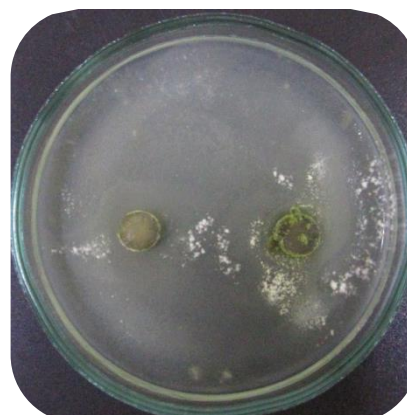
phytopathogen *F. solani*. Hoffmann *et al.*, (2015) reported that isolates of *Trichoderma sp.* micromycetes manifested antifungal activity up to 80 % against *Fusarium sp.* phytopathogen fungi. Tapwal *et al.* (2011) studied in the research that fungi of *Trichoderma* genus had high antagonistic activity against phytopathogen fungi of *Alternaria* and *Fusarium* genera. Al-Askar *et al.*, (2016) reported that *Trichoderma sp.* fungus is considered as biological control agent against *Rhizoctonia solani* pathogenic fungi.



Fusarium vasinfectum



Verticillium dahliae



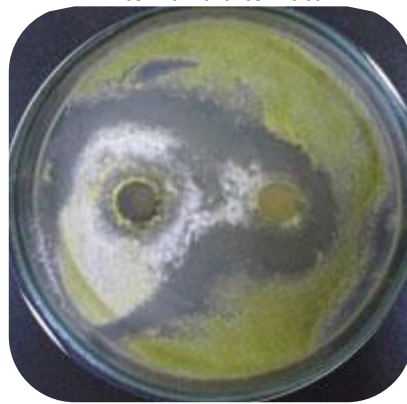
Alternaria alternata



Fusarium semitectum



Aspergillus oryzae



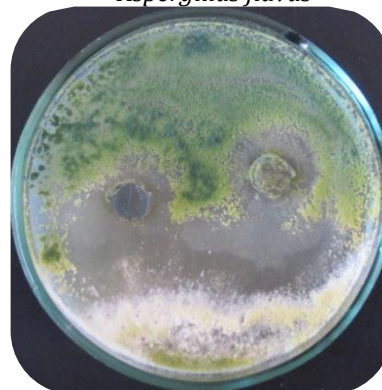
Aspergillus flavus



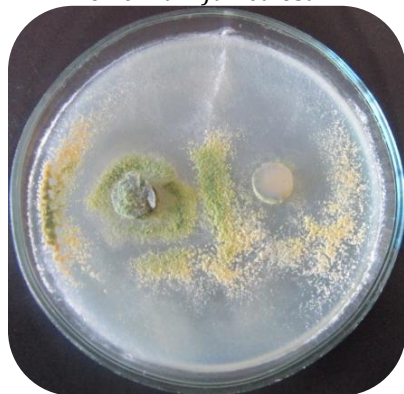
Penicillium funiculosum



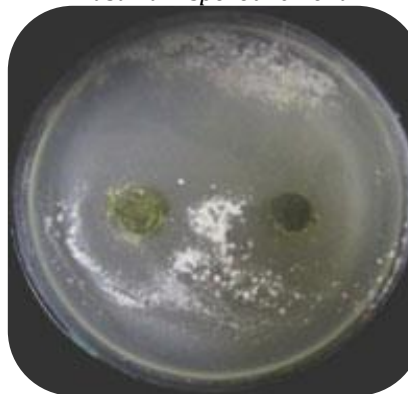
Fusarium sporotrichiella



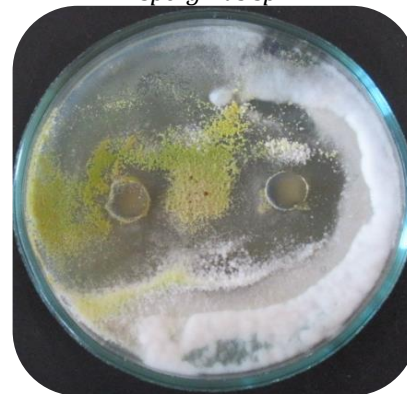
Aspergillus sp.1.



Aspergillus terreus



Penicillium chrysogenum



Penicillium digitatum

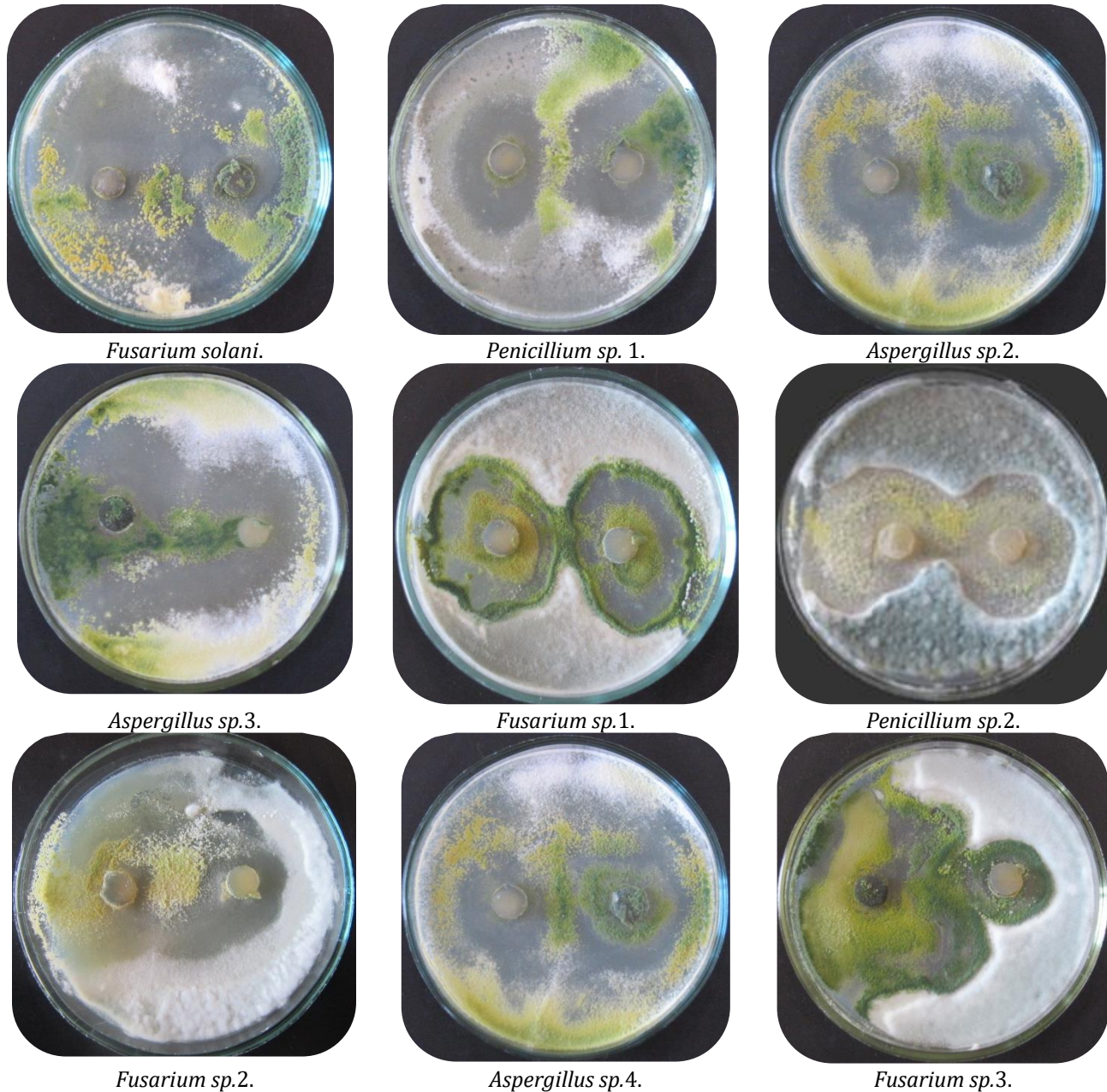


Figure 2. Antifungal activity of *T. harzianum* UzCF 55 fungus relative to phytopathogen fungi.

On the 4th day of the research experiment, *T. harzianum*-55 fungus was found to stop growth ring in relation to phytopathogen micromycetes *Aspergillus oryzae* (60-80 mm), *A. flavus* (55-70 mm), *P. funiculosum* (63-70 mm), *F. sporotrichiella* (70-80 mm), *Aspergillus sp.*, *A. terreus* (80-70 mm), *P. digitatum* (60-85 mm), *P. sp. 1.* (45-50 mm), *A. sp.3* (80-70 mm), *F. sp.1.* (65-70 mm), *F. sp.2* (70-80 mm), *F. sp.3* (45-50 mm) and *P. sp.2* (45-73 mm) (table-1).

CONCLUSION

The use of biological preparations based on the fungal

strain *Trichoderma harzianum*-55 as a biological control agent in the control of disease-causing micromycetes in the cultivation of citrus can be used as the main means of protecting citrus from phytopathogenic micromycetes and diseases they spread.

Thus, the results of the study testify to the development of a drug with a complex effect based on a local micromycet strain that protects against various phytopathogenic microorganisms and diseases they spread in the cultivation of citrus. The creation of biopreparations on the basis of local strains ensures the

efficiency of ecologically clean and high yields from crops due to the suitability of the soil and climatic conditions of the region.

ACKNOWLEDGEMENTS

Authors are grateful to the Institute of Genetics and Plants Experimental Biology, Academy of Sciences of Uzbekistan for providing space and resources to carry out this work.

REFERENCES

- Al-Askar, A., A. Ezzat, K. Ghoneem and W. Saber. 2016. *Trichoderma harzianum* WKY5 and its Gibberellic Acid Control of *Rhizoctonia solani*, Improve Sprouting, Growth and Productivity of Potato. Egyptian Journal of Biological Pest Control, 26: 787-796.
- Albinas L, J. S. 2002. Toxin producing micromycetes on fruit, berries, and vegetables. Annals of Agricultural Environment and Medicin, 9: 183-197.
- Alizadeh, M., Y. Vasebi and N. Safaie. 2020. Microbial antagonists against plant pathogens in Iran: A review. Open Agriculture, 5: 404-440.
- Aristovskaya TV, V. M., Gllerbach MM, Katanskaya GA, Kashkin PN, Klupt S.E., Lozina-Lozinskiy L.K., Norkina S.P., Rumyanseva V.M., Seliber G.L., Skalon I.S., Skorodumova AM, Khetagurova FV, Chastukhin 1962. Big practicum microbiology. Moscow. High school, 491.
- Barkai, GR. 2001. Chemical control. Postharvest diseases of fruits and vegetables. Barkai-Golan R. Amsterdam: Elsevier: 147-188.
- Bekmukhamedova, N. 2020. Antagonistic and growth stimulating activity of the local strain *Streptomyces roseoflavus* 33. plant cell biotechnology and molecular biology: 65-71.
- Bilay VI. 1977. Fusarium. Kiev. *Naukova dumka*. 434.
- Bilay, V. 1982. Methods of experimental mycology. Kiev: Naukova Dumka: 418-430.
- Carmona-Hernandez, S., J. J. Reyes-Pérez, R. G. Chiquito-Contreras, G. Rincon-Enriquez, C. R. Cerdan-Cabrera and L. G. Hernandez-Montiel. 2019. Biocontrol of postharvest fruit fungal diseases by bacterial antagonists: a review. Agronomy, 9: 121.
- Chet, I., N. Benhamou and S. Haran. 1998. *Trichoderma* and *Gliocladium*, Mycoparasitism and Lytic Enzymes. Taylor and Francis London, 153-172.
- Cronje, C., H. Le Roux, M. Truter, I. Van Heerden and H. Phillips. 2002. Long-term effect of replant soil solarisation on growth of replant citrus trees in South Africa. African Plant Protection, 8: 41-49.
- Cuthbert, R. N., J. T. Dick, A. Callaghan and J. W. Dickey. 2018. Biological control agent selection under environmental change using functional responses, abundances and fecundities; the Relative Control Potential (RCP) metric. Biological Control, 121: 50-57.
- da Silva, J. A. T., E. V. de Medeiros, J. M. da Silva, D. d. A. Tenório, K. A. Moreira, T. C. E. d. S. Nascimento and C. Souza-Motta. 2016. *Trichoderma aureoviride* URM 5158 and *Trichoderma hamatum* URM 6656 are biocontrol agents that act against cassava root rot through different mechanisms. Journal of Phytopathology, 164: 1003-1011.
- Díaz, M. A., M. M. Pereyra, E. Picón-Montenegro, F. Meinhardt and J. R. Dib. 2020. Killer Yeasts for the Biological Control of Postharvest Fungal Crop Diseases. Microorganisms, 8: 1680.
- Dukare, A. S., S. Paul, V. E. Nambi, R. K. Gupta, R. Singh, K. Sharma and R. K. Vishwakarma. 2018. Exploitation of microbial antagonists for the control of postharvest diseases of fruits: a review. Critical reviews in food science and nutrition, 59: 1498-1513.
- Eckert, J. W. and I. Eaks. 1989. Postharvest disorders and diseases of citrus fruits. The citrus industry, 5: 179-260.
- Elad, Y., R. Barak, I. Chet and Y. Henis. 1983. Ultrastructural studies of the interaction between *Trichoderma* spp. and plant pathogenic fungi. Journal of Phytopathology, 107: 168-175.
- Elena, M. F., Dimitris D and Dimtris M. 2006. Dimou *Fomitiporia mediterranea* infecting citrus trees in Greece. phytopathologia mediterranea, 45: 35-39.
- Espargham, N., H. Mohammadi and D. Gramaje. 2020. A survey of trunk disease pathogens within citrus trees in Iran. Plants, 9: 754.
- Fakhrutdinov, M., S. Rashidova and R. Tillaev. 2020. Application of "uzchitan" preparation as the growth of citrus plant regulator. International Journal of Scientific and Technology Research, 9: 1188-1190.
- Garibova LV, L. S. 2005. Bases of mycobiology: Morphology and systematic for fungi and ribosomal organisms. Molecules: 80-89.
- González-Candelas, L., S. Alamar, P. Sánchez-Torres, L. Zacarías and J. F. Marcos. 2010. A transcriptomic

- approach highlights induction of secondary metabolism in citrus fruit in response to *Penicillium digitatum* infection. BMC plant biology, 10: 1-17.
- Green, H., J. Larsen, P. A. Olsson, D. F. Jensen and I. Jakobsen. 1999. Suppression of the biocontrol agent *Trichoderma harzianum* by mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* in root-free soil. Applied and Environmental Microbiology, 65: 1428-1434.
- Guarnaccia, V. and P. W. Crous. 2017. Emerging citrus diseases in Europe caused by species of Diaporthe. IMA fungus, 8: 317-334.
- Hammond-Kosack, K., B. Staskawicz, J. Jones and D. Baulcombe. 1995. Functional expression of a fungal avirulence gene from a modified potato virus X genome. Plant Microbe Interaction, 8: 181-185.
- Harman, G. E. 2000. Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant disease, 84: 377-393.
- Harman, G. E., C. R. Howell, A. Viterbo, I. Chet and M. Lorito. 2004. *Trichoderma* species—opportunistic, avirulent plant symbionts. Nature reviews microbiology, 2: 43-56.
- Hernández-Montiel, L. and J. Ochoa. 2007. Fruit rot caused by *Penicillium italicum* on lemon (*Citrus aurantifolia*) in Colima, México. Plant disease, 91: 767-767.
- Hoffmann, C. A., L. F. B. Chagas, D. P. Da Silva, A. F. C. Junior and G. N. Scheidt. 2015. Potencial de antagonismo de isolados de *Trichoderma* sp. contra o isolados de *Fusarium* sp., in vitro. Revista Verde de Agroecologia e Desenvolvimento Sustentável, 10: 37.
- Howell, C. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant disease, 87: 4-10.
- Hsuan, H. M., B. Salleh and L. Zakaria. 2011. Molecular identification of *Fusarium* species in *Gibberella fujikuroi* species complex from rice, sugarcane and maize from Peninsular Malaysia. International Journal of Molecular Sciences, 12: 6722-6732.
- Inbar, J. and I. Chet. 1994. A newly isolated lectin from the plant pathogenic fungus *Sclerotium rolfsii*: purification, characterization and role in mycoparasitism. Microbiology, 140: 651-657.
- Karimov HK, T. B., Azimova NSh, Hamidova Kh.M. 2020. Antagonistic effect of fungi of *Trichoderma* species on phytopathogens. . Proceedings from Science Academy. Tashken, 5: 85-93.
- Kazakov, VS. 2017. Decision on the base of MALDI-TOF mass-spectrometry for express identification of microorganisms.
- Khanchouch, K., A. Pane, A. Chriki and S. O. Cacciola. 2017. Major and emerging fungal diseases of citrus in the Mediterranean Region. Citrus Pathology, 1: 66943.
- Kulyan RV, S. L., Rakhmangulov RS. 2017. Genetic resources of citrus in Russia, the Ukraine and Belarussia: storage and use. Vavilov journal of genetics and breeding. Letters to Vavilov Journal of Genetics and Breeding, 21: 506-514.
- Leila, T, M. E. A. D., Nassira Gaouar, Bouayad Samira, and BoufeldjaTabti. 2014. Antioxidant and Antifungal Activity of Extracts of the Aerial Parts of *Thymus capitatus* (L.) Hoffmanns Against Four Phytopathogenic Fungi of *Citrus sinensis*. Journal of Natural Pharmaceutical Products, 9: 49-54.
- Litvinov, MA. 1967. Determiner of microscopic soil fungi. Leningrad. *Nauka*.106: 303.
- Lorito, M. 1998. Chitinolytic enzymes and their genes. *Trichoderma* and *Gliocladium*, 2: 73-99.
- Mandels, M., F. W. Parrish and E. T. Reese. 1962. Sophorose as an inducer of cellulase in *Trichoderma viride*. Journal of Bacteriology, 83: 400-408.
- Moraes Bazioli, J., J. R. Belinato, J. H. Costa, D. Y. Akiyama, J. G. d. M. Pontes, K. C. Kupper, F. Augusto, J. E. de Carvalho and T. P. Fill. 2019. Biological control of citrus postharvest phytopathogens. Toxins, 11: 460.
- Ochoa, J., L. Hernández-Montiel, H. Latisnere-Barragán, J. L. de La Luz and C. P. Larralde-Corona. 2007. Aislamiento e identificación de hongos patógenos de naranja *Citrus sinensis* l. osbeck cultivada en baja california sur, méxico isolation and identification of pathogenic fungi from orange *Citrus sinensis* l. osbeck cultured in baja california sur, mexico. Cyta. Journal of Food, 5: 352-359.
- Palou, L., J. Usall, J. L. Smilanick, M. J. Aguilar and I. Vinas. 2002. Evaluation of food additives and low-toxicity compounds as alternative chemicals for the control of *Penicillium digitatum* and

- Penicillium italicum* on citrus fruit. Pest management science, 58: 459-466.
- Peever, T., Y. Canihos, L. Olsen, A. Ibanez, Y.-C. Liu and L. Timmer. 1999. Population genetic structure and host specificity of *Alternaria* spp. causing brown spot of *Minneola tangelo* and rough lemon in Florida. *Phytopathology*, 89: 851-860.
- Pereira, C. d. O. F. 2014. Estudo da patogenicidade e controle biológico de *Fusarium* sp. com *Trichoderma* sp.
- Pidoplichko, N.M. And A.A. Milko. 1971. Atlas of mucoral fungi. Academy of science of the Ukraine, Institute of Microbiology and Virusology named after D.K. Zabolotnoy. Kiev.
- Rosado, I. V., M. Rey, A. C. Codón, J. Govantes, M. A. Moreno-Mateos and T. Benítez. 2007. QID74 Cell wall protein of *Trichoderma harzianum* is involved in cell protection and adherence to hydrophobic surfaces. *Fungal Genetics and Biology*, 44: 950-964.
- Safdarpour, F. and G. Khodakaramian. 2019. Assessment of antagonistic and plant growth promoting activities of tomato endophytic bacteria in challenging with *Verticillium dahliae* under in-vitro and in-vivo conditions. *Biological Journal of Microorganism*, 7: 77-90.
- Sánchez-Torres, P. and J. J. Tuset. 2011. Molecular insights into fungicide resistance in sensitive and resistant *Penicillium digitatum* strains infecting citrus. *Postharvest biology and technology*, 59: 159-165.
- Sandoval-Contreras, T., S. Marín, A. Villarruel-López, A. Gschaedler, L. Garrido-Sánchez and F. Ascencio. 2017. Growth modeling of *Aspergillus niger* strains isolated from citrus fruit as a function of temperature on a synthetic medium from lime (*Citrus latifolia* T.) Pericarp. *Journal of food protection*, 80: 1090-1098.
- Sivasithamparam K. and Ghisalberti E L. 1998. *Trichoderma* and *Gliocladium*. 1 (eds Kubicek, C. P. & Harman, G. E.) Taylor and Francis, London. 1998: 139-191.
- Spadaro, D. and S. Droby. 2016. Development of biocontrol products for postharvest diseases of fruit: the importance of elucidating the mechanisms of action of yeast antagonists. *Trends in Food Science & Technology*, 47: 39-49.
- Srinivasa, N., T. P. Devi, S. Sudhirkumar, D. Kamil, J. L. Borah and N. Prabhakaran. 2014. Bioefficacy of *Trichoderma* isolates against soil-borne pathogens. *African Journal of Microbiology Research*, 8: 2710-2723.
- Tafinta, I., K. Shehu, H. Abdulganiyyu, A. Rabe and A. Usman. 2013. Isolation and identification of fungi associated with the spoilage of sweet orange (*Citrus sinensis*) fruits in Sokoto State. *Nigerian Journal of Basic and Applied Sciences*, 21: 193-196.
- Tepper, E., V. Shil'nikova and G. Pereverzeva. 2005. *Practicum on microbiology*. Moscow: Bustard.
- Trouillas, F. P., J. R. Urbez-Torres and W. D. Gubler. 2010. Diversity of diatrypaceous fungi associated with grapevine canker diseases in California. *Mycologia*, 102: 319-336.
- Tsuge, T., Y. Harimoto, K. Akimitsu, K. Ohtani, M. Kodama, Y. Akagi, M. Egusa, M. Yamamoto and H. Otani. 2013. Host-selective toxins produced by the plant pathogenic fungus *Alternaria alternata*. *FEMS microbiology reviews*, 37: 44-66.
- Turaeva BI, K. K., Eshdavlatova G, Kamolov LS. 2019. Low-molecular metabolites of fungus *Trichoderma harzianum* Uz CF-55. *KarSU news. Karshi*, 1: 28-31.
- Turaeva, B., A. Soliev, F. Eshboev, L. Kamolov, N. Azimova, H. Karimov, N. Zukhritdinova and K. Khamidova. 2020. The use of three fungal strains in producing of indole-3-acetic acid and gibberellic acid. *Plant Cell Biotechnology and Molecular Biology*, 21: 32-43.
- Turaeva, B., N. Zukhritdinova, X. Karimov, K. M. Khamidova and Z. Ahmedova. 2016. The antagonistic activity of fungus *T. harzianum* UZ CF-55 against some phytopathogens. *European Journal of Biomedical and Pharmaceutical Sciences*, 3: 70-72.
- Vafaie A, B. B., Jafarie A. 2018. Effect of *Streptomyces* isolates from tomato rhizosphere on *Fusarium oxysporum* sp. *radicis lycopersici*. 23th Iranian Plant Protection Congress: 874.
- Vega, B. and M. M. Dewdney. 2014. QoI-resistance stability in relation to pathogenic and saprophytic fitness components of *Alternaria alternata* from citrus. *Plant disease*, 98: 1371-1378.
- Wells, H. 1988. *Trichoderma* as a biocontrol agent. *Biocontrol of plant diseases*. I: 71-82.
- Zhang, T., Q. Cao, N. Li, D. Liu and Y. Yuan. 2020.

Transcriptome analysis of fungicide-responsive gene expression profiles in two *Penicillium italicum* strains with different response to the sterol demethylation inhibitor (DMI) fungicide prochloraz. BMC genomics, 21: 1-16.

Contribution of Authors:

Bakhora I. Turaeva	:	Conduct trials and wrote manuscript
Azamjon B. Soliev	:	Helped in manuscript write up and research trials
Husniddin K. Karimov	:	Prepared tables, figures and graphs
Nodira S. Azimova	:	Helped in conducting research trials
Guzal J. Kutlieva	:	Helped in manuscript write up
Khurshida M. Khamidova	:	Supervised the study
Nigora Y. Zuxritdinova	:	Conceive the idea of research and contribute in write up