How to Cite:
AL-Khazraji, E. H. A., & Hanawi, M. J. (2022). In vitro antifungal activity of alcohol and aqueous extract of stachys sp on fusarium solani. *International Journal of Health Sciences*, *6*(S5), 12013–12021. https://doi.org/10.53730/ijhs.v6nS5.11811

In vitro antifungal activity of alcohol and aqueous extract of stachys sp on fusarium solani

Enas H.A.AL-Khazraji
Department of Biology, College of Science, Wasit University, Iraq

Mohammed J. Hanawi
Department of Biology, College of Science, Wasit University, Iraq

***Abstract*---**The genus *Stachys* L. (Lamiaceae) includes about 300 species as annual or perennial herbs or small shrubs, spread in temperate regions of Mediterranean, Asia, America and southern Africa. *Stachys* sp has antioxidant activity and destroys free radicals. Antifungal effects of methanolic extracts of four different types of *Stachys* on several bacteria and yeasts were recorded. This study was conducted to evaluate the effect of the alcoholic and aqueous extract of *Stachys* sp. on the growth and spore viability of *Fusarium solani* and the results revealed that the extract affect the daily growth of *Fusarium solani* and the effect was increased with the increasing the concentration. The lowest rate of daily growth was recorded in the case alcohol extract which was 0.203cm at the concentration 25% while the highest one was 0.537cm at the concentration 6.25% as compared with the control 1.00cm. The aqueous and alcohol extract of *Stachys* sp suppressed the spore production (sporogenesis) of *Fusarium* significantly as compared with the control and the effect increased with the increasing of concentration.

***Keywords*---**plant extract, antifungal activity, fungi, *Stachys* L.

Introduction

The fungus *Fusarium* is a large genus of filamentous fungi, part of a group often referred to as hyphomycetes, widely distributed in soil and associated with plants. Most species are harmless saprobes, and are relatively abundant members of the soil microbial community. Some species produce mycotoxins in cereal crops that can affect human and animal health if they enter the food chain .The genus *Fusarium* constitutes a large monophyletic group of several hundred species which includes agriculturally important plant pathogens, endophytes and

saprophytes capable of metabolizing diverse substrates, and emerging pathogens of clinical importance. Molecular phylogenetic analysis of this genus has revealed that it comprises at least 20 clades or 'species complexes' and that it originated in the middle Cretaceous period approximately 91.3 million years ago. (1)

The pathogen is a species complex that can persist in soil for several years and is distributed by wind, equipment, and water. The fungus causes different types of destructive diseases such as wilt, slow decline, and fruit rot on host crops.(2,3) *F. solani* also often causes rots on the crown an scaffold roots. Rot of the fibrous roots is associated with a reduction in the size of the canopy and with wilting, defoliation, dieback, and sloughing of root cortex and water-soaking of root tissues (4). The genus *Stachys* L. (Lamiaceae) includes about 300 species as annual or perennial herbs or small shrubs, spread in temperate regions of Mediterranean, Asia, America and southern Africa. Several species of this genus are extensively used in various traditional medicines. They are consumed as herbal preparations for the treatment of stress, skin inflammations, gastrointestinal disorders, asthma and genital tumors. Previous studies have investigated the chemical constituents and the biological activities of these species(5).

Study was conducted by (6) and revealed that *Stachys lavandulifolia* has antioxidant activity and destroys free radicals . Stachys species have also been studied in biosystematic and chemotaxonomic studies (7,8) *Stachys* species also have several folkloric uses, e.g. the leaf of *S. officinalis* L. Trev. is used as decided to study the constituents of the essential oil from leaf and stem of *Stachys byzanti* a carminative and to relieve headaches(9) . Different study had been revealed that the extract of *Stachys glutinosa* and other medicinal plant have good antimicrobial activity against pathogenic bacteria and fungi (10,11). The aim of this research was to determine the antifungal activities of the *Stachys* extract on *Fusarium solani*.

Material and Methods

Preparation of *Stachys* extracts

Aqueous extract

The extract was prepared by taking 100 of the dry powder of *Stachys* and mixing it with 40 ml of distilled water in a 1000 ml beaker and leaving it for 24 hours at laboratory temperature, after which the mixture was filtered using a kit. Layers of medical gauze for the purpose of getting rid of plankton and then centrifugation using a centrifuge at a speed of 3000 rpm for a period of 10 minutes, then filtering the extract using a 0.2 Millipore Filter to obtain a clear, sterile solution until use (12).

Alcohol extract

This extract was prepared according to the method of (13) by taking 100 gm from the dry material of the plant and placing it in a glass beaker with a capacity of 1000 ml, adding 500 ml of ethyl alcohol with a concentration of 70% and leaving it for 24 hours at room temperature, then the mixture was precipitated using a

centrifuge machine 300 cycles / minute for 15 minutes and filtered using filter paper to evaporate the solution using an air drier device at a temperature of 37 ° C until a thick solution is obtained and this solution is kept in the refrigerator at 4 ° C Until use the concentration of the base solution was calculated by taking a ml of the thick solution and its dry weight was calculated, where it was found that one ml contains 2 g of the working substance, then this amount was saturated with 80 ml of distilled water and a series of The concentrations required for the study are 25mg, 12.5 and 6.25 mg (14).

Effect of *Stachys* extract on growth of *Fusarium solani*

This test was conducted by using *Poison food technique* to evaluate the toxic effect of the extract of the *Stachys* extract on the daily growth rate of the fungus and its vitality. Where the medium of PDA was banned in glass flasks capacity of 500 ml (250 ml culture medium / beaker) sterilized by an autoclave at a temperature of 121 m and pressure 1 kg / cm 2 for a quarter of an hour. Then it was treated with aqueous extract and alcoholic extract, both individually, at concentrations of 25, 12.5 and 6.25, and then poured into petri dishes with a diameter of 9 cm and left to solidify the medium. Then the center of each dish was inoculated with a disk with a diameter of 1.5 cm from fungi cultures grown on a one-week-old medium , The dishes were incubated at (25±2) C, then the daily growth rate of the fungus was calculated in three replicates for each concentration according to the following equation:

$$\text{The daily growth rate of the fungus} = \frac{D2-D1}{2(T2-T1)}$$

Where D Is the diameter of the colony and T is time
The viability of the fungus was investigated by selecting three discs with a diameter of 1.5 cm from the fungal cultures treated with the extract and three untreated discs for comparison, at a distance of 2 cm from the plate. Thus, 1 ml of the sporophyte was transferred to an empty Petri dish, over which the medium was poured and incubated at 25 °C, in order to find out the number of colonies formed by the fungus(15).

Statistical analysis

The data were analyzed according to the Augmented factorial design (Factorial plus added control) in Completely Randomized design CRD (16,17,18). The means were compared using the least significant difference (LSD) test at a probability level of 0.05. The data were analyzed using Genstat v.12.1 software.

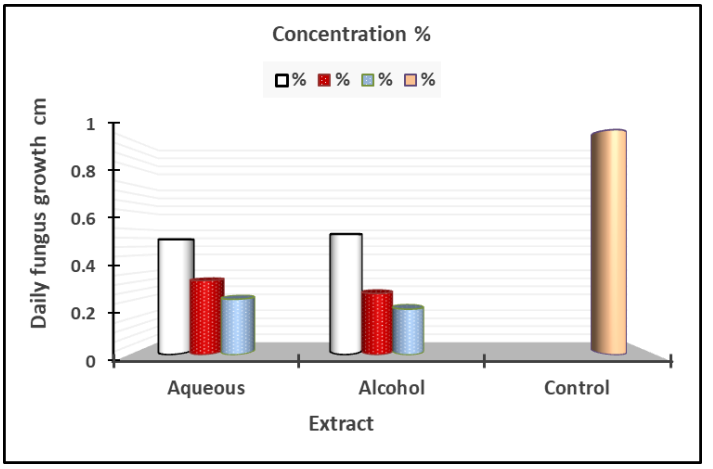
Results

The result of this study that presented in table (1) and figure (1) had been revealed that the effect of the extract against daily growth of *Fusarium solani* was dependent on the concentration of extract and the effect was increased with the increasing of the concentration. The lowest rate of daily growth was recorded in the case alcohol extract which was 0.203cm while the highest one was 0.537cm at the concentration 6.25% as compared with the control 1.00cm. The result also

showed that the alcohol extract was more effective than the water extract on the daily growth of *Fusarium solani* but there is significant differences

Table (1): Effect of *Stachys* extract on daily growth (cm) of *Fusarium solani*

Extract	Concentration (%)			Mean extract
	6.25	12.5	25	
Aqueous	0.513cm	0.330cm	0.247cm	0.338cm
Alcohol	0.537cm	0.273cm	0.203cm	0.363cm
Control	1.00cm			
LSD	0.068			0.039
Mean Conc.	0.525cm	0.301cm	0.225cm	
LSD	0.048			



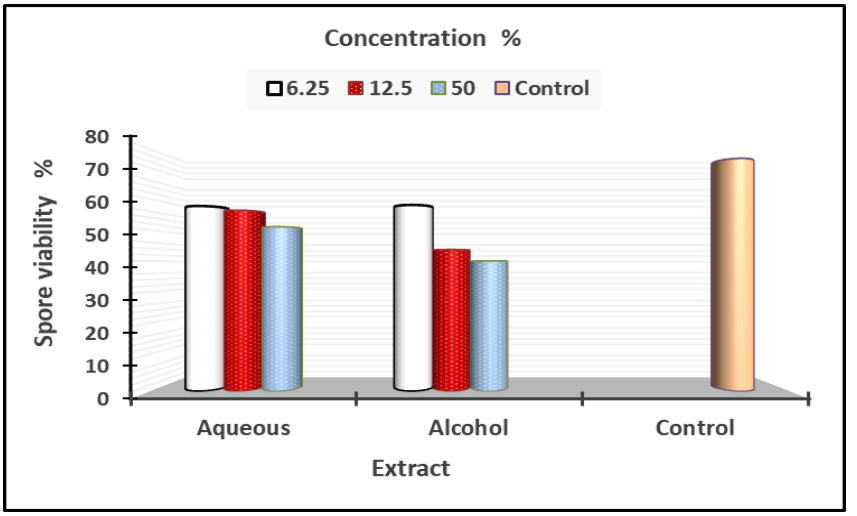
Figure(1): Effect of *stachys* extract on daily growth of *Fusarium solani*

The results of this study that presented in table (2) and figure (2) also indicated that the aqueous and alcohol extract of *Stachys* sp significantly suppressed spore production (sporogenesis) of *Fusarium* as compared with the control and the effect increased with the increasing of concentration . The percentage of spore production were 60 , 45.67, 42% at the concentrations 6.25 , 12.5, 25% respectively in the case of alcohol extract while in the case of aqueous extract were 59.67, 58.33, 53% at the same concentrations respectively. The percentage of spore production was 75% in the control treatment.

The results also revealed that the alcohol extract have more effect significantly than the aqueous extract and the mean percentage of spore production was 49.22 % in the case of alcohol extract as compared with the aqueous one which was 57%. The spore production was decreased with increasing of extract concentration and the mean percentages of spore production were 59.83, 52 , 47.50 at the concentrations 6.25 , 12.5, 25% respectively.

Table (2) Effect of *Stachys* extract on spore viability of *Fusarium solani*

Extract	Concentration (%)			Mean extract
	6.25	12.5	25	
Aqueous	59.67	58.33	53.00	57.00
Alcohol	60.00	45.67	42.00	49.22
Control	75.00			
LSD	3.03			1.75
Mean Conc.	59.83	52.00	47.50	
LSD	2.15			



Figure(2): effect of stachys extract on spore production by fusarium solani

Discussion

The result of our study revealed that both type of extract affect the growth of *Fusarium* and this may be due to the presence of antifungal compound in the extract . Various non-volatile chemical constituents have been reported from different species of the genus Stachys, categorizing into important chemical groups including fatty acids, alkaloids (e.g., stachydrine, turiaine), triterpenes, phytosterols, phytoecdysteroids, diterpenes, iridoids, flavonoids, phenylpropanoid glucosides, acetophenones, phenylethanoid glycosides, lignans, phenolic acids, megastigmanes and polysaccharides . Different study mentioned that large number of phytochemicals were mainly discovered from the aerial parts, leaves and a few were found in stems and roots. (19,20,21)

The results were agree with the results of (22) who found that the leaf ethanolic extract of *Stachys pseudopinardii* have antifungal activity against *Candida albicans* ATCC 10239, *Debaryomyces hansenii* DSM 70238, *Kluyveromyces fragilis* ATCC 8608 and *Rhodotorula rubra* DSM 70403, by disc diffusion and microdilution methods. (23) also reported that the extract of *Stachys lavandulifolia* exhibited good inhibitory effect on *Candida tropicalis* (MIC 0.094 mg/mL).

Another study conducted by (24) had been revealed that the essential oils of *Stachys pubescens* Ten. exhibited an appropriate antifungal activity against *Fusarium oxysporum* and *Botrytis cinerea* and the results of mentioned works are in parallel with results of the our study . The results of this study also revealed that the *Stachys* leaf extract affect the production of spores by the fungus *Fusarium* and this may be due to the presence of phytochemical compounds that serve as antifungal compounds. Various non-volatile chemical constituents have been reported from different species of genus *Stachys*, categorizing into important chemical groups including fatty acids, alkaloids (e.g., stachydrine, turiaine), triterpenes, phytosterols, phytoecdysteroids, diterpenes, iridoids, flavonoids, phenylpropanoid glucosides, acetophenones, phenylethanoid glycosides, lignans, phenolic acids, megastigmanes and polysaccharide, these anti-fungal compounds can inhibit the formation of fungal spores of Fusarium (25,26).

Our results indicated that both aqueous and alcohol extract of *Stachys* affect the sporogenesis by *Fusarium* and this results are parallel with result of (27) who found that the aqueous extract of Rumex sp. significantly suppressed the spores' formation of *Fusarium oxysporum* at the lowest tested concentration (10 mg/ml), while sporogenesis completely inhibited at higher concentrations. Similar study had been revealed that the spore production of *Fusarium solani* was affected by plant extracts and failed to produce spores when treated with aqueous extracts of Rumex sp. and Ziziphus sp. at the concentration of 20% which elevated an evident that plant extracts could provide a potential source of antifungal compounds (28). Another study was conducted by (29) and revealed that the extract of *Cymbopogon citratus* significantly reduced the spore production of *Fusarium oxysporum* f.sp. *ciceris* Padwick with all the tested concentrations and the rate of inhibition ranged between 30.39 and 100%. As well, the spore production was completely inhibited by the Artemisia herba-alba extract at the concentrations of 2.5 and 5 µl/ml.

Conclusions

In conclusion , the stachys extract have an activity as fungicidal on the growth and spore production of *Fusarium solani* and the alcoholic extract more effective than aqueous extract may be due to the presence of phytochemical compounds that serve as antifungal compounds .

References

1. Abu-Taleb , A. M. ; El-Deeb , K. and El-Otibi , F. O. (2011) . Assessment of antifungal activity of *Rumex vesicarius* L. and *Ziziphus spina-christi* (L) wild extract against two phytopathogenic fungi. Afr. J. Microbiol. Res. 5, 1001–1011.
2. Akueshi, C. O. ; Kadiri, C. O. ; Akueshi , E. U. ; Agina, S. E. and Ngurukwem, B. (2002) . Antimicrobial potentials of Hyptis sauvedens Poit (Lamiaccae), Nigeria. J Bot 15: 37-41.
3. Alotibi, F. O. ; Ashour , E. H. and Al-Basher , G. (2020). Evaluation of the antifungal activity of *Rumex vesicarius* L. and *Ziziphus spina-christi* (L) Desf. aqueous extracts and assessment of the morphological changes induced to

certain myco- phytopathogens. Saudi Journal of Biological Sciences. 27 : 2818–2828.
4. Al-Sadi, A. M. ; Al-Ghaithi, A. G. ; Al-Fahdi, N. and Al-Yahyai, R. (2014) . Characterization and pathogenicity of fungal pathogens associated with root diseases of citrus in Oman. Int J Agric Biol 16:371–376.
5. Anessiny, G. and C. Perez (1993) . Screening of plants used agreen line. Folk Medicine for Antimicrobial Activity. J. Eth., 39 : 119-128.
6. Chiej , R. (1984) . The Macdonald Encyclopedia of Medicinal Plants. Macdonald &Co. Ltd., London, 212,297.
7. Dulger , G. and Aki , C. (2009) . Antimicrobial activity of the leaves of endemic *Stachys pseudopinardii* in Turkey. Trop J Pharm Res. 8 (4):371-5.
8. Ekaterina-Michaela, T. ; Christina, B. and Helen, S. (2020). Genus Stachys: A Review of Traditional Uses, Phytochemistry and Bioactivity . journal of Medicines .7, 63.
9. EL-Ansari, M. A. ; Barron, D. ; Abadall, M. F. ; Saleh, N. A. M. and Le Qurer, J. L. E. (1991). Flavonoid constituents of *Stachys aegyptiaca* . Phytochemistry . 30(4) :1169-1173.
10. Fazio , C. ; Paternostro, M. P. ; Passannantl , S. and Piozzi , F. (1994) . Further neo-clerodane diterpenoidsfrom *Stachys rosa*. Phytochemistry. 37(2):501-503.
11. Giorgio, G. ; Chessa, M. ; Manconi P. ; Zanetti, S. ; Deriu A. B. and Tirillini B. (2006). Chemical Composition and Antimicrobial Activities of Essential Oil of *Stachys glutinosa* L. from Sardinia. Natural Product Communications Vol. 1 (12): 1133 – 1136.
12. Işcana, G. ; Demircia , B. ; Demircia, B.D.F. ; Gögera, F.; Kırımera, N. ; Yavuz, B. K. and Kemal, H.C.B.(2012) . Antimicrobial and Antioxidant Activities of *Stachys lavandulifolia* sub sp. *lavandulifolia* Essential Oil and its Infusion. Natural Product Communications Vol. 7 (9): 1242 – 1244.
13. Jalil, A. T., Kadhum, W. R., Khan, M. U. F., Karevskiy, A., Hanan, Z. K., Suksatan, W., ... & Abdullah, M. M. (2021). Cancer stages and demographical study of HPV16 in gene L2 isolated from cervical cancer in Dhi-Qar province, Iraq. *Applied Nanoscience*, 1-7. https://doi.org/10.1007/s13204-021-01947-9
14. Jalil, A. T., Shanshool , M. T. ., Dilfy, S. H. ., Saleh, M. M., & Suleiman, A. A. . (2022). HEMATOLOGICAL AND SEROLOGICAL PARAMETERS FOR DETECTION OF COVID-19. *Journal of Microbiology, Biotechnology and Food Sciences*, e4229. https://doi.org/10.15414/jmbfs.4229
15. Lomer , C. J. and Lomer, C. H. (1997) . Insect pathology manual .241pp.
16. Marini, R.P. (2003). Approaches to analyzing experiments with factorial arrangement of treatments plus other treatments . Hort Science 38(1): 117-120.
17. Marofi, F., Rahman, H. S., Al-Obaidi, Z. M. J., Jalil, A. T., Abdelbasset, W. K., Suksatan, W., ... & Jarahian, M. (2021). Novel CAR T therapy is a ray of hope in the treatment of seriously ill AML patients. *Stem Cell Research & Therapy*, *12*(1), 1-23. https://doi.org/10.1186/s13287-021-02420-8
18. Meremeti, A. ; Karioti, A. ; Skaltsa, H. ; Heilmann, J. and Sticher, O. (2004) . Secondary metabolites from *Stachys ionica*. Biochem. Syst. Ecol. 2004, 32, 139–151.
19. Moghadasi, S., Elveny, M., Rahman, H. S., Suksatan, W., Jalil, A. T., Abdelbasset, W. K., ... & Jarahian, M. (2021). A paradigm shift in cell-free

approach: the emerging role of MSCs-derived exosomes in regenerative medicine. *Journal of Translational Medicine*, *19*(1), 1-21. https://doi.org/10.1186/s12967-021-02980-6

20. Mohammadi, A. ; Nazari, H . ; Imani, S. and Amrollahi, H. (2014). Antifungal activities and chemical composition of some medicinal plants. J. Mycol. Med. 24(2): 1-8.
21. Moutassem , D. ; Belabid , L. ; Bellik, Y. ; Ziouche, S. and Baali, F. (2019). Efficacy of essential oils of various aromatic plants in the biocontrol of *Fusarium* wilt and inducing systemic resistance in chickpea seedlings. Plant Protect. Sci., 55: 202–217.
22. Mukherjee, P. K. ; Leidich, S. D. ; Isham, N. ; Leitner, I. ; Ryder, N. S. and Ghannoum, M. A. (2003). Clinical *Trichophyton rubrum* strain exhibiting primary resistance to terbinafine. Antimicrob Agents Chemother;47(1):82-6.
23. Nemec, S . and Baker, R. (1992) . Observations on *Fusarium solani* naphthazarin toxins, their action, and potential role in citrus plant disease. Proceedings of the 7th Congress of the International Society for Citriculture 2, 832– 7.
24. O'Donnell , K. ; Rooney, A. P. ; Proctor, R. H. ; Brown , D. W. and McCormick, S. P. (2013) . Phylogenetic analyses of RPB1 and RPB2 support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. Fungal Genet. Biol. 52:20–31
25. Piepho, H.P ; Williams, E. and Flecks, M. . (2006) . A note on the analysis of designed experiments with complex treatment structure. HortScience 41(2): 446-452.
26. Piozzi, F. and Bruno, M. (2011) . Diterpenoids from roots and aerial parts of the genus *Stachys*. Rec. Nat. Prod. , 5: 1–11.
27. Ramazanov, N. S. ; Bobayev, I. D.; Yusupova, U. Y. ; Aliyeva, N. K. ; Egamova, F. R . ; Yuldasheva, N. Y. and Syrov, V.N. (2016) . Phytoecdysteroids-containing extract from *Stachys hissarica* plant and its wound-healing activity. Nat. Prod. Res. 31, 593–597.
28. Salah, M. Dawood, ; Ahmed, K. Abdulrazzaq ; Kareem, T. ; Shnawa, and Mohammed J. Hanawi .(2021) . In Vitro Antifungal Activity of *Pleurotus eryngii* against *Trichophyton rubrum*. Indian Journal of Forensic Medicine & Toxicology, October-December , Vol. 15, No. 4.
29. Saleh, M. M., Jalil, A. T., Abdulkereem, R. A., & Suleiman, A. A .(2020). Evaluation of Immunoglobulins, CD4/CD8 T Lymphocyte Ratio and Interleukin-6 in COVID-19 Patients. *TURKISH JOURNAL of IMMUNOLOGY*, *8*(3), 129-134. https://doi.org/10.25002/tji.2020.1347
30. Sarjito, Elveny, M., Jalil, A., Davarpanah, A., Alfakeer, M., Awadh Bahajjaj, A. & Ouladsmane, M. (2021). CFD-based simulation to reduce greenhouse gas emissions from industrial plants. *International Journal of Chemical Reactor Engineering*, 20210063. https://doi.org/10.1515/ijcre-2021-0063
31. Stover, E. d. ; Mike , F. ; Richard , R. ; Xiaoe , Y. and Terence , R. (2001) . Fruit Weight, Cropload, and Return Bloom of 'Empire' Apple Following Thinning with 6-Benzyladenine and NAA at Several Phenological Stages. . Hort Science 36(6):1077–1081.
32. Taghikhani, M. ; Nasri, H. ; Asgari, A. ;Afrough, H. ; Namjoo, A. R. and Ansari-Samani, R. (2012). The renal toxicity of hydroalcoholic extract of *Stachys lavandulifolia* Vahl in Wistar rats. Life Sci J. 9(4): 3025-31.

33. Tombo, G. B. E., Zambrano, E. C., Barreiro, J. L., & Posligua, J. M. (2020). Potential energy of plant biomass: banana, coconut, cacao and corn. *International Journal of Physical Sciences and Engineering*, *4*(1), 11–20. https://doi.org/10.29332/ijpse.v4n1.415
34. Tundis , R . ; Peruzzi , L. and Menichini , F. (2014) . Phytochemical and biological studies of *Stachys* species in relation to chemotaxonomy: A review. Phytochemistry . 102 , 7–39 .
35. Tundis , R . ; Peruzzi , L. and Menichini , F. (2014) . Phytochemical and biological studies of *Stachys* species in relation to chemotaxonomy: A review. Phytochemistry . 102 , 7–39 .
36. Turki Jalil, A., Hussain Dilfy, S., Oudah Meza, S., Aravindhan, S., M Kadhim, M., & M Aljeboree, A. (2021). CuO/ZrO2 nanocomposites: facile synthesis, characterization and photocatalytic degradation of tetracycline antibiotic. *Journal of Nanostructures*. https://doi.org/10.22052/JNS.2021.02.014
37. Vitalina, N., Valentyna, V., Olexandr, S., Iryna, R., & Roman, O. (2022). The influence of digital creative technologies on the development of education and medicine. *International Journal of Health Sciences*, *6*(2), 699–708. https://doi.org/10.53730/ijhs.v6n2.7669
38. Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, *29*(3), 550–561. https://doi.org/10.1002/cae.22202
39. Widjaja, G., Jalil, A. T., Rahman, H. S., Abdelbasset, W. K., Bokov, D. O., Suksatan, W., ... & Ahmadi, M. (2021). Humoral Immune mechanisms involved in protective and pathological immunity during COVID-19. *Human Immunology*. https://doi.org/10.1016/j.humimm.2021.06.011
40. Yaseen, T. and D'Onghia, A. M. (2012). *Fusarium* Spp. Associated To Citrus Dry Root Rot: An Emerging Issue For Mediterranean Citriculture. Acta Horticulturae, (940), 647–655.