Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(4): 3242-3249. **OPEN ACCESS**

In vitro bioassay of the adversarial action of a few microbes confined from compost extricates

Masoud Radmanesh

Department of Agriculture and plant breeding, Islamic Azad University, Sari Branch, Mazandaran, **Iran**

***Correspondence:** masoud_rad0000000@yahoo.com **Received 12-11-2021, Revised: 19-12-2021, Accepted: 20-12-2021 e-Published: 22-12-2021**

Nine types of compost extracts were tested in this study, and they mainly were looked at for their ability to fight the cause of crown gall, Agrobacterium tumefasiens (strain C58). The second step was to separate bacteria from the most effective extracts. For this, twenty-seven bacteria were isolated and studied in the lab to find effective antagonists for this disease. The double layer method compares the bacteria's activity to Agrobacterium rhizogenes K84, the standard antagonist. A study of the antagonistic activity in vitro found that, when the pathogen was incubated with the antagonists at 27°C, they had much power in the lab and slowed down the growth of strain C58 of Agrobacterium tumefasiens with a range of degrees. Four groups of antagonistic isolates were found through statistical analysis. The first group comprises the isolates that did not cause an inhibition zone. The second group comprises isolates that did not show any significant activity compared to the control. The third group comprises the less efficient isolates, and the last group is made up of the most efficient isolates. C5B2 had the largest inhibition zone diameter (30.25 mm), compared to 19.37 mm in control. Pathogen growth was reduced by 38% as compared to control. The compost extract isolates evaluated in this work may be regarded as prospective sources of novel bioactive compounds and attractive candidates for developing new biocontrol agents for the treatment of crown gall disease.

Keywords: compost extracts, antagonists, *Agrobacterium rhizogenes* K84, *Agrobacterium tumefaciens*, inhibition, control.

INTRODUCTION

Crown gall is a bacterial disease produced by Agrobacterium tumefaciens that is economically significant. It affects about 100 distinct families of dicotyledonous plants worldwide (Al-Dahmani, Abbasi et al. 2003, Gholamin and Khayatnezhad 2020, Bi, Dan et al. 2021, Cheng, Hong et al. 2021, Khayatnezhad and Nasehi 2021), including stone fruits, grapevines, roses, certain ornamental species, forest trees, and tomato (Camozzi 2003, Cazorla, Romero et al. 2007, Jia, Khayatnezhad et al. 2020, Si, Gao et al. 2020, Peng, Khayatnezhad et al. 2021). Infected plants, particularly those with tumors on the main roots and collar, are not marketable and must be discarded (Haas and Keel 2003, Gupta, Khosla et al. 2010, Tao, Cui et al. 2021, Wang, Shang et al. 2021).

Crow gall has been commonly seen on bitter almonds in Tunisia (Hoitink and Fahy 1986, Khayatnezhad and Gholamin 2021). The disease has spread swiftly as fruit tree production has expanded and new nurseries without acceptable phytosanitary requirements have been established. Tunisian farmers are currently having difficulty cultivating healthy stone fruit plants in nurseries, owing to a lack of knowledge about this illness and challenges in recognizing defective stocks early on. Despite preventative efforts, crown gall continues to inflict significant damage in nurseries and in the field (HOITINK, Inbar et al. 1991, Khayatnezhad and Gholamin 2020, Xu,

Ouyang et al. 2021).
Biological control has been used effectively for over three decades using the nonpathogenic strain Agrobacterium rhizogenes K84 (Idris, Labuschagne et al. 2007, Gholamin and Khayatnezhad 2020, Li, Mu et al. 2021, Ren and Khayatnezhad 2021). It was the first instance of biocontrol against pathogenic Agrobacterium strains in various hosts and nations throughout the globe (Gholamin and Khayatnezhad 2021, Zhang, Khayatnezhad et al. 2021). Nonetheless, there are certain drawbacks to using K84 (Jenana, Haouala et al. 2009). This strain's failure is primarily due to the transfer of genes governing agrocin 84 synthesis and, as a result, the establishment of resistance to K84.
As a result, it is vital to look for different antagonistic microorganisms with high activity to manage the crown gall. Composts and compost extracts have been shown to prevent plant illnesses caused by pathogens such as fungus (Kerkeni, Horrigue-Raouani et al. 2007, Gholamin and Khayatnezhad 2020, Guo, She et al. 2021). Composts and extracts inhibited growth by a mix of chemical and biological processes. These goods include biological ingredients, particularly microflora (fungi and bacterial species) (Naidu, Meon et al. 2010, Ma, Khayatnezhad et al. 2021, Sun and Khayatnezhad 2021).
In reality, multiple studies have demonstrated the efficiency of microorganisms isolated from composts and compost extracts against various diseases (Gholamin and Khayatnezhad 2020). Bacillus, Pseudomonas, and Serratia bacteria, as well as filamentous fungus of the genus Trichoderma, were the most isolated and were recognized as biocontrol agents (Nelson and Boehm 2002, Hou, Li et al. 2021, Yin, Khayatnezhad et al. 2021). The antifungal activity of microorganisms isolated from compost and compost extracts has received a lot of attention, but there haven't been many research on their antibacterial activity. The purpose of this study was to determine the antibacterial activity of several compost extracts in vitro and then the effect of several bacteria isolated from these compost extracts on Agrobacterium tumefaciens strain C58, as well as to compare their activity to that of the reference antagonist Agrobacterium rhizogenes K84.

MATERIALS AND METHODS

Compost extracts

We employed nine extracts from various composts (C1, C2, C3, C4, C5, C6, C7, C8, and C9) that were predominantly consisted of various animal manures (poultry, sheep, cow, and horse manures) (Table 1). Original composts were created using an aerobic technique at the composting unit of the lab of Mazandaran university, Iran. The extraction process is suspending composts in tap water (1:5, v/v) in a 20-liter plastic container and shaking the mixture everyday for about 10 minutes over a five-day period (Wahyuni, Mudjiharjati et al. 2010). Following incubation, the mixtures were filtered through 250 m of cheesecloth and the resulting extracts were kept at 4°C. They were removed 30 minutes before to usage.

Table 1: Composition of composts used for extracts preparation

Composts	
C1	50%CM+25%SM+25%PM
C2	60%CM+30%SM+10%ground straw
C3	50%CM+25%SM+25%HM
C4	50%CM+20%SM+20%PM+10%ground straw
C5	25%CM+25%SM+25%PM+25%HM
C6	30%CM+30%SM+30%PM+10% ground straw
C7	40%CM+40%SM+20% vegetable wastes
C8	25%CM+25%SM+25%PM+ 15%HM+10% ground straw
C9	25%CM+25%SM+25%PM+25%HM

C1-C9: compost1-compost9; CM: cattle manure; SM: sheep manure; PM: poultry manure; HM: horse manure

Isolation and growth conditions of tested bacteria

Compost extract bacteria: A serial dilution of compost extract up to 10-3 was performed, and 10 L aliquots of this dilution were placed over Glutamate-Mannitol (MG) medium based on yeast agar (Oxoid) (0.5 g. L-1), Glutamic acid (2 g. L-1), Mannitol (5 g. L-1), KH2PO4.3H2O (0.5 g. L-1), NaCl (0.2 (20 g.L-1). Bacterial colonies produced in seeded media were individually resuspended in MG medium after 48 hours of incubation at 27°C. The method was continued until a pure bacterial culture was obtained.
Twenty-seven bacterial isolates with diverse morphological characteristics were selected and designed by the following individuals: C1A (isolate A from compost C1), C1B1 (isolate B1 from compost C1), C1B2 (isolate B2 from compost C1), C1C (isolate C1 from compost C1), C4A, C4B,

C4C, C4D, C5A, C5D, C5B, C5C, C They were kept alive on King's B medium at 27 degrees Celsius. Pure cultures were cultured at -20°C in eppendorf tubes (0.5 mL) containing 50 percent glycerol and 50 percent sterile LB media to ensure long-term preservation of the organism. The API system was used to identify them, which allowed them to be identified (Khayatnezhad and Gholamin 2021).

Agrobacterium rhizogenes K84 and Agrobacterium tumefaciens strain C58:
The olive institute of Sfax contributed the A. tumefaciens strain C58 and the reference antagonistic strain K84 (Tunisia). They were kept alive at 25°C on MG medium.

***In vitro* Bioassay**
Effect of compost extracts on *Agrobacterium tumefaciens*
The antibacterial activity of each extract was evaluated using the double-layer technique against Agrobacterium tumefaciens strain C58 (Ma, Ji et al. 2021). The test consists of individually suspending 10 l of each extract on MG medium on Petri plates and incubating them for 24 and 48 hours at 27°C. The A. tumefaciens strain was streaked over the hardened surface of the MG medium on the same day as the extract incubation. Following incubation, the plates were washed with 70% alcohol and subjected to chloroform vapor for 30 minutes in a laminar flow cabinet. Following evaporation, one ml of A. tumefaciens solution (108 CFU. ml-1) was mixed with three ml of LBA (0.6 percent agar) at 45°C and promptly overlaid to plates containing the extracts. Plates were re-incubated at 27°C for another 24-48 hours to look for the formation of inhibitory haloes around the extracts' spots.

Effect of compost extract isolated bacteria on Agrobactrium tumefaciens
The in vitro sensitivity of A. tumefaciens strain C58 to the isolated antagonist bacteria was tested using the same procedure used for compost extracts (Khayatnezhad and Gholamin 2020). In this example, a bacterial suspension of antagonists (108 CFU mL-1) was produced in sterile distilled water, and 20 L aliquots were spot-inoculated on LBA medium (10 g tryptone, 5 g yeast extract, 5 g NaCl, and 20 g agar in 1 liter of distilled water) and cultured at 25°C for 2 days. After two days, the antagonistic bacteria were exposed to chloroform vapor, and a one-milliliter suspension of A. tumefaciens (108 CFU. ml-1) was combined with three milliliters of LBA (0.6 percent agar) and overlaid on plates containing the bacterial isolates at 45°C.
The hostile microorganisms K84 were used to represent control plates. Plates were subsequently incubated at 27°C for 24 hours, with the emergence of inhibition haloes around the antagonist's spots monitored. The experiment used a totally randomized design with three replicates and was replicated twice.

Experimental design and statistical analysis
The experiment was conducted twice using a totally randomized design with three replicates. The antagonistic bacterium K84 was used as a control plate.
The SPSS program was used to do an analysis of variance (ANOVA) on the data (version 13). The Duncan's test was used to examine the significance of mean differences, and answers were deemed significant at the 5% level (P=0.05).

RESULTS

Effect of compost extracts on the development of *A. tumefaciens*
Figure 1 shows that all of the tested extracts were efficient in suppressing Agrobacterium tumefasiens strain C58 development after 24 hours of incubation at 27°C. There was a substantial variation between the 9 extracts. The C7 extract had the greatest antibacterial activity, with an inhibition zone of 24.57 mm. In comparison to the control, the C1 proved ineffective (19.5 mm). C4 and C5 extracts, on the other hand, were the least effective in suppressing pathogen growth, by 6.09 and 18.96 mm, respectively.
The antibacterial activity of the extracts was boosted when the incubation time was increased to 48 hours. The 24 hour inhibition zones were more essential than the inhibition zones. The C6 extract has an inhibition diameter of 33 mm. However, there was no discernible difference between the extracts and the control K84.

Antibacterial activity of compost extracts strains
Table 2 shows the diameters of the inhibition zones caused by antagonists against the strain C58. The results showed that after 24 hours of incubation at 27°C with the pathogen, compost extract bacteria inhibited A. tumefaciens growth to varying degrees. In reality, when compared to the control K84, statistical studies identified four

groups of hostile microorganisms. C1A, C1B2, C4A, C5B, C8A, C8B, C8C, C8D, and C9A belong to the first category, which includes isolates with no antibacterial activity. The isolates in the second group had the same activity as the control group (C4D, C1B1, C4C and C4B). The third group comprises isolates that had a lower effect than the control (C3A1, C3B, C5C, and C5E), whereas the fourth group contains isolates that had the highest activity than the control (C3A1, C3B, C5C, and C5E) (C5B2, C5D, C5A, C7A1, C3D, C3C, C3A2, C2B, C2A and C1C). With the C5B2 strain, the largest inhibition zone diameter is seen (Figure 2).

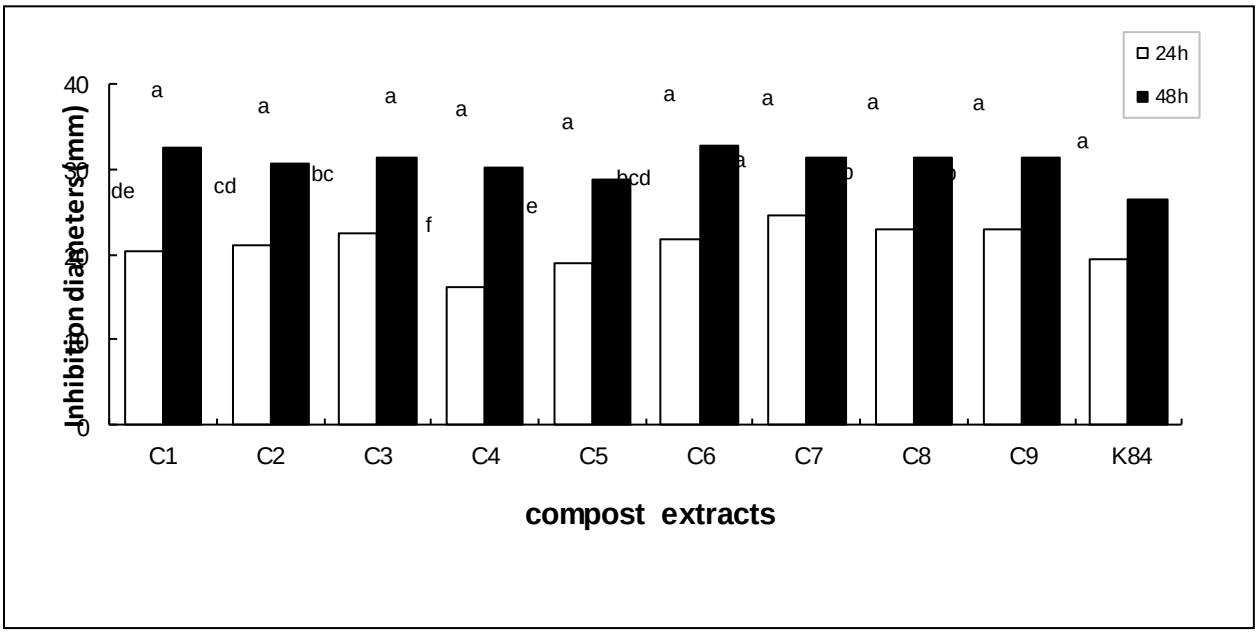

Figure 1: Distances across of restraint zone measured by the diverse compost extricates after hatching at 27°C for individually 24 and 48 hours. Each bar speaks to the cruel of three duplicates. Medications influenced by diverse letters were altogether distinctive agreeing to the Duncan test at the level of 5 %.

Table 2: Restraint zone distance across (mm) actuated by compost extricate microbes against Agrobacterium tumefaciens strain C58 in twofold layer culture, after 24 hours of brooding. Information taken after by diverse letters signify noteworthy distinction (p< 0.05), concurring to Duncan's test.

Antagonists	*A. tumefaciens* strain C58	Antagonists	*A. tumefaciens* strain C58
Control (K84)	19.38b	C1C	24.5a
C4D	16.5 b	C2A	25.75a
C1B1	17 b	C2B	25.0a
C4C	17.75b	C3A2	23.5a
C4B	19.75b	C3C	23.75a
C4A	0 d	C3D	25.5a
C5B	0 d	C7A1	22.0a
C8A	0 d	C5A	27.75a
C8B	0 d	C5B2	30.25a
C8C	0 d	C5D	28.5a
C1A	0 d	C5C	8.75c
C8D	0 d	C5E	6.25c
C1B2	0 d	C3A1	11.0c
C9A	0 d	C3B	10.0c

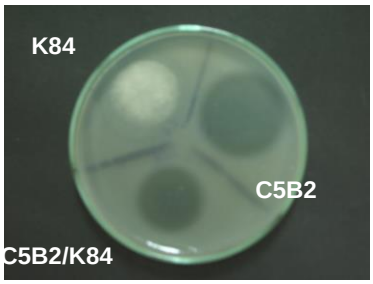

Figure 2: inhibition zones induced by compost extract bacteria (C5B2) compared to the control K84

DISCUSSION

Agrobacterium tumefaciens-caused crown gall is a dangerous disease that causes significant damage in nurseries and in the field (Karasakal, Khayatnezhad et al. 2020, Zheng, Zhao et al. 2021). By isolating non-pathogenic strains of Agrobacterium radiobacter from disease areas and examining their capacity to compete with pathogenic bacteria in mixed inoculations, Pulawska (2010) found and built the first biocontrol system. He discovered that various non-pathogenic strains lowered infection, but that one strain in particular, A. radiobacter strain K84, totally prevented illness. Some strains of A. tumefaciens, however, were resistant to the bacteriocin (agrocin 84) generated in vitro by strain 84. This has prompted researchers to explore for new diseases that are resistant to strain 84. Compost extracts have been shown to inhibit a variety of plant diseases, including bacteria (Sun, Lin et al. 2021).

Animal manure compost extracts were shown to suppress the development of Agrobacterium tumefaciens in this investigation (strain C58). After 24 hours, all of the extracts tested were helpful in suppressing pathogen development. However, the C7 extract (40 percent CM+40 percent SM+20 percent vegetable wastes) had the greatest antibacterial activity, with an inhibition zone of 24.57 mm. The type of the microbes and chemicals generated from those organic products might account for the differences seen in compost extracts.

All of the compost extracts were equivalent to the reference strain K84 in lowering disease development after 48 hours of incubation, although their activity was higher than after 24 hours. The illness reduction rate was 32 percent (C1 and C6). This increase in extract activity is most likely due to the expression of all of the microorganisms in the extracts, which leads to the creation of more antibiotics and inhibitory compounds in the culture medium. Compost extract microorganisms and antibacterial compounds may need additional time to reach their full biological potential.

According to Wahyuni et al., (2010) the efficiency of compost extracts might vary greatly. This might be attributed to changes in extract preparation processes, the source, content, quality, and maturity of the compost, storage period, and potentially other variables. On addition, Penyalver et al. (2001) found that when Agrobacterium rhizogenes K84 is cultivated in an iron-deficient medium, it produces greater quantities of iron-binding chemicals (hydroxamate iron chelator) compared to A. tumefaciens (this is the case of the medium used in this study). This compound might be similar to ALS84, a previously identified antibacterial substance (Karasakal, Khayatnezhad et al. 2020, Huang, Wang et al. 2021, Sun, Lin et al. 2021, Zhu, Liu et al. 2021, Zhu, Saadati et al. 2021). Based on these findings, we may ascribe some of the suppressive action of compost extracts to their iron concentration. Kerkeni et al., (2007) found that all compost extracts employed in these in vitro assays contain more than 0.3 ppm of iron in a prior study.

CONCLUSION

The antibacterial activity of numerous compost extracts and associated bacterial strains was one of the study's key goals. This research also attempted to discover novel options that may be effective in the biological management of Agrobacterium tumefaciens-caused crown gall disease. When compared to the reference strain K84, it is clear that compost extracts and their bacterial isolates have a considerable impact on A. tumefaciens strain C58. Compost extracts and antagonistic bacteria with a wide spectrum of antagonistic activities were evaluated in this research and might be regarded as alternate sources for managing crown gall disease. Future

research should focus on identifying the bioactive compounds of the antagonistic bacteria discovered here, determining their modes of action as biocontrol agents, and testing their capacity to reduce crown gall disease *in vivo*.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

This research has been supported by Iran National Science Foundation (INSF) under grant number 25978542.

AUTHOR CONTRIBUTIONS

Masoud Radmanesh conducted, planned, Analyzed the data, wrote manuscript and interpreted the results and involved in manuscript preparation. All authors read and approved the final version.

REFERENCES

Al-Dahmani, J. H., P. A. Abbasi, S. A. Miller and H. A. Hoitink (2003). "Suppression of bacterial spot of tomato with foliar sprays of compost extracts under greenhouse and field conditions." Plant disease 87(8): 913-919.

Bi, D., C. Dan, M. Khayatnezhad, Z. sayyah HASHJIN, Z. Li and Y. Ma (2021). "Molecular identification and genetic diversity in hypericum l.: a high value medicinal plant using rapd markers markers." Genetika 53(1): 393-405.

Camozzi, R. (2003). "Tea has helpful microbes by the billions." The register-guard, Eugene, Oregon, USA.

Cazorla, F., D. Romero, A. Pérez-García, B. Lugtenberg, A. d. Vicente and G. Bloemberg (2007). "Isolation and characterization of antagonistic Bacillus subtilis strains from the avocado rhizoplane displaying biocontrol activity." Journal of applied microbiology 103(5): 1950-1959.

Cheng, X., X. Hong, M. Khayatnezhad and F. Ullah (2021). "Genetic diversity and comparative study of genomic DNA extraction protocols in Tamarix L. species." Caryologia 74(2): 131-139.

Gholamin, R. and M. Khayatnezhad (2020). "Assessment of the Correlation between Chlorophyll Content and Drought Resistance in Corn Cultivars (Zea Mays)." Helix 10(05): 93-97.

Gholamin, R. and M. Khayatnezhad (2020). "The effect of dry season stretch on Chlorophyll Content and RWC of Wheat Genotypes (Triticum Durum L.)." Bioscience Biotechnology Research Communications 13(4): 1833-1829.

Gholamin, R. and M. Khayatnezhad (2020). "Study of Bread Wheat Genotype Physiological and Biochemical Responses to Drought Stress." Helix 10(05): 87-92.

Gholamin, R. and M. Khayatnezhad (2020). "The Study of Path Analysis for Durum Wheat (Triticum durum Desf.) Yield Components." Bioscience Biotechnology Research Communications 13(4): 2139-2144.

Gholamin, R. and M. Khayatnezhad (2021). "Impacts of PEG-6000-induced Drought Stress on Chlorophyll Content, Relative Water Content (RWC), and RNA Content of Peanut (Arachis hypogaea L.) Roots and Leaves." Bioscience Research 18(1): 393-402.

Guo, L.-N., C. She, D.-B. Kong, S.-L. Yan, Y.-P. Xu, M. Khayatnezhad and F. Gholinia (2021). "Prediction of the effects of climate change on hydroelectric generation, electricity demand, and emissions of greenhouse gases under climatic scenarios and optimized ANN model." Energy Reports 7: 5431-5445.

Gupta, A., K. Khosla, S. Bhardwaj, A. Thakur, S. Devi, R. Jarial, C. Sharma, K. Singh, D. Srivastava and R. Lal (2010). "Biological control of crown gall on peach and cherry rootstock colt by native Agrobacterium radiobacter isolates." The Open Horticulture Journal 3(1).

Haas, D. and C. Keel (2003). "Regulation of antibiotic production in root-colonizing Pseudomonas spp. and relevance for biological control of plant disease." Annual review of phytopathology 41(1): 117-153.

Hoitink, H. A. and P. C. Fahy (1986). "Basis for

the control of soilborne plant pathogens with composts." Annual review of phytopathology 24(1): 93-114.

HOITINK, H. J., Y. Inbar and M. Boehm (1991). "Status of compost-amended potting mixes naturally suppressive to soilborne diseases of floricultural crops." Plant disease 75(9): 869-873.

Hou, R., S. Li, M. Wu, G. Ren, W. Gao, M. Khayatnezhad and F. gholinia (2021). "Assessing of impact climate parameters on the gap between hydropower supply and electricity demand by RCPs scenarios and optimized ANN by the improved Pathfinder (IPF) algorithm." Energy 237: 121621.

Huang, D., J. Wang and M. Khayatnezhad (2021). "Estimation of Actual Evapotranspiration Using Soil Moisture Balance and Remote Sensing." Iranian Journal of Science and Technology, Transactions of Civil Engineering: 1-8.

Idris, H. A., N. Labuschagne and L. Korsten (2007). "Screening rhizobacteria for biological control of Fusarium root and crown rot of sorghum in Ethiopia." Biological control 40(1): 97-106.

Jenana, R. K. B., R. Haouala, M. A. Triki, J.-J. Godon, K. Hibar, M. B. Khedher and B. Henchi (2009). "Composts, compost extracts and bacterial suppressive action on Pythium aphanidermatum in tomato." Pakistan Journal of Botany 41(1): 315-327.

Jia, Y., M. Khayatnezhad and S. Mehri (2020). "Population differentiation and gene flow in Rrodium cicutarium: A potential medicinal plant." Genetika 52(3): 1127-1144.

Karasakal, A., M. Khayatnezhad and R. Gholamin (2020). "The Durum Wheat Gene Sequence Response Assessment of Triticum durum for Dehydration Situations Utilizing Different Indicators of Water Deficiency." Bioscience Biotechnology Research Communications 13(4): 2050-2057.

Karasakal, A., M. Khayatnezhad and R. Gholamin (2020). "The Effect of Saline, Drought, and Presowing Salt Stress on Nitrate Reductase Activity in Varieties of Eleusine coracana (Gaertn)." Bioscience Biotechnology Research Communications 13(4): 2087-2091.

Kerkeni, A., N. Horrigue-Raouani and M. B. Khedher (2007). "Effet suppressif de cinq extraits de compost vis à vis du nematode a galles Meloidogyne incognita Suppressive effect of five compost extracts against the root-knot nematode Meloidogyne incognita." Nematologia mediterranea.

Khayatnezhad, M. and R. Gholamin (2020). "A Modern Equation for Determining the Dry-spell Resistance of Crops to Identify Suitable Seeds for the Breeding Program Using Modified Stress Tolerance Index (MSTI)." Bioscience Biotechnology Research Communications 13(4): 2114-2117.

Khayatnezhad, M. and R. Gholamin (2020). "Study of Durum Wheat Genotypes' Response to Drought Stress Conditions." Helix 10(05): 98-103.

Khayatnezhad, M. and R. Gholamin (2021). "The Effect of Drought Stress on the Superoxide Dismutase and Chlorophyll Content in Durum Wheat Genotypes." Advancements in Life Sciences 8(2): 119-123.

Khayatnezhad, M. and R. Gholamin (2021). "Impacts of Drought Stress on Corn Cultivars (Zea mays L.) At the Germination Stage." Bioscience Research 18(1): 409-414.

Khayatnezhad, M. and F. Nasehi (2021). "Industrial Pesticides and a Methods Assessment for the Reduction of Associated Risks: A Review." Advancements in Life Sciences 8(2): 202-210.

Li, A., X. Mu, X. Zhao, J. Xu, M. Khayatnezhad and R. Lalehzari (2021). "Developing the non-dimensional framework for water distribution formulation to evaluate sprinkler irrigation." Irrigation and Drainage.

Ma, A., J. Ji and M. Khayatnezhad (2021). "Risk-constrained non-probabilistic scheduling of coordinated power-to-gas conversion facility and natural gas storage in power and gas based energy systems." Sustainable Energy, Grids and Networks: 100478.

Ma, S., M. Khayatnezhad and A. A. Minaeifar (2021). "Genetic diversity and relationships among Hypericum L. species by ISSR Markers: A high value medicinal plant from Northern of Iran." Caryologia 74(1): 97-107.

Naidu, Y., S. Meon, J. Kadir and Y. Siddiqui (2010). "Microbial starter for the enhancement of biological activity of compost tea." Int. J. Agric. Biol 12(1): 51-56.

Nelson, E. B. and M. J. Boehm (2002). "Microbial mechanics of compost-induced disease suppression." Biocycle 43(7): 45-47.

Peng, X., M. Khayatnezhad and L. Ghezeljehmeidan (2021). "Rapd profiling in detecting genetic variation in stellaria l. (caryophyllaceae)." Genetika-Belgrade 53(1): 349-362.

Penyalver, R., P. Oger, M. a. M. López and S. K. Farrand (2001). "Iron-binding compounds from Agrobacterium spp.: biological control strain Agrobacterium rhizogenes K84 produces a hydroxamate siderophore." Applied and Environmental Microbiology 67(2): 654-664.

Pulawska, J. (2010). "Crown gall of stone fruits and nuts, economic significance and diversity of its causal agents: tumorigenic Agrobacterium spp." Journal of Plant Pathology: S87-S98.

Ren, J. and M. Khayatnezhad (2021). "Evaluating the stormwater management model to improve urban water allocation system in drought conditions." Water Supply.

Si, X., L. Gao, Y. Song, M. Khayatnezhad and A. A. Minaeifar (2020). "Understanding population differentiation using geographical, morphological and genetic characterization in Erodium cicunium." Indian J. Genet 80(4): 459-467.

Sun, Q., D. Lin, M. Khayatnezhad and M. Taghavi (2021). "Investigation of phosphoric acid fuel cell, linear Fresnel solar reflector and Organic Rankine Cycle polygeneration energy system in different climatic conditions." Process Safety and Environmental Protection 147: 993-1008.

Sun, X. and M. Khayatnezhad (2021). "Fuzzy-probabilistic modeling the flood characteristics using bivariate frequency analysis and α-cut decomposition." Water Supply.

Tao, Z., Z. Cui, J. Yu and M. Khayatnezhad (2021). "Finite Difference Modelings of Groundwater Flow for Constructing Artificial Recharge Structures." Iranian Journal of Science and Technology, Transactions of Civil Engineering.

Wahyuni, W. S., A. Mudjiharjati and N. Sulistyaningsih (2010). "Compost extracts of vegetable wastes as biopesticide to control Cucumber Mosaic Virus." HAYATI Journal of Biosciences 17(2): 95-100.

Wang, C., Y. Shang and M. Khayatnezhad (2021). "Fuzzy Stress-based Modeling for Probabilistic Irrigation Planning Using Copula-NSPSO." Water Resources Management.

Xu, Y.-P., P. Ouyang, S.-M. Xing, L.-Y. Qi, M. khayatnezhad and H. Jafari (2021). "Optimal structure design of a PV/FC HRES using amended Water Strider Algorithm." Energy Reports 7: 2057-2067.

Yin, J., M. Khayatnezhad and A. SHAKOOR (2021). "EVALUATION OF GENETIC DIVERSITY IN Geranium (Geraniaceae) USING RAPD MARKER." Genetika 53(1): 363-378.

Zhang, H., M. Khayatnezhad and A. Davarpanah (2021). "Experimental investigation on the application of carbon dioxide adsorption for a shale reservoir." Energy Science & Engineering n/a(n/a).

Zheng, R., S. Zhao, M. Khayyatnezhad and S. Afzal Shah (2021). "Comparative study and genetic diversity in Salvia (Lamiaceae) using RAPD Molecular Markers." Caryologia 74(2): 45-56.

Zhu, K., L. Liu, S. Li, B. Li, M. Khayatnezhad and A. Shakoor (2021). "Morphological method and molecular marker determine genetic diversity and population structure in Allochrusa." Caryologia 74(2): 121-130.

Zhu, P., H. Saadati and M. Khayatnezhad (2021). "Application of probability decision system and particle swarm optimization for improving soil moisture content." Water Supply.