

Synergistic effects of Indian medicinal plants and transition metal complexes on *Alternaria solani*

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ABSTRACT

Metal complexes were synthesized and characterized using infra-red spectra, magnetic susceptibility and X-ray diffraction analysis. The combined application of these metal complexes with extracts from medicinal plants (*Tamarindus indica*, *Emblica officinalis*, *Vitex negundo* and *Mangifera indica* L.) with antimicrobial properties, showed synergistic effect. Comparative analysis revealed that the antimycotic activity of medicinal plant extracts combined with metal complexes was higher than individual metal complexes or plant extracts alone. Notably, cadmium ferrocyanide + *E. officinalis* extract and nickel ferrocyanide + *T. indica* extract exhibited highest antimycotic activities, respectively, when integrated with metal hexacyanoferrate (II) compounds against *Alternaria solani*. These findings showed the enhanced antimicrobial efficacy through synergistic interactions between metal complexes and medicinal plant extracts, highlighting their potential for sustainable disease management.

Keywords: *Alternaria solani*, *Emblica officinalis*, *Mangifera indica* L., medicinal plants, synergistic effects *Tamarindus indica*, transition metal ferrocyanides, *Vitex negundo*

Nomenclature: *Mn (II)* - $Mn_2[Fe(CN)_6] \cdot 3H_2O$, *Co (II)* - $Co_2[Fe(CN)_6] \cdot 2H_2O$, *Ni (II)* - $Ni_2[Fe(CN)_6] \cdot 5H_2O$, *Cu (II)* - $Cu_2[Fe(CN)_6] \cdot 7H_2O$, *Zn (II)* - $Zn_2[Fe(CN)_6] \cdot 3H_2O$, *Cd (II)* - $Cd_2[Fe(CN)_6]$

INTRODUCTION

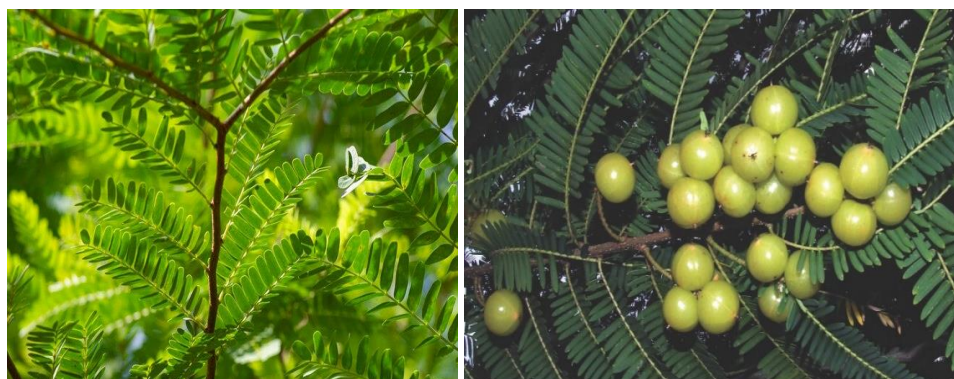
Herbal plants rich in alkaloids, terpenoids and flavonoids (13,15) contribute significantly to therapeutic plant development due to their antibacterial, antifungal, and other bioactive properties (10,17). These compounds, with antioxidant and free radical-scavenging attributes, play a crucial role in mitigating the risk of various diseases (8). The application of phytochemicals on crops protects them from fungi, bacteria, weeds and insects (10,12,23).

The fungal pathogen *Alternaria solani* primarily causes early blight disease in potatoes (11), reduces its yield and quality. Recognizing the significance of medicinal plants in herbal remedies for plants and animals, their use and safe handling (5,9). We may discover novel molecules from medicinal plants to treat bacterial, fungal, and viral infections.

Tamarindus indica (*Caesalpinaceae* family) is used as medical plant for centuries (Figure 1). Its fruits are antimicrobial agent (9). Its seeds are medicinal and anti-cerecarial activities (6). *Emblica officinalis* (Amla family *Euphorbiaceae*) have various medicinal properties: anti-viral, anti-bacterial, anti-cancer, anti-allergy and anti-mutagenic called Amla (Figure 2). It contains various phytochemicals (tannins, alkaloids and phenolic compounds). It is also used to treat antiviral disease symptomatic as cold fever infection, flu

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and respiratory infections in lungs due to its antibiotic and antifungal activity against various bacteria and fungi (4,18). *Vitex negundo* (*Verbenaceae* family (Figure 3) is used in Ayurveda and Indian medical systems. It is analgesic, anti-inflammatory and anti-itching substance. *Mangifera indica* possesses antidiabetic, anti-oxidant, anti-viral, cardiogenic, hypotensive, anti-inflammatory properties. Various effects (antibacterial, antifungal, anthelmintic, antiparasitic, anti tumor, anti-HIV, anti-bone resorption, antispasmodic, antipyretic, antidiarrhoeal, antiallergic, immunomodulation, hypolipidemic properties) have also been studied.



Figures 1-2. Leaves of *Tamarindus indica* and *Emblica officinalis*



Figures 3-4. Leaves of *Vitex negundo* and *Mangifera indica*:

Medicinal values of *T. indica*, *E. officinalis*, *V. negundo* and *M. indica* shows potential of medicinal plants. The medicinal plant contains secondary metabolites (flavonoids, terpenoids, and alkaloids) and provides natural remedies. These substances fight the bacterial and fungal infections in addition to enhancing the resistance of the plant.

In comparison to synthetic pharmaceuticals, medicinal plants are thought to be a safer, more affordable, and more environmentally friendly source of medication (14). Transitional metal complexes have been documented in literature to have a variety of antifungal and antibacterial properties (21,22). There are several complexes of transition metals found to be the essential for various crops as nutrients (4,7). The primary objective of this research is to investigate the antimicrobial potential of transitional metal complexes in combination with secondary metabolites from medicinal plants against bacterial and fungal pathogens. Specifically, the study aims to evaluate the synergistic effects of transitional metal complexes and medicinal plant secondary metabolites on antimicrobial activity (3,19).

METHODS AND MATERIAL

2.1 Collection of fungal cultures

Alternaria solani fungal strain were collected from the Central Potato Research Institute (CPRI), Modipuram, Meerut, India, during January 2024. It was raised on potato dextrose agar (PDA) medium for two days at 25 to 30 °C for 2 days.

2.2 Collection of medicinal plants

Samples of leaves of *T. indica*, *E. officinalis*, *V. negundo*, and *M. indica* were collected in August and September 2023, from Herbal Garden, Patanjali Yogpeeth Haridwar (Altitude 300 m, Latitude 29°54'23" N-29°54'12" N, longitude 78°00'04" E-77°59'54"E. Average annual rainfall 2051 mm and temp 13.3-27.8 °C). The plant species were dried for 15-20 days in shade at 30-35 °C. Each plant species' shade-dried leaves were pulverized in a mixer and kept. The dry leaf powder was extracted 4-5 times with 5 mL methanol solvent/g of plant material. Each extract was concentrated at 40 °C.

2.3 Synthesis of transition metal ferrocyanides

The Kourim method was used to prepare the metal complexes (17), ferrocyanides of manganese, cobalt, nickel, copper, zinc, and cadmium were made, making a total of six transition metal compounds. The potassium ferrocyanide solution (167 mL, 0.1 M) was continuously stirred at room temperature and the necessary metal salt (500 mL, 0.1 M) was added. Afterward, the reaction mixture was heated on water bath for 3-4 h at 80 °C, then kept at room temperature for 24 h. Precipitate was thoroughly purified with distilled water and with vacuum pump additional contaminants were removed. The precipitate was dried in a 60 °C in an oven. The product was dried, ground, and filtered through a 100 mesh sizes. Metal complex coloured granules were stable in air. These were identified using magnetic susceptibility testing (Sherwood Scientific), IR spectra (KBR disc on Bio-red FTIR spectrophotometer) and X-ray diffraction analysis (SCXRD).

Antifungal activity of plant extracts with metal ferrocyanide complexes

The paper disc method was used to test the antifungal activity. Medicinal plant extracts and metal complexes were selected for this study (2). In a sterilised petri dish containing potato dextrose agar (PDA) media, a selected medicinal plant extract (5 mg) and transition metal ferrocyanide (5 mg) were added, and then, using an aspirator, fungal spores were sprayed in entire bottom of petri dish. The same process was done again with new extract and ferrocyanide metal.

Growth Inhibition (%) was calculated as Under (22):

$$\text{Growth Inhibition (\%)} = (Cg - Tg) / Cg \times 100$$

Where, Cg : Growth (mm) in control and Tg: Growth (mm) in therapy.

Each experiment was done in triplicate, and the mean value was used to interpret the findings. Each experiment was done in triplicate, and the mean value was taken into account when interpreting the findings.

Synergistic effects of medicinal plant extracts with metal complexes

A sterile petri dish was filled with 5 mg plant extract and 5 mg metal ferrocyanide. With aspirator, fungus spores were scattered in dish's bottom. This process was applied, when blending different plant extracts and metal complexes.

Statistical analysis

The statistical programme SPSS Statistics IBM Base 22.0 was used to conduct the statistical analysis. In the study, descriptive statistics, paired t-tests, and Shapiro-Wilk tests for normality were used. Understanding and description of the data was done through descriptive statistics and Shapiro-wilk test for confirming the assumption for the parametric methods. Paired t-test was applied to look the significance mean difference between the metal ferrocyanides and medicinal plants.

RESULTS AND DISCUSSION

Antimycotic activity of plant extracts

The analysis aimed to find the fungicidal and antimycotic activity of *T. indica*, *E. officinalis*, *V. negundo* and *M. indica* shows that *E. officinalis* and *T. indica* extract. The order of antimycotic property in these test plants extracts followed the order: *E. officinalis* > *V. gundo* > *M. indica* > *T. indica*.

Synergistic effect of medicinal plant extracts with metal complexes

The combination of Cd (II) metal complex with plant extract of *E. officinalis*, *V. negundo*, *M. indica* and *T. indica* had maximum fungicidal potential (16-20 mm growth inhibition) against *A. solani* fungus (Figure 5). Co (II) and Cu (II) had also significant antifungal potential (10-17 mm), Mn (II) recorded greater fungal growth inhibition from 8-15 mm with all plant extracts. On the other hand, the combinations of Ni (II) and Zn (II) with all plant extracts recorded lowest antifungal activity (5-11 mm).

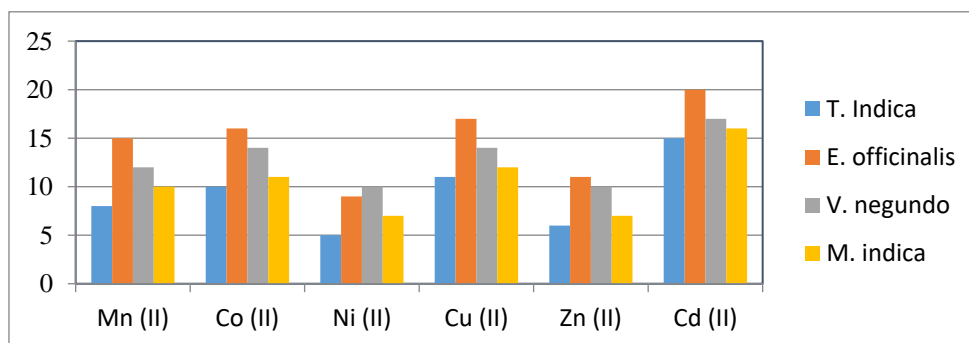


Figure 5. Synergistic effects of medicinal plant extracts with metal complexes

The combination of transition metal complexes with medicinal plant extracts followed the order of Antimycotic activity (Figure 5):

- (i). Mn (II): *E. officinalis*>*V. negundo*>*M. indica*>*T. indica*
- (ii). Co (II): *E. officinalis*>*V. negundo*>*M. indica*>*T. indica*
- (iii). Ni (II): *E. officinalis*>*V. negundo*>*M. indica*>*T. indica*
- (iv). Cu (II): *E. officinalis*>*V. negundo*>*M. indica*>*T. indica*
- (v). Zn (II): *E. officinalis*>*V. negundo*>*M. indica*>*T. indica*
- (vi). Cd (II): *E. officinalis*>*V. negundo*>*M. indica*>*T. indica*

The highest and lowest antimycotic properties were found in Cd (II) and Ni (II), respectively, in metal ferrocyanides. The secondary metabolites' antimycotic activity was enhanced when they interact with metal ferrocyanides. *T. indica* extract with nickel ferrocyanide complex and *E. officinalis* extract with cadmium ferrocyanide complex had the highest and lowest fungicidal capabilities, respectively.

The combination of Cd (II) and *E. officinalis* extract may be used as powerful antimycotic agent for potato early blight. The transition ferrocyanides demonstrated that Cu (II), Co (II), and Cd (II) might be used to explore the likely cause of synergistic action of bioactive chemical compounds present in medicinal plants.

The synergistic effect of methanol extracts of medicinal herbs mixed with metal ferrocyanides against *A. solani* may be due to the porous surface structure and adsorption capacity of metal ferrocyanides. Because these metal ferrocyanides may adsorb active components of medicinal plants on their active sites, the concentration of active components increased at the surface of metal ferrocyanides at active sites for adsorption as well as inside the pores present at their surface. The results indicated that these transition metal complexes may be used as fungicides alone or in combination with other plant botanical fungicides, leading to the creation of ecologically friendly fungicides that can help protect both our environment and our health from soil contamination.

In all metal complexes, the peaks for H₂O molecules and OH- group in the molecular formula were seen at 3423-3830 cm⁻¹, whereas the HOH characteristic bending was shown at 1516-1645 cm⁻¹. The range of peaks for all complexes was 2063-2104 cm⁻¹, which was typical of the cyanide group stretching vibration, and strong peaks at 592-599 cm⁻¹, which was typical of Fe-C stretching vibrations. ν_{Metal-Nitrogen} was also observed at 445-497 cm⁻¹. All synthetic metal complexes have diamagnetic magnetic property because there are paired

electrons present. If there is magnetic moment, the surrounding cations may also contributed to its observation.

These metal hexacyanoferrates' estimated and measured magnetic moment values were in good agreement. In accordance with the d7 structure of Co^{2+} and the reported value of 4.6 BM for the magnetic moment of cobalt ferrocyanide, the presence of three unpaired electrons was indicated in observations for cobalt ferrocyanide.

Five, three, two and one unpaired electrons were present in Mn (II), Co (II), Ni (II), and Cu (II) hexacyanoferrates revealed by μ_{obs} values are 6.21, 4.36, 2.99, 2.45 BM. μ_{obs} respectively, while the value of zinc and cadmium ferrocyanides complexes had 0.81, 0.90 BM. μ_{obs} zero magnetic moments. Method to test the degree of ionization in non-aqueous solution was provided by conductivity measurement of complexes. Molar conductance values of Mn (II), Co (II), Ni (II), Cu (II), Zn (II) and Cd (II), 24.2 (μS), 9.81 (μS), 6.61 (μS), 6.72 (μS), 2.70 (μS), 7.44 (μS) respectively for all the complexes in DMSO indicated that they were non-electrolytes.

Antifungal activity of metal complexes

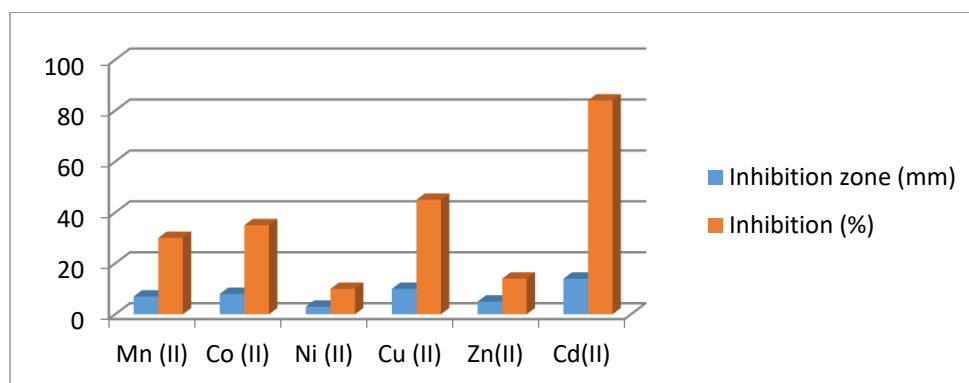


Figure 6. Antifungal activity of metal complexes against *Alternaria solani*

We studied the fungicidal activity of Mn-II, Co (II), Ni (II), Cu (II), Zn (II) and Cd (II) and it was found that Cd (II) and Ni (II) possess maximum and minimum fungicidal property, respectively (Figure 6). The Cd (II) caused maximum growth inhibition 84 % of *A. solani*. Cu (II) caused significant growth inhibition 45 %, while Mn-II and Co (II) had antimycotic potential of 30-35 %. On the other hand, Ni (II) and Zn (II) caused least less growth inhibition (only up to 10 %). The antifungal activity of metal ferrocyanides followed the Order: Cd (II) > Cu (II) > Co (II) > Mn (II) > Zn (II) > Ni (II) (Figure 5).

X-ray diffraction examinations were used to characterize all synthesized metal complexes. For Mn-II, Co (II), Ni (II), Cu (II), Zn (II) and Cd (II) respectively, the d-values of the observed peaks of metal complexes are in good agreement with the published data (PC-PDF file nos. 46-0910, 23-0188, 14-0291, 01-0244, 24-0164, and 01-0433).

D-spacing (\AA) observed for Mn (II) was recorded as 5.0348, 3.56478, 3.0117, 2.3005, 2.2525, and 2.0846 which showed good agreement with the published data PC-PDF file.

The relative intensity was 100 % in 2θ (24.9795) angle in between the d spacing 3.56478 and d-spacing [Å] reported in PCPDF database 3.5570. The d-spacing (Å) observed for Co(II) have been found to be 5.0072, 3.5527, 2.5045, 2.0702 and 2.0165 showed very closely resemblance to the data present in PC-PDF file. The relative intensity was 100 % in case of 2θ 25.0657 angle in between the d spacing 3.5527 and d-spacing [Å] reported in PCPDF database 3.5600. The d-spacing (Å) values of Ni (II) was recorded as 5.0069, 3.5604, 2.5145, 2.2463, 2.0542, 1.7789, 1.676, 1.5891 have showed good agreement with the published data PC-PDF file. The relative intensity was 100 % in case of 2θ (25.0107) angle in between the d spacing 3.5604 and d-spacing [Å] reported in PCPDF database 3.5700. D-spacing (Å) observed for Cu (II) 3.5375, 3.005, 2.491, 2.2372, 2.0424 showed good agreements with the published data PC-PDF file. The relative intensity was 79.69 % in 2θ (25.1752) angle in between the d spacing 3.5375 and d-spacing [Å] reported in PCPDF database 3.5000. D-spacing (Å) observed for Zn (II) 5.4157, 4.5014, 4.0783, 3.1139, 3.002, 2.5141, 2.3810, 2.3186, 2.202, 1.9008 showed good agreement with the published data PC-PDF file. The relative intensity was 100 % in 2θ (16.3677) angle in between the d spacing 5.4157 and d-spacing [Å] reported in PCPDF database 5.4000. D-spacing (Å) observed for cadmium ferrocyanide 4.5415, 3.1096, 2.8178, 2.5412, 2.2726, 2.1153, 1.8541, 1.7976, 1.6073, 1.5575, 1.509, 1.4094 showed good agreement with the published data PC-PDF file respectively. The relative intensity was 39.74 in 2θ (35.3196) angle in between the d spacing 2.5412 and d-spacing [Å] reported in PCPDF database 2.4900 respectively.

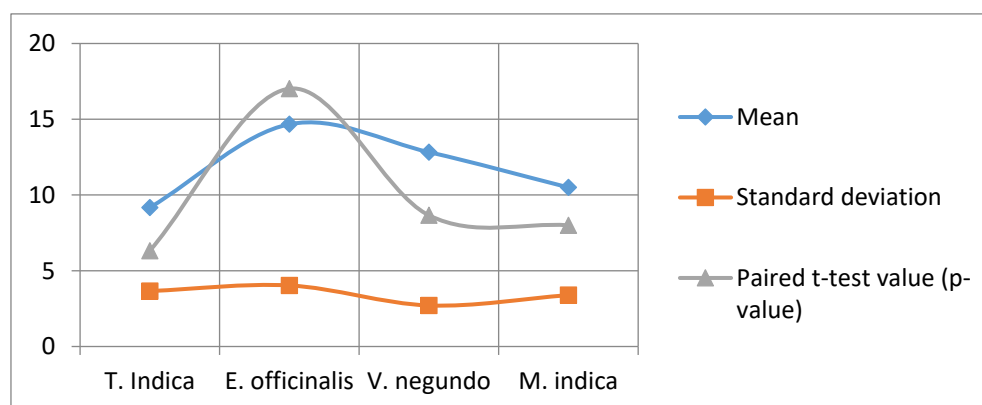


Figure 7. Statistical analysis of Synergistic effect of medicinal plant extracts with metal complexes

Results of paired t-test performed indicated that the significance difference in fungal growth of *A. solani* to *T. Indica* (paired t-test 6.32 (< 0.001), *E. officinalis* (paired t-test 17.02 (< 0.000), *V. negundo* (paired t-test 8.660 (< 0.000), *M. indica* [paired t-test 8.000 (< 0.000)]. There was highly significant p-value in all 4-test medicinal plants extract for Antimycotic activity in combination of transition metal complexes (Figure 7).

CONCLUSIONS

The continuous and recurrent use of synthetic chemicals against plant pathogens deteriorates the environment. Hence there is need to find a sustainable and eco-friendly solution to overcome the above problem, hence, more emphasis has been laid on eco-friendly herbal alternative to fungicides for crop protection.

The various infections and diseases caused by pest and pathogens have been treated by medicinal plants secondary metabolites. The various bioactive chemical compounds in medicinal plants possess specific antimicrobial properties. Hence, these results may be very fruitful to develop and synthesize the plant-based herbal, ecofriendly antimicrobial agents to control various diseases and infections caused by microbes.

There is possibility of using metal complexes as fungicides on their own or in combination with different secondary metabolites of medicinal plants to develop environmentally friendly, safer fungicides. Hopefully, future research will focus on developing unique ways to control pathogenic bacteria and fungus using the synergistic effects of synthetic chemicals and plant botanicals.

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct.

REFERENCES

1. Abad, M.J., Ansuategui M. and Bermejo P. (2007). Active antifungal substances from natural sources. *ARKIVOC* **7**: 116-145.
2. Arora, C. and Bharti, D. (2012). Antifungal potential of transition metal hexacyanoferrates against fungal diseases of mushroom. *Open Journal of Synthesis Theory and Applications* **1**: 23-30.
3. Arora, C. and Kaushik, R.D. (2003). Fungicidal activity of plant extracts from Uttaranchal hills against soybean fungal pathogens, *Allelopathy Journal* **11**: 217-228.
4. Arora, C. Bharti, D. Arora, D. and Tamrakar, V. (2018). A review on phytoconstituents and medicinal properties of *Emblca officinalis*, *Annals of Horticulture Journal* **11(1)**: 1-12.

5. Chau, T.P., Devanesan, S., Ayub, R. and Perumal, K. (2024). Identification and characterization of major bioactive compounds from *Andrographis paniculata* (Burm. f.) extracts showed multi-biomedical applications. *Environmental Research* **242**: 117763.
6. Chaudhary, S., Sharma, S., Lal, M., Sagar, S., Sharma, S. and Kumar, M. (2023). Morphological and pathological variability of *Rhizoctonia solani* isolates from dhaincha-potato crop rotation and their mycelial compatibility relationship. *Indian Phytopathology* **76**: 355-369.
7. Chugh, C.A. and Bharti, D. (2014). Chemical characterization of antifungal constituents of *Emblca officinalis*. *Allelopathy Journal* **34** (1): 155-178.
8. Ghosh S., Sarkar B., Ranadheera C.S. and Thongmee S. (2024) Synergistic effects of plant extracts and nanoparticles for therapy. *Nanotechnology and In Silico Tools Natural Remedies and Drug Discovery* pp. 75-87 Elsevier, Netherlands.
9. Doughari, J.H. (2006). Antimicrobial activity of *Tamarindus indica* L. *Tropical Journal of Pharmaceutical Research* **5**: 597-603.
10. Ferdosi, M.F., Ahmed, H, Khan, I.H. and Javaid, A. (2022). Fungicidal potential of flower extract of *Cassia fistula* against *Macrophomina phaseolina* and *Sclerotium rolfsii*. *Journal of Animal and Plant Sciences* **32**(4): 1028-1034.
11. Fostel, J.M. and Lartey, P. (2000). An Emerging novel antifungal agent. *Drug Discovery Today* **5**: 25-32.
12. Javaid, A., Khan, I.H. (2020). Potential use of *Coronopus didymus* (L.) SM. in parthenium management. *Pakistan Journal of Weed Science Research* **26**(1): 37-45.
13. Javaid, A., Ferdosi, M.F., Khan, I.H., Shoaib, A., Saeed, H.M. and Hassan M.A.U. (2021). Biochemical analysis of flowers of *Vinca major*, a medicinal weed plant of hilly areas of Pakistan. *Pakistan Journal of Weed Science Research* **27**(4): 537-546.
14. Kalra, P., Sharma, S. and Kumar, S. (2011). Antiulcer effects of methanolic extract of *Tamarindus indica* seeds in different experimental models, *Journal of Pharmacy and Bioallied Sciences*, **3** (2): 236-241.
15. Kanwal, Q., Hussain, I., Siddiqui, H.L. and Javaid, A. (2010). Antifungal activity of flavonoids isolated from mango (*Mangifera indica* L.) leaves. *Natural Product Research* **24**(20): 1907-1914.
16. Kazuhide, I. (2014). Micro-and macro-scale measurement of fungal growth under various temperature and humidity conditions. *Evergreen Journal of Novel Carbon Resource Sciences, Green Asia Strategy* **1** (1): 32-39.
17. Khan, I.H. and Javaid, A. (2020). Antifungal activity of leaf extract of *Cannabis sativa* against *Aspergillus flavipes*. *Pakistan Journal of Weed Science Research* **26**(4): 447-453.
18. Kourim, V., Rais, J. and Millon, B. (1964). Exchange properties of complex cyanides – I (Ion – exchange of caesium on ferrocyanides, *Journal of Inorganic and Nuclear Chemistry* **26**: 1111.
19. Rniak, I.G., Bartoszewski, R. and Liczewski, J.K. (2019). Comprehensive review of antimicrobial activities of plant flavonoids, *Phytochemical Reviews* **18**: 241-272.
20. Sahlan, M., Kristabel, D., Pratami, D.K., Adawiyah, R., Anondho W., Lischer, K. and Fauzi, K. (2020). Exploration of the antifungal potential of indonesian propolis from *Cyamopsis tetragonoloba* on *Candida* sp. and *Cryptococcus neoformans*. *Evergreen Joint Journal of Novel Carbon Resource Sciences & Green Asia Strategy* **07** (1): 118-125.
21. Srikamwang, C., Onsa, N.E., Sunanta, P., Sangta, J., Chanway, C.P., Thanakkasaranee, S. and Sommano, S.R. (2023). Role of microbial volatile organic compounds in promoting plant growth and disease resistance in horticultural production. *Plant Signaling & Behavior* **18**(1): 2227440.
22. Zahed, M.M., Diab, M.A., El-Sonbaty, A.Z., Nozha, S.G., Issa, H.R., El-Mogazy, M.A. and Morgan, S.M. (2024). Antibacterial, antifungal, DNA interactions, and antioxidant evaluation of Cu (II) Co (II), Ni (II), Mn (II) and UO₂ (II) mixed ligand metal complexes: Synthesis, characterization and molecular docking studies. *Materials Science and Engineering, B*. **299**: 116998.
23. Zafar, R., Ahmad, S., Javaid, A., Khan, I.H., Iqbal, M., Ferdosi, M.F.H. (2022). Insecticidal effect of ethanolic leaf extract of *Conocarpus lancifolius* Engl. against khapra beetle. *International Journal of Biology and Biotechnology* **19**(4): 511-515.