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Diversity and correlation of entomopathogenic and associated fungi with soil factors

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ABSTRACT

Ecological consideration is of key importance in finding fungi and other entomopathogens for managing insect pests. The probability of finding entomopathogenic fungi is increased by knowing the soil characteristics supporting fungal survival and diversity. Many opportunistic fungi are closely associated with EPF in soil. Diversity and occurrence of fungi were carried out from soil samples (145) and dead insects (225) collected from natural and cultivated areas of south Punjab. The relative research for the presence and abundance of EPF in samples of soils collected from cultivated to non-cultivated hilly lands show that fruit orchid can be considered as a richer in these fungal species. The EPF was mainly isolated from the collected (225) insect cadavers belonging to six insect orders out of which only 94 were positive for any category of fungus isolated. Insects from Coleoptera were reported with maximum occurrence (44.68%) for harboring any kind of the fungus followed by Lepidoptera (36.17%). *Aspergillus niger* (27.50%) was the most occurring taxa among all isolates, while *Fusarium oxysporium* was dominantly occurring specie (17.02%). It can be concluded that orchard soils that are least disturbed (tillage, weeding, etc) and supplied with ample moisture should be preferred for sampling in order to isolate the entomopathogens. Furthermore, insect cadavers from coleoptera and lepidoptera should be preferred for collection for the sake of entomopathogenic fungi.

1. Introduction

Microorganisms play a key role in the natural ecosystem, particularly in the soil environment, enhancing productivity, improving soil structure and ecosystem functioning, and the health of the plants (Acosta-Martinez et al., 2007). In soils the development of microorganisms mainly depends upon the physical (soil texture) and chemical properties (pH, E.C., C/N ratio), agrotechnical factors, fertilization and organic matter content which are the main source of nutrients and energy for microorganisms (Johansson et al., 1999). One of the best alternatives to synthetic insecticides is entomopathogenic fungus adapted to local conditions. Entomopathogenic fungus control offers a successful, cost-effective, and less labor-intensive which makes it a better alternative to chemical control.

Insect pathogenic fungi abundance, biological activity, and epizootics mainly depend on climatic conditions (Stuart et al., 2006; Karar et al., 2021). EPF such as *Beauveria bassiana*, *Metarhizium anisopilae*, and *Isaria furmosea* are well studied and have great potential against insects (Evans et al., 2018). As an ideal microbial

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control agent, these fungi have some special approaches against fruit fly including repellency, oviposition deterrence, and high mortality (Karar et al., 2021; Dimbi et al., 2009). The effectiveness and consistency of performance are strongly relying on the abiotic and biotic factors (Fernandes et al., 2010). Entomopathogenic fungi formulation contains nutrition, inert material adjuvants offer a high degree of pathogenicity against different limiting abiotic factors (Barreto et al., 2016), desiccation, and ultraviolet radiation (Fernandes et al., 2007).

The occurrence and distribution of EPF in various regions of Pakistan are poorly understood. The present study was planned to isolate native EPF isolates from the soils of cultivated, non-cultivated habitats, forest and hilly areas. The survey was conducted in different regions of Punjab on different habitats (forest, fruits, vegetables, and crop fields). In this survey insect cavaders and soil samples are collected were EPF isolates where isolated from insect bait method using *Galleria mellonella*. The present study aimed at isolating entomopathogenic fungi from local soils and insect cadavers providing a potent strain with greater adaptability and virulence.

2. Materials & methods

2.1. Insect sampling and isolation

Insect cadavers with fungal growth were collected from vegetables, orchids, and non-cultivated fields near Multan from November 2019 to March 2020. Cadavers were treated from the sodium hypochlorite 0.3% for the 60 s, rinsed it with dH_2O for 4–5 times. After that cadavers were placed on the Potato Dextrose Agar @39 g in dH_2O per liter. Inoculated petri dishes were placed in an incubator at 25 °C for 3–5 days. The fungal growth which developed around the cadavers were identified and purified through repeated culturing on PDA. The nature of entomopathogenic or opportunistic fungi was determined after macroscopic examination, and fungal spore and colony characters were further considered for identification. Any fungal growth observed was preserved for morphological identification (based on slide characters) under suitable magnification using taxonomic keys (Bartnet and Hunter, 1999).

2.2. Soil sampling

Soil samples were collected from November 2019 to March 2020. Sampling was carried out using the soil auger at the depth of 5–20 cm after the removal of small plants and soil litter from the top layer. Randomized sampling was done by taking 5 subsamples, mixing these to form a composite sample. Soil samples were shade dried and sieved to remove any debris. When the moisture was removed, the soil was ground into a fine powder and sieved again and preserved for further use.

2.3. EPF isolation

Finely powdered soil, 10 g was shifted into rearing vials (1.5 × 6 cm) and 5 larvae of *Galleria mellonella* were buried into this soil for 5 days. The soil in vials (5 replications) was incubated at 20–22 °C in dark. After 5 days, *G. mellonella* larvae shifted on the moist filter paper for fungal sporulation. Any growth on the larvae was observed with microscopic examination. Larvae were then transferred to PDA plates to permit any fungal particle to grow into a colony. The resultant culture was purified by repeated culture.

2.4. Statistical analysis

The incidence of EPF in changed habitats was associated using chi-squred test. The pathogenicity in bioassay, and death percentage in control test was very little (0.08%). so, it was excluded from analysis. The mortality percentage of the cavaders from every isolate was corrected by using Abbott's (1925) formula. The Minitab v13.2, which is a statistical software was used for the analysis of the data (Minitab 2002 Software Inc., Northampton, MA, USA).

3. Results

Soil is one the best reservoir that harbors several fungal taxa, including entomopathogenic as well as opportunistic fungi. Entomopathogenic fungi have a great tendency to thrive in soil especially low in soil pH, EC, and temperature while high in moisture contents (Table 1). However, some opportunistic fungi can also survive in soils. The insect pathogenic fungus requires good moisture content for its viability. The 145 soil samples from 29 sites in Punjab which were collected during the survey, exhibited the great diversity of fungi. Those fungi belong to different genera (Table 2) and among them 40 were pathogenic to the insect.

The distribution and strength of EPF in soil samples cultivated from and non-cultivated mountainous lands showed that orchids had a more species diversity of these fungi. Sun and Liu (2008) found about 25 species in soil of field crops and 20 in orchard soils, indicating that the environment had a substantial effect on the occurrence of fungal species. The most common specie of all isolates was *Aspergillus niger* (27.50%), *Aspergillus flavus* (22.50%), and *Mucor varians* (21.50%).

The EPF were found in a significant number of cadavers obtained from the insect belonging to six insect orders. 225 insect cadavers were collected out of which only 94 were positive for any category of fungus isolated (Table 3). Insects from Coleoptera were reported with maximum occurrence (44.68%) for harboring any kind of the fungus followed by Lepidoptera (36.17%). The fungus *Fusarium oxysporium* was the most dominantly occurring species (17.02%) isolated, followed by *F. solani* (15.96%). Among the entomopathogenic fungi, *B. bassiana* was most occurring (7.45%) followed by the fungus *M. anisopliae* distributed with 4.26% occurrence (Table 4).

4. Discussions

Soil inhabiting EPF plays a major role in managing many soil-associated insect populations and pervasive element of many terrestrial ecosystems (Quesada Moraga et al., 2007; Meyling and Eilenberg, 2007). The same fungal isolates perform the role as a potential biocontrol agent under cultivated agro-systems (Meyling and Eilenberg, 2006). Ecological and soil characteristics had a greater contribution to the richness of entomopathogenic taxa. Soil properties like soil texture, pH, E.C., and temperature play a key role in the occurrence of EPF. The probability of occurrence of *I. fumosorosea* and *M. anisopliae* is increased in sandy soils, while clay soil also offer the similar habitat for *I. fumosorosea*, *M. anisopliae* and *B. basssiana* (Tkaczuk et al., 2014). Organic soils provide the particular environment to promote the diversity of entomopathogenic fungi and the fungal population (Klingen et al., 2002; Uzman et al., 2019). Pinruan *et al.* (2007) found 147 fungal species in rotting palm material, with 79 ascomycetes in 50 genera (53%), 65 anamorphic taxa in 53 genera (45%), and 3 basidiomycete species in 3 genera (2%) being new to science.

Insect cadavers or infected insects are one good source to isolate the entomopathogenic fungus. Similarly, opportunistic fungi also invade insect cadavers taking advantage of weaker conditions

Table 1
Physical and geographical characteristics of soil samples collected for isolation of entomopathogenic fungi.

Field category	Sample code	Crop	Temperature		Soil Characters			Soil Texture			Geographical quadrats		
			Air	Soil	Moisture%	pH	E.C. (mS)	Clay %	Silt%	Sand%	Altitude (m)	Latitude	Longitude
Field crops	JC209	Cotton	31	33.1	9.98	8.28	0.38	13.63	31.81	54.54	126	30.04276	71.85388
	MC109	Cotton	28	29.1	10.73	6.89	1.62	6.67	16.67	76.67	122	30.14935	71.45893
	JDC209	Desi cotton	34	33.3	4.2	5.9	0.22	35	20	45	126	30.05056	71.87638
	MSo99	Sorghum	30	27.8	6.87	6.47	1.61	12.5	9.37	78.12	122	30.15461	71.44747
	JSoii209	Sorghum	34	32.6	13.83	8	0.28	10	30	60	126	30.08371	71.85750
	JSoi209	Sorghum	29	32.1	23.1	6.8	0.19	36.36	22.72	40.9	126	30.04102	71.85751
	MR109	Rice	28	26.4	22.3	7.4	0.98	4	36.9	59.1	122	30.15469	71.47980
	JRi209	Rice	31	32.4	22.69	8.4	0.44	6.25	37.5	56.25	126	30.04176	71.85990
	JRii209	Rice	34	33	20.41	7.8	0.76	5.5	33.33	61.11	126	30.08382	71.89574
	MRo109	Rose	28	26.6	19.76	6.91	1.71	12.12	18.18	69.69	122	30.15399	71.47667
	JMa209	Maize	34	33.8	11.17	6.5	0.55	4.54	22.72	72.72	126	30.04261	71.85388
Fruit crops	JMi209	Mango	29	34.5	11.98	5.9	0.3	10	30	60	126	30.04294	71.84362
	MM99	Mango	30	28.1	13.52	5.5	0.58	6.67	13.33	83.33	122	30.15477	71.44756
	JMii209	Mango	31	32.7	6.9	6.1	0.3	6.25	37.5	56.25	126	30.08381	71.37682
	MD99	Date palm	30	26.2	10.79	6.51	1.79	6.06	18.18	75.75	122	30.15335	71.44866
	JD209	Date palm	31	32.6	13.99	7.7	1.1	31.81	27.27	40.9	126	30.05962	71.87157
	JL209	Lemon	29	33.1	14	6.5	0.21	13.63	31.81	54.54	126	30.05973	71.87362
	JO209	Orange	29	32.7	5.68	6.78	0.33	20.83	33.33	5.16	126	30.05757	71.87625
	MO109	Orange	28	28.5	14.64	6.61	1.72	10	20	70	122	30.15397	71.47662
Vegetable crops	JP209	Pumpkin	31	32.9	12.55	7.9	0.18	4.54	27.27	68.8	126	30.04151	71.36159
	JCa209	Cauliflower	34	32.5	20.4	7.39	0.78	10	20	70	126	30.04261	71.85889
	MRi109	Ridge gourd	28	27.1	10.34	6.62	2.03	3.33	23.33	73.33	122	30.15497	71.48085
	MSp109	Spinach	28	27.6	16.94	6.97	1.85	12.25	25	62.5	122	30.15503	71.4808
Hilly area	JG209	Grass	34	32.4	21.43	7.8	0.56	16.6	33.33	50	126	30.04668	71.87269
	JSe209	Sesbania	34	32	8.72	7.76	0.32	6.25	31.25	62.5	126	30.04913	71.87628
	MSe109	Sesbania	28	28.5	18.33	5.93	1.67	9.09	12.12	78.7	122	30.15469	71.47988
	MBl99	Wild Black berry	30	28.5	10.54	6.64	1.48	6.45	16.12	77.14	122	30.15125	71.44767

Table 2
Frequency distribution of occurrence of entomopathogenic fungi from different soil types.

Nature of fungi	Fungal isolates	Field	Fruit	Vegetable	Hilly areas	χ^2	*P*	Distribution frequency (%)	Total isolates (n)
Entomopathogenic	*Beauveria bassiana*	0	1	0	1	1.94	0.58	05.00	2
	Metarhizium anisopliae	0	1	0	0	2.27	0.51	02.50	1
	Trichoderma harzianum	1	1	1	2	1.06	0.78	12.50	5
Opportunistic	*Aspergillus flavus*	2	1	4	2	2.88	0.41	22.50	9
	Asprgillus niger	2	2	5	2	1.91	0.58	27.50	11
	Fuasrium oxysporum	1	1	1	0	0.97	0.80	07.50	3
	Mucor varians	2	1	2	3	2.01	0.57	20.00	8
	Penicillium chrysogenum	1	0	0	0	3.59	0.30	02.50	1
Total isolates		**15**	**21**	**17**	**15**	-	-	-	40
Percent occurrence (%)		37.50	52.50	42.50	37.50	-	-	100	-

Table 3
Fungal occurrence in infected insects collected from various localities (n=94)

Nature of fungi	Fungal species	Insect count collected (n) from different orders						χ^2	*P*	Distribution frequency (%)	Total isolates (n)
		Lep.	**Dip.**	**Col.**	**Hemi.**	**Hym.**	**Ortho.**				
Entomopathogenic	*Beauveria bassiana*	5	0	0	0	1	1	13.1	0.0224	7.45	7
	Metarhizium anisopliae	2	0	1	0	1	0	3.2	0.6691	4.26	4
	Paecilomyces lilacianus	1	0	1	0	0	1	3.62	0.6053	3.19	3
Opportunistic	*Aspergillus flavus*	1	1	2	1	1	1	0.51	0.9917	11.70	11
	Aspergillus fumigatus	1	2	2	1	1	1	1.29	0.9359	12.77	12
	Asprgillus niger	4	2	1	1	1	3	7.01	0.2202	12.77	12
	Fusarium oxysporium	3	2	1	1	2	3	5.4	0.3639	17.02	16
	Fusarium solani	2	0	3	2	1	2	4.05	0.5429	15.96	15
	Penicillium capsulatum	3	1	1	1	2	1	1.67	0.8931	9.57	9
	Rhizopus stolonifers	2	1	1	0	0	1	3.35	0.6455	5.32	5
Total		34	23	42	19	31	15			100.00	94
Percent occurrence (%)		36.17	24.47	44.68	20.21	32.98	15.96				

of insect near to death. Previously it has been reported that the occurrence of some EPF is more reliant on soil than plants, but some fungi are more associated with the vegetation (Zahid et al., 2020; Saeed et al., 2019; Steinwender et al., 2015; Nishi and Sato, 2019: Farooq et al., 2021). The EPF population is influenced by different cultivation (Tkaczuk et al., 2012; Kolczarek and Jankowski, 2014; Trizelia et al., 2015). *M. anisopliae* is prevailing in all habitats with cultivated plants, while *B. bassiana* prefers soil from orchards and natural sites (Jarmuł-Pietraszczyk et al., 2011; Medo and Cagan, 2011; Keyser et al., 2015).

Local soils and infect insects or the cadavers are the optimum media to harbor entomopathogenic fungi. The biggest advantage of using local resources is the greater adaptability to environmental conditions. Extreme weather conditions are the reason that

Table 4
Morphological characteristics of different opportunistic and entomopathogenic fungi isolated from soil and insect samples.

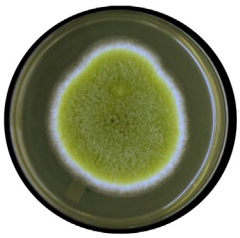
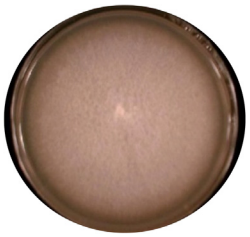
Species	Visual	Characters
Aspergillus flavus		Colony color is greenish-yellow to olive and has white borders; Spores have a thick mycelial mat, septate hyphae having large conidiophores sizes of 3-6 μm.
Aspergillus fumigatus		Colony color is bluish-green with multiple shades ending in a white border; spores are velvety and powdery; hyphae are septate with smooth walled conidiophores.
Asprgillus niger		The colony appears to be in pale yellow starting from white at the start; producing radial fissures and mat like mycelial growth.
Fusarium oxysporium		The initial colony color is white; turns greyish red to brown, septate hyphae, mycelia are aerial, and sizes ranges from 8 to 14 μm.
Fusarium solani		Colonies are white and cottony in the start later turn into blue-green or bluish brown. Microconidia shapes are oval, reniform, elongated oval, size 44–78 × 3.3–5.6 μm.
Rhizopus stolonifers		The colony is whitish to brown, fluffy, and cottony and hyphae are septate sparsely septate.
Beauveria bassiana		Colony color is bone white to pale yellow; the texture is velvety; powdery to cottony, hyphae are narrow and septate and conidiophores are single or aggregate with dense clusters.

Table 4 (*continued*)

Species	Visual	Characters
Metarhizium anisopliae		Colony color white to green spores is olivaceous green, cylindrical, and 2.5-3.5 μm long.
Paecilomyces lilacianus		Colony color appears in faint violet color; septate hyphae and conidiophores size from 3 to 4 μm.
Penicillium chrysogenum		Spores are dry forming chains with filamentous hyphae; usually colorless and branched with septate hyphae and brushed shape conidiophore.
Mucor varians		Hyphae of Mucor is filamentous, aseptate or coenocytic.
Trichoderma harzianum		They formed 1-2 concentric rings with green conidial production. Conidia (typically 3 to 5 μm in diameter).

leads to the failure of entomopathogenic fungi with its effect on spore health, germination, and virulence. The entomopathogens isolated from indigenous resources have the potential to be incorporated into an effective pest management system. Once formulated into ready to use the product, these entomopathogenic fungi will be able to successfully control the fruit fly infestations under all agricultural systems ranging from fruits, vegetable crops to forest systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Acosta-Martinez, V., Mikha, M.M., Vigil, M.F., 2007. Mi- crobial communities and enzyme activities in soils under alternative crop rotations compared to wheat–fallow for the Central Great Plains. Appl. Soil Ecol. 37, 41–52.

Barnett, H.L., Hunter, B.B., 1999. Illustrated Genera of Imperfect Fungi, 4th Edn. APS Press, The American Phytopathological Society, St. Paul, MN. p 218.

Barreto, L.P., Luz, C., Mascarin, G.M., Roberts, D.W., Arruda, W., Fernandes, É.K.K., 2016. Effect of heat stress and oil formulation on conidial germination of

Metarhizium anisopliae ss on tick cuticle and artificial medium. J. Invertebr. Pathol. 138, 94–103.
Dimbi, S., Maniania, N.K., Ekesi, S., 2009. Effect of *Metarhizium anisopliae* inoculation on the mating behavior of three species of African Tephritid fruit flies, *Ceratitis capitata*, *Ceratitis cosyra* and *Ceratitis fasciventris*. Biol. Control 50, 111–116.
Evans, H.C., Elliot, S.L., Barreto, R.W., 2018. Entomopathogenic fungi and their potential for the management of *Aedes aegypti* (Diptera: Culicidae) in the Americas. Mem. Inst. Oswaldo Cruz 113, 206–214.
Farooq, Maqbool, M.M., Bashir, M.A., Ullah, M.I., Shah, R.U., Ali, H.M., Farraj, A.A., Elshikh, M.S., Hatamleh, A.A., Bashir, S., Wang, Y.-F., 2021. Production suitability of date palm under changing climate in a semi-arid region predicted by CLIMEX model. Journal of King Saud University - Science. https://doi.org/10.1016/j.jksus.2021.101394.
Fernandes, É.K.K., Keyser, C.A., Chong, J.P., Rangel, D.E.N., Miller, M.P., Roberts, D.W., 2010. Characterization of *Metarhizium* species and varieties based on molecular analysis, heat tolerance and cold activity. J. Appl. Microbiol. 108, 115–128.
Fernandes, E.K.K., Rangel, D.E.N., Moraes, Á.M.L., Bittencourt, V.R.E.P., Roberts, D.W., 2007. Variability in tolerance to UV-B radiation among *Beauveria* spp. isolates. J. Invertebr. Pathol. 96, 237–243.
Jarmuł-Pietraszczyk, J., Kamionek, M., Kania, I., 2011. Occurrence of Entomopathogenic Fungi in Selected Parks and Urban Forests Of The Warsaw District Ursynow. Ecol. Chem. Eng. A 18, 1571–1574.
Johansson, M., Stenberg, B., Torstensson, L., 1999. Micro- biological and chemical changes in two arable soils after long-term sludge amendments. Biol. Fertility Soils 30, 160–167.
Karar, H., Bashir, M.A., Haider, M., Haider, N., Hassan, M., Alajmi, R.A., Metwally, D. M., 2021a. Hemipterous bug (dysdercus koenigii) (hemiptera: pyrrhocoridae) and boll rot disease of cotton (gossypium hirsutum) grown in the field. Saudi J. Biol. Sci. https://doi.org/10.1016/j.sjbs.2021.03.066.
Karar, H., Bashir, M.A., Khaliq, A., Jaffar Ali, M., Atalla Alajmi, R., Metwally, D.M., 2021b. Successful management strategies for Agonoscelis sp. (Heteroptera: Pentatomidae) in Alfalfa. Saudi J. Biol. Sci. https://doi.org/10.1016/j.sjbs.2021.03.013.
Keyser, C.A., Henrik, H., Steinwender, B.M., Meyling, N.V., 2015. Diversity within the entomopathogenic fungal species *Metarhizium flavoviride* associated with agricultural crops in Denmark. BMC Microbiol. 15, 249.
Klingen, I., Eilenberg, J., Meadow, R., 2002. Effects of farming system, field margins and bait insect on the occurrence of insect pathogenic fungi in soils. Agric. Ecosyst. Environ. 91, 191–198.
Kolczarek, R., Jankowski, K., 2014. Occurrence of entomopathogenic fungi in soils from Festuca pratensis Huds. Crop. J. Ecol. Eng. 15.
Medo, J., Cagáň, Ľ., 2011. Factors affecting the occurrence of entomopathogenic fungi in soils of Slovakia as revealed using two methods. Biol. Control 59, 200–208.
Meyling, N.V., Eilenberg, J., 2006. Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. Agric. Ecosyst. Environ. 113, 336–341.
Meyling, N.V., Eilenberg, J., 2007. Ecology of the entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae in temperate agroecosystems: potential for conservation biological control. Biol. Control 43 (2), 145–155.
Nishi, O., Sato, H., 2019. Isolation of Metarhizium spp. from rhizosphere soils of wild plants reflects fungal diversity in soil but not plant specificity. Mycology 10, 22–31.
Quesada Moraga, E., Navas-Cortés, J.A., Maranhao, E.A.A., Ortiz-Urquiza, A., Santiago-Álvarez, C., 2007. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycol. Res. 111, 947–966.
Saeed. S., M.A., Bashir, Khan., K.A., Sajjad. A., Alvi. A.M., Atta. S. and Ansar.J. 2019. Assemblage of pollinator communities in four widely isolated natural reserves of Southern Punjab, Pakistan. Saudi Journal of Biological Sciences. Volume 26, Issue 4, May 2019, Pages 860-865.
Steinwender, B.M., Enkerli, J., Widmer, F., Eilenberg, J., Kristensen, H.L., Bidochka, M. J., Meyling, N.V., 2015. Root isolations of Metarhizium spp. from crops reflect diversity in the soil and indicate no plant specificity. J. Invertebr. Pathol. 132, 142–148.
Stuart, R.J., Barbercheck, M.E., Parwinder, S.G., Taylor, R.A.J., Hoy, C.W., 2006. Population biology of entomopathogenic nematodes: concepts, issues and models. Biological Con- trol 38, 80–102.
Sun, B.-D., Liu, X.-Z., 2008. Occurrence and diversity of insect-associated fungi in natural soils in China. Appl. soil Ecol. 39, 100–108.
Tkaczuk, C., Król, A., Majchrowska-Safaryan, A., Nicewicz, Ł., 2014. The occurrence of entomopathogenic fungi in soils from fields cultivated in a conventional and organic system. J. Ecol, Eng, p. 15.
Tkaczuk, C., Krzyczkowski, T., Wegensteiner, R., 2012. The occurrence of entomopathogenic fungi in soils from mid-field woodlots and adjacent small-scale arable fields. Acta Mycologica 47 (2).
Trizelia, T., Armon, N., Jailani, H., 2015. The diversity of entomopathogenic fungi on rhizosphere of various vegetable crops. Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia., 998–1004
Uzman, D., Pliester, J., Leyer, I., Entling, M.H., Reineke, A., 2019. Drivers of entomopathogenic fungi presence in organic and conventional vineyard soils. Appl. Soil Ecol. 133, 89–97.
Zahid A, Fozia, M. Ramzan, M. Amjad Bashir, M. Ahsan Khatana, M. Tahir Akram, S. Nadeem, M. Saad Qureshi, W. Iqbal, M. Umar, S. Walli, R. Muhammad Sabir Tariq, S. Atta, D.A. Al Farraj, M.T. Yassin, Effect of humic acid enriched cotton waste on growth, nutritional and chemical composition ofoyster mushrooms (Pluerotus ostreatus and Lentinus sajor-caju), Journal of King Saud University – Science (2020), Volume 32, Issue 8, December 2020, Pages 3249-3257 10.1016/j.jksus.2020.08.016