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Effect of Mint Leaves Powder (Mentha sp.) on Fungi Associated with Sunflower Helianthus annus L. Seeds

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ABSTRACT

Sunflower Helianthus annus is one of the economically significant crops. During poor storage conditions, seed-borne fungi develop. As a result, it is polluted with mycotoxins and its quality is reduced for various uses Chemical and non-chemical methods to control fungal contamination were used. The current study examined the fungi associated with local products seeds and the antifungal activity of mint leaves powder (MLP) on seeds-borne isolates. Alternaria sp., Aspergillus sp., Cladosporium sp., Fusarium sp., Mucor sp., Penicillium sp., Pythium sp., Rhizopus sp., Trichoderma sp., were isolated from untreated seeds by moistened chamber method. Mixing MLP with sunflower seeds decreased the number of fungal isolates. Aspergillus, Mucor, Penicillium, Pythium, and Trichoderma disappear from tested seed samples after month. Also the seed germination% increase after mixing with MLP (not significant), it indicates that the treatment did not affect the embryo's vitality, the significant characteristic for the seeds that will be used for planting.

Kewords: Antifungal, Leaves powder, Mint, Sunflower seed.

1. Introduction

Helianthus annuus, (Asteraceae), the common sunflower is a large annual genus and the fruits are a source of edible oil and traditional nuts in bad storage conditions; fungi can grow on seeds and peels.

Sunflower stems and leaves effect by several phytopathogens in addition to crop diseases. Vaidehi 2002 and Morar et al. 2004, explain that sunflower seeds are highly held numerous fungi which attack the plants at different periods of development and then during harvesting and storage [1, 2].

In Pakistan, the workers reported 55 isolates related mainly to Hyphomycetes the various isolates, nine species of Aspergillus were isolated from seed samples [3].

These isolates produce different groups of aflatoxins, which are natural toxins and dangerous to animals and man [4].

Control of seed-borne fungi is the aim of several studies. It is important to use safe methods for human health and

friend to the environment. A traditional method uses powder of the medical plant leaves [5].

Mentha sp. is one of the widely used herbs. The plant has several pharmaceutical applications [6].

Because rousted sunflower fruits are traditionally a type of nuts consumed commonly in Erbil society, the current study aims to identify the fungi associated with local seeds (from markets) and explain their health impact as mycotoxin producers.

2. Materials and Methods

2.1 Preparation of mint leaves powder

The leaves of Mint Mentha sp. were collected from the home gardens. They were washed thoroughly by tap water, then by distilled water. The leaves were dried in the lab environment. After perfect dryness (no farther decrease in the wight) an electrical blender was used to transfer them into powder. Leaves powder was passed through a one mm sieve. The powder was kept in a glass jar with a lid for the following work. Fifty seeds were quietly mixed with powder in the container.

2.2 Seeds examination

About 200gm of sunflower seeds were collected from several markets in Erbil city. Five seeds were placed on two layers of blotting paper in the bottom of plastic petri-dish; then, 3 ml sterile distilled water was added to saturate papers [7].

Ten plates (50 seeds) were used for seed free from mint powder beside 50 seeds in ten plates for mint powder contaminated seeds. The twenty plates were incubated at lab temperature 25°C±2. The washing method was followed for detecting the total colony-forming unit (CFU). Five seeds were soaked in 10ml of sterile distilled water. The vials (replicate) were shaken gently for 2 minutes, then one ml of water was transferred to a sterile Petri-dish. Fifteen ml of TPA medium was powered in the Petri-dish and was mixed by rotating movement. Plates were incubated in the lab environment and were checked after five days to count the colony's spots. The CFU was counted for untreated seeds after six months of mixing with mint powder.

2.3. Fungal identification

The developing fungi were identified directly or after culturing on Typha pollen agar (TPA) medium [8]. Fungi were identified based on morphological characteristics after [9 and 10] the occurrence percentage were calculated according to Al-Bader [11].

$$O\% = (\text{no. of observation/no. of samples}) \times 100$$

The seed germination percentage was conducted to check the effect of Mint sp. powder on seed viability. Twenty-five seeds were put on double layers of filter paper (no. 1) in 90-mm glass petri dishes. The filtered papers were moistened with 5ml distilled water added and were kept in nylon bags in laboratory conditions.

3. Results and Discussion

Fourteen species related to nine genera were isolated from untreated sunflower seeds viz *Alternaria sp., Aspergillus sp., Cladosporium sp., Fusarium sp., Mucor sp., Penicillium sp., Pythium sp., Rhizopus sp, and Trichoderma sp*., Table1.

Table 1: The occurrence percentage of fungal isolate.

	Fungi	Occurrence%
1	*Alternaria alternate*	6%
2	*A. tenuissina*	4%
3	*Aspergillus flavus*	8%
4	*As. Fumigates*	14%
5	*As.terreus*	4%
6	*Cladosporium cladosporoides*	18%
7	*Fusarium* sp.	2%
8	*F.moniliform*	4%
9	*F. oxysporum*	4%
10	*Mucor mucedo*	6%
11	*Penicillium notatum*	10%
12	*Pythium sp.*	4%
13	*Rhizopus stolonifera*	6%
14	*Trichoderma viridi*	10%

Several fungi have been recognized in sunflower seeds [12] listed 48 species of fungi belonging to 19 genera from Iraqi cultivated seeds. The importance of seed fungal contamination significantly increases when intended for human use, or animal feeding [13,14, 15]

Alternaria, Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus, and Trichoderma are commonly recorded on seeds of several crops [16, 17]. Members related to these genera are well-known toxins producers, Table 2.

Several physical and eco-friendly methods were implicated in avoiding or reducing chemical treatments of seed-borne contamination. Plant extracts and natural compounds have

proven to treat seed-borne pathogens. They are widely used due to their broad-spectrum [25].

Table 2: Toxins of the common isolates of sunflower seeds.

Fungi	Toxins	Ref.
Alternaria	alternariol, alternariol monomethyl ether, altenuene, altertoxins I, II, III, tenuazonic	[18]
Aspergillus	ochratoxin A (OTA), aflatoxins (AFs), as well as less-prominent toxins like patulin	[19]
Cladosporium	cladosporin, isocladosporin,emodin, epi- and fagicladosporic acid, and ergot alkaloids	[20]
Fusarium	HT-2 toxin, T-2 toxin, nivalenol, fusarenon-X, deoxynivalenol, zearalenone, fusaric acid	[21]
Penicillium	ochratoxin A, citrinin, patulin, cyclopiazonic acid, citreoviridin, penitrem A, roquefortine, and secalonic acids.	[22]
Trichoderma	Trichothecenes, trichodermin and harzianum A (HA)	[23]
Mucor	Mucoricin, 3-nitropropionic acid	[24]
Rhizopus	3-nitropropionic acid	[24]

In the current study, the powder of mint leaves showed a high effect. It eliminated the occurrence of *Aspergillus, Mucor, Penicillium, Pythium,* and *Trichoderma* from tested seed samples after month, Table 3. *Alternaria, Cladosporium, Fusarium,* and *Rhizopus* disappear after (5,4,3,4) months, respectively.

Table 3: Period (month) of fungal disappearance from seeds after mixing with MLP. Alt.=Alternaria, Asp.=Aspergills, Cla.=Cladosporium, Fus.=Fusarium, Muc.=Mucor, Pen.=Penicillium, Pyt.=Pytheium,Rhi.=Rhizopus,Tri.=Tricoderma.

Fungi	Alt	Asp	Cla	Fus	Muc	Pen	Pyt	Rhi	Tri
Mounts	5	1	4	3	1	1	1	4	1

The use of natural compounds has been intensively investigated in recent years. The use of plant essential oils extracted is one of the practical and trusted methods. Mint leaves and their oil significantly affect postharvest fungi [26]. Antifungal efficacy in terms of the period of the disappearance of fungal growth showed two groups of fungi. The sensitive isolates of Aspergillus, Penicillium, Mucor, Pythium, and Trichoderma beside the resistance isolates Alternaria, Cladosporium, and Fusarium.

The seed's germination percentage did not decrease after mixing with mint leaves powder, Table 4. Increasing of germination rate (not significant) indicates that the treatment did not affect the embryo's vitality, which is an essential characteristic for the seeds that will be used for planting.

Table 4: The seed's germination percentage of untreated seed, after one month and after six months

Time / Treatme	untreated	1st month	6th month
	80%	84%	86%

The total colony count showed a high reduction. The mean of three plates at the three-time of the exam (untreated seeds, one month after mixing with mint powder, and six months after mixing) were 21,14, 2 colonies, respectively. Fungi that occur after six months are probably associated with the seed's embryos rather than the seed's coat.

The compounds of mint oil showed a significant antifungal effect. The main compounds are menthol, the major component of the essential oil, followed by menthyl acetate and menthofuran [27].

The components of mint oil were used to control fungal phytopathogens. They affect *Macrophomina*, which causes root rot of cotton [28].

4. Conclusion

The results showed that mint leaves powder significantly affects surface contamination. This result needs further studies to identify the volatile compounds of mint leaves and test their efficacy against fungal phytopathogens.

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