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ANTIFUNGAL INHIBITORY ACTIVITY OF *THYMUS VULGARIS L.* AND *ARTEMISIA HERBA-ALBA* POWDER AND ITS CONSTITUENT PHYTOCHEMICALS AGAINST *ASPERGILLUS OCHRACEUS* AND *FUSARIUM GRAMINEARUM* GROWTH

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Abstract

Aspergillus ochraceus and *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schwein. (Petch)], are ones of the most destructive diseases of wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.) worldwide. Numerous strategies for diseases resistance breeding, chemical and bio-control are in use, including marker-assisted selection for resistance QTL and using plants extracts for inhibiting the presence of mycotoxins. The most destructive consequences of these fungi are evidenced through a reduction in grain quality, and the presence of mycotoxins, the most common of which are ochratoxin and deoxynivalenol (DON). Thus, there is great interest among researchers in inhibiting and controlling these fungi. To this end, a study was devised as follows. Powder of grinded *Thymus vulgaris* and *Artemisia herba-alba* plants were applied to potato sucrose agar (PSA) at concentrations of (1, 3, and 5%). This treatment resulted in inhibiting the fungal growth with thyme powder by (35.52, 66.5, 78.50, 39.5, 68.70, 81.6) for both fungi and concentrations, respectively. Treatment of artemisia powder at the same concentrations inhibited fungal growth by (20.5, 50.2, 65.8, 23.6, 51.5, and 67.7%) for both fungi and concentrations, respectively. Phytochemical extract of thyme and artemisia plants at concentrations of (0.5%, 1%, and 2%) inhibited the growth of fungi by a certain percentage as follows. Fungal growth inhabitation was decreased by (70.3, 92.6, 100%, 75.5, 94.1, and 100%) for both fungi strains respectively with thyme extract. Alcoholic extract of artemisia inhibited and decreases their growth by (41.5, 85.7, 100, 55.7, 88.7 and 100%), respectively.

Key words*:* antifungal activity; *Thymus vulgaris*; *Artemisia herba-alba*; *Aspergillus ochraceus; Fusarium graminearum.*

Introduction

Aspergillus ochraceus and Fusarium graminearum have become increasingly recognized as important pathogens in critically plant diseases. Damage produced by them includes yield reduction, contamination of mycotoxins, discolored, shriveled "tombstone" kernels and decreases in quality of seed. These fungi and their mycotoxins also reducing the test weight and lowering the market grade, Khaeim Hussein (2013).

Fungi that colonize cereal crops are divided into two main categories depending on their environmental requirements: field fungi and storage fungi. Before harvesting, fungi colonize crops with a moisture content of about 25%, which is equivalent to 95% relative moisture content or more. Cereals can be infected also in the storage if these conditions are existed, by including *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp., *Helminthosporium* spp. *Trichoderma* spp. *Rhizopus and Mucor*. Storage fungi infect moisture-bearing grains of 65-90% relative moisture. The separation between these fungi sorts is not conclusive and of the most common grain storage fungi are *Aspergillus flavus* and *A.* o*chraceus* as well as other types of *Aspergillus* spp. *Fusarium* spp. *Penicillium* spp. *Candidus* spp. and some types of *Rhizopus* spp. and *Mucor* spp. Most of these fungi produce mycotoxins, Baldawi (2007); Nakheel (2011); and Luma A. Alabadi, *et al.,* (2018).

Claviceps purpurea is one of the oldest known

fungal toxins. It causes severe poisoning of humans and animals, Alexopoulos (1972). It was spread epidemically in Europe in the Middle Ages as a result of eating bread made of polluted grains, IFST (2006); and Baldawi (2007). Fungal toxins emerged as a real problem during World War II. It was observed that the consumption of contaminated grain with the mold caused symptoms such as skin necrosis, bleeding, liver and kidney failure, and death cases of many people and animals. Scientists have noticed the seriousness of this problem only after the famous incident in Britain in the early 1960s after the discovery of the unknown disease, and the discovery of the aflatoxins, Nakheel (2011), and Wafaa Sahib (2018).

Fungi that produce toxin belong to six major species: *Aspergillus, Penicillium, Fusarium, Claviceps, Stachybotrys, Neotyphodium*. They were distinguished because of their ability to produce high concentrations of fungal mycotoxins, and their ability to live in a variety of different environments, CAST (2003). *Aspergillus, Penicillium,* and *Fusarium* are the most abundant and productive of the mycotoxins in grain storage. *Aspergillus* and *Fusarium* attack grains in the field and continue with grains during storage, CAST (2003); and FAO (2011). The most serious and essentially dangerous fungal toxins that pollute the stored grain and poultry components are aflatoxins, oxytoxins, and stregmastocin produced from *Aspergillus* spp. This additional to stearin, patulin, oxytocin, and styroferride produced by *Penicillium* spp., and tricotisins, zeralins, feminine and monifluoramin produced from *Fusarium* spp., Ezekiel *et al.,* (2011).

Thymus vulgaris is one of the very important medicinal plants. It contains many active substances such as volatile oils by (1%) and (10%) tannin. The active substance is present throughout the whole plant body. Thyme extract and its extracted oil are effective against many plant pathogens. They use also to inhibit the growth of *Rhizopus oryzae* and to prevent the formation of sporangia, Riccioni, and Orzali (2011); Mota *et al.* (2012).

Artemisia herba-alba is also an important medicinal plant. The active ingredient exists in leaves and delicate stems such as Misin, Santonin, Absinthin, and Thugone, which are not specified in the plant. Santoaica powder and its volatile oil have been used to inhibit the growth and prevent the stuttering of many plant pathogens such as *Fusarium*, Sbayou *et al.* (2014), Mehani *et al.* (2016) and El-Muostafa *et al.* (2017).

This study aims to use *Thymus vulgaris* L. and *Artemisia herba-alba* powder and their constituent phytochemicals to inhibit *Aspergillus ochraceus* and *Fusarium graminearum* growth.

Materials and Methods

Fungal Isolation

Samples of maize and wheat grains were brought to isolate the target fungi. Grains were sterilized with sodium hypochlorite (NaClO) solution (6%) that was diluted to free chlorine (1%) for 3 minutes and then washed with sterile water then they dried with filter paper. These grains were planted in a medium of Potato Sucrose Agar (PSA) (200 g potatoes, 10 g sucrose, 20 g agers per 1 liter of water) by 5 grains of maize and 10 grains of wheat for each dish. These dishes were incubated at (25 ± 2°) for 5 days. Afterward, they counted and examined under a compound microscope.

Purification of fungal isolates

Suspected species of *Aspergillus ochraceus* and *Fusarium graminearum* that producers of oxytocin and (DON) respectively in the stored grain were targeted. They were grown in Sabouroud Dextrose Agar (SD) (65 g suspended in 1 liter distilled water) and (PDA) (39 g powder in 1 liter distilled water) and incubated under (25 + 2° C) for (5 – 7) days.

Determination the best effective percentage of plant powders in inhibiting fungal growth on the PSA

Three percentages were tested to determine the effective inhibitory effect of thyme and artemisia. They were (10, 30 and 50 g/L). Samples of leaves and stalks of thyme plant and whole artemisia plant were dried. These samples were grinded using Wiley mill standard model mill No.3 Arther Thomas co. The powders were sieved and placed in polythene bags and kept till use. These powders were tested according to the food poisoning method, Dixit *et al.* (1976). Concentrates were applied to the sterile and refrigerated food medium (45-50 m) and poured into (9 cm) sterile dishes. Four replicates were used for each treatment. After hardening, dishes were inoculated in the center with a diameter of (6 mm) for each dish of fungal colonies. They were incubated at (25 ± 1°C). The results were calculated using the mean of two orthogonal diameters from each colony, after filling the comparison colony for the entire dish. The percentage of inhibition was calculated using the following equation, Abdullah (2003) :

$$\text{Inhabitaton}\% = \frac{\text{Cont Colony Average} - \text{Cont Colony Average}}{\text{Cont Colony Average}} \times 100$$

Determination the best effective percentage of plant extracts in inhibiting fungal growth on the PSA

Three percentages of the alcoholic extracts of thyme

and Artemisia were tested, which are (0.5, 1, and 2%). These concentrations were applied to the sterile and cooled medium after the alcoholic extract was prepared as follows: (50 g) of the powder of each plant (Thyme and Artemisia) were taken and placed in a glass flask of (500 ml). 100 mL ethyl alcohol was added to it. The shutter of the beaker was closed and then shacked for 24-hours using an electric shaker. The extract was filtered through the Whatman No.1 filter paper in the Buechner funnel with air discharge. The filtered solution was concentrated with a rotary evaporator at a temperature of (45-50 m) to remove the solvent. Extracts were placed in sterile glass bottles and draw with aluminum foils to be opaque. Cans were tagged with plant name and extraction date and kept frozen until use, Ketabchi *et al.,* (2011).

Results and Discussions

Evaluation of the efficiency of different concentrations of thyme and artemisia powder in inhibiting the growth of *A. ochraceus* and *F. graminearum*

Table 3 presents the effect of different percentages of thyme and artemisia powder on *A. ochraceus* and *F. graminearum* growth. The inhibitory percentage increased as the applied treatment parentage increased and the highest inhibitory rate was recorded for both fungi with a percentage of (5%). The thyme powder of the concentration of (1%) produced an inhibitory ratio for the growth of *A. ochraceus* and *F. graminearum* by 35.52% and 39.5%, respectively. The inhibition rate at (3%) concentration reached 66.5% and 68.70% for both fungi, respectively. At a concentration of 5%, the inhibition rate was 78.5% and 81.6%, respectively. Using artemisia extract of (1%) concentration, the inhabitation rates were 20.5% and 23.6%, respectively. The concentration of (3%), the inhibition ratio was 50.20% and 51.50% for both fungus, respectively. The 5% concentration inhibits fungal growth by 65.80 and 67.7% for both fungi, respectively. Thyme, its extracts, and volatile oil have been used to inhibit many pathogenic fungi, Riccioni, and Orzali (2011).

Evaluation of the effect of different concentrations of the water and alcoholic extracts of the thym E, and artemisia plants in inhibiting *A. ochraceus* and *F. graminearum* growth

The alcoholic extracts of both plants inhibited fungal growth in various percentages depends on the concentration, table 2. Inhibition rate increased as the concentration of the extract increase and the highest inhibitory rate for both fungi is (2%). The alcoholic extract of the thymus plant of the (0.5%) concentration reduced *A. ochraceus* and *F. graminearum* by 70.3% and 75.5%, respectively. The concentration of (1%) decreased fungal growth rate by 92.60% and 75.10% for both strains, respectively. The (2%) completely prevented fungal growth rate by 100%.

The alcohol extract of the artemisia plant at (0.5%) concentration inhibited fungal growth rate by 41.50% and 55.70% of both fungi, respectively. The (1%) concentration, inhibited both fungi by 85.70% and 88.70%, respectively. The alcoholic extract of (2%) concentration completely inhibited fungal growth by 100% for both fungi.

Table 1 : Effect of different concentrations of thyme and artemisia powder on *A. ochraceus* and *F. graminearum* growth.

Treatments	Thyme 1%	Thyme 3%	Thyme 5%	Artemisia 1%	Artemisia 3%	Artemisia 5%	LSD 0.05
Inhabitation rate of *A. ochraceus*	35.52	66.50	78.50	20.50	50.20	65.80	08.69
Inhabitation rate of *F.gramnearum*	39.50	68.70	81.60	23.60	51.50	67.70	09.77

Table 2 : Effect of different concentrations of alcohol extract of thyme and artemisia on *A. ochraceus* and *F. graminearum*.

Treatments	Concentration *A. ochraceus*	Inhabitation % of	Inhabitation rate of *F.gramnearum*
Alcoholic extract of the thyme plant	0.5	70.30	75.50
	1	92.60	94.10
	2	100.0	100.0
Alcoholic extract of the artemisia plant	0.5	41.50	55.70
	1	85.70	88.70
	2	100.0	100.0
LSD value 0.05	----------	10.50	08.51

Conclusion

Application of extracted powders and the alcoholic solutions of thyme and artemisia to the nutritional medium in the laboratory were effective in inhibition of *A. ochraceus* and *F. graminearum* growth in various rates. Therefore, fungal growth and mycotoxins production dramatically reduced. The alcoholic extract of the concentration of (2%) of the thyme and application plants completely inhibited the growth of the fungi.

Recommendation

It is recommended to apply some plant extracts such as thyme

and application powder to the stored grain. In order to reduce the negative effects of pollution of fungi causing the contamination of these grains and hives and thus prevent the growth of fungi and their production ochratoxin and (DON). Thus increasing the nutritional value as a natural substance and eliminating the side effects of methods of inhibition of fungi and reduction of other toxins such as chemical and physical methods.

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