J. Plant Prot. and Path., Mansoura Univ., Vol.9 (9): 567 – 571, 2018

Antimicrobial Activity of *Artemisia herba-alba* Extract against Pathogenic Fungi of Pigeon Droppings

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ABSTRACT

Pigeon droppings represent the most important source of pathogenic fungi of plants and other living organisms. The occurrence of fungi in samples of feral pigeon droppings was screened. Moreover, the antifungal properties of *Artemisia herba-alba* extract against these fungi was investigated. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of this extract were determined. The results revealed that *Alternaria alternata* recorded the highest frequency in the collected samples while, *Cladosporium sphaerospermum* was the least isolated fungus. *Artemisia herba-alba* extract showed the highest antifungal effect against *C. sphaerospermum* fungus by MIC and MFC values of 1.5% and 3%, respectively. However, *A. alternata*, *Aspergillus niger*, *Fusarium oxysporum* and *Rhizopus stolonifer* were the most resistant fungi to the extract by MIC value (6%). The highest MFC value (10%) was showed against *A. niger*, *Mucor circinelloides*, *Penicillium citrinum* and *R. stolonifer* fungal isolates.

Keywords: Feral pigeon droppings, pathogenic fungi, *Artemisia herba- alba* extract, antifungal properties.

INTRODUCTION

Pigeon droppings considered one of the most important damage caused by birds to the different crops. Large number of mycotic infections was detected in all type of birds. Theses fungal diseases include Aspergillosis, Candidasis, Dactylariosis, Cryptococcosis, Histoplasmosis and Torulopsis (Dhama *et al*., 2013). About 50% of birds are carriers for fungi which potentially pathogenic to birds themselves and human (Dynowska *et al*., 2015). Feral pigeons, *Columbia livia* are known to transmit pigeon ornithosis, encephalitis, cryptococcosis, toxoplasmosis, salmonella and other diseases (Weber, 1979). Birds and their droppings can carry over 60 diseases which can be transferred to the humans just by being around them (Singh *et al*., 2012). Droppings of the feral pigeon could be a potential carrier in the spread of pathogenic yeasts and mold fungi into the environment (Maryam *et al*., 2013). Several pathogens and parasites were detected in the fecal samples of this pigeon as *Trichomonas gallinae*, coccidia, helminths, ectoparasites and fungi (Schreiber *et al*., 2015). On the other hand, birds in general are susceptible to fungi due to their anatomy of the respiratory and nervous systems and not have enough antifungal activity in their serum (Shivachandra *et al*., 2004). Pathogenic fungi as *Botrytis cinerea*, *Fusarium graminearum* and *F. oxysporum* also causing significant damage and severe losses in crops as tomato, cotton, banana and other plants (Ralph *et al*., 2012). *Aspergillus* species also responsible of different plant diseases with the consequence of possible accumulation of mycotoxins. The aflatoxin producing by *A. flavus* and *A. parasiticus* and ochratoxinogenic *A. niger*, *A. ochraceus* and *A. carbonarius* species are encountered in the agricultural products. These fungal species can contaminate agricultural products at different stages including pre-harvest, harvest, processing and handing causes great losses in crop production (Perrone *et al*., 2007). So that, these pathogenic fungi or fungal infections require more attention for diagnosis and control (Dhama *et al*., 2011). Recent studies have shown the importance of natural products which derived from plants as fungicidal agents (Morcia and Terzi, 2011). Natural extracts have great potential to be used for safe and environmentally treatment of pathogenic fungi. It can be used as alternative to the chemical treatments for avoid the environmental pollution (Morcia *et al*., 2015).

The *Artemisia herba-alba*, was a desert wormwood has been used in folk medicine since ancient times and used to treat arterial hypertension and diabetes (Zeggwagh *et al*., 2008). It is suggested to be important as fodder for sheep and livestock (Benmansour and Taleb, 1998). Moreover, it has been used as analgesic, antibacterial and hemostatic agents (Laid *et al*., 2008). The extract of this plant has many therapeutic properties. It is used for its antiseptic properties against infectious diseases of fungal origin and against dermatophytes. It showed high antifungal potential against wide spectrum of pathogenic fungi as *Aspergillus versicolor*, *A. ochraceus*, *A. niger* and *Penicillium* species (Peda *et al*., 2015).

The objective of this study is the isolation and identification of fungi which associated with pigeon droppings and also investigating the antifungal effect of *A. herba-alba* extract against these fungi.

MATERIALS AND METHODS

Plant materials

Leaves and stems of wormwood, *Artemisia herba-alba* were collected from Sinai desert, Egypt in February 2016. The collected plant parts were thoroughly washed with water to remove dirt and dried at the room temperature. After, the plant parts were grinded well by using electric grinder (Laouini *et al*., 2016).

Extract preparation

Grinded plant materials of *A. herba-alba* (150 g) was immersed in ethanol 95%. The obtained extract was filtered through filter paper and then solvent was evaporated by using a rotary evaporator (Kamel *et al*., 2015). Dried crude extract was prepared in 1.5, 3, 6 and 10% concentrations by mixing with distilled water (Sanjoy *et al*., 2013).

Pigeon dropping fungi

Sixty fresh droppings samples of the feral pigeon, *Columba livia* were collected from different fields at Zagazig district, Sharkia Governorate, Egypt in a period of 9 months (April to December 2016) and taken to the laboratory. Droppings were suspended in saline solution and then spread onto the surface of Potato Dextrose Agar medium (PDA) and incubated at 28ºC for 7 days. Fungal colonies were enumerated and purified by using single spore technique (Maryam *et al*., 2013). The fungal isolates were identified according to the morphological characteristic on the culture media PDA and MEA (Malt Extract Agar) (Torbati *et al*., 2016).

Determination of antifungal activity

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *A. herba-alba* extract against the fungal isolates of pigeon dropping were determined. The plant extract was serially diluted to 1.5, 3, 6 and 10% concentrations from the crude extract. These concentrations were prepared in glass bottles (100 ml) capacity, each bottle contained 50 ml sterilized potato dextrose broth medium used as diluent. Sporangial suspension concentration of each fungal isolate was estimated using a hemocytometer and adjusted to 2×10^6 spores/ml (Abril *et al*., 2008). Ten ml from the fungal suspension was inoculated into each bottle separately. Bottles without extract were prepared as control to ensure the ability of fungi to grow in the medium. All bottles were incubated at 27°C for 10 days. The lowest concentrations without visible growth in comparing with the control were defined as the MIC (Eloff, 1998). After determination of the MIC values, 20 ml was subcultured from each well that showed no visible growth and from the control bottles (extract-free medium) onto PDA plates. The plates were incubated at 27°C until growth was appeared in the growth control plates. The lowest extract concentration that did not yield any fungal growth on the solid medium defined as MFC indicating 99.5% killing of the original inoculum (Espinel-Ingroff *et al*., 2002). All experiments were performed in triplicate.

RESULTS

Frequencies of different fungi species isolated from pigeon dropping

Total count of fungal species which isolated from sixty dropping samples of feral pigeon was calculated. The frequency of occurrence was also recorded for each species. As presented in Table 1. *Alternaria alternata* was the most common fungus in the dropping samples; it showed the highest number (96 colonies) and also the highest frequency of occurrence. It was followed by *Penicillium citrinum* (n = 82), *Aspergillus flavus* (n = 74), *Fusarium oxysporum* (n = 42), *Aspergillus niger* (n = 29), *Mucor circinelloides* (n = 25), *Aspergillus oryzae* (n = 17), *Rhizopus stolonifer* (n = 13), *Cylindrocarbon magnusianum* (n = 11) and *Nigrospora sphaerica* (n = 7). *Cladosporium sphaerospermum* recorded the lowest number (n = 4) and only once appeared in the dropping samples so it showed also the lowest frequency of occurrence.

Antifungal activity of *Artemisia herba-alba* extract

The antifungal potential of *A. herba-alba* extract was investigated against fungal species isolated from the feral pigeon dropping. Data in Table 2. indicated that the MIC of this extract ranged between 1.5 - 6%. While the MFC was in the range of 3 – 10%. The extract exhibited higher antifungal activity against *C. sphaerospermum* and *C. magnusianum* with MIC value 1.5% and MFC 3 and 6% for the two fungi, respectively. While it showed the same or slightly lower antifungal potential against *A. alternata*, *A. niger*, *F. oxysporum* and *R. stolonifer* with MIC value 6%. The same MFC value 6% was recorded for *A. alternata* and *F. oxysporum* and 10% for *A. niger* and *R. stolonifer*. Moderate effect was achieved by the extract against the other fungi; *A. flavus*, *A. oryzae*, *M. circinelloides*, *N. sphaerica* and *P. citrinum* with the same MIC 3% and MFC between 6 – 10%.

Table 1. Frequencies of fungi species isolated from feral pigeon dropping

Fungal species	No. of isolates	Frequency of occurrence
Alternaria alternata	96	18 H
Aspergillus flavus	74	16 H
Aspergillus niger	29	7 L
Aspergillus oryzae	17	4 R
Cladosporium sphaerospermum	4	1 R
Cylindrocarbon magnusianum	11	3 R
Fusarium oxysporum	42	9 L
Mucor circinelloides	25	7 L
Nigrospora sphaerica	7	2 R
Penicillium citrinum	82	11 M
Rhizopus stolonifer	13	5 L

H = high frequency (15 – 18)   M = moderate frequency (11 -14)
L = low frequency (5 – 10)   R = rare frequency (less than 4)

Table 2. Antifungal activity of *Artemisia herba-alba* extract

Fungi	MIC	MFC
Alternaria alternata	6.0	6.0
Aspergillus flavus	3.0	6.0
Aspergillus niger	6.0	10.0
Aspergillus oryzae	3.0	6.0
Cladosporium sphaerospermum	1.5	3.0
Cylindrocarbon magnusianum	1.5	6.0
Fusarium oxysporum	6.0	6.0
Mucor circinelloides	3.0	10.0
Nigrospora sphaerica	3.0	6.0
Penicillium citrinum	3.0	10.0
Rhizopus stolonifer	6.0	10.0

MIC: minimum inhibitory concentration
MFC: minimum fungicidal concentration

DISCUSSION

Pathogenic fungi which associated with pigeon droppings is a matter of concern due to causing plant diseases and infection of humans and animals. Current study was done to evaluate mold fungi frequency in some samples of feral pigeon droppings and also investigate the antifungal potential of *Artemisia herba-alba* extract against these fungi. Our results indicated that *Alternaria alternata* was the most predominant fungus in the samples followed by *Penicillium citrinum*, *Aspergillus flavus*, *Fusarium oxysporum*, *Aspergillus niger*, *Mucor circinelloides*, *A. oryzae*, *Rhizopus stolonifer*, *Cylindrocarbon magnusianum*, *Nigrospora sphaerica* and *Cladosporium sphaerospermum*. These findings are in accordance with Maryam *et al*. (2013) showed that pigeon droppings associated different pathogenic fungal species including *Penicillium* spp. (n = 30), *Apergillus* spp. (n = 25), *Mucor* spp. (n = 18), *Rhizopus* spp. (n = 14), *Paecilomyces* spp. (n = 11), *Fusarium* spp. (n = 4) and *Cladosporium* spp. (n = 2). Moreover, Khosravi (1997) confirmed that *Aspergillus*, *Mucor* and *Penicillium* spp. were the most frequently isolates on the droppings of *Columba livia* pigeon. A total 44 fungal isolates belonging to *Aspergillus* spp. were isolated from eighteen samples of the dropping of blue rock pigeon. *Rhizopus* spp. (29.55%) was the predominant isolate while, *Penicillium* spp. (2.27%) was the least isolated fungus (Joshi *et al*., 2015). However, Abbas *et al*. (2017) indicated that *Penicillium* (19%) achieved the highest frequency on the dropping of pigeon. It was followed by *Mucor* (9%), *Rhizopus* (7%), *A*. niger (6%), *A. fumigatus* (5%), *A. flavus* (4%), *Cladosporium* (3%) and *Alternaria* (2%). On the other hand, Abulreesh *et al*. (2015) isolated *Cryptococcus neoformans* from 38 samples of pigeon droppings. Twenty species related to sixteen genera of fungi other than this fungus were recovered from the samples. Costa *et al*. (2010) also isolated this fungus and other species including *C. laurentii*, *Candida* spp., *Rhodotorula mucilaginosa* and *Trichosporon* sp. from the pigeon droppings. *Rhodotorula rubra* was recovered from feces of some birds indicates that it feed on sewage waters (Seo *et al*., 2006). The decline or variation in frequency of pathogenic fungi on bird droppings may be due to changing of the environmental conditions (Isfahani *et al*., 2001). In the present study, *A. herba-alba* extract showed the highest fungicidal activity against *C. sphaerospermum* and *C. magnusianum* fungi by minimal inhibitory concentration (MIC) value of 1.5% and minimum fungicidal concentration (MFC) values of 3 and 6%, respectively. While, *A. niger* and *R. stolonifer* were the most resistant fungi to this extract by MIC and MFC of 6 and 10%, respectively. These results were concurred with Kyeong *et al*. (1993) reported that, *A. herba-alba* extract has a high antifungal activity against wide spectrum of fungal species as *Aspergillus nidulance*, *Fusarium solani* and *Pleurotus ostreatus*. Moreover, the essential oils of this plant exhibited high potential action against *Aspergillus fumigatus*, *A. versicolor*, *A. niger*, *Trichoderma viride* and *Penicillium funiculosum* by MIC values of 0.12, 0.06, 0.25, 0.06 and 0.03 mg / ml, respectively (Janackovic *et al*., 2015). On the other hand, Dellavalle *et al*. (2011) revealed that other plant extracts as *Rosmarinus officinalis* and *Cynara scolymus* has amazing fungicidal activity against *Alternaria* spp. by MIC value 1.25 µg ml^{-1} and MFC of 1.25 and 2.5 µg ml^{-1}, respectively. MIC_s of less than 100 µg ml^{-1} suggest strong antifungal activity (Webster *et al*., 2008). In the current study, it is strongly observed that *A. herba-alba* extract recorded the same value 6% of the MIC and MFC against *A. alternata* and *F. oxysporum* fungi. This finding was supported by other obtained by Amini *et al*. (2012) stated that the oils of *Thymus vulgaris*, *T. kotschyanus* and *Zataria multiflora* have their MIC similar to MFC for *Pythium aphanidermatum* and *Fusarium graminearum* fungi. On the other hand, that is a variations in the degree of the extract antifungal activity. For example, McGuffin (1997) reported that *A. herba-alba* has moderate antifungal properties. Whereas, our results showed that it has a strong antifungal action against most fungal isolates. In general, plant does not contain an immune system so it synthesizes bioactive organic compounds, antifungal proteins and peptides to defend themselves from the pathogens as fungi (Broekaert *et al*., 1997; Morrisey and Osbourn, 1999; Selitrennikoff, 2001). The antimicrobial effect of it might not be due to the action of a single active compound, but the synergistic effect of all these compounds (Davicino *et al*., 2007). The quantity and quality of these compounds depends on the species of plant, plant tissue and also the environmental factors (Demo and Oliva, 2008).

CONCLUSION

The present study concluded that pigeon droppings is one of the most important damage caused by birds to the different crops reduce its quality and also potential source of pathogenic fungi which represent a real risk to plants. The extract of *Artemisia herba- alba* is considered a new alternative bioactive agent that inhibit the growth of these fungi. These findings support future research into the antimicrobial properties of *A. herba-alba* extract for their potential application in plant protection and other fields.

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النشاط ضد الميكروبي لمستخلص الشيح على الفطريات الممرضه في فضلات الحمام البري

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تسبب فضلات الطيور ضررا بالغا للمحاصيل الزراعيه حيث أنها تقلل من جودتها كما أنها تحمل الكثير من الفطريات الممرضه. أجريت هذه الدراسه بهدف عد و حساب تكرار الفطريات الممرضه في عينات فضلات الحمام البري. تم أيضا إختبار التأثير المضاد لمستخلص الشيح عند التركيزات 1,5٪ و 3٪ و 6٪ و 10٪ ضد هذه الفطريات تحت الظروف المعمليه. أوضحت النتائج أن فطر ألترناريا ألترناتا هو الأكثر تواجدا في هذه العينات و كان فطر كلادوسبوريوم سفائيروسبيرمم هو الاقل ظهورا و تواجدا. حقق مستخلص الشيح أعلى تأثير مضاد ضد فطري كلادوسبوريوم سفائيروسبيرمم و سليندروكاربون ماجنيسيانم بأقل تركيز مثبط 1,5٪ بينما كانت الفطريات ألترناريا ألترناتا و أسبراجيللس نايجر و فيوزاريم أوكسيسبوريم و ريزوباس ستولونفير هم الأكثر مقاومه لهذا المستخلص بأعلى تركيز مثبط 6٪.