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Preliminary Antifungal Study of Some Essential Oils of Three Medicinal Plants Against Fusarium Oxysporum f. sp. *ciceris*, and Aspergillus Niger

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Abstract: The aim of this study is to investigate preliminary antifungal activity of three essential oils by disc diffusion method, the essential oils of three plants used in traditional Sudanese medicine namely *Mentha spicata* (Lamiaceae), *Cymbopogon schoenanthus* L. (Poaceae), *and Citrus reticulata* (Rutaceae), was evaluated for their ability to inhibit the growth of selected phytopathogenic fungi *Fusarium oxysporum* f. sp. *ciceris* (causing wilt in chick pea) and the standard fungi *Aspergillus niger* was used for comparison. Essential oils were extracted by hydrodistilation using Clevenger apparatus. The experiment was carried out using disc diffusion method in different concentrations of essential oil on PDA culture at 25°C. The result showed essential oil of studied plants in all concentrations had completely inhibited growth for tested fungi.

Keywords: Antifungal, Essential Oil, Disc Diffusion, Phytopathogen

1. Introduction

Aromatic plants have great importance for food, cosmetics and pharmaceutical industries due to its essential oil content. The essential oils are aromatic substances which are obtained from various plant parts by steam or hydro distillation. They have been used since ancient times and many of which exhibit antibacterial [1], antifungal [2] [3] and antiviral [4] activity. Despite many of essential were substituted by synthetic ones, the demand for natural products is increasing. Pesticide residues in agricultural products and the incidence of resistance in plant pathogens against chemical pesticides demonstrates, the need for non-chemical methods including natural metabolites. Application of natural compounds on phytopathogens almost returns to the time the plant disease were ascribed. However, the search for natural products with antimicrobial activity was accelerated from a few past decades [5].

For example, monoterpene limonene has shown to possess deterrent and insecticide properties and corvine is used as a sprout inhibitor [6] [7]. Essential oils are complex and highly variable mixtures of constituents that belong to two groups: terpenoids and aromatic compounds. Hydrocarbons are almost always present in monoterpenes [8].

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition. Aromatherapy is the therapeutic use of fragrances or at least mere volatiles to cure, mitigate or prevent diseases, infections and indispositions by means of inhalation. [9].

The genus *Mentha* includes 25–30 species that grow in the temperate regions of Eurasia, Australia and South Africa. The mint species have a great importance, both medicinal and commercial. Indeed, leaves, flowers and stems of *Mentha spp.* are frequently used in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavour. In addition, *Mentha spp.* has been used as a folk remedy for treatment of nausea, bronchitis, flatulence, anorexia, ulcerative colitis, and liver complaints due to its anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, and anticatharrhal activities [10].

As a medicinal part, the leaf or the plant, and the drug are used officinal in many national pharmacopoeia. Owing to their complex chemical composition, the preparations made of *Mentha* species, show anti-inflammatory, antimicrobial, spasmodic, carminative, antioxidant properties. In the traditional and conventional medicine these preparations are used for better digestion, they improve the secretion of the gall. In addition, the essential oils isolated from *Mentha* species, are used externally, in the preparations which alleviate pains in the muscles and in the treatments against neuralgia, but the largest quantities are used in cosmetic and food industry for production of different sweets and beverages [11] [12].

Cymbopogon is an important genus of the Poaceae family is widely distributed in the tropical and subtropical regions of Africa, Asia and America. Comprised of 144 species, this genus is famous for its high content of essential oils which have been used for cosmetics, pharmaceuticals, and perfumery applications [13] [14]. Half bar, the common name of *Cymbopogon schoenanthus* (L.) spreng, is a common weed grows in southern Egypt and Northern parts of Sudan [15].

Citrus reticulata, comon name is mandarin is an evergreen tree growing up to 4.5 m by 3 m. this plant possess antioxidant effect [16] [17] and antimicrobial [17]. It is a source of natural compounds such as citruscridone [18] citriolide-A [19] isolimonexic acid methyl ether, in addition to the previously isolated limonin, deacetylnomilin, obacunone and ichangin, the marginal antimalarial activity of isolimonexic acid methyl ether is reported [20]. It has phenolic [17] and flavonoids among its components [21].

Fusarium oxysporum has been reported as soil born and seed borne pathogens containing more than 150 form species infected specific host [22] [23] [24]. *Fusarium oxysporum f. sp. ciceris* is an important fungal pathogen causing wilt in chickpea (*Cicer arietinum* L.) in many countries, and their losses ranging from 10-40% worldwide [25] [26] and is capable of causing 100% yield loss. In Sudan chickpea is one of the important legume crops and it is grown under irrigation system as a winter crop.

The aim of this study is to investigate preliminary antifungal activity of three essential oils by disc diffusion method.

2. Methods

2.1. Plant Materials

Plants were collected from different area, chamel's hay and mint from the experimental farm of our Agrotechnology Department, Shambat. And mandarin plant was bought from local market, Khartoum.

The plant names, and the plant parts used for the study are shown in (Table 2).

2.2. Method of Extraction

Plants Essential oils were extracted by Hydrodistillation using Clevenger Apparatus for 3 hours and dried using anhydrous sodium sulphate.

2.3. Preparation of Essential Oils for Bioassay

To prepare the stock solutions of essential oil, the essential oil was dissolved in methanol. Sample with known weights were further diluted with 10% of the methanol solvents used to prepare test samples, where the final concentration of the solvent was 0.625% (6.25 µl/ml).

2.4. Preparation of Fungal Suspension

F. oxysporum f. sp. *ciceris* obtained from Microbial Type Culture Collection, Central lab of Plant Pathology, Plant Protection Directorate. This fungus was grown on Sabouraud dextrose agar plate at 28°C and maintained with periodic sub-culturing at 4°C. The fungal cultures of *F. oxysporum* f. sp. *ciceris*, were maintained on Sabouraud dextrose agar (SDA), incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline,, to produce a suspension containing about 10^8 to 10^9 CFU/ml. The suspension was stored in the refrigerator at 4°C till used. While the suspension culture of the standard fungi *Aspergillus niger* was obtained from Microbiology and Parasitology Department (Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan.).

2.5. Biological Assay of Antifungal Activities

The determination of the antifungal effect of the essential oil was tested according to the disc diffusion method [27] [28]. Fungal suspension was diluted with sterile physiological solution to 10^8 cfu/ ml (turbidity = McFarland standard 0.5). Sterilized filter paper discs (Whatman No. 1, 6 mm in diameter) were placed on the surface of the SDA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position and plates were used in triplicate for each treatment. The diameters (mm) of the inhibition zones were measured.

3. Results and Discussion

The mean diameter of inhibition zones produced by the candidate essential oils against the studied fungi is presented as screening antifungal activity (100 µl/ml). The results were expressed in terms of the diameter of the inhibition zone according to Mukhtar and Ghori [29]. The result (in Table 1) showed that the candidate essential oil was more active against the *A. niger* (the inhibition zones ranging between (21-25 mm)), and ranging (15-19 mm) for *F. oxysporum* f. sp. *ciceris*. The Camel's hay essential oil was more active against both of them (*A. niger*. 25 mm; *F. oxysporum* f. sp. *ciceris,* 19 mm), the essential oils of mandarin and mint were more active against *A. niger*, (21 mm) while it was active against *F. oxysporum* f. sp. *ciceris*, (15 mm).

The inhibitory concentration of plants essential oil against fungal strains varied from plant extract to another. Moreover, the mean diameter of inhibition zones value of the same plant essential oil has changed according to the test organism (Table 2). The highest inhibition zone dimeter value, (27 mm), was obtained by camel's hay against *Aspergillus niger*. However, the result showed that the essential oil of studied plants in all concentrations had shown growth inhibition activity against *F. oxysporum* f. sp. *ciceris*.

The essential oil concentrations that considered to be more active on *F. oxysporum* f. sp. *ciceris* was (100/50/25 µl/ml) of camel's hay, (50/25 µl/ml) of mint, and (50/25/12.5 µl/ml) of mandarin. In the other hand the active concentrations against *A. niger*, was (100/50/25/12.5 µl/ml) in the camel's hay, and only one concentration (100 µl/ml) in mint and mandarin. The lowest concentration showed activity (6.25 µl/ml) found in camel's hay against *A. niger*, (17 mm), but in mint and mandarin the growth inhibition zone was (16 mm) on *F. oxysporum* f. sp. *ciceris*.

Recently the search for natural plant products with antimicrobial activity has been accelerated due to the appearance of chemical pesticides residues and the development of pesticides resistant. Thus the search for non-chemical methods, including plant for treatment of phytopathogens has gain revival interest.

However, it is difficult to manage fusarium wilt through application of chemicals because of the pathogen persists in soil as well as on seed and each form species has many races [23] [24] [25] [30].

There is a good evidence in the literature that the plant extract and essentials of some families possess moderate to good antifungal activates, among them is Lamiaceae family [5], Poacea [31]. The studied plants were used for the first time to inhibit growth of *F. oxysporum* f. sp. *ciceris*.

This result of the antifungal screening was similar to the previous research of El-Assiuty *et al*. [5] who reported that n-hexane extract of *C. schoenanthus* inhibited the growth of *Fusarium verticillioides* and *Aspergillus flavus*. Their results were comparable to our findings found of Camel's hay essential oil against F. oxysporum f. sp. *ciceris,* and *A. niger.*

The essential oil of mint had ability to inhibit growth of *F. oxysporum* f. sp. *radices - cucumerinum* [5]. The essential oil of *Citrus reticulata* had capacity to inhibit the production and germination of the spores of fungi e. g. *Fusaruim spp*. [32] [33].

The result of our MIC study showed that mandarin essential oils was the most active against *F. oxysporum* f. sp. *ciceris* up to 12.5 µl/ml while both mint and Camel's hay essential oils were more active at 25 µl/ml (Table 2). The result of the inhibitory activity of the studied essential oils against both fungi depended on concentration. This result was in line with the result of Simin, *et al*. [5] who reported antifungal activity of mint essential oil on *Fusarium oxysporum f. sp. radicis-cucumerinum*, that the effect of growth inhibitory of plant essential oils depend on the amount of essential oil and the incubation time. And also the effects depend on species of fungi [34].

Table 1. *Antifungal activity of essential oils (µl/ml) of selected medicinal plants against tested fungi.*

Family/Botanical Name/Vernacular name/Common name	Part used	Concentration µl/ml	DZ (mm)	
			***F. oxysporum* f. sp. cicer**	***A. niger***
Lamiaceae, *Mentha spicata*. Nanaa, Mint.	Leaves	100	15	21
Poaceae, *Cympobogon schouansus*. Mahareib, Hamarib. Chamel's hay	Leaves	100	19	25
Rutaceae *Citrus reticulata* Mandarin	Fruit peels	100	15	21

Table 2. *Antifungal activity of essential oils at different concentrations (50-6.25 µl/ml).*

Family/Botanical Name/Vernacular name/Common name	Concentrationµl/ml	Zone of inhibition (mm)	
		***F. oxysporum* f. sp. cicer**	***A. niger***
Lamiaceae, *Mentha spicata*. Nanaa, Mint	50	26	18
	25	26	18
	12.5	17	16
	6.25	16	13
Poaceae, *Cympobogon schouansus*. Mahareib, Hamarib. Camel's hay	50	20	27
	25	20	21
	12.5	15	21
	6.25	11	17
Rutaceae *Citrus reticulata* Mandarin	50	23	13
	25	22	11
	12.5	23	11
	6.25	16	10

4. Conclusion

It seems that the inhibitory activity of the essential oil of the three plant species was concentration dependant which showed a promising result to be promoted as antifungal candidate for plant disease management. The results indicated that the essential oils of studied plants had biological activity against *F. oxysporum* f. sp. *ciceris,* and *Aspergillus niger*, which may explain the traditional uses of the oils against plant fungal diseases, and that mean the essential oil of studied plant had a potential to be applied as antifungal agent. So more in vitro studies to comparison standard fungicide with this result, and more fields' experiments and investigation should be carried out to verify that result in vitro test and to determine the applicable dose, and also to develop a new and safe antifungal agent as a natural fungicides.

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Conflicts of Interest

The authors declare no conflict of interest.

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