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STUDY OF ALKALOIDS, PHENOLS AND TERPENES OF *MENTHA SPICATA* AS A FUNGICIDE AGAINST *RHIZOCTONIA SOLANI, SCLEROTINIA SCLEROTIORUM* AND *FUSARIUM OXYSPORUM*

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ABSTRACT
The present study aimed to evaluate the antifungal activity of alkaloids, phenols and terpenes of *Mentha spicata* and their effect on bio-efficiency of *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*, which isolated from infected cucumbers, chili pepper and eggplant respectively. Three concentration (5, 10, and 15 mg / ml) was prepared from crude extract, phenols, alkaloids and terpenes of *Mentha spicata*, then tested against *F. oxysporum*, *R. solani* and *S. sclerotiorum*, by using food poisoning method and fungal growth inhibition percentage was calculated individually. So bio-efficiency of fungi after treated with plants extract was studied such as radial mycelia growth, wet and dry mycelia weight. Results exhibited that *M. spicata* crude extracted at 5, 10 and 15% (v/v) concentrations had inhibitory growth actions 100% against *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Results demonstrated the fungus growth inhibition percentage increases with increasing concentration of phenols, alkaloids and terpenes. *R. solani* growth inhibition percentage rate was the highest by phenols, alkaloids and terpenes 94% at 5 and 10% concentrations, while at 15% concentrations, phenols, terpenes and alkaloids had completely inhibitory mycelium growth of all Fungus. Effect of phenols, alkaloids and terpenes on bio-efficiency of *F. oxysporum*, *R. solani* and *S. sclerotiorum* showed that the pathogenicity, mycelia growth rate, wet weight and dry weight percentage significantly reduction. Alkaloid was most effective in reducing the pathogenicity percentage of fungi to 12% compared to phenols and terpenes *R. solani* mycelia growth rate percentage was most affected by phenols, and terpenes, as the growth rate dropped to 68 and 65.66% respectively, while *S. sclerotiorum* mycelia wet weight percentage was most affected by alkaloids and terpenes, as the mycelia wet weight percentage dropped to 70 and 68% respectively, also mycelia dry weight percentage of *S. sclerotiorum* was significantly most affected by phenols, alkaloids and terpenes which recorded 57.33%.

KEYWORDS: antifungal activity, alkaloids, phenols, terpenes, *Mentha spicata* ,*Fusarium oxysporum, Rhizoctonia solani, Sclerotinia sclerotiorum.*

INTRODUCTION

Plant diseases are the main cause of the destruction of natural resources in agriculture. Specifically, the plant pathogenic fungi are considered the most damage to agricultural crops because of the high virulence and thus the main factor to huge economic losses. The dispersal of many phytopathogenic fungi, like *Phythium*, *Botrytis*, *Rhizoctonia*, *Phytophthora* and *Fusarium*, has extended during the previous few years because of changes introduced in farming, with damaging effects on crops of economic importance. In addition, not solely growing crops but also stored fruits are prey to fungal infections (Pandya *et al*., 2010). Chemical pesticides are among the most effective means and in the rapid reduction of plant diseases and thus reducing the losses. In any case, the unreasonable utilization of these chemical pesticides to control pathogenic growths for the plant, bringing about regularly unfriendly outcomes due to the subsequent harm to the earth and people, where it collects in the dirt and water, and also in leafy foods, and that expanded requests to diminish of utilization (Nicholson, 2007). An increasing need a day to use pesticides easily biodegradable and with high selectivity, because the chemical pesticides and theirresidues cause significant problems for the environment. As of late, the attention has expanded to scan for new sorts of pesticides that effect against a set number of target species and biodegradable into non-dangerous compounds (Gupta and Dikshit, 2010). One of the most encouraging intends to accomplish this objective is by the utilization of integrated disease management which based on the natural plant products derived from plants to control fungi that cause plant diseases, and these compounds is called botanical pesticides which are biodegradable faster than chemical pesticides which can degrade within a few days, and sometimes within a few hours, as well as most of them are selective against certain pathogenic fungi for the plant more than chemical pesticides, thus are considered to be eco-friendly (Sanjay and Tiku, 2009). Therefore, This study aimed to evaluate the effect of crude, phenols, alkaloids, and terpenes plant extracts from *Mentha spicata L.* in the inhibition of phytopathogens growth of *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum.*

MATERIALS & METHODS

Isolation and identification

A total of 250 samples of infected plants were collected from greenhouses in Baghdad. They were distributed as follows: 100 samples of cucumber plants, 75 samples of chili pepper plants and 75 samples eggplant plants. The infected plants were placed in polyethylene bags marked in the collection area, the glass house number and date of collection. After that, the infected plants were carried to the laboratory to isolate and diagnose the pathogen. *Fusarium oxysporum, Rhizoctonia solani and Sclerotinia sclerotiorum* isolated from the infected plants and died seedlings (cucumbers, chili pepper and eggplant respectively). Infected plants and died seedlings were placed under a running water tap for one hour individually, in order to clean them from sticking soil. After that the roots and crown district were sliced to little pieces (5mm) and its surface was sanitized by drenching in sodium hypochlorite (1% free chlorine) for 2min, flushed with sterile distilled water, And then placed in PDA plates and incubated for 4 days at 27±2 C°. The isolates were diagnosed as *F. oxysporum, R. solani* and *S. sclerotium* according to the morphological characteristic (Watanabe, 2002). All fungal isolates were cultured and maintained on autoclaved PDA supplemented with 100mg of chloramphenicol and incubated for 7days at 27 ±2C°. All Petri dishes used in the experiments had a diameter of 90 mm, were filled with 20 ml PDA, and were singly sealed with Parafilm, also slant culture were prepared and are all preserved in a refrigerator at 4C° (Burmeister, 2008).

Pathogenicity test

Fungal suspensions was prepared by taking mycelial plug (5mm from the growing margin of three days old cultures on PDA) from each of *F. oxsporum*, *R. solani* and *S. sclerotiorum* isolate and transferred to flask (250ml) containing 100ml autoclaved PDB, incubated for ten days at 27±2 C° on shaker incubator at 85 rpm and the mycelium developed in every flask (fluid culture) was isolated from fluid medium by filtration through Whatman filter paper (no.1) and washed with sterile distilled water. Five grams of fresh mat of each fungus was blended in 50 ml of sterile distilled water to produce a stiky suspension, and then these suspensions were used to inoculate the seeds. Local cress seeds untreated with fungicide were surface sterilized by immersion in sodium hypochlorite (1% free chlorine) for 2min, rinsed 3 times with sterile distilled water, dried by sterile filter paper, after that the sterile seeds were soaked in the fungal suspensions (for 12 hours and left to dry at room temperature before sowing, then inoculated seeds were placed on the sterile filter paper inside a petri dish containing moisture (by putting drops of sterile distil water), and leaves for 7 days at room temperature, then the percentage of seed germination was account according to this formula.

$$P = \frac{\text{Number of non germinating seeds}}{\text{Total number of seeds planted}} X\ 100$$

P = % pre-emergence infected seedlings

Collection of the studied plant

Mentha spicata leaves and stem were purchased from local market, then ground to semi powdered state by using grinder, then labeled and stored in the clean containers into refrigerator until use.

Preparation of plant extracts

Crude extract

According to, Deshmurkh and Borle (1975) 10g of dried leaves and stems of *Mentha spicata* successively extracted in a soxhlet extraction for 24 hours with the solvent (200 ml of 80% ethanol), And then extracted was placed in the oven at 50C° for two days in order to remove the solvent, Then extract the remaining were scraped and saved in refrigerator until utilize.

Phenols extract

The extraction was made depending on Ribereau-Gayon (1972) and Harborne (1984), 200 g of dried plant materials were separated into 2 identical quantities, one was blended with 300 ml of D.W. and the other one was blended with 300 ml of 1% HCl. After that samples were homogenized in electrical shaker for 5 min., and warmed by centrifuge. The supernatants were mixed with equal volume of butanol and saturated with amount of Nacl in separating funnel, 2 layers were appeared: the lower one (aqueous layer) was extracted with amount of butanol and concentrated by using oven. The upper layer was dried by electrical oven at 40°C. Dry material for both layers was disbanded with 5ml of 96% ethanol, the 2 layers were dried by electrical oven at 50°C, and then kept in the refrigerator until utilize.

Alkaloids extract

The extraction was prepared according to the method of Harborne (1984), and modifications of Al-Samrraei (1983) and Al-Mansour (1995) were considered. Dried plant materials (100g) were mixed with (350ml) of (4:1) ethanol: distilled water and homogenized by electrical shaker, and by muslin filtrated. The filtrate was concentrated to quarter of authentic volume through a filter paper in Bouknner funnel, after that the acidity of the filtrate was adjusted to become between 1 and 2 pH by adding drops (2% H_2SO_4). Then the filtrate was taken away with chloroform 3 times in the separating funnel; The pH of extract was adjusted to be between 9 and 10 by adding drops of NH_4OH in order to precipitate alkaloids, then extracted with chloroform-methanol (1:3) twice and with chloroform once, 2 layers arise, lower layer and upper layer (aqueous layer) was dried by electrical oven individually, and dried residue was extracted with methanol. Then saved in the refrigerator until utilize.

Terpenes extract

Based on Harborne (1984), 10g of dried plant material (leaves) sequentially extracted in a soxhlet extractor for 24 hours with (200 ml) chloroform. And to get rid of the solvent, the extract was placed inside electrical oven at 40C° for some times, then scarped and saved in refrigerator until utilize.

The isolated compound's indicators

Phenolic indicators

Ferric chloride and Potassium ferricyanide reagent

The purpose of this test is to detect general phenols. This test is accomplished by taking 2 equal size of aqueous solution of ferric chloride 1% and potassium ferric cyanide 1%. After that mixed with little amount of Phenols extract,

blue-green color appeared indicating that the test is positive (Harborne, 1984).

Alkaloid indicators

Tannic acid reagent

This acid was used to precipitate alkaloids (Al-Salami, 1998). 1% tannic acid was prepared, and then 1-2 ml of reagent was added to 5ml of the extract. The appearance of turbid white is a sign of the presence of alkaloids.

Terpenoid indicators

Acetic anhydride reagent

According to Al-Bid (1985), 1 ml of the extract was added to 1-2 drops of chloroform then 1 drop of anhydride acetic acid, and finally 1 drop of concentrated H_2SO_4. The appearance of turbid white is a sign of the existence of terpenes, and after a period of time the color change to be black-blue.

Evaluation of anti-fungal activity of the plant extracts

Food poisoning technique was used to determine inhibitory concentration (IC) {anti-fungal activity} of the studied plant extracts for plant pathogens *Fusarium oxysporum, Rhizoctonia solani and Sclerotinia sclerotiorum*, based on (Wang *et al*., 2005) as follows: Various volumes of the phenols, terpenes and alkaloids were prepared and each of these volumes was mixed apart with 100 ml of autoclaved PDA (Potato Dextrose Agar) when the temperature of autoclaved PDA temperature becomes approximately 45C° under sterile conditions (inside hood), so as to obtain the following concentrations of each extract (5, 10, 15%), then the mixture was shaken well and poured into sterilized petri dishes (90mm) and left to solidify in a sterile conditions. Mycelial plugs 5mm were cut from the growing margin of four days old cultures *Fusarium oxysporum, Rhizoctonia solani and Sclerotinia sclerotiorum* (which was the most virulence) and were transferred to the center of PDA plate containing plant extracts concentration (0, 5 and 15%) (v/v) individually, with three replicates per concentration and control. Radial mycelia growth rate of the assay fungus was measured after 7days 27 ±2C°, and the effect of each toxic extracts was expressed as percent inhibition of radial mycelia growth (growth inhibition %) as follows (Burmeister, 2008).

$$\text{Growth inhibition } \% = \frac{\text{CM} - \text{TM}}{\text{CM}} X100$$

CM= Control mycelium growth rate
TM= Treatment mycelium growth rate

Evaluate the effect of plant extracts on bio-efficiency of fungi:

The evaluation has been achieved through re-isolate *F. oxysporum, R. solani* and *S. sclerotiorum* selectively, by taken 5mm mycelia plugs from each the cultures that treated previously with plant extracts (phenols, terpenes and alkaloids) at 10% concentrations, then cultured on the PDA media free from plant extracts and incubated for 7 days at 27∓2 C°, after that was measured following bio-efficiency indicators (Thomson, 1989).

Pathogenicity test and mycelia growth rate

These tests have been accomplished, as already mentioned.

Wet and dry weight of fungal mycelium:

The fungal mycelium fresh and dry weight were prepared by taking two mycelial plugs (10mm from the growing margin of 7 days old cultures on PDA) from each *Fusarium oxysporum, Rhizoctonia solani and Sclerotinia sclerotiorum* and transferred to flask (250ml) containing 100ml PDB autoclaved, incubated for ten days at 27±2 C° on shaker incubator at 85 rpm. After that, growth culture was filtrated, wet or fresh weight recorded and dry weight was calculated after oven drying at 60 ^{0}C for 24 hours, then wet and dry weight percentage for each *Fusarium oxysporum, Rhizoctonia solani and Sclerotinia sclerotiorum* individually based on this formula (Djilani *et al*., 2006). Three replicates per treatment with the control, has been working in this experiment.

$$\text{Wet or dry mycelium weight\%} = \frac{\text{C} - \text{T}}{\text{C}} \text{ X } 100$$

C= Control wet or dry mycelium weight
T = Treatment wet or dry mycelium weight

RESULTS & DISCUSSION

Isolation and identification of fungi

Three species of fungi *F. oxysporum, R. solani* and *S. sclerotiorum* were isolated from Chili pepper, cucumber and eggplant respectively; purified, and identified after collection, depending on morphological characters and microscopically characters (Watanabe, 2002). Martin and Bull, (2002) mentioned that the early detection and diagnosis of the pathogen in plants and soils is essential for development of an effective disease control strategy.

Pathogencity test of fungi

Results of pathogenicity test showed that *S. sclerotiorum* was the highest pathogenic to the cress seeds compared with *F. oxysporium* and *R. solani* which recorded 89 %, 78 % and 59 % respectively (Figure 1).

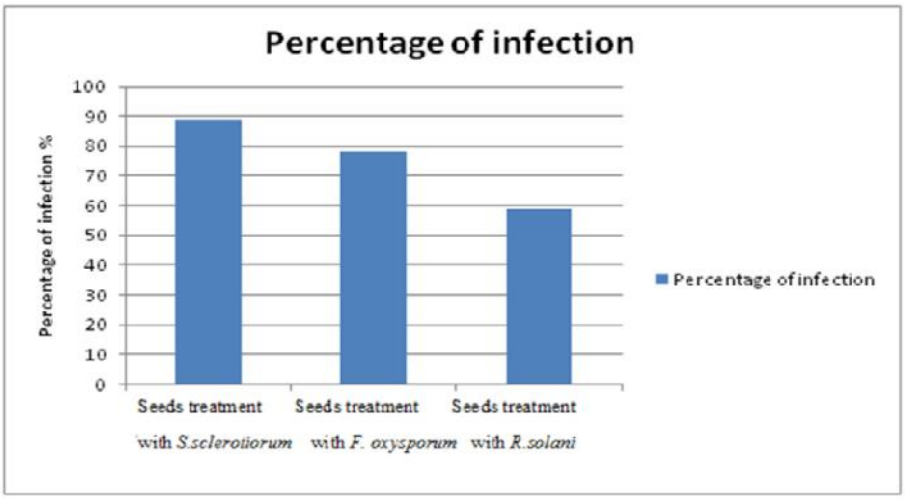

FIGURE 1: Percentage of infection

AL-jaafri *et al.* (2010) found that percentage of pathogenicity of *R. solani* 48 % and *F. oxysporum* 45.33%, compared with the present results which were *R. solani* 59% and *F. oxysporum* 78 % that means compatible with my results. While my results differed from Sharma *et al*. (2014), where they found that the pathogenicity of *S. sclerotiorum* against *Brassica juncea* seeds which ranged between 16.7 to 46.9%. The ability of cause's disease is due to the nature of the fungus which secretion of some toxic compounds that lead to kill of embryos and production cellulase, chitinase and proteinase that cause rot seeds (Rasmussen *et al*., 1989; Agrios, 2005). *S. sclerotiorum* produce a large assortment of cell wall degrading enzymes (CWDE), by secreting a wide variety of these CWDE's, *Sclerotina sp.* can macerate tissues, and break down cell wall components that greatly facilitates penetration of the host tissue. Breakdown of tissue also releases nutrients for use by the fungus (Bolton *et al*., 2006).

Plant Extracts

Crude plant extracts

The results showed that the yield of crude extracted from 10 gm of *M. spicata* were 2.5 gm. In a previous study were obtained 2 g crude extract of 10 g of *M. spiacta* (Fatiha *et al*., 2012), but Ullah *et al*., (2012) have pointed out that 1gm crude extract was obtained from 10 gm of *M. spiacta*

Phenol, Alkaloid and Terpene plant extracts

Phenols, alkaloids and terpenes were extracted from leaves and stem of *M. spicata*. Figure (2) showed that the phenol achieved higher yield 8% than terpene and alkaloids.

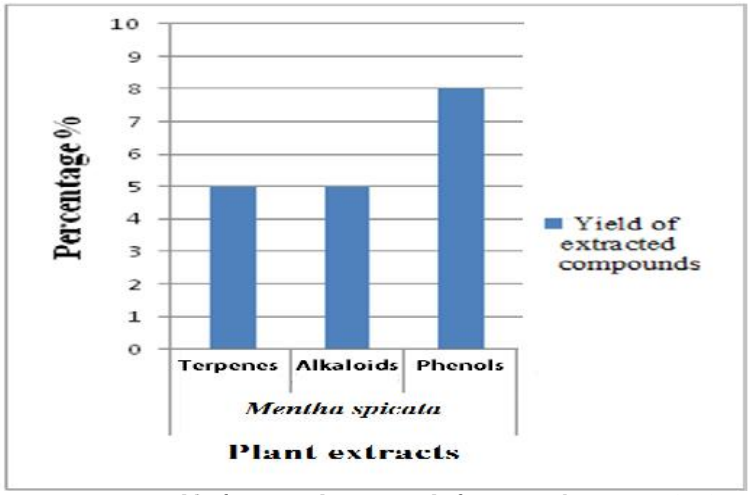

FIGURE 2: Yield of extracted compounds from *Mentha spicata* (gm).

Evaluation of anti-fungal activity of plant extracts

Crude plant extracts anti-fungal activity:

Results showed that crude extracted at 5, 10 and 15 % (v/v) concentrations from *M. spicata* had inhibitory actions 100 % against *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* mycelium growth (Figure 3).

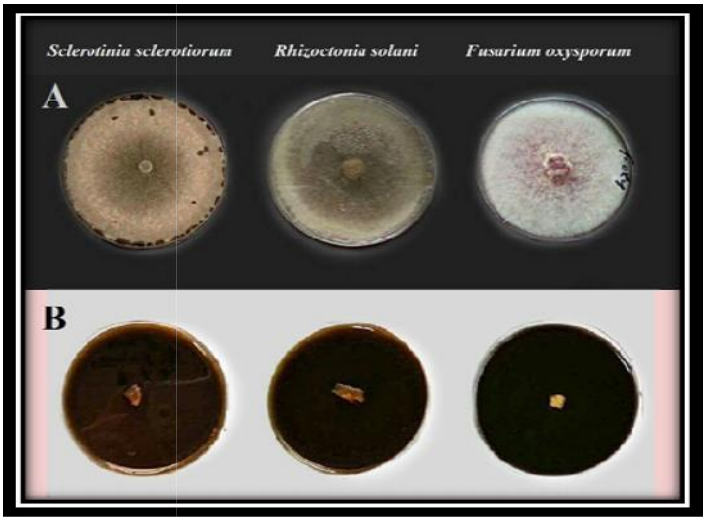

FIGURE 3: Effect of crude plant extracts on fungi at 5, 10 and 15 % (v/v) concentrations, A= control; B treated with crude plant extracts.

My results are consistent with the results of Wagle and Budathoki, (2012), which found that the growth inhibition of *Fusarium solani* was 100% at 5 and 10% concentration of *Mentha arvensis* crude extracts at 5% and 10% concentrations. Also, *Mentha longifolia* crude extract at 4 and 6% concentrations had inhibition 100% *Fusarium moniliforme* and *Alternaria citri* growth (Yazgi *et al*., 2015). High inhibitory efficacy of crude extract are attributable to the synergistic action of the components of crude extract, and other words, the plant have more than one active compounds influence together as synergistically and complementary against pathogenic fungi, this feature is not formed in the manufacturer materials (Al-Daami, 2001; Yazdani *et al*., 2011).

TABLE 1: Percentage of growth inhibition of *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* by using phenols, alkaloids and terpenes extracted from *Mentha spicata* at 5 and 10 % concentration.

	% growth inhibition at 5% concentration			% growth inhibition 10% concentration			Means	LSD P ≤ 0.05
Fungus	Phenol	Alkaloid	Terpene	Phenol	Alkaloid	Terpene		
F. oxysporum	85.33 CDb ±2.88	82.66 Db ±3.78	82.66 Da ±3.78	92.00ABb ±1.00	88.00BCa ±1.73	96.00Aa ±0.00	87.78	4.65
R. Solani	90.66 Da ±1.15	94.00 Ca ±0.00	84.00 Ea ±1.73	100.00 Aa ±0.00	98.33ABa ±1.15	97.00Ba ±0.00	94.00	1.72
S. sclerotium	68.00 Cc ±3.00	71.33BCc ±2.08	74.00 Bb ±0.00	84.33 Ac ±0.57	86.00 Ab ±1.73	86.33Ab ±2.517	78.33	3.48
Means	81.33	82.66	80.22	92.11	90.77	93.11	86.7	1.82
LSD P ≤ 0.05	4.98	4.98	4.80	1.33	3.12	2.90	1.82	

Capital and small letter s indicate to comparison in row and column respectively, similar letters are non-significantly differences between means at (p≤ 0.05), using (LSD test)

Phenol, Alkaloid and Terpene plant extracts anti-fungal activity

Results presented that the percentage of growth inhibition differed based to type; concentrations of plant extract components and fungal species. *F. oxysporum, R. solani* and *S. sclerotiorum* growth was inhibited by plant extracts components (phenol, alkaloid and terpenes) at whole concentrations that applied in this study. Results of growth inhibition percentage revealed significant differences among *F. oxysporum, R. solani* and *S. sclerotiorum* with each other, also showed that the growth inhibition percentage of *F. oxysporum, R. solani* and *S. sclerotiorum* increases with increasing concentration of phenols, alkaloids and terpenes (Table 1). *R. solani* growth inhibition percentage was the highest by phenols, alkaloids and terpenes, recorded rate 94 %, followed by *F. oxysporum* and *S. sclerotiorum* which recorded 87.78 % and 78.33 % respectively. Results in Table (1) revealed no significant difference between the terpenes and phenols in the rate of growth inhibition percentage of *F. oxysporum, R. solani* and *S. sclerotiorum* at the concentration of 10%, which recorded 93.11 and 92.11% respectively, whereas at 5% concentration there was no significant difference between alkaloids and phenols in the rate of growth inhibition percentage of *F. oxysporum, R. solani* and *S. sclerotiorum*. Results showed that phenols, terpenes and alkaloids extracted of *M. spicata* had completely inhibitory mycelium growth of *F. oxysporum, R. solani* and *S. sclerotiorum* at 15% concentrations. Bajpai and Kang, (2009) noted that the use of *Magnolia liliflora* terpenes at different concentrations (0.5, 1, 2, 3, 4 and 5 %) has inhibited the growth of *F. oxysporum, R. solani* and *S. sclerotiorum* with a range of 38 to 65.6 %, where the percentage of growth inhibition increases with increased concentration. Contradictory to the present findings, the wild basil terpenes at 5% was completely

inhibition mycelia growth of *Sclerotium rolfsi* (Benini *et al*., 2010). Gwinn, *et al.* (2010) reported that mint phenol at 10 % concentration was completely inhibition mycelia growth of *R. solani*, and this is consistent with the current study. In an Iraqi study, Al-Tikriti, (2011) who found that the best inhibition growth of *F. semitectum* was using thyme terpenes at the concentration of 1%, where recorded 71.2 %. Inhibition growth of *F. semitectum*, *R. solani* and *S. sclerotiorum* was completely by *Conocarpus lancifolius* phenol at 10% concentration (Al-Shatti *et al*., 2014). Phenols, terpenes and alkaloids extracted of *Melia azedarach, Cassia siamea, Murraya koenigii, Jatropha curcas* and *Delonix regia* showed high inhibitory activity agains *Aspergillus niger* growth at 10, 15 and 20% concentration (Danish *et al.*, 2015). In recent study, Qu *et al.* (2016) found that phenol had a great antifungal activity against *Pythium aphanidermatum, Botrytis cinerea* and *Rhizoctonia cerealis* at 10% concentration. Total alkaloids extracted from *Siparuna sessiliflora* displayed a minimal inhibition concentration (MIC) against *Fusarium oxysporum* at 0.2% concentration (Guevara *et al.*, 2016). Lopez-Meneses *et al.* (2017) revealed that essential oils (terpenes compounds) extracted from Cinnamon leaf and lemongrass Guatemala high inhibition growth of *Fusarium verticillioides* and *Alternaria tenuissima* at 0.5 and 1% concentration. Of the above studies, we note that most of them agree with the results of our current study, on the one hand, the increasing concentration of plant extracts leads to an increase in the growth inhibition of fungi, and on the one hand, the rate of inhibition of growth vary depending on the type of plant extract and fungus type. The antifungal action of terpenes generally depends upon their hydrophilic or lipophilic character (Knobloch *et al*., 1989). Also, Hiroshi (1994) mentioned that terpenes influence the membrane enzymes activities and interfere with respiratory pathways. These oils also caused degeneration of fungal hyphae, and hyphae appeared empty of cytoplasmic content materials (Zambonelli *et al*., 1996). Phenols influence on fungi depends on concentration. At low concentrations, phenols impact the enzymatic effectiveness, but at higher concentrations, they lead to protein denaturation (Prindle and Wright, 1977). The effect of phenols on fungal cells is due to the sensitivity of the cytoplasmic membrane, which is mainly composed of phospholipid bilayer for phenol, leading to increased permeability and finally damage mitochondrial membranes (Freiesleben and Jäger, 2014). Many studies emphasized that the antifungal effect of phenols are depended on their hydrophobicity and partition in the fungal plasmatic membrane (Trillas *et al*., 2006).

Evaluate plant extracts effect on bio-efficiency of fungi:

Pathogenicity of Fungi:

As shown in Table (3) the results indicated that the pathogenicity percentage of *F. oxysporum*, *R. solani* and *S. sclerotiorum* significantly reduced by phenols, alkaloids and terpenes of *Mentha spicata* compared with control.

TABLE 3: Influence of phenols, alkaloids and terpenes extracted from *Mentha spicata* at 10% concentration in pathogenicity percentage of *Fusarium oxysporum, Rhizoctoni solani* and *Sclerotinia sclerotiorum*

% Pathogenicity						
Fungus	Control	phenol	Alkaloid	Terpene	Means	LSD P ≤ 0.05
F. oxysporum	78.00 Ba ±5.00	18.00 Aa ±9.00	18.00 Aa ±0.00	24.00Aa ±5.19	20.00	11.98
R. Solani	59.00 Cb ± 0.00	27.00 Aa ±0.000	9.00 Ba ±0.00	9.00Bc ±0.000	15.00	0.00
S. sclerotium	89.00 Cc ± 6.00	27.00 Aa ±0.00	9.00 Ba ±9.00	18.00Bb ±0.00	18.00	10.38
Means		24.00	12.00	17.00	17.66	3.29
LSD P ≤ 0.05	5.33	10.38	10.38	5.99		

Capital and small letter s indicate to comparison in row and column respectively, similar letters are Non-significantly differences between means at (p≤ 0.05), using (LSD test)

While, phenols, alkaloids and terpenes revealed no significant difference between them in reduction the pathogenicity percentage of *F. oxysporum*, but alkaloids and terpenes significantly reduced the pathogenicity percentage of *R. solani* and *S. sclerotiorum* more than phenols. Also, the results showed that alkaloids were most effective in reducing the pathogenicity percentage of *F. oxysporum*, *R. solani* and *S. sclerotiorum* compared to phenols and terpenes. Contradictory to the present findings, Al-Husaini (2007) in their study, reported that *Mentha spicata* terpenes reduction pathogenicity percentage of *Phytophthora infestans* to 50 %, while our results were ranged between 9 to 24 %. In another study, Rowaished and Moniam, (2006) observed that *Azadirachta indica* alkaloids reduction percentage of infection of *F. oxysporum* to 18.6 % against Papaya seeds and this result similar to results of present study. *Mentha spicata* phenols had reduced pathogenicity percentage of *F. oxysporum* to 14.3 % against tomato seeds (Al-Husaini, 2007). In a similar result, AL-kaisi *et al*. (2011) found that the use of *Thymus vulgaris* terpenes at a concentration of 10% reduced the pathogenicity percentage of *Rhizoctonia solani* against seeding of *Raphanus sativus* to (29.4%). Okwu and Uchendu, (2009) mentioned that phenols, alkaloids and terpenes which extracted form plants possess antifungal and bactericidal properties. The effect of plant extracts on the reduction of fungal pathogenicity may be attributed to its ability to inhibit enzymatic activity, close ion channels, overlap with neurotransmission and lead to lack of electrical coordination (ataxia) in influenced fungus (Enyiukwu *et al*., 2014).

Fungal mycelia growth rate

Results presented that the percentage of mycelia growth rate differed based to type plant extracts components (phenol, alkaloid and terpenes) and fungal species. *F. oxysporum, R. solani* and *S. sclerotiorum* mycelia growth

rate percentage was reduction by all plant extracts components (phenol, alkaloid and terpenes) that applied in this study Table (4). Mycelia growth rate percentage revealed significant differences among *F. oxysporum*, *R. solani* and *S. sclerotiorum* with each other. *R. solani* mycelia growth rate percentage was most affected by phenols, and terpenes, as the growth rate dropped to 68 and 65.66% respectively, while *S. sclerotiorum* mycelia growth rate percentage was most affected by alkaloids and terpenes, as the growth rate dropped to 65.33 and 64% respectively; but we note no significant difference between the phenols, alkaloids and terpenes in reducing mycelia growth rate percentage of *F. oxysporum*, also, results showed that mycelia growth rate percentage of *F. oxysporum* was significantly less affected by phenols, alkaloids and terpenes (Table 4). plant extracts components (phenol, alkaloid and terpenes) seem to inhibit fungal growth through different mechanisms involving the inhibition of extracellular fungal enzymes (cellulases, pectinases, laccase, xylanase,…), inhibition of fungal oxidative phosphorylation, nutrient deprivation (metal complexation, protein insolubilisation) (Chérif *et al.*, 2007). Xiao *et al*., (2014) mentioned that total alkaloids of Cinchona had excellent antifungal activity, which inhibited growth rate of *F. oxysporum* to 60.53%. The total phenolic from *Mentha spicata* exhibited high antifungal activities (minimum inhibitory concentration) at 16 µg/mL concentration against *Aspergillus niger* and *Microsporum audouinii* (Alaklabi, *et al.*, 2016).

TABLE 4: Influence of phenols, alkaloids and terpenes extracted from *Mentha spicata* at 10% concentration on mycelia growth rate inhibition (%) of *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotinia sclerotiorum* on free PDA

% mycelia growth rate inhibition					
Fungus	phenols	Alkaloids	Terpenes	Means	LSD P ≤ 0.05
Fusarium oxysporum	37.33 Ac ± 4.50	31.33 Ab ± 5.13	37.33 Ab ± 5.13	35.33	9.85
Rhizoctonia Solani	68.00 Aa ±6.00	57.66 Ba ± 3.05	65.66 ABa ± 4.04	63.77	9.05
Sclerotinia sclerotium	54.33 Bb ± 3.78	65.33 Aa ± 3.21	64.00 ABa ±7.00	61.22	9.90
Means	53.22	51.44	55.66	53.44	2.71
LSD P ≤ 0.05	9.69	7.82	11.04		

Capital and small letter s indicate to comparison in row and column respectively, similar letters are Non-significantly differences between means at (p≤ 0.05), using (LSD test)

Wet and dry weight of mycelium

As shown in Table (5) the results indicated that the mycelia wet weight percentage of *F. oxysporum, R. solani* and *S. sclerotiorum* significantly reduced by phenols, alkaloids and terpenes of *Mentha spicata. R. solani* mycelia wet weight percentage was most affected by phenols, and terpenes, as the mycelia wet weight percentage dropped to 58 and 59% respectively, while *S. sclerotiorum* mycelia wet weight percentage was most affected by alkaloids and terpenes, as the mycelia wet weight percentage dropped to 70 and 68% respectively; but we note no significant difference between the phenols, alkaloids and terpenes in reducing mycelia wet weight percentage of *F. oxysporum*, also, results showed that mycelia wet weight percentage of *F. oxysporum* was significantly less affected by phenols, alkaloids and terpenes which recorded 33.77%, while mycelia wet weight percentage of *S. sclerotiorum* was significantly most affected by phenols, alkaloids and terpenes which recorded 64.66% (Table 5).

TABLE 5: Influence of phenols, alkaloids and terpenes extracted from *Mentha spicata* at 10% concentration on mycelia wet weight reduction % of *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotinia sclerotiorum* on free PDB.

% Wet Weight reduction					
Fungus	Phenol	Alkaloid	Terpene	Means	LSD P ≤ 0.05
Fusarium oxysporum	39.00 Ab ±4.58	30.00 Ac ±4.35	32.33 Ac ±5.85	33.77	9.945
Rhizoctonia Solani	59.00 Aa ±3.00	52.00 Bb ±1.00	58.00 Ab ±2.64	56.33	4.756
Sclerotinia sclerotium	56.00 Ba ±2.64	70.00 Aa ±1.00	68.00 Aa ±1.73	64.66	3.826
Means	51.33	50.66	52.77		1.55
LSD P ≤ 0.05	7.01	5.28	7.68		

Capital and small letter s indicate to comparison in row and column respectively, similar letters are Non-significantly differences between means at (p≤ 0.05), using (LSD test)

In case of mycelia dry weight percentage, the results revealed that phenols, alkaloids and terpenes of *Mentha spicata* significantly reduced the mycelia dry weight percentage of *F. oxysporum, R. solani* and *S. sclerotiorum*. Phenols were most effective in reducing mycelia dry weight percentage *R. solani* and *S. sclerotiorum* compared to *F. oxysporum* which recorded 55.33, 54.00 and 33.66% respectively; while alkaloids and terpenes were most effective in reducing mycelia dry weight percentage of *S. sclerotiorum* which recorded 58.33 and 59.66 respectively,

but we note no significant difference between the phenols, alkaloids and terpenes in reducing mycelia dry weight percentage of *F. oxysporum*, also, results showed that mycelia dry weight percentage of *F. oxysporum* was significantly less affected by phenols, alkaloids and terpenes which recorded 31.99%, while mycelia dry weight percentage of *S. sclerotiorum* was significantly most affected by phenols, alkaloids and terpenes which recorded 57.33% (Table 6).

TABLE 6: Influence of phenols, alkaloids and terpenes extracted from *Mentha spicata* at 10% concentration on mycelia Dry weight reduction% of *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotinia sclerotiorum* on free PDB.

% Dry Weight					
Fungus	Phenol	Alkaloid	Terpene	Means	LSD P ≤ 0.05
Fusarium oxysporum	33.66 Ab ±5.85	30.00 Ac ±2.64	32.33 Ac ±4.93	31.99	9.34
Rhizoctonia Solani	55.33 Aa ±2.51	44.66 Bb ±3.21	50.66 Ab ±2.08	50.21	5.28
Sclerotinia sclerotium	54.00 Ba ±3.46	58.33 ABa ±1.52	59.66 Aa ±1.52	57.33	4.70
Means	47.55	44.33	47.55		2.22
LSD P ≤ 0.05	8.37	5.11	6.42		

Capital and small letter s indicate to comparison in row and column respectively, similar letters are Non-significantly differences between means at (p≤ 0.05), using (LSD test)

In general, the high concentration of plant extracts reduces the biomass (wet and dry weight of the mycelium) of the fungus by breaking the fungus mycelium, where these extracts penetrate the fungus mycelium and destroy the cell membrane; the cells then decompose and die (Thanaboripat *et al*., 2007). In results similar to our current study, Haikal, (2005) found pronounced decrease in fungal biomass, when used phenols of *Ziziphus spina* against *Fusarium solani* at 5, 10, 15, 20 and 25% concentrations. So, Hadi *et al*. (2013) studied antifungal activity of *M. piperita* against *Penecillium digitatum* and found that *M. piperita* alkaloid remarkably inhibition of the mycelial weight. *Moringa oleifera* terpenes have remarkably inhibition of the dry mycelial weight of *R. solani, F. oxysporum, F. solani* and *Sclerotinia rolfsii* at 10% concentration (El–Mohamedy and Abdalla, 2014). Saeed, *et al.* (2016) mentioned that leaf extracts of *Syzigium cumini* and *Melia azedarach* reduced *Sclerotium rolfsii* biomass up to 97% and 86% respectively.

CONCLUSION

This study concludes that alkaloids, phenols and terpenes of *Mentha spicata* have a high antifungal activity and can be used to control plants pathogenic fungi *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotinia sclerotiorum.*

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