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Evaluation of two aqueous plant extracts in protection of wheat against phytopathogen fungus, *Helminthosporium rostratum*

Ashour Eman ^{1,*} and Al-Otibi Fatimah ²¹ Department of Microbiology, Faculty of Agriculture, Mansoura University, Mansoura, Egypt.² Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 22452, Riyadh, 11495, Kingdom of Saudi Arabia.

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Abstract

In the field and greenhouses, chemical fungicides is the most commonly tool used for controlling fungal disease. Although chemical usage has been very effective in controlling fungal plant diseases, some major problems threaten to limit the continued use of fungicides. In the present research, extracts of *Ziziphus* and *Rumex* plants were chosen based on both traditional usages suggestive of antifungal activity. In addition, toxic fungal constituents of higher plants are generally thermo-labile, therefore, an aqueous extract was preferred for prevention any adverse effects. Phytochemicals of both plant extracts were detected by using HPLC, various phenolic and flavonoids compounds were detected. *Ziziphus* and *Rumex* extract concentrations that inhibited 50% of fungal growth (IC₅₀) were 25 and 50 mg/ml, respectively. *In vivo*, results of morphological parameters of plant growth revealed that aqueous of tested plant extracts had allelopathy effects on seed germination. Increasing of plant roots length was appeared in the case of *Ziziphus* coated seed (either uninfected or infected). Both *Ziziphus* and *Rumex* aqueous extracts had positive synergistic effect where shoot lengths significantly increased. Dry weight of wheat spike significantly increased in *Ziziphus* and *Rumex* extracts treatments. Total proteins content of wheat plant were higher in all treatments than infected wheat plant with *H. rostratum*, on contrary, proline content. The results serve and support the new trend in biological control, where the aqueous extracts of *Rumex* and *Ziziphus* plants clearly reduced the effectiveness of fungal infection and improved all growth parameters of wheat seedlings (especially in the case of tri-treatments).

Keywords: *Rumex*; *Ziziphus*; Aqueous extracts; Fungi; *Helminthosporium*; Antifungal activity

1. Introduction

Rumex vesicarius is an edible plant and eaten fresh, or cooked. Green leafy vegetables are good sources of vitamins, minerals, and fibers. Moreover, *R. vesicarius* L. has many important medicinal uses [1]. In addition, *Ziziphus spina-christi* (L.) is a deciduous tree and native to the warm-temperate and subtropical regions. It has very nutritious fruits and usually eaten fresh. It significantly contributes to the improvement of human health in terms of cure and prevention of diseases [2].

Among the most frequently occurring fungal species in the rhizosphere, *Helminthosporium* is cosmopolitan fungus found in soils. It causes leaf blight disease of wheat. If infection occurs early in the crop cycle and conditions remain favorable for development, complete defoliation is possible; major reductions in yield and severely shriveled kernels will then results [3]. Control of such diseases mainly depends on fungicidal treatments. However, the use of synthetic fungicides may cause hazards to human health and may directly increase environmental pollution. In addition, some fungicides may not readily be biodegradable and tend to persist for years in the environment. Moreover, some fungal strains have developed resistance to these chemicals.

* Corresponding author: Ashour Eman Email: eashour@mans.edu.eg

In addition, wheat, *Triticum aestivum*, is one of the most important field crops in the Kingdom of Saudi Arabia. Agriculture and Water ministries estimate that 87,280 hectares of wheat will be cultivated, and that production will reach 500,000 MT in 2019/20 [4]. Unfortunately, this crop species was attacked by certain pathogens, especially fungi, in all wheat-growing regions of the Kingdom, which affected its growth and yield.

In recent years, public pressure to reduce the use of synthetic fungicides in agriculture has increased. Concerns have been raised about both the environmental impact and the potential health risk related to the use of these synthetic compounds. Consequently, natural products as plant extracts provide unlimited opportunities for discovering novel antifungal agents of natural origin, high efficacy, restricted toxicity and safety for humans, animals, host plants and ecosystems with low production cost.

Because of these associated problems, researchers are now trying to use environmentally safe alternative methods for fungal control such as biological control or extract of many allelopathic plants.

Plant extracts, especially aqueous, were preferred in the present study for many reasons and referring to the previous researches. Firstly, although much of the antifungal research conducted to date has assessed ethanol or methanol extracts while few studies have utilized aqueous extracts, despite the fact that aqueous extracts a closer approximation of the traditional medicine plants usage.

Most of the researches carried out were based on the use of solvents less polar than water such as hexane, diethyl ether, ethyl acetate, acetone, ethanol and methanol. Therefore, it is not known, from the literature, the extent to which water extracts of various herbs contain valuable bioactive ingredients. However, a water extract was found recently to be richer in polar phenols than acetone, ethanol and methanol extracts of the same plant material [5].

In addition, alcoholic extracts provide a more complete extraction, including less polar compounds, and many of these extracts have been found to possess antifungal properties. However, success with aqueous extracts have been observed [6].

Furthermore, in cases where alcohol extracts have previously shown antifungal potential, should the aqueous extract of the same plant also appear activity it would suggest that the active components might be the more polar compounds. Moreover, polar plant secondary metabolites often rapidly degraded in soil, they generally have no mammalian toxicity, and they can have an effective role in sustainable agriculture [7].

Thus, the present study focused firstly on phytochemical properties of chosen plants to use their aqueous extracts as environmentally safe alternative method for controlling plant pathogenic fungi. Then, the antifungal activity of the aqueous plants extracts against plant pathogenic fungus tested *in vitro* and apply effective concentration *in vivo*.

2. Material and methods

2.1. Plant material

The used plants were *Rumex vesicarius* L. and *Ziziphus spina-christi* L. Wild, which collected from Riyadh region, Kingdom of Saudi Arabia.

2.2. Fungal strain

Phytopathogenic fungal strain, *Helminthosporium rostratum* was kindly obtained from College of Food and Agricultural Sciences, King Saud University, Riyadh, King of Saudi Arabia (KSA). Fungal Culture was grown regular by sub-culturing on Czapek Dox agar medium and maintained on PDA slants at 4 °C.

2.3. Preparation of aqueous plant extracts

The used plant parts were the leaves from *Z. spina-christi* and the aerial parts (shoot) from *R. vesicarius*. Thoroughly cleaned plant materials were shade dried and then collected, as fresh dry plant materials, for usage.

The fresh dry plant materials (both *Rumex* and *Ziziphus*) were crushed then 25 g were soaked in 300 ml distilled water overnight in closed container at room temperature, then transferred on rotary shaker another overnight. The macerates were filtered by gravity twice, first through cotton and then through layers of tissue paper. Finally, the remaining filtrates were left over night in cleaned metallic trays for drying and then collected whole the dried solid materials in

clean and dry glass container and keep it close in the refrigerator till use. The plant aqueous extract concentrations were prepared as gram per milliliter. The crude aqueous extracts were firstly centrifuged to remove any suspended solid materials, subsequently, was sterilized by filtration using a bacteriological sterile Millipore filter 0.45 μm then 0.22 μm pore size (Sartorius 18081-E, Satolab RF). Each stock sterile filtered extract was preserved aseptically in refrigerator until needed.

2.4. Growth inhibition assay

The agar dilution method described by Sisti *et al.*, [8], with slight modification, was used for determining the inhibition of mycelial growth of tested fungus by both plant extracts. Sterilized aqueous extract was mixed aseptically with molted cooled (40-45 °C) Czapek Dox agar medium, the plates left awhile for solidification. Subsequently, for the bioassay, 6 mm disc taken from the edge of activity growing colonies of the tested fungal strain was placed in the center of Czapek Dox agar plates that containing specific concentration of plant extract (as treated) or containing the basal medium only served as a Control. All plates were incubated for 7 days at 25 ± 2 °C. Each assay was replicated three times. Radical mycelial growth was evaluated by calculating the mean of two perpendicular colony diameters for each replicate. The values were expressed in millimeters diameter and was calculated as percentage of mycelial growth inhibition according to the following formula:

$$\text{Growth Inhibition (\%)} = \frac{\text{DC}-\text{DT}}{\text{DC}} \times 100$$

Where: **DC**= colony diameter of control (untreated), and **DT** = colony diameter of treated ones [9].

2.5. Greenhouse experiment

2.5.1. Preparation of conidial suspension

Conidial suspension of tested phytopathogen, *Helminthosporium rostratum*, was obtained from 10 day-old culture and mixed with sterile distilled water to obtain a homogenous conidial suspension of 10^6 spore/ml. The fungal inoculum was thoroughly added to the pot soil surface at the rate of 0.5 % (v/w) per pot, and then covered with a thin layer of sterilized soil.

2.5.2. Preparation of wheat seeds

Wheat seeds were surface disinfested in 4 % sodium hypochlorite solution for 10 min and rinsed thoroughly with sterile distilled water. For preparation of infected seeds by *H. rostratum* and plant extract coated seeds, to Petri dishes containing sterilized wheat seeds, conidial suspension and 1 % of aqueous plant extracts were added, respectively, then left to dry and used in sowing. Coated wheat seeds by chemical fungicide (Vitavax) were obtained from National Center for Research on Agriculture and Livestock in Riyadh, KSA.

2.5.3. The treatments of pot experiment

The experiment design in a randomized complete block with five replicates of each treatment was carried out in the greenhouse at college of Science, King Saud University. Ten wheat seeds per pot were planted in 15 cm diameter plastic pots containing 1000 g sterilized soil. The soil consisted of peat moss and sand in a 1:1 ratio. The moisture content was maintained at 60-70 % of the soil water holding capacity by continual irrigation with distilled water. All pots were incubated in the greenhouse, five replicates of each treatment.

The treatments of pot experiment were as follows (14 treatments):

Control (Untreated)	FI + VCS
FI (Fungal Infection)	FI + RE
VCS (Vitavax Coated Seeds)	FI + ZE
RE (Rumex Aqueous Extract)	FI + RCS
ZE (Ziziphus Aqueous Extract)	FI + ZCS
RCS (Rumex Coated Seeds)	FI + RE + RCS
ZCS (Ziziphus Coated Seeds)	FI + ZE + ZCS

At the end of the experiment (after eight weeks from planting), the plants were gently picked up and removed adhering soil particles. Some parameters were recorded on the fresh plants, and then the plants were dried at 70 °C in an aerated oven to constant weight, ground to fine powder and kept for chemical analysis.

Wheat seeds germination (%) was recorded in pots before picking up the plants. Then, different morphological parameters of plant growth [root length (cm), shoot length (cm), leave number, spike weight (g), plant fresh weight (g), plant dry weight (g)] were assessed. In addition, biochemical activities of wheat plants (total proteins and the amino acid proline) were determined.

2.6. Chemical Analysis

2.6.1. Total proteins determination

The total protein contents were measured by using Folin-Ciocalteu reagent according to the procedure by Daughaday *et al.*, [10].

2.6.2. Proline determination

Free proline was estimated according to the method described by Bates *et al.*, [11].

2.6.3. Phytochemical analysis

Total content of phenolic and flavonoid compounds of the *Rumex* and *Ziziphus* plant samples were analyzed according to method of Goupy *et al.*, [12] by using HPLC analysis.

2.7. Data Analysis

Statistical analyses of the data were undertaken using STATISTIX 10 computer program (analytical software). The experiments were analyzed by one-way analysis of variance (AVONA) and the significance of the differences between means was calculating using the Duncan test. All values given are means of at least 3 replicates. The results were presented as mean \pm standard error (SE).

3. Results

3.1. Phytochemical Characterization of Plant Extracts

Data represent in Figure (1) indicate that various phenolic compounds were detected in both plant extracts but qualitatively and quantitatively varied. Catechol, caffeic acid and cinnamic acid were found only in *Rumex* extract, while coumarin, chrysin and syringic acid were detected in *Ziziphus* extract only. Salicylic and benzoic content scored highest values (623.54 and 401.99 $\mu\text{g/g}$ respectively) in *Rumex* extract. Otherwise, ferulic and benzoic acid recorded highest values in *Ziziphus* extract, 331.48 and 234.67 $\mu\text{g/g}$ respectively.

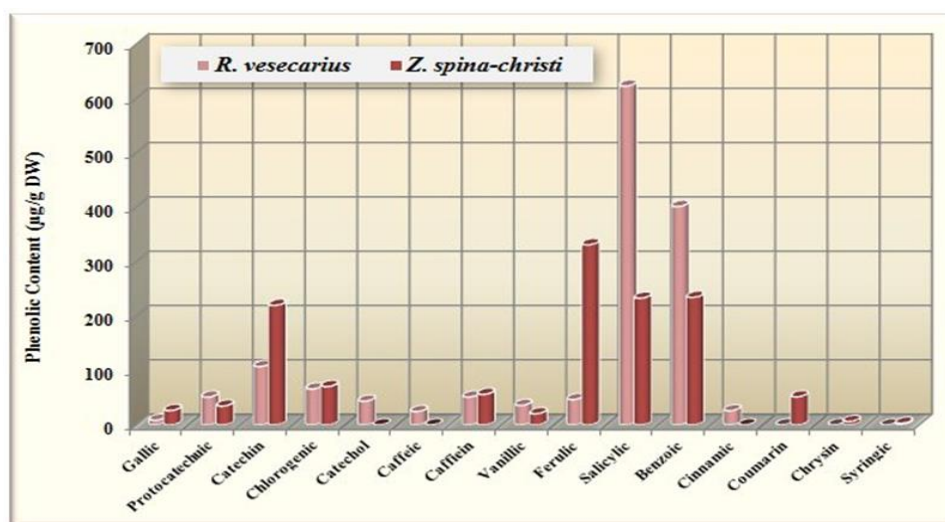


Figure 1 Phenolic compositions ($\mu\text{g/g}$) of plant extracts

Among all detected flavonoid compounds, rutin, quercitrin, narenginin and kampferol were detected in both *Rumex* and *Ziziphus* plants. Rutin content were reached to 1559.01 and 1069.55 $\mu\text{g/g}$ in *Ziziphus* and *Rumex* extract, respectively. In addition, rosmarinic was found only in *Rumex* and hispertien was detected only in *Ziziphus* (Figure 2).

Most of the phenolic and flavonoids compounds were detected in both *Rumex* and *Ziziphus* extracts. Nevertheless, when consider the total content of flavonoids pattern (its derivatives), *Ziziphus spina-christi* appeared richer flavonoids where its total contents reached to 2632.91 $\mu\text{g/g}$, mostly two fold its quantity in *Rumex vesicarius* (1240.15 $\mu\text{g/g}$). However, total phenolic content of both plant species showed a slight variation, where found 1486.98 and 1288.49 $\mu\text{g/g}$ of *R. vesicarius* and *Z. spina-christi*, respectively.

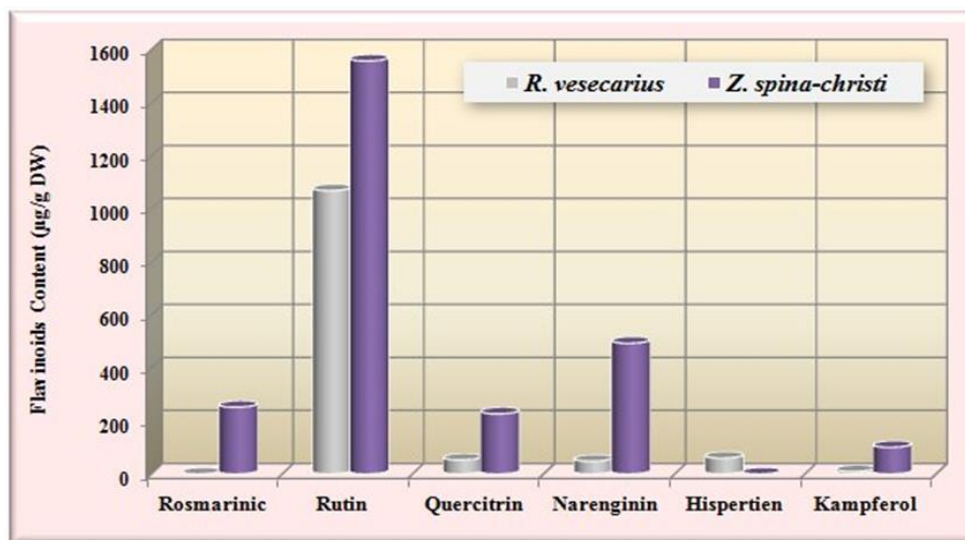
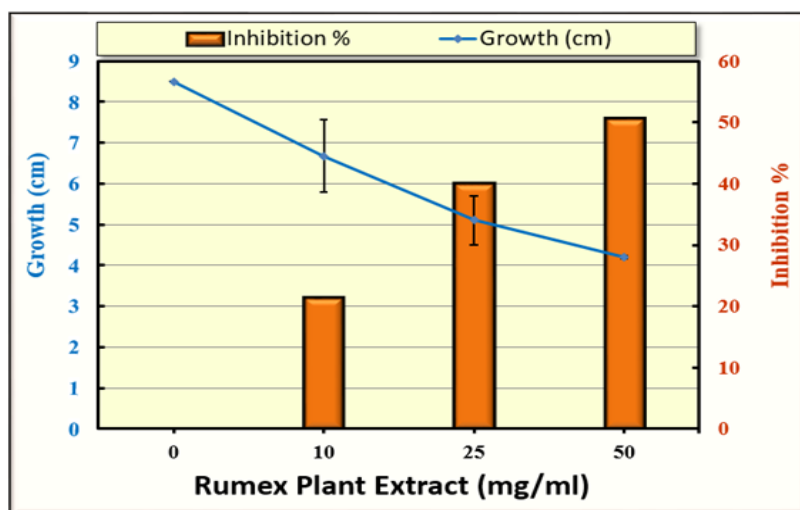


Figure 2 Flavonoid compositions ($\mu\text{g/g}$) of plant extracts

3.2. Effect of aqueous plant extracts on fungal growth *in vitro*

Antifungal activity of the aqueous extracts of *R. vesicarius* and *Z. spina-christi* at various concentrations (10, 25, 50 mg/ml) was evaluated separately on Czapek Dox agar medium against saprophytic fungus, *H. rostratum* for the possibility of enhancing their application in soil for inhibiting such mycophytopathogen. Data illustrated in Figure (3) reveal that lowest concentration (10 mg/ml) of *Rumex* and *Ziziphus* extract significantly reduced the growth of *H. rostratum*. Then fungal growth obviously decreased by increasing of plant extract concentrations. *Rumex* and *Ziziphus* extracts concentration that reduced $\sim 50\%$ of fungal growth (IC_{50}) were 50 and 25 mg/ml, respectively. *Ziziphus* extract appeared a significant degree of activity higher than *Rumex* extract against fungal growth.



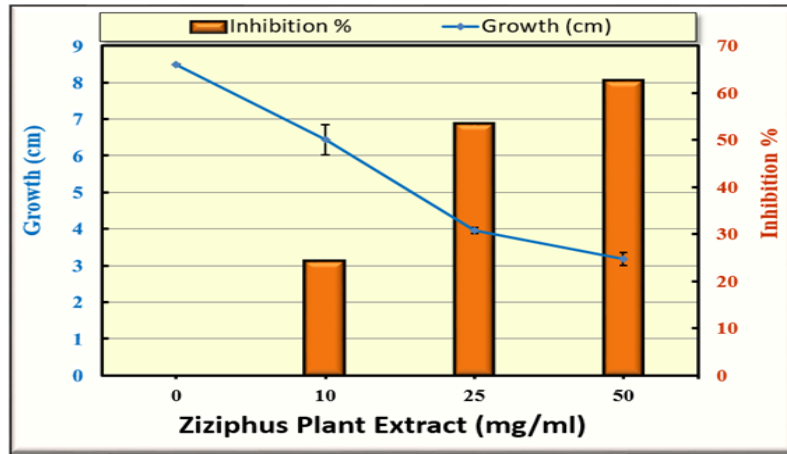


Figure 3 Influence of aqueous plant extracts at various concentrations on mycelial growth diameter (cm) of *Helminthosporium rostratum* on Czapek Dox agar medium after 7 days of incubation at 25 °C. Data represented as mean ± standard error.

Aqueous extracts of Rumex and Ziziphus were not only reduced the fungal colony diameter but also caused obvious changes in colony morphotype. The plates of *H. rostratum* in Figure (4) indicate that fungal hyphae formed very weak and the hyphae grew in the vertical expansion. Colony's color became darker and pigmented comparing to untreated ones.

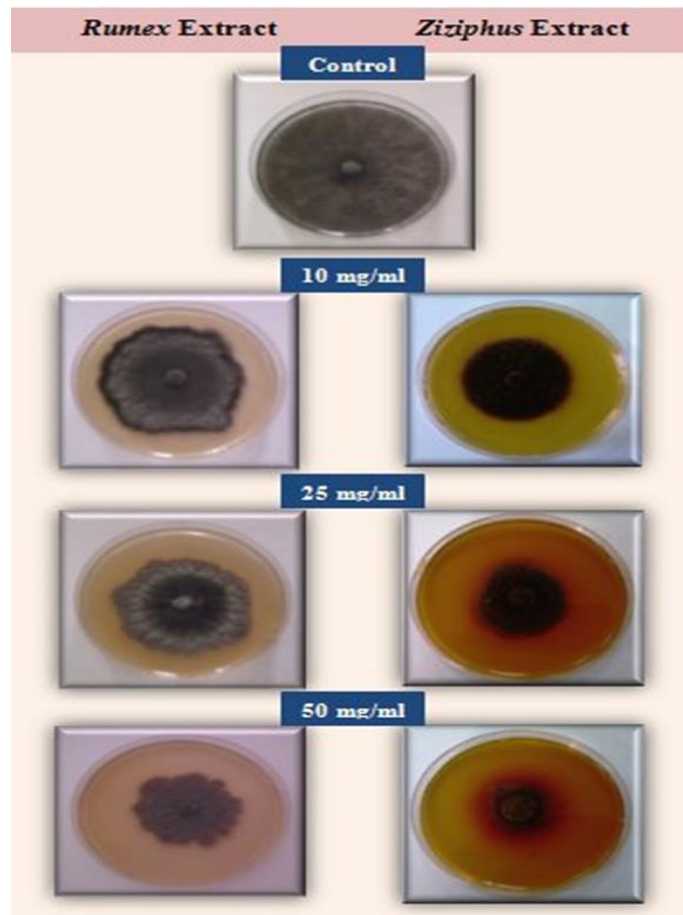


Figure 4 Photographs showing changes in growth of *Helminthosporium rostratum* in plates of Czapek Dox agar medium amended with different concentrations of aqueous plant extracts.

3.3. Antifungal activity of aqueous plant extracts *in vivo*

To assess the efficiency of *Rumex* and *Ziziphus* aqueous extracts in protection of wheat plant seedlings against plant-pathogenic fungus, *H. rostratum*. The experiment designed and conducted in pots under greenhouse conditions for eight weeks with 14 treatments. According to the above obtained results of the most effective concentration of both *Rumex* and *Ziziphus* aqueous extracts against *H. rostratum* growth, 50 mg of each aqueous extract (either per pot or for seeds coating or both) and compared with synthetic fungicide-coated (vitavax) wheat seeds.

Data represent in Table (1) show the allelopathy effects of aqueous extracts in pots on seed germination (%) of wheat plants which was 100 % in the cases of bi-treatment FI+RCS and FI+ZCS. However, seed germination percent was recorded the lowest value (60 %) in the cases of *Rumex* and *Ziziphus* extracts treatments (FI+RE & FI+ZE).

Among all treatment, roots length of wheat plants were significantly varied. *Ziziphus* coated seed (either uninfected or infected) showed significant high values (17.54 and 17 cm respectively) comparing with vitavax coated seed (either uninfected or infected) 17.2 and 16.0 cm respectively. However, all treatments with *Rumex* aqueous extract of wheat plants caused significantly shorten in roots length.

The shoot lengths of all infected wheat treatments with both *Ziziphus* and *Rumex* aqueous extracts were significantly increased comparing with the infected wheat treatment and ranged 16.11-30.37 cm. It obviously found that shoot length of tri-treatments in both cases of *Ziziphus* (FI+ZE+ZCS) and *Rumex* (FI+RE+RCS) aqueous extracts were the most effective treatment in the reduction or prevention fungal infection. These results explain that there is a positive synergistic effect for shoot length in the combined treatments against fungal infection.

The impact of fungal infection obviously appeared in lowering leaves number per plant. On contrary, wheat coated seed with vitavax gave highest number of leaves per plant. However, the means values of leaves number for all treatments slightly varied but there were no significant differences.

All the treatments clearly affected in weight of wheat spike and ranged from 0.021 to 0.240 g (Table 1). Due to fungal infection, wheat spike significantly reduced and gave a lowest value. However, all fungal infection treatments in the presence of both *Ziziphus* and *Rumex* extract, spike weight significantly increased.

Data presented in Table (1) show that plant wet weight significantly varied among various treatments. The wet weight of wheat seedling ranged from 0.099 to 0.382 g, about four fold higher. The lowest value recorded in the case of single-treatment of *H. rostratum* infection (FI) while the highest values of seedling wet weight significantly exhibited 0.382, 0.371 and 0.342 g in the case of tri-treatment FI+RE+RCS, wheat coated seed with vitavax (VCS) and bi-treatment FI+VCS, respectively. The obtained results of wheat seedling wet weight emphasize that plant extracts' treatments reduced deterioration caused by the fungal infection. Dry weight of wheat seedling' data over all treatments, appear to be more or less in the same trend of the wet weight' data (Table 1).

Data illustrated in Figure (5) show influence of various treatments on protein and proline content of wheat plant in pots experiment. The obtained results revealed that protein content ranged from 3.26 to 1.07 g/100 g DW. Protein content significantly increased in all treatments comparing with untreated one (control), except case of infected wheat plant with *H. rostratum* (FI), had the lowest protein content value. In contrary, tri-treatment FI+ZE+ZCS was the most effective treatment that significantly scored the highest protein content value. Both *Rumex* (RE) and *Ziziphus* (ZE) aqueous extract significantly reduced protein content. However, wheat seed coated with each plant extract (RCS & ZCS) treatments slight increased protein content. Data also indicated that there was a significant improves in protein content in plant treated or seed coated with chemical fungicide, vitavax. These obtained results explain that there was the positive allelopathic effect for protein content in the combined treatment against fungal infection. In addition, proline content significantly increased in fungal infected plant (FI) and recorded a highest value 3.83 g/100 DW. *Rumex* and *Ziziphus* aqueous extracts or seed coated led to significant depression in proline content. The lowest value of proline content (0.64 g/100 g DW) found in tri-treatment FI+ZE+ZCS. The observations of protein and proline imply that certain biochemical alterations in the host might be associated with defense mechanism and enhanced the growth.

Table 1 Effectiveness of various treatments on growth parameters of wheat plant in pots experiment.

Treatments	Germination (%)	Root Length (cm)	Shoot Length (cm)	Leaves (No.)	Spike Weight (g)	Plant Wet Weight (g)	Plant Dry Weight (g)
Control	86 ^{cde}	18.80 ^a	19.28 ^f	3.83 ^b	0.037 ^{fg}	0.136 ^{de}	0.081 ^{ef}
FI	92 ^{abcd}	14.30 ^{de}	16.11 ^g	3.00 ^c	0.021 ^g	0.099 ^e	0.055 ^f
VCS	98 ^{ab}	17.20 ^b	25.11 ^{bc}	5.17 ^a	0.130 ^b	0.371 ^a	0.180 ^{ab}
RE	84 ^{de}	13.74 ^{def}	21.36 ^{def}	3.83 ^b	0.070 ^{bcd}	0.237 ^b	0.159 ^{bcd}
ZE	92 ^{abcd}	14.22 ^{de}	20.88 ^{ef}	4.17 ^b	0.039 ^{efg}	0.167 ^{cd}	0.093 ^e
RCS	98 ^{ab}	14.74 ^{cde}	22.57 ^{cde}	4.17 ^b	0.028 ^{fg}	0.153 ^{de}	0.104 ^e
ZCS	98 ^{ab}	17.54 ^{ab}	21.54 ^{def}	4.00 ^b	0.034 ^{fg}	0.140 ^{de}	0.091 ^e
FI + VCS	94 ^{abc}	16.00 ^{bcd}	25.00 ^{bc}	3.83 ^b	0.096 ^{bc}	0.342 ^a	0.167 ^{abc}
FI + RE	60 ^f	13.50 ^{ef}	20.68 ^{ef}	4.00 ^b	0.059 ^{def}	0.146 ^{de}	0.097 ^e
FI + ZE	60 ^f	13.34 ^{ef}	27.77 ^{ab}	4.00 ^b	0.073 ^{cd}	0.253 ^b	0.148 ^{cd}
FI + RCS	100 ^a	14.20 ^{de}	24.02 ^{cd}	4.00 ^b	0.040 ^{efg}	0.232 ^b	0.140 ^{cd}
FI + ZCS	100 ^a	17.00 ^{bc}	22.99 ^{cde}	4.00 ^b	0.046 ^{defg}	0.225 ^{ab}	0.133 ^d
FI + RE + RCS	82 ^e	11.82 ^f	25.18 ^{bc}	3.83 ^b	0.130 ^b	0.382 ^a	0.193 ^a
FI + ZE + ZCS	90 ^{bcde}	14.64 ^{de}	30.37 ^a	4.00 ^b	0.240 ^a	0.269 ^b	0.150 ^{bcd}

Control = untreated; **FI** = Fungal Infection; **VCS** = Vitavax Coated Seeds; **RE** = *Rumex* Extract; **ZE** = *Ziziphus* Extract; **RCS** = *Rumex* Coated Seeds and **ZCS** = *Ziziphus* Coated Seeds. Means in the same column followed by the same letter are not significantly different based on LSD at $p = 0.05$ according to Duncan's multiple range test.

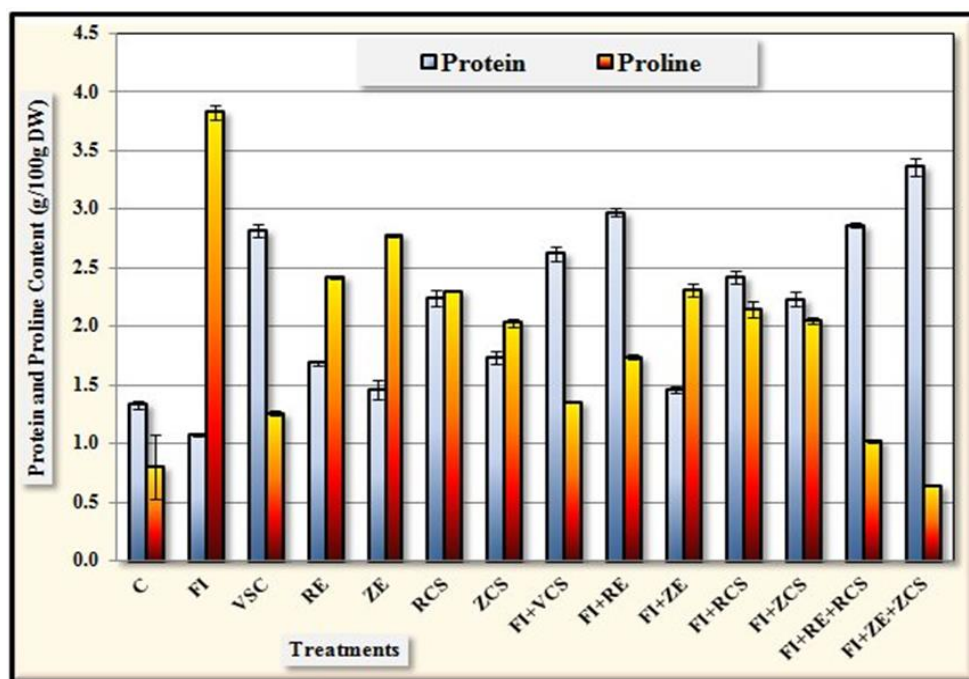


Figure 5 Influence of various treatments on protein and proline content (g/100 g) of wheat plant in pots experiments. Data represented as mean \pm standard error. C, untreated; FI, Fungal Infection; VCS, Vitavax Coated Seeds; RE, *Rumex* Extract; ZE, *Ziziphus* Extract; RCS, *Rumex* Coated Seeds and ZCS, *Ziziphus* Coated Seeds.

4. Discussion

The phytochemicals of *Ziziphus* and *Rumex* species revealed that a number of cyclopeptide and alkaloids, flavonoids, terpenoids and their glycosides found in various amounts in this study. The leaves of these plants contain betulinic and ceanothic acids, various flavonoids, saponins, erols, tannins and triterpenes [13-18]. The aqueous extract of *Z. spina-christi* was shown to contain butic acid and ceanothic acid, cyclopeptides, as well as saponins, tannins, glycosides and flavonoids [13]. In addition, Said *et al.*, [15] isolated three compounds from the seeds of *Ziziphus spina-christi* that identified as *p*-hydroxybenzoic acid, kaempferol, quercetin-3-O- α -L-rhamnosyl- β -D-glucopyranoside (rutin). It also indicated that the *Ziziphus*' fruits contained high level of total phenolic compounds, 7.55 mg/g as gallic acid [17]. Moreover, whole *Rumex vesicarius L.* parts extract found to contain high amount of Quercetin 82.452 μ g/g [16]. Phenolic and antioxidant compounds were detected in leaves and roots of *R. dentatus*, and these compounds are *p*-hydroxybenzoic acid, syringic acid, vanillin, benzoic acid, ferulic acid and cinnamic acid [18].

Phytochemical compounds have a broad-spectrum antifungal activity and mostly are non-toxic to plant and mammalian cells. For instance, plant extract thionins-rich inhibit the growth of about 20 different fungal plant pathogens *in vitro* [19]. Where their mode of action, thionins are known to form cation-selective ion channels [20] by binding to phosphatidylserine head groups in lipid bilayer membranes [21], which causes permeabilization and oxidative burst followed by cell death [22].

On plates, activity of the aqueous extracts of *R. vesicarius* and *Z. spina-christi* appeared against growth of saprophytic and plant pathogenic fungus, *H. rostratum*. The closer articles to the present circumstances as well as in line trend with present data, Haikal [23] who found that aqueous extract of *Ziziphus spina-christi* was inhibitory to *Fusarium* growth. In addition, study of Bazaid and Elmougy [24] was evaluated antifungal activity of the leaf juices of *Ziziphus in vitro*.

Effects of aqueous extracts of varied wild plants against some plant pathogenic microorganisms was observed [25], while, ethanol extract of *Ziziphus spina-christi* showed no activity against the fungal isolates, *Aspergillus niger* and *Candida albicans* [14]. Moreover, the antifungal activity vary with the plants species and material used. This difference in antifungal efficacy is due to the phytochemicals which may also specifically found in certain taxa of plants and vary in presence among different parts of plant tissues [26, 27].

On the other hand, phytochemical analysis of *Rumex* and *Ziziphus* indicated above that these plants have sufficient amounts of phenols, phenolic acids and flavonoids (Figs. 1 & 2). These compounds possess high levels of antimicrobial

activity; explain their interaction with bio-membrane and serves as plant defense mechanisms against pathogenic microorganisms. Phenolic toxicity to microorganisms is due to the site(s) and number of hydroxyl groups present in the phenolic compound [28, 29]. Over each, the beneficial antimicrobial effect of plant materials basically results from the secondary metabolites present in the plant and is not usually attributed to a single compound but a combination of these metabolites [30, 31].

Several phytochemical compounds, which have inhibitory effects on microorganisms *in vitro*, should be undergone *in vivo* experimentation to assess the efficacy in controlling plant disease comparing to artificial fungicides. In the current study, both aqueous extract of *Rumex* and *Ziziphus* showed varying antifungal activities. Therefore, it would be interesting to apply the effective concentration in pot experiments. In pot experiment, the obtained data revealed that all treatments of synthetic fungicide, Vitavax, mostly reduced the pathogenic fungal growth which is reflected on improvement all growth parameters of wheat seedlings. This result is consistent with many literatures, for instance, Sharma-Poudyal *et al.*, [32] found that Vitavax increased germination of wheat seeds and reduced seedling infection by *Cochliobolus sativus*. Whereas, the use of artificial fungicides is not encouragement because of their costs and harmful effects on the environment. Moreover, chemical fungicide of disease management may lead to the appearance of resistant fungal strains. For these reasons, there is widely recognized that biological control offers suitable alternatives tool for plant disease management [33-35].

The obtained results serve and support the new trend in biological control, where indicated that aqueous extracts of *Rumex* and *Ziziphus* clearly reduced the pathogenic fungal growth and improved all growth parameters of wheat seedlings; specially, tri-treatments (FI+RE+RCS & FI+ZE+ZCS) were the most effective treatments in the reduction or prevention wheat fungal infection.

The observations of protein and proline imply that certain biochemical alterations in the host might be associated with defense mechanism and enhanced the growth. Due to fungal infection, plant seedlings have been stimulated to produce elevated levels of total protein contents in host tissues, which indicated the possibility of involvement of these proteins as a response in the disease defense [36, 37].

Moreover, it has been reported that proline increase proportionately and accumulation faster in plant tissues than other amino acids as a part of the stress signal influencing adaptive responses [38]. That is means accumulation of proline is a common physiological change in plants as response to environmental stress as well as a wide range of biotic and abiotic stresses [39, 40].

Many researchers using other fungal species and different plant extracts found similar effects. The studies used plant extract of different species of *Rumex* against phytopathogenic fungi *in vivo* have been conducted. Gyung *et al.*, [41] found that *Rumex acetocella* roots reduced the development of barley powdery mildew caused by *Blumeria graminis*. In addition, Kim *et al.*, [35] reported that *Rumex crispus* roots effectively suppressed the development of cucumber powdery mildew caused by *Podosphaera xanthii* in growth chamber. Haikal [23] evaluated the potential of aqueous extract of *Ziziphus spina-christi* against the pathogenic fungus *Fusarium solani*.

Such medicinal plants have therapeutic properties due to biosynthesis of various complex phytochemical substances as phenolic, alkaloids and terpenoids. Synergistic interaction among the multiple phytochemicals may responsible for the overall bioactivity of a given medicinal plant [42].

5. Conclusion

The present study aimed to evaluate the antifungal activity of aqueous plant extracts, in hope to find out new natural compounds to be applied as alternative products to synthetic fungicides and develop such types of natural fungicides in biocontrol of many agricultural plant pathogens causing drastic losses to crops. Both plants *Rumex vesicarius* L. and *Z. spina-christi* are a wild edible plant; cheap and significantly contribute to the improvement of human health in terms of cure and prevention of diseases. Therefore, their extracts serve and suitable to be non-toxic to mammals and their high efficacy for defending plants against plant myco-pathogens that would make them a good alternative in integrated plant protection, in addition may help in reducing the human health hazard associated with certain synthetic fungicides.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have read and agree to submit this manuscript to World Journal of Advanced Research and Reviews.

Informed consent

This research does not involve any information about any individual person or group of persons.

Statement of ethical approval

The present research does not contain any studies performed on animals/humans subjects.

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