

Full Length Research Paper

Assessment of antifungal activity of *Rumex vesicarius* L. and *Ziziphus spina-christi* (L.) Willd. extracts against two phytopathogenic fungi

Amira M. Abu-Taleb^{1*}, Kadriya El- Deeb² and Fatimah O. Al-Otibi³

¹Botany Department, Faculty of Science, Cairo University, Egypt.

²Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt.

³Botany and Microbiology Department, Faculty of Science, King Saud University, Riyadh, Saudi Arabia.

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The preliminary phytochemical screening of extracts of *Rumex vesicarius* L. and *Ziziphus spina-christi* (L.) Willd. showed the presence of compounds that are biologically active against the two root rot pathogens *Drechslera biseptata* and *Fusarium solani* *in vitro*. The relative efficacy of this action, however, differed according to the extracted plant, solvent used, extract concentration, the target fungus and phase of growth. Ethanolic extract ranked first, followed by the remaining aqueous layer fraction. Eight flavonoid subfractions (rutin, quercetin, myricetin, apigenin, quercetin-3-O-galactoside, luteolin, kaempferol and kaempferol-3-O-robinoside) and six flavonoid subfractions (apigenin-7-O-glucoside, quercitrin, quercetin, isovitexin, rutin and quercetin-3-O-lucoside-7-O-rhamnoside) were isolated from the remaining aqueous layer fraction of *R. vesicarius* and *Z. spina-christi*, respectively. Generally, spore production and germination as well as cellulolytic and pectolytic activity of *F. solani* were affected by plant extracts more than that of *D. biseptata*. *F. solani* failed completely to produce spores when treated with ethanolic extract of *Z. spina-christi* at the concentration of 20%. However, growth of *D. biseptata* was more sensitive to plant extracts than that of *F. solani*. Maximum activity of plant extracts was observed against spore production. It was evident that plant extracts could provide potential source of antifungal compounds.

Key words: *Rumex vesicarius*, *Ziziphus spina-christi*, antifungal activity, *Drechslera biseptata*, *Fusarium solani*.

INTRODUCTION

Root rot diseases caused by fungi are worldwide spread in crop growing areas, causing significant economic losses. The use of chemical compounds toxic to fungi is the most commonly known means of controlling fungal diseases in the field and greenhouse (Washington and McGee, 2000; Fravel et al., 2005). Although this method has been effective in controlling plant fungal diseases, some major environmental problems are involved. Therefore, there is need to explore alternative agents for the control of fungal diseases in plants (Hadizadeh et al., 2009). In recent years, research on the antimicrobial

activity of medicinal plants has attracted global attention. Application of plant extracts for controlling plant diseases is non-pollutive, cost effective, non hazardous and does not disturb ecological balance. Species of *Rumex* (Family Polygonaceae) and *Ziziphus* (Family Rhamnaceae) have received keen interest in phytochemical, pharmacological and microbiological research due to their potential medicinal values. They produce a diverse of bioactive compounds which were found to possess antimicrobial properties (Panduraju et al., 2009; Abalaka et al., 2010). Antimycotic activity of the ethanol extracts prepared from *Urtica dioica* L., *Citrullus colocynthis* L. Schrad, *Ziziphus spina-christi* L. and *Nerium oleander* L. floral parts was noted against *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium solani* and *Rhizoctonia solani* using agar dilution bioassay (Hadizadeh et al., 2009). *F. solani*, *Aspergillus*

*Corresponding author. E-mail: agabry2008@yahoo.com. Tel: 00 201 23 592 823.

flavus and *Aspergillus niger* were susceptible to crude extracts of *Polygonum persicaria*, *Rumex hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Polygonum plebejum* and *Rheum australe* with MICs 0.75, 2.15, and 1.75 µg/ml, respectively (Hussain et al., 2010).

Although many reports showed that *Rumex vesicarius* L. and *Ziziphus spina-christi* (L.) Willd. were potentially a good source of antimicrobial compounds, the use of these compounds for application in crop protection was not as many as in medical field. The objective of the present study is to determine the phytochemical constituents and to evaluate their potential to control *in vitro* two root rot pathogens *Drechslera biseptata* and *F. solani*.

MATERIALS AND METHODS

Chemical studies

Plant materials

The plants used in this study were *R. vesicarius* L. and *Z. spina-christi* (L.) Willd. The tested plants were collected in February 2004 from Riyadh region, Kingdom of Saudi Arabia. The collected plants were identified and authenticated by Dr. Nagat Bokhary, Department of Botany and Microbiology, Faculty of Science, King Saud University.

Preparation of plant extracts

The used parts in this study were the aerial parts (shoot) from *R. vesicarius* and the leaves from *Z. spina-christi*. Thoroughly washed plant materials were shade dried and then ground using a blender. Known weight of the dried material of each plant was successively extracted with 95% ethanol at room temperature until ethanol became colorless (The mixture was stirred daily for regular infusion). After filtration, solvent in combined filtrates was evaporated under reduced pressure using Rotavapour at 60°C until the extract was dried. Dry fractions were stored at 4°C until use.

The ethanolic extract was divided into 3 portions; one was taken for phytochemical screening, second for successive extraction by petroleum ether followed by chloroform and the rest was suspended in Tween-80 for measuring antifungal activity.

Phytochemical screening

Qualitative phytochemical screening of plant extracts was carried out using the methods of Rizk (1982) to test for the presence of carbohydrates and/or glycosides, anthraquinones, steroids and/or terpenes, coumarins, saponins and alkaloids; Geissman (1962) to test for the presence of flavonoids and Stahl and Schild (1981) to test for the presence of tannins.

Isolation of active compounds

After successive extraction of the crude ethanol extract with petroleum ether and then by chloroform, three fractions were obtained petroleum ether, chloroform and the remaining aqueous layer. Each fraction was dried under reduced pressure using Rotavapour at 60°C. The antifungal activity of each fraction was assessed.

It is well known that phenolic compounds play an important role in the bioactivity of plant extracts (Veldhuizen et al., 2006); so flavonoids of the remaining aqueous layer fraction, which showed marked antifungal activity in this study, were isolated and purified by using the thin layer chromatography technique. Similar flavonoid subfractions were collected and identified by using the UV, FT-IR, ¹HNMR, MS, FAB-MS spectra (Ahmed and Rahman, 1994).

Microbiological studies

Source and maintenance of fungi

Virulent root rot causing isolates of *D. biseptata* (Sacc. & Roun) Richardson & Fraser and *F. solani* (Mart.) Sacc. were obtained from the Faculty of Science of King Saud University and King Abdel Aziz University, respectively. Both isolates were maintained on potato dextrose agar (PDA) medium.

Antifungal activities

The selected extract was diluted in water to give the required concentration (×2) by the aid of 10% Tween 80, then sterilized by passing through 0.45 µm bacterial filter. Known volume of the diluted extract was added to equal volume of PDA medium (double strengthened) to give the required concentration 1, 10 or 20% based on the weight of dried powder of the plant materials used in preparing ethanol extract. Control was made by addition of the same volume of Tween 80 to extract free medium. After pouring the medium into Petri dishes, the plates were inoculated with 10 mm disc cut from the margin of actively growing mycelium of the pathogen and incubated at 25°C. The colony diameter was measured and the number of produced spores was counted by a haemocytometer after 7 days incubation period (El-Abyad et al., 1983). Germination of conidia was studied employing microscope slide covered with droplets of the required concentration of plant extract (ethanol or the remaining aqueous layer fraction only) containing conidia (El-Abyad et al., 1983). The plates containing the slides were incubated at 25°C until the germination percentage reached about 50% in the control and fix this time for the other treatment.

Production of cell wall degrading enzymes (cellulase and pectate lyase)

Cell wall degrading enzymes of pathogenic origin have an important role in destruction of host cells during pathogenesis (Ramos et al., 2010); so it is important to study the efficacy of the tested materials (extracts) against the phytopathogenic fungi through their effect on two important cell wall degrading enzymes (cellulase and pectate lyase).

Surface disinfected seeds of *Cucurbita maxima* Duchense (winter squash) were sown in pots containing autoclaved vermiculite. The pots were placed in the greenhouse and watered daily. Cell walls prepared from fresh tissues of 10-day-old hypocotyls were used as a carbon source for growth and enzyme production by *D. biseptata* and *F. solani* (Nevins et al., 1967).

Conical flasks (100-mL) each containing 30 mL of the medium described by Bateman et al. (1969) were amended with 0.5 g of dried cell wall as a carbon source. Cell wall amended medium was autoclaved, cooled and then supplemented with the selected plant extract (ethanol or the remaining aqueous layer fraction only) to produce the following concentrations 1, 10 and 20%. A 6 mm diameter agar disc bearing hyphae of either *D. biseptata* or *F. solani* cut from 7-days-old colonies was transferred to each flask and incubated at 25°C for 9 days. At the end of incubation period,

Table 1. Phytochemical screening of *Rumex vesicarius* and *Ziziphus spina-christi* extracts.

Active compounds	<i>R. vesicarius</i>		<i>Z. spina-christi</i>	
	EtOH	Aq L	EtOH	Aq L
Crystalline sublimate	-	-	-	-
Volatile substances	-	-	-	-
CHO and/or glycosides	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Saponins	-	-	+	+
Alkaloids	-	-	-	-
Coumarins	-	-	-	-
Steroids and/or triterpens	+	-	+	-
Anthraquinones	+	+	+	+

+, Present; -, Absent; EtOH, Ethanol; Aq L, Aqueous layer.

growth culture was filtered and centrifuged at 15000 ×g for 20 min. at -5°C. The supernatant was used as crude enzyme source.

The activity of cellulase enzyme was assayed by incubating 0.5 mL of the crude enzyme with 4.5 mL of 1% (w/v) carboxy methyl cellulose in 0.1 M potassium phosphate buffer (pH 7) at 30°C for one hour. Reaction was stopped by boiling for 10 min. The increase in liberated reducing groups was measured photometrically by the method described by Miller (1959). Enzyme unit= amount of enzyme which produce one mg of glucose in one hour under the condition of the experiment.

Pectate lyase was assayed by incubating reaction mixtures containing 0.5 mL 2% (w/v) citrus pectin, 1.5 mL of 0.05 M Tris-HCl buffer (pH 8), 0.1 mL of 0.02 M CaCl₂ and 0.5 mL of cell wall degrading enzymes source at 30°C for one hour. The reaction was stopped by boiling the mixture for 5 min; and after cooling, the activity was measured photometrically as the increase in absorbance at 235 nm. One unit of pectate lyase was defined as an increase of one absorbance unit in one hour at 30°C (Longland, 1992).

Statistical analysis

In order to clarify the effect of plants, extracts, extract concentrations and types of fungi as well as their interaction on the studied parameters, multi-way analysis of variance (MANOVA) were used. Duncan's test of homogeneity was used to compare between different studied groups at the corresponding conditions. The data were analyzed by aid of Statistical Package for the Social Sciences (SPSS) VERSION 18. All values given in this work are means of 3 or 4 replicates.

RESULTS AND DISCUSSION

Chemical studies

Preliminary phytochemical screening of *R. vesicarius* L. and *Z. spina-christi* (L.) Willd. extracts revealed the presence of carbohydrates and/or glycosides, tannins, flavonoids, anthraquinones and steroids and/or triterpenes, whereas saponins were found in *Z. spina-christi* only (Table 1). Panduraju et al. (2009) indicated

the presence of carbohydrates, anthraquinone glycosides, cardiac glycosides, saponin glycosides, flavonoids, tannins, steroids, cysteine, glutamic acid, proline, phenylalanine and histidine in leaves extracts of *R. vesicarius*. Phytochemical tests revealed that the extract of *Zizyphus oxyphylla* contained alkaloids, anthraquinones, flavonoids, glycosides, phenols, resins, saponins and tannins (Nisar et al., 2007). Abalaka et al. (2010) isolated cardiac glycosides, polyphenols, saponins and tannins from *Zizyphus mauritiana* and *Z. spina-christi*. The remaining aqueous layer fractions of *R. vesicarius* and *Z. spina-christi* were the most efficient among ethanolic extract fractions against both pathogens of the present study. The active compounds of this fraction were identified and represented in Table (1). Eight flavonoid subfractions were isolated from the remaining aqueous layer of *R. vesicarius* (R1-R8) and identified as rutin, quercetin, myricetin, apigenin, quercetin-3-O-galactoside, luteolin, kaempferol and kaempferol-3-O-robinoside.

Compound R1

Yellow powder, gave reaction with alkalies and aluminium chloride solution indicating the possible presence of flavonoidal compound. This, together with positive Molish's test, suggested its glycosidic nature. The UV spectral data confirmed the flavonoidal nature of the compound. Location of the hydroxyl groups on the skeleton was established by utilizing the UV shift reagents and comparison with published data (Mabry et al., 1970).

Compound R2

Yellow powder, mp 215-218°C; ¹H-NMR (400MHZ, DMd₂SO-d₆) δ:12.56 (1H, s,5-OH), 10.81,9.67, 9.18

(each 1H, 7,3', 4'-OH), 7.54hg (2H, dd, J=8.5, 2,0 HZ, H-2',6'), 6.85(1H,d,J=8.8HZ, H-5'), 6, 41(1H, d,J=1.8 HZ,H8), 6.18(1H, d, J=1.8HZ, H-6), 5.30 (1H,d, J=7.0 HZ. GLc H-1, 4.40 (1H, brs, Rha H-1), 3, 80-3.04(sugar H), 1.00(3H, d, J=6.0, Rha CH₃). ¹³C-NMR (100MHZ, DMSO-d₆) 156.2(C-2), 133.1(C-3), 177.1(C-4), 160.6(C-5), 98.2(C-6), 164.0(C-7), 93.2(C-8), 121.1(C-1'), 115.1(C-2'), 144.4(C-3'), 148.2(C-4'), 116.1(C-5'), 121.5(C-6'), 101.2(C-1''), 74.0(C-2''), 76.2(C-3''), 70.2(C-4''), 76.2(C-5''), 67.1(C-6''), 100.3(C-1'''), 70.3(C-2'''), 70.5 (C-3'''), 71.8 (C-4'''), 68.1(C-5''') and 17.4(C-6''').

Compound R3

Yellow powder, gave reaction with alkalies and aluminium chloride solution indicating the possible presence of flavonoidal compound. The UV spectral data confirmed the flavonoidal nature of the compound. Location of the hydroxyl groups on the skeleton was established by using the UV shift reagents and comparison with authentic sample.

Compound R4

¹H-NMR: δ: 6.72 (S, H-3), 6.18(d, J=2.1, H-6), 6.46 (d, J=2.1, H-8), 7.88 (d, J=8.8, H-2'), 6.91(d, J=8.8, H-3'), 7.88 (d, J=8.8, H-6'), 12.92(S, OH-S). ¹³C-NMR: 163.7(C-2), 102.4(C-3), 181.2(C-4), 161.0(C-5), 98.4(C-6), 164.2(C-7), 93.4(C-8), 157.1(C-9), 103.4(C-10), 120.9 (C-1'), 128.2(C-2'), 115.6(C-3'), 161.2(C-4'), 115.5(C-5') and 128.1(C-6').

Compound R5

¹³C-NMR: 165.0(C-2), 103.7(C-3), 182.1(C-4), 160.4(C-5), 99.2(C-6), 164.1(C-7), 94.4(C-8), 158.2(C-9), 104.8(C-10), 123.0(C-1'), 115.3(C-2'), 145.8(C-3'), 150.0(C-4'), 117.0(C-5'), 120.4(C-6').

Compound R6

¹³C-NMR: 146.4(C-2), 135.2(C-3), 175.5(C-4), 160.7(C-5), 98.2(C-6), 165.5(C-7), 93.5(C-8), 156.0(C-9), 103.0(C-10), 121.0(C-1'), 130.5(C-2'), 115.2(C-3'), 159.2(C-4'), 115.2(C-5'), 129.5(C-6').

Compound R7

Yellow powder, mp 316-317C; ¹H-NMR (400 MHZ, CD₃OD) δ: 7.62(1H, d, J=2.2HZ, H-2'), 7.53(1H, dd, J=8.4, 2.2 HZ, H-6'), 6.78(1H, d, J=8.4 HZ, H-5'), (1H, d, J=1.8 HZ, H-8), 6.07(1H, d, J=1.8HZ, H-6). ¹³C-NMR

(100MHZ, DMSO-d₆) 146.2 (C-2), 135.2(C-3), 175.1(C-4), 159.4 (C-5), 98.1 (C-6), 163.2 (C-7), 93.0(C-8), 122.2(C-1'), 115.3(C-2'), 145.2(C-3'), 147.2(C-4'), 115.3(C-5') and 120.2(C-6').

Compound R8

Yellow powder, gave reaction with alkalies and aluminium chloride solution indicating the possible presence of flavonoidal compound. This together with negative Molish's test suggested its aglycone nature. The UV spectral data confirmed the flavonoidal nature of the compound. Location of the hydroxyl groups on the skeleton was established by using the UV shift reagents and comparison with published data (Mabry et al., 1970).

On the other hand, six flavonoid subfractions (Z1-Z6) were isolated from the remaining aqueous layer fraction of *Z. spina-christi* and identified as apigenin-7-O-glucoide, quercitrin, quercetin, isovitexin, rutin and quercetin-3-O lucoside-7-O-rhamnoside.

Compound Z1

Yellow powder, gave reaction with alkalies and aluminium chloride solution indicating the possible presence of flavonoidal compound. This together with positive Molish's test suggested its glycosidic nature. The UV spectral data confirmed the flavonoidal nature of the compound. Location of the hydroxyl groups on the skeleton was established by using the UV shift reagents and ¹H-NMR and comparison with published data (Mabry et al., 1970).

Compound Z2

Yellow powder, mp 181-182C ¹H-NMR (400 MHZ, CD₃OD) δ : 7.57 (1H, d, J =1.9 HZ, H-2'), 7.53 (1H, dd, J=8.2, 1.9 HZ, H-6'), 6.77 (1H, d, J =8.2 HZ, H-5'), 6.29 (1H, d, J =1.8 HZ, H-8), 6.10 (1H, d, J =1.8 HZ, H-6), 5.01(1H, d, J =2.0 HZ, H-1''), 4.22-3.14 (sugar H), 1.02 (3H, d, J =6.0 HZ, H-6''), ¹³C-NMR (100 MHZ, CD₃OD) 158.0 (C-2), 136.2 (C-3), 179.2(C-4), 158.7 (C-5), 99.3 (C-6), 165.4 (C-7), 94.1(C-8), 122.2 (C-1'), 116.0 (C-2'), 146.3 (C-3'), 149.2 (C-4'), 116.2(C-5'), 122.4(C-6'), 103.1 (C-1''), 71.6 (C-2''), 72.2 (C-3''), 73.2 (C-4''), 71.6 (C-5'') and 17.3(c-6'').

Compound Z3

Yellow powder, mp 316-317C ¹H-NMR (400 MHZ, CD₃OD) δ: 7.62(1H, d, J=2.2HZ, H-2'), 7.53(1H, dd, J=8.4, 2.2 HZ, H-6'), 6.78 (1H, d, J=8.4 HZ, H-5'), (1H, d, J=1.8 HZ, H-8), 6.07(1H, d, J=1.8 HZ, H-6). ¹³C-NMR

(100MHZ, DMSO-d₆) 146.2 (C-2), 135.2 (C-3), 175.1 (C-4), 159.4 (C-5), 98.1 (C-6), 163.2 (C-7), 93.0 (C-8), 122.2 (C-1'), 115.3 (C-2'), 145.2 (C-3'), 147.2 (C-4'), 115.3 (C-5') and 120.2 (C-6').

Compound Z4

Yellow powder, mp 220-222 C; ¹H-NMR (400 MHZ, CD₃OD) 7.7 (2H, d, J=8 HZ, H-2, H-6'), 6.95 (2H, d, J=8 HZ, H-3, H-5'), 6.70 (1H, s, H-3), 6.5 (1H, s, H-8). MS m/z 312, 284, 283, 165 and 118 (no molecular ion peak at 432) (Harborne et al., 1975).

Compound Z5

Yellow powder, mp 215-218C; ¹H-NMR (400MHZ, DMSO-d₆) δ:12.56 (1H, s, 5-OH), 10.81, 9.67, 9.18 (each 1H, 7,3', 4'-OH), 7.54 (2H, dd, J=8.5, 2.0 HZ, H-2', 6'), 6.85 (1H, d, J=8.8HZ, H-5'), 6.41 (1H, d, J=1.8 HZ, H-8), 6.18 (1H, d, J=1.8HZ, H-6), 5.30 (1H, d, J=7.0 HZ, GLc H-1, 4.40 (1H, brs, Rha H-1), 3.80-3.04 (sugar H), 1.00 (3H, d, J=6.0, Rha CH₃), ¹³C-NMR (100MHZ, DMSO-d₆) 156.2 (C-2), 133.1 (C-3), 177.1 (C-4), 160.6 (C-5), 98.2 (C-6), 164.0 (C-7), 93.2 (C-8), 121.1 (C-1'), 115.1 (C-2'), 144.4 (C-3'), 148.2 (C-4'), 116.1 (C-5'), 121.5 (C-6'), 101.2 (C-1''), 74.0 (C-2''), 76.2 (C-3''), 70.2 (C-4''), 76.2 (C-5''), 67.1 (C-6''), 100.3 (C-1'''), 70.3 (C-2'''), 70.5 (C-3'''), 71.8 (C-4'''), 68.1 (C-5''') and 17.4 (C-6''').

Compound Z6

Yellow powder, gave reaction with alkalis and aluminium chloride solution indicating the possible presence of flavonoidal compound. This together with positive Molish's test, suggested its glycosidic nature. The UV spectral data confirmed the flavonoidal nature of the compound. Location of the hydroxyl groups on the skeleton was established by using the UV shift reagents and ¹H-NMR and comparison with published data (Mabry et al., 1970).

Shahat et al. (2001) also isolated four flavonoid compounds from *Z. spina-christi* viz., rutin, hyperoside, quercetin and quercetin-3-O-[beta-xylosyl-(1-2)-alpha-rhamnoside]4'-O-alpha-rhamnoside.

Microbiological studies

The results of the present investigation clearly indicate that ethanolic extract of *R. vesicarius* and *Z. spina-christi* and its fractions petroleum ether, chloroform and the remaining aqueous layer fraction were effective in controlling growth (Figure 1) and sporulation (Figure 2,) of *D. biseptata* and *F. solani* particularly at the high concentrations. According to multiway analysis of variance

(MANOVA), growth and sporulation were significantly affected by the types of fungi ($F_{1,176} = 58.4$, $P < 0.000$ for growth and $F_{1,128} = 3806.5$, $P < 0.000$ for sporulation), plant ($F_{1,176} = 18.9$, $P < 0.000$ for growth and $F_{1,128} = 5.8$, $P < 0.018$ for sporulation) and extracts ($F_{3,176} = 170.7$, $P < 0.000$ for growth and $F_{3,128} = 1413.7$, $P < 0.000$ for sporulation) as well as level of extract concentrations ($F_{3,176} = 279.7$, $P < 0.000$ for growth and $F_{3,128} = 7889.5$, $P < 0.000$ for sporulation). Significant inhibitory effect of ethanolic extract and the remaining aqueous layer fraction prepared from the selected plants on spore germination (Figure 3), cellulase activity (Figure 4) and pectate lyase activity (Figure 5) was obvious. This effect was confirmed statistically according to multiway analysis of variance (MANOVA). The inhibitory effect of the selected extracts was dependent upon the types of fungi ($F_{1,64} = 914.8$, $P < 0.000$, $F_{1,64} = 772.9$, $P < 0.000$ and $F_{1,64} = 5653.5$, $P < 0.000$ for spore germination, cellulase and pectate lyase, respectively), plants ($F_{1,64} = 47.1$, $P < 0.000$, $F_{1,64} = 195.9$, $P < 0.000$ and $F_{1,64} = 1014.5$, $P < 0.000$ for germination, cellulase and pectate lyase, respectively) and extracts ($F_{1,64} = 67.1$, $P < 0.000$, $F_{1,64} = 20.3$, $P < 0.000$ and $F_{1,64} = 121.9$, $P < 0.000$ for germination, cellulase and pectate lyase, respectively) as well as level of extract concentrations ($F_{3,64} = 389.8$, $P < 0.000$, $F_{3,64} = 1172.1$, $P < 0.000$ and $F_{3,64} = 2656.1$, $P < 0.000$ for germination, cellulase and pectate lyase, respectively). The marked antifungal activity of the plant extracts in this investigation is in line with previous studies (Chitra et al., 2001; Akhter et al., 2006; Alam et al., 2004; Mahesh and Satish, 2008; Panduraju et al., 2009).

In the present work, the remaining aqueous layer fraction prepared from ethanolic extract of *R. vesicarius* at the concentrations of 10 and 20% changed the black color of *D. biseptata* to grey at the center and white at the margin (top view), while the reverse of the colony appeared orange in color (Figure 6). However, the other extracts of the selected plants had no obvious effect on the colony color of both pathogens.

The antifungal activity of the selected plants may be attributed to their constituents of biologically active compounds (Table 1). Several studies have been conducted to understand the mechanism of action of plant extracts and essential oils, but it is still unclear (Hadizadeh et al., 2009). However, some researchers attributed the antimicrobial activity to the phenolic compounds. The amphipathicity of these compounds can explain their interactions with biomembranes causing the inhibitory effect (Veldhuizen et al., 2006). Omidbeygi et al. (2007) suggest that extract components cross the cell membrane, interacting with enzymes and proteins of the membrane, so producing a flux of protons towards the cell exterior which induces changes in the cells and, ultimately, their death. Sharma and Tripathi (2006) concluded that essential oils and plant extracts may act on the hyphae of the mycelium, provoking exit of components from the cytoplasm, the loss of rigidity and

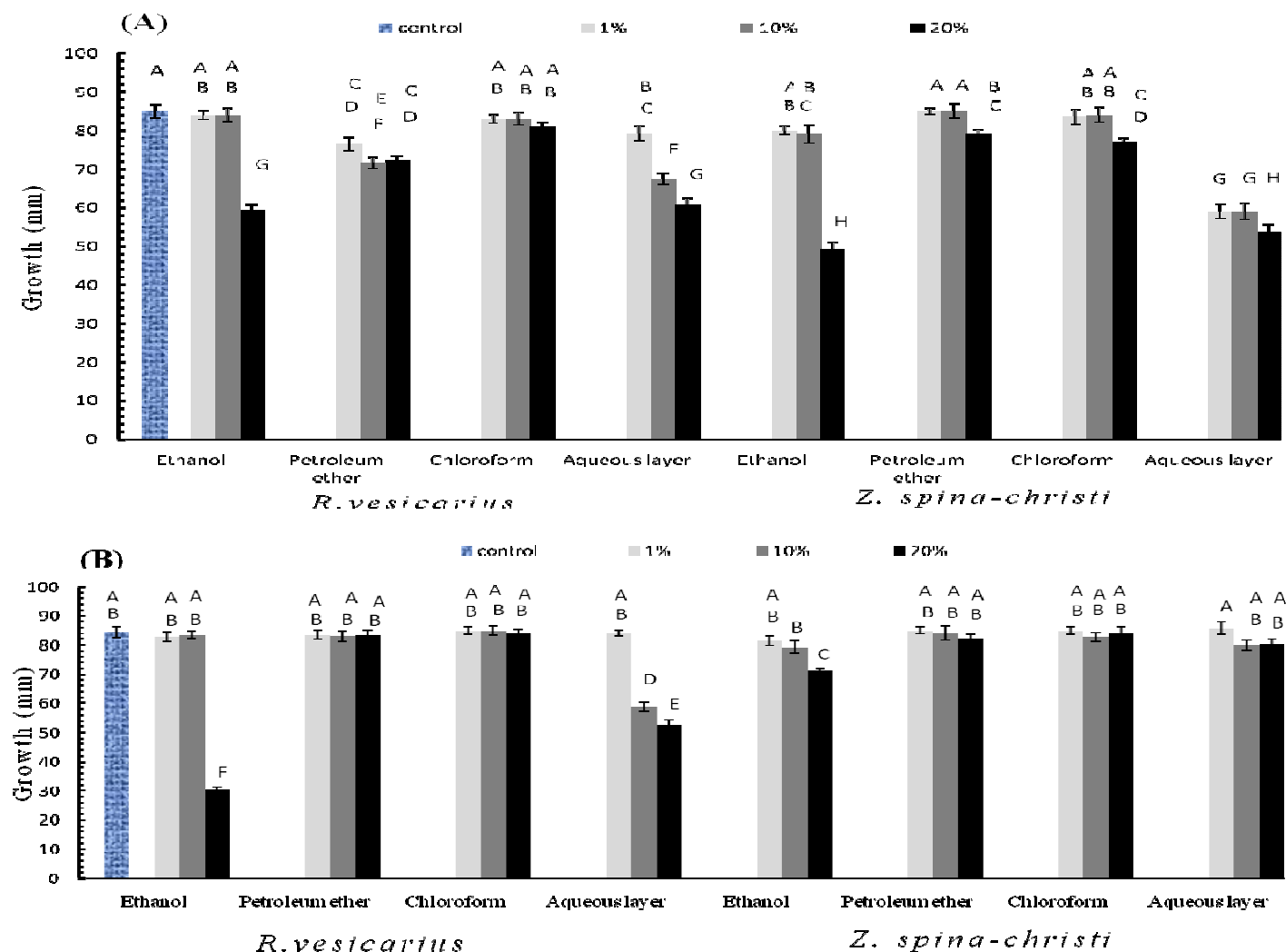


Figure 1. Growth (mm) of *Drechslera biseptata* (A) and *Fusarium solani* (B) after incubation for 7 days at 25°C on PDA medium supplemented with various concentrations of *Rumex vesicarius* and *Ziziphus spina-christi* extracts. Data represented as mean \pm S.E. Bars marked with the same letters are insignificantly different ($P > 0.05$), where as those with different letters are significantly different.

integrity of the hypha cell wall, resulting in its collapse and death of the mycelium.

It is evident from the results of the current study that susceptibility of pathogens to plant extracts depends upon plant species (Bagwan, 2001), solvent used for extraction and extract concentration (Abou-Jawdah et al., 2002), as well as the organism tested and phase of growth (Kumaran et al., 2003). The variation in antifungal activity of the selected plants may be attributed to the difference in chemical nature and concentration of the active constituents of each plant (Hadizadeh et al., 2009). In our investigation, saponins were found in *Z. spina-christi* only. In addition, the remaining aqueous layer fractions of the two selected plants contained different types of flavonoid subfractions. The flavonoids compounds, iso quercitrin and rutin (Pepeljnjak et al., 2005), luteolin (Sartori et al., 2003), isovitexin (Othman et

al., (1996), quercetin and kaempferol (Santas et al., 2010), quercetin, rutin and apigenin (Basile et al., 2000), and quercetin, quercetin-3-O- α -L-arabinofuranoside, quercetin-3-O- β -D-arabinopyranoside, quercetin-3-O- β -D-glucoside, quercetin-3-O- β -D-galactoside and quercetin-3-O- β -D-arabinopyranoside (Metwally et al., 2010) appeared to have antifungal activity. Generally these results are in agreement with that of the present investigation.

At the same time, the different types of extracts of the same plant exhibited variation in the intensity of antifungal activity. It may be said that as each type of the compounds was extracted by certain solvents, that resulted in variable activity of the different extracts of the same plant (Javaid et al., 2008). In this work steroids and/or triterpenes were found only in ethanolic extract of both plants (Table 1). According to antifungal activity,

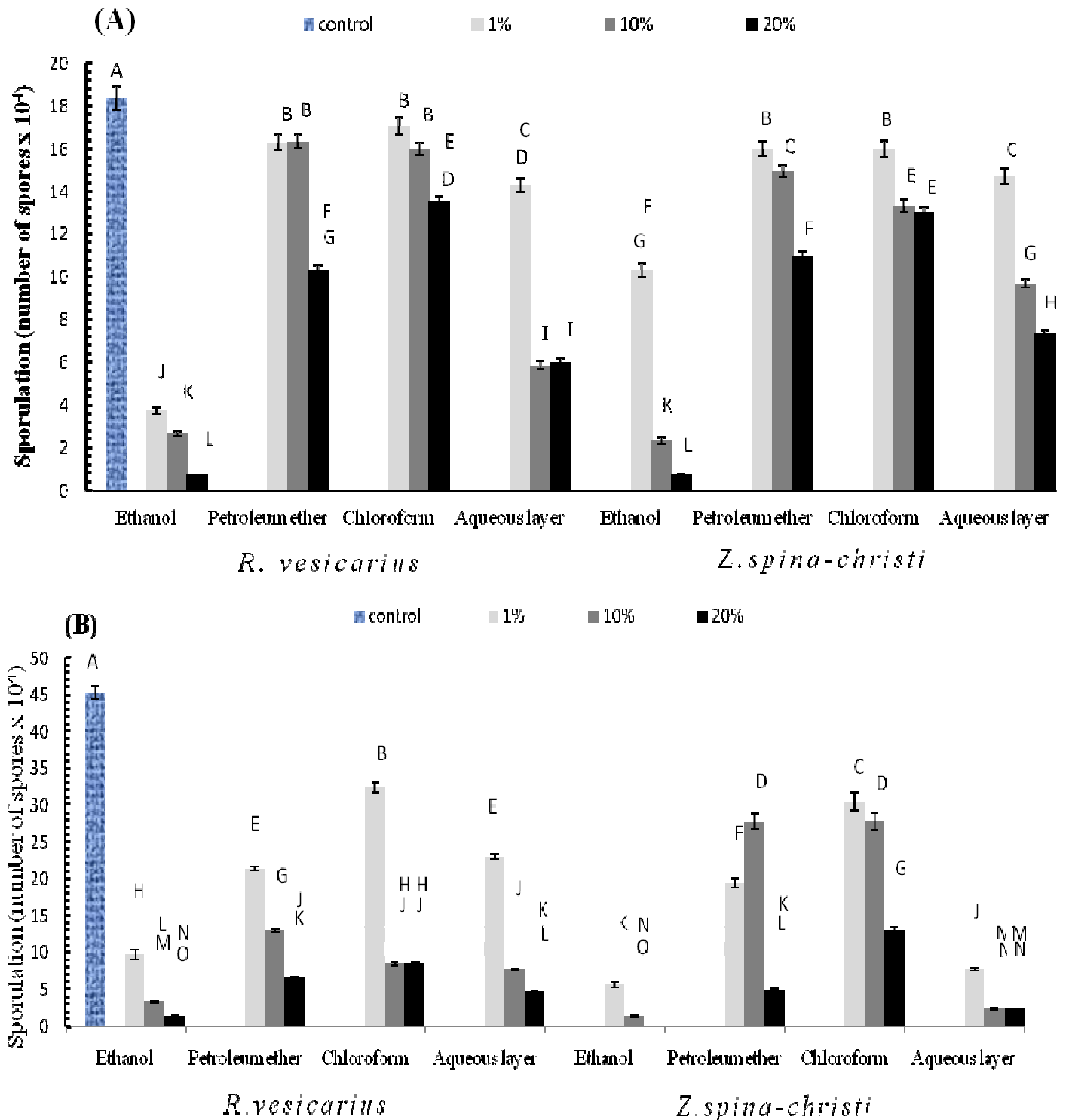


Figure 2. Sporulation (number of spores $\times 10^4$) of *Drechslera biseptata* (A) and *Fusarium solani* (B) after incubation for 7 days at 25°C on PDA medium supplemented with various concentrations of *Rumex vesicarius* and *Ziziphus spina-christi* extracts. Data represented as mean \pm S.E. In the same column, means marked with the same letters are insignificantly different ($P > 0.05$), whereas those with different letters are significantly different.

ethanolic extract ranked first followed by the remaining aqueous layer fraction. Panduraju et al. (2009) showed that methanolic extract of *R. vesicarius* possessed

significant antimicrobial activity in a concentration dependent manner more than water or petroleum ether extract. Synergism due to the presence of large number

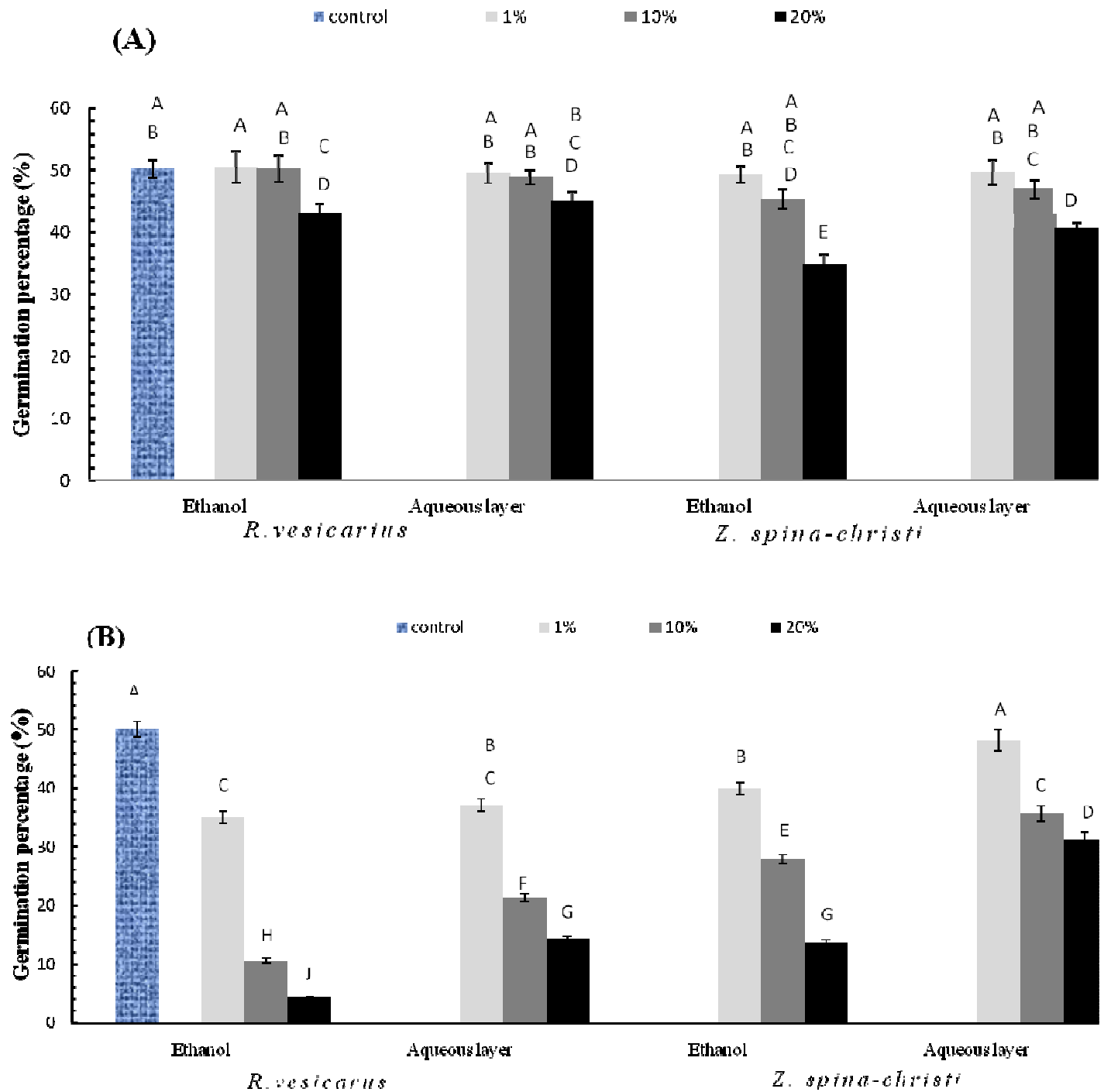


Figure 3. Effect of different concentrations of *Rumex vesicarius* and *Ziziphus spina-christi* extracts on percentage germination (%) of conidia of *Drechslera biseptata* (A) and *Fusarium solani* (B). Data represented as mean \pm S.E. In the same column, means marked with the same letters are insignificantly different ($P > 0.05$), whereas those with different letters are significantly different.

of biologically active compounds in the crude ethanolic extract and the remaining aqueous layer fraction (Table 1) may enhance their antifungal activity and explain our results. Arima et al. (2002) concluded that the combination of quercetin and quercitrin, quercetin and morin, and quercetin and rutin showed higher antifungal activity

than either any one of these flavonoids alone. They added that although rutin did not show activity of its own, the antimicrobial activities of quercetin and morin were enhanced in presence of rutin.

It was obvious from the current study that conidial production and germination as well as enzymes activity of

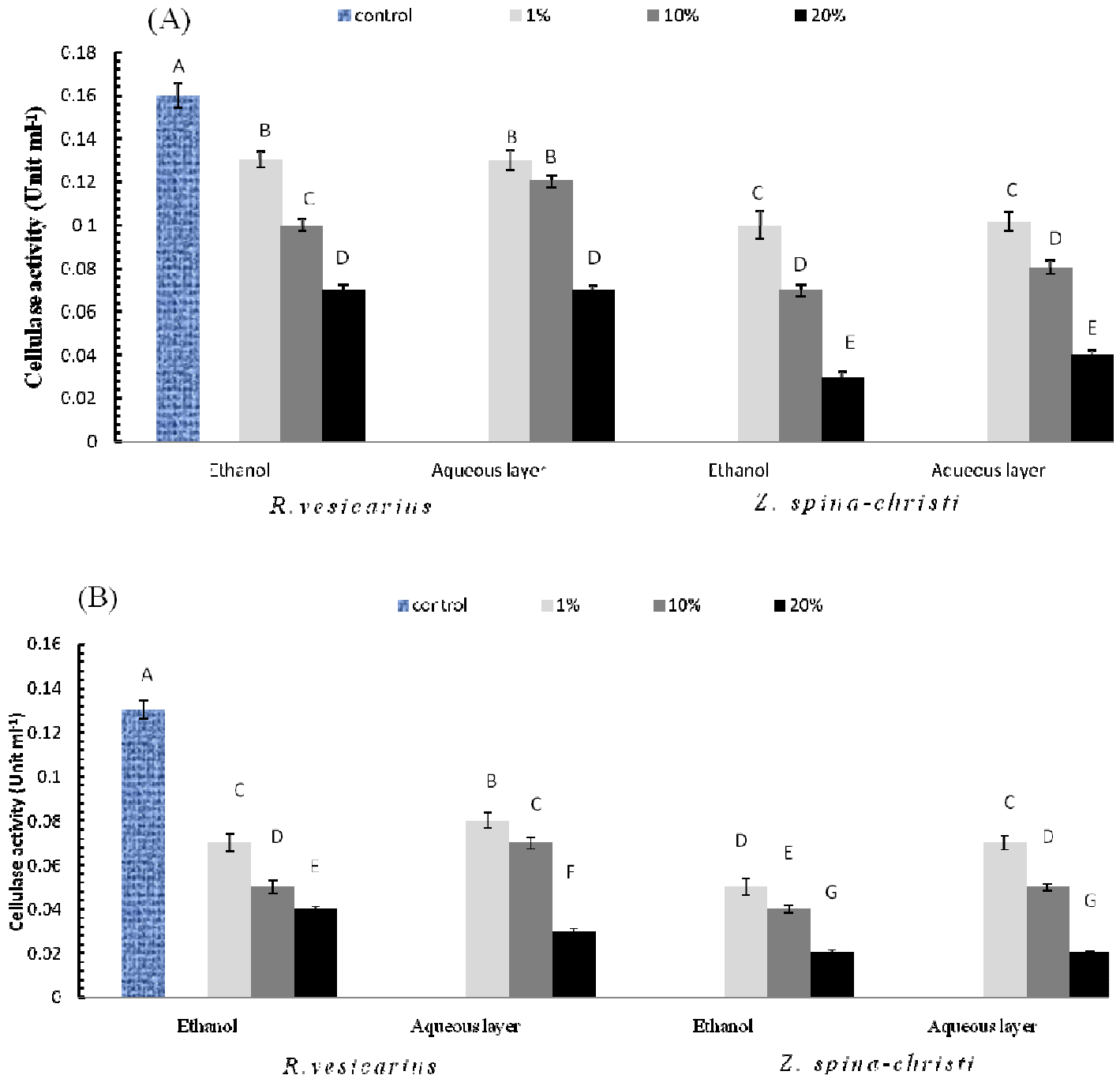


Figure 4. Inhibitory effect of *Rumex vesicarius* and *Ziziphus spina-christi* extracts on cellulolytic activity (unit ml⁻¹) of *Drechslera biseptata* (A) and *Fusarium solani* (B). Data represented as mean ± S.E. In the same column, means marked with the same letters are insignificantly different (P>0.05), whereas those with different letters are significantly different.

F. solani were generally more sensitive to the selected extracts than that of *D. biseptata*. *F. solani* failed completely to produce spores when treated with ethanolic extract of *Z. spina-christi* at the concentration of 20%. On the other hand, growth of *D. biseptata* was generally more susceptible to plant extracts than that of *F. solani*. *Z. spina-christi* extracts induced high antifungal activity

particularly against *D. biseptata*. Mahesh and Satish (2008) supported this observation and concluded that *Ziziphus mauritiana* and *Tinospora cordifolia* recorded significant antifungal activity against *D. turcica*.

The finding of the present investigation is an important step towards crop protection strategies against the two important root rot pathogens *D. biseptata* and *F. solani* by

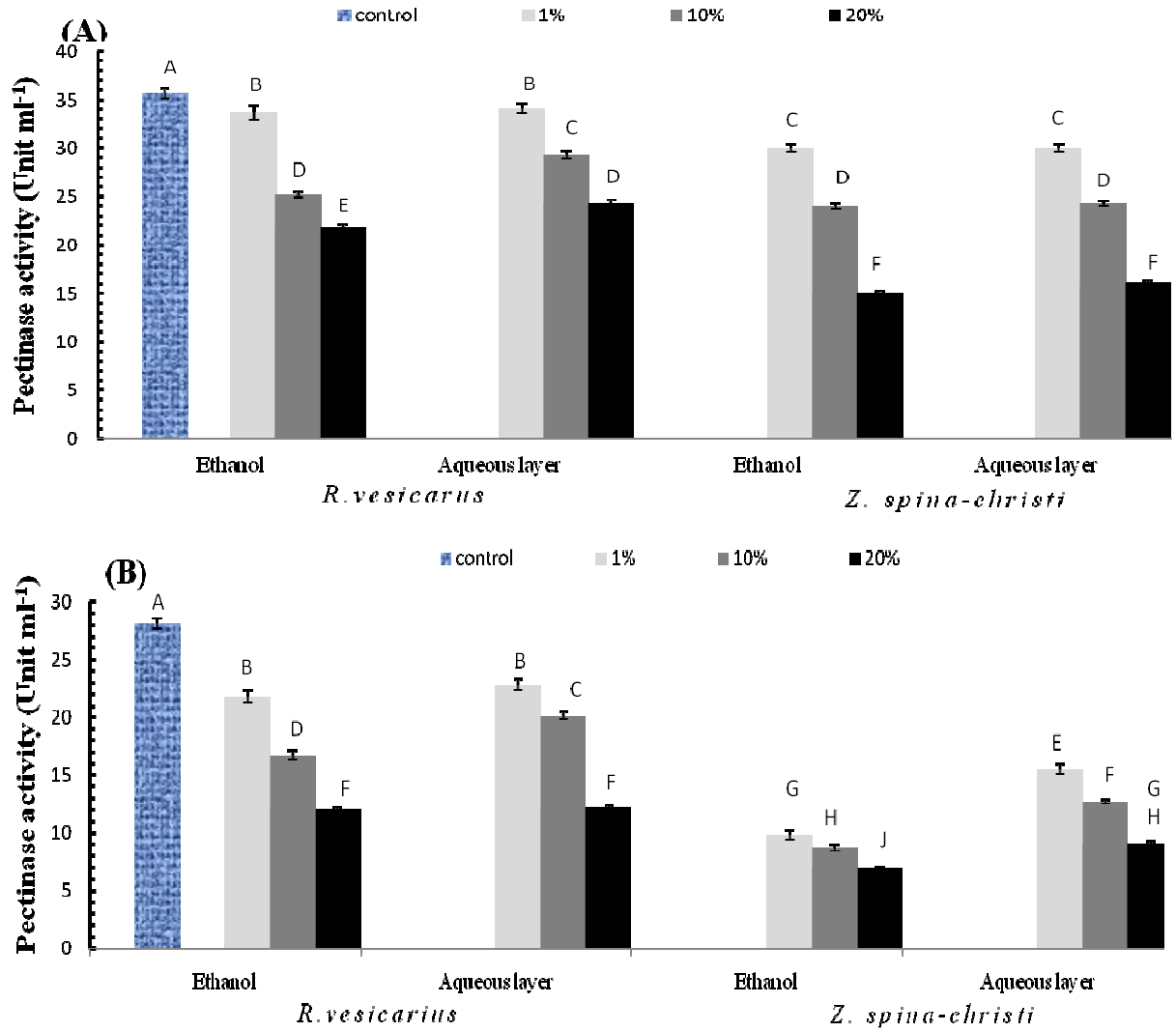


Figure 5. Effect of different concentrations of *Rumex vesicarius* and *Ziziphus spina-christi* extracts on Pectinolytic activity (unit ml⁻¹) of *Drechslera biseptata* (A) and *Fusarium solani* (B). Data represented as mean \pm S.E. In the same column, means marked with the same letters are insignificantly different ($P > 0.05$), whereas those with different letters are significantly different.

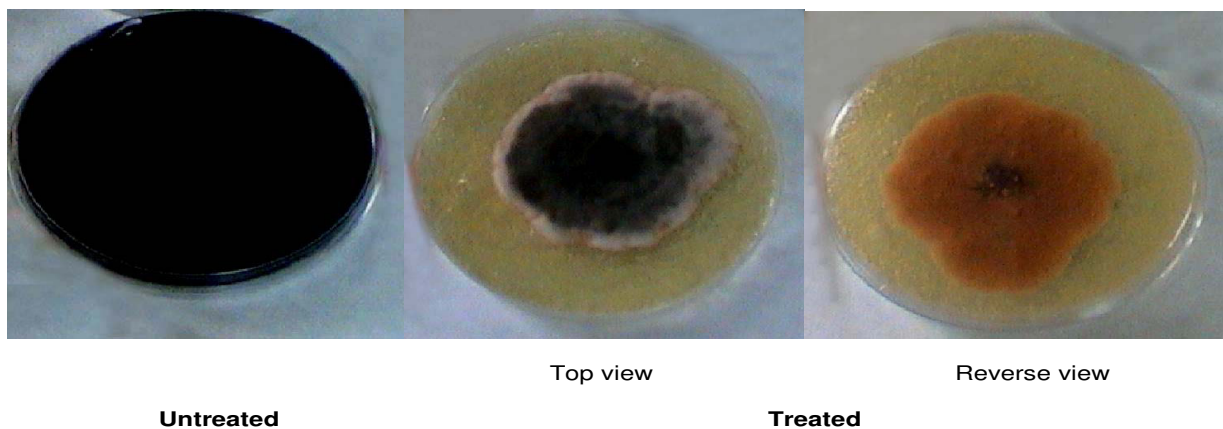


Figure 6. *Drechslera biseptata* grown on PDA medium amended with the remaining aqueous layer fraction of ethanol extract prepared from *Rumex vesicarius* for 7 days at 25°C.

using natural products.

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