Pak. J. Bot., 53(3): 1133-1139, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-3(17)

ANTIFUNGAL ACTIVITY OF METHANOLIC LEAF EXTRACT OF *CARTHAMUS OXYCANTHA* AGAINST *RHIZOCTONIA SOLANI*

MUHAMMAD RAFIQ, ARSHAD JAVAID* AND AMNA SHOAIB

Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan
**Corresponding author's email: arshad.iags@pu.edu.pk, arshadjpk@yahoo.com*

Abstract

Potato is a globally important crop whose production is severely damaged by black scurf disease (stem cankers and tuber blemishes) caused by soil-borne fungus, *Rhizoctonia solani.* This study was undertaken to assess antifungal potential of *Carthamus oxycantha* extracts and detection of likely antifungal components by GC-MS. In laboratory bioassays, antifungal activity of methanolic extracts of leaf, stem, root and inflorescence of *C. oxycantha* was checked using a range of concentrations from 1.562 to 200 mg mL^{-1}. The leaf extract significantly suppressed fungal growth. Methanolic leaf extract was subjected to GC-MS analysis. A total of 95 compounds were found present in this extract. Predominant compound was D-ribofuranose, 5-deoxy-5-(methylsulfinyl)-1,2,3-tris-O-(trimethylsilyl)- (13.312%) followed by benzoic acid, 4-hydroxy-3-methoxy-, methyl ester (11.888%), bis(2-ethylhexyl) phthalate (9.842%), 4-hydroxy-2,2',4',6'-tetrachlorobiphenyl, trimethylsilyl ether (6.837%) and pentanedioic acid (4.926%). Besides, a number of free fatty acids and fatty acid methyl esters with known antifungal potential were also identified as minor compounds.

Key words: *Rhizoctonia solani*, *Carthamus oxycantha*, Natural fungicides, Potato.

Introduction

Black scurf of potato is a divesting disease of potato in the most potato cultivating regions around the globe. This disease is caused by *Rhizoctonia solani* (Kanetis *et al.*, 2016; El-Zaidi *et al*., 2018). The ability of *R. solani* to survive in soil and plant debris for longer period of time because of their persistent sclerotia makes this disease more alarming for the crop (Sedláková *et al.*, 2013). The optimum temperature (20–25°C) and high moisture content favor the growth of the pathogen. The pathogen affects the crop from seedling to harvesting. Black spots or sclerotia formed on potato tubers have negative effect on quality and marketing of the crop. Mostly this disease is managed by chemical methods (Lahlali & Hijri, 2010), which are not effective to completely manage this disease and in return may cause environmental pollution (Kurzawińska & Mazur, 2008). Cultivation of non-host crops in rotation is another strategy opted to minimize disease incidence and severity for 3–5 years, but rotation is not easy to carry out in the major potato growing regions (Bakali & Martín, 2006).

Plant extracts as phytobiocides could be an alternative option to control black scurf disease due to their quick degradation, narrow range of activity and nontoxic effects on the environment (Bakali & Martín, 2006). Many recent studies have shown potential use of botanicals for management of various fungal pathogens such as *Fusarium oxysporum, Macrophomina phaseolina, Sclerotium rolfsii* and *Alternaria* spp. (Sana *et al*., 2017; Akhtar & Javaid, 2018; Javaid *et al.*, 2018a, b; Khurshid *et al.*, 2018). Earlier studies also revealed antifungal activity of extracts of *Azadirachta indica, Eucalyptus camaldulensis, Allium cepa, Allium sativum, Lantana camara, Capparis deciduas, Dodonaea viscosa* and *Peganum harmala* extracts against *R. solani* (Naz, 2006; Sharma & Kama, 2009; Atiq*et al*., 2014; Khan *et al*., 2016).

Carthamus oxycantha M. Bieb. is a problematic weed for major crops but is a major source of compounds used in medicines (Ahmad *et al*., 2010). It has been reported as a significant medicinally important weed due to the presence of anti-hyperlipidaemic attributes and can be used to increase blood circulation (Ahmad *et al*., 2009). Apart from its medicinal importance, the weed is also known to exhibit allelopathy against weeds and crops plants (Hesammi, 2012; Siyar *et al*., 2018). However, studies about antifungal activity of *C. oxycantha* are lacking. Thus, this study was carried out to check the antifungal effect of *C. oxycantha* extracts against *R. solani* isolated from black scurf disease affected potato tuners.

Materials and Methods

Pathogen's isolation: Potato tubers suffering from black scurf disease were procured from three vegetable markets of Lahore, Pakistan. Symptomatic potatoes were treated with 1% sodium hypochlorite to remove surface microbial flora. The infected parts were sliced and inoculated on 2% malt extract agar at 25°C. After 7 days incubation, the colonies appeared around the incubated potato slices were sub-cultured on fresh malt extract agar.The fungus was identified as *Rhizoctonia solani* on the bases of macroscopic and microscopic characteristics (Lakshman *et al*., 2016).

Pathogenicity test: Potato tubers were surface sterilized with 1% NaOCl and washed with sterilized water. The inoculum from fresh culture of *R. solani* was taken with the help of an inoculating needle and put on the surface of the tubers. The pathogen was allowed to establish on the potato by incubating at 25°C. The symptoms were observed after 7 days and the pathogen was again isolated for the re-confirmation.

Extract preparation: *C. oxycantha* was collected from Lahore. Different parts of the plant *viz.* root, stem, leaves, and flowers were separated. After drying in sun and crushing, 200 g of each part were soaked in 1 L of 80% methanol for 15 days. The solvent was filtered with

muslin cloth and filter papers followed by evaporation at 45°C on a rotary evaporator. The remaining leaf, stem, root and inflorescence materials after complete evaporation of the solvent in an oven at 45°C were weighed 8.2 g, 9.3 g, 8.4 g and 7.9 g, respectively and saved in autoclaved beakers for further experimentation (Javaid *et al*., 2018b).

Laboratory bioassays: For stock solution preparation, 1.2 g extract of each plant part (leaf, stem, root, and inflorescence) was dissolved in 1 mL dimethyl sulphoxide (DMSO). Autoclaved malt extract broth was added to make the volume up to 6 mL. Half the amount (3 mL) was poured into pre-sterilized test tubes (1 mL in each). The volume of the remaining 3 mL was again raised to 6 mL by adding 3 mL malt extract broth. Likewise, the growth medium was serially diluted to get 1.562 to 200 mg mL^{-1} concentrations. Each dilution was used for bioassays with 3 replications. The suspension of *R. solani* was prepared in autoclaved distilled water and its 20 µL were used to inoculate each test tube. Incubation was done at 25°C for 7 days. Thereafter, the fungal biomass from all the test tubes was collected on filter papers, dried and weighed (Javaid *et al*., 2018c).

Identification of compounds by GC-MS: GC-MS analysis of methanolic leaf extract was carried out following Rafiq *et al.,* (2017).

Statistical analysis

Data were subjected to ANOVA followed by mean separation using LSD Test at P = 0.05 using software Statistix 8.1.

Results and Discussion

A significant reduction of 27-37% in fungal growth was recorded due to leaf extract. Likewise, stem and inflorescence extracts also reduced fungal biomass by 16-35% and 11-30%, respectively. However, generally antifungal effects of these extracts were insignificant as compared to corresponding control treatments. Root extract did not show any antifungal activity (Figs. 1 & 2).

Based on the highest antifungal potential, leaf extract was further analyzed through GC-MS. The GC-MS revealed the presence of 95 compounds in the extract (Table 1, Fig. 3). The major compounds included D-ribofuranose, 5-deoxy-5-(methylsulfinyl)-1,2,3-tris-O-(trimethylsilyl) (13.312%), benzoic acid, 4-hydroxy-3-methoxy-, methyl ester (11.888%), bis(2-ethylhexyl) phthalate (9.842%), 4-hydroxy-2,2',4',6'-tetrachlorobiphenyl, trimethylsilyl ether (6.837%), pyridine, 2-pentyl- (6.698%), pentanedioic acid (4.926%), 4-hydroxybutanoic acid (4.630%), olean-18-en-3-ol, O-TMS, (3. beta.) (3.800%), phenol, 4-ethenyl-2,6-dimethoxy (3.768%), 5-amino-8-hydroxyquinoline (3.234%), 2-methylidene-6,10,14-trimethylpen 2-methylidene-6,10,14-trimethylpentadecanoic acid silylated (2.439%), galactopyranose (2.176%), l-isoleucine, N-trifluoroacetyl (2.100%), 13-retinoic acid, (Z)- (1.731%), and 9-octadecenamide, (Z)- (1.729%). Among the major compounds, bis (2-ethylhexyl) phthalate is known to possess antifungal activity against various fugal species (El-Sayed, 2012). This compound is a known synthetic plasticizer. However, it has been isolated from a number of plants and other organisms including *Aloe vera, Euphorbia cyparissias, E. seguieriana, Alchornea cordifolia, Calotropis gigantea* and roots of *C. oxycantha* (Toth-Soma *et al.*, 1993; Lee *et al.*, 2000; Habib & Karim, 2009; Javaid *et al*., 2019).

Among the minor compounds, compounds namely octadecanoic acid; azelaic acid; *cis*-13-octadecenoic acid; *n*-hexadecanoic acid; dodecanoic acid; tetradecanoic acid and pentadecanoic acid belonged to fatty acids group. Fatty acids may be saturated or unsaturated (Moss *et al*., 1997). These compounds possess a number of biological activities including antifungal activity (Pohl *et al*., 2011). Linolenic and linoleic acids demonstrated antifungal activity against *Rhizoctonia solani, Pyrenophora avenae* and *Pythium ultimum* (Walters *et al*., 2004). Fatty acids isolated from cuticle of an insect *Sarcophaga carnaria* showed antifungal activity against various entomopathogenic fungi (Golebiowski *et al*., 2014). Antifungal fatty acids directly interact with cell membranes of fungi by entering in lipid bi-layer and causing increased fluidity of the membrane that results in disorganization of the cell membrane and ultimately causes cell disintegration (Avis & Bélanger, 2001). Antifungal fatty acids can replace synthetic agrochemicals which are being used to control fungal pathogens globally (Liu *et al*., 2008).

Various identified minor compounds belonged to fatty acid methyl esters (FAME) group. These included eicosanoic acid, methyl ester; heneicosanoic acid, methyl ester; *cis*-13-eicosenoic acid, methyl ester; pentadecanoic acid, methyl ester; tetracosanoic acid, methyl ester; 9,12-octadecadienoic acid (Z,Z)-, methyl ester and 13-docosenoic acid, methyl ester, (Z)- (Table 1). Most of the compounds of this group are known to exhibit antifungal activity (Javaid *et al.*, 2018b c). Pinto *et al.*, (2017) reported that antifungal activity of FAME of corn, soybean and maize against *Paracoccidioides brasiliensis, Candida glabrata*, *C. parapsilosis* and *C. krusei*was mainly due to 9,12-octadecadienoic acid (Z,Z)-, methyl ester (also known as methyl linoleate). Likewise, FAME isolated from seeds of *Annonacornifolia* showed inhibitory effects against a number of strains of *P. brasiliensis* (Lima *et al.*, 2011). FAME of *Salicornia brachiata* (family Chenopodiaceae) also showed antifungal activity against a number of fungi (Chandrasekaran *et al.*, 2007). FAME of *Excoecaria agallocha* showed antifungal activity against various clinically important fungal species (Agoramoorthy *et al.*, 2007).

This study concludes that leaf extract of *C. oxcantha* possess a number of antifungal constituents especially bis(2-ethylhexyl) phthalate, free fatty acids and fatty acid methyl esters that inhibited the growth of *R. solani.*

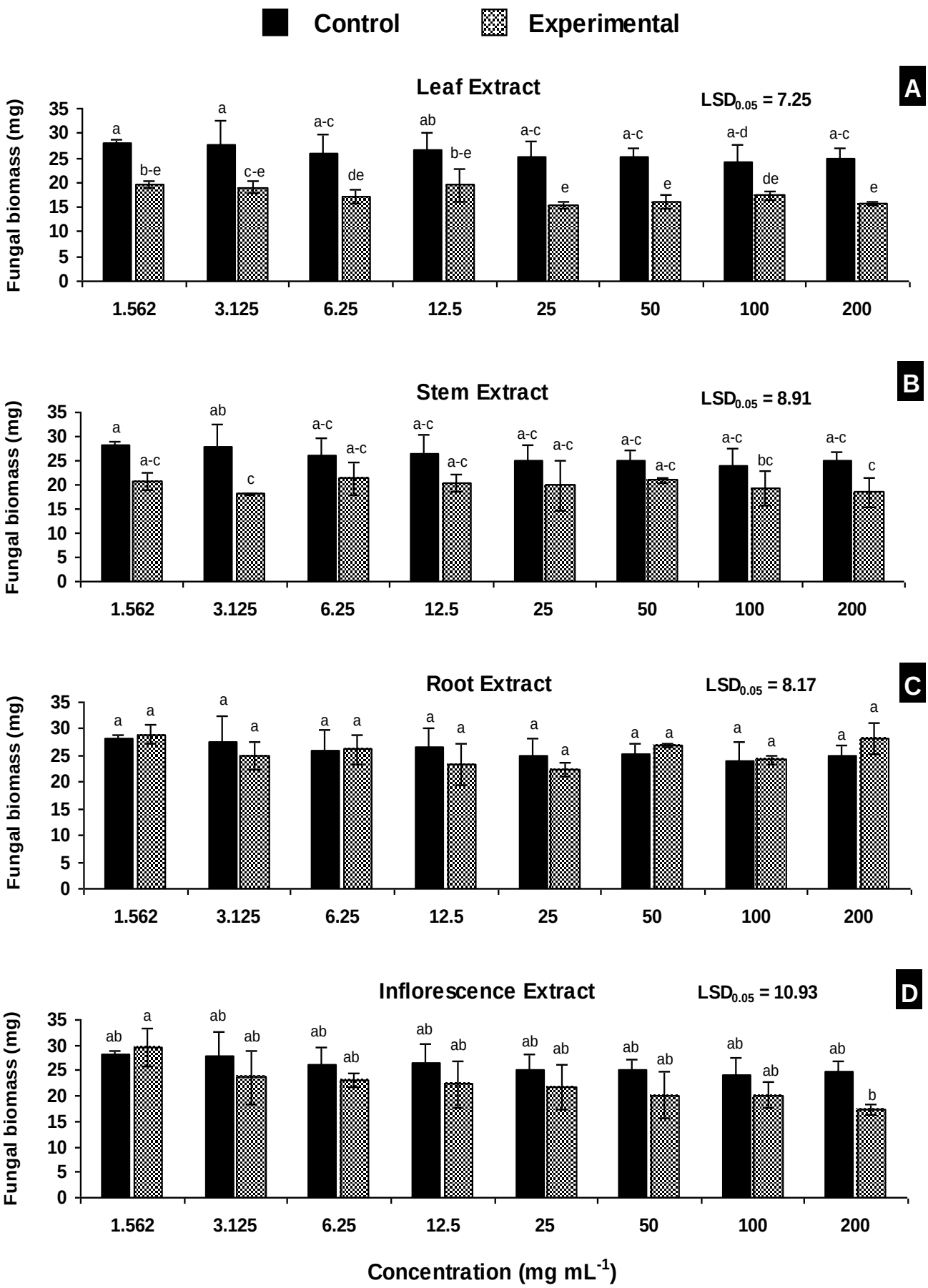

Fig. 1. Effect of different concentrations of methanolic extracts of leaf, stem, root and fruit of *Carthamusoxycantha* on growth of *Rhizoctoniasolani*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($p \leq 0.05$) as determined by LSD Test.

Table 1. Compounds identified from methanolic leaf extract of *Carthamus oxycantha* through GC-MS analysis.

Comp. No.	Names of compounds	Formula	Weight	Retention time (min)	Peak area (%)
1.	D-Ribofuranose, 5-deoxy-5-(methylsulfinyl)-1,2,3-tris-O- (trimethylsilyl)-	$C_{15}H_{36}O_5SSi_3$	412.16	8.42	13.312
2.	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	$C_{10}H_{12}O_4$	196.07	8.48	11.888
3.	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.28	15.12	9.842
4.	4-Hydroxy-2,2',4',6'-tetrachlorobiphenyl, trimethylsilyl ether	$C_{15}H_{14}Cl_4OSi$	377.96	16.12	6.837
5.	Pyridine, 2-pentyl-	$C_{13}H_{13}N$	183.10	5.65	6.698
6.	Pentanedioic acid	$C_5H_8O_4$	132.11	7.45	4.926
7.	4-Hydroxybutanoic acid	$C_4H_8O_3$	104.10	5.91	4.630
8.	Olean-18-en-3beta-ol	$C_{33}H_{58}O$	498.43	19.53	3.800
9.	Phenol, 4-ethenyl-2,6-dimethoxy-	$C_{10}H_{12}O_3$	180.08	8.85	3.768
10.	5-Amino-8-hydroxyquinoline	$C_9H_8N_2O$	160.17	16.36	3.234
11.	2-Methylidene-6,10,14-trimethylpen2-methylidene-6,10,14-trimethylpentadecanoic acid silylated	$C_{22}H_{44}O_2Si$	368.31	11.73	2.439
12.	Galactopyranose	$C_6H_{12}O_6$	180.15	17.98	2.176
13.	l-Isoleucine, N-trifluoroacetyl-	$C_8H_{12}F_3NO_3$	227.08	6.49	2.100
14.	13-Retinoic acid, (Z)-	$C_{20}H_{28}O_2$	300.43	15.27	1.731
15.	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.27	14.11	1.729
16.	Dehydroabietic acid	$C_{20}H_{28}O_2$	372.25	14.38	1.698
17.	Phosphoric acid, bis(trimethylsilyl)monomethyl ester	$C_7H_{21}O_4PSi_2$	256.07	5.42	1.220
18.	Decanedioic acid, dibutyl ester	$C_{18}H_{34}O_4$	314.25	12.96	1.052
19.	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.46	13.26	0.977
20.	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354.35	14.95	0.885
21.	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256.24	10.71	0.878
22.	Benzoic acid, 3-[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_7H_6O_2$	282.11	8.83	0.856
23.	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666.17	10.72	0.846
24.	10-Undecenoic acid	$C_{11}H_{20}O_2$	184.27	12.91	0.753
25.	Bohlmann k2631	$C_{15}H_{20}O_2$	232.15	11.92	0.582
26.	Undecanedioic acid	$C_{11}H_{20}O_4$	216.27	11.85	0.486
27.	2-Aminoethanol, N-acetyl-	$C_4H_9NO_2$	103.12	5.64	0.475
28.	Pimelic acid	$C_7H_{12}O_4$	160.16	9.09	0.468
30.	Octadecanoic acid	$C_{18}H_{36}O_2$	284.27	12.90	0.406
31.	2-O-Glycerol-α-d-galactopyranoside, hexa-TMS	$C_{27}H_{66}O_8Si_6$	686.34	14.00	0.376
32.	Triethylene glycol	$C_6H_{14}O_4$	150.17	8.30	0.364
33.	Androst-4-ene-3, 17-dione, 15-hydroxy-, (15.alpha.)-	$C_{19}H_{26}O_3$	302.19	14.56	0.358
34.	1-Monomyristin	$C_{17}H_{34}O_4$	302.45	14.29	0.314
35.	*n*-Tetracosanol-1	$C_{24}H_{50}O$	354.39	14.23	0.309
36.	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	$C_{14}H_{20}O_2$	196.07	8.08	0.295
37.	Hexacosane	$C_{26}H_{54}$	366.42	13.76	0.273
38.	Azelaic acid	$C_9H_{16}O_4$	188.10	9.34	0.273
39.	Pantothenic acid	$C_9H_{17}NO_5$	219.23	11.98	0.251
40.	3-(4-Hydroxyphenyl)-1-propanol	$C_9H_{12}O_2$	152.19	9.74	0.224
41.	Phloretic acid	$C_9H_{10}O_3$	166.17	10.33	0.215
42.	Propanetriol, 2-methyl	$C_4H_{10}O_3$	106.12	7.88	0.183
43.	Glycerol	$C_3H_8O_3$	92.09	5.55	0.163
44.	2-Linoleoylglycerol	$C_{21}H_{38}O_4$	354.53	15.97	0.152
45.	4-Coumaric acid	$C_9H_8O_3$	164.16	11.57	0.132
46.	5-O-Coumaroyl-D-quinic acid	$C_{16}H_{18}O_8$	388.10	17.32	0.122
47.	2'-Hydroxy-6'-methoxyacetophenone	$C_9H_{10}O_3$	166.17	8.23	0.110

Table 1. (Cont'd.).

Comp. No.	Names of compounds	Formula	Weight	Retention time (min)	Peak area (%)
48.	Xylitol	$C_5H_{12}O_5$	152.14	10.07	0.102
49.	L-Valine	$C_5H_{11}NO_2$	117.14	5.76	0.093
50.	Octahydro-1H-cyclopenta[b]pyridin-4-ol	$C_8H_{15}NO$	141.12	4.26	0.083
51.	Decanedioic acid, bis(2-ethylhexyl) ester	$C_{26}H_{50}O_4$	426.37	16.35	0.079
52.	Stigmastanol	$C_{29}H_{52}O$	416.73	19.62	0.068
53.	Diethylene glycol	$C_4H_{10}O_3$	106.12	6.00	0.060
54.	N,N-Bis(2-hydroxyethyl)-p-toluidine	$C_{11}H_{17}NO_2$	195.13	14.38	0.056
55.	Propanedioic acid	$C_3H_4O_4$	104.06	5.63	0.055
56.	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326.32	13.86	0.042
57.	Oleic acid, butyl ester	$C_{22}H_{42}O_2$	338.32	13.83	0.038
58.	6-Phthalazinecarboxylic acid, 1,2,3,4-tetrahydro-2,3-dimethyl-1,4-dioxo-	$C_{11}H_{10}N_2O_4$	234.06	7.57	0.030
59.	Eicosane	$C_{20}H_{42}$	282.33	11.88	0.025
60.	Fluoxymesterone	$C_{20}H_{29}FO_3$	336.21	13.42	0.023
61.	Niacin	$C_6H_5NO_2$	123.03	6.13	0.017
62.	*cis*-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.26	12.78	0.007
63.	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.24	11.65	0.007
64.	Dodecanoic acid	$C_{12}H_{24}O_2$	200.18	8.72	0.001
65.	Dodecane	$C_{12}H_{26}$	170.20	5.55	0.001
66.	Benzeneacetic acid	$C_8H_8O_2$	136.05	6.08	0.001
67.	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340.33	14.42	0.001
68.	Hexadecanoic acid, ethyl ester	$C_{18}H_{34}O_2$	282.26	11.85	0.001
69.	1-Hexacosene	$C_{26}H_{52}$	364.41	13.09	0.001
70.	Squalene	$C_{30}H_{50}$	410.39	16.48	0.001
71.	Cetene	$C_{16}H_{32}$	224.25	8.98	0.001
72.	*cis*-13-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324.30	13.75	0.001
73.	Benzaldehyde, 3-hydroxy-4-methoxy-	$C_8H_8O_3$	152.05	7.50	0.001
74.	9-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268.24	11.28	0.001
75.	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.21	10.24	0.001
76.	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.03	5.96	0.001
77.	Phosphoric acid, dioctadecyl ester	$C_{18}H_{39}O_4P$	350.26	13.01	0.001
78.	Hexadecanoic acid, butyl ester	$C_{20}H_{40}O_2$	312.30	13.05	0.001
79.	Picoxystrobin	$C_{18}H_{16}F_3NO_4$	367.10	12.96	0.001
80.	γ-Sitosterol	$C_{29}H_{50}O$	414.39	19.41	0.001
81.	Salicylic acid	$C_7H_6O_3$	138.03	6.61	0.001
82.	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382.38	15.96	0.001
83.	Methyl stearate	$C_{19}H_{38}O_2$	298.29	12.69	0.001
84.	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370.31	14.28	0.001
85.	Tetradecane	$C_{14}H_{30}$	198.23	7.38	0.001
86.	3-Chloropropionic acid, heptadecyl ester	$C_{20}H_{39}ClO_2$	346.26	13.69	0.001
87.	Arecoline	$C_8H_{13}NO_2$	155.09	5.87	0.001
88.	Nonadecanoic acid	$C_{19}H_{38}O_2$	298.29	13.49	0.001
89.	Octadecane	$C_{18}H_{38}$	254.30	10.52	0.001
90.	*n*-Butyl laurate	$C_{16}H_{32}O_2$	256.24	10.43	0.001
91.	Tetradecanoic acid, 13-oxo-, methyl ester	$C_{15}H_{28}O_3$	256.20	14.88	0.001
92.	Clonitazene	$C_{20}H_{23}ClN_4O_2$	386.15	14.71	0.001
93.	13-Docosenoic acid, methyl ester, (Z)-	$C_{23}H_{44}O_2$	352.33	14.84	0.001
94.	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.22	10.94	0.001
95.	1-Nonadecene	$C_{19}H_{38}$	266.30	11.84	0.001

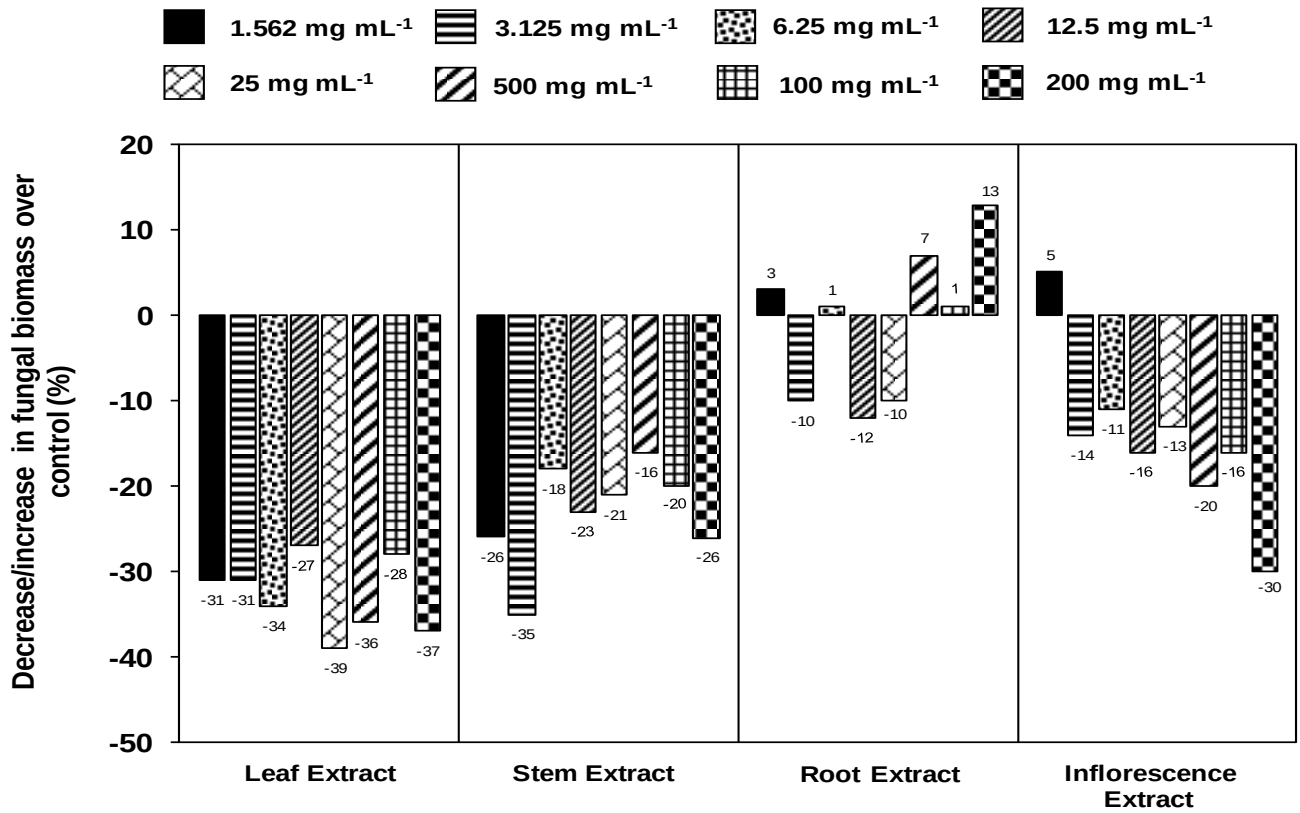

Fig. 2. Percentage decrease in biomass of *Rhizoctoniasolani* due to different concentrations of methanolic leaf, stem, root and fruit extracts of *Carthamusoxycantha.*

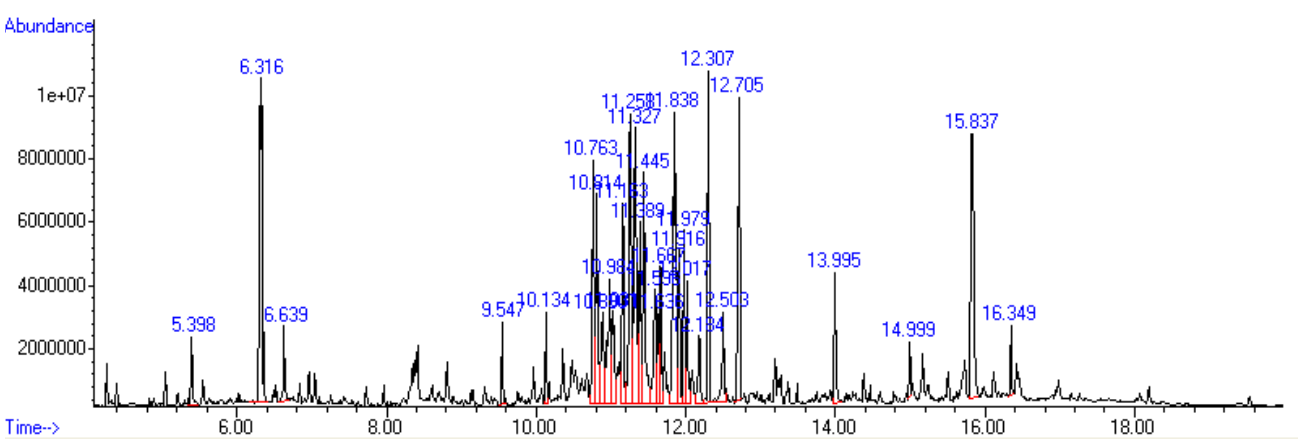

Fig. 3. GC-MS chromatogram of methanolic leaf extract of *Carthamusoxycantha*.

References

Agoramoorthy, G., M. Chandrasekaran, V. Venkatesalu and M.J. Hsu. 2007. Antibacterial and antifungal activities of fatty acid methyl esters of the blindyour- eye mangrove from India. *Braz. J. Microbiol.*, 38: 739-742.

Ahmad, M., I. Waheed, M. Khalil-ur-Rehman, U. Niaz and S. Saeed-ul-Hassan. 2010. A review on *Carthamus oxycantha*. *Pak. J. Pharm.*,23: 37-41.

Ahmad, S.S., A. Wahid, E. Bukhsh, S. Ahmad and S.R. Kakar. 2009. Antihyperlidemic properties of *Carthamus oxyacantha*. *Pak. J. Sci*., 61: 116-121.

Akhtar, R. and A. Javaid. 2018. Biological management of basal rot of onion by *Trichoderma harzianum* and *Withania somnifera*. *Planta Daninha*, 36: e017170507.

Anjani, K. 2005. Genetic variability and character association in wild safflower (*Carthamus oxycantha*). *Ind. J. Agric. Sci.*, 75: 516-518.

Atiq, M., A. Karamat, N.A. Khan, M. Shafiq, M. Younas, R.T. Iqbal, R. Bashir, M.J. Zaib, U. Nawaz and H.U. Khan. 2014. Antifungal potential of plant extracts and chemicals for the management of black scurf disease of potato. *Pak. J. Phytopathol*., 26: 161-167.

Atiq, M., A. Karamat, S. Sahi, A. Habib, A. Nawaz and W. Hussain. 2013. Screening of potato varieties / cultivars against black scurf. *Pak. J. Phytopathol.*,25: 48-51.

Avis, T.J. and R.R. Bélanger. 2001. Specificity and mode of action of the antifungal fatty acid *cis*-9-heptadecenoic acid produced by *Pseudozyma flocculosa*. *Appl. Environ. Microbiol.*, 67: 956-960.

Bakali, A.M.E. and M.P. Martín. 2006. Black scurf of potato. *Mycologist*, 20: 130-132.

Chandrasekaran, M., K. Kannathasan and V. Venkatesalu. 2007. Antimicrobial activity of fatty acid methyl esters of some members of Chenopodiaceae. *Z. Naturforsch.*, 63c: 331-336.

El-Sayed, M.H. 2012. Di-(2-ethylhexyl) phthalate, a major bioactive metabolite with antimicrobial and cytotoxic activity isolated from the culture filtrate of newly isolated soil Streptomyces (*Streptomyces mirabilis* strain NSQu-25). *World Appl. Sci. J.*, 20: 1202-1212.

El-Zaidi, M., M.A. Mahmoud, M.R. Al-Othman and N.S Abdulazim. 2018. Molecular identification and characterization of *Rhizoctonia solani* AG-3 isolates causing black scurf of potato. *Pak. J. Bot*., 50: 415-420.

Golebiowski, M., A. Urbanek, A. Oleszczak, M. Dawgul and P. Stemnowski. 2014. The antifungal activity of fatty acids of all stages of *Sarcophagacarnaria* L. (Diptera: Sarcophagidae). *Microbiol. Res.*, 169: 279-286.

Habib, M.R. and M.R. Karim. 2009. Antimicrobial and cytotoxic activity of di-(2-ethylhexyl) phthalate and anhydrosophoradiol-3-acetate isolated from *Calotropis gigantea* (Linn.) flower. *Mycobiology*, 37: 31-36.

Hesammi, E. 2012. Allelopathic effects of *Carthamus oxycantha* and *Chenopodium mural* on germination and initial growth of *Phasaeolous vulgaris*. *Int. J. Farm. Allied Sci.*, 1: 54-56.

Javaid, A., I.H. Khan and A. Shoaib. 2018a. Management of charcoal rot of mungbean by two *Trichoderma* species and dry biomass of *Coronopus didymus*. *Planta Daninha*, 36: e018182795.

Javaid, A., U. Latif, N. Akhtar, D. Ahmed and S. Perveen. 2018b. Molecular characterization of *Fusarium moniliforme* and its management by methanolic extract of *Coronopus didymus*. *Pak. J. Bot*., 50: 2069-2075.

Javaid, A., G..R. Shahzad, N. Akhtar and D. Ahmed. 2018c. Alternaria leaf spot disease of broccoli in Pakistan and management of the pathogen by leaf extract of *Syzygium cumini*. *Pak. J. Bot*., 50: 1607-1614.

Javaid, A., F. Anjum and N. Akhtar. 2019. Molecular characterization of *Pyricularia oryzae* and its management by stem extract of *Tribulus terrestris. Int. J. Agric. Biol.,* 21: 1256-1262.

Kanetis, L., D. Tsimouris and M. Christoforou. 2016. Characterization of *Rhizoctonia solani* associated with black scurf in Cyprus. *Plant Dis.*,100: 1591-1598.

Karima, S., S. Farida and Z.M. Mihoub. 2013. Antimicrobial activity of an Algerian medicinal plant: *Carthamus caeruleus* L. *Pharmacogn. Commun.*, 3: 7176.

Khan, I., S. Alam, H. Hussain, B. Shah, A. Naeem, W. Ullah, W.A. Khan, M. Adnan, K. Junaid, S.R.A. Shah, N. Ahmed and M. Iqbal. 2016. Study on the management of potato black scurf disease by using biocontrol agent and phytobiocides. *J. Entomol. Zool. Stud.*, 4: 471-475.

Khurshid, S., A. Javaid, A. Shoaib, S. Javed and U. Qaiser. 2018. Antifungal activity and GC-MS analysis of aerial parts of *Cenchrus pennisetiformis* against *Fusarium oxysporum* f. sp. *lycopersici*. *Planta Daninha*, 36: e017166627.

Kurzawińska, H. and S. Mazur. 2008. The usefulness of chitosan and Pythiumoligandrum in potato tuber protection against *Helminthosporium solani*. *Folia Hort.*, 20: 67-74.

Lahlali, R. and M. Hijri, 2010. Screening, identification and evaluation of potential biocontrol fungal endophytes against *Rhizoctonia solani* AG3 on potato plants. *FEMS Microbiol. Lett.*, 311: 152-159.

Lakshman, D.K., P.P. Jambhulkar, V. Singh, P. Sharma, and A. Mitra. 2016. Molecular identification, genetic diversity, population genetics and genomics of *Rhizoctonia solani*. In: *Perspect. Plant Pathol. Genomic Era*. P. Chowdappa, P. Sharma, D. Singh and A.K. Mitra (eds.), Today and Tomorrow's Printers and Publications, New Delhi, India. pp. 5555-5589.

Larkin, R.P. and S. Tavantzis. 2013. Use of biocontrol organisms and compost amendments for improved control of soil-borne diseases and increased potato production. *Am. J. Potato Res.*, 90: 261-270.

Lee, K.H., J.H. Kim, D.S. Lim and C.H. Kim. 2000. Antileukaemic and anti-mutagenic effects of di-(2-ethylhexyl) phthalate isolated from *Aloe vera* Linn. *J. Pharm. Pharmacol.*, 52: 593-598.

Lima, L.A.R.S., S. Johann, P.S. Cisalpino, L.P.S. Pimenta and M.A.D. Boaventura. 2011. *In vitro* antifungal activity of fatty acid methyl esters of the seeds of *Annona cornifolia* A. St. Hil. (Annonaceae) against pathogenic fungus *Paracoccidioides brasiliensis*. *Rev. Soc. Bras. Med. Trop.*, 44: 777-780.

Liu, S., R. Weibin, L. Jing, X. Hua, W. Jingan, G. Yubao and W. Jingguo. 2008. Biological control of phytopathogenic fungi by fatty acids. *Mycopathologia*, 166: 93-102.

Moss, G.P., P.A.S. Smith and D. Tavernier. 1997. *IUPAC Compendium of Chemical Terminology. Pure & App. Chem.,* 67(2nd ed.). International Union of Pure and Applied Chemistry. pp. 1307-1375.

Naz, F. 2006. Integrated management of black scurf of potato. University of Arid Agriculture, Rawalpindi, Pakistan.

Pinto M.E.A., S.G. Araújo, M.I. Morais, N.P. Sá, C.M. Lima, C.A. Rosa and L.A.R.S. Lima. 2017. Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. *An. Acad. Bras. Ciênc.*, 89: 1671-1681.

Pohl, C.H., L.F.J. Kock and V.S. Thibane. 2011. Antifungal free fatty acids: a review. In: *Science against Microbial Pathogens: Communi. Curr. Res. & Technol Advan.*, Méndez-Vilas (Ed.), Formatex Research Center. pp. 61-70.

Rahul, S.N., K. Khilari and S.K. Jain. 2016. Management of black scurf of potato caused by *Rhizoctonia solani* with organic amendments and their effect on different parameter of potato crop. *J. Pure Appl. Microbiol.*,10: 2433-2438.

Rauf, C.A., M. Ashraf and I. Ahmad. 2007. Management of black scurf disease of potato. *Pak. J. Bot*., 39: 1353-1357.

Raza, M.A., F. Mukhtar and M. Danish. 2015. *Cuscuta reflexa* and *Carthamus oxyacantha*: potent sources of alternative and complimentary drug. *Springer Plus*, 4: 76.

Sanam, N., A. Javaid and A. Shoaib. 2017. Antifungal activity of methanolic leaf extracts of allelopathic trees against *Sclerotium rolfsii*. *Bangladesh J. Bot*., 46: 987-993.

Sedláková, V., J. Dejmalová, P. DoleŽal, E. Hausvater, P. Sedlák and P. BaŠtová. 2013. Characterization of forty-four potato varieties for resistance to common scab, black scurf and silver scurf. *Crop Prot.*, 48: 82-87.

Sharma, B. and P. Kamar. 2009. *In vitro* antifungal potency of some plant extracts against *Fusarium oxysporum*. *Int. J. Pharm.*, 3: 63-65.

Sharma, B.P. and B.K. Ram. 2007. Participatory black scurf disease management on potato in Nepal. *Nepal Agri. Res. J.*, 8: 56-62.

Siyar, S., A. Majeed, Z. Muhammad, R. Ullah and S. Islam. 2018. Allelopathic management of some noxious weeds by the aqueous extracts of *Parthenium hysterophorus* and *Carthamus oxyacantha*. *Pol. J. Nat. Sci*., 33: 223-231.

Taskova, R., M. Mitova, H. Najdenski, I. Tzvetkova and H. Duddeck. 2002. Antimicrobial activity and cytotoxicity of *Carthamus lanatus*. *Fitoterapia*, 73: 540-543.

Toth-Soma, L.T., S. Gulyas and Z. Szegletes. 1993. Functional connection between and extracellular secretion in species of *Euphorbia* genus. *Acta Biol. Hung.*, 44: 433-443.

Walters, D., L. Raynor, A. Mitchell , R. Walker and K. Walker. 2004. Antifungal activities of four fatty acids against plant pathogenic fungi. *Mycopathologia*, 157: 87-90.

(Received for publication13 March 2019)