Pak. J. Bot., 50(5): 2069-2075, 2018.

MOLECULAR CHARACTERIZATION OF *FUSARIUM MONILIFORME* AND ITS MANAGEMENT BY METHANOLIC EXTRACT OF *CORONOPUS DIDYMUS*

ARSHAD JAVAID[1*], UMAIR LATIF[1], NAUREEN AKHTAR[1], DILDAR AHMED[2] AND SHAGUFTA PERVEEN[1]

[1]*Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan*
[2]*Department of Chemistry, Forman Christian College Lahore, Pakistan*
[*]*Corresponding author's email: arshadjpk@yahoo.com*

Abstract

Bakanae disease caused by different species of *Fusarium,* is one of the most emerging diseases of rice in Asia which is responsible for significant yield losses of this staple crop. The present study was designed to isolate and identify causal fungus and then the pathogen management by methanolic extract of a brassicaceous weed, *Coronopus didymus*. Based on cultural and morphological studies, isolated pathogen was identified as *Fusarium moniliforme* J. Sheld. Identity of the isolated pathogen was further confirmed by analysis of rDNA Internal Spacer Sequence (ITS) analysis. For preparation of extract, 200 g of *C. didymus* was soaked in 1.0 L of methanol for 14 days. Thereafter, material was filtered, filtrates were evaporated on a rotary evaporator and different concentrations (0.5 to 4%) of methanolic extract were prepared. These concentrations caused 23-48% reduction in the fungal biomass as compared to control. The inhibitory effect of 1.5% and higher concentrations was significant. Methanolic extract was subjected to GC-MS analysis and thirteen compounds were identified. Benzyl nitrile (34.22%), Eucalyptol (23.62%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (12.80%), *n*-Hexadecanoic acid (9.40%), Benzoic acid (6.89%), and 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (5.18%) were the major constituents among the identified compounds.

Key words: Antifungal activity, *Coronopus didymus, Fusarium moniliforme,* Natural fungicides.

Introduction

Rice bakanae disease is a wide spread disease of almost all rice growing countries, especially Asian countries including Pakistan. The pathogen of bakanae disease is soil-borne (Dawe *et al.*, 2010) as well as seed-borne (Iqbal *et al.*, 2013). Cultural practices are manipulated to suppress losses due to bakanae disease or to reduce the rate of disease development. These include growing of healthy seeds, managing the time of planting, improving general health of the plant, and water and nutrient management (Gupta *et al.*, 2015). However, these cannot give complete eradication of the disease. Crop rotation and use of higher doses of nitrogen fertilizers are other methods but these are uneconomical (Iqbal *et al.*, 2013).

Numerous fungicides are used to control the rice diseases and to increase the per acre yield ability of rice but consistent use of these chemicals is very expensive, causes many environmental hazards and also destroys the beneficial and non-target microorganisms (Amutharaj *et al.*, 2013). Residual toxicity and fungicides resistance in pathogen are also the major problems related to heavy application of pesticides. Biological control is the best alternative method used to suppress the activity of pathogens as biocontrol agents are highly selective in action, biodegradeable, non-phytotoxic, environment friendly and widely used in disease management (Sena *et al.*, 2013).

Extracts obtained from different plants such as eucalyptus, garlic, neem, ginger, tobacco, turmeric as well as tea, lemon grass, thyme and eucalyptus oils are commonly used as botanical pesticides (Das *et al.*, 2010; Gurjar *et al.*, 2012). Many weeds extracts can also be used for management of plant pathogens (Akhtar & Javaid, 2017; Khurshid *et al.*, 2017). Crude plant extracts can be directly used or the substance responsible for antifungal activity can be isolated from the crude plant extracts (Jabeen & Javaid, 2010; Javaid *et al.*, 2018). Many recent studies have shown that the use of crude plant extracts are highly effective to control plant pathogens and diseases (Ali *et al.*, 2017; Banaras *et al.*, 2017; Javaid *et al.*, 2017a). Members of Brassicaceae family are known to possess antifungal properties against soil-borne fungal plant pathogens (Busnardo *et al.*, 2010; Iqbal & Javaid, 2012). However, generally cultivated species of this family have been used for investigation of antifungal activity while such studies using weeds are scarce (Javaid & Iqbal, 2014). Therefore, the present study was conducted to characterize the pathogen of bakanae disease and its management using methanolic extract of *C. didymus*, a weed of Brassicaceae.

Materials and Methods

Isolation of fungal pathogen: During October, 2015, a survey of different rice fields of Lahore, Pakistan was conducted to study bakanae disease. Samples of rice plants infected with bakanae disease were collected from the fields at University of the Punjab and Thokar Niaz Baig, Lahore. For the isolation of pathogen, one infected portion each from ten different plants from each field was selected randomly. Approximately 2 mm^2 leaf portions containing diseased as well as some healthy portion were surface sterilized in 1% sodium hypochlorite solution for 5 minutes. After several washings with sterilized water, these pieces were transferred aseptically on the Petri plates containing malt extract agar (MEA) medium and potato dextrose agar (PDA) medium. Inoculated Petri plates were incubated at 27°C for 5 days. Mycelia growing out from the plant tissues were transferred to fresh media plates to obtain pure cultures.

Identification of fungal pathogen: Seven days old pure fungal cultures were examined for morphological and cultural characterization. Cultural characteristics included colony colour, texture, diameter, margins, sporulation

pattern and presence or absence of zones. For microscopic characterization, colour, septation, shape and size (length and width) of micro and macro conidia, conidial septation, branching and attachment to conidiophores; shape, colour, length and septation of the conidiophores were recorded.

Morphological based identification was verified by nucleotide sequence analysis of Internal Transcribed Spacer (ITS) Sequence of rDNA. Total genomic DNA isolation was carried out following the method of Akhtar *et al.,* (2014). Isolated DNA was subjected to agarose gel electrophoresis for quality and integrity assessment along with DNA size marker. The Polymerase Chain Reaction (PCR) was carried out for ITS sequence analysis. Primer pair ITS1 Forward (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 Reverse (5'-TCCTCCGCTTATTGATATGC-3') was used for the amplification of ITS region of rDNA (White *et al.,* 1990). Resulting nucleotide sequence was analysed using bioinformatics tools.

Confirmation of Koch's pathogenecity postulates: To confirm the pathogenicity of the fungus, inoculum of *Fusarium moniliforme* was prepared by transferring the mycelium from actively growing fungus culture to sterilized boiled seeds of millet in 250 mL volume flasks and incubating for 10 days at 27°C. This inoculum was mixed in sterilized loamy soil and watered to establish the fungal pathogen in the soil. Rice seeds were surface sterilized with 1% sodium hypochlorite solution (NaOCl) for 5 minutes, followed by thorough washing with sterilized water. These seeds were sown in infected soil, kept in plant growth chamber at 35°C, 75% humidity and 12 h duration of light daily.

***In vitro* management of the pathogen:** Mature plants of *C. didymus* were collected, washed thoroughly under tap water and dried in sun. The dried plant parts were crushed thoroughly. For the extraction of methanol soluble compounds, 200 g crushed plant material was soaked in 1.5 L of methanol for fifteen days at room temperature in air tight bottles. The extract was filtered through muslin cloth followed by filter paper. The filtrate was evaporated under vacuum in a rotary evaporator at 45°C until 100 mL of the filtrate left. Then extract was transferred to clean beaker and left open in an electric oven at 45°C to completely evaporate the solvent and finally crude methanolic extract (18 g) was obtained.

For *In vitro* bioassays, stock solution was prepared by dissolving 8.1 g crude methanolic extract in 5 mL of DMSO (dimethyl sulfoxide) and final volume was raised up to 18 mL with autoclaved distilled water. The control solution was prepared by dissolving 5 mL DMSO in 13 mL autoclaved distilled water. Eight concentrations viz. 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5% and 4% were prepared by adding 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 mL of stock solutions and 3.5, 3, 2.5, 2, 1.5, 1, 0.5 and 0 mL of control solution, respectively, to 41 mL malt extract medium flask to raise the total volume 45 mL. Each concentration treatment was replicated three times. For control treatment, 4 mL of control solution was added to 41 mL of malt extract medium. This quantity was equally divided into three replicates. All the flasks were inoculated with 5 mm mycelial plugs from actively growing pure culture of *F. moniliforme*. The flasks were incubated at 27°C. After 10 days, fungal biomass in each flask was filtered on pre-weighed filter papers and dried in an electric oven at 60°C to get the dry fungal biomass (Sana *et al.,* 2016).

GC-MS analysis: Agilent technologies GC: 7890a and MS: 5975c was used for GC-MS analysis. The capillary column of the instrument (HP-5 MS) was 30 m × 250 µm long with film thickness of 0.25 µm that was composed of 95% dimethyl polysiloxane. Helium was used as a carrier gas (flow rate of 1 mL min^{-1}). The instrument was set to inlet temperature at 240ºC. Microwave oven was raised 10ºC min^{-1} to 310ºC for 5 min and run time was finished in 30 min. The temperatures of MS transfer line and source were kept at 150ºC and 240ºC, respectively. Volume of sample used in injection was 1 µL. For compound identification, electron impact ionization (71 eV) was utilized and data was assessed through Total Ion Count (TIC). The acquired spectrums of the components were examined with the databank of known components spectrum. Turbo-Mass-OCPTVS-Demo SPL software was used to measure peak areas and data processing.

Statistical analysis: All the data were analyzed by analysis of variance followed by LSD at 5% level of significance using computer software Statistix 8.1.

Results and Discussion

Pathogen characterization: Morphological characterization was carried out on pure fungal culture. Colony diameter of 10 days old pure culture was 4.32 cm when grown at 27 ± 2°C. Colony colour was initially white turning light yellow upon maturity. Texture of the colony was cottony and growth zones were absent. Culture exudates were not observed (Fig. 1). Macroconidia were long ranging in length from 22–28 µm. The apical cells of macroconidia were tapered and elongated while basal cells were foot-shaped. The macroconidia were septate with 5–7 vertical septa. Microconidia were sub-globose in shape with 0-1 septation. Conidophores were rough walled and hyaline in colour. On the basis of morphological characters, the isolated pathogen was identified as *Fusarium moniliforme*, a well-known pathogen of bakanae disease of rice or foot rot of rice (Nirenberg, 1976). Pure culture of the fungal pathogen was deposited to Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan under the accession No. FCBP1487. Earlier, Amatulli *et al.,* (2010) isolated *F. moniliforme* from 98% of rice plants infected with bakanae disease. They isolated 79 isolates that all belonged to the *G. fujikuroi* complex, and 73 were *F. moniliforme.* These findings confirmed the earlier report of this pathogen (Moretti *et al.,* 2007; Zainudin *et al.,* 2008).

In earlier times, mycologists who identified the *Fusarium* species on the basis of morphological characters emphasized that *F. moniliforme* was the only species that cause bakanae disease of rice (Nirenberg 1976; Nelson *et al.,* 1983). However, modern studies suggested that other closely related *Fusarium* species in the section Liseola may also cause the same infection

(Amoah *et al.*, 1996; Desjardins *et al.*, 2000). To resolve this problem molecular characterization is conducted to verify the morphological based identification. Therefore, in the present study, nucleotide sequence analysis of ITS region of rDNA was carried out. Universal primer pair, ITS1/ITS4 amplified approximately 650 bp DNA fragment (Fig. 2). Resulting nucleotide sequence was deposited to GenBank (Fig. 3). BLAST analysis showed 99-100% pair wise sequence homology with already reported strains of *F. moniliforme/ F. verticillioides* (synonym) in GenBank database including 64fusa (HQ625614), F3 (EF158026) and P6-26 (GU723435).

Fig. 1. Purified culture of *Fusarium moniliforme*.

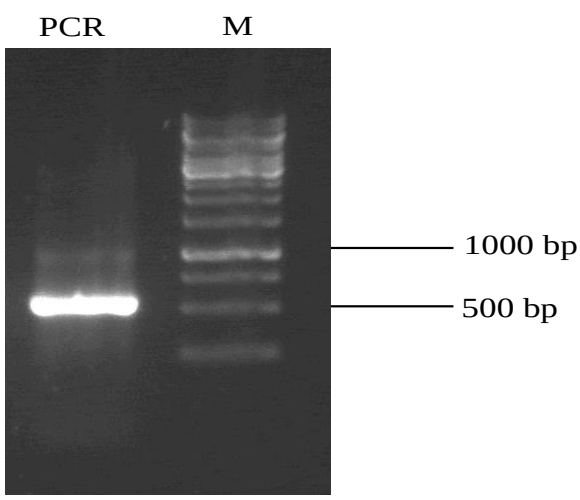


Fig. 2. PCR amplification of ITS region of rDNA. M: DNA marker.

Pathogenicity was confirmed upon isolation of the same pathogen from artificially inoculated plants. After seven days of inoculation, pathogen produced the symptoms on the plants closely related to bakanae disease of rice (Fig. 4). *F. moniliforme* was re-isolated from these experimentally infected plants and showed 100% similarity with the pathogen isolated from the field infected plants.

Antifungal activity of methanolic extract: The highest fungal biomass (300 mg) was recorded in control. The two lowest concentrations of methanolic extract of *C. didymus* (0.5% and 1%) did not show any pronounced antifungal activity against *F. moniliforme.* However, further increase in concentration of methanolic extract significantly ($p \leq 0.05$) reduced fungal biomass over control. There was 29–48% reduction in biomass of *F. moniliforme* due to 1.5% to 4% methanolic extract (Fig. 5A&B). There was a polynomial relationship between fungal biomass and extract concentration with $R^2 = 0.7179$ (Fig. 5C). Earlier, Iqbal and Javaid (2012) found that *C. didymus* extracts were highly effective against *Sclerotium rolfsii*, the cause of collar disease of bell pepper (*Capsicum annuum* L.). Likewise, Riaz *et al.,* (2010) reported that soil amendment with leaves of *C. didymus* can reduce corm rot disease of gladiolus caused by *Fusarium oxysporum* f. sp. *gladioli.* Some other workers have also reported that extracts of all parts of *C. didymus* possess antimicrobial activity (Usman *et al.*, 2007; Jindal *et al.*, 2012).

GC-MS analysis of methanolic extract: Thirteen compounds were identified in methanolic extract of *C. didymus*. These included Eucalyptol (23.62%), Benzyl nitrile (34.22%); Benzoic acid (6.89%); Benzene, (isothicyanatomethyl)- (0.57%); 3-tert-Butyl-4-hydroxyanisole (1.78%); 2-Methyl-E, E-3, 13-octadecadien-1-ol (0.57%); Hexadecanoic acid, methyl ester (2.48%); *n*-Hexadecanoic acid (9.40%); 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (5.18%); Phytol (0.73%); 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (12.80%); Piperine (1.05%) and Stigmasta-5,22-dien-3-ol, acetate, (3 beta.)- (0.66%) (Table 1, Fig. 6). Structures and mass chromatograms of these compounds are shown in Figs. 7-9. Among the identified compounds, Eucalyptol was among the major compounds. It is known to exhibit antifungal properties against a number of fungal species namely *Fusarium sporotrichioides, F. subglutinans, F. verticillioides, F. cerealis, F. proliferatum, F. oxysporum, Aspergillus carbonarius, A. tubingensis, Penicillium* sp. and *Alternaria alternata* (Morcia *et al.*, 2012). Another frequently occurring compound in methanolic extract was 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-. Fatty acid methyl esters are known to possess antifungal properties (Agoramoorthy *et al.*, 2007). Seeds of *Annona cornifolia* contains various fatty acid methyl esters chiefly linoleic, olei and palmitic acid methyl esters which repressed the growth of various strains of *Paracoccidioides brasiliensis*, a clinical pathogenic fungus (Lima *et al.*, 2011). Likewise, fatty acid methyl esters extract of *Salicornia brachiata* possessed the highest antifungal activity among the four halophytes of family Chenopodiaceae (Chandrasekharan *et al.*, 2008). Similarly, fatty acid methyl ester extract of *Excoecaria agallocha* rich in palmitic acid methyl ester (56.02%) retarded the growth of *Candida albicans, C. tropicalis, C. krusei* and *C. parapsilosis* (Agoramoorthy *et al.*, 2007).

The present study concludes that *F. moniliforme* is the cause of bakanae disease of rice. Methanolic extract of *C. didymus* can retard the growth of this fungus possibly because of presence of eucalyptol and 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-.

GGGAAAAAGAATTAACTCCAACCCCTGTGACATACCTATACGTTGCCTCGGCGGATCAGC
CCGCGCCCCGTAAAACGGGACGGCCCGCCCGAGGACCCCTAAACTCTGTTTTTAGTGGAA
CTTCTGAGTAAAACAAACAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGC
ATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCA
TCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCG
TCATTTCAACCCTCAAGCTCAGCTTGGTGTTGGGACTCGCGGTAACCCGCGTTCCCCAAA
TCGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAATCATACACCTCGTTACTGGTAA
TCGTCGCGGCCACGCCGTAAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAA
TACCCGCTGAACTTAAGCATATCAATAAGCCGGAGGAAA

Fig. 3. ITS sequence alignment of *Fusarium moniliforme*.

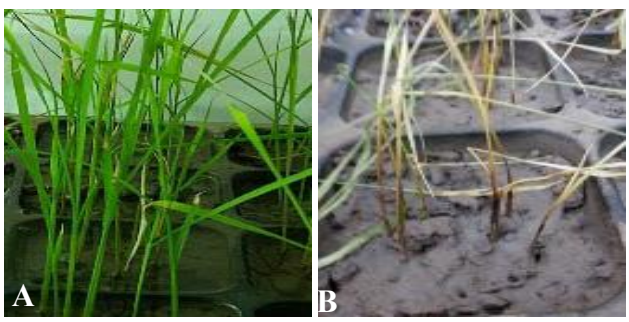


Fig. 4. Pathogenicity test. Healthy (A) and diseased plants showing the symptoms of bakanae disease of rice (B).

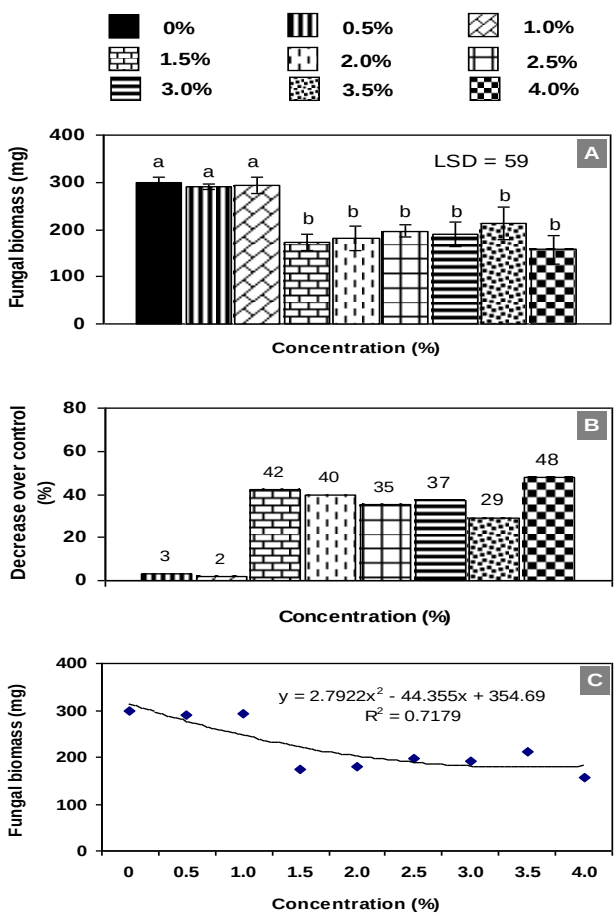


Fig. 5. A: Effect of different concentrations of methanol extract of *Coronopus didymus* on biomass of *Fusarium moniliforme,* B: Percentage reduction in fungal biomass due to different concentrations of methanolic extract over control, C: Relationship between extract concentration and fungal biomass. 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.

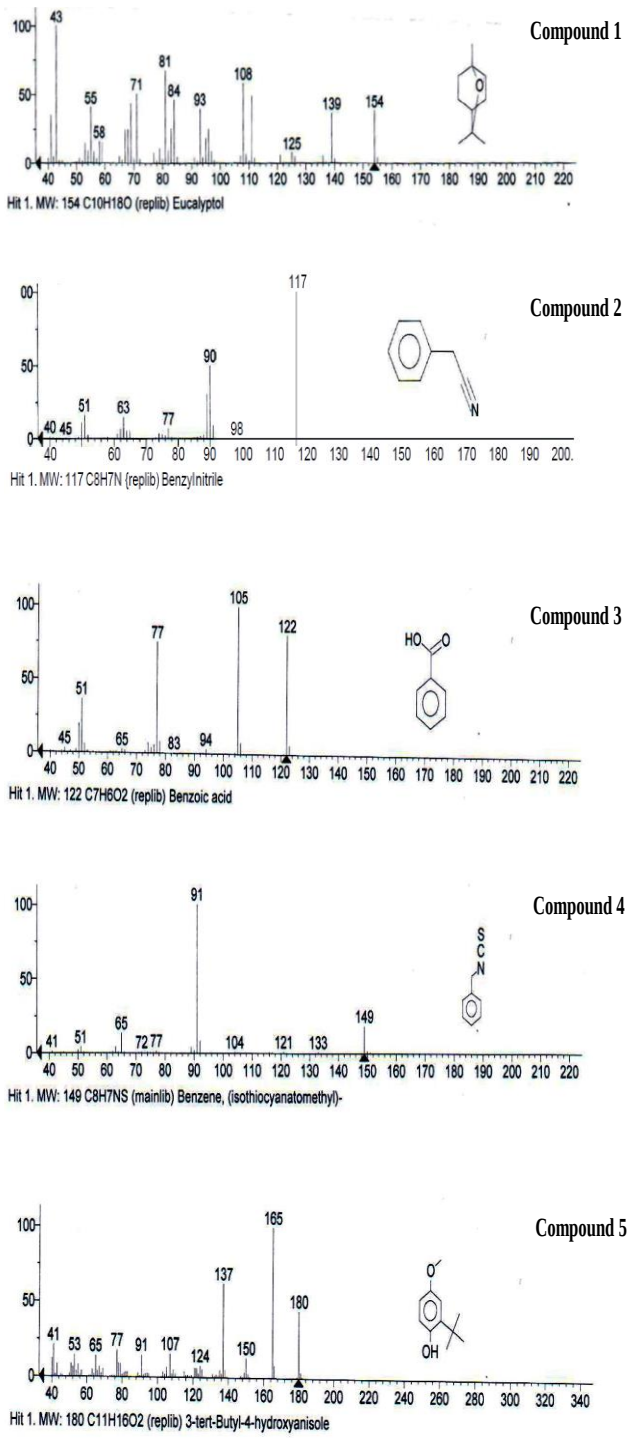
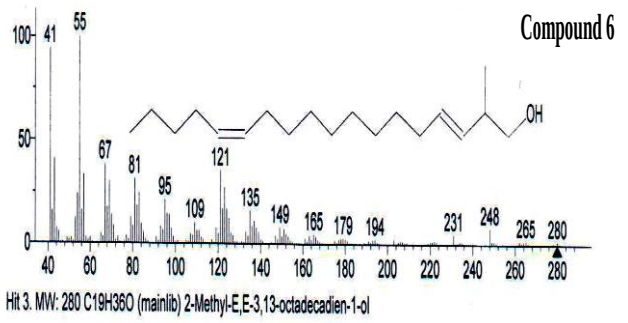
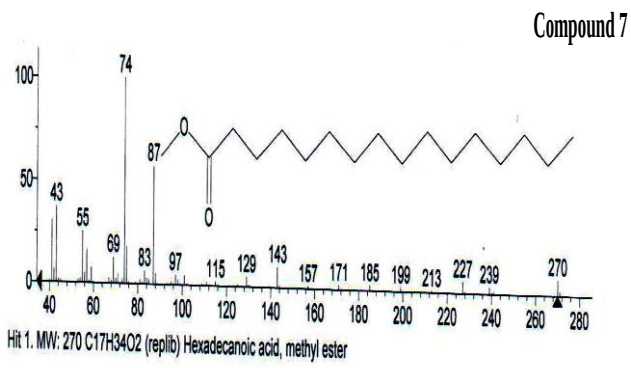
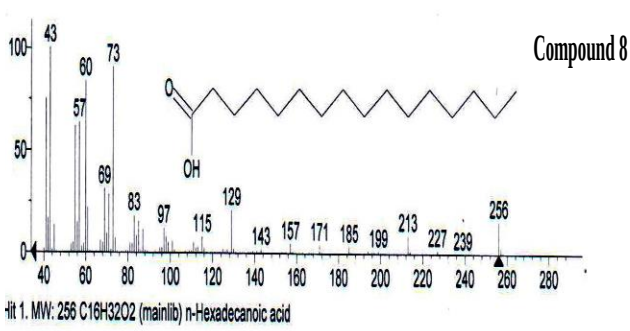


Fig. 7. Mass chromatograms and structures of compounds 1-5 identified in methanolic extract of *Coronopus didymus* through GC-MS analysis.







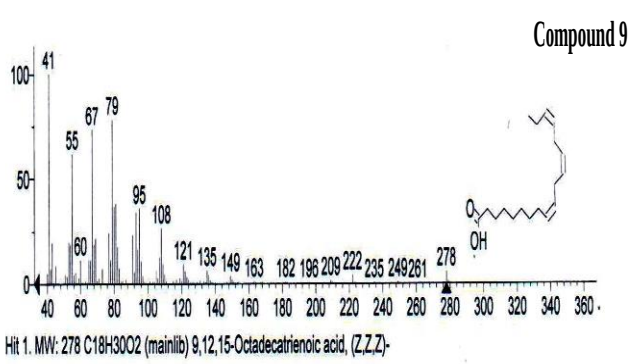
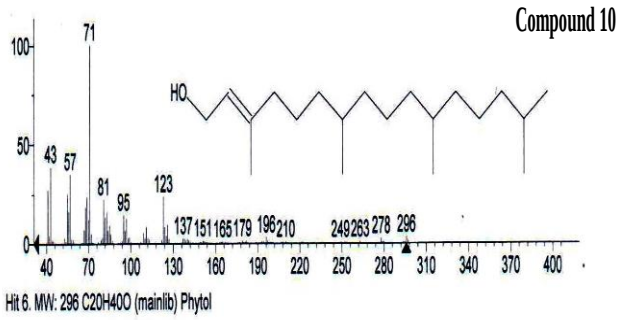
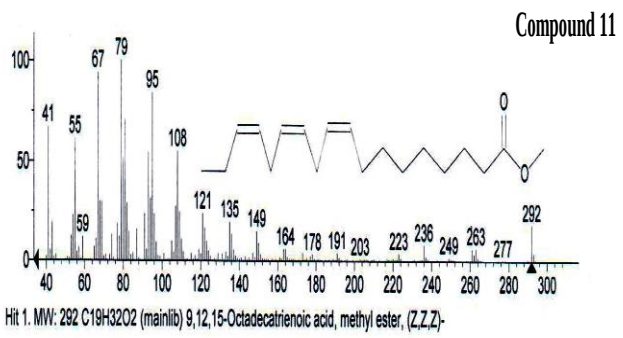
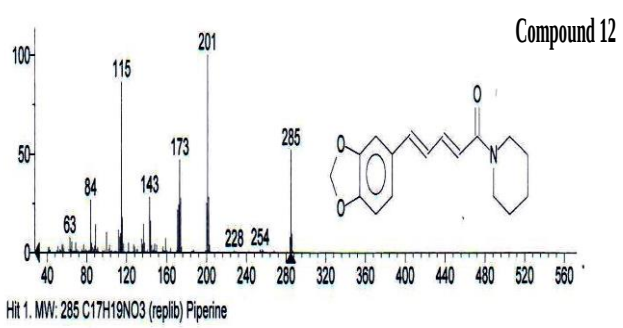


Fig. 8. Mass chromatograms and structures of compounds 6-9 identified in methanolic extract of *Coronopus didymus* through GC-MS analysis.







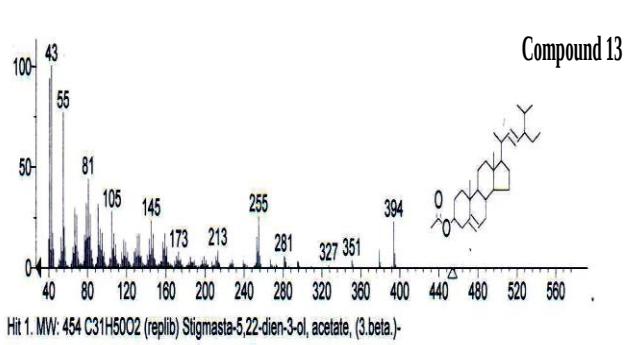


Fig. 9. Mass chromatograms and structures of compounds 10-13 identified in methanolic extract of *Coronopus didymus* through GC-MS analysis.

Table 1. GC-MS analysis of methanolic extract of *Coronopus didymus.*

Comp. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1.	Eucalyptol	$C_{10}H_{18}O$	154	4.57	23.62
2.	Benzyl nitrile	C_8H_7N	117	6.41	34.22
3.	Benzoic acid	$C_7H_6O_2$	122	7.68	6.89
4.	Benzene, (isothicyanatomethyl)-	C_8H_7NS	149	9.25	0.57
5.	3-tert-Butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$	180	11.79	1.78
6.	2-Methyl-E,E-3,13-octadecadien-1-ol	$C_{19}H_{36}O$	280	14.57	0.57
7.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	15.39	2.48
8.	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	16.09	9.40
9.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	17.10	5.18
10.	Phytol	$C_{20}H_{40}O$	296	17.21	0.73
11.	9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	17.79	12.80
12.	Piperine	$C_{17}H_{19}NO_3$	285	23.82	1.05
13.	Stigmasta-5, 22-dien-3-ol, acetate, (3 beta.)-	$C_{31}H_{50}O_2$	454	24.70	0.66

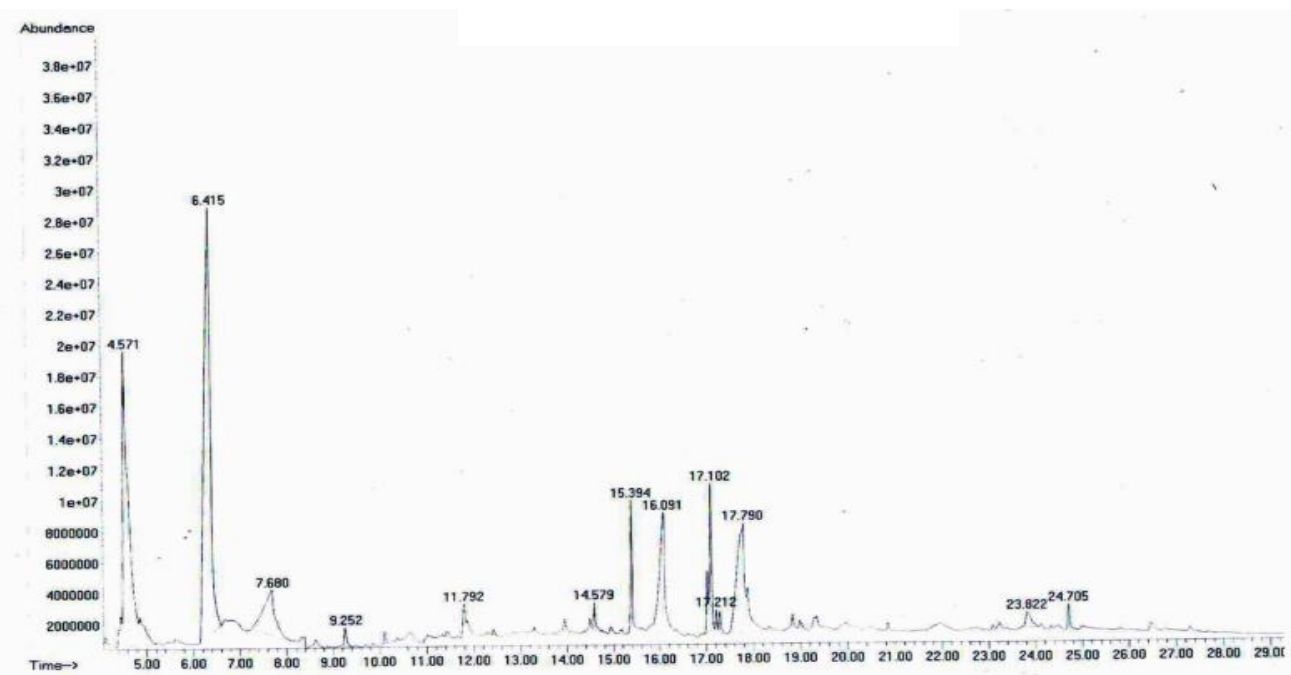


Fig. 6. GC-MS chromatogram of methanolic extract of *Coronopus didymus*.

References

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

Akhtar, N., A. Shoaib, S. Munir, A. Ali and S. Khurshid. 2014. Isolation, identification and enzyme production profile of *Aspergillus niger*. *Pak. J. Anim. Plant Sci.*, 24: 1438-1443.

Akhtar, R. and A. Javaid. 2018. Biological management of basal rot of onion by *Trichoderma harzianum* and *Withania somnifera. Planta Daninha,* 36: Article No. e017170507. DOI: 10.1590/S0100-83582018360100009, pages 1-7.

Ali, A., A. Javaid and A. Shoaib. 2017. GC-MS analysis and antifungal activity of methanolic root extract of *Chenopodium album* against *Sclerotium rolfsii*. *Planta Daninha,* 35: Article No. e017164713. DOI: 10.1590/S0100-83582017350100046, pages 1-8.

Amatulli, M.T., D. Spadaro, L. Gullino and A. Garibaldi. 2010. Molecular identification of *Fusarium* spp. associated with bakanae disease of rice in Italy and assessment of their pathogenicity. *Plant Pathol.,* 59: 839-844.

Amoah, B.K., M.V. MacDonald, H.N. Rezanoor and P. Nicholson. 1996. The use of random amplified polymorphic DNA technique to identify mating groups in the Fusarium Section Liseola. *Plant Pathol.,* 45: 115-125.

Amutharaj, P., C. Sekar and S.E. Natheer. 2013. Development and use of different formulations of *Pseudomonas fluorescens* siderophore for the enhancement of plant growth and induction of systemic resistance against *Pyricularia oryzae* in lowland rice. *Int. J. Pharma Bio Sci.*, 4: 831-838.

Banaras, S., A. Javaid, A. Shoaib and E. Ahmed. 2017. Antifungal activity of *Cirsium arvense* extracts against a phytopathogenic fungus *Macrophomina phaseolina. Planta Daninha,* 35: Article No. e017162738, DOI: 10.1590/S0100-83582017350100014, pages 1-9.

Busnardo, T.C.P.M., C. Padoani and C. Burger. 2010. Anti-inflamatory evaluation of *Coronopus didymus* in the pleurisy and paw odema models of mice, *J. Ethnopharmacol.*, 128: 519-525.

Chandrasekharan, M., K. kannathasan and V. venkatesalu. 2008. Antimicrobial activity of fatty acid methyl esters of some members of chenopodiaceae. *Zeitschrift für Naturforschung C,* 63: 331-336.

Das, K., R.K.S. Tiwari and D.K. Shrivastava. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *J. Med. Plants Res.,* 4: 104-111.

Dawe, D., S. Pandey and A. Nelson. 2010. Emerging trends and spatial patterns of rice production. *In:* Pandey, S., D. Byerlee, D. Dawe, A. Dobermann, S. Mohanty, S. Rozelle and B. Hardy. (Eds.), Rice in the global economy: Strategic research and policy issues for food security. International Rice Research Institute, Los Baños Philippines. pp. 15-35.

Desjardins A.E., H.K. Manandhar, R.D. Plattne, G.G. Manandhar, S.M. Poling and Maragos C.M. 2000. *Fusarium* species from Nepalese rice and production of mycotoxins and gibberellic acid by selected species. *Appl. Environ. Microbiol.,* 66: 1020-1025.

Gupta, A.K., I.S. Solanki and Y.Singh. 2015. Bakanae disease of Rice-An emerging disease in Asia. *J. Anim. Plant Sci.*, 25: 1499-1514.

Gurjar, M.S., S. Ali, M. Akhtar and K.S. Singh. 2012. Efficacy of Plant extracts in plant disease management. *Agric. Sci.,* 3: 425-433.

Hema, R., S. Kumaravel and K. Alagusundaram. 2011. GC/MS determination of bioactive components of *Murraya koenigii*. *J. Am. Sci.,* 7: 80-83.

Iqbal, D. and A. Javaid. 2012. Bioassays guided fractionation of *Coronopus didymus* for its antifungal activity against *Sclerotium rolfsii*. *Nat. Prod. Res.*, 26: 1638-164.

Iqbal, M., N. Javed and W. Wakil. 2013. Studies on the chemical control of bakanae disease (*Fusarium moniliforme*) of rice in Pakistan. *Pak. J. Phytopathol.*, 25: 146-154.

Jabeen, K. and A. Javaid. 2010. Antifungal activity of *Syzygium cumini* against *Ascochyta rabiei,* the cause of chickpea blight. *Nat. Prod. Res.*, 24: 1158-1167.

Javaid, A. and D. Iqbal. 2014. Management of collar rot of bell pepper (*Capsicum annuum* L.) by extracts and dry biomass of *Coronopus didymus* shoot. *Biol. Agric. Hort.,* 30: 164-172.

Javaid, A., L. Afzal and A. Shoaib.2017a. Antifungal potential of a brassicaceous weed *Sisymbrium irio* against *Macrophomina phaseolina. Planta Daninha* 35: Article No. e017164280, DOI: 10.1590/S0100-83582017350100057, pages 1-9.

Javaid, A., L. Niaz and A. Shoaib. 2017b. Effect of incorporation of leaf biomass of *Coronopus didymus* on management of basal rot disease of onion and plant physiology. *Int. J. Agric. Biol.,* 19: 445-452.

Javaid, A., G.R. Shahzad, N. Akhtar and G. Ahmed. 2018. 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.

Jindal, A., P. Kumar and G. Singh. 2012. *In vitro* antimicrobial activity of *Tribulus terrestris* L. *Int. J. Pharm. Pharmaceut. Sci.*, 4: 270-272.

Khurshid, S., A. Shoaib, A. Javaid, F. Akhtar, M. Shafique and M. Qaisar. 2017. Management of Fusarium wilt of tomato by soil amendment with *Cenchrus pennisetiformis* under chromium stress. *Physiol. Mol. Plant Pathol*., 97: 58-68.

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

Morcia, C., M. Malnati and V. Terzi. 2012. *In vitro* antifungal activity of terpinen-4-ol, eugenol, carvone, 1,8-cineole (eucalyptol) and thymol against mycotoxigenic plant pathogens. *Food Addit. Contaminants: Part A,* 29: 415-422.

Moretti, A., S. Somma, A.M. Picco, M. Rodolfi and A. Ritieni. 2007. Biological, molecular and toxin characterization of *Fusarium* species isolated from bakanae diseased rice plants. In: *Proc. 4th Int. Temperate Rice Conf., 2007*. Novara, Italy. pp. 164-165.

Nirenberg, H.I. 1976. Untersuchungen uber die morphologische and biologische differenzierung in Fusarium Sektion Liseola. Mitt. Biol. Bundesansi. Land-Forstwirtsch. *Berlin Dahlem,* 169: 1-117.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. Fusarium Species: An Ilustrated Manual for Identification. The Pennsylvania State University Press, University Park. pp. 193.

Riaz, T., S.N. Khan and A. Javaid. 2010. Management of *Fusarium* corm rot of gladiolus (*Gladiolus grandiflorus* sect. Blandus cv. Aarti) by using leaves of allelopathic plants. *Afr. J. Biotechnol.,* 8: 4681-4686.

Sana, N., A. Shoaib and A. Javaid.2016. Antifungal potential of leaf extracts of leguminous trees against *Sclerotium rolfsii. Afr. J. Trad. Complemen. Altern. Med.,* 13: 54-60.

Sena, A.P.A., M.V.C.B. Chaibub, G.B. Cortes, V.L. Silva, A.S. Prabhu and L.G. Araujo. 2013. Increased enzymatic activity in rice leaf blast suppression by crude extract of *Epicoccum* sp. *Trop. Plant Pathol.,* 68: 543-548.

White, T.J., T. Bruns and S. Lee. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: (Eds.): Taylor, J., A. Innis, D.H. Gelfand, J.J. Sninsky. PCR Protocols, pp. 315-322. Academic Press, San Diego, CA, USA.

Zainudin, N., A.A. Razak and B. Salleh. 2008. Bakanae disease of rice in Malaysia and Indonesia: etiology of the causal agent based on morphological, physiological and pathogenicity characteristics. *J. Plant Prot. Res.,* 48: 475-485.

(Received for publication 16 June 2017)