



Article

JVAID, A.^{1*}
AFZAL, L.¹
SHOAIB, A.¹

ANTIFUNGAL POTENTIAL OF A BRASSICACEOUS WEED *Sisymbrium irio* AGAINST *Macrophomina phaseolina*

Potencial Antifúngico da Planta Daninha Brassicácea *Sisymbrium irio* Diante de *Macrophomina phaseolina*

ABSTRACT - *Macrophomina phaseolina*, a soil-borne plant pathogen, has the ability to cause diseases in about 500 plant species. Unfortunately, so far no registered fungicide is available against this fungal pathogen. In the present study, different extracts of *Sisymbrium irio*, a weed of family Brassicaceae, were tested for evaluation of their antifungal activity against *M. phaseolina*. In screening bioassays, antifungal activity of methanolic extracts of 1% to 6% concentrations of different parts (leaf, stem, root and fruit) of the weed was assessed against the fungal pathogen. Methanolic leaf and root extracts significantly reduced fungal biomass up to 59% and 69% over control, respectively. Methanolic leaf and root extracts were further partitioned using four organic solvents namely *n*-hexane, chloroform, ethyl acetate and *n*-butanol in order of increasing polarity. Antifungal activity of different concentrations of these fractions (3.125, 6.25, ..., 200 mg mL⁻¹) was assessed against the pathogen. Chloroform and *n*-hexane fractions of methanolic leaf extract showed highly pronounced activity resulting in 35-75% and 15-87% reduction in fungal biomass over corresponding control treatments, respectively. Likewise, the highest concentration (200 mg mL⁻¹) of chloroform, ethyl acetate and *n*-butanol fractions of methanolic root extract reduced fungal biomass by 75%, 70% and 87%, respectively. The present study concludes that chloroform and *n*-butanol fractions of methanolic leaf and root extracts of *S. irio*, respectively, contain potent antifungal constituents for management of *M. phaseolina*.

Keywords: antifungal activity, London rocket, plant extracts.

RESUMO - *Macrophomina phaseolina*, um patógeno vegetal do solo, tem a capacidade de causar doenças em cerca de 500 espécies de plantas. Infelizmente, até agora nenhum fungicida registrado foi disponibilizado contra esse patógeno fúngico. No presente estudo, diferentes extratos de *Sisymbrium irio*, uma planta daninha da família Brassicácea, foram testados para avaliação de sua atividade antifúngica contra *M. phaseolina*. Em bioensaios de triagem, a atividade antifúngica de extratos metanólicos de concentrações de 1% a 6% de diferentes partes (folha, caule, raiz e fruta) da planta daninha foi avaliada contra o patógeno fúngico. Os extratos metanólicos de folhas e raízes reduziram significativamente a biomassa fúngica em até 59% e 69% acima do controle, respectivamente. Os extratos metanólicos de folha e de raiz foram divididos utilizando quatro solventes orgânicos: *n*-hexano, clorofórmio, acetato de etilo e *n*-butanol, em ordem de polaridade crescente. A atividade antifúngica de diferentes concentrações dessas frações (3,125, 6,25, ..., 200 mg mL⁻¹) foi avaliada contra o patógeno. As frações de clorofórmio e *n* hexano do extrato metanólico de folha mostraram uma atividade

* Corresponding author:
<arshadjpk@yahoo.com>

Received: May 11, 2016
Approved: June 30, 2016

Planta Daninha 2017; v35:e017164280

¹ Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.

*altamente pronunciada, resultando em 35-75% e 15-87% de redução da biomassa fúngica em relação aos tratamentos de controle correspondentes, respectivamente. Da mesma forma, a maior concentração (200 mg mL⁻¹) de frações de clorofórmio, acetato de etilo e n-butanol de extrato metanólico de raiz reduziu a biomassa fúngica em 75%, 70% e 87%, respectivamente. O presente estudo conclui que as frações de clorofórmio e n-butanol de extratos metanólicos de folhas e raízes de *S. irio*, respectivamente, contêm potentes componentes antifúngicos para o manejo de *M. phaseolina*.*

Palavras-chave: atividade antifúngica, *London rocket*, extratos vegetais.

INTRODUCTION

Macrophomina phaseolina is one of the most destructive soil-borne Ascomycetous fungus of family Botryosphaeriaceae that is devoid of a sexual stage. Under favorable environmental conditions, the fungus causes charcoal rot, dry rot, stem blight, leaf blight and damping off in more than 500 plant species belonging to about 100 angiospermic families (Ijaz et al., 2013). Cereals, legumes, oil seed crops, horticultural crops as well as woody plants could not escape from this devastating pathogen (Ijaz et al., 2013; Yildiz and Benlioglu, 2014; Sun et al., 2016). It primarily infects its host plants through black and multi-celled (50 to 200 individual cells) microsclerotia which are hyphal cells aggregates formed by joining melanin material (Holliday, 1995). Microsclerotia may plug the vascular vessels with consequences in plant wilting (Islam et al., 2012). When the infected plant is decayed, the microsclerotia are carried to the soil and persist there for more than three years (Dhinga and Sinclair, 1977). Infected plant debris in soil gives microsclerotia a survival home (Holliday, 1995). Sclerotia can bear a temperature above 30 °C in dry soil and under wet conditions mycelium hardly survives over 7 days and the sclerotia survival period is not exceeded over 2 months (Premamalini et al., 2012). Adaptation of *M. phaseolina* to a broad range of osmotic and pH is due to the higher content of a distinct set of carbohydrate esterases (Islam et al., 2012). This aggressive pathogen is hard to manage through cultural or chemical options (Afouda et al., 2012) and a biological strategy could be effective through exploring the role of indigenous plants (Javaid et al., 2015).

Brassicaceae is one of the largest angiosperm families distributed all over the world with 338 genera and more than 3,709 species (Al-Shehbaz et al., 2006). London rocket (*Sisymbrium irio*) is an annual winter weed of family Brassicaceae, native to northern Africa. It grows along roadsides, fence and ditches in Punjab, Pakistan. It is also recognized with other names like desert mustard, rocket mustard etc. and produces many small flowers, self-compatible, self-pollinated, and disseminates through seeds (Wilkin and Hannah, 1998). The nutritional composition of this species, including protein, fat, and carbohydrate and minerals, is considered to be beneficial as forage (Shehata, 2014). This weed has significance as folk medicinal plant due to its antimicrobial, analgesic, antipyretic and antioxidant potential (Lev, 2003; Al-Jaber, 2011). The phytochemical assessment of the weed has revealed that it is comprised of many secondary metabolites like flavonoids, alkaloids, oils, and glucosinolates (Khan et al., 1991; Al-Qudah and Abu Zarga, 2010; Shah et al., 2013). Previous literature has confirmed the antimicrobial activity of phenolic and flavonoid compounds of Brassicaceous plants (Mothana and Lindequist, 2005; Song et al., 2007; Grosso et al., 2011). However, *S. irio* has rarely been explored for its antifungal activity. The present study was therefore carried out to investigate the antifungal activity of methanolic extracts of different parts of this weed against *M. phaseolina*.

MATERIALS AND METHODS

Screening bioassays

Two hundred grams of thoroughly crushed materials of each part viz. leaf, stem, fruit and root of *S. irio* were soaked in 1,000 mL methanol separately in air tight jars for 14 days. Cheese cloth followed by qualitative filter paper were used to filter soaked materials, and solvent was removed on a rotary evaporator at 45 °C under reduced pressure. For a complete evaporation of

the solvent, each extract was poured in 100 mL beaker and finally a thick paste of 14.29 g, 10 g, 11 g and 10.87 g leaf, stem, root and fruit extracts, respectively, was obtained (Javaid and Akhtar, 2015).

Methanolic extract (9.45 g) of each of the four plant parts was dissolved in 6 mL Dimethyl sulphoxide (DMSO) and a final volume (21 mL) was raised by distilled autoclaved water to prepare stock solutions. Similarly, a control solution was prepared by adding 6 mL DMSO in 15 mL distilled water. For each treatment, 74 mL malt extract (ME) were autoclaved in a 250 mL volume flask and cooled at room temperature. Six concentrations viz. 1, 2, ..., 6% were prepared by adding 1, 2, 3, 4, 5 and 6 mL of stock solution with 5, 4, 3, 2, 1 and 0 mL of the control solution, respectively, to raise the volume of the medium in 80 mL in each flask. This volume was divided into four equal parts in 100 mL flasks. To prepare the control treatment, 6 mL of the control solution were mixed with 74 mL of malt extract to maintain the same quantity of DMSO in all the treatments, and divided into four equal parts of 20 mL each to serve as replicates. Mycelial discs of *M. phaseolina* were prepared from the tips of 7 days old fungal culture by using a sterilized 5 mm diameter cork borer and one disk was transferred to each flask. Flasks were incubated at 27 ± 1 °C for 7 days. After the incubation period, pre-weighed filter papers were used to filter the fungal biomass and a dried biomass was obtained by drying it at 60 °C (Javaid and Akhtar, 2015).

Fractionation of methanolic leaf and root extracts

Two kilograms of dried and powdered leaves and one kilogram of roots were extracted with methanol (2 x 7 L) at room temperature for two weeks. Following filtration, a dried crude methanolic extract of leaf and root was obtained by evaporating the solvent on a rotary evaporator.

The crude methanolic leaf extract was suspended in water (200 mL) in a separatory funnel and extracted with *n*-hexane (500 mL) until whole *n*-hexane soluble compounds from the mixture were separated. *n*-hexane phase was evaporated under vacuum and a 9.1 g dried fraction was collected. Afterward, a successive extraction of residual extract was done with chloroform (500 mL), ethyl acetate (500 mL), and *n* butanol (500 mL) and yield of 24.3 g, 1.9 g and 6.2 g of chloroform, ethyl acetate and *n*-butanol fraction, respectively, were achieved. The leftover aqueous extract was evaporated and 19.7 g of this fraction were obtained (Javaid and Rauf, 2015).

The crude methanolic root extract was dissolved in 200 mL water and fractionated by successive solvent extractions with *n*-hexane (500 mL several times), chloroform (500 mL), ethyl acetate (500 mL) and *n*-butanol (500 mL), respectively, to yield *n*-hexane fraction (4.1 g), chloroform fraction (2.7 g), ethyl acetate fraction (3.0 g), *n*-butanol fraction (2.5 g), and aqueous fraction (3.2 g).

Bioassays with fractions of methanolic extracts

In vitro, antifungal activity of various fractions from methanolic leaf and root extracts were assessed against *M. phaseolina*. A stock solution of 200 mg mL⁻¹ was prepared by homogenizing 1.2 g of each fraction of methanolic leaf and root extracts in 1 mL DMSO and 5 mL of ME broth. Lower-grades of 100, 50, 25, 12.5, 6.25, and 3.125 mg mL⁻¹ were prepared by adding ME broth. To make a control set parallel to various extract concentrations, a serial double dilution was done after dissolving 1 mL of DMSO in 5 mL malt extract broth. Antifungal assays were performed in 10 mL glass test tubes, each filled with 1 mL of the growth medium. Inoculum of *M. phaseolina* was prepared in distilled water and 10 µL of this inoculum were added to each test tube and these were incubated at room temperature. A triad set of each treatment was maintained. After 7 days, fungal biomass in each test tube was filtered, dried and weighed (Javaid and Rauf, 2015).

Statistical analysis

All the data were analyzed by analysis of variance (ANOVA) followed by the LSD method ($P \leq 0.05$) using computer software Statistix 8.1.

RESULTS AND DISCUSSION

Screening bioassays

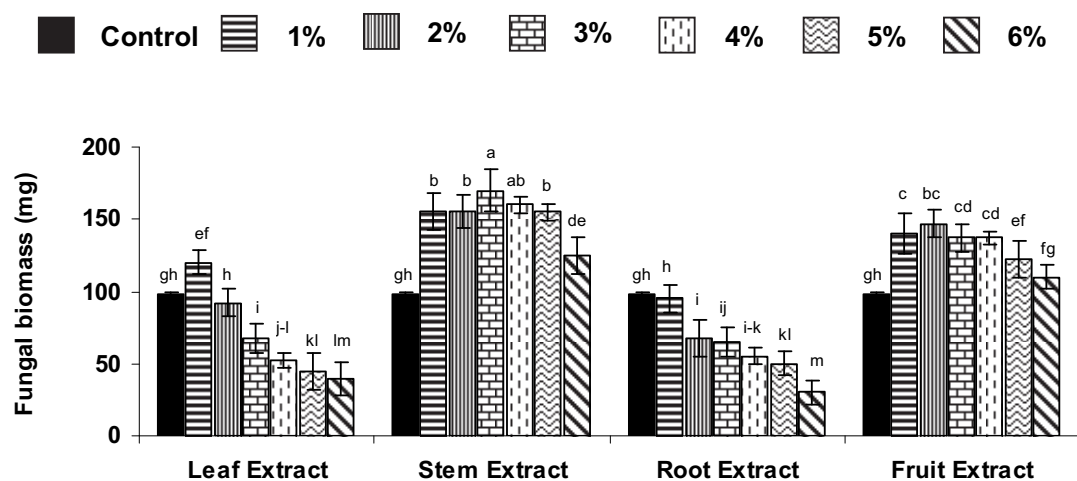
Analysis of variance (ANOVA) revealed that the effect of different plant parts of *S. irio* (P), extract concentration (C) as well the interactive effect of P x C were significant for biomass of *M. phaseolina* (Table 1).

Data regarding the effect of methanolic extract of different parts of *S. irio* on biomass of *M. phaseolina* is presented in Figure 1. There was a great variability in antifungal activity among extracts of different parts of the test plant species. In case of leaf extract, maximum fungal biomass (120 mg) was recorded in the lower most extract concentration, i.e., 1% that was significantly greater than fungal biomass in control by 23%. By contrast, all other extract concentrations variably reduced fungal biomass by 6-59%. There was a gradual reduction in fungal biomass due to an increase in the extract concentration from 2% to 6%. An adverse effect of 3-6% concentrations on fungal biomass was significant as compared to control. Likewise, all the concentrations of root extract except 1% significantly reduced fungal biomass by 31-69%. An inhibitory effect of the extract was concentration dependant. In contrast, both stem and fruit extracts stimulated the fungal growth to variable extents. Different concentrations of stem and fruit extracts enhanced the fungal biomass by 59-64% and 13-51%, respectively. Generally, lower concentrations (1-5%) were more stimulatory than the highest concentration (6%). There are many similar examples in the literature supporting the findings of the present study

Table 1 - Analysis of variance (ANOVA) for the effect of different concentrations of methanolic leaf, stem, root and fruit extracts of *Sisymbrium irio* on biomass of *Macrophomina phaseolina*

Sources of variation	df	SS	MS	F values
Treatments	27	189468	7017	73*
Plant parts (P)	3	130218	43405	450*
Concentration (C)	6	26480	4413	46*
P x C	18	32769	1820	19*
Error	84	8100	96.4	
Total	111	197567		

* Significant at P≤0.001.



Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as LSD Test.

Figure 1 - Effect of different concentrations of methanol leaf, stem, root and fruit extracts of *Sisymbrium irio* on biomass of *Macrophomina phaseolina*.

(Carpinella et al., 2003; Singh et al., 2009; Javaid et al., 2015). Variation in antifungal activity may be attributed to different quantities of effective antifungal constituents or the presence of different types of compounds in different plant parts (Rauf and Javaid, 2013). A similar variability in antifungal activity among plant parts has also been demonstrated in *Datura metel*, *Coronopus didymus* and *Imperata cylindrica* against *Fusarium oxysporum*, *Macrophomina phaseolina* and *Sclerotium rolfsii* (Iqbal and Javaid, 2012; Javaid and Saddique, 2012; Javaid et al., 2015).

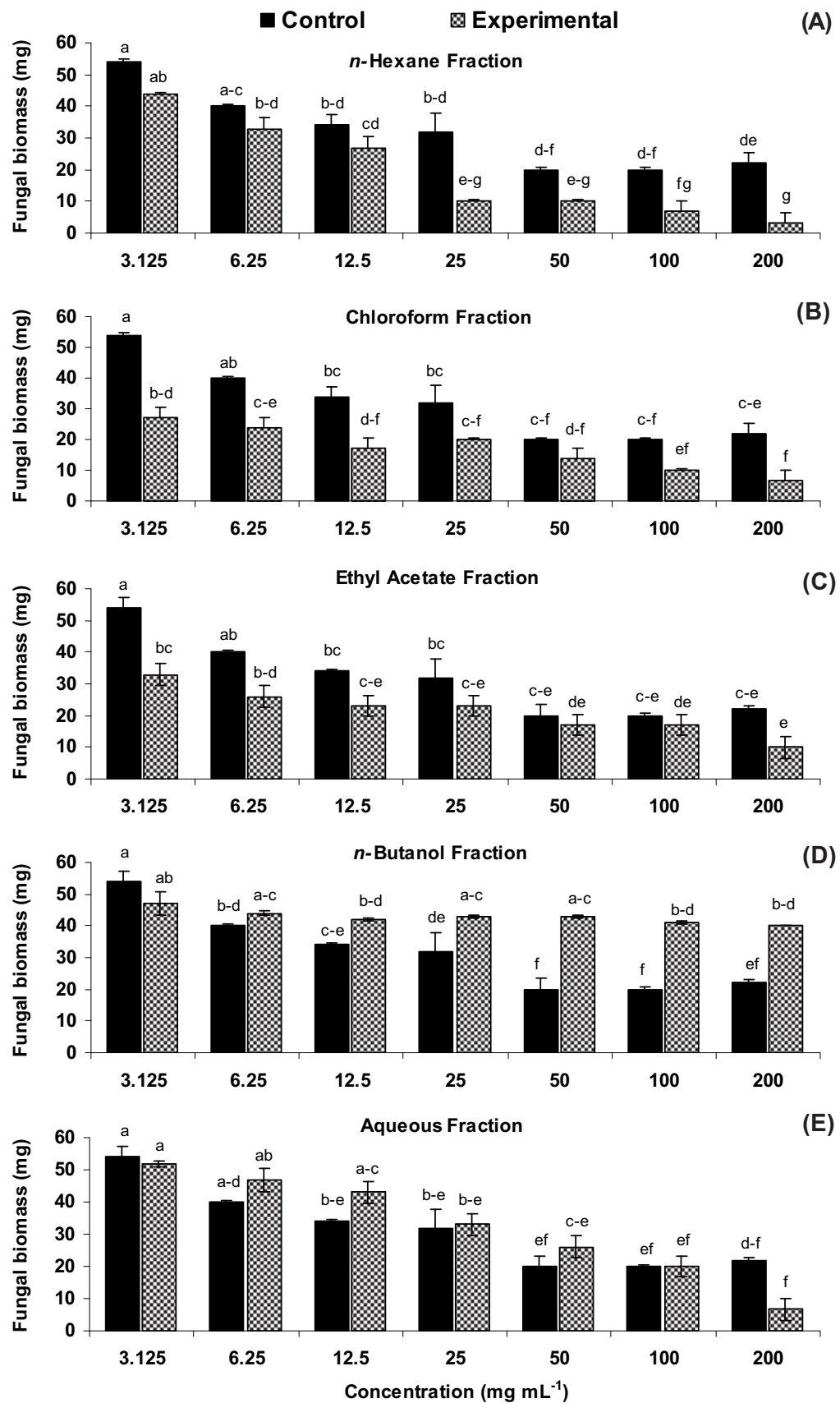
Bioassays with fractions of methanolic leaf extract

DMSO used for dissolving various fractions of methanolic leaf extract also inhibited the fungal growth and its effect was increased with increasing concentrations (Figure 2). Fungal biomass varied from 20 mg to 54 mg in different concentrations of DMSO in control. A similar inhibitory effect of DMSO has also been reported against other fungal species, namely *Alternaria alternata*, *Fusarium oxysporum* and *Ascochyta rabiei* (Javaid and Samad, 2012; Javaid and Munir, 2012; Javaid and Rauf, 2015). All the concentrations of *n*-hexane, chloroform and ethyl acetate fractions reduced the fungal biomass to variable extents with respect to the corresponding control treatments having the same concentration of DMSO in the growth medium. The effect of lower concentrations (3.125-12.5 mg mL⁻¹) of *n*-hexane fraction was less pronounced with 15-20% reduction in fungal biomass over the respective control treatments. Higher concentrations of this fraction (25-200 mg mL⁻¹) were much more inhibitory, resulting in 67-87% reduction in fungal biomass over control (Figure 2A). The adverse effect of different concentrations of chloroform fraction was generally significant ($P \leq 0.05$) where 35-75% reduction in fungal biomass over control was recorded (Figure 2B). The antifungal activity pattern of different concentrations of ethyl acetate fraction was similar to that of chloroform fraction. However, this fraction was comparatively less effective against *M. phaseolina* as compared to chloroform fraction. There was a 35-75% reduction in fungal biomass over control due to different grades of ethyl acetate fraction of leaf extract (Figure 2C). By contrast, all the grades of *n*-butanol fraction except the lower most one (3.125 mg mL⁻¹) variably stimulated the fungal growth, resulting in an enhanced fungal biomass over control. The effect of lower concentrations (6.25-25 mg mL⁻¹) was insignificant where a 10-38% increase in fungal biomass over control was recorded. On the other hand, higher concentrations (50-200 mg mL⁻¹) significantly ($P \leq 0.05$) enhanced the fungal biomass by 43-48% over the corresponding control treatments (Figure 2D). The highest extract concentration (200 mg mL⁻¹) of aqueous fraction significantly ($P \leq 0.05$) reduced fungal biomass by 70% over the corresponding control treatment (Figure 2E). A similar variable antifungal potential of different organic solvent fractions of methanolic extracts of *Withania somnifera*, *C. didymus*, *Chenopodium album* and *D. metel* has also been reported against *S. rolfsii*, *A. rabiei*, *F. oxysporum* f. sp. *cepae* and *M. phaseolina* (Iqbal and Javaid, 2012; Javaid and Munir, 2012; Javaid and Saddique, 2012; Rauf and Javaid, 2013). This possibly happened because of dissimilar polarity compounds in various organic solvents with different antifungal activities.

Bioassays with fractions of methanolic root extract

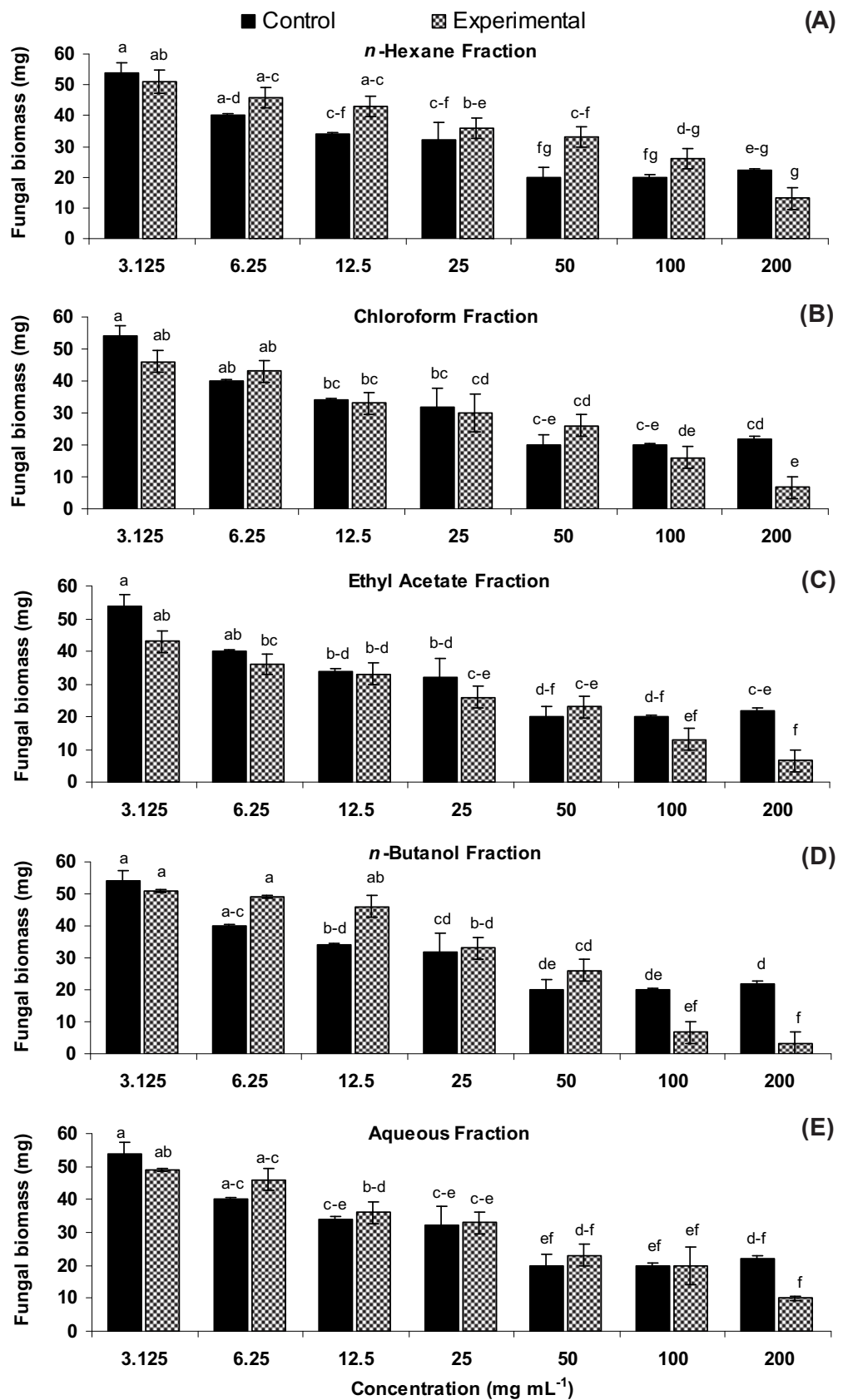
The antifungal activity of different fractions of methanolic root extract of *S. irio* against *M. phaseolina* was less pronounced than the corresponding fractions of leaf extract. In general, lower concentrations (3.125-100 mg mL⁻¹) either stimulated or showed an insignificant effect on fungal growth. Conversely, the highest concentration (200 mg mL⁻¹) of all the fractions of methanolic root extract exhibited a significant ($P \leq 0.05$) adverse effect on fungal growth. There was a 41%, 75%, 70%, 87% and 54% reduction in fungal biomass due to 200 mg mL⁻¹ concentration of *n*-hexane, chloroform, ethyl acetate, *n* butanol and aqueous fraction of methanolic root extract, respectively (Figure 3A-E). The production of glucosinolates by the Brassicaceous plants has already been explored (Sun et al., 2011) and the potential of such compounds has been suggested against different fungal pathogens (Al-Gendy et al., 2010). In addition, flavonoides present in *S. irio* (Khan et al., 1991; Al-Qudah and Abu Zarga, 2010), may also be responsible for antifungal activity against *M. phaseolina* (Kanwal et al., 2010).

The present study concludes that root and leaf extracts of *S. irio* contain potent antifungal constituents for the management of *M. phaseolina*. Further studies are required to identify



Values with different letters show a significant difference ($P \leq 0.05$) as determined by the LSD Test.

Figure 2 - Effect of different concentrations of *n*-hexane, chloroform, ethyl acetate, *n* butanol and aqueous fraction of methanolic leaf extract of *Sisymbrium irio* on growth of *Macrophomina phaseolina*.



Values with different letters show a significant difference (P<0.05) as determined by the LSD Test.

Figure 3 - Effect of different concentrations of *n*-hexane, chloroform, ethyl acetate, *n* butanol and aqueous fraction of methanolic root extract of *Sisymbrium irio* on growth of *Macrophomina phaseolina*.

effective antifungal constituents from chloroform and *n*-butanol fractions of methanolic leaf and root extracts of *S. irio*, respectively.

REFERENCES

- Afouda L.C.A. et al. Biological control of *Macrophomina phaseolina* on cowpea (*Vigna unguiculata*) under dry conditions by bacterial antagonists. **Int J Biol Chem Sci.** 2012;6:5068-77.
- Al-Jaber N.A. Phytochemical and biological studies of *Sisymbrium irio* L. Growing in Saudi Arabia. **J Saudi Chem Soc.** 2011;15:345-50.
- Al-Gendy A.A. et al. Glucosinolates, volatile constituents and biological activities of *Erysimum corinthium* Boiss. (Brassicaceae). **Food Chem.** 2010;118:519-24.
- Al-Qudah M.A., Abu Zarga M.H. Chemical composition of essential oils from aerial parts of *Sisymbrium irio* from Jordan. **J Chem.** 2010;7:6-10.
- Al-Shehbaz I.A., Beilstein M.A., Kellogg E.A. Systematics and phylogeny of the Brassicaceae (Cruciferae): An overview. **Plant Syst Evol.** 2006;259:89-120.
- Carpinella H. et al. Antifungal effects of different organic extracts from *Melia azedarach* L. on phytopathogenic fungi and their isolated active components. **J Agric Food Chem.** 2003;51:2506-11.
- Dhinga O.D., Sinclair J.B. **An annotated bibliography of *Macrophomina phaseolina* 1905–1975.** Viçosa, MG: Universidade Federal de Vicoso, 1977.
- Grosso C. et al. Chemical composition and biological screening of *Capsella bursa-pastoris*. **Rev Bras Farmac.** 2011;21:635-43.
- Holliday P. **Fungus diseases of tropical crops.** New York: Dover Publications, 1995. p.254-5.
- Ijaz S., Sadaqat H.A., Khan M.N. A review of the impact of charcoal rot (*Macrophomina phaseolina*) on sunflower. **J Agric Sci.** 2013;151:222-7.2013.
- Iqbal D., Javaid A. Bioassays guided fractionation of *Coronopus didymus* for its antifungal activity against *Sclerotium rolfsii*. **Nat Prod Res.** 2012;26:1638-44.
- Islam M.S. et al. Tools to kill: genome of one of the most destructive plant pathogenic fungi *Macrophomina phaseolina*. **BMC Genomics.** 2012;13:1-16.
- Javaid A., Munir R. Bioassay guided fractionation of *Withania somnifera* for the management of chickpea blight pathogen *Ascochyta rabiei*. **Int J Agric Biol.** 2012;14:797-800.
- Javaid A., Saddique A. Control of charcoal rot fungus *Macrophomina phaseolina* by extracts of *Datura metel*. **Nat Prod Res.** 2012;26:1715-20.
- Javaid A., Samad S. Screening of allelopathic trees for their antifungal potential against *Alternaria alternata* strains isolated from dying back *Eucalyptus* spp. **Nat Prod Res.** 2012;26:1697-702.
- Javaid A., Akhtar R. Antifungal activity of methanolic root extract of *Withania somnifera* against *Fusarium oxysporum* f. sp. *cepae*. **Afr J Trad Complement Altern Med.** 2015;12:22-7.
- Javaid A., Rauf S. Management of basal rot disease of onion with dry leaf biomass of *Chenopodium album* as soil amendment. **Int J Agric Biol.** 2015;17:142-8.
- Javaid A. et al. Management of *Macrophomina phaseolina* by extracts of an allelopathic grass *Imperata cylindrica*. **Pak J Agric Sci.** 2015;15:37-41.
- Kanwal Q. et al. Antifungal activity of flavonoids isolated from mango (*Mangifera indica* L.) leaves. **Nat Prod Res.** 2010;24:1907-14.
- Khan M.S.Y., Javed K., Khan M. Chemical constituents of the aerial parts of *Sisymbrium irio*. **J Indian Chem Soc.** 1991;68:532.

- Lev F. “*Sisymbrium irio*” Medicinal substances in Jerusalem from early times to the present day Archaeopress. Oxford: 2003. 62p.
- Mothana A.A.R., Lindequist U. Antimicrobial activity of some medicinal plants of the island Soqatra. **J Ethnopharmacol.** 2005;96:177-81.
- Premamalinea T. et al. Fungal keratitis caused by *Macrophomina phaseolina* – A case report. **Med Mycol Case Rep.** 2012;1:123-6.
- Rauf S., Javoid A. Antifungal activity of different extracts of *Chenopodium album* against *Fusarium oxysporum* f. sp. *cepae* the cause of onion basal rot. **Int J Agric Biol.** 2013;15:367-71.
- Shah S. et al. Pharmacognostic standardization and pharmacological study of *Sisymbrium irio* L. **Am J Res Comm.** 2013;1:241-53.
- Shehata H.F.S. Ecology and nutritive status of *Sisymbrium irio* L. in the Nile delta, Egypt. **Egypt J Exp Biol.** 2011;10:127-42.
- Singh K. et al. Phenolic acid analysis and biological activity of methanolic extracts of some medicinal plants against some phytopathogenic fungi. **Int J Alt Med.** 2009;6:10-20.
- Song N. et al. Several flavonoids from *Capsella bursa-pastoris* (L.) Medik. **Asian J. Tradit Med.** 2007;2:218-22.
- Sun B. et al. Variation of glucosinolates in three edible parts of Chinese kale (*Brassica alboglabra* Bailey) varieties. **Food Chem.** 2011;124:941-7.
- Sun S. et al. Occurrence of charcoal rot caused by *Macrophomina phaseolina*, an emerging disease of adzuki bean in China. **J Phytopathol.** 2016;164:212-6.
- Wilkin D., Hannah L. ***Sisymbrium irio* (Brassicaceae) London Rocket.** Santa Barbara: Botanic Garden for Channel Islands National Park, 1998.
- Yildiz A., Benlioglu S. *Macrophomina phaseolina* and *Rhizoctonia solani* isolates to strawberry stolons. **Phytoparasitica.** 2014;42:367-9.