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IN VITRO AND *IN VIVO* EVALUATION OF ANTIFUNGAL ACTIVITIES OF SOME ANTAGONISTIC PLANTS AGAINST CHARCOAL ROT CAUSING FUNGUS *MACROPHOMINA PHASEOLINA*

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Antifungal potential of twenty antagonistic plants was assessed against the most damaging phytopathogenic fungus *Macrophomina phaseolina*. All the test plants inhibited the growth of *M. phaseolina* significantly to varying levels. The maximum inhibition was observed with *Carum copticum* (83.5%), *Azadirachta indica* (76.1%) and *Nigella sativa* (70.4%) at 10% concentration. The powders of *Olea europaea*, *Cassia angustifolia*, *Ocimum americanum* and *Lawsonia inermis* caused more than 50% reductions in the growth of the fungus. Percentage inhibition was found to be significantly higher at higher concentrations of all the plants as compared to lower ones. The treatment of seeds with decoctions of these plants improved seedling emergence of mungbean in a dose responsive manner. The maximum seedling emergence was observed when the seeds were treated with *C. copticum* (83.3%) followed by *A. indica* (80.0%) at 10%. The study demonstrates the potent antifungal activities of these plants with potential practical applications in the treatment of charcoal rot disease of Mungbean.
Keywords: Antifungal plants, charcoal rot, *Macrophomina phaseolina*, *Vigna radiata*

INTRODUCTION

The low yield of mungbean in Pakistan can be attributed to legions of biotic and abiotic constraints. Among biotic factors, diseases are the most destructive. The losses due to diseases to pulse crops have been estimated up to 44 percent, depending upon the crop variety (Bashir and Malik, 1988). Mungbean is attacked by about 26 diseases in the world. Among these, charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid, is of prime importance in reducing crop yield especially in arid regions of the world. The pathogen is distributed in diverse climatic conditions from arid to tropical regions with broad host range (Cottingham, 1981; Abawi and Pastor-Corrales, 1990; Iqbal and Mukhtar, 2014). *M. phaseolina* is a soil and seed borne pathogenic fungus; produces cushion shaped black sclerotia. Its prevalence can be enhanced by different physiological and ecological factors such as low moisture contents, high temperature, heat etc. (Dhingra and Sinclair, 1978) and disease severity is correlated with viable sclerotia present in the soil. There are more than 500 hosts of the fungus including legume and cereal plants (Dhingra and Chagas, 1981).

Charcoal rot infects plants at almost all growth stages. Dark lesions appear on the epicotyls and hypocotyls followed by seedling death due to obstruction of xylem vessels. In plants, the pathogen causes red to brown lesions on roots and stems with production of dark mycelia and black microsclerotia and ultimately the plant becomes defoliated and wilted (Abawi and Pastor-Corrales, 1990) and may cause up to 100% yield losses (Bashir and Malik, 1988). Traditionally, fungal diseases including charcoal rot are controlled by synthetic fungicides (Wang *et al*., 2014a, b) which not only increase agricultural costs but also are riddled with potential hazards to the environment and human health. Chemical fungicides affect soil born fungal diseases (Sovler *et al*., 2012). A possible alternative to solve such problems is the use of plants capable of producing antifungal substances (Koch *et al*., 2013; Paredes *et al*., 2013; Corato *et al*., 2014). The use of antifungal plants may be useful to poor rural farmers as they cannot afford commercial fungicides. The use of such plants in protecting crops against fungal pathogens may also inhibit the development of resistance in the pathogen populations due to certain antifungal compounds contained in them. Myriad of antagonistic plants have been reported to possess antimicrobial and nematicidal activities against a multitude of plant pathogens (Liu *et al*., 2013a; Svecová *et al*., 2013; Trigui *et al*., 2013a; Vogt *et al*., 2013). The possible use of plants with antifungal activities for the control of *M. phaseolina* is an area that has not been fully exploited. The objective of the present study was, therefore, to evaluate twenty antagonistic plants (Table 1) found in the country for their antifungal activity against *M. phaseolina*, as no information is available on the antifungal activity of these plants against the fungus.

Table 1. Antagonistic plants used for their antifungal potential against *M. phaseolina*

Common Name	Botanical Name	Family	Location of collection	Plant part used
Neem	*Azadirachta indica*	Meliaceae	Faisalabad	leaves
Cannabis	*Cannabis sativa*	Cannabaceae	Islamabad	leaves
Olive	*Olea europaea*	Oleaceae	Rawalpindi	leaves
Mint	*Mentha piperita*	Lamiaceae	Islamabad	leaves
Chinaberry	*Melia azedarach*	Meliaceae	Dera Ghazi Khan	leaves
Oleander	*Nerium indicum*	Apocynaceae	Rawalpindi	leaves
Ajowan	*Carum copticum*	Apiaceae	Islamabad	seed
Sodom apple	*Calotropis procera*	Asclepiadaceae	Bahawal Pur	leaves
Hoary basil	*Ocimum americanum*	Lamiaceae	Islamabad	seed
Fennel	*Foeniculum vulgare*	Apiaceae	Islamabad	seed
Indian rosewood	*Dalbergia sissoo*	Fabaceae	Dera Ghazi Khan	leaves
Fenugreek	*Trigonella foenum-graecum*	Fabaceae	Islamabad	leaves
Golden shower tree	*Cassia fistula*	Fabaceae	Rawalpindi	leaves
Dill	*Anethum graveolens*	Apiaceae	Islamabad	seed
Black seed	*Nigella sativa*	Ranunculaceae	Islamabad	seed
Chamomile	*Matricaria chamomilla*	Asteraceae	Islamabad	flower
Senna	*Cassia angustifolia*	Caesalpinaceae	Islamabad	flower
Whitetop Weed	*Parthenium hysterophorus*	Asteraceae	Islamabad	leaves
Henna	*Lawsonia inermis*	Lythraceae	Sargodha	leaves
Tobacco	*Nicotiana tabacum*	Solanaceae	Rajan Pur	leaves

MATERIALS AND METHODS

***Isolation, purification and identification of M. Phaseolina*:** The fungus used in the study was isolated from stem bark tissues of mungbean bearing fungal sclerotia and characteristic charcoal rot symptoms on Chloroneb Mercury Rose Bengal Agar (CMRA) medium (Meyer *et al*., 1973) and identified on the basis of standard key (Barnett and Hunter, 1972).

***Multiplication of M. phaseolina for pot assay*:** Sorghum seeds were water soaked overnight, air dried under room temperature and placed in conical flasks. The mouth of each flask was plugged with cotton wool, wrapped in aluminum foil and autoclaved at 15 psi (121°C) for 20 min. After cooling, the seeds in flasks were inoculated with 4 mm mycelial plugs from a 7-day old culture of *M. phaseolina* and incubated at 25±1°C for 15 d. The flasks were shaken at alternate days for uniform colonization of the grains. The inoculum thus produced was used in pot assay.

***Collection of plant materials*:** Leaves or seeds or flowers of twenty antagonistic plants belonging to 13 families used in the studies were collected from different locations of Pakistan (Table 1).

***Preparation of plant materials for experimental use*:** Leaves or seeds or flowers of test plants were surface sterilized for 2 min. in 70% ethanol. Samples were then rinsed twice in sterilized distilled water, dried under room temperature for 21 days and triturated separately and used for *in vitro* evaluation against *M. phaseolina*. For preparation of decoctions, 10, 25, 50 and 100 g dried material of each test plant was boiled in 1 L of water for 5 min to get concentrations of 1, 2.5, 5 and 10 percent. The decoctions were squeezed through double cheesecloth sheets and filtered through Whatman no. 1 filter paper. The decoctions were further passed through Millipore filter of 0.2 µm pore size to avoid the bacterial contamination and stored at 4°C until use.

***In vitro evaluation of plants*:** The pulverized material of each plant sample was added to 250 mL of Potato Dextrose Agar (PDA) medium at the rate of 2.5, 6.25, 12.5 and 25 g to get concentrations of 1, 2.5, 5 and 10 percent. The resulting suspensions were agitated for 10 min, autoclaved for 20 min, filtered through four layers of sterile cheesecloth and dispensed into 9-cm diameter Petri dishes. PDA without plant material served as control. The amended agar plates were inoculated in the center with 5 mm discs from the actively growing periphery of a 5-day old colony of *M. phaseolina* on PDA and incubated at 25±1°C for 7 d in a completely randomized design. The un-amended agar medium plates served as control. Each treatment was replicated 10 times. Seven days after incubation, the colony diameter of the fungus was measured from each treatment and the inhibition of mycelial growth was determined by the following formula.

$$\% \text{ Mycelial inhibition} = \frac{\text{Colony diameter of control} - \text{Colony diameter of treated}}{\text{Colony diameter of control}} \times 100$$

***Evaluation of plant materials in pot assay*:** Seeds of mungbean cv. NM-92 were surface sterilized for 10 min in 5% commercial sodium hypochlorite solution, washed in sterilized distilled water and air dried. The seeds were then soaked in different concentrations of decoctions of test plats for 2 h and air dried for 3 h in a laminar flow chamber. Seeds treated with sterilized distilled water served as control. The treated seeds were planted in soils amended with sorghum seeds colonized with *M. phaseolina* @ 2g/kg of soil. Ten seeds were planted in each pot. Each treatment was replicated five times. The pots were kept under field conditions in a completely randomized design in an iron cage. Data on seedling emergence was recorded after 20 d and percent increase over control was reckoned (Irshad *et al*., 2012; Mukhtar *et al*., 2013a).

***Statistical analysis*:** The experiment was repeated twice. Percent reduction in mycelial growth and increase in seedling emergence were calculated over controls prior to statistical analysis (Hussain *et al*., 2011a; Mukhtar *et al*., 2013b, 2014). All the data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278 (www.vsni.co.uk). The differences among means were compared by Fisher's protected least significant difference test at ($P \leq 0.05$).

RESULTS AND DISCUSSION

***Effects of plant materials on the growth of M. Phaseolina*:** The powders of all the tested plants significantly suppressed the growth of *M. phaseolina* ($P > 0.001$). However, plant species showed variations in their inhibitory effects against the fungus. Of all the plants, *C. copticum* proved to be the most effective in suppressing the growth of the pathogen at all concentrations followed by *N. sativa* and *A. indica* whereas those of *N. indicum*, *F. vulgare* and *T. foenum-graecum* appeared to be the least effective. The powders of remaining plants were found intermediate in suppressing the growth of the fungus. Similarly, concentrations also had significant inhibitory effect on the growth ($P > 0.001$), being the maximum at 10% concentration of the powders. As the concentration of plant materials lowered, the magnitude of inhibition of growth of the fungus also decreased significantly showing a direct relationship between concentrations and growth inhibitions. The individual

Table 2. Mean percent inhibition in mycelial growth of *Macrophomina phaseolina* after treatments with different antagonistic plants

Plant	% inhibition over control[a]				Mean[b]
	1%	2.5%	5%	10%	
A. indica	43.2 uvw	44.4 w	**58.8 CD**	**76.2 H**	55.65 l
C. sativa	14.2 d	17.6 e	25.2 gh	31.2 l-p	22.05 c
O. europaea	36.4 q	38.2 qr	48.2 xy	64.0 **F**	46.7 k
M. piperita	28.2 jk	30.4 lmn	37.6 qr	39.2 rs	33.85 g
M. azedarach	24.4 g	26.8 hij	38.8 rs	49.4 yz	34.85 gh
N. indicum	8.2 ab	11.0 c	17.2 e	27.4 ij	15.95 a
C. copticum	57.4 C	62.6 EF	71.0 G	83.6 I	68.65 n
C. procera	30.8 l-o	31.6 l-p	41.8 tu	47.2 x	37.85 i
O. americanum	30.2 klm	32.2 m-p	49.2 xyz	54.8 **B**	41.6 j
F. vulgare	7.2 a	9.6 bc	18.2 e	31.2 l-p	16.55 ab
D. sissoo	17.0 e	18.8 e	30.0 kl	38.2 qr	26.00 de
T. foenum-graecum	8.0 ab	8.6 ab	21.4 f	32.0 l-p	17.5 b
C. fistula	26.8 hij	33.0 p	38.8 rs	44.4 w	35.75 h
A. graveolens	10.8 c	14.6 d	31.8 l-p	44.2 vw	25.35 d
N. sativa	48.2 xy	**52.2 A**	**60.6 DE**	**70.4 G**	57.85 m
M. chamomilla	17.8 e	21.4 f	28.2 jk	39.4 rs	26.7 ef
C. angustifolia	27.2 hij	31.0 l-p	42.2 tuv	55.2 B	38.9 i
P. hysterophorus	32.4 nop	32.6 op	40.8 st	49.4 yz	38.8 i
L. inermis	30.4 lmn	38.8 rs	44.0 vw	50.6 **zA**	40.95 j
N. tabacum	18.6 e	25.4 ghi	28.2 jk	38.6 r	27.7 f

[a] Values are means of ten replicates. Means sharing common letters regarding concentrations do not differ significantly at $P \leq 0.05$ according to Fisher's protected least significant difference test.

[b] Overall mean inhibition in colony diameter of *M. phaseolina* after treatment with each antagonist plant.

LSD value for Plants = 1.098 and for interaction between plants and concentrations = 2.196

growth inhibitions at four concentrations of test plants are given in Table 2. The reduction in fungal growth is attributable to the presence of antifungal compounds in plant extracts including glycoside, steroid, saponin, medicagenic acid, 3-O-B-D glycopyranoside, (3-GleMA) ajone, tannins, sesquiterpenes, lactones, terpenoids and phobol esters (Johnson and Nunley, 2000; Tiwari and Singh, 2004). Various phenolic acids, namely protocatechuic, ferulic, *p*-coumaric, *p*-hydroxybenzoic, caffeic and syringic acids have also been isolated from a number of plants which have been reported to possess antifungal activities against *M. phaseolina* and other pathogenic fungi (El-Khatib *et al*., 2003; Batish *et al*., 2007; Trigui *et al*., 2013b).

***Effects of plant extracts on seedling emergence of mungbean*:** The decoctions of all the test plants when used as seed treatment significantly enhanced seedling emergence ($P > 0.001$). Of all the test plants, *A. indica* showed maximum increase in emergence of mungbean plants over control followed by *C. copticum* and *N. sativa*. Per contra, *L. inermis*, *C. fistula* and *M. piperita* appeared to be the least effective in reducing the damage of the pathogen. The maximum individual increase in emergence of mungbean (83.4%) was achieved with 10% concentration of *C. copticum*. The minimum of 20% increase in plant emergence was obtained with *L. inermis* at 1% concentration. Significant effects ($P > 0.001$) of concentrations were also observed. Maximum plant emergence was recorded at 10% concentration of decoctions. As the concentration decreased, the effects also diminished. The effect of concentrations was found to be directly proportional to seedling emergence. The individual percent increases of seedling emergence at four concentrations of the test plants are given in Table 3.

The enhanced seedling emergence is attributed to the deposition of chemical compounds around seed surface which prevented penetration of the pathogen. These chemicals might have caused lyses of sclerotia and triggered plant growth hormones which resulted in increased emergence and decreased disease incidence.

Investigations on the mechanisms of disease suppression by plant products have suggested that the active principles present in them may either act on the pathogen directly (Baraka *et al*., 2006; Liu *et al*., 2013b), or induce systemic resistance in host plants resulting in reduction of disease development (Narwal *et al*., 2000; Paul and Sharma, 2002; Kagale *et al*., 2004; Hussain *et al*., 2011b; Kayani *et al*., 2012; Mukhtar *et al*., 2013c; Chen *et al*., 2014).

Variations in antifungal activities have also been observed

Table 3. Mean percent increase in seedling emergence of mungbean after treatments with different antagonistic plants

Plant	% increase in seedling emergence of mungbean[a]				Mean[b]
	1%	2.5%	5%	10%	
A. indica	50.0 hi	53.4 ij	66.6 kl	80.0 mn	62.5 h
C. sativa	30.0 bcd	33.4 cde	40.0 efg	40.0 efg	35.85 b-f
O. europaea	36.8 def	36.6 def	46.4 ghi	60.0 jk	44.95 g
M. piperita	33.4 cde	35.0 de	37.4 ef	40.0 efg	36.45 c-f
M. azedarach	26.2 abc	26.6 abc	36.6 def	43.4 fgh	33.2 bc
N. indicum	30.0 bcd	36.6 def	40.0 efg	43.4 fgh	37.5 def
C. copticum	46.8 ghi	50.0 hi	63.4 k	83.4 n	60.9 h
C. procera	26.6 abc	30.0 bcd	46.6 ghi	50.0 hi	38.3 ef
O. americanum	30.0 bcd	40.0 efg	53.4 ij	63.4 k	46.7 g
F. vulgare	23.4 ab	26.6 abc	36.6 def	46.8 ghi	33.35 bc
D. sissoo	23.4 ab	30.0 bcd	40.0 efg	46.6 ghi	35.00 b-e
T. foenum-graecum	26.6 abc	33.4 cde	43.4 fgh	53.2 ij	39.15 f
C. fistula	26.6 abc	26.6 abc	33.4 cde	43.4 fgh	32.5 ab
A. graveolens	26.6 abc	33.4 cde	43.4 fgh	50.0 hi	38.35 ef
N. sativa	46.6 ghi	53.4 ij	60.0 jk	76.8 mn	59.2 h
M. chamomilla	26.6 abc	30.0 bcd	36.8 def	43.4 fgh	34.2 bcd
C. angustifolia	33.4 cde	36.8 def	50.0 hi	73.2 lm	48.35 g
P. hysterophorus	26.8 abc	30.0 bcd	43.4 fgh	53.2 ij	38.35 ef
L. inermis	20.0 a	23.2 ab	33.2 cde	40.0 efg	29.1 a
N. tabacum	26.8 abc	30.0 bcd	43.2 fgh	46.6 ghi	36.65 c-f

[a] Values are means of five replicates. Means sharing common letters regarding concentrations do not differ significantly at $P \leq 0.05$ according to Fisher's protected least significant difference test.

[b] Overall mean increase in seedling emergence of mungbean after treatment with each antagonist plant.

LSD value for Plants = 3.655 and for interaction between plants and concentrations = 7.309

among the twenty antagonistic plants. The differences in the toxicity of the plants might be due to the differences in the chemical composition of the plants and concentration of toxic components indicating more antifungal potential at higher concentrations than lower ones.

***Conclusions*:** The present study demonstrates that of the twenty antagonistic plants *C. copticum*, *A. indica*, *N. sativa*, *Olea europaea*, *Cassia angustifolia*, *Ocimum americanum* and *Lawsonia inermis* possessed potent antifungal activities with potential practical applications in the treatment of charcoal rot disease of mungbean. The antifungal activity was found in the plant powders as well as in their decoctions. These antifungal plants outstrip synthetic fungicides on account of their ready availability, cost effectiveness, non-phytotoxicity, biodegradability and being ecofriendly. As these plants and plant materials are commonly found in the country and can therefore, be effectively utilized as a surrogate to chemical fungicides particularly by small-scale farmers to protect crops against fungal attack in the organic production of crops.

REFERENCES

Abawi, G.S. and M.A. Pastor-Corrales. 1990. Root rots of beans in Latin America and Africa: diagnosis, research methodologies and management strategies. CIAT, Colombia.

Baraka, M.A., S.A. Omar, El-Barougy, Ebtehage and A.H. Zian. 2006. Controlling seedling damping-off, root rot and wilt diseases of lupine (*Lupinus albus* L.). Agric. Res. J. Suez Canal Univ. 6:57-68.

Barnett, H.L. and B.B. Hunter. 1972. Illustrated Genera of Imperfect Fungi. Burgress Publishing Company, Minneapolis MN, 241p.

Bashir, M.A. and B.A. Malik. 1988. Diseases of major pulse crops in Pakistan. Trop. Pest Manage. 34:309-314.

Batish, D.R., K. Lavanya, H.P. Singh and R.K. Kohli. 2007. Phenolic allelochemicals released by *Chenopodium murale* affect the growth, nodulation and macromolecule content in chickpea and pea. Plant Growth Regul. 51:119-128.

Chen, J., X. Zou, Q. Liu, F. Wang, W. Feng and N. Wan. 2014. Combination effect of chitosan and methyl jasmonate on controlling *Alternaria alternata* and enhancing activity of cherry tomato fruit defense mechanisms. Crop Prot. 56:31-36.

Corato, U.D., E. Viola, G. Arcieri, V. Valerio, F.A. Cancellara and F. Zimbardi. 2014. Antifungal activity of liquid waste obtained from the detoxification of steam-exploded plant biomass against plant pathogenic fungi. Crop Prot. 55:109-118.

Cottingham, C. 1981. Numbers and distribution of sclerotia of *Macrophomina phaseolina* in soils of South Carolina. Plant Dis. Rep. 65:355-356.

Dhingra, O.D. and D. Chagas. 1981. Effect of soil temperature, moisture and nitrogen on competitive saprophytic ability of *Macrophomina phaseolina*. Trans. Brit. Mycol. Soc. 77:15-20.

Dhingra, O.D. and J.B. Sinclair. 1978. Biology and pathology of *Macrophomina phaseolina.* Minas Gerais, Brazil; Universidade Federal de Vicosa. 166p.

El-Khatib, A.A., A.K. Hegazy and H.K. Galal. 2003. Allelopathy in the rhizosphere and amended soil of *Chenopodium murale*. Weed Biol. Manag. 41:35-42.

Hussain, M.A., T. Mukhtar and M.Z. Kayani. 2011a. Assessment of the damage caused by *Meloidogyne incognita* on okra (*Abelmoschus esculentus*). J. Anim. Plant Sci. 21:857-861.

Hussain, M.A., T. Mukhtar and M.Z. Kayani. 2011b. Efficacy evaluation of *Azadirachta indica*, *Calotropis procera*, *Datura stramonium* and *Tagetes erecta* against root-knot nematodes *Meloidogyne incognita*. Pak. J. Bot. (Special Issue) 43:197-204.

Iqbal, U. and T. Mukhtar. 2014. Morphological and pathogenic variability among *Macrophomina phaseolina* isolates associated with mungbean (*Vigna radiata* L.) Wilczek from Pakistan. Sci. World J. Article ID 950175, 9 pages.

Irshad, U., T. Mukhtar, M. Ashfaq, M.Z. Kayani, S.B. Kayani, M. Hanif and S. Aslam. 2012. Pathogenicity of citrus nematode (*Tylenchulus semipenetrans*) on *Citrus jambhiri*. J. Anim. Plant Sci. 22:1014-1018.

Johnson, B.A. and J.R. Nunley. 2000. Treatment of Seborrheic Dermatitis. Am. Fam. Physican. 61:1-12.

Kagale, S., T. Marimuthu, B. Thayumanavan, R. Nandakumar and R. Samiyappan. 2004. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. Physiol. Mol. Plant Pathol. 65:91-100.

Kayani, M.Z., T. Mukhtar and M.A. Hussain. 2012. Evaluation of nematicidal effects of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against root-knot nematodes, *Meloidogyne incognita*. Crop Prot. 39:52-56.

Koch, E., M. Enders, C. Ullrich, D. Molitor and B. Berkelmann-Löhnertz. 2013. Effect of Primula root and other plant extracts on infection structure formation of *Phyllosticta ampelicida* (asexual stage of *Guignardia bidwellii*) and on black rot disease of grapevine in the greenhouse. J. Plant Dis. Protect. 120:26-33.

Liu, J., S. Xie, J. Feng and J. Cai. 2013a. Protective effect of *Dryopteris crassirhizoma* extracts in the control of the root-knot nematode *Meloidogyne incognita*. J. Plant Dis. Protect. 120:34-40.

Liu, J., S. Xie, J. Feng and J. Cai. 2013b. Effects of chloroform extract of *Dryopteris crassirhizoma* on the ultramicroscopic structures of *Meloidogyne incognita*. Sci. World J. 2013, Article ID 313482, 6 pages.

Meyer, W.A., J.B. Sinclair and M.N. Khare. 1973. Biology of *Macrophomina phaseolina* in soil studies with selective media. Phytopathol. 63:613-620.

Mukhtar, T., M.A. Hussain and M.Z. Kayani. 2013b. Biocontrol potential of *Pasteuria penetrans*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Meloidogyne incognita* in okra. Phytopathol. Mediterr. 52:66-76.

Mukhtar, T., M.A. Hussain, M.Z. Kayani and M.N. Aslam. 2014. Evaluation of resistance to root-knot nematode (*Meloidogyne incognita*) in okra cultivars. Crop Prot. 56:25-30.

Mukhtar, T., M.Z. Kayani and M.A. Hussain. 2013a. Response of selected cucumber cultivars to *Meloidogyne incognita*. Crop Prot. 44:13-17.

Mukhtar, T., M.Z. Kayani and M.A. Hussain. 2013c. Nematicidal activities of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against *Meloidogyne incognita*. Ind. Crop Prod. 42:447-453.

Narwal, S., A. Balasubrahmanyam, P. Sadhna, H. Kapoor and M.L. Lodha. 2000. A systemic resistance inducing antiviral protein with N-glycosidase activity from *Bougainvillea* × *buttiana* leaves. Ind. J. Exp. Biol. 39:600-603.

Paredes, C.C., P.B. Balbás, A. Gómez-Velasco, Z.N. Juárez, E.S. Arreola, L.R. Hernández and H. Bach. 2013. Antimicrobial, antiparasitic, anti-inflammatory, and cytotoxic activities of *Lopezia racemosa*. Sci. World J. 2013, Article ID 237438, 6 pages.

Paul, P.K. and P.D. Sharma. 2002. *Azadirachta indica* leaf extract induces resistance in barley against leaf stripe disease. Physiol. Mol. Plant Pathol. 61:3-13.

Soyler, D., E. Canıhoş, N. Temel and M. Hajyzadeh. 2012. Determination of chemical fungicide against soil borne fungal diseases of capers (*Capparis ovata* Desf. var. herbacea) during early stages. Pak. J. Agri. Sci. 49:345-348.

Svecová, E., S. Proietti, C. Caruso, G. Colla and P. Crinò. 2013. Antifungal activity of *Vitex agnus-castus* extract against *Pythium ultimum* in tomato. Crop Prot. 43:223-230.

Tiwari, S. and A. Singh. 2004. Toxic and sub-lethal effects of oleadrin on biochemical parameters of freshwater air breathing murrel, *Chant punctatus* (Bloch.). Ind. J. Exp. Biol. 42:413-418.

Trigui, M., A.B. Hsouna, I. Hammamib, G. Culioli, M. Ksantini, S. Tounsi and S. Jaoua. 2013a. Efficacy of *Lawsonia inermis* leaves extract and its phenolic compounds against olive knot and crown gall diseases. Crop Prot. 45:83-88.

Trigui, M., A.B. Hsouna, S. Tounsi and S. Jaoua. 2013b. Chemical composition and evaluation of antioxidant and antimicrobial activities of Tunisian *Thymelaea hirsute* with special reference to its mode of action. Ind. Crop Prod. 41:150-157.

Vogt, V., D. Cifuente, C. Tonn, L. Sabini and S. Rosas. 2013. Antifungal activity *in vitro* and *in vivo* of extracts and lignans isolated from *Larrea divaricata* Cav. against phytopathogenic fungus. Ind. Crop Prod. 42:583-586.

Wang, W., L. Yan, R. Meng, J. Zhao, X. Zhang, X. Han and Z. Ma. 2014a. Sensitivity to fluopicolide of wild type isolates and biological characteristics of fluopicolide-resistant mutants in *Pseudoperonospora cubensis*. Crop Prot. 55:119-126.

Wang, W., P. Zhang, R. Meng, J. Zhao, Q. Huang, X. Han, Z. Ma and X. Zhang. 2014b. Fungitoxicity and synergism of mixtures of fluopicolide and pyraclostrobin against *Phytophthora infestans*. Crop Prot. 57:48-56.