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Etiology, Pathogenicity and Management of Collar Rot in Cockscomb (*Celosia argentea*)

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Abstract

Collar rot induced by *Macrophomina phaseolina*, recently appeared as one of the most significant constraints to the production of Cockscomb (*Celosia argentea* L.), an ornamental plant. Symptoms include, wilting of the plant, chlorosis of lower leaves, shrinking of stem at collar region, which becomes black at later stage and affected plant become shredded, consequently a clear mycelial growth of the fungus can be seen if the stem of the affected plant is split open. In this study, the causal fungus was isolated from the diseased tissues (collar and stem regions, externally and internally). Then the pathogenicity test of isolated pathogens was confirmed through Koch's postulates. Considering the management trials, five fungicides (Carbendazim, Thiophenate-M, Ridomil Gold, Antracol and Score) and five different plant extracts (*Azadirachta indicia* L., *Melia azedarach* L., *Eucalyptus cameldulensis* L., *Syzygium cumini* L. and *Moringa oleifera* L.) were evaluated against the collar rot disease pathogen (*Macrophomina phaseolina*) by poison food technique at different concentrations *viz*., 100, 150, 200 and 250 µg/mL for fungicides and 10, 15 and 20% for plant extracts. Out of five fungicides used, Topsin M and Antracol were found to be most effective in reducing the mycelial growth of *M. phaseolina* by (83%) and (76%) followed by Ridomil Gold, Score and Carbendazim (46, 39 and 29% reduction). In case of plant extracts, *A. indicia* and *E. cameldulensis* extracts were more effective by reducing the mycelial growth of *M. phaseolina* by 77 and 74%, respectively, followed by *M. oleifera*, *M. azedarach*, and *S. cumini* (61, 45 and 18% reduction). © 2015 Friends Science Publishers

Keywords: Cockscomb; Collar rot; Plant extracts; Fungicides; *Macrophomina phaseolina*

Introduction

Cockscomb (*Celosia argentea* L.) an important summer annual flowering plant belonging to family *Amaranthaceae*, is used in landscaping worldwide. In Pakistan, this flower is called as *Kalgha* and is sown during July and August as an ornamental plant in lawns. Flowers are of red, yellow, pink and golden color. In Pakistan, ornamental plants are cultivated on an area of 450 hectares (MINFAL, 2011).

Macrophomina phaseolina (Tassi. Goid.) is the cause of collar rot disease in many plants. The fungus is seed and soil borne (Dhingra and Sinclair, 1975: Sadashivaiah *et al*., 1986). It has wide host range and is responsible for losses in more than 500 cultivated as well as wild plant species worldwide (Indra *et al.,* 1986). In Pakistan, this pathogen is known to attack more than 67 plant species of economic importance such as rice, cotton, maize, okra, cucurbits etc. (Shahzad *et al.,* 1988). *M. phaseolina* also causes charcoal rot in many crops; for example in legumes it is responsible for progressive wilting followed by defoliation that results in vigor and yield reduction (Meyer *et al.,* 1974).

During September, 2011 Collar rot of Cockscomb was reported by the Department of Estate care in the lawns of University of Agriculture, Faisalabad. Typical Collar rot symptoms of the disease were observed (wilting, chlorosis of lower leaves, shrinking and shredding of stem and roots) Fig. 1a and 2a). This disease occurs in cockscomb during the month of September during warm (30°C) and dry weather (less than 25% soil moisture).

The present studies were undertaken with a view to investigate the cause of the disease and for finding some novel molecules targeted at reducing the fungal growth *in vitro*.

Materials and Methods

Present studies were conducted in the experimental area as well as in the Disease Diagnostic Laboratory of the Department of Plant Pathology, University of Agriculture, and Faisalabad.

Isolation, Purification and Identification of Fungi

Cockscomb plants exhibiting the typical symptoms of collar rot were collected from different locations in the University of Agriculture, Faisalabad *i.e*., Faculty of Agriculture, Faculty of Veterinary Science, Faculty of Agricultural

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Engineering, Administration Block, D-ground, Tipu Hall, Ayyub Hall, Tariq Hall, Qazzafi Hall, Sir Sayyed Hall and Youngwala. These were taken to Plant Disease Diagnostic Laboratory for microscopic examination, isolation and purification of the pathogen to further establish the cause of observed symptoms. Small pieces of collar portion of symptomatic plants were cut and surface sterilized by dipping in 70% ethyl alcohol for one minute, subsequently washed with sterilized distilled water and then placed on sterilized filter paper for drying. The Potato Dextrose Agar media was prepared and autoclaved at 121ºC for 15 min. Media was poured into sterilized petri plates under aseptic conditions. After solidification of media, the surface sterilized pieces of diseased plants (collar region, stem, roots etc.) were inoculated onto the medium under aseptic conditions (laminar flow chamber) and the petri plates were incubated at 25ºC. The fungal colonies were observed on each petri plates after 5-7 days, following purification by hyphal tip method on the same media, the plates were left in the incubator till spore production (apprx.10 days, at 25±1ºC under 12 h light and dark cycles; Rehman *et al*., 2012).

For spore development, the isolated/purified fungus was identified as *M. phaseolina* on the basis of morphological characteristics. Morphological study has shown that the fungus consisted long celled hyphae which are brownish in color (Fig. 2b), Sclerotial bodies were of black color and different size (Dhingra and Sinclair, 1973; Aboshosha *et al.*, 2007).

Pathogenicity was confirmed on 25-30 days old cockscomb plants grown in pots containing sterilized soil inoculated with 5 mL spore suspension (10^5/mL). In control plants no culture of *M. phaseolina* was added. The soil moisture content was maintained at 40% and pots were kept at 35ºC under controlled conditions (Jha and Dubey, 1998). The plants were assessed for collar rot symptom development 30 days post inoculation (Kalamalakannan *et al*., 2005).

Evaluation of Fungicides and Plant Extracts

Five fungicides (Antracol, Carbendazim, Ridomil Gold, Topsin M and Score) and extracts of five plants Neem (*Azadirachta indicia* L.), Bukain, Sufaida (*Eucalyptus cameldulensis* L.), Jaman (*Syzygium cumini* L.) and Sohanjna (*Moringa oleifera* L.) were evaluated *in vitro* to assess their effect on colony development of isolated fungi by using poisoned food technique (Naz *et al*., 2006). Each of the fungicides was tested at 100, 150, 200 and 250 µg/mL concentration. For preparation of stock solution (10000X), one gram or equivalent of each fungicide was dissolved in 100 mL of sterilized water. Then solution of given concentrations i.e., 100, 150, 200 and 250 µg/mL were prepared by diluting the stock solution in desired volume of the solvent.

For preparing plant extracts, fresh leaves were taken, washed thoroughly with tap and sterilized water and air dried under laminar flow. These were then ground with the help of pestle and mortar. Two hundred grams of each of the five powdered medicinal plants were soaked in two liters of methanol in air tight glass jars separately for 7 days at room temperature. Afterwards, extracts were obtained by filtering muslin cloth and filter papers (Whatmann No. 10) and evaporating the solvent (under vacuum through rotatory evaporators) to reduce the volume up to 20 mL. Then 20 mL of the extracts was poured in open wide mouth pots and put in the air dried oven at 40ºC to completely evaporate the methanol (Sultana *et al.,* 2009; Rauf and Javed, 2013) and re-dissolved finally in 1 mL of methanol containing 500 µg of dried solvable plant extract (designated as 100%). Extracts of Neem, Bukain (*Melia azedarach* L.), Sufaida, Jaman and Sohanjna were applied by poison food technique at final concentrations of 10, 15 and 20% in required volume of growth media.

After solidification of the media, the plates were inoculated by placing 7 days old cultures of isolated fungi. The experiments were carried out by making three replications of each treatment; the plates without fungicide and plant extracts served as controls. The plates were incubated at 28°C. After 3, 5 and 7 days of incubation data on colony diameter were recorded for each Petri plate including control and were subjected to analysis, determining the efficacy of the treatment in terms of percentage fungal growth reduction.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and Duncun's multiple range (DMR) test at 5% level of significance for comparing the difference among treatments (Steel *et al*., 1997).

Results

Identification of Isolated Fungal Pathogen

Fungus grows as whitish mycelial mates on PDA medium then starts turning grayish in color. After 6-7 days of inoculation, colony appears as black mats of fungus due to appearance of asexual sclerotial bodies (Dhingra and Scinclair, 1973). Microscopic studies have shown that the fungus consists of long celled hyphae which appear brownish in color upon maturation with septate mycelium, Sclerotial bodies black colored and of different size (Fig. 2b).

In Vitro Assays for Evaluating the Effect of Different Fungicides and Plant Extracts on *M. phaseolina*

Five fungicides and ethanolic extracts from five different plants were evaluated through poisoned food technique. The effect of the tested compounds was evaluated in terms of percentage growth inhibition in colony diameter as

Fig. 1A: Diseased plant (right) and healthy plant (left) of cockscomb in lawn; (**B**) Root shredding/collar rot due to attach of *M. phaseolina*

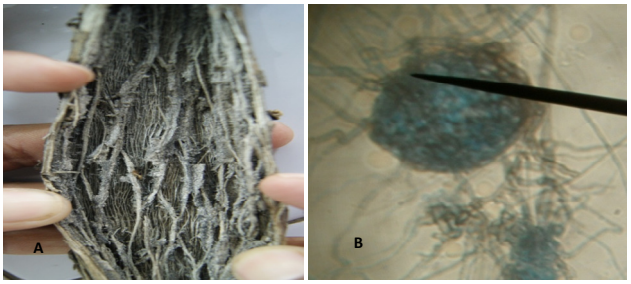

Fig. 2A: Internal side of diseased stem portion; (**B**) Sclerotia of the fungus with mycelial appendages

compared to the untreated control. Present studies indicated that various tested concentrations of all the fungicides (100, 150, 200 and 250 µg/mL) and plant extracts (10, 15 and 20%) could successfully inhibit the mycelial growth of *M. phaseolina*. The data were recorded after 7 days of incubation in terms of colony diameter (in centimeters) as the outward radial growth of the fungus. The effectiveness of tested fungicides and plant extracts in reducing mycelial growth of *M. phaseolina* varied considerably.

It was noticed that all the applied fungicides could significantly inhibit the *in vitro* mycelial growth of *M. phaseolina* as compared to those left as controls. Topsin M at 250 µg/mL concentration was found to be the most effective as it could inhibit the fungal growth by 100% (no growth was observed) followed by Antracol (0.566 cm) at 250 µg/mL, with an overall decrease of 17 and 25% over control. Score and Carbendazim were less effective and statistically at par with each other in reducing the mycelial growth of *M. phaseolina* (over all 68 and 71%). Ridomil Gold was least effective (54% reduction) among all the tested fungicides. On over all bases it was noticed that colony diameter was decreased with the increased concentrations (Table 1).

Similarly, all the plant extracts at various concentrations (10, 15 and 20%) inhibited the mycelial growth of *M. phaseolina* and their effectiveness also varied considerably. After seven days of incubation Neem and Sufaida extracts were most effective at 20% concentrations in reducing the mycelial growth of *M. phaseolina* with value of 0.36 cm and 0.60 cm, with 91 and 86% overall reduction for all tested concentrations, respectively. Both of these plant extracts worked even at low concentrations. Whereas, Sohanjna, Jaman and Bukain extracts were statistically at par at 20 % concentrations, with an overall mean colony growth of 1.81, 2.32 and 2.47 cm, with a value of 41, 52 and 55% decrease over control for all concentrations (Table 2).

Discussion

Present studies were designed to investigate the cause of charcoal rot disease in cockscomb in terms of causal organism with special emphasis on designing some disease control measures. As an outcome of this study, the fungal pathogen associated with this disease could be identified as *M. phaseolina* at species level. *M. phaseolina* is pathogenic to a wide range of cultivated and wild plant species around the world. The expression of symptoms depends on environmental conditions: more severe symptoms are observed under warmer conditions. Cockscomb is an important ornamental plant that faces a number of fungal bacterial and nematode diseases. Collar rot/charcoal rot cocks comb is very destructive disease that infects at collar portion resulting in complete plant death.

In the present studies, commercially available fungicides Topsin M (thiophenate methyl), Antracol (propineb), Ridomil (metalaxyl), Score (difenoconazole), Carbendazim and extracts of several plants *viz.* Neem, Bakain, Sufaida, Jaman and Moringa were evaluated *in-vitro* against *M. phaseolina* fungus. Topsin M and Antracol were found affective in inhibiting the mycelial growth of *M. phaseolina*. Topsin M (Thiophanate-methyl) is a broad spectrum protectant and curative fungicide for effective disease management in various crops. It has been registered in several advanced countries (Canada, New Zealand, the USA and the UK) for food and non-food uses, and control of soil-borne diseases of ornamental plants (Melkebeke, 1997). Usually it has been recommended to use it at 30-50 g a.i./ha concentration and it has been found effective against a variety of fungal pathogens such as eyespots and other diseases in cereals, scabs, *Monilia* disease and *Gloeosporim* rot in apples and pears. Also, it has been found effective in controlling cankers in fruit trees, powdery mildews on pome fruits, vegetables, cucurbits, strawberries, vines, roses, etc.; and as well as *Botrytis* and *Sclerotinia* based infections. Also, leaf spot diseases on beet, oilseed rape, celery, celeriac, etc., club root on brassicas, dollar spot due to *Corticium*, and *Fusarium* spp in turfs , grey mould in vines, rice blast (*Pyricularia oryzaei*) in rice and sigatoka disease in bananas. Also, Thiophanate-methyl has been successfully used in almonds, pecans, tea, coffee, peanuts, soya beans, tobacco, chestnuts, sugar cane, citrus fruit, figs, hops, mulberries, and many other horticultural crops (EFSA, 2012).

Whereas second best fungicide in the present studies is Antracol (propineb) it is also a broad spectrum protectant as

Table 1: Efficacy of various concentrations of fungicides on the mycelial growth (cm) of *M. phaseolina* after 7 days

Treatment	Mycelial growth (cm)				
	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL	Mean
Score	2.700±0.17b	1.966±0.15c	1.866±0.17c	1.866±0.18c	2.0750±0.17B
Topsin M	0.866±0.16fg	0.833±0.16fgh	0.366±0.18j	0.000±0.18k	0.5167 ±0.17E
Carbendazim	2.733±0.16b	1.900±0.16c	2.066±0.17c	2.000±0.18c	2.1750±0.17B
Ridomil Gold	1.433±0.17d	2.066±0.18c	1.966±0.18c	1.166±0.19e	1.6583±0.18C
Antracol	1.066±0.20ef	0.800±0.21ghi	0.600±0.22hij	0.566±0.22ij	0.7583±0.21D
Control	3.090±0.17a	3.066±0.15a	3.023±0.17a	3.130±0.18a	3.0775±0.17A
Mean	1.9650±0.17A	1.7722±0.17B	1.6483±0.18C	1.4550±0.19D	

Table 2: Efficacy of various concentrations of plant extracts on the mycelial growth (cm) of *M. phaseolina* after 7 days

Treatments	Mycelial growth (cm)			
	10%	15%	20%	Mean
Sohanjna	2.683±0.17b	1.900±0.17c	0.866±0.18c	1.8163±0.17B
Neem	1.892±0.18ef	0.833±0.18ef	0.366±0.19g	1.0303±0.18D
Jaman	2.999±0.18b	2.900±0.18c	1.066±0.18c	2.3216±0.18B
Bukain	3.453±0.19d	2.066±0.19c	1.900±0.20c	2.4730±0.19C
Eucalyptus	1.966±0.22e	0.899±0.23ef	0.600±0.24fg	1.1517±0.23D
Control	4.496±0.17a	4.490±0.17a	4.486±0.18a	4.4906±0.17A
Mean	2.9148±0.18A	2.1813±0.19B	1.5473±0.20C	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall means

well as curative fungicide, propineb belongs to propylene-(bis)- dithiocarbamate group, besides its broad spectrum antifugal behavior it also has a pronounced positive influence on growth development and vigor of crops cultivated under Zn deficiency. *M. phaseolina* infections in mungbean could be efficiently controlled using Antracol (Iqbal *et al*., 2004). Previously, sensitivity of *M. phaseolina* isolated from the infected mungbean plants was studied against five different fungicides *i.e*., Antracol, Benlate, Captan, Ridomil and Trimiltox, and among these Antracol gave suitable best results. However, in the present studies we included Topsin M, which owing to its promising results in controlling the sclerotia and mycelial growth of the fungus, proved to be an efficient contribution to the currently available pesticides that can be readily utilized against this fungus for controlling Charcoal rot in Cockskomb under our local conditions. Pesticides belonging to thiophanate-methyl group bind to tubulin thereby block mitosis, cause inhibition of respiratory activities, reduction in DNA biosynthesis and are therefore considered useful in control many fungal diseases (Martijn and Dobrat , 1988). Our studies also validate a few related studies in which Topsin M (Thiophenate-methyl) was the most effective amongst the eight *in vitro* tested fungicides in inhibiting sclerotial growth of *M. phaseolina* (El-Dahab *et al*., 1980,). Additionally, Topsin M can also be used through soil drenching for destroying the sclerotia and mycelia of pathogen in soil. Alternatively, as the sclerotia of fungus are inhabiting in soil, soil fumigation can also be a good option for destroying the sclerotia in soil with methyl bromide and metam sodium (sodium salt of methyldithiocarbamate), which can cause 80–90% pathogen mortality in field experiments (Sheikh and Ghaffar, 1975).

Similarly, in the present studies, all the plant extracts viz., Neem, Sufaida, Sohanjna, Jaman and Bukain were effective at various concentrations (10, 15 and 20%) in inhibiting the *in vitro* mycelial growth of *M. phaseolina*. The data were recorded in terms of radial fungal growth determination in centimeters similarly as stated above (in centimeters) after 7 days of incubation. The effectiveness of tested plant extracts varied considerably in reducing mycelial growth of *M. phaseolina*. After seven days of incubation Neem and Sufaida extracts were most effective at 20% concentrations in reducing the mycelial growth with values of 0.366 and 0.600 cm, with an overall 91 and 86% reduction, respectively. Both of these plant extracts worked even at lower concentrations. Whereas, Sohanjna, Jaman and Bukain extracts were statistically at par in reducing the mycelial growth of *M. phaseolina* at 20% concentrations. Maximum colony diameter of *M. phaseolina* was observed in control treatments in which no plant extracts were applied. Previously, different extracts from four tested plant species i.e., *S. cumini*, *E. citriodora*, *A. indica* and *M. azedarach* have also been reported to effectively inhibit the growth of *M. phaseolina* (Rehman *et al*., 2012). However, among these extract of *Azadirachta indica* was found to be most effective. Plant extracts are known for their beneficial usage in food industry, Agriculture for pest and disease management (Stephen *et al*., 2010; Javaid and Rehman, 2013). Also in human medicine, these have been exploited for developing energy, skincare and other therapeutic products especially against bacterial and fungal pathogens (Atawodi and Atawodi, 2009; Serrone and Nicolleti, 2013).

In the present studies, extracts from Neem leaves proved best in reducing the *in vitro* mycelial growth of the collar rot causing fungi *M. phaseolina*. Traditionally, Neem plant parts and products such as neem seeds, neem kernel powder, neem leaf extracts, neem bark powder, neem oil,

ethanolic and methanolic extracts and neem plant parts, are known for a wide range of effects in plants, arthropods, animals and as well as humans (Atawodi and Atawodi, 2009; Verma *et al*., 2011; Kumar *et al*., 2011). These include the usage in Agriculture as biopesticides, insecticides, insect repellants and bioactive molecules for stimulating the microbial populations in plants, endophytic fungi and plant growth promoting microflora (Kharwar *et al*., 2009; Rajagopal and Suryanarayanan, 2002). In animals these are having anti-pyretic, anti-malarial, anti-tumor, anti-cancer, anti-ulcer, anti-diabetic, anti-fertility, CNS and cardiovascular effects (Ram *et al*., 2002; Srivastava *et al*., 2012; Serrone and Nicoletti, 2013). Neem plant extract is known to have above 100 compounds, principally Limnoids belonging to the tetranortriterpinoids group especially 'Azadirachtin' and its analogs (Verma *et al*., 2007; 2011). Previously, Neem oil (2.5 and 5 percent concentration) has been successfully used to inhibit the *invitro* growth of early blight and wilt pathogens of tomato (*Alternaria solani* and *Fusarium* spp) by 61.17%. At 10% concentration it gave 100% inhibition of all the tested pathogens (Vir and Sharma, 1985; Hassanein *et al*., 2008). It has been reported that the use of aqueous extracts of *Azadirachta indica* reduced the biomass of *M. phaseolina* up to 85% (Ashraf and Javaid, 2007). Neem seeds and leaves extracts have antimicrobial activity with notable effects on some fungal phytopathogens showing fungistatic behavior *e.g.* when tested against powdery mildew fungi (*Sphaerotheca fuliginea*) gave 11% percent reduction in conidial germination in liquid culture (Coventary and Allan, 2001). Similarly, Neem extracts have been commercially exploited to produce several bioproducts like Biosal and Nimokil readily usable against several insects and plant pathogens in agriculture as important component of organic farming, organic pesticides and insecticides, in integrated pest management, and also as cosmetic industry beauty products and curatives for dermal infections in humans and animals (Ogbuevo *et al*., 2011; Stephen *et al*., 2010).

In our studies, Sufaida extracts proved effective in controlling the *invitro* growth of collar rot pathogen *M. Phaseolina*. Eucalyptol, a Sufaida derived molecule is a potent antimicrobial agent (Falahati *et al*., 2005) crude extracts, essential oils, methanolic extracts and volatile compounds have profound antimicrobial activities against several human as well as plant pathogens (Mahmoud *et al*., 2011; Hassani *et al*., 2013; Kumar and Navaratnam, 2013). Bacterial pathogens like, *Helicobacter pylori*, *Staphylococcus aureus* ATCC 29737, *Klebsiella pneumoniae* ATCC 10031, *S. epidermidis* ATCC 12228, *Salmonella paratyphi*-A serotype ATCC 5702, *Bacillus subtilis* ATCC 6633, *Pseudomonas* spp., *S. typhi E. coli* and *S. aureus* are inhibited by Sufaida derived compounds (Adeniyi *et al*., 2009; Naveed *et al*., 2013). Also, Sufaida compounds were found effective against some fungal pathogens like storage fungi *Aspergillus flavus* Link and *A. parasiticus* Speare (Vilela *et al*., 2009). Similarly fungi like *A. niger* ATCC 16404, *Candida albicans* ATCC 10231, *Proteus vulgaris* PTCC 1182 and *Trichomonas vaginalis* are also inhibited by Sufaida extracts (Yousefi *et al*., 2012; Hassani *et al*., 2013).

Conclusion

M. phaseolina was found associated with collar rot of cockscomb and its pathogenicity was confirmed. For the *in vitro* management fungicides like Antracol and Topsin M, and ethanolic extracts of Neem and Sufaida were found to be most effective in inhibiting the mycelial growth of *M. phaseolina* at various concentrations in plate assays using poisoned food technique. Hence, it is recommended that these fungicides and plant extracts must be evaluated further for assessing their ability to control collar rot disease in cocks comb under field conditions. To our knowledge this is the first confirmation of *M. phaseolina* related collar rot in Cockscomb reported from Pakistan.

References

Aboshosha, A.A., S.I. Atta Alla, A.E. El-Korany and E.E. Argawy, 2007. Characterization of *Macrophomina phaseolina* isolates affecting sunflower growth in El-Behera Governorate, Egypt. *Int. J. Agric. Biol*., 9: 807–815

Adeniyi, C.B., T.O. Lawal, G.B. Mahady, 2009. *In vitro* susceptibility of *Helicobacter pylori* to extracts of *Eucalyptus camaldulensis* and *Eucalyptus torelliana*. Pharm *Biol.* 47:99–102

Ashraf, H. and A. Javaid, 2007. Evaluation of antifungal activity of Meliaceae family against *Macrophomina phaseolina*. *Mycopathology*, 5: 81–84

Atawodi, S.E., J.C. Atawodi, 2009. *Azadirachta indica* (neem): A plant of multiple biological and pharmacological activities. *Phytochem. Rev*.8: 601–620

Coventary, E., and E.J. Allan, 2001. Microbiological and Chemical analysis of Neem (*Azadirachta indica*) extracts: New data on antimicrobial activity. *Phytoparasitica*, 29: 441–450

Dhingra, O.D. and J.B. Sinclair, 1973. Location of *Macrophomina phaseolina* on soybean plants related to cultural characteristics and virulence. *Phytopathology*, 63: 934–936

Dhingra, O.D. and J.B. Sinclair, 1975. Survival of *Macrophomina phaseolina* sclerotia in soil: Effect of soil moisture, carbon: nitrogen ratio, carbon sources, and nitrogen concentrations. *Phytopathology*, 65: 236–240

El-Dahab, M.K.A., A.M. Tarabeih and S.E. Muhammad, 1980. Studies on sun flower diseases in Egypt. *J. Phytopathol*., 12: 113–122

European Food Safety Authority (EFSA), 2012. Reasoned opinion on the modification of the existing MRLs for thiophanate-methyl and carbendazim in apples and pears. *EFSA Journal*; 10: 2685

Falahati M, N.O. Tabrizib, F. Jahaniani, 2005. Anti dermatophyte activities of *Eucalyptus camaldulensis* in comparison with Griseofulvin. *Iran J. Pharmacol and Therapeutics*: 80–83

Hassanein, N.M., M.A. Abou Zeid, K.A. Youssef and D.A. Mahmood, 2008. Efficacy of leaf extracts of Neem (*Azadirachta indica*) and Chinaberry (*Melia azedarach*) against early blight and wilt diseases of tomato. *Aust. J. Basic Appl. Sci.*, 2: 763–777

Hassani, S., G. Asghari, H. Yousefi, A. Kazemian, M. Rafieiean, and H.Y. Darani, 2013. Effects of different extracts of *Eucalyptus camaldulensis* on *Trichomonas vaginalis* parasite in culture medium. *Adv. Biomed. Res*., 2: 47

Indra, K., T. Singh, C.C. Machado and J.B. Sinclair, 1986. Histopathology of soybean seed infected by *Macrophomina phaseolina. Phytopathology*, 76: 532–535

Iqbal, S.M., Z. Ahmad, A. Ghafoor and M. Bashir, 2004. *In vitro* evaluation of fungicides against *Macrophomina phaseolina*. *Pak. J. Agric. Res.,* 18: 99–100

Javaid, A. and H.A. Rehman, 2013. Antifungal activity of leaf extracts of some medicinal trees against *Macrophomina phaseolina*. *J. Med. Plant Res*., 5: 2868–2872

Jha, A.K. and S.C. Dubey, 1998. Effect of plant age and weather parameters on collar rot of okra caused by *Macrophomina phaseolina*. *J. Mycolo. Plant Pathol*., 28: 351–353

Kalamalakannan, A., L. Mohan, V. valluvaparidasan, P. Mareeswari and R. Karuppiah, 2005. First report of *M. phaseolina* root rot (*M. phaseolina*) on medicinal coleus (*Coleus forskohlii*) in India. *New Dis. Report.,* 11: 48

Kharwar, R.N., V.C Verma. , A. Kumar, S.K. Gond, J.K. Harper, W.M. Hess, E. Lobkovosky, C. Ma, Y. Ren, G.A. Strobel, 2009. Javanicin, an antibacterial nephthaquinone from an endophytic fungus of neem, *Chloridium* sp. *Curr Microbiol*.58: 233–238

Kumar, R., R.S. Tiwari and O. N. Srivastava, 2011. Scalable synthesis of aligned carbon nanotubes bundles using green natural precursor: neem oil. *Nanoscale Res Lett*. 6: 92

Kumar, V.S. and V. Navaratnam, 2013. Neem (*Azadirachta indica*): Prehistory to contemporary medicinal uses to Humankind. *Asian Pac. J. Trop. Biomed*. 3: 505–514

Mahmoud, D.A., N.M. Hassanein, K.A. Youssef and M.A. Abou Zeid, 2011. Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. *Braz. J. Microbiol.* 42: 1007–1016

Martijn, A. and W. Dobrat, 1988. CIPAC Handbook volume D, pp: 166–168

Melkebeke, T., 1997. Validation of an analytical method for residues of thiophanate-methyl in grapes. Project No. NOTOX 189045, NOTOX. Unpublished.

Meyer, W.A., J.B. Sinclair and M.N. Khare, 1974. Factors affecting charcoal rot of soybean seedlings. *Phytopathology,* 64: 845–849.

MINFAL, 2011. *Government of Pakistan*. Ministry of Food and Agriculture Economic Wing) Islamabad. Pakistan. www.minfa.gov.pk.

Naveed, R., I. Hussain, A. Tawab, M. Tariq, M. Rahman, S. Hameed, M.S. Mahmood, A.B. Siddique and M. Iqbal, 2013. Antimicrobial activity of the bioactive components of essential oils from Pakistani spices against *Salmonella* and other multi-drug resistant bacteria. BMC Complement *Altern Med*., 13: 265.

Naz, F., C.A. Rauf, I.U. Haque and I. Ahmad, 2006. Management of *Rhizoctonia solani* with plant diffusates and chemicals. *Pak. J. Phytopathol*., 18: 36–43

Ogbuevo, I.P., V.U. Odoemenam, H.O. Obikaonu, M.N. Opara, Emenalom O.O., M.C. Uchegbu, I.C. Okoli, B.O. Esonu, M.U. Floeje, 2011. The growing importance of neem (*Azadirachta indica* A. Juss) in agriculture, industry, medicine and environment: A Review. *Res. J. Medic. Plant*.5: 230–245

Rajagopal, R. and T.S. Suryanarayanan, 2002. Isolation of endophytic fungi from leaves of neem *(Azadirachta indica). Curr Sci*.,78: 1375–1378

Ram, M.S., Ilavazhagan G., Sharma S.K., Dhanraj S.A., B. Suresh, 2002. Anti-microbial activity of a new vaginal contraceptive NIM-76 from neem oil (*Azadirachta indica*). *J. Ethnopharmacol*., 71: 377–382

Rauf, S. and A. Javaid, 2013. Antifungal activity of different extracts of *Chenopodium album* against *Fusarium oxysporum* f. sp. *cepae*, the cause of onion basal rot. *Int. J. Agric. Biol*., 15: 367–371

Rehman, A., S. Mehboob, C. Akem, A. Batool and M. Idrees, 2012. First report of Anthracnose/collar rot caused by *Colletotrichum dematium* on Statice in Pakistan. *Afr. J. Biotechnol*., 11: 9950–9954

Sadashivaiah, A.S., K.G. Ranganatfaiah and D.M. Gowda, 1986. Seed health testing of *Helianthus annuus* with special reference to *Macrophomina phaseolina*. *Ind. Phytopathol.*, 39: 445–447

Serrone, P.D. and M. Nicoletti, 2013. Antimicrobial Activity of a Neem Cake Extract in a Broth Model Meat System. *Int. J. Environ. Res. Public Health*. 10: 3282–3295

Shahzad, S., A. Sattar and A. Ghaffar, 1988. Addition to the hosts of *Macrophomina phaseolina. Pak. J. Bot*., 20: 151–152

Sheikh, A.H. and A. Ghaffar. 1975. Population study of sclerotia of *Macrophomina phaseolina* in cotton fields. *Pak. J. Bot*., 7: 13–17

Srivastava, P., N. Yadav , R. Lella, A. Schneider , A. Jones , T. Marlowe, G. Lovett, K. O'Loughlin, H. Minderman, R. Gogada, D. Chandra, 2012. Neem oil limonoids induces p53-independent apoptosis and autophagy. *Carcinogenesis*., 33: 2199–2207

Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1997. *Principles and Procedures of Statistics*: *A Biometric Approach,* 3rd edition. McGraw Hill Book Company, New York, USA

Stephen, O.D., L.C. Charles, M.M. Kumudini, E.W. David, T. Nurhayat and K.S. Kevin, 2010. Natural Toxins for Use in Pest Management. *Toxins*, 2: 1943–1962

Sultana, B., A. Farooq and A. Muhammad, 2009. Effect of extraction Solvent/Technique on the antioxidant activity of selected medicinal plant extracts. *Molecule*, 14: 2167–2180

Verma V.C., Gond S.K., Kumar A., Kharwar R.N., Boulanger L.A., and Gary A. G.A. Strobel, 2011. Endophytic Fungal Flora from Roots and Fruits of an Indian Neem Plant *Azadirachta indica* A. Juss., and Impact of Culture Media on their Isolation. *Ind. J. Microbiol*. 51: 469–476

Verma V.C., S.K. Gond, A. Kumar, R.N. Kharwar, and G.A. Strobel, 2007. The endophytic mycoflora of bark, leaf and stem tissues of *Azadirachta indica* A. Juss (Neem) from Varanasi (India). *Microb. Ecol*., 54: 119–125

Vilela G.R., Steffen de Almeida G, D'Arce M.A.B.R., Duarte Moraes H.D., Brito J.O., G.F. das Silva M.F., Silva S.C., Piedade S.M.S., Calori-Domingues M.A., and E. Micotti da Gloria, 2009. Activity of essential oil and its major compound, 1,8-cineole, from Eucalyptus globulus Labill., against the storage fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. *J. Stored Prod. Res*. 45: 108–111

Vir, D. and R.K. Sharma, 1985. Efficacy of fungicides: XXXII. Evaluation of Neem oil for control of plant pathogens. *Ansi. Farm Chem*., 1: 23–24

Yousefi, H., A. Kazemian, M. Sereshti, E. Rahmanikhoh, E. Ahmadinia and M. Rafiaian, 2012. Effect of *Echinophora platyloba*, *Stachys lavandulifolia* and *Eucalyptus camaldulensis* plants on *Trichomonas vaginalis* growth *in vitro*. *Adv. Biomed. Res*., 1: 1–3

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