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MANAGEMENT OF CAUSAL ORGANISM OF COLLAR ROT OF BELL PEPPER (*SCLEROTIUM ROLFSII*) BY ORGANIC SOLVENTS EXTRACTS OF *DATURA METEL* FRUIT

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A B S T R A C T

Present study was undertaken to investigate the antifungal potential of methanolic fruit extract of *Datura metel* L. (Syn*. Datura alba* Nees.) and its various solvent fractions against *Sclerotium rolfsii* Sacc., the cause of devastating collar rot disease of bell pepper (*Capsicum annuum* L.). In an *in vitro* study, conducted in 250-mL conical flasks, different concentrations of methanolic fruit extract (0.5, 1.0, 1.5, …, 4.0%) significantly reduced the fungal biomass by 69–94%. Methanolic fruit extract was successively partitioned among *n*-hexane, chloroform, ethyl acetate and *n*-butanol in order of increasing polarity. Different concentrations (3.125, 6.25, 12.50, 25, 50, 100, 200 mg mL^{-1}) of these fractions were used against the test pathogen. Pronounced variability in antifungal activity was recorded with respect to extracting solvent and the extract concentration. Chloroform fraction exhibited the highest antifungal activity resulting in 36–50% reduction due to different concentrations. Ethyl acetate was found comparatively less inhibitory where only 8–35% reduction in fungal biomass was observed due to different concentrations of the extract. *n*-hexane, *n*-butanol and aqueous fractions generally stimulated the fungal growth to variable extents. The present study concludes that *S. rolfsii* can be controlled by natural antifungal compounds in chloroform fraction of methanolic fruit extract of *D. metel*.

Keywords: Bell pepper, collar rot, *Datura* fruit extract, *Sclerotium rolfsii*.

INTRODUCTION

Bell pepper (*Capsicum annum* L.), locally known as Shimla Mirch, is the world's known vegetable of family Solanaceae (Dias *et al*., 2013). Total cultivated area of bell pepper in Pakistan is about 62,700 ha and annual production is 150,300 t (Anonymous, 2013). Bell pepper is a rich source of protein, carbohydrate, dietary fiber, vitamin C and E, provitamin A, ascorbic acid and carotenoids. It contains various antioxidant compounds especially polyhydroxy phenols and flavonoids which help to reduce harmful oxidation reactions and may prevent cardiovascular, cancer and neurological disorders (El- Ghoraba *et al*., 2013). Collar rot caused by *Sclerotium rolfsii* is a highly destructive disease of bell pepper. This pathogen is widespread and causes significant economic losses to more than 500 plant species in the world (Punja, 1985; Deepthi and Reddy, 2013). Initial symptoms are gradually yellowing and wilting of leaves. It produces a large amount of white cottony thread like material known as mycelium, which can grow up the stem of host plant and also spread out across the soil to infect other plants. Small, spherical, tan to brown sclerotia are formed profusely on mycelium (Kokub *et al.,* 2007). Control of collar rot disease of bell pepper is one of the challenging matters. The most conventional methodology in over 50 years is the use of synthetic fungicides, cultural practices and growing resistant varieties for controlling collar rot of bell pepper. Though, fungicides have shown some promising results in suppressing collar rot disease but fungicide residues could lead to environmental pollution and human health hazards (Gurjar *et al*., 2012). In addition, there is gradual increase in resistant pathogens to chemical fungicides (Parveen *et al*., 2013). Management by using natural antifungal plant products is an

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ecofriendly strategy for management of fungal plant diseases (Jabeen *et al.,* 2011; Rauf and Javaid, 2013; Javaid and Iqbal, 2014). Allelopathic plants like herbs and spices are important to investigate for natural antimicrobial properties (Zahid *et al.,* 2012).

Datura metel L. belongs to family *Solanaceae* is the most mysterious and powerful sacred plant. It performed as sedative, anodyne, anti-asthmatic, antispasmodic, anti-tussiue, bronchodilator, and hallucinogenic (Monira and Munan, 2012; Jyothi and Taskeen, 2013). A number of phytochemicals have been reported in *D. metel*. These phytoconstituents are alkaloids, flavonoids, phenols, tannins, saponins, sterols, hyoscyamine and scopolamines (Dabur *et al*., 2004). Although insecticidal, antibacterial and antifungal properties of *D. metal* are known (Gnanamani *et al*., 2003; Dabur *et al*., 2004; Chakkaravarthy *et al*., 2011), however, studies regarding management of *S. rolfsii* are lacking. This study was, therefore, undertaken to explore the antifungal potential of methanolic fruit extract of *D. metel* and its various organic solvent fractions for the management of *S. rolfsii,* the causal organisim of collar rot of bell pepper.

MATERIALS AND METHODS

Isolation of *Sclerotium rolfsii*: *S. rolfsii* was isolated from a bell pepper plant infected with collar rot disease. The diseased portions were cut into small pieces surface sterilized with 1% sodium hypochlorite solution for one minute and thoroughly rinsed with distilled water. These pieces were placed on malt extract agar medium in 9-cm Petri plates and incubated at 25 °C for 10 days. After 10 days, Petri plates were filled with mycelium and small tan brown saclerotia. Culture was purified and maintained on malt extract agar at 25 °C.

Bioassays with methanolic fruit extract: Dried fruit of *D. metel* (5 kg) was crushed and dipped in methanol (10 L) for 10 days. After that, material was filtered through doubled layered muslin cloth. Residues were re-extracted with methanol and filtered through a double layered muslin cloth followed by filter paper sheets. Solvent was evaporated under reduced pressure on a rotary evaporator at 45 °C to yield crude methanolic extract of *D. metel* fruit.

Stock solution was made by dissolving 14.4 g of crude methanolic extract in 5 mL dimethyl sulphoxide (DMSO) and raised the volume up to 18 mL by adding sterilized distilled water. A control solution was made by mixing 5 mL DMSO and 13 mL distilled water. Malt extract broth (76 mL) was autoclaved in 250-mL flask at 121 °C. Nine treatments (0.5, 1.0, 1.5,…,4.0%) were made by adding 4 mL of mixture of stock and control solutions in appropriate quantities to make final volume up to 80 mL, which were divided in four replications of 20 mL each in 100 mL flasks. Fungal inoculation was done by taking virulent strain of *S. rolfsii* through 5 mm cork borer from tips of seven days old fungal culture. Petri plates were incubated at 25 °C in an incubator for one week. Thereafter, fungal biomass was weighed by filtering through pre-weighed filter papers and dried at 60 °C.

Bioassay with different fractions of methanolic fruit extract: Crude gummy methanolic fruit extract of *D. metel* was mixed with 500 mL distilled water. The mixture was transferred to a separating funnel and extracted successively with *n*-hexane, chloroform, ethyl acetate and *n*-butanole on the basis of increasing polarity. After extraction, all the solvents were evaporated under vacuum in a rotary evaporator and 58 g *n*-hexane, 19.9 g chloroform, 5.63 g ethyl acetate, 15.37 g *n*-butanole and 125.69 g aqueous fractions were obtained.

Antifungal activity of all the fractions was investigated *in vitro* against *S. rolfsii*. For this purpose, 1.2 g of each of the five above mentioned fractions of methanolic fruit extract were dissolve separately in 0.5 mL DMSO followed by addition of 5.5 mL malt extract broth to prepare a 200 mg mL^{-1} solution. This stock solution was serially double diluted to make lower concentrations of 100, 50, 25,12.50, 6.25, 3.125 by adding malt extract broth. For control, 0.5 mL DMSO was mixed with 5.5 mL malt extract broth and serially double diluted to get various concentrations of DMSO corresponding to various concentrations of DMSO in extract treatments. Each treatment was replicated three times with 1 mL medium in each 10-mL volume test tube. Tubes were inoculated by taking standard droplets of 10 µL from fungal suspension. Test tubes were incubated for seven days at 25 °C. After seven days, fungal biomass was filtered, dried and weighed.

Statistical analysis: All the data were analyzed by analysis of variance (ANOVA) followed by Tukey's HSD test using computer software Statistics 8.1.

RESULTS AND DISCUSSION

Antifungal activity of methanolic fruit extract: Data regarding the effect of methanolic fruit extract of *D. metel* on biomass of *S. rolfsii* is demonstrated in Fig. 1. All the concentrations of methanolic extracts significantly ($P \leq 0.05$)

declined biomass of *S. rolfsii.* In general, fungal biomass was inversely proportional to the extract concentrations. There was 69–94% reduction in biomass of *S. rolfsii* due to different concentrations (0.5–4.0%) of methanolic fruit extract of *D. metel*.

Antifungal activity of different fractions of methanolic fruit extract: In different control treatments, fungal biomass was gradually decreased with increase of DMSO concentration (Table 1). There are also earlier reports that DMSO can reduce growth of some other fungal species namely *Alternaria alternata* (Fr.) Keissl.*, Macrophomina phaseolina* (Tassi) Goid. and *Ascochyta rabiei* (Pass.) Lab. (Javaid and Samad, 2012; Javaid and Munir, 2012; Naqvi *et al.,* 2012). In the present study, different concentrations of DMSO were used in control to compare the effect of DMSO in different treatments of extracts.

Table 1. Effect of different concentrations of n-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fraction of methanolic fruit extract of *Datura metel* on biomass of *Sclerotium rolfsii*.

Methanolic extract fraction	Conc. of DMSO (mL mL^{-1})	Extract conc. (mg mL^{-1})	Fungal biomass (mg)
Control	0.0026	0	40 b-d
	0.0052	0	37 b-d
	0.0104	0	30 d
	0.0208	0	27 d
	0.0416	0	20 d
	0.0833	0	20 d
	0.1666	0	17 d
n-hexane	0.0026	3.125	70 a
	0.0052	6.25	63 ab
	0.0104	12.5	43 a-d
	0.0208	25	40 b-d
	0.0416	50	37 b-d
	0.0833	100	27 d
	0.1666	200	27 d
Chloroform	0.0026	3.125	23 d
	0.0052	6.25	20 d
	0.0104	12.5	17 d
	0.0208	25	17 d
	0.0416	50	10 d
	0.0833	100	10 d
	0.1666	200	10 d
Ethyl acetate	0.0026	3.125	33 cd
	0.0052	6.25	23 d
	0.0104	12.5	23 d
	0.0208	25	23 d
	0.0416	50	23 d
	0.0833	100	17 d
	0.1666	200	17 d
n-butanol	0.0026	3.125	60 a-c
	0.0052	6.25	37 cd
	0.0104	12.5	30 d
	0.0208	25	27 d
	0.0416	50	20 d
	0.0833	100	17 d
	0.1666	200	17 d
Aqueous	0.0026	3.125	70 a
	0.0052	6.25	60 a-c
	0.0104	12.5	40 b-d
	0.0208	25	30 d
	0.0416	50	27 d
	0.0833	100	23 d
	0.1666	200	23 d

In vertical columns, values with different letters show significant difference ($P \leq 0.05$) as determined by Tukey's HSD test.

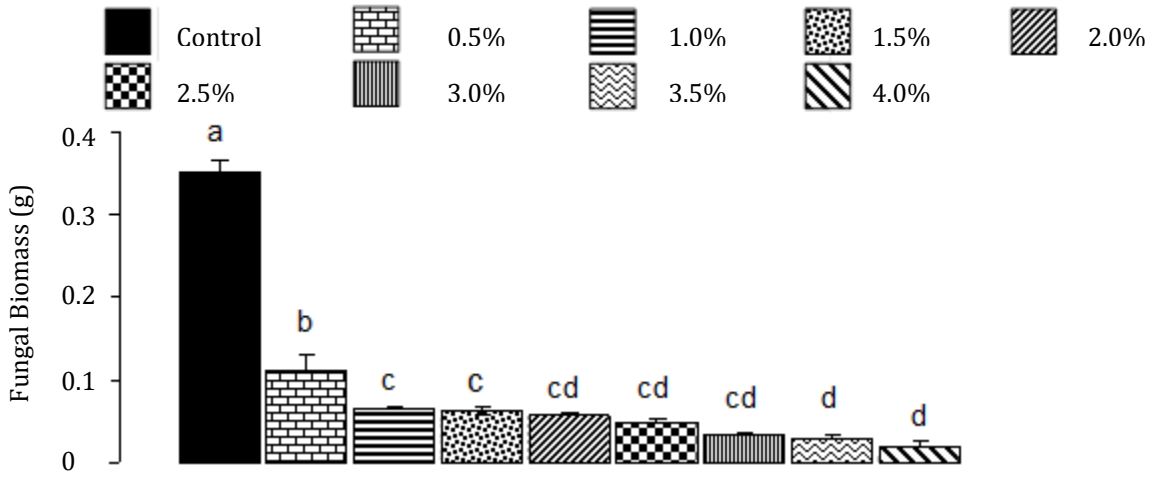

Fig. 1: Effect of different concentrations of methanol fruit extract of *Datura metel* on biomass of *Sclerotium rolfsii.* Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD method.

Higher concentrations of DMSO adversely affected the fungal growth. In general, there was a gradual reduction in fungal biomass with the increase in DMSO concentration in the growth medium. Some previous studies also showed that higher concentrations of DMSO are inhibitory to growth of *Alternaria alternata* and *Fusarium oxysporum* (Schlecht.) Snyder & Hansen (Javaid and Samad, 2012; Rauf and Javaid, 2013). In order to avoid the effect of DMSO on fungal growth in different extract treatments, a series of control treatments was included with different concentrations of DMSO corresponding to various concentrations of DMSO in extract treatments.

The comparison of different concentrations of various fractions of methanolic fruit extract showed that the percentage fungal biomass inhibition was more pronounced due to chloroform fraction as compare to other ones. Different concentrations of chloroform fraction (3,125 mg mL^{-1} to 200 mg mL^{-1}) suppressed the fungal biomass by 36% to 50% as compared to corresponding control treatments. Ethyl acetate fraction exhibited comparatively lower antifungal activity than chloroform fraction. There was up to 17% reduction in fungal biomass due to this fraction over corresponding control treatments. In contrast, *n*-hexane, *n*-butanol and aqueous fractions did not suppress the fungal growth significantly over control. The variation in antifungal activity of different organic fractions may be attributed to the different chemical nature of the fractions. It is probable that different type of compounds dissolve in different organic fractions due to different polarities of the extracting solvents. Similar variation in antifungal activity of different fractions of methanolic extracts of *Syzygium cumini* (L.) Skeels, *Coronopus didymus* (L.) Sm., *Withania somnifera* (L.) Dunal and *Chenopodium album* L. has been reported against other fungal plant pathogens (Iqbal and Javaid, 2012; Javaid and Munir, 2012; Javaid and Samad, 2012; Rauf and Javaid, 2013). It is further reported that higher amount of total phenolic content in chloroform compared with other organic solvent extracts could be responsible for higher antifungal activity of chloroform fraction than other fractions of methanolic fruit extract of *D. metel* (Hossain *et al.,* 2013). In addition, they also identified a number of other bioactive substances including 2,2-dimethyl-3-hexanone, 2-heptanol, 2-decanol, 2,4-decadienal, 2,4-decadienal(E-E), 2-oxo-ME and hyoscyamine, which might be responsible for antifungal activity. Four different bioactive components namely acetic acid, trifluoro-, 2,2-dimethylpropyl ester, 4-Trifluoroacetoxyoctane and 1,4-yclohexadiene, 1-methyl- with antimicrobial properties have been isolated from methanolic of flower of *D. metel* (Kiruthika and Sornaraj, 2011). Antifungal activity of *D. metel* leaves and flower organic (petroleum ether, chloroform, methanol) and aqueous extracts has also been reported against different formae speciales of *Fusarium oxysporum* (Vadlapudi and Kaladhar, 2012; Rienz *et al*., 2013).

CONCLUSIONS

Present study concludes that chloroform fraction of methanolic fruit extract of *D. metel* is highly effective

against *S. rolfsii.* Further studies are required to isolated and identify various antifungal compounds from this fraction.

REFERENCES

Anonymous. 2013. Agricultural Statistics of Pakistan, Ministry evaluation of irrigation management analysis in of Food and Agriculture, Islamabad. p. 23

Chakkaravarthy, V.M., T. Ambrose, S. Vincent, R. Arunachalam, M.G. Paulraj, S. Ignacimuthu, G. Annadurai. 2011. Bioefficacy of *Azadirachta indica* A. Juss and *Datura metel* Linn. leaves extracts in controlling *Culex quinquefasciatus* (Diptera: Culicidae). J. Entomol. 8: 191-197.

Dabur, R., M. Ali, H. Singh, J. Gupta, G.L. Sharma. 2004. A novel anti-fun-gal pyrrole derivative from *Datura metel* leaves. Pharmazie 59: 568-570.

Deepthi, K.C., and N.P.E. Reddy. 2013. Stem rot disease of groundnut (*Arachis hypogaea* L.) induced by *Sclerotium rolfsii* and itsnagemen. Int. J. Life Sci. Biotechnol. Pharmol. 2: 26-38.

Dias, G.B., V.M. Gomes, T.M. Moraes, U.P. Zottich, G.R. Rabelo, A.O. Carvalho, M. Moulin, L.S. Gonçalves, R. Rodrigues, M. Da Cunha. 2013. Characterization of *Capsicum* species using anatomical and molecular data. Genet. Mol. Res. 12: 6488-6501.

El-Ghoraba, A.H., Q. Javed, F.M. Anjum, S.F. Hamed, H.A. Shaabana. 2013. Pakistani bell pepper (*Capsicum annum* L.): Chemical compositions and its antioxidant activity. Int. J. Food Properties 16: 18-32.

Gnanamani, A., K.S. Priya, N. Radhakrishnan, M. Babu. 2003. Antibacterial activity of two plant extracts on eight burn pathogens. J. Ethnopharmacol. 86: 59-61.

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

Hossain, M.A., K.M. Al-sabari, A.M. Weli, and Q. Al-Riyami. 2013. Gas chromatography–mass spectrometry analysis and total phenolic contents of various crude extracts from the fruits of *Datura metel* L. J. Tai. Uni. Sci. 7: 209-215

Iqbal, D., A. Javaid. 2012. Bioassays guided fractionation of *Coronopus didymus* for its antifungal activity against *Sclerotium rolfsii*. Nat. Prod. Res., 26:1638-1644.

Jabeen, K., A. Javaid, E. Ahmad and M. Athar. 2011. Antifungal compounds from *Melia azedarach* leaves for management of *Ascochyta rabiei* – the cause of chickpea blight. Nat. Prod. Res. 25: 264-276.

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: 164-175.

Javaid, A. and R. Munir. 2012. Bioassay guided fractionation of *Withania somnifera* for the management of chickpea blight pathogen *Ascochyta rabiei.* Int. J. Agric. Biol. 14: 797-800

Javaid, A. and S. Samad. 2012. Screening of allelopathic trees for their antifungal potential against *Alternaria alternata* strains isolated from dying back *Eucalyptus* spp. Nat. Prod. Res. 26: 1697-1702

Jyothi, V.A., and S. Taskeen. 2013. A review: *Datura metel*. Int. J. Pharma. Res. Rev. 1: 74-85.

Kiruthika, K.A. and R. Sornaraj. 2011. Screening of bioactive components of the flower *Datura metel* using the GC-MS technology. Int. J. Pharm Technol. Res. 3: 2025-2028

Kokub, D., F. Azam, A. Hassan, M. Ansar, M.J. Asad and A. Khanum. 2007. Comparative growth, Morphological and molecular characterization of indigenous *Sclerotium rolfsii* strains isolated from different locations of Pakistan. Pak. J. Bot. 39: 1849-1866.

Monira, K.M. and S.M. Munan. 2012. Review on *Datura metel*: A potential medicinal plant. Global. J. Res Med. Plants Indigenous Med. 1: 123-132.

Naqvi, S.F., A. Javaid and A. Shoaib. 2012. Evaluation of antifungal activity of methanolic extracts of *Dicanthium annulatum* for the management of *Macrophomina phaseolina.* Afr. J. Microbiol. Res. 6: 5882-5886.

Parveen, S., A.H. Wani, A.A. Ganie, S.A. Pala and R.A. Mir. 2014. Antifungal activity of some plant extracts on some pathogenic fungi. Arch. Phytopathol. Plant Prot. 47: 279-284

Punja, Z.K. 1985. Biology, ecology and control of *Sclerotium rolfsii*. Ann. Rev. Phytopathol. 23: 97-127.

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.

Rienz, A., M. Daami-Remadi, A. Ladhari, F. Omezzine, I. Rinez and R. Haouala. 2013. Antifungal activity of *Datura metel* L. organic and aqueous extracts on some pathogenic and antagonistic fungi. Afr. J. Microbiol. Res. 7: 1605-1612.

Vadlapudi, V. and D.S.V.G. Kaladhar. 2012. Antimicrobial study of plant extracts of *Datura metel* L. against some important disease causing pathogens. Asian Pacific J. Trop. Dis. 94-97

Zahid, N.Y., N.A. Abbasi, I.A. Hafiz, A. Hussain and Z. Ahmad. 2012. Antifungal activity of local fennel (*Foeniculum vulgare* Mill) extract to growth responses of some soil diseases. Afr. J. Microbiol. Res. 6: 46-51.