INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 10, No. 1, p. 327-334, 2017

RESEARCH PAPER OPEN ACCESS

Fungistatic potential of *Sonchus asper* against *Botrytis cinerea* and *Rhizoctonia solani*

Tayyaba Ejaz, Khajista Jabeen*, Sumera Iqbal, Aroosa Naeem

Department of Botany, Lahore College for Women University Jail Road, Lahore, Pakistan

Key words: Antifungal, *Botrytis cinerea*, Methanolic extract, MIC, *Rhizoctonia solani*

http://dx.doi.org/10.12692/ijb/10.1.327-334 Article published on January 31, 2017

Abstract

Sonchus asper L. was evaluated for its antifungal potential against *Botrytis cinerea* Pers. and *Rhizoctonia solani* Kuhn. Various concentrations viz. 1%, 2%, 3%, 4%, 5% of methanolic extract of aerial part of *S. asper* were tested against *B. cinerea* and *R. solani*. All the applied concentrations of tested extracts significantly inhibited the growth of tested fungi up to 37%-74%. Methanolic extract of aerial parts was partitioned with *n*-hexane, ethyl acetate, chloroform and *n*-butanol. These isolated organic fractions were further serially diluted from 100 mg to 0.78 mg mL^{-1} to evaluate their Minimum Inhibitory Concentration (MIC). A synthetic fungicide (Puslan, 72 WP) was used as reference and data was recorded at interval of 24, 48 and 72 hours. Ethyl acetate and Puslan (Synthetic fungicides) were found highly effective against both the test fungi with MIC of 0.78 mg after 72 hrs of incubation period. Chloroform and *n*-hexane fractions also inhibited the fungal growth.

* **Corresponding Author:** Khajista Jabeen ✉ khaijsta_1@hotmail.com

Introduction

Botrytis cinerea Pers. belongs to class Ascomycetes is a destructive air borne pathogen. *B. cinerea* possesses a necrotrophic lifestyle and caused disease in a large number of crop plants worldwide (Williamson *et al.,* 2007). This pathogenic fungus produces abundant hyaline conidia on grey color conidiophore. Under unfavorable conditions sclerotia formation is also reported in *B. cinerea* as survival structures (Yua and Suttona, 1999). *B. cinerea* also known as grey mould as it causes gray mould decay on hundreds of dicot plants (Elad *et al.,* 2004). This disease affects the leaves, stems, flowers, fruits, tubers and roots even tubers and roots of many important crop plants including potatoes and carrots (York, 2007).

Rhizoctonia solani Kuhn. belongs to Basidiomycetes is a common soil borne pathogen. It is the imperfect state of the Basidiomycete fungus that does not produce sexual spores and exists as vegetative mycelium and sclerotia in nature (Townsends and Willets, 1957; Webster, 1980; Agrios, 1988). It causes damping off, black scurf, seed decay, stem cankers and fruit & foliage decay in variety of crop plants. This fungus occurs worldwide and is capable of attacking several host plants, causing seed decay, damping-off, stem cankers, fruit decay, and foliage diseases (Parameter, 1970).

Many commercial synthetic fungicides are also being used to control both of these fungi. These include mancozeb, anilinopyrimidines, phenylpyrroles, hydroxyanilides, benzimidazoles and dicarboximides (Myresiotis *et al.,* 2007; Marta e*t al.*, 2011; Mishra *et al.*, 2012). However natural antifungal complexes have been found to be reasonably much harmless than synthetic compounds in terms of harmfulness in food stuffs. For this reason there is high prospect to discover biologically active compounds that can be a potential fungicide (Hanekamp and Kwakman, 2004). Plant extracts possess strong antifungal properties to control various pathogenic fungi (Nagamalleswari *et al.,* 2013).

Sonchus asper L. (Sowthistle) is an annual herb to occasional biennial, C3, herbaceous plant; it reproduces only from seeds (Parker, 1972).

S. asper contains glycosides, ascorbic acid, flavonoids, carotenoids and also have antioxidant, anticancer and antifungal properties (Shimizu *et al.,* 1989; Giner *et al.,* 1993; Manez *et al.,* 1994). According to researches *S. asper* is pharmacologically important and used in aliments of various disorders like cough, asthma, bronchitis, gastrointestinal infection and kidney disorders (Rivera and Oben, 1993; Ahmad *et al.,* 2006; Rehman, 2006; Zabihullah *et al.,* 2006; Kareru *et al.,* 2007; Hussain *et al.,* 2008; Jan *et al.,* 2009; Qureshi *et al.,* 2009).

The current study is thus conducted to investigate the antifungal activity of mentholic extract of aerial parts (leaves + stem) of *Sonchus asper* against two of the most devastating fungi i.e. *Botrytis cinerea* and *Rhizoctonia solani*.

Materials and methods

Collection of experimental material

Test plant (*S. asper*) was collected from Lahore College for Women University, Lahore, Pakistan. The plant material was dried under sunlight and ground into powder form. Dried plant material was stored in air tight plastic jars. Culture of *B. cinerea* was prepared by isolating from infected onion bulb. This culture was further subcultured and maintained on 2% MEA (Malt Extract Agar) medium and was stored in incubator at 20 ^{o}C. *R. solani* culture was prepared by sprinkling contaminated soil on PDA medium. This culture was retained on 2% MEA and stored at 4^{o}C in refrigerator.

Antifungal bioassay

Forty gram dried aerial parts of *S. asper* was soaked in 200 mL methanol for one week. Afterwards the soaked test plant material was filtered with the help of autoclaved muslin cloth. The filtrate was evaporated at room temperature until the volume is reduced to 4 mL. 20% stock solution was prepared by adding 196 mL of sterilized distilled water in 4 mL of methanolic *S. asper* extract. This stock solution was further used to prepare different concentrations of extract. ME (2%) was prepared by dissolving 4 g of malt extract in 200 mL of distilled water.

This solution was then autoclaved at 121 °C for 30 minutes. The 5% w/v concentration of the extract in the medium was prepared by adding 50 mL of stock solution in 150 mL of ME solution. The lower concentrations of 4%, 3%, 2% and 1% were prepared by adding 40, 30, 20, and 10 mL of the stock solutions to 160, 170, 180 and 190 mL of ME solutions respectively. These solutions were autoclaved and cooled up to 50 °C. Chloromycetin capsules were added in each concentration to avoid bacterial growth. All the concentrations were replicated thrice (Jabeen *et al.,* 2014).

In vitro bioassays were conducted with methanolic extract of *S. asper*. *B. cinerea* and *R. solani* and mycelial discs (5 mm) were prepared from the tips of seven days old fungal culture by using sterilized cork-borer. These discs were placed into each flask which is then covered tightly with foil paper to avoid contamination and these flasks were incubated at 25 ± °C for 7 days. After 7 days the test fungal biomasses of each flask were filtered, dried in electric oven and weighed on electric balance. Percentage growth inhibition of the fungal biomass was calculated by using the following formula:

$$\text{Growth inhibition (\%)} = \frac{\textit{Growth in Treatment} - \textit{Growth in Control}}{\textit{Growth in Control}} \times 100$$

Partitioning of plant material

One hundred grams of dried *S. asper* was soaked in 250mL of methanol at room temperature for one week. The extract was evaporated at room temperature a gummy mass of 23.7 g was obtained after evaporation which is the methanolic extract of aerial part. This extract was then portioned with *n*-hexane and water using separating funnel. The aqueous fraction of methanolic extract was partitioned with *n*-butanol, ethyl acetate and chloroform according to their polarity (Waheed *et al.,* 2016). This partitioning resulted in gummy mass of *n*-butanol (0.2g), ethyl acetate (0.2 g), chloroform (4.4 g) and *n*-hexane (6.35 g).

Evaluation of Minimum Inhibitory Concentration (MIC)

The antifungal potential of four organic t fractions viz. *n*-butanol, ethyl acetate, chloroform and *n*-hexane aerial parts of *S. asper* and a reference synthetic fungicide Puslan 72% WP were tested against *B. cinerea* and *R. solani* by (MIC) bioassay. MIC assay was conducted in test tubes by serial dilution method (Shahbaz *et al.,* 2015). The maximum and minimum applied concentrations for MIC assay were 100 mg mL^{-1} -0.78 mg mL^{-1}. ME medium was freshly prepared and added to seven days old fungal cultures of *B. cinerea* and *R. solani* to reach a final conidia/spore concentration $1x10^5$ mL^{-1}.

Hundred microliter of this suspension was added in each test tube and these test tubes were incubated at 25 ±°C at room temperature. Test tube containing DMSO and distilled water was used as control. The MIC of each fraction was observed visually after 24, 48 and 72 hours by using inverted microscope.

Statistical analysis

All data was statistically analyzed by applying ANOVA followed by DMRT (Duncan's Multiple Range Test) at ($P \leq 0.05$) (DMR) (Steel *et al.,* 1997).

Result and discussion

The analysis of variance (ANOVA) show significant effects of aerial parts of *Sochus asper* solvent extracts and their different concentrations on growth of both the test fungi *Botrytis cinerea* and *Rhizoctonia solani*.

Antifungal activity of methanol extract of aerial part of Sonchus asper against Botrytis cinerea and Rhizoctonia solani.

According to the results *S. asper* shows high antifungal activity against *B. cinerea*. All the concentrations of this extract significantly reduced the colony growth of *Botrytis cinerea* by 74% to 46% (Fig. 1). The maximum reduction in dry weight of *B. cinerea* was observed in 5% concentration which caused 74% reduction in biomass of the test fungus. Minimum reduction (46%) in dry weight of *B. cinerea* was observed in *B. cinerea* in 1% with 46%.

Upadhyay *et al.* (2013) reported that methanolic fraction of *S. asper* possesses strong antifungal activity against *A. niger* and this activity is correlated with the presence of bioactive flavonoids, saponins and phenolic compounds.

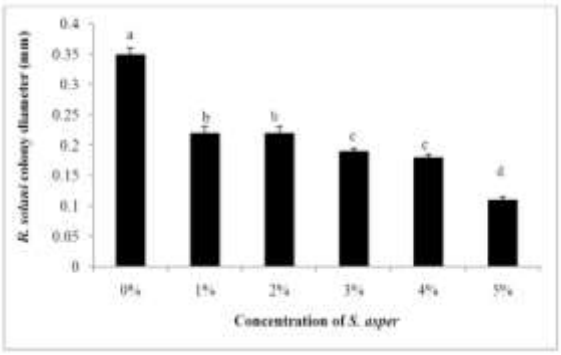

Fig. 1. Effect of methanolic extract of aerial part of *S. asper* on *B. cinerea*.

Methanol extract of aerial part of *S. asper* also show antifungal activity against *R. solani* but in relatively less amount as compared to *B. cinerea*. All the applied concentration effectively retards the growth of test fungal biomass (Fig. 2). The highest reduction in dry weight of *R. solani* was observed in 5% i.e. 69% followed by 3% and 4% concentrations. Earlier Khan and co-workers in (2010) reported that *S. asper* extracts were found effective against *Candida albicans* and *A. flavus*. So this information makes our results valid. Different concentrations of methanolic extract of *S. asper* showed variable activities against test fungi. Priya *et al.* (2012) suggested that the reason of variation in activities of concentrations might be attributed to the solvent, chemical composition of test plant, solubility and temperature.

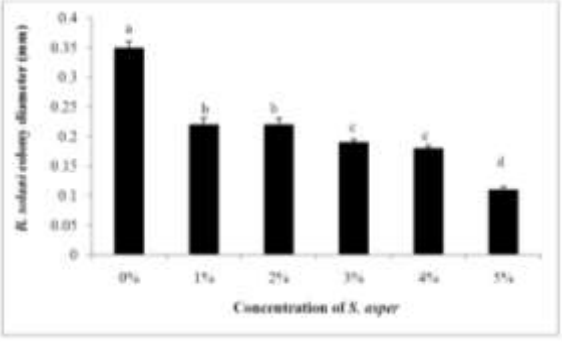

Fig. 2. Effect of methanolic extract of aerial part of *S. asper* on *R. solani*.

Minimum Inhibitory Concentration (MIC) Assay

The minimum inhibitory concentration (MIC) of the four organic solvent fractions *n*-hexeane, chloroform, ethyl acetate and *n*-butanol along with synthetic fungicide (Puslan) was tested against *B. cinerea* and *R. solani* (Table 1 & 2). In case of *B. cinerea*, chloroform and Puslan effectively suppressed the germination of spore and mycelial growth of the test fungi with MIC of 0.78 mg mL^{-1}. Complete inhibition in fungal mycelial growth was observed after 72 hrs of incubation period. Mycelial growth was also observed in all concentrations of water and DMSO which act as the control, so both of these controls set did not play any role in reducing the mycelial growth.

The MIC results of *R. solani* revealed that among all the tested organic fractions ethyl acetate and synthetic fungi were found to be most effective in suppressing the test fungal growth. Complete inhibition in spore germination of R. *solani* was observed in all of their concentrations after 72 hrs of incubation period. Other applied fractions of *n*-heaxane, *n*-butanol and chloroform were found least effective. It might be possible that the compound with antifungal property were soluble in chloroform and ethyl acetate which be further extracted for the production of fungicides. Jimoh *et al.* (2011) evaluated *S. asper* and *S. oleraceus* antimicrobial and antioxidant activity of methanol, acetone and aqueous extracts.

The results suggested that presence of saponins, alkaloids, flavonoids and proanthocyanidins are potential secondary metabolites in the tested *Sonchus* species which possibly a reason of its strong antioxidant antimicrobial properties. There are many literature reports which support our findings that *S. asper* possesses antifungal activity against various fungi. Like (Hussain *et al.,* 2010) chemically screened *S. asper* and suggested that it contains flavonoids, glycosides, ascorbic acid, riboflavin, thiamine, niacin and carotenoids and which are might be responsible of antioxidant, anticancer, anti-inflammatory and antifungal potential of *S. asper*. Elkhay *et al.* (2009) phytochemically analyzed the roots of *S. oleraceus* which gave 13-dihydrourospermal A,

loliolide, ursolic acid, 15-O-β-glucopyranosyl-11β, β-sitosterol- 3-O-glucopyranoside and lupeol. These compounds showed strong antimicrobial potential against various important microbes. Macias *et al.* (1992) suggested that electrophilic and nucleophilic systems are existing in plants which act in coordination with protein relate to the high antifungal activity of plants.

On the basis of these findings present study concluded that of *Sonchus asper* possessed pronounced antifungal potential against both phytopathogenic fungi *Botrytis cinerea* and *Rhizoctonia solani*, but the exact compounds are yet to be discovered and according to the results those compounds might be present in potential fractions of current finding.

Table 1. MIC values of isolated organic fractions from *Sonchus asper* and Puslan) against *Botrytis cinerea* after 24, 48 and 72 hrs incubation period. Mycelium present (+), Mycelium Absent (-).

Fractions	Concentration (mg mL^{-1})							
	100	50	25	12.5	6.25	3.125	1.56	0.78
After 24 hours incubation								
Control (DMSO)	-	-	-	-	-	-	+	+
Control (H_2O)	+	+	+	+	+	+	+	+
n-Butnaol	-	-	-	-	-	-	-	+
Ethyl acetate	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-
n-Hexane	-	-	-	-	-	-	-	-
Puslan	-	-	-	-	-	-	-	-
After 48 hours incubation								
Control (DMSO)	-	-	-	-	-	+	+	+
Control (H_2O)	+	+	+	+	+	+	+	+
n-Butnaol	-	-	-	-	-	-	+	+
Ethyl acetate	-	-	-	-	-	-	-	+
Chloroform	-	-	-	-	-	-	-	-
n-Hexane	-	-	-	-	-	-	-	+
Puslan	-	-	-	-	-	-	-	-
After 72 hours incubation								
Control (DMSO)	+	+	+	+	+	+	+	+
Control (H_2O)	+	+	+	+	+	+	+	+
n-Butnaol	-	-	+	+	+	+	+	+
Ethyl acetate	-	-	-	+	+	+	+	+
Chloroform	-	-	-	-	-	-	-	-
n-Hexane	-	-	-	-	-	+	+	+
Puslan	-	-	-	-	-	-	-	-

Table 2. MIC values of isolated organic fractions from *Sonchus asper* and Puslan against *Rhizoctonia solani* after 24, 48 and 72 hrs incubation period. Mycelium present (+), Mycelium absent (-).

Fractions	Concentration (mg mL^{-1})							
	100	50	25	12.5	6.25	3.125	1.56	0.78
After 24 hours incubation								
Control (DMSO)	-	-	-	-	-	-	+	+
Control (H_2O)	+	+	+	+	+	+	+	+
n-Butnaol	-	-	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-
n-Hexane	-	-	-	-	-	-	-	-
Puslan	-	-	-	-	-	-	-	-
After 48 hours incubation								
Control (DMSO)	-	-	-	-	-	+	+	+
Control (H_2O)	+	+	+	+	+	+	+	+
n-Butnaol	-	-	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	+	+
n-Hexane	-	-	-	-	+	+	+	+
Puslan	-	-	-	-	-	-	-	-
After 72 hours incubation								

Fractions	Concentration (mg mL^{-1})							
	100	50	25	12.5	6.25	3.125	1.56	0.78
Control (DMSO)	+	+	+	+	+	+	+	+
Control (H_2O)	+	+	+	+	+	+	+	+
n-Butnaol	-	-	-	-	-	-	+	+
Ethyl acetate	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	+	+	+	+
n-Hexane	-	-	+	+	+	+	+	+
Puslan	-	-	-	-	-	-	-	-

References

Agrios GN. 1988. Plant pathology, 3rd ed. Academic Press. INC. San Diego, New York, Berkeley, Boston, London, Sydney, Tokyo Toronto.

Ahmad M, Khan MA, Manzoor S, Zafar M, Sultana S. 2006. Check list of medicinal flora of Tehsil Isakhel, District Mianwali Pakistan. Ethnobotanical Leaflets **10,** 41-48.

Elad Y, Williamson B, Tudzynski P, Delen N. 2004. Botrytis spp. and diseases they cause in agricultural systems – an introduction. Botrytis: Biology, Pathology and Control, Kluwer Academic Publishers, Dordrecht, Netherlands 1-6.

Elkhayat ES. 2009. Cytotoxic and antibacterial constituents from the roots of *Sonchus oleraceus* L. growing in Egypt. Pharmacognosy Magazine. **5(20),** 324-328.

Giner RM, Ubeda A, Just MJ, Serrano A, Manez S, Rios JL. 1993. A chemotaxonomic survey of *Sonchus* subgenus *Sonchus*, Biochemical Systematics and Ecology **21,** 6-17.

Hanekamp JC, Kwakman J. 2004. Beyond Zero-Tolerance: A novel and global outlook on food-safety and residues of pharmacological active substances in foodstuffs of animal origin, Directorate-General Enterprise and Industry, European Commission Brussels.

Hussain J, Muhammad Z, Ullah R, Khan FU, Khan IU, Khan N, Ali J, Jan S. 2010. Evaluation of the chemical composition of *Sonchus eruca* and *Sonchus asper*. Journal of American Science **6(9),** 231-235.

Hussain K, Shahazad A, Zia-ul-Hussnain U. 2008. An ethnobotanical survey of important wild medicinal plants of Hattar, District Haripur Pakistan. Ethnobotanical Leaflets **12,** 29-35.

Jabeen K, Zubairi T, Iqbal S. 2014. Management of *Botrytis cinerea* (grey mold disease) by methanolic extract of *Pongamia pinnata* L. Mitteilungen Klosterneuburg **64,** 105-113.

Jan G, Khan MA, Gul F. 2009. Ethnomedicinal Plants Used against Jaundice in Dir Kohistan valleys (NWFP) Pakistan. Ethno botanical Leaflets **13,** 1 029-1041.

Jimoh FO, Adeolu AA, Afolayan AJ. 2011. Comparison of the nutritive value, antioxidant and antibacterial activities of *Sonchus asper* and *Sonchus oleraceus*. Records of Natural Products. **5(1),** 29-42.

Kareru PG, Kenji GM, Gachanja AN, Keriko JM, Mungai G. 2007. Traditional medicine among the Embu and Mbeere peoples of Kenya. African Journal of Traditional: Complementary and Alternative Medicines **4,** 75-86.

Khan RA, Khan MR, Sahreen S, Bokhari J. 2010. Cytotoxic and antibacterial constituents from the roots of *Sonchus oleraceus* L. growing in Egypt. African Journal of Biotechnology **9(25),** 3883-3887.

Koche DK, Shirsat RP, Imran S, Nafees M, Zingare AK, Donode KA. 2008. Ethno medicinal survey of Nigeria Wildlife Sanctuary, District Gondia (MS). India- Part II. Ethno botanical Leaflets **12,** 532-537.

Macías FA, Galindoadoand JCG, Massanet GM. 1992. Potential allelopathic activity of several sesquiterpene lactone model. Phytochemistry **31,** 1969-1977.

Manez S, Recio MC, Giner RM, Sanz MJ, Terencio MC, Peris JB, Stubing G, Rios JL. 1994. A chemotaxonomic review of the subtribe Crepidinae based on its phenolic constituents. Biochemical Systematics and Ecology **22,** 297.

Marta A, Julie B, Nelleman C, Kiersgaard M, Rosenkjold PJ, Christiansen S, Hongard KS, Halla U. 2011. Exposure to widely used Mancozeb causes thyroid hormone disruption in rat dams but no behavioral effects in the offsprings. Journal of Toxicological Sciences **10,** 1093-1094.

Mishra RK, Adholeya A, Sardana HR. 2012. Integrated pest management: strategies for onion and garlic. The energy and resources institute, New Delhi.

Myresiotis CK, Karaoglanidis GS, Tzavella-Klonari K. 2007. Resistance of *Botrytis cinerea* isolates from vegetable crops to anilinopyrimidine, phenylpyrrole, hydroxyanilide, benzimidazole and dicarboximide fungicides. Plant Diseases **91,** 407-413.

Nagamalleswari K, Yasodamma N, Binny RJA. 2013. Phytochemical screening, antibacterial and antifungal studies of *Pittosporum floribundum* wight & arn. leaf, bark, fruit and seed extracts. International Journal of Pharma and Biosciences **4,** 464-474.

Parameter JR. 1970. *Rhizoctonia solani,* Biology and pathology, University of California Press, Berkeley 3-5.

Parker KF. 1972 An illustrated guide to Arizona weeds, The University of Arizona Press, Tucson, Arizona **338**.

Priya, GS, Radhika R, Siddhuraju P. 2012. Antioxidant and antimicrobial activity of traditional Indian leafy vegetables: *Mukia maderaspatana* and *Solanum trilobatum*. International Journal of Pharmacy and Pharmaceutical Sciences **4(2),** 513-521.

Qureshi R, Waheed A, Arshad M, Umbreen T. 2009. Medicoethanobotanical inventory of tehsil Chakwal, Pakistan. Pakistan Journal of Botany **41,** 529-538.

Rehman EU. 2006 Indigenous knowledge on medicinal plants, village Barali Kass and its allied areas, district Kotli Azad Jammu and Kashmir, Pakistan. Ethnobotanical Leaflets **10,** 254-264.

Rivera ND, Oben DCC. 1993. Ethnopharmacology of Murcia (SE Spain). Conference International Ethnomedecine **2,** 7.

Shahbaz H, Jabeen K, Iqbal S. 2015. Evaluation of antifungal activity of *Solanum nigrum* against *Aspergillus niger*, the cause of black rot disease of onion. Pakistan Journal of Phytopathology **27(2),** 121-125.

Shimizu S, Miyase T, Ueno A, Usmanghani K. 1989. Sesquiterpene lactone glycosides and ionone derivative glycosides from *Sonchus asper*. Phytochemistry **28,** 3399.

Steel RGD, Torrie JH, Dickey DA. 1997. Principles and Procedures of Statistics: A Biometrical Approach. New York, USA: Mc Graw Hill Book Co., Inc (ISBN 0070610282).

Townsend BB, Willets HJ. 1957. The development of *Sclerotia* of different fungi. Transactions of the British Mycological Society **37,** 213-22.

Upadhyay H, Kumar A, Gupta MK, Sharma A, Rahal A. 2013. Validation of medicinal values of traditionally used *Sonchus asper* (prickly sow thistle) leaves for the treatment of skin ailments. Advancement in Medicinal Plant Research **1(1),** 29-35.

Waheed N, Jabeen K, Iqbal S, Javaid A. 2016. Biopesticidal activity of *Calotropis procera* L. against *Macrophomina phaseolina* African Journal of Traditional: Complementary and Alternative Medicines **13(6),** 163-167.

Webster J. 1980. Introduction to Fungi, 2nd ed. Cambridge University Press, Cambridge UK.

Williamson B, Tudzynski B, Tudzynski P, Van KJA. 2007. *Botrytis cinerea*: the cause of grey mould disease. Molecular Plant Pathology **8(5),** 561-580.

York SH. 2007. *Grey mould (Botrytis cinerea):* Plant health care fact sheet. Central Science Laboratory, United Kingdom.

Yua H, Suttona JC. 1999. Density dynamics of *Gliocladium roseum* in relation to biological control of *Botrytis cinerea* in red raspberry. Canadian Journal of Plant Pathology **21(1),** 23-32.

Zabihullah Q, Rashid A, Akhter N. 2006. Ethanobotnical survey in Kot Manzary Baba valley Malakand Agency, Pakistan. Pakistan, Pakistan Journal of Plant Sciences **12,** 115-121.