Comparative antimycotic activity of some phyto extracts against *Alternaria alstroemeriae*, a rot pathogen of common bean

NAYEEMA JAN, SHAIESTA HASSAN, MANSOOR A. MALIK, ABDUL HAMID WANI, MEESA JAN, MOHD YAQUB BHAT, AASIA RASHID BHAT

J. Mycopathol. Res. 60(4) : *629-633, 2022;*
ISSN 0971-3719

Department of Botany,
University of Calcutta,
Kolkata 700 019, India

J. Mycopathol. Res. **60(4)** : 629-633, 2022; (ISSN 0971-3719)
doi:10.57023/JMycR.60.4.2022.629

Comparative antimycotic activity of some phyto extracts against *Alternaria alstroemeriae*, a rot pathogen of common bean

NAYEEMA JAN, SHAIESTA HASSAN, MANSOOR A. MALIK, ABDUL HAMID WANI, MEESA JAN, MOHD YAQUB BHAT*, AASIA RASHID BHAT**
Section of Mycology, Plant Pathology and Microbiology, Department of Botany, University of Kashmir, Srinagar- 190006
***Department of Botany, Govt. P. G. College, Rajuri*

Received : 09.09.2022 Accepted : 12.10.2022 Published : 26.12.2022

Common beans collected from different sites of Kashmir valley were found infected with *Alternaria alstroemeriae* causing pod rot. The diseased pods appeared dark brown to black. The decayed tissues became soft and watery. The causal pathogen was isolated and cultured on PDA medium for cultural and microscopic examination. Its pathogenicity was confirmed following Koch's postulates. Results revealed that the pods were infected with *Alternaria alstroemeriae* resulting in rot of beans. Efficacy of ethanolic, methanolic and aqueous extracts of different plants against the isolated pathogen was evaluated. Amongst the different phytoextracts, ethanolic extract of *Salvia moorcroftiana* proved most effective in inhibiting the mycelial growth of the pathogen (showing mycelial growth inhibition of 86.66%) whereas the aqueous extract of *Inula racemosa* proved least effective (showing mycelial growth inhibition of 9.23%) at the standard concentration Ethanolic extracts of *S.moorcroftiana* proved more effective than methanolic and aqueous phytoextracts. Higher concentrations showed more efficacy than lower concentrations.

Key words: Antifungal activity, ethanolic extract, methanolic extract, microscopic characteristics

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an economically important vegetable crop grown throughout the world including India where it is locally known as Rajmash. It possesses high nutritional value due to its high content of protein, vitamins, iron, zinc, fiber, flavonoids and antioxidants (Choung *et al*. 2003; Costa *et al*. 2006). It can be grown under a wide range of climatic conditions including tropical, sub-tropical and temperate regions (Popelka *et al*. 2004). It is cultivated over an area of 2000 ha in Jammu and Kashmir with an annual production of 400 tons (Masoodi and Masoodi, 2003).

Pathogenic fungi that cause yield and quality losses of common bean throughout the world include *Fusarium oxysporum* (Schltdl.) Fr., *F. solani* (Mart) Sacc., *Macrophomina phaseolina* (Tassi) Goid and *Rhizoctonia solani* Kuhn (Schwartz *et al*. 2005; Naseri and Mousavi, 2008). In general, synthetic fungicides are used to control pathogenic fungi. However, their use is increasingly limited owing to their harmful effect on human health and environment (Harris *et al*. 2001). These restrictions have led to focus on the search for novel and eco-friendly approaches to control pathogenic fungi. In recent years, plant extracts have been evaluated for their efficacy to control the fungal pathogens and many plants have been reported with high frequency of their antifungal activity from different parts of the world (Baka, 2010; Koka *et al*. 2020).

The present study was performed to evaluate the antimycotic activity of methanolic, ethanolic and aqueous extracts of some plants against the pathogenicfungus *Alternaria alstroemeriae* E.G. Simmons and C.F. Hill, causing rot of common bean in Kashmir valley. Knowledge about the bio-management strategies will help to reduce the yield losses of common bean.

MATERIALS AND METHODS

Isolation and identification of the pathogenic fungus

Infected samples of common bean were collected from different areas of Kashmir valley and

* Correspondence: myaqub35@gmail.com

transported to Plant Pathology and Mycology Laboratory for further investigation. For isolation of fungi, the samples were first washed with tap water to remove debris and then immersed in 0.5% sodium hypochlorite (NaOCl) for 1-2 min. This was followed by washing the samples with double distilled water twice and then drying them on sterile filter paper. The pods were cut to small 2-3 mm pieces from which 3 pieces were placed in 90 mm diameter Petri plates containing 2.5% Potato Dextrose Agar (PDA) medium to which 10 mgL^{-1} rifampicin and 200 mgL^{-1} of ampicillin was added. This was followed by incubating the plates in an incubator at 25±2°C for about seven days. Subculturing of the fungal growth on each plate was done and the plates were then put in an incubator at a temperature of 25±2°C.

The isolated fungus was identified on the basis of cultural and microscopic characteristics (Fawole and Oso, 1995). A clean slide was taken and a drop of lactophenol cotton blue stain was placed over it with the help of a dropper.

Fungal mycelial portions were then taken from the Petri plates and spread very well on the slide with the help of mounted needles. This was followed by putting a cover slip over the slide which was then examined under the microscope. Microscopic characteristics of the fungi like hyphae type and reproductive structures etc. were observed under microscope and recorded (Bukar *et al*. 2009). Koch's postulates were followed to test the pathogenecity of the fungus. The culture of the pathogenic fungus was deposited in fungal collection centre of Kashmir University Herbarium (KASH) with with the accession number KASH-2622.

Preparation of phytoextracts

Different concentrations of methanolic, ethanolic and aqueous extracts of three plants viz. *Inula racemosa* Hook. f., *Salvia moorcroftiana* Wall. Ex Benth. and *Euphorbia wallichii* Hook. f. were prepared and evaluated for their efficacy in the inhibition of mycelial growth of *Alternaria alstroemeriae,* a pathogenic fungus isolated from common bean. 200 g leaves of the fresh plant material of three test plants were taken and washed with sterilized distilled water followed by grinding in mortar and pestle using 200 ml of methanol, ethanol and water in order to prepare plant extracts (Bhat and Sivaprakasan, 1994). The material was then homogenized for 5 minutes followed by filtration through muslin cloth and Whatman's filter paper No. 1. Centrifugation at 5000 rpm was carried out for 10 minutes to prepare standard concentration. Then other concentrations, S/2 and S/5 were prepared by addition of appropriate amount of methanol, ethanol and water to the standard concentration. The different concentrations were then evaluated for their antifungal activity by food poisoning technique (Adams and Wong, 1991).

The effect of different concentrations of plant extracts was evaluated for their efficacy on the mycelial growth inhibition as per the formula

$$\text{Mycelial growth Inhibition } \% = \frac{dc - dt}{dc} \times 100$$

Where dc=average diameter of fungal colony in control, and dt= average diameter of fungal colony in treatment group (Edington *et al*.1971).

Statistical analysis

All experiments were performed in triplicates. The data collected during these investigations were subjected to appropriate statistical analysis using SPSS statistical software (version 16.0). The data was statistically analysed by one way analysis of variance (ANOVA) and comparison of the means was done by Duncan multiple comparison tests at $P < 0.05$.

RESULTS AND DISCUSSION

Pathological characterization of the fungus

The fungus was identified on the basis of cultural and microscopic characteristics to be *Alternaria alstroemeriae*. Upon culturing it on PDA, the colonies were olive-green initially, but later on turned brown and finally black in color. The colonies were cottony, smooth, and the aerial mycelium was absent (Fig.1a & b).Conidia were 26.3±4 µm in length and 9.2±1.5 µm wide (Fig.1c & d). Mycelium was hyaline, septate and branched (Fig. 1e). All the pods treated with the fungus developed dark lesions initially, but later on they became watery and soft and hence developed rot (Fig. 1f) whereas the control pods did not show any symptoms of the disease (Fig. 1g).

Table1: Effect of different concentrations of the plant extracts on the mycelial growth of the fungus:

Plant extract	Type	Mycelial inhibition (mm)			
		S	S/2	S/5	Control
Euphorbia wallichii	Meth.	12±0.2^{a}(73.33)	21±0.1^{b}(53.33)	26±0.2^{c}(42.22)	45±0.5^{d}
	Eth.	7±0.1^{a}(84.44)	14±0.2^{b}(68.88)	21±0.2^{c}(53.33)	45±0.5^{d}
	Aq.	26±0.1^{a}(25.71)	30±0.2^{b}(14.28)	32±0.1^{c}(8.57)	35±0.3^{d}
Inula racemosa	Meth.	13±0.2^{a}(71.11)	23±0.2^{b}(48.88)	35±0.2^{c}(22.22)	45±0.5^{d}
	Eth.	8±0.1^{a}(82.22)	20±0.2^{b}(55.55)	30±0.1^{c}(33.33)	45±0.5^{d}
	Aq.	59±0.2^{a}(9.23)	60±0.5^{b} (7.69)	63±0.3^{c}(3.07)	65±0.3^{d}
Salvia moorcroftiana	Meth.	7±0.3^{a}(84.44)	15±0.2^{b}(66.66)	23±0.1^{c}(48.88)	45±0.5^{d}
	Eth.	6±0.1^{a}(86.66)	12±0.3^{b}(73.33)	20±0.3^{c}(55.55)	45±0.5^{d}
	Aq.	13±0.1^{a}(35.0)	14±0.1^{b}(30.0)	16±0.2^{c}(20.0)	20±0.1^{d}

Meth. = Methanolic extract; Eth. = Ethanolic extract; Aq. = Aqueous extract; values are represented as mean±SD.Figures in parenthesis indicate the inhibition in mycelial growth (%). Values followed by same alphabets are not statistically different ($p \leq 0.05$)

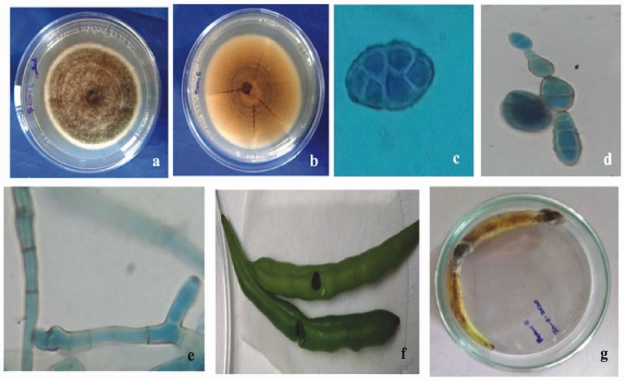

Fig.1: (a) Growth of *Alternaria alstroemeriae* in Petri plate (b) Reverse side of culture plate (c) Single conidia (d) Conidia in chain (e) Mycelium (f) Healthy pods into which mycelial disc was inoculated (g) Pod after seven days of inoculation.

Effect of various phytoextracts

All the phytoextracts showed significant inhibition on the mycelial growth of *Alternaria alstroemeriae* (Fig. 2a-i). At the standard concentration (S), among the different phytoextracts, the extract of *Salvia moorcroftiana* proved most effective (showing mycelial growth inhibition of 84%, 86.66% and 35.0% for methanolic, ethanolic and aqueous extracts respectively) followed by *Euphorbia wallichii* (showing mycelial growth inhibition of 73%, 84.44% and 25.71% for methanolic, ethanolic and aqueous) and *Inula racemosa* (showing mycelial growth inhibition of 71%, 82.22% and 9.23% for

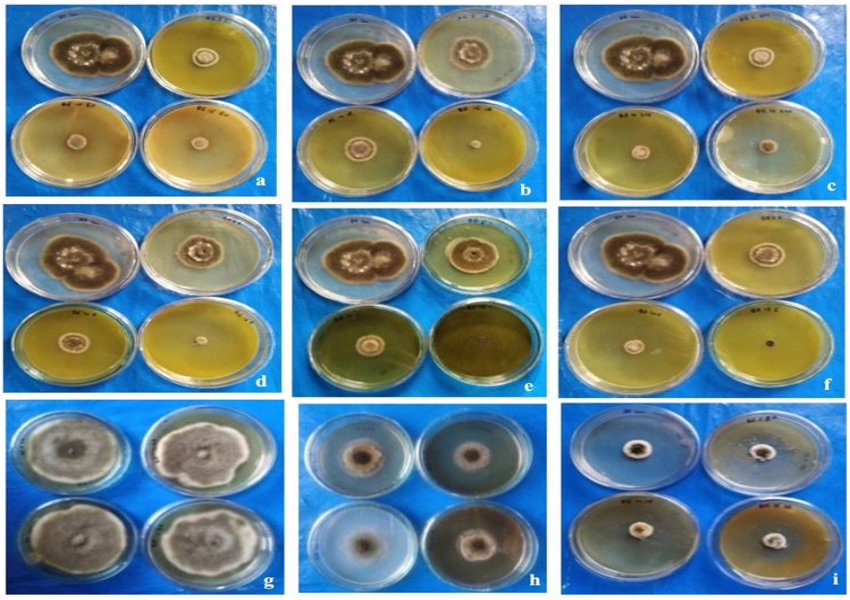

Fig. 2 : Treatment with methanolic extract of *Euphorbia wallichii* (a), *Inula racemosa* (b) and *Salvia moorcroftiana* (c); with ethanolic extract of *Euphorbia wallichii* (d) *Inula racemosa* (e) and *Salvia moorcroftiana* (f); with aqueous extract of *Euphorbia wallichii* (g), *Inula racemosa* (h) and *Salvia moorcroftiana* (i).

methanolic, ethanolic and aqueous respectively). Similar order of the inhibition was followed for the methanolic, ethanolic and aqueous extracts of the three test plantsat the concentrations S/2 and S/5 (Table1). Ethanolic extract of all the three plants proved the most effective followed by methanolic and aqueous extract respectively. Higher concentrations of plant extract proved more effective in inhibiting the mycelial growth as compared to lower concentrations.

In the present study, the rot causing fungus, *Alternaria alstroemeria* was isolated from infected samples of common bean. The causal pathogen was identified on the basis of cultural and microscopic characteristics. The efficacy of different plant extracts against the isolated fungal pathogen *Alternaria alstroemeriae* was also studied. All the phytoextracts showed significant inhibition on the mycelial growth of *Alternaria alstroemeriae*. Ethanolic extract of all the three plants proved the most effective followed by methanolic and aqueous extract respectively which is in accordance with results obtained by Ekpo and Etom (2009; Timothy *et al*. 2012). The efficacy of different plant extracts in reducing the growth of different pathogenic fungi has been reported earlier (Taskeen-Un-Nisa *et al*. 2011; Raji and Raveendran, 2013; Znini *et al*. 2013; Ounchokdee *et al*. 2016; Parveen *et al*. 2016; Zatla *et al*. 2017). Abass (2007) reported antimycotic activity of the leaf extracts of *Lawsonia inermis* at different concentrations on some plant pathogenic fungi. The antifungal activity of aqueous, ethanolic and acetone extracts of *Allium sativum*, *Allium cepa*, *Allium porrum*, *Ocimum basilicum* and *Allium sativum* against *Aspergillus niger*, *Colletotrichum gloeosporioides* and other different fungi has been reported by many workers (Irkin and Korukluoglu, 2007; Ogbebor *et al*. 2007). Wahab *et al*. (2020) and Webster *et al*. (2008) reported that *Fragaria virginiana*, *Epilobium angustifolium* and *Potentilla simplex* show a promising antimycotic potential. The antifungal activity of some medicinal plants such as *Amaranthus spinosus*, *Barbeya oleoides*, *Clutia lanceolata*, *Lavandula pubescens*, *Maerua oblongifolia* and *Withania somnifera* against some plant pathogenic fungi was reported by Baka (2010). Anti mycotic activity of extracts obtained from *Borago officinalis*, *Orobanche crenata*, *Plantago lanceolata*, *Plantago coronopus*, *Sangui sorba minor*, *Silene vulgaris*, *Sonchus asper*, *Sonchus oleraceus* and *Taraxacum officinale*, against some postharvest fungal rot causing pathogens *Monilinia laxa*, *Botrytis cinerea*, *Penicillium expansum*, *Penicillium digitatum*, *Penicillium italicum*, *Aspergillus carbonarius* and *Aspergillus niger* under *in vitro* and *in vivo* conditions were observed (Gatto *et al*. 2011). Koka *et al*. (2020) evaluated the effect of ethanolic and aqueous extracts of *Prunella vulgaris* and *Paeonia suffruticosa* against some fungi causing diseases on tomato and brinjal.

The effect of plant extracts on *Alternaria alstroemeriae* has not been reported so far.It is concluded from this study that the ethanolic, methanolic and aqueous plant extracts of *Salvia moorcroftiana*, *Euphorbia wallichiana* and *Inula racemosa* can possibly be used in the management of plant pathogenic fungi to prevent bio-deteriorations in an eco-friendly way and to develop alternative biopesticides. However, further investigation is needed in this field.

ACKNOWLEDGEMENT

The first author is highly grateful to University Grants Commission, New Delhi for providing funding in the form of Senior Research Fellow to carry out this research.

REFERENCES

Abass, M.H. 2007. Study on antifungal activity of Henna leaves (*Lawsoniainermis*) extracts on some plant pathogenic fungi. The Agriculture Informative Magazine of Dumshik Univ.**23**: 113-125.

Adams, P.B., Wong, J.A.L. 1991. The effect of chemical pesticides on the infection of sclerotia of *Sclerotinia minor* by the biocontrol agent *Sporidesmium sclerotivorum. Phytopathology* **81**: 1340-1343.

Baka, Z.A.M. 2010. Antifungal activity of six Saudi medicinal plant extracts against five phyopathogenic fungi. *Arch.Phytopathol.Pflanzenschutz*.**43**: 736-743.

Bhat, N.M., Sivaprakasan, K. 1994. Antifungal activity of some plant extracts. In:*Crop Innovation techniques and management* (Eds.Sivaprakasan, K. and I. Seetharaman) New Delhi: Kalyani publishers, pp. 335-339.

Bukar, A., Mukhtar, M.D., Adamu, S. 2009. Isolation and identification of postharvest spoilage fungi associated with sweet oranges (*Citrus sinensis*) traded in kano metropolis. *Bayero J. Pure Appl. Sci.***2**:122 – 124.

Choung, M.G., Choi, B.R., An, Y.N., Chu, Y.H., Cho, Y.S. 2003. Anthocyanin profile of Korean cultivated kidney bean (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.***51**: 7040–7043.

Costa, G.E.A., Queiroz-Monici, K.S., Reis, S.M.P.M., Oliveira, A.C. 2006.Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chem.* **94**:327–330

Edington, L.V.; Khew, K. L., Barron, G. L. 1971. Fungitoxic spectrum of benzimidazole compounds. *Phytopathology* **61**: 42-44.

Ekpo, M.A., Etim P.C. 2009. Antimicrobial activity of ethanolic and aqueous extracts of *Sidaacuta* on microorganisms from skin infections. *J. Med. Plant Res*.**3**: 621-624.

Fawole, M.O., Oso, B.A. 1995. *Laboratory Manual of Microbiology.*1st ed. Nigeria: Spectrum Books Ltd, Ibadan, pp. 34-35.

Gatoo, M.A., Ippolito, A., Linsalata, V.,Cascarano, N.A., Nigro, F.,Vanadia, S., Venere, D.D. 2011. Activity of extracts from wild edible herbs against postharvest fungal diseases of fruits and vegetables. *Postharvest Biol.Technol*.**61**: 72-82.

Harris, C.A., Renfrew, M.J., Woolridge, M.W. 2001.Assessing the risk of pesticide residues to consumers: recent and future developments. *Food Addit.Contam*. **18**:1124-1129.

Irkin, R., Korukluoglu, M. 2007. Control of *Aspergillus niger* with garlic, onion and leak extracts. *Afr. J.Biotechnol*.**6**: 384-387.

Koka, J.A. Wani, A.H., Bhat, M.Y. 2020.Evaluation of different plant extracts for effective management of fungal rot of tomato and brinjal in Kashmir Valley. *Braz. J.Biol. Sci*.**7**: 229-238.

Masooodi, M.A., Massoodi, S.D. 2003.*Agriculture in J&K, A Perspective in Production of Vegetables*.Mohisarw Book Series, Srinagar, Kashmir, India. Pp. 111-126.

Naseri, B., Mousavi, S.S. 2008. Root rot pathogens in field soil, roots and seeds in relation to common bean (*Phaseolus vulgaris*), disease and seed production. *Int.J.Pest Manag*. **61**: 60-67.

Ogbebor, N.O., Adekunle, A.T., Enobakhare, D.A. 2007. Inhibition of *Colletotrichum gloeosporioides* (Penz.)Sacc. causal organism of rubber (*Heveabrasiliensis* Muell.Arg.) leaf spot using plant extracts. *Afr. J. Biotechnol*. **6**: 213-218.

Ounchokdee, U.,Rueangrit, S., Dethoup, T. 2016. Antifungal activity profile of *Piper longum* fruit extract against plant pathogenic fungi. *J.Biopestic*. **9**: 97-103.

Parveen, S., Wani, A.H., Bhat, M.Y., Koka, J.A., Wani, F.A. 2016. Management of postharvest fungal rot of peach (*Prunus persica*) caused by *Rhizopuss tolonifer* in Kashmir Valley, India.*Plant Pathol. Quaran*. **6**: 19–29.

Popelka, J., Carlos, N.T.,Higgins, T.J.V. 2004. Gene technology for grain legumes: can it contribute to the food challenge in developing countries. *Plant Sci*. **167**: 195-206.

Raji, R., Raveendran, K. 2013. Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger*. *Asian J.Plant Sci.Res*. **3**: 13–15.

Schwartz, H.F., Steadman, J.R., Hall, R., Forster, R.L. 2005.*Compendium of bean diseases*. Minnesota, USA: The American Phytopathological Society.

Taskeen-un-Nisa, Wani, A.H., Bhat, M.Y., Pala, S.A., Mir, R.A. 2011.*In vitro* inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*. *J. Biopestic*.**4**: 53-56.

Timothy, S.Y.,Wazis, C.H.,Adatti, R.G., Maspalma, I.D. 2012. Antifungal activity of aqueous and ethanolic leaf extracts of *Cassia alata* Linn. *J.Appl. Pharm. Sci*. **2**: 182-186.

Wahab, H. A., Malek, A., Ghobara, M. 2020 Efects of Some Plant Extracts, Bioagents, and Organic Compounds on Botrytis and Sclerotinia Molds *Acta Agrobotanica*. **73 :** 1-11, DOI: https://doi.org/10.5586/aa.7321

Webster, D.,Taschereau, P.,Belland, R.J., Sand, C., Rennie, R.P. 2008.Antifungal activity of medicinal plant extracts; preliminary screening studies. *J. Ethnopharmacol*.**115**: 140-146.

Zatla, A.T., Dib, M.E.A.,Djabou, N.,Ilias, F., Costa, J., Muselli, A. 2017. Antifungal activities of essential oils and hydrosol extracts of *Daucuscarota* sub sp. *Sativus* for the control of fungal pathogens, in particular gray rot of strawberry during storage. *J. Essen. Oil Res*. **29**:391-399.

Znini, M.,Cristofari, G., Majidi, L., El- Harrak, A.,Paolini, J., Costa, J. 2013.*In vitro* antifungal activity and chemical composition of *Warionia saharae* essential oil against three apple phytopathogenic fungi. *Food Sci.Biotechnol*. **22**: 113–119.