INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 4, p. 145-150, 2018

RESEARCH PAPER OPEN ACCESS

In vitro antifungal activity of selected indigenous plant extracts against *Colletotrichum capsici*

Abdul Sattar[1*], Abid Riaz[1], Inam-ul-Haq[1], Muhammad Kausar Nawaz Shah[2]

[1]*Department of Plant Pathology, PMAS-Arid Agriculture University Rawalpindi, Pakistan*

[2]*Department of Plant Breeding and Genetics, PMAS-Arid Agriculture University Rawalpindi, Pakistan*

Key words: *Colletotrichum capsici*, Polygonum, Neem, Poisoned food technique, Growth Inhibition.

http://dx.doi.org/10.12692/ijb/12.4.145-150 Article published on April 08, 2018

Abstract

Anthracnose of chilli caused by *Colletotrichum capsici* is a wide spread problem limiting the profitable cultivation of chilli throughout the major chilli growing regions of the Punjab, Pakistan. Presently chilli anthracnose is managed by using number of fungicides like Captan and Thiram which results in environmental damage and have human hazardous effects. An experiment on antifungal efficacy of five plant extracts *viz.*, Polygonum (*Polygonum amplexicaule*), Neem (*Azadirachta indica*), Dodonaea (*Dodonaea viscosa*), Clove (*Syzygium aromaticum*) and Eucalyptus (*Eucalyptus globulus*) was done through poisoned food technique by setting three concentrations at0.05%, 0.1% and 0.2% in *in vitro* conditions against *C. capsici*. The results revealed significant antifungal activities of tested plant extracts at all applied concentrations. Polygonum gave 98.5% growth inhibition of my celial growth of tested pathogen at 0.2% concentration and found highly effective from all tested plant extracts as compared to control. Neem ranked second by showing 88.75% growth inhibition after Polygonum at 0.2% concentration. Eucalyptus and Dodonaea also showed significant growth inhibition percentage in ranges of 78.25-44% and 8.5-22.5% respectively. Clove was found least effective among the plant extracts and showed growth inhibition in ranges of 7-18% at all concentrations. Present study suggests that Polygonum and Neem could be tried for the eco-friendly management of the disease.

* **Corresponding Author:** Abdul Sattar ✉ abdulsattarkoli@yahoo.com

Introduction

Chilli (*Capsicum annuum*) is one of the major cultivated spice crops in versatile climates of the world. It belongs to Solanaceae family and is considered valuable cash crop around the world and also in Pakistan (Hussain and Abid, 2011). In Pakistan during 2016, area under pepper chilli cultivation was 75000 ha with a total production of 191 thousand tons (MINFAL, 2016). Several biotic and abiotic factors affect the productivity of the chilli pepper crop worldwide. Among the biotic factors, numerous fungal, bacterial, nematodes and viruses result into devastating diseases which deteriorate the quality and quantity of the produce and are often difficult to control due to number of limitations (Nono-womdim, 2001).

Chilli anthracnose the most devastating fungal disease in chilli caused by *Colletotrichum capsici* is a major problem in chilli pepper production which infects the ripened fruits and result into fruit rot, occur frequently around the world in chilli growing areas (Poulos, 1992). Attack of *Colletotrichum capsici* results up to 84% fruit yield loss (Pakdeevaraporn *et al.,* 2005).Presently Chilli anthracnose is managed by using number of chemicals like Captan and Thiram fungicide which results in environmental damage and have human hazardous effects (Harris *et al.,* 2001) but it is now realized that chemical fungicides cause serious environmental problems and are toxic to non-target organisms (Anon, 2005).

Pakistan is a country with versatile climates ranged from coastal areas to the mountains of Himalayans and Karakoram and full of floral diversity. Plant extracts obtained from plants are one of several non-chemical control alternatives that are inspiring great interest due to their availability, non-toxicity and environment friendly nature. Many plant extracts have antifungal potential due to presence of number of chemical compounds like terpenoids, saponins, alkaloids, flavonoids (Arif, 2009). This study was designed to evaluate the antifungal effect of five indigenous plants though their extracts in *in vitro* conditions by poisoned food techniqueat different Concentrations.

Materials and methods

Plant materials

The nomenclature of the plant materials used is shown in the Table 1. Polygonum roots were collected from the hilly areas of the Punjab and Khyber Pakhtunkhwa. Dodonaea plant leaves was collected from the Margalla hills of Islamabad region while Neem and Eucalyptus leaves were harvested from the plants located in plain areas of Punjab (Jhelum and Gujarat) while clove buds were obtained from local vegetable market (Rawalpindi). The raw material (leaves) of Dodonaea and Neem, roots of Polygonum while buds of clove were dried under shade and crushed in porcelain mortar to turn into powder form.

Plant extraction

Sixty grams of respective ground samples were put into flat bottom flasks to which 180 mL of methanol (Riedel-de Haen, Germany) or di ethyl ether was added. After adding the solvent flasks were placed at magnetic stirrer for four hours at temperature of 25°C. The supernatant was separated with the help of filter paper (Whatman 5B). After filtration solvents were evaporated by keeping them under fan for about ten hours. Samples were defused with 10mL of methanol. The extracts were sterilized by passing them through a Milipore filter paper (45 pm) before adding into media at different concentrations.

Fungal cultures

Colletotrichum capsici cultures were isolated from major chilli growing areas of the Punjab in the crop season 2015-2016 and 7-10 days old cultures were used in the study to obtain mycelia disc (5mm) for placing on PDA media.

Treatment adjustments

After preparing pure plant extracts their concentrations were adjusted at 0.05%, 0.1% and 0.2 % by mixing it at mentioned percentage in 100 ml PDA media prepared and allowed to cool up to warmth temperature before pouring into Petri plates. Extracts were passed through membrane filter to

avoid any microbial contamination. Four replications of each treatment along with control were taken.

Poisoned food technique

Plant extracts were subjected to antifungal activity assay at above mentioned treatments through poisoned food technique on the procedure given by Mohana *et al.,* (2010) with some minor modification. Briefly, 5 mm disc of 7-10 days old culture of tested pathogen was placed on a PDA media already adjusted above mentioned treatments and replications along with control and allowed to incubate at 25°C for 12 days. Average growth rate of the pathogen was also calculated by measuring the fungal diameter at interval of 48 hours for twelve days. The antifungal activity of the extracts was calculated in terms of fungal growth percentage inhibition (%)by using the formula,Growth percentage inhibition (%) = ((Dc-Dt)/Dc)×100

Where:

Dc: Diameter of fungal growth in control Dt: Diameter of fungal growth treatment

Statistical analysis

SPSS v 16.0 Statistical software was used to analyze the data; all experiments were conducted in a completely randomized design with four replications and means were separated by least significant difference (LSD) test at $P<0.05$.

Results and discussion

All tested plant extracts were applied at three i.e. 0.05%, 0.1%, 0.2% concentrations and produce significant results at each level. Maximum antifungal activity (98.5%, Fig.3)was shown by Polygonum at 0.2% concentration while lowest (07%, Fig. 1) antifungal activity was recorded in case of Clove at 0.05%. Other plant extracts expressed results in between these max and min ranges of growth inhibition percentage. Antifungal activity shown by Polygonum ranged between 98.5-89% (Fig. 1,2 and 3) on PDA media.

Table 1. Nomenclature and other characteristics of plants used in biological control of *Colletotrichum capsici.*

Sr No.	Common name	Botanical name	Place of collection	Part used
1	Polygonum	*Polygonum amplexicaule*	From Hilly areas of Punjab and Khyber Pakhtunkhwa	Roots
2	Sanatha	*Dodonaea viscosa*	Margalla hills	Leaves
3	Clove	*Syzygium aromaticum*	Peer-Wadhai Vegetable market	Buds
4	Neem	*Azadirachta indica*	From the plane areas of Punjab districts	Leaves
5	Eucalyptus	*Eucalyptus globulus*	From the plane areas of Punjab districts	Leaves

After Polygonum, Neem plant extract gave the maximum control by showing antifungal activity ranged from 89-55%while by Eucalyptus the antifungal activity was shown in range of 78%-44%. Dodonaea produced growth inhibition percentage in ranges from 8.5-22.5%while Clove inhibit the pathogen's growth least in ranges of 7.5-20.5%as compared to control group of the experiment.

Analysis of data showed that all concentration of the Polygonum plant extract found highly significant as compared to other plant extracts except Neem and Eucalyptus at 0.2% (Fig.3). While performance of other plant extracts remain significantly different from each other at different levels.

A perusal of the literature discovered that many scientists have reported the antimicrobial activity of plant extracts against different fungal species (Tzortzakis, 2007) and antifungal activity of the *P. amplexicaule, D. viscosa, S. aromaticum, A. indica* and *Eucalyptus spp.* Against number of pre and post harvest fungal pathogens (Thippeswamy *et al.,* 2013, Sattar *et al.,*2014, Begum and Nath, 2015). However, no reports are available on inhibitory activity of these plants against *C. capsici* recovered from major chilli growing areas of the Punjab in Pakistan and the present investigation reports the antifungal potential of the indigenous plant extracts against the targeted pathogen to sort eco-friendly management strategy in future.

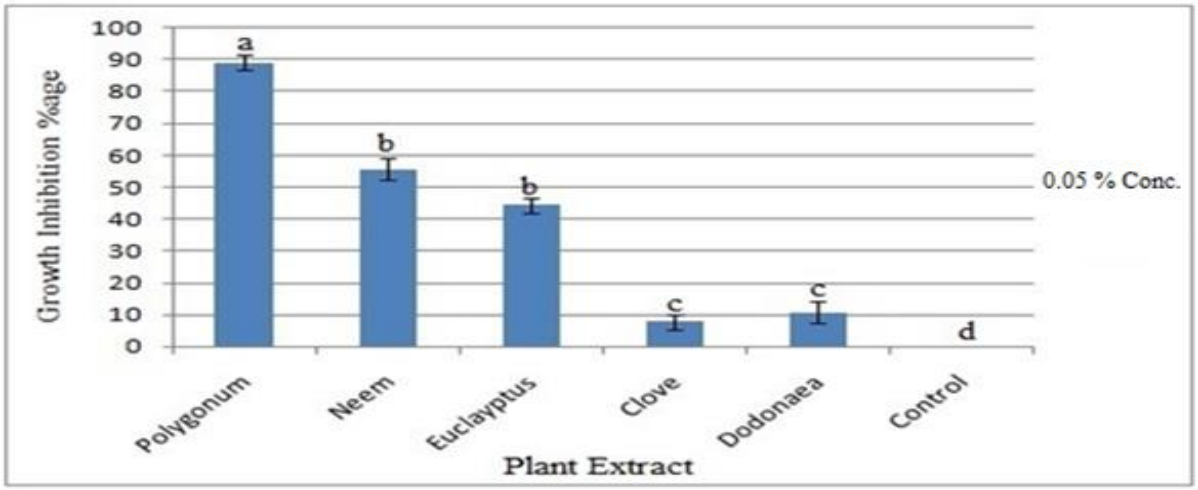

Fig. 1. Effect of different plant extracts on *in vitro* my celial growth of *Colletotrichum capsici* at 0.05% concentration through poisoned food technique. Values presented are means of growth inhibition percentage of plant extracts and error bars represent the standard deviation of the means of four independent experimental replications. Means followed by the same letter are not significantly different at 5% level by least significant difference test.

Present findings are in accordance with the findings of Sattar *et al.,*(2014) who reported that plant extracts of Polygonum roots inhibit the complete growth of *Penicillium expansum* when applied at different levels of applications. Similarly Tiwari *et al.,* in 2008 conducted a detailed study on four most aggressive isolates of the *C. capsici* collected from different region of West Bengal and found that Neem plant extract showed significant antifungal activity against the targeted pathogen in *in vitro* conditions. Bander *et al.,* in 2011 reported the antifungal effect of eucalyptus and clove against five aggressive species of *Trichophyton* ranging from 20-55% fungal growth retardation in *in vitro* conditions. Prakash *et al.,* (2012) reported the antifungal potential ranging from 2-18% against five pathogenic fungal species i.e. *Curvularia lunata, Fusarium oxysporum, Aspergillus flavus, Aspergillus niger* and *Pencillium citrinum*. Results of plant extracts in this study are supported and justifiable by the findings many of above mentioned scientists who reported the antifungal potential of the subjected tested plant extracts.

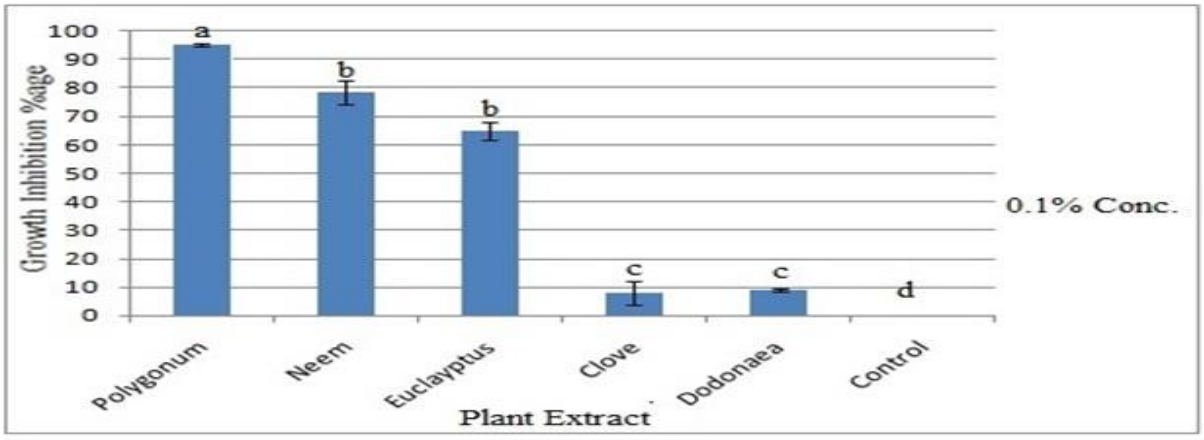

Fig. 2. Effect of different plant extracts on *in vitro* mycelial growth of *Colletotrichum capsici* at 0.1% concentration through poisoned food technique. Values presented are means of growth inhibition percentage of plant extracts and error bars represent the standard deviation of the means of four independent experimental replications. Means followed by the same letter are not significantly different at 5% level by least significant difference test.

The differences in performance of the plant extracts by showing variation in growth inhibition percentage of *C. capsici* is understandable by the reports of Kurucheve *et al.,* (1997)that the variation in antifungal potential of plant extracts may be due to qualitative and quantitative differences in antifungal principles. The high growth inhibition of the pathogen was may be observed due to the presence of high quantity of active antifungal compounds like tannins, flavonoids, glycosides and alkaloids (Harborne, 1984).Statistical analysis of the results revealed significance of the data at 5% level of significance.

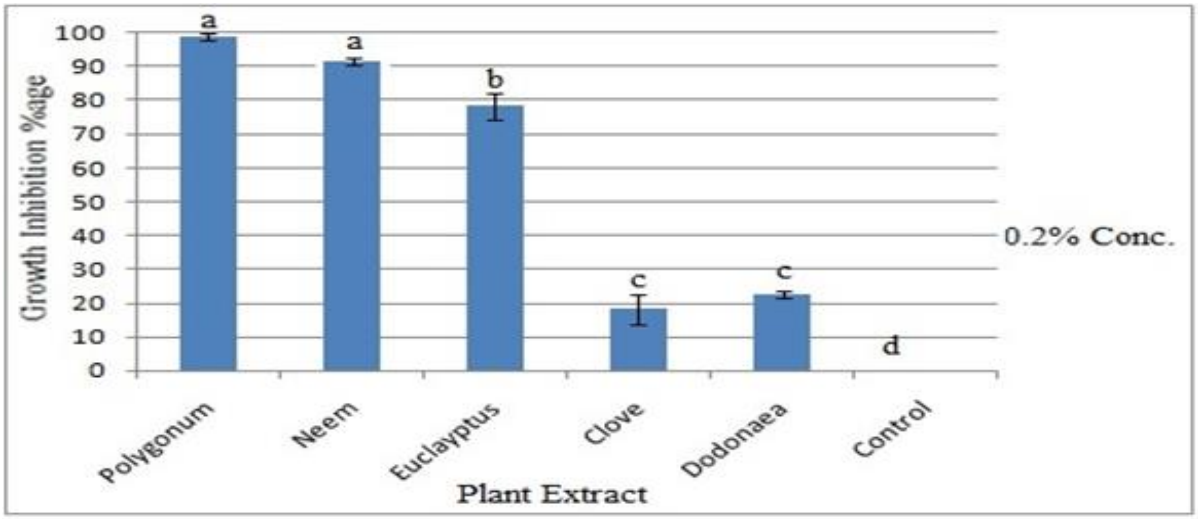

Fig.3. Effect of different plant extracts on *in vitro* mycelial growth of *Colletotrichum capsici* at 0.2% concentration through poisoned food technique. Values presented are means of growth inhibition percentage of plant extracts and error bars represent the standard deviation of the means of four independent experimental replications. Means followed by the same letter are not significantly different at 5% level by least significant difference test.

References

Anon. 2005. Pest control background. International Journal of Pest Control **45(2),**232-233.

Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, Lavekar GS, Dabur R. 2009. Natural products – antifungal agents derived from plants. Journal of Asian Natural Products Research. **11**, 7, July 2009, 621–638.

Bander KI. 2011. Anti-Fungal activity of some plant extracts against Trichophyton species. Tikrit Medical Journal 2011; **17(1),** 81-87.

Begum S, Nath PS. 2015.Eco-friendly management of anthracnose of chilli caused by Colletotrichum capsici. Journal of Applied and Natural Science **7(1),** 119–123.

Harborne JB. 1992. Phytochemical Methods: A guide to modern techniques of plant analysis. 2nd edition Chapman and Hall, New York, 288p.

Harris CA, Renfrew MJ, Woolridge MW. 2001. Assessing the risk of pesticide residues to consumers: recent and future developments. Food Additives and Contaminants **18,** 1124-1129.

Hussain F, Abid M.2011. Pest and diseases of chilli crop in Pakistan: A review. International journal of biotechnology **8,** 325-332.

Kurucheve V, Gerard EJ, Jayaraj J. 1997. Screening of higher plants for fungitoxicity against Rhizocotinia solani in vitro. Indian journal of Phytopathology**50(2),** 2350241.

Lavekar Dabur R.2009. Natural products – antifungal agents derived from plants Journal of Asian Natural Products Research **11,**7, July 2009, 621–638.

MINFAL. (Ministry of National Food Security & Research). http://www.mnfsr.gov.pk/

Mohana DC, Raveesha KA. 2010.Antimycotic, antibiodeteriorative and antiaflatoxigenic potency of 2-hydroxy-4-methoxybenzaldehyde isolated from Decalepis hamiltonii on fungi causing biodeterioration of maize and sorghum grains. Journal of Mycology and Plant Pathology **40(2),** 197-206.

Nono-Womdim R. 2001.An overview of major virus diseases of vegetable crops in Africa and some aspects of their control. In: Proceedings of Plant Virology in sub Saharan Africa, 4-8 June 2001 IITA, Nigeria, 213-230p.

Pakdeevaraporn P, Wasee S, Taylor PWJ, Mongkolporn O.2005.Inheritance of resistance to anthracnose caused by Colletotrichum capsici in Capsicum. Plant Breeding, **124(2),** 206-208.

Poulos JM. 1992. Problems and Progress of Chilli Pepper Production in the Tropics.

Prakash NKU, Selvi CR, Sasikala V, Dhanalkshmi S, Prakash SBU. 2012. Phytochemistry and Bio-Efficacy of a Weed, Dodonaea Viscosa. International Journal of Pharmacy and Pharmaceutical Sciences. **4,** Issue 2, 2012.

Sattar A, Riaz A, Ahmed S, Hassan I. 2014.Efficacy of Selected Plant Extracts for Inhibition of Penicillium expansum Growth on Apple Fruits. Pakistan Journal of Phytopathology **26(01),**2014.63-66.

Thippeswamy S, Mohana DC, Abhishek RU, Manjunath K. 2013. Effect of plant extracts on inhibition of Fusarium verticillioides growth and its toxin fumonisin B1 production. Journal of Agricultural Technology 2013 **9(4),** 889-900.

Tiwari PK, Anil K, Awadhiya GK, Thrimurty VS. 2008. Efficacy of bioagents, neem based plant products and plant extracts against Colletotrichum capsici. In-dian Journal of Plant Pathology **36(1)** 94-97.

Tzortzakis NG. 2007. Maintaining post harvest quality of fresh produce with volatile compounds. Innovative Food Science Emerging Technology, **8**:111-116.

Viuda-martos M, Ruiz-navajas Y, Fernaadez-lopez J, Perez-alvarez JA. 2007. Antifungal activities of thyme, clove and organo essential oils. Journal of Food Safety **27,** 91-101.