Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

EFFICACY OF SELECTED PLANT EXTRACTS FOR INHIBITION OF *PENICILLIUM EXPANSUM* GROWTH ON APPLE FRUITS

[a]Abdul Sattar*, [a]Abid Riaz, [b]Sheeraz Ahmed, [c]Imran Hassan
[a] *Department of Plant Pathology, PMAS-Arid Agriculture University Rawalpindi, Pakistan.*
[b] *Department of Biochemistry, PMAS-Arid Agriculture University Rawalpindi, Pakistan.*
[c] *Department of Horiculture, PMAS-Arid Agriculture University Rawalpindi, Pakistan.*

A B S T R A C T

Penicillium expansum is an important postharvest pathogen that not only causes decay on apple and pear fruit but also produces the carcinogenic mycotoxin patulin in spoiled and processed fruit. Although synthetic fungicides are effective to protect fruit decay, their potential effects on human health and the environment are a concern. Plant extracts are one of several non-chemical control alternatives that are of inspiringly great interest due to their availability, non-toxicity and environment friendly nature. In this study, evaluation of antifungal activity against *P. expansum* from four plants (Garlic, Clove, Dodonaea (Sanatha) and Polygonium) by means of solvent extraction with methanol was done. Conidial suspension (100 µL) was applied to each injury site on apple fruits. Lesion diameter of the treated fruits was observed daily for 8 days. The antifungal activity of *Polygonium* was found highly effective than other three .Growth inhibition activity of *P. expansum* was dependent. In these results, methanol was found to be an appropriate solvent for use in extracting active compounds from plants presenting antifungal activity against *P. expansum.* Methanol extracts of *Polygonium at* 50 µL/wound was the most effective for inhibiting mycelial growth of the fungus.

Keywords: *Postharvest pathogen, antifungal activity, solvent extraction*.

INTRODUCTION

Apple is the most important fruit species in the world and is produced in many countries, almost in all continents (Mehmood *et al.,* 2008). The worldwide production of apple was amounted to 75,635,283 t in 2011 (FAO, 2013), The total area, reported under apple cultivation in Pakistan is about 111600 hectares with a production of 3666300 tons (Anonymous, 2009). The biggest planetary producers are China, USA, Iran and Turkey. Italy is European leader in apple production (Lana and Marko, 2009). *P. expansum* (blue mould) causes fruit rot, a serious postharvest disease of apples and is the main producer of the mycotoxin, patulin. Synthetic fungicides are effective to protect against fruit decay. However, their adverse effects on human health and the environment are the main concern. Since fruit protection by synthetic fungicides is less acceptable by consumers, the demand for alternative means is pressing (Ryu and Holt, 1993). Plant extracts are one of several non-chemical control alternatives that are inspiring great interest due to their availability, non-toxicity and environment friendly nature (Aqil *et al.,* 2010). Many plant extracts have potential as natural antimicrobial agents that can be applied to agricultural produces, foods and pharmaceutics (Horburg, 1998; Maoz and Neeman, 1998) because they contain a phytochemical that exhibits antimicrobial and cytotoxic effects on microorganisms (Feldberg *et al.,* 1988). Examples include allyl isothiocyanate from mustard which is able to inhibit the growth of *P. expansum* (Mari *et al.,* 2002), phenolic compounds in thyme oil which exhibited antimicrobial effects on food-borne bacteria (Cosentino *et al.,* 1999). These extracts are of Generally Recognized As Safe (GRAS) status. This study was aimed to examine the antifungal properties of four plant extracts against *P. expansum* Link. The comparative study of their activities at different doses and days was done.

* Corresponding Author:
Email: abdulsattar_sunny@yahoo.com

MATERIALS AND METHODS

Plant Materials: The nomenclature of the plant materials used is shown in the Table 1. All of these plants were collected from adjacent areas of the Margalla hills and from vegetable market located at Peer Wadhai (Rawalpindi). The raw material of *Dodonaea viscosa* and polygonium were dried under shade and then crushed in porcelain mortar while other samples were dried in a ventilated chamber and ground in blender (Monilex).

Plant Extraction: Twenty grams of respective ground samples were put into flat bottom flasks to which 60 mL of methanol (Riedel-de Haen, Germany) or di ethyl ether were added. After adding the solvent flasks were placed at magnetic stirrer for four hours at temperature of 25 ^{0}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).

Cultures: A previously isolated culture of *P. expansum* was maintained at 25 °C for 7-14 days on PDA medium. Conidial suspension from this fungal culture was prepared by washing the conidial mass from pure colony in to 10mL of sterilized distilled water. Then 1mL from the suspension was poured into the test tube which contains 9mL of sterilized distilled water. The concentration of the suspension was adjusted at 10^6 spores per mL by using haemacytometer (Neubauer, Germany).

Preparation of Fruits for inoculation: Apple fruits used in the research were taken from the fruit market by taking care of the following parameters:

- No fruit had any physical injury
- All fruits of same variety were used
- All selected fruits were free of symptoms of post-harvest diseases
- All selected fruits were of equal size

After selection all sampled fruits were soaked in 10% of sodium hypochlorite and washed twice with distilled water then kept dry under ambient condition. Each fruit was wounded with a sterilized cork borer of 3 mm diameter and 6 mm depth, for a total of 3 wounds per fruit. Prepared conidial suspension (100 µL) was added with the help of micro pipette to each wound. An hour after those three replications was subjected to each dose of treatment. 10, 25 and 50 µL plant extract was added to each group of three replications. The treated fruits were kept under the ambient condition at a temperature of 25°C and lesion diameter was observed daily for 8 days.

Table 1. Particulars of the Plants Used in Biological Control of *P. expansum.*

Sr.	Plant Name	Botanical Name	Place of Collection(Source)	Parts Used
1	Polygonium	*Polygonium amplexicaule*	Department of Biochemistry PMAS-AAUR	Leaves, petals
2	Dodonaea (Sanatha)	*Dodonaea viscosa*	Margalla hills	Leaves
3	Clove	*Syzygium aromaticum*	Peer-wadhai Vegetable market	Buds
4	Garlic	*Allium sativum*	Peer-wadhai Vegetable market	Underground stem

RESULTS AND DISCUSSION

Antimicrobial activity of plant extracts has been studied against different type of fungi for years, but in a more intensified way in the last few decades. Many plant extracts have potential as natural antimicrobial agents that can be applied to agricultural produces, foods and pharmaceutics (Horburg, 1998; Maoz and Neeman, 1998) In this study antifungal activity of four different plant extracts, extracted with methanol as extraction solvent had been checked against growth of the *P. expansum* on apple fruits in laboratory conditions at constant temperature of 25°C. From the selected 4 different plant extracts each extract was applied at three different doses (10, 25 and 50 µL) and showed different antifungal properties. All extracts were applied through solution contact method on apple fruits previously inoculated with conidial suspension of the above mentioned test fungus. All plant extracts showed different behavior against the growth inhibition of *P. expansum* at different doses in contrast to control group, effect of each extract was analyzed separately.

All apples were kept under ambient condition in incubator and lesion diameter was daily measured. *P. amplexicaule* showed most effective results in comparison with other three plant extracts and did not allow the pathogen to grow for five days of inoculation (Fig.1) and at second *Dodonaea viscosa* showed good antifungal response as compared to

other two remaining extracts, garlic was the least effective plant extract against the growth of fungus. *Polygonium amplexicaule* which was applied at three doses shows more effective results at 50µl as compared to other two doses of 10 and 25µl (Fig.1).

Dodonaea viscosa was the second most effective plant extract examined in this study which show growth inhibition of pathogen at different doses and most effective results were obtained at dose of 50µL than other two doses of 10 and 25 µL (Fig.2) while garlic extract show least growth inhibition of the pathogen (Fig.3). It can be concluded that *Polygonium amplexicaule* and *Dodonaea viscosa* can be used as bio fungicides for the control of blue mold of apple on apple fruits and will be safe to use. Both these plants are found in the adjacent area of Rawalpindi and Islamabad and are easily available. The impact of plant extracts on other fungi will be studied to investigate if they may be more useful for the control of other fungal diseases.

However, the extract should be added to an appropriate eluted solvent that is non-toxic to the host fruit and capable of maintaining stability of the active compounds during storage and should be used in appropriate concentration and doses where required. The antimicrobial activity of the essential oils present in the plant extracts depends on the state (liquid or gas) at which they are stable and solution application method is the most effective method (Zafar et al,. 2004).

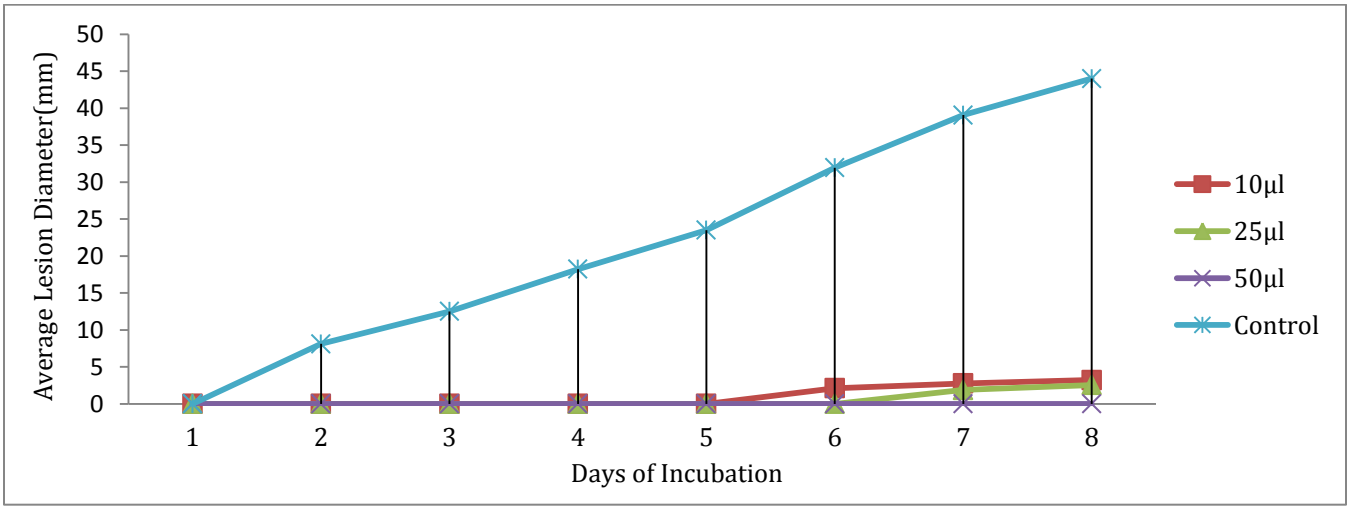

Figure 1. Effect of Polygonium on the growth of *P. expansum* at different doses for eight days of inoculation.

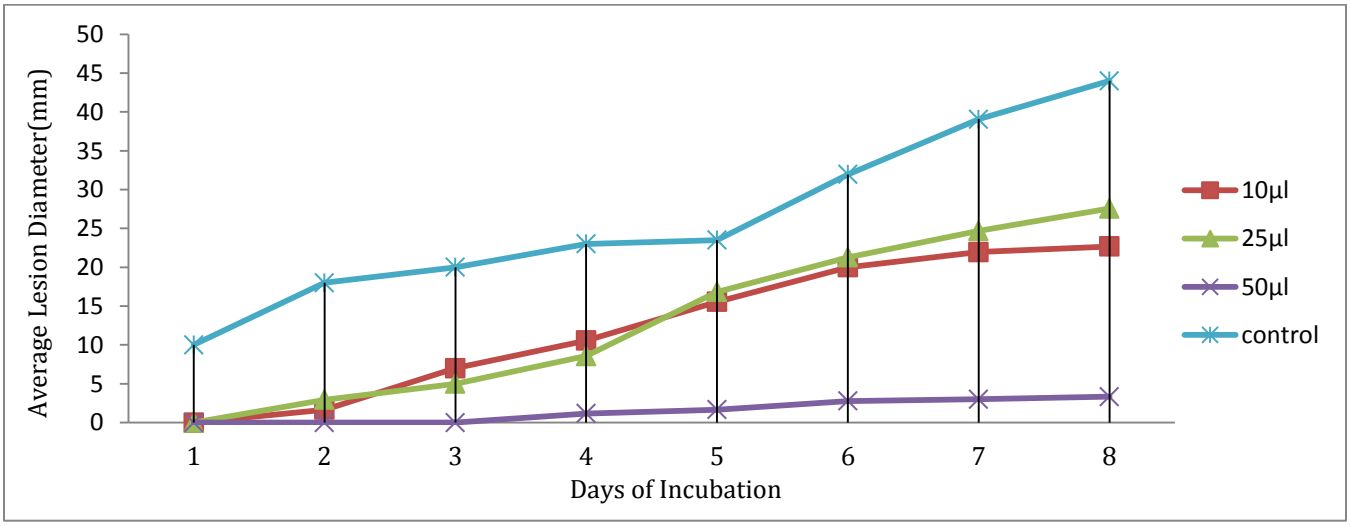

Figure 2. Effect of *D. viscosa* on the growth of *P. expansum* at different doses for eight days of inoculation.

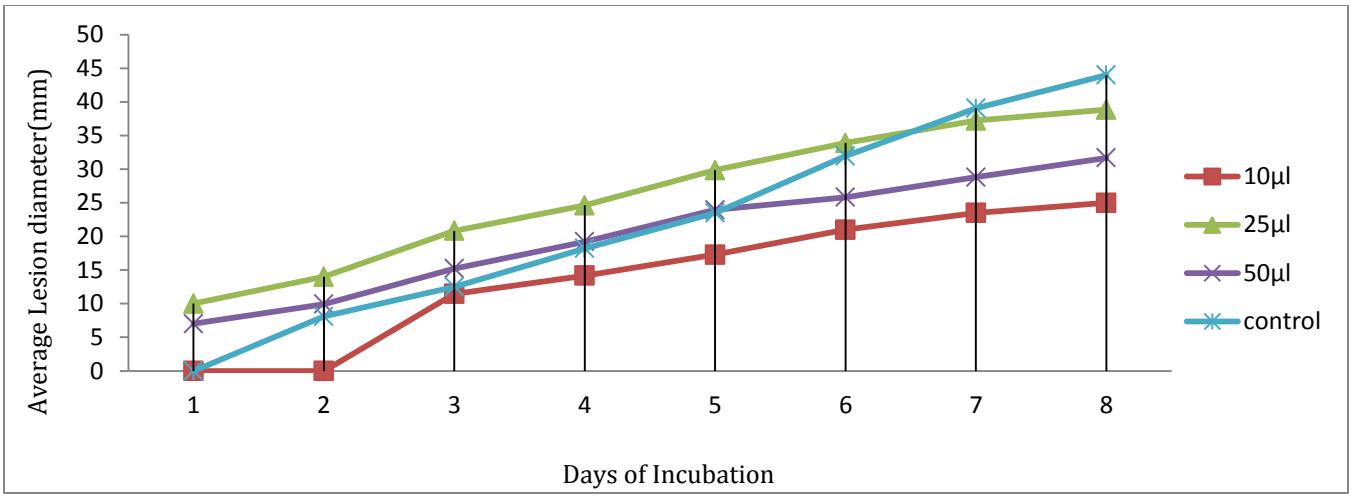

Figure 3. Effect of Garlic on the growth of *P .expansum* at different doses for eight days of inoculation.

REFERENCES

Anonymous 2009. Agriculture statistics of Pakistan .MINFAL, Govt. of Pak.

Aqil, F., M. Zahin, I. Alunad, M. Owais and M. Sajjad. 2010. Antifungal Activity of Medical Plant Extracts and Phytocompounds: A Review. *In*: Combating Fungal Infections: Problem and Remedy, Alunad, I., M. Owais and M. Shahid (Eds.). Springer-Verlag, Berlin Heidelberg. 449-485.

Cosentino, S., C.I. Tuberoso, B. Pisan, M. Satta, V. Mascia, E. Arzedi and F. Palmas, 1999. *In vitro* antimicrobial activity and chemical composition of sardinian Thymus essential oils. Lett. Applied Microbiol. 29: 130-135.

FAO. 2013. (http://faostat.fao.org) as of 1-March-2013

Feldberg, K.S., S.C. Chay, A.N. Kotik, M. Nadler, Z. Neuwirth, D.C. Sunderstrom and N.H. Thompson, 1988. *In vitro* mechanism of inhibition of bacterial cell growth by Allicin. Antimicrob. Agents Chemother., 32: 1763-1768.

Horburg, H. 1998. Influence of volatile extracts on storage pathogens of carrots *in vitro.* Vaxtskyddsnotise, 64: 87-89.

Lana, I. and J. Marko. 2009. Analysis and planning of apple production as factor of rural development support. Econ. Anal. 42: 78-85.

Maoz, M. and I. Neeman. 1998. Antimicrobial effect of aqueous plant extracts on the fungi *Microsporum canis* and *Trichophyton rubrum* and on three bacterial species. Lett. Appl. Microbiol. 26: 61-63.

Mari, M., O. Leoni, R. Lori and T. Cembali, 2002. Antifungal Vapor-phase Activity of Allyl-isothiocyanate against *Penicillium expansum* on Pears. Plant Pathology 51: 231-236.

Mehmood, Z., Z. Alm, A. Mohammad, B. Nizakat, B. Amal and I. Ihsanullah, 2008. Effect of pasteurization and chemical preservatives on the quality and shelf stability of apple juice. Am. J. Food Technol., 3: 147-153.

Ryu, D. and D. Holt, 1993. Growth inhibition of *Penicillium expansum* by several commonly used food ingredients. J. Food Prot. 56: 862-867.

Zafar, I., S. Mussarat, H. Farrakh, B. Sheraz, I. Mohammad, Z. Shahida and A. Bashir, 2002. Antifungal properties of some indigenous plants from Peshawar valley. Asian J. Plant Sci. 1: 708-709.