International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2021; 9 (1):103-106
ISSN:2320-7817(p) | 2320-964X(o)

Research Article Open Access

In Vitro anti-fungal activity of *Tinospora cordifolia* and *Adhatoda vasica* against post-harvest fungal pathogens in Apple fruits

Baviskar RN

Department of Botany, ICLES' M. J. College, Vashi, Navi Mumbai. (M.S.) India.
Email: - baviskar_ramesh@yahoo.co.in

Manuscript details:

Received: 16.02.2020
Accepted: 27.03.2021
Published: 31.03.2021

Cite this article as:
Baviskar RN (2021) *In Vitro* anti-fungal activity of *Tinospora cordifolia* and *Adhatoda vasica* against post-harvest fungal pathogens in Apple fruits *Int. J. of Life Sciences*, 9 (1):103-106.

Available online on http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

The main aim of the present study was to find out antifungal activities of natural medicinal herbs on fungal pathogens. Ethanolic extracts of *Tinospora cordifolia* and *Adhatoda vasica* was observed at concentrations of different ranging from 500, 1000, 1500, 2000, 2500 and 3000µg/ml. were tested against dominant fungal pathogens for antifungal activity *in vitro* on *Penicillium expansum*, *Aspergillus flavus,* and *Mucor piriformis* isolated from naturally infected apple fruit. Both plants material were collected from different places of Maharashtra and identified. Ethanolic extracts of both medicinal herbs were assessed for antifungal susceptibility using dilution method. Known antifungal properties were used as positive control. The water extracts were used as control and it was observed that the ethanolic extracts concentrations were more effective and showed antifungal activity against the test pathogens. The results are revealed that *Tinospora cordifolia* showed 99% mycelial growth inhibition at 3000µg/ml against *Penicillium expansum*, *Aspergillus flavus,* whereas *Adhatoda vasica* showed 100% mycelial growth inhibition at 3000µg/ml against *Mucor piriformis*. Hence, the results of the present investigation indicate that the plant extracts possess antifungal properties that can be exploited as an ideal treatment for future plant disease management to eliminate fungal spread.

Keywords: *Tinospora cordifolia* , *Adhatoda vasica*, Plant extracts, Post-harvest pathogens, Disease management.

INTRODUCTION

Apple (*Pyrus malus* L.) is the pomaceous belonging from family Rosaceae and most important temperate fruit of the North Western Himalayan region. It is one of the most equally important agricultural crops, not only because of its economic importance, but also for the nutritional value. It is predominantly grown in Jammu and Kashmir, Himachal Pradesh and Uttar Pradesh. It accounts for about 90% of the production of which is extensively used as fruit. Apple is infested by *Penicillium expansum*,

Aspergillus flavus, and *Mucor piriformis* after harvesting during storage. The various fungicides are used to control of blue mould of apple disease. In earlier perception farmer used various systemic fungicides to control fungal diseases, their indiscriminate use may cause environmental hazards. Therefore, its management is equally important to increase the yield and maintain quality of post harvest apple fruits using various plant solvents extracts is the alternative for fungicides. Apple caused by various dominant fungal pathogens (*Penicillium expansum, Aspergillus flavus, Alternaria alternata, Aspergillus fumigates*, *Botrytis cinerea, Venturia inaequalis, Erwinia amylovora, Botryospaeria obtusa, Leptodontium elatius, Rhizopus arrhizus, Mycosphaerella pomi, Mucor piriformis* and *Monilinia fructigena*) among these *Penicillium expansum*, *Aspergillus flavus,* and *Mucor piriformis* is very serious pathogen was considered to be managed using ethanolic plant extract. Very few research works have been carried out on severe disease management using plant extracts. Various plants are known to have antifungal and antibacterial properties and these are used as promising bio-control agents (Datar, 1988; Nene and Thapiyal, 1993; Gangawane 2008; Khandare *et.al*.,2007; Dahiwale *et al*., 2009).

Ecofriendly herbal extracts agents have shown to be great potential as an alternative to synthetic fungicides (Janisiewicz and Korsten, 2002; Zhang *et a*l., 2005). Recently, some antimicrobial activity of higher plant products that are biodegradable and safe to human health (Kumar *et al*., 2008) has attracted the attention of microbiologists in the control of plant disease, but the actual use of these products for the management of postharvest pathogens of fruits generally, and in particular for apple fungal pathogens is, however, still limited. The purpose of our research is to test the possibility of using ethanolic extracts from *Tinospora cordifolia* and *Adhatoda vasica* to control or inhibits the pathogens causing post-harvest diseases in apple fruit.

MATERIALS AND METHODS

Collection of diseased fruits:

APMC fruit Market Vashi, Navi Mumbai were surveyed in September to December 2019, to observe the common post-harvest disease symptoms in apple fruits. The prominent symptoms observed were the growth of bluish, black and brown - mold on the fruits. Random samples were collected from the apple fruits and brought to the Research Laboratory, Department of Botany, K. V. Pendharkar College, Dombivli (E)-421203 (M.S.) India for further studies. They were washed with sterile distilled water and disinfected with 0.1% mercuric hypochlorite, and cultured on PDA medium for 8 days at 27±2ºC temperature under aseptic conditions, for identification, isolation of single spore and propagation under the laboratory conditions at 25^0C.

Test Pathogens:

After eight days colony character, culture pattern was studied and identified *Penicillium expansum*, *Aspergillus flavus,* and *Mucor piriformis* using literatures. Single-spores were isolated from apple fruits and grown on potato dextrose agar (PDA) at 25^0C for 8 days. Spores were harvested by flooding the media surface with sterile distilled water and kept in the refrigerator for further studies and propagation.

Collection and Preparation of plant extracts:

Tinospora cordifolia and *Adhatoda vasica* were collected from different places of Maharashtra and washed under running tap water. Plant material was dried overnight in the laboratory electric oven at 40^0C. One hundred grams of leaves material were crush by an electric mixer, and preserved in labeled glass bottles that were sealed until use.

The extraction technique was used modification of Ruch's (2001) method. Fifty grams each of the oven dried & powered material from *Tinospora cordifolia* and *Adhatoda vasica* were treated with 100 ml of 95% ethanol with constant stirring for 30 minutes. After stirring, the solutions were filtered through 4 layered of muslin cloth and Whatman's filter paper no. 1 and evaporate at 60^0C temperature for 60 minutes in evaporating dish. The dark spongy materials from the evaporating dish were removed and dried in an oven at 37^0C for 2 days. The dried powder from the oven was stored in small, sterilized 5 ml screw-capped glass bottles and kept in the refrigerator at 4^0C until further usage.

Preparations of plant extract dilutions:

The *Tinospora cordifolia* and *Adhatoda vasica* powdered extracts were removed from the refrigerator and brought to the laboratory for the preparation of extract dilutions. Aliquots of 0.5g, 1.0g, 1.5g, 2.0g, 2.5g and 3.0g of each powder were

mixed with distilled water to make dilutions of 500, 1000, 1500, 2000, 2500 and 3000µg/ml.

***In vitro* screening:**

PDA medium was incorporated into 250 ml conical flasks and autoclaved for 20 min at 15lbs. After autoclaving the flasks were cooled down to about 45ºC. Five ml of each plant extract, (500, 1000, 1500, 2000, 2500 and 3000µg/ml from *Tinospora cordifolia* and *Adhatoda vasica* was pipette out and mixing properly with 20ml aliquots of the amended media were dispensed into three 9cm Petri-dishes. The experiment was performed under aseptic conditions and replicated thrice. One ml each of *Penicillium expansum*, *Aspergillus flavus,* and *Mucor piriformis* spore suspensions were pipetted on to the centre of the amended PDA extracts. Inoculated plates were incubated at 25ºC for 8 days. The inoculated Petri-dish without the extract concentrations, served as control. The fungal Colony diameter was determined by measuring the average radial growth.

The mycelia growth inhibition zone **(P)**, was measured using the formula of Francisco (2010) as follows:

$$P = \frac{(gC - gT)}{gC} \text{ X } 100$$

Where **C** is the growth of colony diameter of the control and **T** is of the treatments.

RESULTS AND DISCUSSIONS

The post-harvest fungi, identified on basis of their cultural and morphological characteristics and tested for the anti microbial activity of the plant extracts were *Penicillium expansum*, *Aspergillus flavus,* and *Mucor piriformis.* Mixing culture PDA media with all concentrations, 0µg/ml (control), 500, 1000, 1500, 2000, 2500 and 3000µg/ml of the plant extracts of the *Tinospora cordifolia* showed significant results (Table.1) when compared with the control. *Penicillium expansum* showed a reduction in colony development ranging from an average of 47.50%, 54.53%, 61.00%, 79.50%, 85.51 and 98.25% at concentrations of 500, 1000, 1500, 2000, 2500 and 3000µg/ml respectively. *Aspergillus flavus* recorded mycelial growth inhibition of 50.53%, 54.50%, 61.00%, 73.50%, 87.55% and 99.15% at similar plant extract concentrations respectively. The mycelial growth inhibition observed in *Mucor piriformis* were 54.42%, 61.00%, 72.15% 80.00%, 89.50% and 100% respectively at concentrations in the ascending order. The control treatments showed no inhibition zones. From Table 1 it is also observed that the 3000µg/ml showed the excellent result in inhibiting the mycelial growth in the entire 3 fungal organism studied.

Result on the efficacy of *Adhatoda vasica* extract on the post-harvest pathogens in apple is presented in similar trend as the *Tinospora cordifolia* extract was observed in its microbial inhibition activity except that at 3000µg/ml, all the 3 fungi, namely, *Penicillium expansum*, *Aspergillus flavus,* and *Mucor piriformis* recorded almost 100% inhibition of mycelial growth.

The impacts of different *Tinospora cordifolia* and *Adhatoda vasica* concentrations on the percentage of mycelial growth inhibition of the fungi are presented in Table 1. From the data, it is observed that, the concentration of 3000µg/ml gave the significant inhibition of mycelial growth with both the extracts.

Table 1. Mycelial growth Inhibition (in percentage) by the ethanolic extracts of the test plants at different concentrations

Concentration (µg/ml)	***Tinospora cordifolia***			***Adhatoda vasica***		
	Penicillium expansum	*Aspergillus flavus*	*Mucor piriformis*	*Penicillium expansum*	*Aspergillus flavus*	*Mucor piriformis*
500	47.50	50.53	54.42	51.50	49.00	54.37
1000	54.53	54.50	61.00	63.00	55.50	62.11
1500	61.00	61.00	72.15	78.50	63.00	75.00
2000	79.50	73.50	80.00	89.75	75.00	86.50
2500	85.51	87.55	89.50	94.00	87.50	95.00
3000	98.25	99.15	100	100	100	100

As compared to earlier investigators studies have depicted and co-relate the results in which leaf extract

of different plants inhibited the growth of *Fusarium*, *Alternaria* and *Helminthosporium* (shinde et.al., 2009) and also the results compared with earlier studies showed that the effect of plant extracts against the fungi *Penicillium digitatum* include garlic (Obagwa, 2002), Neem (Mossini,*.et al*,2009), *Withania somnifera* (Samson, 1984), Mustard and Horseradish (McOnie,1964).

Conflicts of interest: The author stated that no conflicts of interest.

REFERENCES

Dahiwale MA, Baviskar RN and Suryawanshi NS (2009) Integreted management of carbendazim resistant *Altrenaria alternata* causing fruit rot of Pomeranate*. Life Sci J Bioinfolate* 6 (1):44-45.

Datar VV (1988) Antifungal activity of plant extracts against *Fusarium* Sp. Causing rhizome rot of ginger paper presented in National Seminar on Management of Crop-Diseases with Plant products/biological agent. *Agril College and Res Inst Madurai India* PP 28.

Francisco DH (2010) *Lippia graveolens* and *Carya illinoensis* Organic Extracts and there *in vitro* Effect Against *Rhizoctonia Solani* Kuhn. *American J of Agricultural and Biological Sci* 5 (3): 380-384.

Gangawane LV (2008) Glimses of phytopathology for sustainable Agriculture. *Indian Phytopath*. 61 (1):2-8.

Janisiewicz WJ and Korsten L (2002) Biocontrol of post harvest diseases of fruits *Annu Rev Phytopathology.* 40: 411-441.

Khandare KR Waghmare MB Khandhare NK Patil AV and Kamble SS (2007) Effect of biopesticides in combination with systematic and Non systemic fungicides on growth of some pathogenic fungi. *Bioinfolet* 4(1): 52-56.

Kumar A Shukla R Singh P Prasad CS and Dubey NK (2008) Assessment of *Thymus vulgaris* L. essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities. *Int Food Sci Emerg* 4: 575-580.

McOnie KC (1964) The latent occurrence in citrus and other hosts of a *Guignardia* easily confused with *G. citricarpa*, the black spot pathogen. *Phytopathology* 54: 40- 43.

Mossini SAG, C Carla and C Kemmelmeier (2009) Effect of neem leaf extract and Neem oil on *Penicillium* growth, sporulation, morphology and ochratoxin a production. *Toxins* 1: 3-13.

Nene YL and Thapiyal PN (1993) Fungicides in plant diseases control, *Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi.*

Obagwa J Korsten L (2002) Control of citrus and green and blue molds with garlic extracts*. Plant Pathology*.109,221-225.

Ruch Ba Worf R (2001) Processing of neem for plant protection simple and sophisticated standardized extracts. Abstracts of the .*Work shop Neem and Pheromones University of Uberaba Brazil March 29-30 Augusts,* P.499.

Samson JA (1984) Tropical *fruits*- Tropical agricultural series. *Longman Inc., New York* pp 64-118.

Shinde SL, Junne SB, Wadje and Baig MMV (2009) The diversity of antibacterial compounds of *Terminalia* sp. (Combrataceae). *Pakistan J of Biological Sci* 12 (22): 1483-1486.

Zhang H Zheng X (2005) Biological control of postharvest blue mold of oranges by *Cryptococcus laurentii* (Kufferath) Skinner. *Biocontrol* 50: 331-342.