RESEARCH ARTICLE

Screening of Antifungal Activity of *Holarrhena antidysenterica* and *Maduca longifolia* against fungal pathogens in Apple fruit

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ABSTRACT

The present study aims to investigate the effect of some easily available medicinal plants on fungal pathogens. *Holarrhena antidysenterica* and *Maduca longifolia* ethanolic extracts was observed at different concentrations ranging between 500, 1000, 1500, 2000, 2500, 3000 µg/ml were tested for antifungal activity *in vitro* on *Penicillium expansum*, *Aspergillus fumigatus* and *Alternaria alternata* isolated from naturally infected apple fruit. Both plants were collected from different places of Maharshtra and identified. Ethanolic extracts of plants were assessed for antifungal susceptibility using dilition method. Known antifungal agents were used as positive control. The water extracts 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 reveled that *Holarrhena antidysenterica* showed 99% mycelial growth inhibition at 3000µg/ml against *Penicillium expansum* and *Aspergillus fumigatus* whereas *Maduca longifolia* showed 100% mycelial growth inhibition at 3000µg/ml against *Alternaria alternata*. Hence, the plant extracts possess antifungal properties that can be exploited as an ideal treatment for future plant disease management to eliminate fungal spread.

Keywords: Ethanolic extracts, *Holarrhena antidysenterica*, *Maduca longifolia*, Post-harvest pathogens.

INTRODUCTION

Apple (*Pyrus malus* L.) is an important temperate fruit of the North Western Himalayan region. It accounts for about 90% of the production of which is extensively used as fruit. Apple is infested by *Penicillium expansum, Aspergillus fumigatus* and *Alternaria alternata* after harvesting during storage. The various fungicides are used to control of blue mould disease. In earlier sense 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 using various plant solvents extracts is the alternative for fungicides. Apple caused by various pathogens viz. *Venturia inaequalis, Erwinia amylovora, Botryospaeria obtusa, Leptodontium elatius, Rhizopus arrhizus, Botrytis cinerea, Alternaria alternata, Aspergillus fumigatus Penicillium expansum*

and *Aspergillus flavus* among these *Penicillium expansum, Aspergillus fumigatus* and *Alternaria alternata* is very serious pathogen was considered to be managed using ethanolic plant extract. Very few research works have been carried out on 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).

Environmentally friendly plant extracts agents have shown to be great potential as an alternative to synthetic fungicides (Janisiewicz and Korsten,2002; Zhang *et. a*l., 2005). Recently, the antimicrobial activity of some 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 control of postharvest pathogens of fruits generally, and in particular for apple pathogens is, however, still limited. The purpose of our research is to test the possibility of using extracts from *Holarrhena antidysenterica* and *Maduca longifolia* to control or inhibits the pathogens causing post-harvest diseases in apple fruit.

MATERIALS AND METHODS

APMC fruit market Vashi, Navi Mumbai was surveyed in September to December 2015, to observe the common post-harvest disease symptoms in apple fruits. The prominent symptoms observed were the growth of green, gray and bluish -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 water and disinfected with 0.1% mercuric hypochlorite, and cultured on PDA medium for 8 days at 27±2^{0}C temperature under aseptic conditions, for identify-cation, single-spore isolation, and propagation under the laboratory conditions at 25^{0}C. After eight days colony character, culture pattern were studied and identified *Penicillium expansum, Aspergillus fumigatus* and *Alternaria alternata* using literatures. Single-spores were isolated from apple fruits and grown on potato dextrose agar (PDA) at 25^{0}C 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. *Holarrhena antidysenterica* and *Maduca longifolia* were collected from different places of Maharash-tra and washed under running water. They were dried overnight in the laboratory electric oven at 40^{0}C. 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 used was a modification of Ruch's (2001) method. Fifty grams each of the oven dried and powered material from *Holarrhena antidysenterica* and *Maduca longifolia* 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^{0}C degree for 60 min. in evapor-ating dish. The dark spongy materials from the evaporating dish were removed and dried in an oven at 37^{0}C for 2 days. The dried powder from the oven was stored in small, sterilized 5ml screw-capped glass bottles and kept in the refrigerator at 4^{0}C until further usage. The *Holarrhena antidysen-terica* and *Maduca longifolia* powder 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 3000 µg/ml. 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 45^{0}C. Five ml of each plant extract, (500, 1000, 1500, 2000, 2500 and 3000 µg/ml from *Holarrhena antidysenterica* and *Maduca longifolia* was pipetted 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 fumigatus* and *Alternaria alternata* spore suspensions were pipetted on to the centre of the amended PDA extracts. Inoculated plates were incubated at 25^{0}C for 8 days. The Petri-dish inoculated without the extract concentrations, served as control. 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:

$$P = \frac{gC - gT}{gc} \times 100$$

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 fumigatus,* and *Alternaria alternata.* Mixing culture PDA media with all concentrations, 0µg/ml (control), 500, 1000, 1500, 2000, 2500 and 3000µg/ml of the plant extracts of the *Holarrhena antidysenterica* showed significant results (Table.1) when compared with the control. *Penicillium expansum* showed a reduction in colony development ranging from an average of 48.88%, 58.88%, 67.77%, 75.55%, 85.55 and 97.77% at concentrations of 500, 1000, 1500, 2000, 2500 and 3000µg/ml respectively. *Aspergillus fumigatus* recorded mycelial growth inhibition of 55.55%, 65.55%, 72.22%, 81.11%, 94.44 and 100% at similar plant extract concentrations respectively. The mycelial growth inhibition observed in *Alternaria alternata* were 54.44%, 62.22%, 70.00% 84.11%, 94.44 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 all the 3 fungi studied.

Result on the efficacy of *Maduca longifolia* extract on the post-harvest pathogens in apple is presented in similar trend as the *Holarrhena antidysenterica* extract was observed in its microbial inhibition activity except that at 3000µg/ml, all the 3 fungi, namely, *Penicillium expansum, Aspergillus fumigatus,* and *Alternaria alternata* recorded almost 100% inhibition of mycelial growth.

The impacts of different *Holarrhena antidysenterica* and *Maduca longifolia* 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.

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).

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

Concentration (µg/ml)	***Holarrhena antidysenterica***			***Maduca longifolia***		
	Penicillium expansum	*Aspergillus fumigatus*	*Alternaria alternata*	*Penicillium expansum*	*Aspergillus fumigatus*	*Alternaria alternata*
500	48.88	55.55	54.44	53.33	50.00	53.33
1000	58.88	65.55	62.22	61.11	57.77	61.11
1500	67.77	72.22	70.00	75.55	65.55	74.44
2000	75.55	81.11	84.11	87.77	72.22	87.77
2500	85.55	94.44	92.44	96.66	88.88	95.55
3000	97.77	100	100	100	100	100

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