

Biological control of postharvest fungal pathogens of sweet oranges by *Plumeria latex*

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ABSTRACT

Plant extracts could be useful in the management of fungal decay in postharvest conditions. This study investigated the phytochemical profile and effects of *Plumeria latex* against the postharvest fungal pathogens of sweet oranges (*Citrus sinensis* L Osbeck). Polar and non polar solvent extractions revealed alkaloids, glycosides and terpenoids as the major phytoconstituents present in *Plumeria latex*. Postharvest fungal pathogens of oranges such as *Aspergillus niger*, *A. fumigatus*, *A. terreus*, *Penicillium digitatum* and *Rhizopus arrhizus* were tested against various extracts of *Plumeria latex*. Antifungal assay of the extracts recorded significant inhibitory activity against *Aspergillus terreus* and *Penicillium digitatum* by the petroleum ether extract. Being the most effective on all species, *Plumeria obtusa* was found to have potential antifungal properties followed by *P. rubra* after five days of incubation. Based on these results, application of *Plumeria latex* can be considered a useful strategy to be included in an integrated approach for controlling postharvest fungal disease of oranges.

Key words: Antifungal, biological control, latex, Oranges, Phytochemical, *Plumeria*, Postharvest

INTRODUCTION

Postharvest decays are caused by latent or wound induced fungal infections which causes as great as 25-50% loss and remain an important challenge in sustainable food production [1]. Oranges (*Citrus sinensis*) are usually stored after harvest and postharvest decay is the major factor limiting their shelflife. Increasing the storage time of the fruit can reduce the economic losses due to decay. Increasing pathogen resistance to key fungicides, lack of replacement fungicides, consequent restrictions on fungicide use and high cost of chemicals requires alternative methods which are safer and ecofriendly to reduce postharvest decays. Further, use of fungicides generates health concerns due to their carcinogenic and teratogenic properties [2, 3].

Postharvest disease control methods must be environmentally friendly, safer for human health, economically viable, and able to extend shelf life [4]. Biological control by natural compounds appears to be feasible and may present an alternative to synthetic fungicides. Using antagonistic microorganisms, compounds of natural origin and physical measures are some of the effective alternatives for biological control of postharvest diseases. Utilization of natural antimicrobial agents could represent a low-cost alternative therapy as microorganisms become resistant to conventional antimicrobial agents. Chemical studies of antimicrobial plant extracts could reveal new substances with potential usefulness as antimicrobial drugs or as models for the development of new drugs.

Latex producing plants secrete endogenous milk like fluid in a network of laticifer cells in which subcellular organelles intensively synthesize proteins and secondary metabolites [5]. Plant latex contains great varieties of defense chemicals and defense proteins [6]. The genus *Plumeria* (Apocynaceae) has been reported to have antimicrobial properties [7-10]. The aim of the present work was to evaluate the effectiveness of solvent extracts of *Plumeria latex* in controlling postharvest fungal pathogens of sweet oranges (*C. sinensis*).

MATERIALS AND METHODS

Latex collection:

Liquid exudates from the cut stalk of leaves and branches were collected from *Plumeria* species and the latex was spread in thin layers over glass sheets for drying in hot air oven at 45°C for 24 hours. The dried latex was pulverized and successively extracted with chloroform, petroleum ether, methanol and aqueous solvents (1:7). The solvents of the filtrates were distilled off with a rotary vacuum evaporator and analyzed for its phytochemical properties.

Phytochemical analysis:

Phytochemical analysis of the extracts was done by following the methods described previously [11-13].

Test for Alkaloids (Hager's test):

To the 0.5 ml of the extract, few drops of 0.1% picric acid were added. Formation of the yellow color which indicates the presence of the alkaloids

Test for Anthraquinones:

2 ml of chloroform was added to 1ml of the extract and the resulting mixture was shaken for 5 min using vortex mixer followed by filtration. The filtrate was shaken with equal volume of 10 % ammonia. The bright pink color in the aqueous layer indicates the positive result.

Test for Flavonoids (Ammonia test):

1 ml of the extract was taken in the test tube and ammonia solution was added (1:5) followed by the addition of conc. sulphuric acid. Appearance of yellow color and its disappearance on standing indicates the positive test for flavonoids.

Test for Glycosides (Keller Kiliani test):

5 ml of each extract was added with 2 ml of glacial acetic acid which was followed by the addition of 2 ml of glacial acetic acid, 1 drop of ferric chloride solution and 1 ml of concentrated sulphuric acid. Formation of brown ring at interface confirms the presence of glycosides.

Test for Phenols (Ferric chloride test):

0.5 ml of the extract was added with few drops of neutral ferric chloride (0.5%) solution. Formation of dark green color indicates the presence of the phenolic compounds.

Test for Phlobatanins:

1 ml of hydrochloric acid (1%) was added to the extract and boiled in hot water bath. Formation of red precipitate indicates the presence of phlobatannins.

Test for Saponins (Froth test):

1 ml of the extract was taken in a test tube and distilled water (2 ml) was added to it. The test tube was then kept in boiling water bath for boiling and was shaken vigorously. Existence of froth formation persisted for next one hour confirms the presence of saponins.

Test for Steroids:

2 ml of acetic anhydride was added to 0.5 ml of the extract and then added 2 ml of sulphuric acid. Change of colour from violet to blue or green indicates the presence of steroids.

Test for Tannins (Ferric chloride test):

1 ml of the extract was added with 5 ml of distilled water and kept for boiling in hot water bath. After boiling, sample was cooled down and to this 0.1% ferric chloride solution was added. Appearance of brownish green or blue black coloration confirms the presence of tannins.

Test for Terpenoids:

5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of concentrated sulphuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpenoids.

Antifungal Assay:

Postharvest fungal pathogens of oranges were isolated and agar well diffusion method was followed to determine the effect of *Plumeria* latex extracts against the fungal pathogens of sweet oranges using Sabouraud's dextrose agar

(SDA). 100 μ l of the spore suspension (10^6 spores/ml) was swabbed on the surface of SDA plates. 50 μ l of the different extracts were added to the well (5mm) and the plates were kept undisturbed for 30 minutes for the pre diffusion of the extracts. The plates were incubated at $28\pm 2^\circ$ C for 5 days and the zone of inhibition was measured using Himedia scale every day. The experiment was done on three replicates and the test was conducted twice. The data was analyzed by using Duncan's multiple range test with a significant value $p = 0.05$.

RESULTS AND DISCUSSION

Phytochemical properties of latex extracts of *P. obtusa*, *P. rubra* and *P. stenophylla* are represented in table-1. Alkaloids, glycosides and terpenoids were present as the major phytoconstituents irrespective of solvents and species studied. The other major phytochemicals were phenols, saponins and steroids. Phenols were found to be the major phytoconstituent along with alkaloids, glycosides and terpenoids in *P. rubra*.

A total of five fungal strains (*Aspergillus niger*, *A. fumigatus*, *A. terreus*, *Penicillium digitatum* and *Rhizopus arrhizus*) from infected oranges were tested against various solvent extracts of *Plumeria* latex. Assays of biological control of fungal pathogens of sweet oranges were performed at $28\pm 2^\circ$ C for 3-5 days. The results obtained with antifungal assay were particularly interesting since the pathogens were inhibited only by petroleum ether extract where as other solvent extracts were completely failed to control the growth of fungal pathogens (Fig: 1-5). Highest antifungal activity was observed with *P. obtusa* against *A. terreus* followed by *P. digitatum*. A significant activity against *A. fumigatus*, *P. digitatum* and *A. terreus* was found with *P. rubra* (Plate: 1-5).

The use of biologically based compounds, such as latex, essential oils obtained from medicinal plants was suggested as a feasible approach for reducing post-harvest diseases in harvested fruits and vegetables [14]. Such alternative method for controlling postharvest diseases will help to combat fungicide resistant strains of pathogens and to avoid pesticides residues from the environment and commodities thus minimizing effects on non-target microorganisms.

Antimicrobial activities of latex of other plants were reported by previous studies [15-18]. Chitinolytic enzymes degrade fungal cell walls and are important in the biological control of the postharvest pathogens [19] and chitinase activity of *Plumeria rubra* has been reported [20].

Fig: 1: Antifungal activity of *Plumeria* latex against *A. fumigatus*

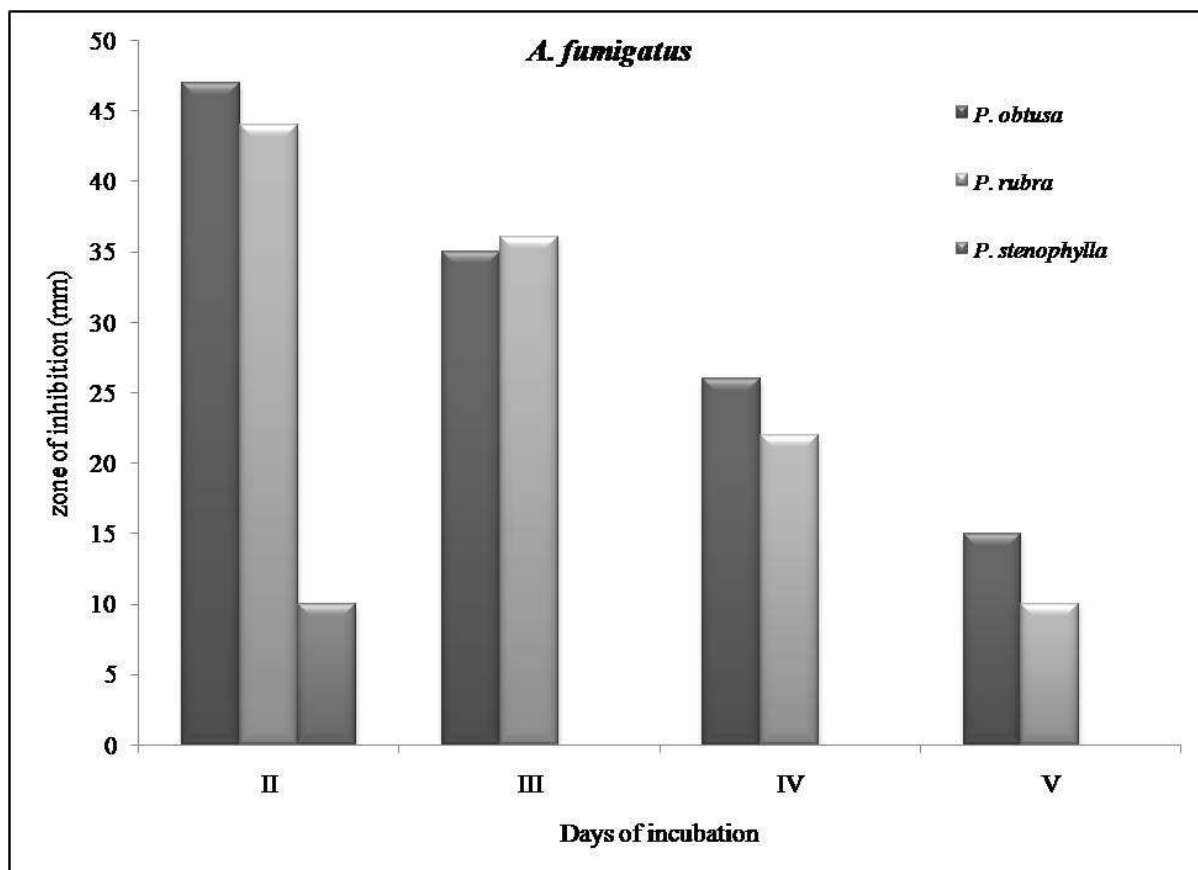


Fig: 2: Antifungal activity of *Plumeria* latex against *A. niger*

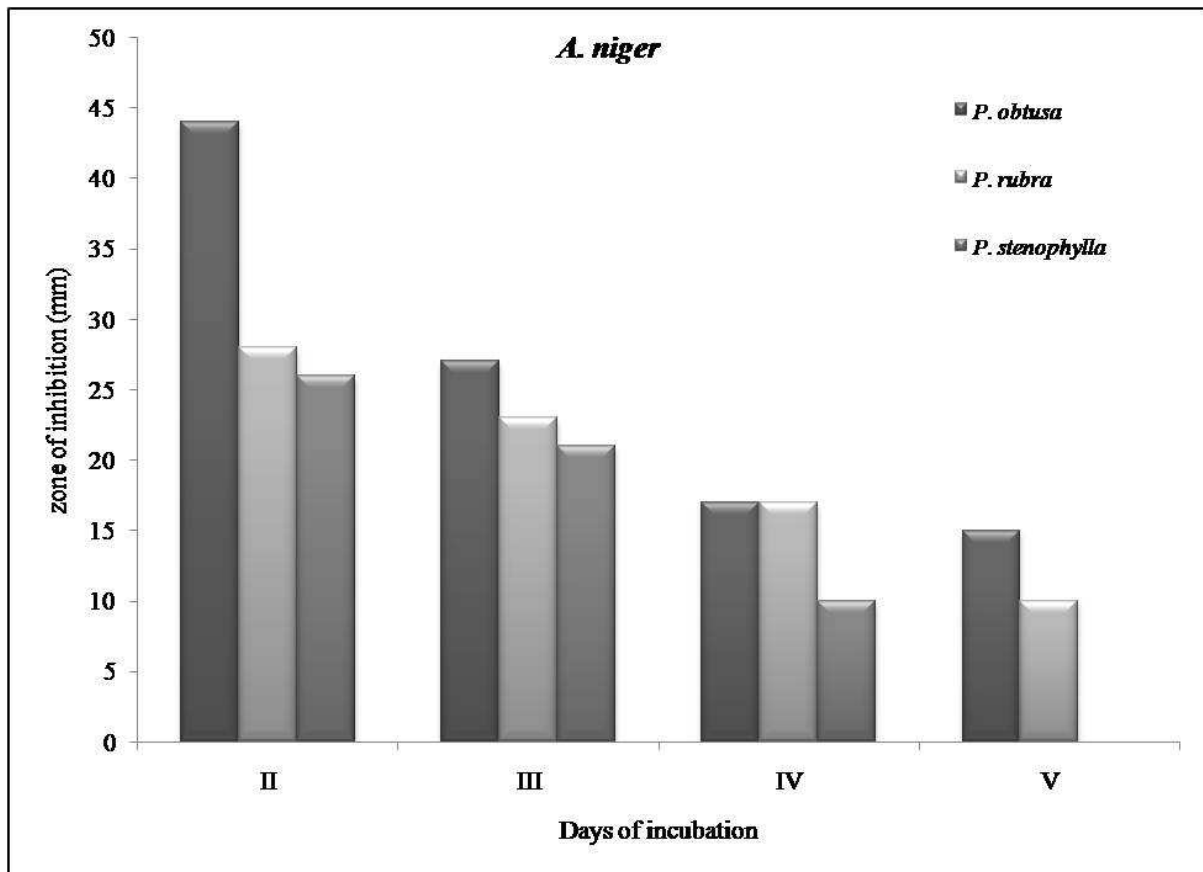


Fig: 3: Antifungal activity of *Plumeria* latex against *A. terreus*

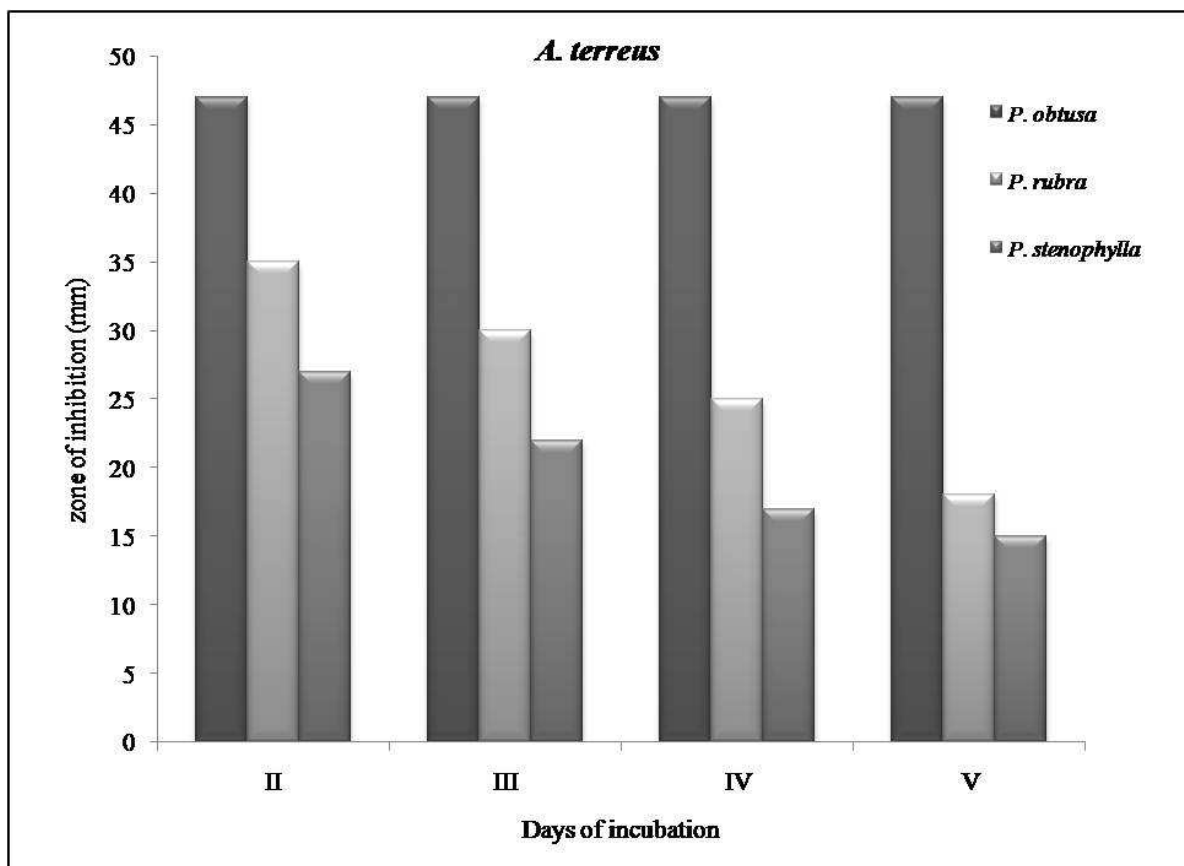
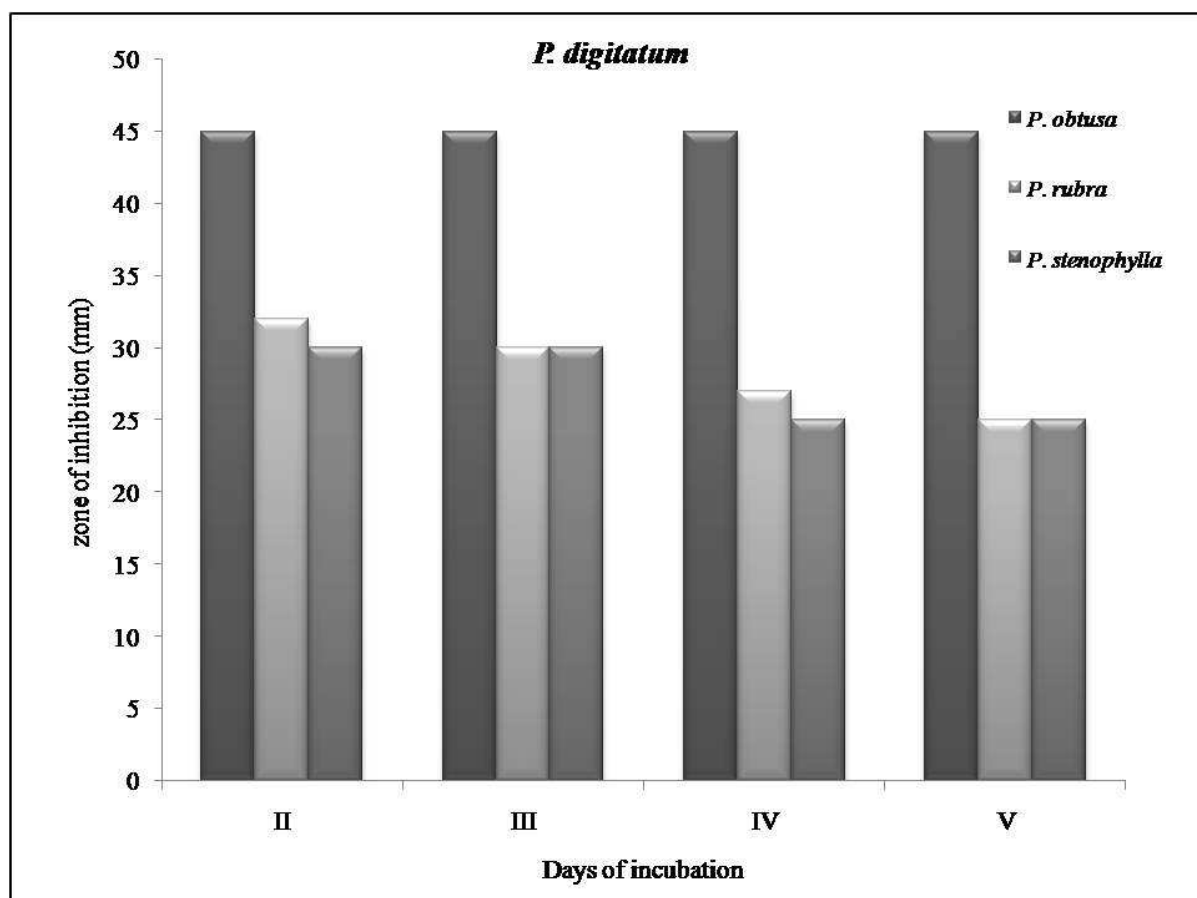


Fig: 4: Antifungal activity of *Plumeria* latex against *P. digitatum*

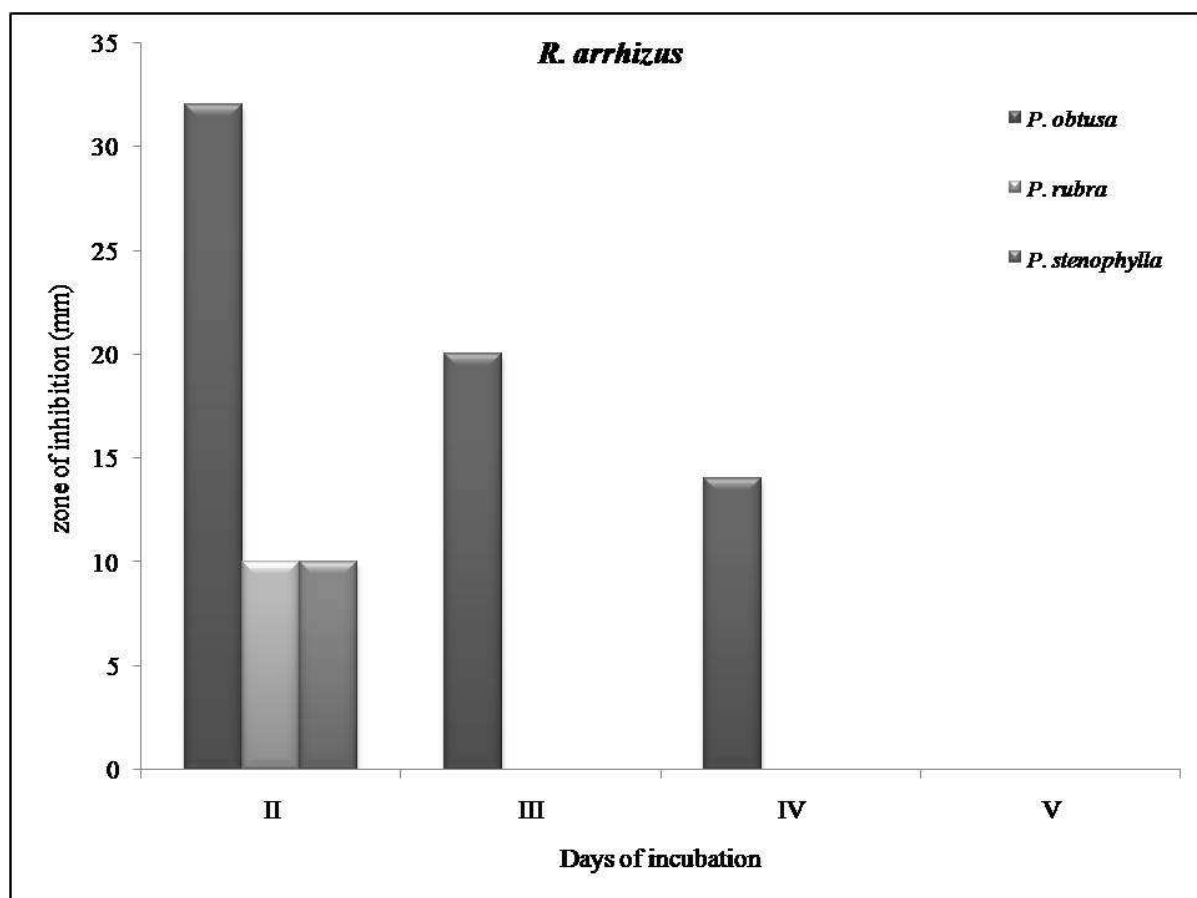
Differences in the sensitivity of fungi to latex extracts suggest its variable potency between the fungi tested. *Aspergillus niger* causes decay on stored citrus fruits and is usually controlled by benzimidazole fungicides or imazalil [21]. In the present study, three species of *Aspergillus* were tested and the highest inhibitory activity was seen with *P. obtusa*. Flavonoids inhibit the activity of enzymes [22, 23]. Tannins coagulate the cell wall proteins, while saponins alter the permeability of the cell wall thus facilitating the entry of toxic materials or leakages of vital constituents from the cell. Antifungal activity of *P. obtusa* could be attributed by the presence of tannins, flavonoids and saponins in the petroleum ether extract. In general, *P. obtusa* and *P. rubra* were effective against most of the pathogens tested. Anti-inflammatory activity of latex of *P. rubra* was reported earlier [24]. *Rhizopus arrhizus*, a fast growing filamentous fungus is another postharvest pathogen of vegetables and fruits. In the present study, *R. arrhizus* was controlled till the fourth day of incubation by *P. obtusa* latex.

Table -1: Phytochemical Profile of Latex of *Plumeria* spp

Plant	Extract	Phytochemical analysis									
		Alk	Ant	Fl	Gly	Phe	Phl	Sap	Ste	Tan	Ter
<i>P. obtusa</i>	Aqu	+	-	+	+	+	-	-	-	+	+
	Met	+	-	-	+	+	-	+	+	+	+
	Chl	+	-	+	+	+	-	+	+	-	+
	Pet	+	-	+	+	-	-	+	+	+	+
<i>P. rubra</i>	Aqu	+	-	+	+	+	-	-	-	+	+
	Met	+	-	-	+	+	-	+	+	-	+
	Chl	+	-	+	+	+	-	+	+	-	+
	Pet	+	-	+	+	+	-	+	+	+	+
<i>P. stenophylla</i>	Aqu	+	-	+	+	+	-	-	-	+	+
	Met	+	-	+	+	+	-	+	+	+	+
	Chl	+	-	+	+	+	-	+	+	-	+
	Pet	+	-	-	+	-	-	+	+	-	+

Alk-alkaloids; Ant-anthraquinones; Fla-flavonoids; Gly-glycosides; Phe-phenolics; Phl-phlobatannins; Sap-saponins; Ste-steroids; Tan-tannins; Ter-terpenoids

Aqu-aqueous; Met-methanol; Chl-chloroform; Pet-petroleum ether

Fig: 5: Antifungal activity of *Plumeria* latex against *R. arrhizus*

Green mold of oranges is one of the most important diseases of oranges caused by *Penicillium digitatum* [25-29]. Applications of sodium bicarbonate, imazalil, thiabendazole, pyrimethanil, fludioxonil and sodium o-phenylphenate are used to manage postharvest green and blue molds of citrus [30]. Latex extract of *P. obtusa* exhibited significant inhibitory effect on the growth of *P. digitatum*. However *P. rubra* and *P. stenophylla* were also able to control the growth of green mold revealing the potential activity of the *Plumeria* species. Significant activities of citral and garlic extracts against *P. digitatum* were observed by previous studies [31-33].

CONCLUSION

This study represents the phytochemical and antifungal properties of different organic solvent extracts of *Plumeria* latex. Most of the postharvest fungal pathogens of oranges were effectively controlled by the petroleum ether latex extracts of various species of *Plumeria* and this alternative strategy has the potential of environmentally safe. Further, the present study suggests the use of *Plumeria* latex extracts as one promising strategy for postharvest disease control in sweet oranges.

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