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Control of Post-harvest Fungal Diseases of Guava by Essential Oil of *Azadirachta indica*

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ABSTRACT

Six post harvest fungal pathogens viz. *Pestalosia psidii* (T1), *Gloesporium psidii* (T2), *Rhizoctonia solani* (T3), *Fusarium sp*. (T4), *Alternaria alternata* (T5), and *Geotrichum candidum* (T6)isolated from guava fruits were screened against essential oil of neem at 0, 1, 5, 10, 30 and 50% concentration by using poisoned food technique. The result indicated that neem oil was effective in inhibiting the mycelia growth against all the tested postharvest fungal pathogen of guava. It was observed that the inhibitory effect of oil is proportional to its concentration. The treatment with 1% oil did not significantly affect the radial growth of the fungus but at 5 and 10% conc. showed moderate antifungal activity against all the postharvest fungal growth tested. Neem oil showed the most significant reduction at 50% concentration. Hence the result of the present investigation indicated that essential oil possessed antifungal activity in controlling the growth of postharvest fungal pathogen of guava under laboratory condition and can be exploited as an ideal treatment for postharvest fungal disease management of guava.

Keywords: Antifungal activity, Essential oil, Guava, Postharvest fungal pathogen

INTRODUCTION

Guava (*Psidium guajava* Linn.) is an important fruit crop of subtropical countries. In India, guava is cultivated on 204.8 thousand hectares of land, and production is about 2462.3 million tons (Anonymous 2011), and it is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Droby 2006, Zhu 2006). Fungal infection on the fruit may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer. Fruits contain high levels of sugars and nutrients, and their low pH makes them vulnerable to fungal decaying (Singh and Sharma 2007). Over the years, the protection of agricultural crops and products was achieved almost entirely through the use of synthetic chemicals. These chemicals, though valued for their effectiveness in controlling various post harvest diseases of fruits, are costly and their continued or repeated applications may disrupt equilibrium of ecosystems, leading to dramatic disease outbreaks, widespread development of pathogens resistant to one or more chemicals, toxicity to non-target organisms and environmental problems. Sometimes, they accumulate in the food chain as residues. Furthermore, pesticide residues in food possess more carcinogenic risks than insecticides and herbicides (Lee et al. 2009). These highlighted the need to develop alternative control strategies or innovative crop protection and postharvest methods of fruit rot control with a reduced use of conventional fungicides or without synthetic chemicals (Kim et al. 2003). Biologically active essential oils represent a rich potential source of an alternative and perhaps environmentally more

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acceptable disease management compounds. With a broad range of natural fungicidal plant volatiles, numerous opportunities exist to explore their usefulness in controlling post-harvest diseases. The general antifungal activity of essential oils is well documented (Pitarokili et al. 1999; Meepagala et al. 2002) and there have been some studies on the effects of these oils on post-harvest pathogens (Bishop and Thornton 1997). The advantage of essential oils is their bioactivity in the vapour phase, a characteristic which makes them attractive as possible fumigants for stored product protection. Therefore, it seemed opportune to assay the *in vitro* fungicidal activity of neem oil against six fungi well known as postharvest fruit decaying agents of guava.

MATERIALS AND METHODS

Essential oil

Neem oils were tested for antifungal activity on six postharvest fungal pathogens isolated from guava fruit. Essential oils used in this study were obtained from Karnataka Aromas, Essential Oil Distillery, Bangalore, Karnataka.

Isolation of fungal pathogen from guava fruit

Fungal pathogens were isolated from infected guava fruits. The diseased parts were cut into small pieces (2-3mm) and surfaced sterilized with 0.1% mercuric chloride solution for 30 seconds. The pieces were then washed three times with sterilized distilled water and aseptically transferred on to clean sterilized Petridishes containing solidified potato dextrose agar medium. The Petridishes were incubated in inverted position at 28±1°C and observed after 3-4 days. Fungal hyphae, growing out from the infected fruit pieces associated with post harvest disease of guava were identified microscopically (Burnett and Hunter, 1999) and purified on PDA slants. Pure culture was maintained by periodic sub culturing (Aneja 2004).

Pathogenicity test

The pathogens were isolated, identified and cultures were used to confirm pathogenicity on guava fruits. The fruits were washed with distilled water and allowed to dry under a fan and then surface sterilized with 0.1% mercuric chloride solution. The Wounds were made in the fruit with the help of sterilized cork borer (2mm). The wounds were inoculated with pathogen containing spore load of $1x10^4$ conidia/ml (Granger and Horne 1924). The inoculated fruits were wrapped in sterilized paper and incubated at 28±1°C. The artificially inoculated fruits were examined daily and extent of damage was recorded. The pathogens were re-isolated and disease symptoms were clearly evident, the culture and symptom were compared with original.

Antifungal activity

The fungistatic activities of the neem essential oil and constituents were evaluated via the poisoned food technique as described by Dhingra and Sinclair (1995) with the following modification: a final concentration of 0.5% (v/v) Tween-20® (Sigma-Aldrich Corp. St. Louis, Mo., U.S.A) was incorporated into the agar after autoclaving to enhance the oil solubility. Different oil concentrations (1, 5, 10, 30, 50%, v/v) were mixed into each of the three replicated plates of PDA (20 mL/plate), just before it solidified. PDA, with 0.5% (v/v) Tween-20 but no oil, was used as a positive growth control. The plates were then centrally inoculated with single 6 mm diameter mycelia plug of pathogen from the PDA plate and incubated at room temperature. The inoculated plates were observed at the 24hrs intervals for four days, and mean values were recorded. This experiment was conducted three times, and the activity was expressed as the mean value of radial growth.

RESULTS AND DISCUSSION

Seven post harvest fungal diseases of guava were isolated from guava fruits (*Pestalosia psidii*, *Gloesporium psidii*, *Rhizoctonia solani*, *Fusarium* sp., *Alternaria alternata*, *Cladosporium* sp. and *Geotrichum candidum*) as shown in Table 1 and essential oil of neem were evaluated against six of them except *Cladosporium.* The inhibitory effect of oil is proportional to its concentration. The treatment with 1% oil did not significantly affect the radial growth of the pathogens, but at 5 and 10% showed moderate antifungal activity against all the postharvest fungal growth tested. The effectiveness of neem oil for the control of fungal diseases was shown when 20 and 30% conc. were used in the assay resulting in reduced mycelial

Table 1. Identification of common pathogens associated with post harvest diseases of guava.

S. No.	Microorganism	Diseases/symptom	Characteristics
1	*Pestalosia psidii*	Fruit canker (Fig. a, b, c) Rust coloured necrotic spots. Later the lesion increases and extends to the pulp. The centre of infection gets depressed.	Acervuli dark, discoid or cushion shaped, subepidermal, conidiophores short, simple, conidia dark, several celled, with hyaline, pointed one cells, ellipsoid to fusiod, with two or morehyaline, apical appendages.
2	*Gloesporium psidii*	Anthracnose (Fig. d, e, f) Small water-soaked spots enlarge to form circular dark-brown lesion. At later stage the centre becomes sunken, dot like acervuli with salman-coloured spore mass may appear on the surface	Acervuli disc shaped or cushion shaped, waxy sub epidermal, typically with dark spines or setae at the edge or among the conidiophores. Conidiophores simple, elongate conidia hyaline. 1-celled, ovoid or oblong.
3	*Rhizoctonia solani*	Fruit rot (Fig. g, h, i) Lesions may first appear as water soaked areas that become light brown, greenish brown or reddish brown, oval to linear on fruit	Asexual fruit bodies and spores lacking, sclerotia brown or black, variables in form, frequently small and loosely formed hyphae or mycelium is brown, with long cells, septa of branch set off from main hyphae.
4	*Fusarium* sp.	Fruit rots (Fig. j, k, l) A dark brown spots increased in size. At later soft stage the fruit rots	Mycelium extensive and cottony in culture, often with some tinge of pink, purple or yellow conidia hyaline, variable, principally of two kinds. Macro conidia several-celled, slightly curved or bent at pointed ends, typically canoe shaped micro-conidia 1-celled ovoid or oblong.
5	*Alternaria alternata*	Fruits spots (Fig. m, n, o) Brown dry fruit spots on the surface invade the inner pulp later stage	Colonies grow rapidly; appear glassy with minute speck of black conidia. Conidia formed in long often branches chain. Obvoidal-obclavate in shape
6	*Cladosporium sp.*	Dark mould (fig. p, q, r) The fruit become flat or wrinkled on the affected side. A sparse surface growth of grey-green fungus is seen on the fruit kept at room	Conidiophores dark, branched variously near the apex or middle portion, clustered on single; conidia dark, 1 or 2 celled variables in shaped and size, ovoid to cylindrical and irregular, some typically lemon-shaped
7	*Geotrichum candidum*	Dairy mold (Fig. s, t, u) Growth appearing first as firm, felt like mass that later becomes soft and creamy	The hyphae are septate and in common species are dichotomously branched. The asexual spores are arthrospores (oidia), which may appear rectangular if from submerged hyphae and oval it from aerial hyphae.

growth but at higher concentration i.e at 50% concentration the mycelial growth was drastically reduced. At 50% conc. the radial growth of *Rhizoctonia solani* was found to be only 6.08mm followed by *Pestolosia psidii* (9mm) and *Alternaria alternata* (12mm) as compared to their respective control.

The use of synthetic fungicide has been cautioned due to their pollutive effect, non biodegradability and residual toxicities. Most of these chemical fungicides have become a popular target of conservationists and are treated to be one of the most vital man-made pollutants (Khoshoo 1980). Search is on for the development of plant disease control agents, which are non-toxic, biodegradable and eco-friendly. Essential oils show antifungal activity against a wide range of fungi (Kurita et al. 1981; Srivatsava et al. 1993; Singh, 1997; Dubey et al. 2000). In the present study, the oil tested inhibited the growth of all fungus at different concentrations; the essential oils from neem had given promising results against postharvest fungal pathogens isolated from Guava. These results confirm the antimicrobial activity of essential oil used in the present study. It is indicated at all concentration mycelial growth of six post harvest fungal pathogen was reduced as compared with their respective control. The most significant reduction was found at 50% conc. used in the assay.

This study indicated that plant essential oil possessed antifungal activity and can be exploited as ideal treatment for future postharvest fungal disease management programs eliminating the fungal spread. Overall effect of essential oil on mycelia inhibitor of six postharvest fungal pathogens isolated from guava fruit is shown in Fig.

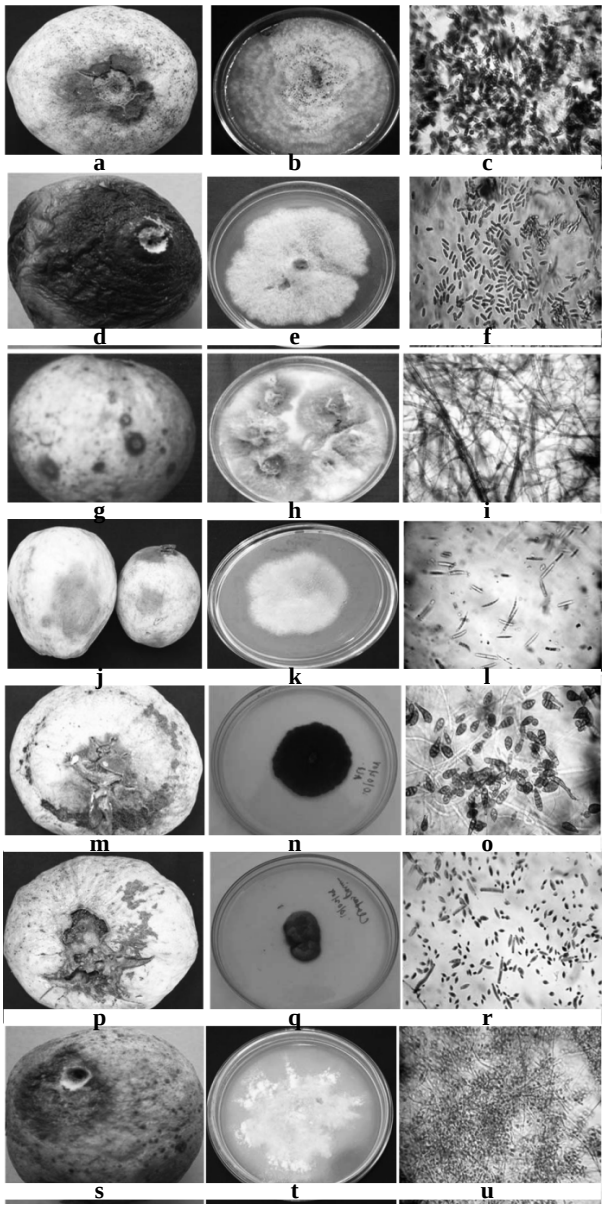

Fig. 1a-u : Pathogen isolated from guava fruits

2. Amongst the different concentration of essential oil tested 30 and 50% seems to be the most effective

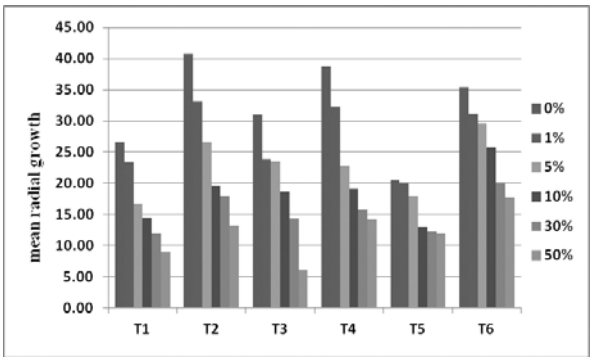

Fig. 2. Effect of different concentration of neem oil on mycelial growth of postharvest fungal pathogens of guava

range. Recently, there has been a great interest in essential oil from aromatic plants for controlling plant pathogens. The information obtained in the present study suggests that essential oil show promising results in controlling the postharvest fungal pathogens of guava under laboratory condition.

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