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EFFECT OF pH AND ORGANIC ACIDS ON GROWTH AND SPORULATION OF A FUNGUS ISOLATED FROM ROTTEN MANDARIN ORANGE (*CITRUS RETICULATA*)

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ABSTRACT: Among the various citrus fruits, mandarin orange (*Citrus reticulata*) is relished as fresh fruit as well as processed to make jam, juices and squash. However, postharvest fungal diseases cause significant economic losses to the citrus industry during storage, transport and marketing. Moreover, food products prepared from rotten orange may be of poor organoleptic and nutritional qualities due to discoloration, off flavor, off odor and spoilage. In this context isolation of fungal pathogen from rotten mandarin orange and understanding the growth and sporulation of the fungus at varying pH is our primary objective. Moreover, the effect of some commonly used chemical preservatives on its growth is our another objective for their effective control. To achieve this target predominant fungal pathogen was first isolated and confirmed morphologically from rotten fruits. Effect of pH on vegetative biomass after seven days was peculiar. Though pH was inversely related to dry weight of mycelium at both extremities, pH 7 was found to be congenial for mycelial growth. With the concomitant increase of pH from 3 to 10, sporulation decreased in a regular fashion. Of the three conventional chemical preservatives, benzoic acid was the most potent requiring lowest amount for complete inhibition of growth.

KEYWORDS: *Aspergillus,Citrus reticulata*, mycelial biomass, sporulation, chemical preservatives.

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1. INTRODUCTION

Citrus fruit cultivation and trading is the third most important fruit industry in India followed by mango and banana. Among the different citrus fruits grown in India, mandarin orange (*Citrus reticulata*), sweet orange (*C. sinensis*) and acid lime (*C. aurantifolia*) occupy the first three positions producing 41, 23 and 23%, respectively, of the total citrus fruit production [1]. India is the ninth largest mandarin orange producing country in the world. It occupies nearly 40% of the total area for citrus fruit cultivation in the country. Mandarin orange is cultivated in Satpura Hills, Darjeeling Hills, Coorg of Karnataka, North-Eastern Hilly areas, Nilgiri Hills and North-Western states of India. Both tropical and subtropical climates with low humidity and low rainfall ranging from 75-250 cm and 10-35°C temperature are most favourable for mandarin orange production. Orange is rich in Vitamins (A & B), phosphorus, citric acid and is consumed fresh or processed to make juice, jam, squash and syrup. Mandarin orange cultivated in Nagpur, the winter capital of the Indian state of Maharashtra, is world famous and has a great potential for export. However post harvest loss is a major concern. Losses from farm to retail level may range from 25-30% depending on season and commodity [2]. Maximum losses occur in the retail market. Post harvest diseases cause economic losses to the farmers resulting in increased cost of production, harvesting, packing, marketing and transportation. Oranges are best harvested on clear, sunny days when humidity is low. After harvesting these are washed, waxed, sized, sorted, graded, packed and stored before sending to the retail market. Physical damage during harvesting, faulty post harvest chemical treatment, improper application of fungicides, bruises on the fruits due to improper storage container and rough handling of packed fruits and environmental condition of storage room are some of the important reasons of post harvest fungal diseases of citrus fruits. Post harvest preservation of fruits is necessitated when production is higher than consumer demand and market price is low. Reduction in post harvest losses would ensure supply of sufficient quantity of quality products to the growing consumer demands. Fungal attack of oranges is very common. Green mould, blue mould and stem-end rot are some major post harvest diseases of mandarin orange. This paper deals with a mould isolated from infected mandarin orange collected from retail market and the effect of pH and organic acids on the growth and sporulation of the mould.

2. MATERIALSAND METHODS

Collection of samples

Rotten oranges were randomly collected in sterile polythene bags lined with soft tissue paper from retailers of fruit market in Kolkata (22° 34' 10" N latitude and 88° 22' 10" E longitude) during the month of January, 2016 and transported to the laboratory for further analysis.

Isolation of fungi from rotten orange

Ten rotten fruits with similar symptoms were selected, washed with sterile distilled water and surface sterilized with 70% ethanol. A block of 5mm x 5mm area with sufficient depth covering both the peel and bulb was cut from each fruit with a sterile scalpel. Cut portions were aseptically transferred to

previously prepared and dried czapek dox agar plates separately and incubated at 28°C for five days. The different colony morphotypes were recorded and the predominant fruit rot causing pathogen was selected based on their maximum appearance. The organism was purified by repeated streaking on czapek dox agar.

Identification of the pathogenic fungi

The pathogen was identified by studying its colony morphology as well as structure of hyphae and reproductive structures after lactophenol cotton blue staining.

Effect of pH on growth

Czapek dox broth containing 10mg/ml streptomycin and 12µg/ml ampicillin was adjusted with 1N HCl and 1N NaOH to pH ranging from 5 to 10. A 2mm diameter agar block cut from the edge of a 5 days old fungal colony was inoculated aseptically to 50ml of czapek dox broth and incubated at 28°C for seven days. Mycelial mass was separated by filtering the culture broth through pre-weighed whatmann number 1filter paper followed by washing with sterile distilled water. The filter paper laden with mycelial mass was then oven dried at 80°C until constant weight. Mycelial growth was measured by subtracting the two weights.

Effect of pH on sporulation

Antibiotic amended czapek dox agar plates of pH ranging from 5 to 10 were inoculated by placing 2 mm dia agar block at the centre followed by incubation at 28°C for ten days. Spores formed on the agar plate were washed with 20 ml sterile distilled water and counted by haemocytometer.

Effect of chemical preservatives on growth

Effect of three chemical food preservatives viz., benzoic acid, citric acid and potassium metabisulphate on fungal growth was monitored by growing 2mm dia agar blocks on czapek dox agar plates supplemented with different concentrations (100 to 1000 µg/ml) of the preservatives in each plate. Inhibition of growth was monitored up to seven days by measuring the diameter of the growing fungal colony.

3. RESULT AND DISCUSSION

The symptoms of fungal disease on the collected orange samples were visible on the epicarp of fruits. The spots were large, circular or irregular in shape and dark brown to black in colour (Figure 1). The mature colony which is black due to similar coloured sporangia of the predominant fungal pathogen isolated from the infected tissues of the rotten mandarin orange is presented in the figure 2.

Figure 1. Fungal spot on epicarp of mandarin orange

Figure 2. Colony of the predominant mould from rotten mandarin orange

The fungal hyphae were thin, hyaline and septate. Sporangiophore was aseptate bearing sporangial head at the top (figure 3). Phialides were double- layered and spores black in colour. Colony and hyphal morphology of the tested fungus is similar to that of *Aspergillus* sp. *Aspergillus* sp. has been found to be readily growing in deproteinized juice produced during the process of green crop fractionation (Rathore, 2016). *Aspergillus* is responsible for secondary rot of plants and food products (Perrone *et al*., 2007). It can contaminate plant products before harvesting or during post-harvest processing. Orange based products like juices, syrups or squash prepared from rotten orange is of inferior nutritional and sensory qualities. Onion bulbs have been found to be heavily infested with *Aspergillus* sp. during storage (Chougule and Andoji, 2016).

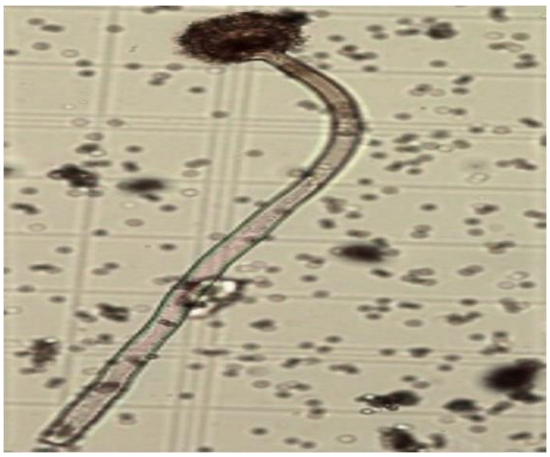

Figure 3. Sporangiophore bearing sporangia of a mould isolated from rotten mandarin orange

The effect of pH on growth of the *Aspergillus* sp. is presented in figure 4. Highest fungal biomass was recovered at pH 3 followed by pH 7. pH 3 to 8 were found to be favourable for the growth of the fungus while more alkaline pH of 9 and 10 were found to be congenial for their growth. Similar result was obtained by Abubakar *et al*., who reported highest biomass of *A. parasiticus* at pH 4 and lowest at pH 10.

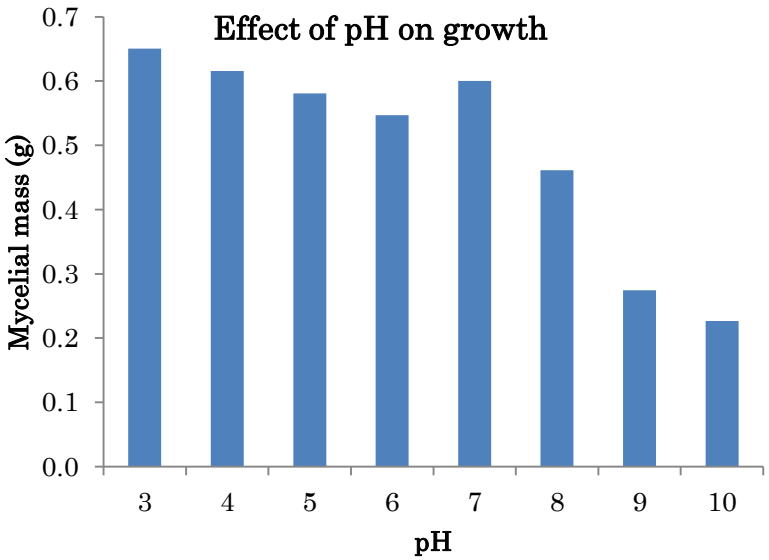

Figure 4. Effect of pH on growth of *Aspergillus* sp. isolated from rotten mandarin orange

The effect of pH on sporulation of the fungus presented in figure 5 shows that spore count decreases directly with the increase of pH. pH 5.0 produced maximum spores (6.30×10^7). The lowest amount of spore formation (1.14×10^7) was recorded at pH 10.0. This result is also supported by Abubakar *et al*.

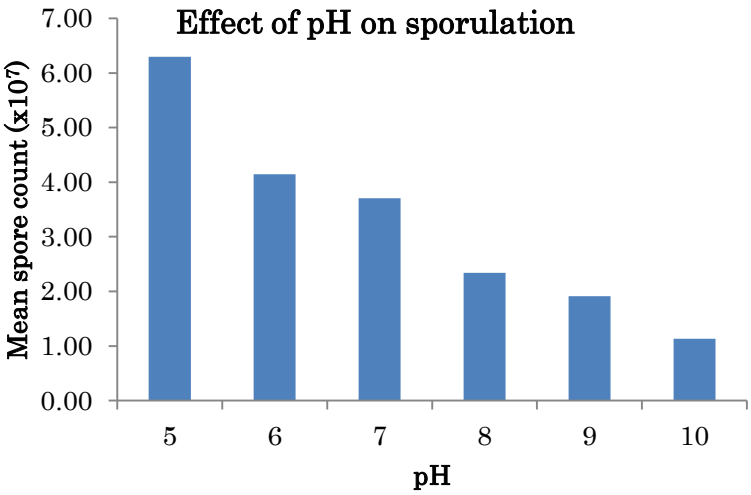

Figure 5. Effect of pH on sporulation of *Aspergillus* sp. isolated from rotten mandarin orange

Effect of benzoic acid on growth of the fungus is shown in the figure 6a. Growth inhibition of the fungus ranged from 48 to 100 percent for the concentrations used in the study. However inhibition pattern was not in ascending order for a particular concentration of benzoic acid with increase in incubation period. Using 100 µg/ml of benzoic acid maximum growth inhibition was observed after 5 days while for 200-500 µg/ml highest growth inhibition was seen after 3 days of inhibition. Complete inhibition of the fungal growth was seen even after 7 days of incubation at a concentration of 700µg/ml or higher.

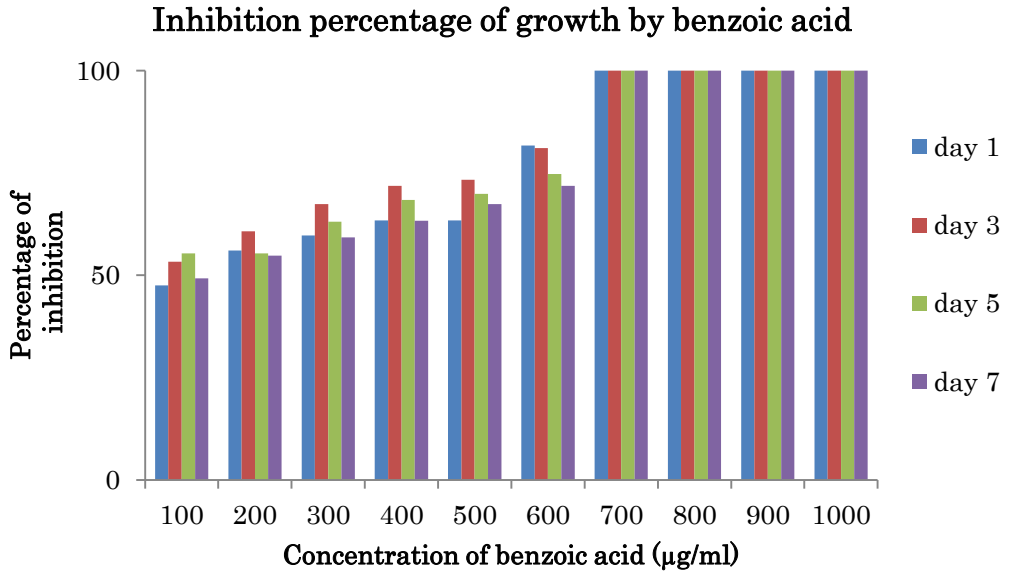

Figure 6a. The effect of benzoic acid on growth of the fungus isolated from rotten mandarin orange

Inhibition percentage of the fungus ranged from 0 to 100 percent using potassium metabisulphate (Figure 6b). No fungal growth was visible at or above 700 µg/ml potassium metabisulphate after 1 day of incubation. However, after 3 days of incubation, all plates showed visible fungal colony as

none of the working concentration completely inhibited fungal growth. 1000 µg/ml potassium metabisulphate caused maximum percentage of inhibition showing values of 76, 57 and 38 after 3, 5 and 7 days of incubation, respectively.

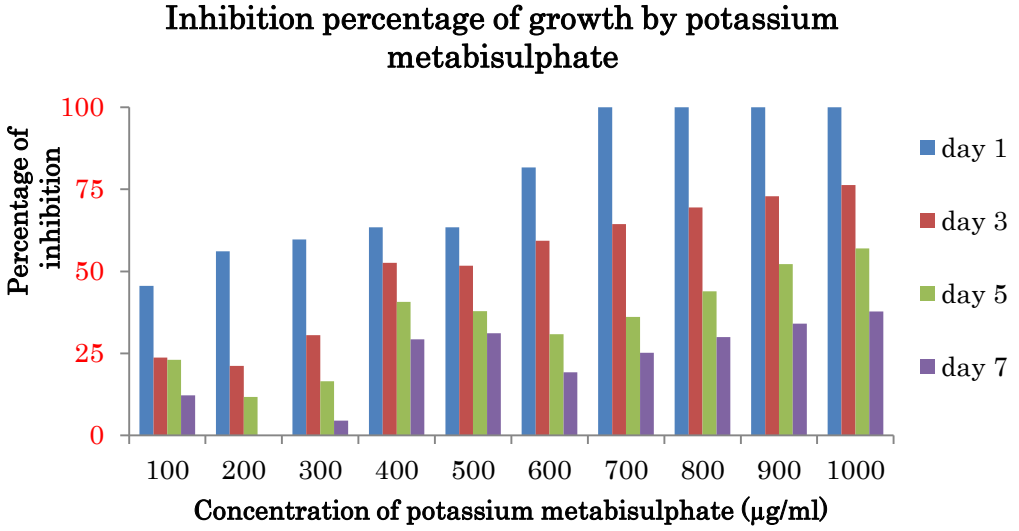

Figure 6b. The effect of potassium metabisulphate on growth of the fungus isolated from rotten mandarin orange

Inhibition percentage of growth using citric acid ranged from 46-100, 13-100, 42-100 and 11-100 after 1, 3, 5 and 7 days of incubation, respectively (Figure 7c).

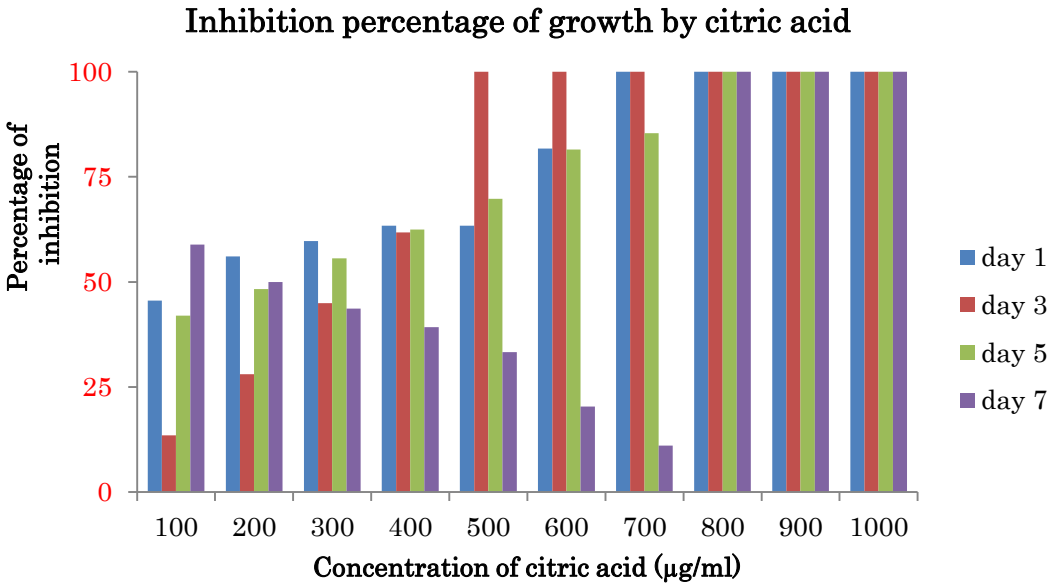

Figure 6c. The effect of citric acid on growth of the fungus isolated from rotten mandarin orange

Table 1. Minimum concentration (µg/ml) of antifungal agents for complete growth inhibition

Antifungal agent	Days of incubation			
	1	3	5	7

Benzoic acid	700	700	700	700
Potassium metabisulphate	700	>1000	>1000	>1000
Citric acid	700	500	800	800

So it can be concluded that *Aspergillus* sp. is the predominant fungal pathogen of rotten mandarin orange. The pathogen grows best at acidic to near neutral pH while sporulates maximally at acidic pH. Benzoic acid is the most potent chemical preservative to inhibit the growth of the fungus.

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