



In vitro INHIBITORY EFFECT OF CERTAIN CHEMICAL PRESERVATIVE AGENTS AGAINST AFLATOXIN PRODUCING *Aspergillus flavus* ISOLATED FROM INFECTED GROUNDNUT (*Arachis hypogoea* L.)

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ABSTRACT

Aflatoxins are most potent carcinogens in animal and human populations and interfere with the functioning of the immune system. In the present study, the fungi infected Groundnut (*Arachis hypogoea* L.) were collected from the farmer's field of Periya Olaipadi village in Tiruvannamalai district, Tamil Nadu,

India to check the presence of aflatoxin producing *Aspergillus flavus*. All the groundnut seeds were screened for per cent of fungal infection and aflatoxigenic fungi *Aspergillus flavus* MF1 and *Aspergillus flavus* MF2 strains which are responsible for aflatoxin production on food grains were isolated and characterized. The effect of various concentrations of chemical food preservatives like Sodium benzoate, Sodium chloride, Propionic acid and Sodium propionate on growth of aflatoxigenic fungi were studied in the present research against the aflatoxin producing *Aspergillus flavus*. From this present research, it was concluded that the mycelial growth of the aflatoxin producing



fungi *Aspergillus flavus* (MF1) and *Aspergillus flavus* (MF2) was decreased with increase in the concentration of Sodium propionate, Sodium benzoate, Sodium chloride and Propionic acid. We also recommend that the addition of chemical preservatives on the groundnut based packed food products will prevent the contamination caused by the toxic aflatoxin producing fungi *Aspergillus niger*.

Key words: Aflatoxin, *Aspergillus niger*, Sodium propionate, Sodium benzoate, Sodium chloride and Propionic acid.

1. INTRODUCTION:

Fungal deterioration of stored seeds and grains is a chronic problem in the Indian storage system because of the tropical hot and humid climate. *Aspergillus* sp. are the most common fungal species that can produce mycotoxins in food and feed stuffs. Mycotoxins are well known for their health-hazardous effects in human beings and animals. Mycotoxins are toxic secondary metabolites produced by fungi and contaminate various agricultural commodities either before harvest or under post-harvest conditions (Williams *et al.*, 2004). Most *Aspergillus flavus* and *Aspergillus parasiticus* can produce polypeptide derived secondary metabolites called aflatoxins which are highly toxic, mutagenic, and carcinogenic to animals. They may also be involved to some degree in primary liver cancer in humans. Aflatoxins have been implicated in hepato cellular carcinoma, acute hepatitis, Reye's syndrome, cirrhosis in malnourished children and kwashiorkor (Reddy *et al.*, 2009).

Preservatives are most commonly used to control mold growth in baked goods and help to reduce or prevent wastage of food through spoilage caused by microorganisms. Longer shelf life enables a greater variety of products to be kept in store and in the home. Sofos and Busta (1991) reported that the chemical preservatives can control the growth of molds by preventing the metabolism, by denaturing the protein of the cell, or by causing physical damage to the cell membrane. Propionic acid, an amino carboxylic acid ($\text{CH}_3\text{CH}_2\text{-COOH}$), is a naturally occurring organic acid and is an oily liquid with a slightly pungent, disagreeable rancid odour. Its salts are white, free-flowing powders with a slight cheese like flavour (Doores, 1993; Sung – Jo Kang *et al.*, 2001). Propionates were selected on the basis that higher MW fatty acids had a higher antimicrobial effect. This acid or its salt can be used to prevent the bacterial spoilage of bread known as rope caused by certain *Bacillus* sp. (Legan, 1993). Several studies have also reported the effects of propionic acid and its salt on mold growth. Concentrations of propionate ranging from 8 to 12 per cent have been reported effective in controlling mold growth on the surface of bakery products (Doores, 1993; Passone, 2007).

2. MATERIALS AND METHODS

2.1. Collection of aflatoxin contaminated Groundnut

The aflatoxin contaminated Groundnut (*Arachis hypogaea* L.) was collected from the farmer's field of Periya Olaijadi village in Tiruvannamalai district, Tamil Nadu, India for the present research.

2.2. Screening of Groundnut for per cent of fungal infection

2.2.1. Percent of fungal infection in Groundnut

As the storage fungi can withstand very high osmotic pressure, a high osmotic Sucrose medium was used as a selective medium for screening the oil seed Groundnut for percent of fungal infection. The groundnut was unsterilized and surface sterilized with 0.1 % mercuric chloride solution for 50 seconds and washed with 7.5 % sodium chloride were placed in the Sucrose medium by direct plating technique using sterile forceps, a market paper template i.e., blue print was kept under the plates for even distribution of seeds. The number of groundnut was 10 seeds respectively. The number of groundnut paced in the plates depended on the size of the seeds. The plates were incubated at room temperature at 30 °C and observed for the growth of colony for 3rd day to 5th day. The infection per cent of unsterilized and surface sterilized seeds were determined by using the below given formula.

$$\text{Infection percent} = \frac{\text{Number of seeds infected}}{\text{Total number of seed plated}} \times 100$$

2.3. Isolation and characterization of aflatoxigenic fungi

2.3.1. Isolation

The fungal cultures found in infected seeds were observed under low power and high power magnification of the compound microscope. Among the various species isolated, *Aspergillus flavus* was identified as predominant in the infected seeds and used for further studies. The per cent fungal infection was high in groundnut seeds and the aflatoxigenic fungal cultures were predominant on aflatoxigenic fungal culture were isolated using Sucrose medium from groundnut seeds and named as *Aspergillus flavus* MF1 and *Aspergillus flavus* MF2.

2.3.2. Morphological and microscopic examination of the aflatoxigenic fungal isolates

The fungal isolates plated on Sucrose medium to study morphological characters viz., colony colour, texture, colour on the reverse side of plates, colony diameter and morphological characters viz., morphological characters, conidia, conidial heads and phialides.

2.4. Effect of food preservatives on inhibition of mycelial growth

The inhibitory effect of different concentrations of various chemical preservatives (Sodium chloride, Propionic acid, Sodium benzoate and Sodium propionate) on the mycelial growth of *Aspergillus flavus* (MF1 and MF2) were studied in this present research. Sabouraud's Dextrose broth was prepared and 50 ml of the broth was transferred to 100 ml of Erlenmeyer flasks in different concentration. Various concentrations of food preservatives viz., 0.0 %, 1.0 %, 2.0 %, 3.0 %, 4.0 % and 5.0 % were tested in the present research for the inhibition of mycelial growth. The Sabouraud's dextrose broth was prepared and distributed in Erlenmeyer flasks with different concentration of chemical preservatives. The chemical preservatives inoculated Sabouraud's Dextrose Broth was inoculated with a 4 mm of *Aspergillus flavus* (MF1 and MF2) disc separately. After the incubation period, the Sabouraud's Dextrose broth was filtered and dry weight of the *Aspergillus flavus* mycelial mat was recorded.

3. RESULTS AND DISCUSSION

3.1. Screening of Groundnut seeds for percent of fungal infection

The per cent of fungal infection in surface sterilized and unsterilized Groundnut seeds were enumerated and the findings were furnished in Table - 1. Among the 10 groundnut seeds, the aflatoxin infection per cent in the surface sterilized groundnut seeds was 40.0 % and unsterilized groundnut seeds was 70.0 %. The fungal cultures found in infected seeds were observed under low power or high power magnification of the compound microscope and identified. Among the various species, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus* and *Penicillium sp.* were identified in the infected groundnut seeds and the majority of the contamination was caused by *Aspergillus flavus* so it was selected for the further research.

Table – 1: Per cent of fungal infection in Groundnut

Sample Seed	Number of seeds plate ⁻¹	Unsterilized seeds		Surface sterilized seeds		Mean percent of infection
		Number of seeds infected	Per cent of infection	Number of seed infection	Per cent of infection	
Groundnut	100	70.0	70.0	40.0	40.0	55.0

3.2. Isolation and characterization of aflatoxigenic fungal strains

The *Aspergillus flavus* found in infected groundnut seeds were observed under microscope. The fungi *Aspergillus flavus* was predominant in the infected seeds and isolated for further studies. The Aflatoxigenic *Aspergillus flavus* MF1 and *Aspergillus flavus* MF2 strains were isolated from groundnut seeds using the selective medium and examined for colony morphology and conidial characters and are presented. Based on the morphological characters viz., colony colour (yellow green becoming brownish with age), texture (velvety), colour on reverse side of the plates (cramish yellow), colony diameter (4.0 to 4.8 cm) and through microscopic examination of conidial characters such as conidiophores (coarsely roughened), conidial (globose and single nucleate without side roughened walls), conidial heads (split into loosely organising), phialides (uniseriate in arrangement) the fungal isolate MF1 and MF2 strains were identified as *Aspergillus flavus*.

3.3. Effect of food preservatives on the inhibition of *Aspergillus flavus* growth

3.3.1. Effect of Sodium propionate on *Aspergillus flavus* growth

The effect of different concentrations of Sodium propionate (0.0 to 2.5 %) on the inhibition of growth and aflatoxin production by *Aspergillus flavus* (MF1 and MF2) stains were studied and the results are presented in Fig - 1. The mycelial growth and release of aflatoxin of *Aspergillus flavus* (MF1 and MF2) were influenced by various concentration of the Sodium propionate ranging from (0 % to 2.5 %) the reaction of mycelia growth was recorded in the strains tested. Highest *Aspergillus flavus* mycelial growth (MF1 - 320 mg and MF2 - 305 mg) was observed at 0.0 per cent because of the absence of preservatives and the *Aspergillus flavus* mycelial lower growth (MF1 - 180 mg and MF2 - 172 mg) was recorded at 2.5 per cent due to the maximum concentration of sodium propionate.

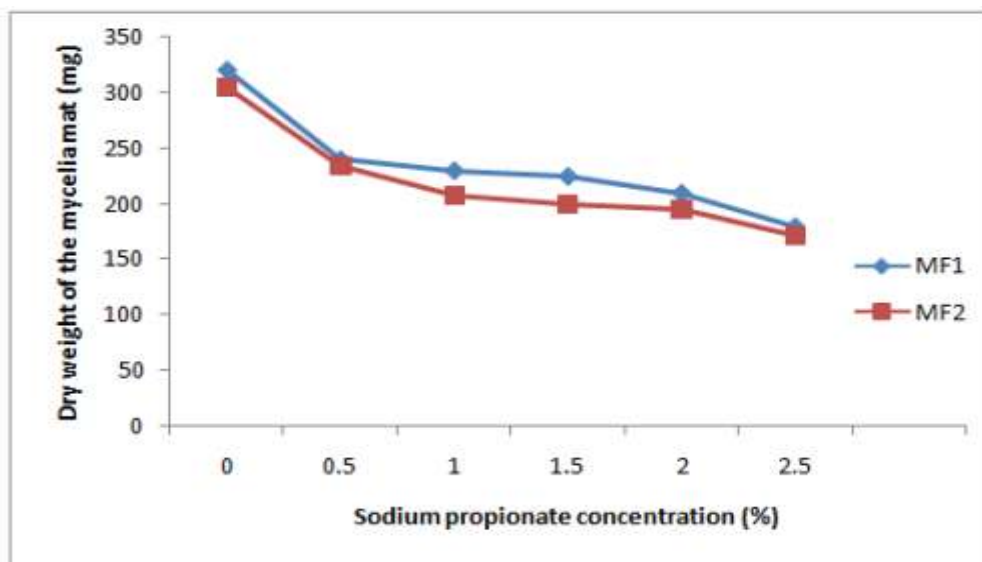


Fig – 1: Effect of Sodium propionate on inhibition of *Aspergillus flavus* mycelial growth

The growth of the *Mucor* sp. was highly controlled when compared to *Rhizopus stolonifer*, *Aspergillus niger* and *Penicillium chrysogenum*. The results clearly showed that the Calcium propionate was effective in the control of food product spoilage fungi (Grundy, 1996). Sauer and Burrughs (1993) observed that the mold free shelf life of corn was extended for 1 week by 0.5 - 1.0 per cent calcium propionate. In the United States, preservation of food products for a few days was generally accomplished by the addition of sodium or calcium propionate. However, some species of *Penicillium* are resistant and can grow in media containing 5 per cent propionic acid (Doores, 1993; Prem Kumar et al., 2015).

3.3.2. Effect of Sodium benzoate on *Aspergillus flavus* mycelial growth

The effect of different concentrations of Sodium benzoate (0 % to 5.0 %) on the inhibition of growth and aflatoxin production of *Aspergillus flavus* (MF1 and MF2) stains were studied and the results are presented in Fig – 2. The *Aspergillus flavus* mycelial growth and release of aflatoxin of *Aspergillus flavus* (MF1 and MF2) were influenced by various concentration of the Sodium benzoate ranging from the reaction of *Aspergillus flavus* mycelia growth was recorded in the strains tested. The higher mycelia growth (MF1 - 305 mg and MF2 - 360 mg) was found at 0.0 per cent and lower *Aspergillus flavus* growth (MF1 - 105 mg and MF2 - 102 mg) was recorded at 5.0 per cent. Thanaboripat et al. (1996) and Ogjehor and Ikenebomeh (2004) reported that the sodium benzoate at 10 µg kg⁻¹ reduced the growth of *Aspergillus flavus* by 13 per cent. Ali and Sayed (2000) reported that the aflatoxin B1 produced by *Aspergillus flavus* in Sabouraud's dextrose broth was completely inhibited at 0.33 per cent of Sodium benzoate.

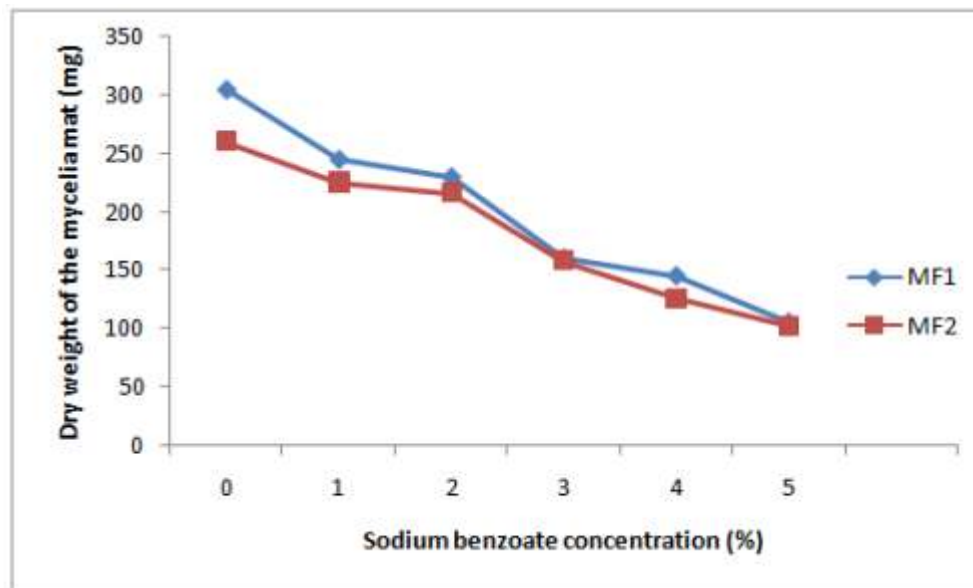


Fig - 2: Effect of Sodium benzoate on inhibition of *Aspergillus flavus* mycelial growth

3.3.3. Effect of Sodium chloride on *Aspergillus flavus* mycelial growth

The effect of different concentrations of Sodium chloride (0 % to 5.0 %) on the inhibition of *Aspergillus flavus* mycelial growth and aflatoxin production of fungal MF1 and MF2 strains were studied and the results were presented in Fig – 3. The *Aspergillus flavus* mycelial growth and release of Aflatoxin by *Aspergillus flavus* (MF1 and MF2) strains were influenced by various concentrations of Sodium chloride tested with increase in the concentration of Sodium chloride ranging from 0 % to 5.0 %. The

reduction of *Aspergillus flavus* mycelial growth was recorded in the strains tested. Higher *Aspergillus flavus* mycelial growth (MF1 - 330 mg and MF2 - 308 mg) was found at 0.0 per cent and lower growth (MF1 - 150 mg and MF2 - 125 mg) was recorded at 5.0 per cent. The result of the present research was similar with the findings of Reddy et al. (2009); Saranraj and Geetha (2012); Darwina et al. (2012); Prem Kumar et al. (2015).

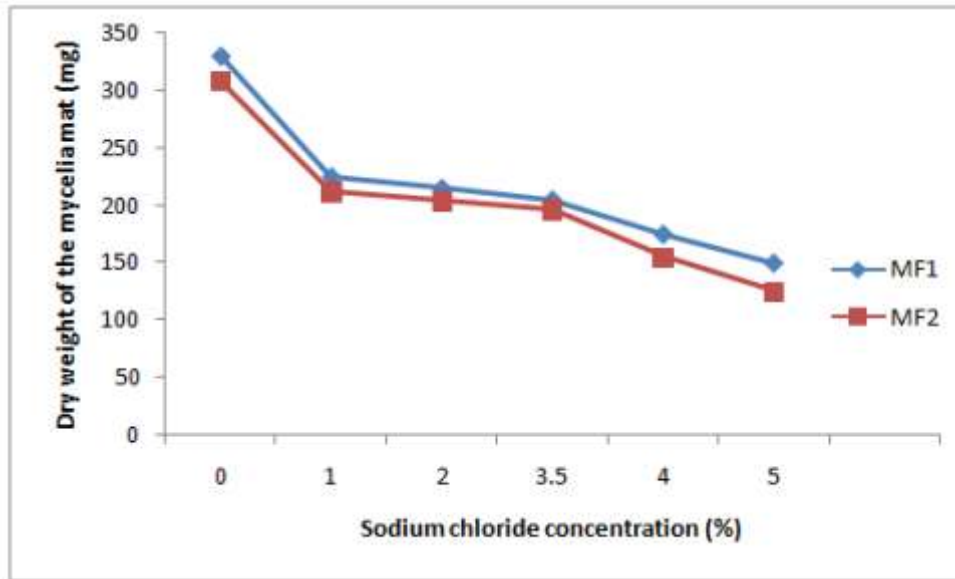


Fig – 3: Effect of sodium chloride on inhibition of mycelial growth

3.3.4. Effect of Propionic acid on mycelial growth

The effect of different concentrations of Propionic acid (0 % to 5.0 %) on the inhibition mycelial growth of the fungal strain MF1 and MF2 were studied and the results were presented in Fig - 4. The mycelial growth and release of aflatoxin by *Aspergillus flavus* (MF1 and MF2) were influenced by various concentrations of Propionic acid from 0.0 % to 5.0 %. The increase in concentration reduced the *Aspergillus flavus* mycelial growth and aflatoxin production in the strains tested. Higher mycelia growth (MF1 - 305 mg and MF2 - 290 mg) was found at 0 % and lower growth (MF1 - 165 mg and MF2 - 150 mg) was recorded at 5.0 %. Preservation potential and fungicidal activity of propionic acid have been demonstrated by Odoemelum and Osu (2009); Jageethadevi et al. (2012); Kanchana et al. (2015).

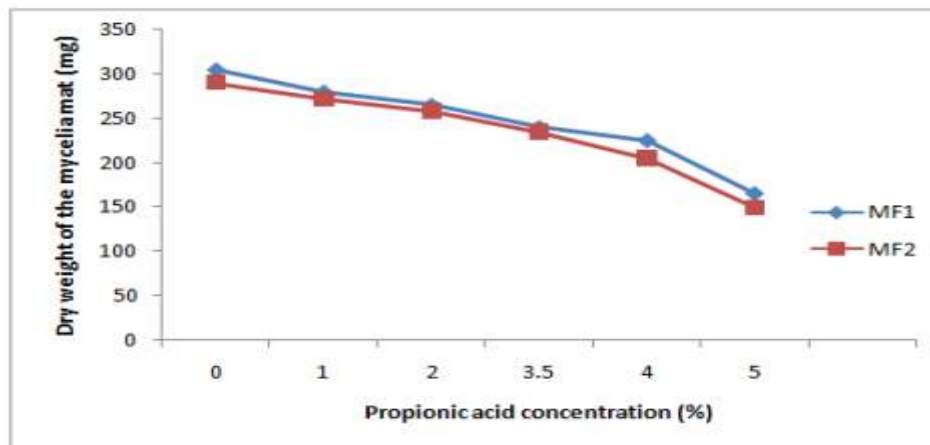


Fig – 4: Effect of Propionic acid on inhibition of mycelial growth

4. CONCLUSION

From this present research, it was concluded that the fungi *Aspergillus flavus* was frequently found in the fungi infected groundnut. It was also concluded that the mycelial growth of the aflatoxin producing fungi *Aspergillus flavus* (MF1) and *Aspergillus flavus* (MF2) was decreased with increase in the concentration of various chemical preservatives like Sodium propionate, Sodium benzoate, Sodium chloride and Propionic acid. We also recommend that the addition of chemical preservatives on the groundnut based packed food products will prevent the contamination caused by the toxic aflatoxin producing fungi *Aspergillus niger*.

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