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# Effect of different chemical seed sterilizing agents on Aspergillus flavus

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#### Abstract

Seven different chemicals *viz.*, sodium hypo chloride, sodium bicarbonate, calcium chloride, sodium chloride, boric acid, propionic acid and neem leaf extract were used as seed sterilizing agents to study their effect on *Aspergillus flavus*. The seeds of groundnut variety GG-20 (susceptible) were treated with different chemicals and inoculated with spore suspension of *A. flavus* were observed for per cent seed infection, per cent seed germination and per cent mortality of germinated seeds. Neem leaf extracts and propionic acid were considered as better chemical seed sterilizing agents for reduction of seed infection. These also resulted in higher seed germination and less mortality of germinated seeds.

Keywords: Aspergillus flavus, groundnut, seed sterilizing agents

#### Introduction

Groundnut is the cash crop in many Asian countries. It plays an important role in the diets of rural populations because of its high contents of protein (21-30%), oil (41-52%), and carbohydrate (11-27%). It is also rich in calcium, potassium, phosphorus, magnesium and vitamin E. Protein meal, a by-product of oil extraction, is an important ingredient in livestock feed. Groundnut haulms are nutritious and widely used for feeding livestock.

Groundnut crop suffers from many pathogens. Many workers have detected different mould fungi and their toxin production ability in stored grains, which deteriorate the stored products (Afzal *et al.*, 1979 and Vedahayagam *et al.*, 1989) <sup>[1, 11]</sup>. Among them, *Aspergillus flavus* is the most serious disease causing mould fungus at pre- and post- harvest/storage of crop, and is the most common species in Africa and Asia (Clinton, 1960) <sup>[3]</sup>. The toxin of *A. flavus* species belongs to a highly toxic group of mycotoxins known as aflatoxin. *A. flavus* is present throughout the groundnut growing areas in the world. *A. flavus* causes the yellow mould disease and also causes the disease – *A. flavot*, which is associated with the presence of toxins produced by *A. flavus*.

Aflatoxin producing fungi, *A. flavus*, can infect groundnut during the crop season and even after harvest. Pre-harvest infection by *A. flavus* and consequent aflatoxin contamination is more important in the semi-arid tropics, especially when drought occurs just before harvest. Poor harvesting and storage conditions can lead to rapid development of the fungi and thus high production of the toxin. Groundnut is also stored (as shelled seed) in poorly ventilated areas, which favor fungal development. Since, there is meager genetic tolerance and recommended cultural practices, which inhibit A. flavus growth and aflatoxin contamination in preharvest groundnut, efforts are being made to prevent this problem after harvest using new antifungal materials from natural sources and chemical preservatives (Onyeagba *et al.*, 2004 and Haciseferogullary *et al.*, 2005)<sup>[8, 5]</sup>. In the present investigation an attempt has been made to find out best seed sterilizing chemical for reduction of *Aspergillus flavus* infection.

#### **Materials and Methods**

The seeds of groundnut variety GG-20 (susceptible) were used to test the effect of different chemical seed sterilizing agents on *A. flavus*. Ten healthy seeds were dipped into different chemicals *viz.*, sodium hypo chloride, sodium bicarbonate, calcium chloride, sodium chloride, boric acid, propionic acid and neem leaf extract at different concentrations for 5 minutes. These were then treated with 5 ml of standardized spore suspension of *A. flavus* ( $10^6$  spores/ml) and were placed on blotter paper. The seeds were observed after ten days of inoculation for per cent seed infection, per cent seed germination and per cent mortality of germinated seeds. Untreated seeds were used as control.

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#### **Results and Discussion**

The seeds of groundnut variety GG-20 were used to test the effect of different chemical seed sterilizing agents at different concentrations *viz.*, sodium hypochlorite (0.1, 0.2, 0.3, 0.4%), sodium bicarbonate (0.5, 1.0, 1.5, 2.0%), calcium chloride (0.5, 1.0, 1.5, 2.0%), sodium chloride (1.0, 1.5, 2.0, 2.5%), boric acid (2.0, 2.5, 3.0, 3.5%), propionic acid (3.0, 4.0, 5.0, 6.0%) and neem leaf extract (1.0, 2.0, 3.0, 4.0%) through blotter paper technique. After 10 days of incubation the data for various parameters *viz.*, percent seed infection, percent seed germination and percent mortality of germinated seeds was recorded and are presented in Table 1.

# Seed infection (%)

The data on per cent seed infection recorded after 10 days of incubation revealed that there was a significant difference among different treatments. Significantly lower seed infection (36.67%) was recorded with sodium hypochlorite at 0.40% concentration which was statistically at par with propionic acid 6.00% and neem leaf extract 4.00% (46.67%); whereas maximum seed infection was recorded with 1.00% sodium chloride (93.33%).

The data also indicated that there was a reduction in seed infection with increase in concentration of all the seed sterilizing agents.

# Seed germination (%)

The data on per cent seed germination recorded after 10 days of incubation revealed that there was a significant difference among different treatments. Maximum seed germination (76.67%) was recorded when neem leaf extract was used at 4.00% concentration, followed by seed germination with 3.00% neem leaf extract, 6.00% propionic acid, 0.10% sodium hypochlorite, and 0.50% calcium chloride respectively. However, minimum seed germination (20.00%) was recorded with 0.50% sodium bicarbonate, which was statistically at par with 2.50% sodium chloride, showing germination of 30.00%.

The data also revealed that seed germination increased with increase in concentration of sodium bicarbonate, propionic acid and neem leaf extract; while adverse effect on seed germination was recorded with sodium hypochlorite, calcium chloride, sodium chloride and boric acid treatments.

# Mortality of germinated seeds (%)

The data on per cent seed mortality of germinated seeds revealed significant difference among different treatments. The minimum seed mortality (24.44%) was recorded with 0.40% sodium hypochlorite, which was statistically at par with neem leaf extract at 4.00% (30.36%), 3.00% (31.75%), 2.00% (37.78%), propionic acid 6.00% (31.75%), sodium chloride 2.00% (38.89%), 2.50% (36.11%), 1.50% (44.44%), calcium chloride 0.50% (36.51%) and sodium bicarbonate 2.00% (41.11%). The maximum seed mortality of germinated seed was recorded with propionic acid 3.00% (85.00%), which was statistically at par with boric acid 3.50% (75.56%), sodium bicarbonate 0.50% (72.22%),and sodium hypochlorite 0.10% (79.37%).

Overall data indicates that the seed treated with neem leaf extracts at 4.00% concentration had less seed infection, higher seed germination and low mortality of germinated seeds; followed by propionic acid at 6.00% concentration.

These findings are confirmed by the results of Kavita and Reddy (2000) [6], who reported that sodium chloride and propionic acid completely inhibited the aflatoxin production and had no adverse effect on the germination and viability of groundnut and maize seeds. Patkar et al. (1995)<sup>[9]</sup> showed comparatively greater reduction in incidence of A. flavus by propionic acid treatment during the storage of rice, sorghum and groundnut. Mukherjee and Nandi (1998) [7] tested the effect of Allyl-isothiocyanate and propionic acid on the seeds of maize, groundnut and soyabean. Phytotoxicity was recorded with allyl-isothiocyanate treated seed at the lowest concentration whereas; phytotoxicity was fairly low in propionic acid treated seed even at the higher concentration. Verma *et al.* (2000) <sup>[12]</sup> showed that propionic acid was the most effective in the prevention of the growth of A. *flavus* and subsequent aflatoxin content. Vaidya and Vir (1989)<sup>[10]</sup> noted that post harvested spray and dip treatment with propionic acid and sodium metabisulfite were effective in controlling A. niger and A. flavus. Bansal and Sobti (1990)<sup>[2]</sup> concluded that Azadirachta indica leaf extract reduced the incidence of A. flavus and increased seed germination in vitro. Salts, propionic acid, asafoetida and Azadirachta indica leaf extract were better in preventing seed infection and aflatoxin contamination in groundnut (Ghewande and Nagaraj, 1987)

 Table 1: Effect of different chemical seed sterilizing agents on seed infection (%), seed germination (%) and mortality of germinated seeds (%)

 by A. flavus on groundnut variety GG-20 after 10 days of incubation\*

Sr. No.	Name of chemical	Conc. %	Seed infection (%)	Seed germination (%)	Mortality of germinated seeds (%)
1	Sodium hypo chlorite	0.10%	68.83 (86.67)	52.75 (63.33)	63.14 (79.37)
		0.20%	50.83 (60.00)	44.98 (50.00)	55.15 (67.22)
		0.30%	46.90 (53.33)	43.06 (46.67)	44.98 (50.00)
		0.40%	37.21 (36.67)	41.05 (43.33)	29.45 (24.44)
2	Sodium bicarbonate	0.50%	66.12 (83.33)	26.06 (20.00)	63.22 (72.22)
		1.00%	63.41 (80.00)	37.21 (36.67)	48.23 (55.56)
		1.50%	58.98 (73.33)	39.13 (40.00)	46.30 (52.22)
		2.00%	56.97 (70.00)	48.83 (56.67)	39.82 (41.11)
3	Calcium chloride	0.50%	56.77 (70.00)	52.75 (63.33)	37.12 (36.51)
		1.00%	46.90 (53.33)	48.83 (56.67)	43.06 (46.67)
		1.50%	46.90 (53.33)	48.83 (56.67)	50.15 (58.89)
		2.00%	43.06 (46.67)	44.98 (50.00)	51.90 (61.67)
4	Sodium chloride	1.00%	81.11 (93.33)	39.13 (40.00)	50.15 (58.89)
		1.50%	61.20 (76.67)	37.21 (36.67)	41.74 (44.44)
		2.00%	61.20 (76.67)	35.20 (33.33)	38.49 (38.89)
		2.50%	54.76 (66.67)	32.99 (30.00)	36.74 (36.11)
5	Boric acid	2.00%	63.41 (80.00)	44.98 (50.00)	43.06 (46.67)
		2.50%	61.20 (76.67)	43.06 (46.67)	48.83 (56.67)
		3.00%	58.98 (73.33)	41.14 (43.33)	51.90 (61.67)

	3.50%	56.97 (70.00)	39.13 (40.00)	65.14 (75.56)
	3.00%	66.21 (83.33)	41.14 (43.33)	71.12 (85.00)
Propionic acid	4.00%	61.20 (76.67)	44.98 (50.00)	50.75 (60.00)
	5.00%	56.97 (70.00)	48.83 (56.67)	46.90 (53.33)
	6.00%	43.06 (46.67)	52.75 (63.33)	34.26 (31.75)
	1.00%	66.12 (83.33)	43.06 (46.67)	44.98 (50.00)
Neem leaf extract	2.00%	52.75 (63.33)	46.90 (53.33)	37.89 (37.78)
	3.00%	46.90 (53.33)	52.75 (63.33)	34.26 (31.75)
	4.00%	43.06 (46.67)	61.20 (76.67)	33.34 (30.36)
Control		89.96 (100.00)	21.14 (13.33)	89.96 (100.00)
S.Em.±		3.12	2.65	4.65
C.D. at 5%		8.89	7.56	13.27
C.V.%		9.41	10.61	16.77
	Neem leaf extract Control S.Em.± C.D. at 5%	Propionic acid         3.00%           4.00%         5.00%           6.00%         6.00%           1.00%         2.00%           3.00%         4.00%           Control         3.00%           S.Em.±         C.D. at 5%	$\begin{array}{c ccccc} & 3.00\% & 66.21 & (83.33) \\ \hline & 3.00\% & 61.20 & (76.67) \\ \hline & 5.00\% & 56.97 & (70.00) \\ \hline & 6.00\% & 43.06 & (46.67) \\ \hline & 1.00\% & 66.12 & (83.33) \\ \hline & 1.00\% & 66.12 & (83.33) \\ \hline & 2.00\% & 52.75 & (63.33) \\ \hline & 3.00\% & 46.90 & (53.33) \\ \hline & 4.00\% & 43.06 & (46.67) \\ \hline & & 89.96 & (100.00) \\ \hline & & S.Em.\pm & & 3.12 \\ \hline & C.D. at 5\% & & 8.89 \\ \hline \end{array}$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

\* Average of three replication

Figures in parenthesis are retransformed value

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