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Studies on aflatoxin contamination in copra and post-harvest management of copra quality

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Abstract: Post harvest management of copra quality is a challenging task owing to the improper handling of copra by farmers/ traders, lack of awareness on mycotoxin contamination and also to the consequent health hazards due to metabolites produced by the harbored molds on copra. Improper handling of copra after harvest is of concern in the present day coconut scenario due to the threat posed by aflatoxin producing molds that infest copra, thus spoiling the healthy food chain and subsequently the coconut trade.
Key words: Mycoflora, Coconut, contamination

Introduction

Coconut (*Cocos nucifera. L.*) is an important plantation crop in India. The crop is being grown in an extent of 18, 39,800 ha with a production of 12,597.3 nuts annually. Among different States, Andhra Pradesh ranks fourth in area and in production with respect to coconut cultivation in our country (Directorate of Economics & Statistics, AP). The crop is often called 'Kalpavriksha' due to its multifarious uses. However, the economic end product *i.e.*, copra is the source of coconut trade in the country. Copra is a source for oil, cake and other edible products. The copra is usually dried by air-drying, forced air-drying and by kiln drying methods. However, improper handling of any of these methods may lead to contamination with mycoflora that deteriorate the quality of copra and thus trade.

Awareness on aflatoxin contamination

Opinion survey on traders' awareness regarding mycoflora responsible for copra rotting and possible aflatoxin contamination in East Godavari district of Andhra Pradesh revealed that all the traders (100%) were aware of the fact that mycoflora play a vital role in rotting of copra. Of them, 72% of the traders are unaware that copra can be contaminated by aflatoxin. They mostly opined that lack of visual indication on the copra is the sole reason for their being unaware of aflatoxin contamination. On the other hand, the remaining (28%) traders attributed the bitterness in taste of copra to the possible aflatoxin contamination due to molds. The dialogue on copra deterioration due to molds revealed that all the traders are well aware of the fact that copra quality is dictated by the extent of mold infection under improper storage conditions. Most of the traders felt that extraction of copra will be carried out at farmers' level and so the chances of mold infestation will be more since the farmers are unaware of the fact that improper drying would lead to fungal contamination.

Association of mycoflora with copra

Isolation studies revealed that Aspergillus flavus, A. niger, Rhizopus spp, Drechslera spp, Botryodiplodia spp and Penicillium spp are the commonly associated mycoflora on copra during storage (Table-1 & Plate-1). Aspergillus flavus was predominant among the mycoflora with percent colonies ranging from 68 to 92 per each sample. This is followed by Penicillium spp with a range of 61 to 69 per cent colonies. Aspergillus niger was recorded to a tune of 46 to 64 per cent colonies whereas other species of Aspergillus were recorded to an extent of 47 to 57 per cent. It is a known fact that copra stored under different moisture conditions show different mycoflora on their surface.

Table-1: Nature and extent of fungal infection on copra collected from traders, East Godavari district, Andhra Pradesh

Fungus	Number of colonies /sample (%)
Aspergillus flavus	68 – 92
Penicillium spp	61 – 69
Aspergillus niger	46 – 64
Aspergillus spp	47 – 67
Rhizopus spp	33 – 44
Drechslera spp	20 – 31
Botryodiplodia spp	11 – 18

Plate-1: Mycoflora associated with copra during improper storage condition

Under high moisture conditions, *Aspergillus* spp rapidly penetrate into the copra meat causing its discoloration, thereby resulting an increase in free fatty

acid levels. On the other hand, copra stored at 5% moisture levels remained moderately free from infection (Susamma & Menon, 1983). Monsoon periods favor the development of fungal population in copra (Susamma *et.al.,* 1980*).* The ill effects of consuming aflatoxin tainted cake and oil have been mainly observed in poultry and milch cattle (Bhat *et. al.* 1978). It is even recognized that when animals consume feed contaminated with aflatoxins, the toxin is metabolized in the body and possibly get into the milk as aflatoxin M1, or may be found in animal products such as meat, eggs etc, which are consumed by man. So, building up of awareness among traders, retailers' regarding aflatoxin contamination as a negative common property resource is essential. Stringent legislative measures have to be enforced so as to make the traders store their collected copra under proper conditions thereby preventing the rot from these aflatoxin producing molds.

***In vitro* antagonistic studies:** Dual culture studies carried out with the three species of *Trichoderma* isolated from soils of coconut gardens revealed that *T.viride, T.harzianum* and *T.hamatum* were found very effective in inhibiting the mycelial growth of *A.flavus* under in vitro conditions (Plate-2). Of them, *T.hamatum* was found very effective in controlling both the isolates. This was followed by *T.harzianum* and *T.viride* with insignificant differences in percent of inhibitions (Table-2). A clear inhibition zone was noticed with all the three *Trichoderma* species and the inhibition zone prevailed up to 1 week. This was followed by mycoparasitism. *Trichoderma* spp are well-known biocontrol agents against several soil borne plant pathogens and are widely used under field conditions to combat aflatoxin problems in groundnut.

Table-2: Dual culture studies between *Trichoderma* spp and *A.flavus*

Antagonist	Per cent inhibition of A.flavus copra isolate	Remarks
Trichoderma viride	84.4b	Inhibition zone was observed after 4 days followed by mycoparasitism.
T.harzianum	83.3[b]	
T.hamatum	88.8[a]	

* Numbers in each column followed by the different letter are significantly different.

Values represent the means of 6 replicates.

Plate-2: *In vitro* inhibition of linear spread of A.flavus by *Trichoderma* spp.

Further *in vitro* studies carried out to determine the efficacy of the *Trichoderma* spp against *A.flavus* isolates revealed that volatile metabolites of 30 day old cultures of *T.viride, T.harzianum* and *T.hamatum* were inhibitory to *A.flavus*. While, 0 and 15 day old cultures of all the three *Trichoderma* spp were infective in inhibiting the mycelial growth of *A.flavus* through volatile metabolites (Plate-3). Among the *Trichoderma* spp of 30 days old, maximum inhibition of *A.flavus* isolates was obtained with *T.viride* (66.67%), followed by *T.harzianum* and *T.hamatum* with an inhibition of 61.11% (Table-3).

In case of non-volatile metabolites, an increasing trend of inhibition of *A.flavus* was noticed with an increase in the concentration of culture filtrate of the *Trichoderma* spp Among the *Trichoderma* spp, maximum inhibition of the mycelial growth of *A.flavus* was obtained by *T.hamatum* (57.6%) (Plate-4), followed by *T.harzianum* (42.3%) and *T.viride* (30.7%) at 100% concentration of the culture filtrate (Table-5). However, inhibition of the test fungus to a notable extent was also achieved by all the three *Trichoderma* spp at culture filtrate concentrations of 75%, 50% and 20% respectively.

Plate-3: Antagonistic activity of *Trichoderma* spp on *A.flavus* by productive of volatile metabolites

Plate-4: Antagonistic activity of *Trichoderma* spp on *A.flavus* by productive of non-volatile metabolites

Table-3: *In vitro* inhibition effect of volatile metabolites of *Trichoderma* spp on *A.flavus*

Antagonist	Per cent inhibition of ***A.flavus***		
	Age of ***Trichoderma*** spp		
	0 days	15 days	30 days
T.viride	0	0	66.67[a]
T.harzianum	0	0	61.11[b]
T.hamatum	0	0	61.11[b]

* Numbers in each column followed by the different letters are significantly different.

Values represent the mean of 6 replicates.

Table-5: *In vitro* inhibition effect of non-volatile metabolites of *Trichoderma* spp on *A.flavus*

Antagonist	Per cent inhibition of ***A.flavus***				
	Concentration of culture filtrate of antagonist				
	10%	20%	50%	75%	100%
T.viride	3.81[b]	7.6[c]	11.5[c]	30.7[c]	30.7[c]
T.harzianum	3.84[b]	23.0[b]	23.0[b]	38.4[b]	42.3[b]
T.hamatum	19.2[a]	34.6[a]	46.1[a]	48.0[a]	57.6[a]

* Numbers in each column followed by the different letters are significantly different.

Values represent the mean of 6 replicates.

Studies on the inhibition effect of chemical preservatives viz., Menadione, Potassium meta bisulphite, Benzoic acid, Sodium benzoate, L-Ascorbic acid, Propionic acid and Acetic acid (glacial) on A.flavus strains (AF2 --- Highly virulent

and Aggressive strain, AF3 ------- Moderately virulent strain) revealed that all the assayed chemicals reduced the linear growth of the aflatoxin producing molds from moderate to significant levels at 500ppm concentration and also to a certain extent at 100ppm concentration (Table-6). A significant positive correlation was noticed among majority of the chemicals with respect to increase in dosage from 100ppm to 500ppm with respect to A.flavus strain inhibition in terms of linear growth (Plate-5). The inhibition of linear growth of the virulent, aggressive strain of A.flavus (AF2) ranged from 6.67 % to 100%, whereas, the other moderately virulent strain, AF3 of A.flavus was inhibited to an extent of 7.00% to 100%. Since, chemicals are targeted against all the strains i.e. right from aggressive to moderately to less aggressive strains, the current discussion on the average percent inhibition of A.flavus is apt in the present study. Among the different chemicals assayed, maximum inhibition of A.flavus strains was obtained by Menadione (100%) followed by Potassium meta bisulphate and Benzoic acid with inhibition of 77.23% and 63.33% respectively. The preservatives, Sodium Benzoate and Ascorbic acid also performed well in inhibiting the A.flavus strains by more than 50% i.e. 57.78% and 53.89% respectively. However, the efficacy of Propionic acid is also notable with an inhibition of 43.33% on A.flavus strains. On the other hand, Glacial acetic acid had a mild inhibitory effect with an inhibition of 6.84% on A.flavus strains. The same chemical preservative even did prove ineffective against both the aflatoxin producing molds at 100ppm with no inhibitory effect (Table-6).

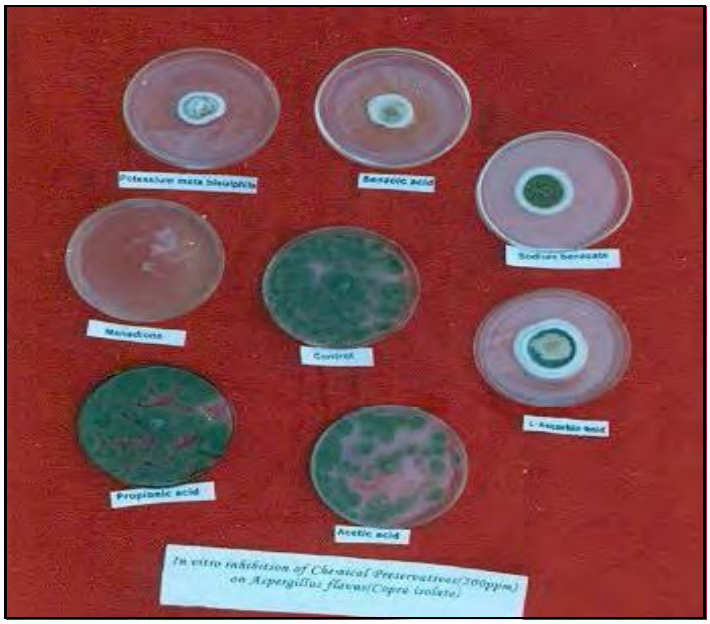


Plate-5: *In vitro* inhibition of chemical preservation on the linear growth of *A.flavus* at 500 ppm concentration

Table-6: Effect of chemical preservatives on the linear growth of *Aspergillus flavus* strains on PDA incubated at 28°c for 96 hrs.

Chemical	AF_2 (*A.flavus*)		AF_3 (*A.flavus*)		Average inhibition (%)
	% Inhibition at		% Inhibition at		of *A.flavus* strains at
	100 ppm	500 ppm	100 ppm	500 ppm	500 ppm
Menadione	77.78(20[a])	100(0[a])	85.56(13[a])	100(0[a])	100
Potassium meta bisulphite	60.00(36[b])	77.78(20[b])	64.44(32[b])	76.67(21[b])	77.23
Benzoic acid	54.44(41[c])	64.44(32[c])	58.89(37[c])	62.22(34[c])	63.33
Sodium benzoate	54.44(41[c])	53.33(42[d])	54.44(41[d])	62.22(34[c])	57.78
L-Ascorbic acid	55.55(40[c])	53.33(42[d])	53.33(42[d])	54.44(41[d])	53.89
Propionic acid	0.00(90[d])	22.22(70[e])	0(90[e])	64.44(32[c])	43.33
Acetic acid glacial	0.00(90[d])	6.67(84[f])	0(90[e])	7.00(83.7[e])	6.84
Control	(90mm)				

* Means followed by similar letters are not different statistically (P = 0.05) by Duncan's multiple range test. ** Values in parentheses are the linear growths of fungi in millimeters.

Post-harvest management of copra quality is a challenging task owing to the improper handling of copra by farmers/ traders, lack of awareness on mycotoxin contamination and also to the consequent health hazards due to metabolites produced by the harbored molds on copra. Improper handling of copra after harvest is of concern in the present day coconut scenario due to the threat posed by aflatoxin producing molds that infest copra, thus spoiling the healthy food chain and subsequently the coconut trade. Among the mycoflora that invade copra, *Aspergillus flavus* Link contamination is a potential hazard to the coconut industry and trade because of its secondary metabolite production, the aflatoxins. Aflatoxins are secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* Speare that are carcinogenic, hepato-toxic and teratogenic in nature (Coulibaly, 1987). There are several reports of aflatoxin contamination of copra. Phillippine coconut industry which exports copra cake used as feed ingredient in Europe, identified Aflatoxin B_1 level to a tune of

7-70 ppb and therefore gave a regulation that any move by European country to restrict the level of Aflatoxin B1 in feed stuffs would pose difficulty for the local coconut product given the climatic conditions in the country. Entry of aflatoxins into food chain is considered to be the most devastating problem not only through coconut, but also through cereal grains, spices, dry fruits, pulses, vegetables, cheese, bread and oil seeds; which are the prominent aflatoxin producing potential sources of *A.flavus* and *A.parasiticus* (Mc Donald, 1976).

Detoxification of coconut products that were prone and contaminated by aflatoxins *viz.,* coconut milk, coconut jam, coconut syrup, coconut skim milk, coconut flour, dessicated coconut (products from kernel) and coconut oil, coconut cake (products of copra) is a futile exercise as none of the detoxification procedures available can detoxify to the fullest extent. Detoxification methods of the present day include Ammoniation, Photolysis (Shantha and Sreenivasamurthy, 1977), extraction with sodium chloride (Shantha and Sreenivasamurthy, 1975), filtration (Basappa and Sreenivasamurthy, 1979), by hydrogen peroxide, by sunlight (Shantha *et.al.,* 1986), urea and formaldehyde (Codifer *et.al.,* 1976) are widely used against groundnut aflatoxin contamination (Read, 1989). However, detoxification of copra products after aflatoxin contamination should be the last resort and efforts to tame the aflatoxin problem in food and feedstuffs of copra should be directed towards managing the problem in the initial stages of contamination of the copra.

Copra preservation by chemical preservatives is generally accomplished by a mechanism of drying i.e. employing a relatively dry environment against microbes that are responsible for spoilage. A microbe in a non-saline environment is able to exchange water through its membrane easily, but in a saline environment, which is induced by adding chemical preservatives, an isotonic situation is attained thereby resulting in slower microbe growth and probably even death (Jay, J.M.2002). In the present study, Menadione (Vitamin K3) effectively checked the A.flavus growth (100% inhibition) probably due to its auto-oxidation effect. Addition of Menadione to the rapeseed and soybean oils resulted in the accelerated process of auto-oxidation of these oils. Further, Menadione when added to vegetable oils, influences the dissolution of natural tocopherols as well as their dimerization (Kupczyk and Gogolewski, 2001). The microbial spoilage by yeasts such as Zygosaccharomyces bailii and Saccharomyces cerevisiae can usually be retarded by a weak organic acid like Benzoic acid which acts by cytostatic action which is a consequence of inhibition of macroautophagy coupled with nitrogen starvation (Reut Hazan et.al., 2004).

Salts in general when dissolved in water, act as weak acids, In solution, sodium benzoate, propionates of sodium and calcium transform into benzoic acid and propionic acid respectively and finally check the mold growth. The chemicals benzoic acid and sodium benzoate are the most common food preservaties in fruit juices, syrups, jams, jellies, pickles and fruit cocktails etc. Yeasts are also inhibited by benzoates to a greater extent than are molds and bacteria. Ascorbic acid or Vitamin C and their salts are highly soluble in water and safe to use in foods. On the other hand, Acetic acid is a

general preservative inhibiting many species of bacteria, yeasts and to a lesser extent molds (Sareen, 2003). The present study also supports the same with Acetic acid showing no efficacy in checking the linear growth of *A.flavus* strains, the aflatoxin producing molds.

Compatibility studies between Trichoderma spp that are isolated viz., T.viride, T.harzianum and T.hamatum from copra godowns and chemical preservatives that inhibit growth of A.flavus revealed that Sodim benzoate, Ascorbic acid and Potassium meta bisulphate are safe with regard to the growth and multiplication of Trichodrerma spp and can be used in conjunction with biocontrol management of copra spoilage. On the other hand, Menadione, Propionic acid, Benzoic Acid and acetic acid reduced the growth of Trichoderma spp at 500-ppm concentration (Plate-6). All these chemicals are relatively safe at 100 ppm with respect to Trichoderma spp growth inhibition (Table-7).

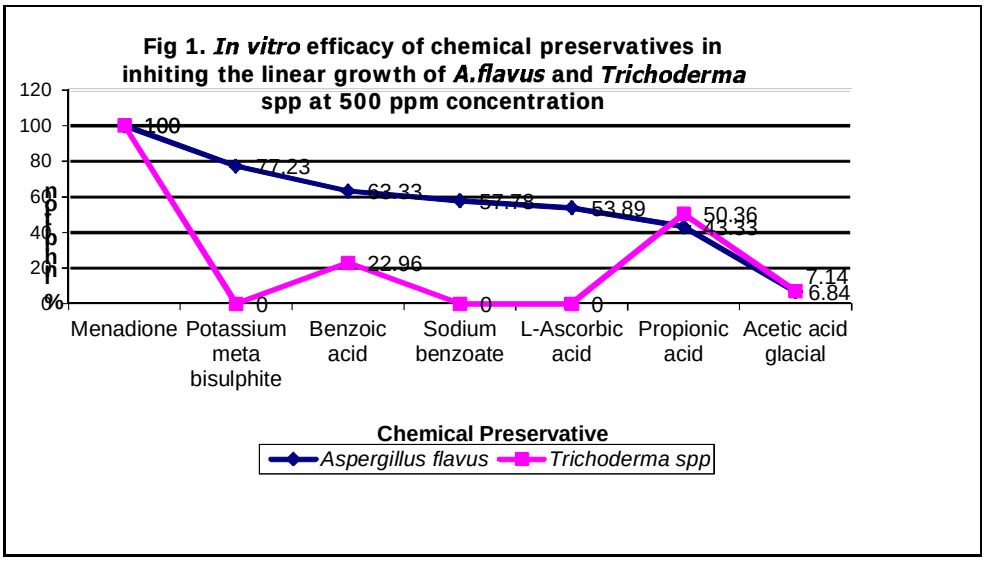
Table-7: Effect of chemical preservatives on the linear growth of ***Trichoderma*** strains on PDA incubated at 28°c for 96 hrs.

Chemical	*T.viride*		*T.harzianum*		*T.hamatum*		Average inhibition (%)
	% inhibition at		% inhibition at		% inhibition at		of *Trichoderma* spp at
	100ppm	500ppm	100ppm	500ppm	100ppm	500ppm	500 ppm
Menadione	68.89(28[a])	100(0[a])	68.89(28[a])	100(0[a])	65.56(31[a])	100(0[a])	100
Potassium meta bisulphate	0.00(90[d])	0.00(90[e])	0(90[c])	0(90[e])	0(90[c])	0(90[e])	0
Benzoic acid	0.00(90[d])	11.11(80[c])	0(90[c])	30.0(63[c])	0(90[c])	27.77(65[c])	22.96
Sodium benzoate	0.00(90[d])	0(90[e])	0(90[c])	0(90[e])	0(90[c])	0(90[e])	0
L-Ascorbic acid	0.00(90[d])	0(90[e])	0(90[c])	0(90[e])	0(90[c])	0(90[e])	0
Propionic acid	18.89(73[b])	53.33(42[b])	5.55(85[b])	44.44(50[b])	11.11(80[b])	53.33(42[b])	50.36
Acetic acid glacial	0.67(89.4[c])	7.11(83.6[d])	0(90[c])	6.66(84[d])	0.28(89.74[c])	7.66(83.10[d])	7.14
Control	(90mm)						

* Means followed by similar letters are not different statistically (P = 0.05) by Duncan's multiple range test. ** Values in parentheses are the linear growths of fungi in millimeters.

Comparison was drawn with respect to chemicals that inhibited *A.flavus* growth and *Trichoderma* spp growth at 500 ppm concentration under *in vitro* conditions.

The results indicated that Menadione though effective in controlling *A.flavus* population, is also adverse in terms of *Trichoderma* spp growth. The results with respect to Potassium meta bisulphate, Benzoic acid, Sodium benzoate and Ascorbic acid are encouraging in the sense that only *A.flavus* growth was reduced whereas *Trichoderma* spp growth is almost unaffected under in-vitro conditions (Fig-1). However, the efficacy of Propionic acid in terms of reduction of both *A.flavus* strains and *Trichoderma* spp is almost on par. With regard to Acetic acid, a poor mold inhibitor is also doubly disadvantageous with its inhibitory effect on *Trichoderma* spp.



The compatibility studies of chemical preservatives on Trichoderma spp is only a study taken up keeping in view the precautions to be adopted while applying preservatives to the copra. Potassium meta bisulphate, Benzoic acid, Sodium benzoate and Ascorbic acid can be recommended to be applied on copra as well as in godowns where copra is stored, the soils of which may inhabit Trichoderma spp. Whereas, in order to have a dual check of mold growth by biocontrol agents as well, the chemical Menadione has to be applied only to the copra and not to the godowns as a general spray.

Preservation of food products containing chemical food preservatives is usually based on the combined synergistic activity of several additives, intrinsic product parameters like composition, acidity, water, processing temperature, storage atmosphere and temperature.

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by aflatoxin producing molds that infest copra, thus spoiling the healthy food chain and subsequently the coconut trade. Among the mycoflora that invade copra, Aspergillus flavus Link contamination is a potential hazard to the coconut industry and trade because of its secondary metabolite production, the aflatoxins. Aflatoxins are secondary metabolites produced by Aspergillus flavus and Aspergillus parasiticus Speare that are carcinogenic, hepato-toxic and teratogenic in nature (Coulibaly, 1987). There are several reports of aflatoxin contamination of copra. Phillippine coconut industry which exports copra cake used as feed ingredient in Europe, identified Aflatoxin B1 level to a tune of 7-70 ppb and therefore gave a regulation that any move by European country to restrict the level of Aflatoxin B1 in feed stuffs would pose difficulty for the local coconut product given the climatic conditions in the country. Entry of aflatoxins into food chain is considered to be the most devastating problem not only through coconut, but also through cereal grains, spices, dry fruits, pulses, vegetables, cheese, bread and oil seeds; which are the prominent aflatoxin producing potential sources of A.flavus and A.parasiticus (Mc Donald, 1976).

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Microbial spoilage of food is reported to be upto a tune of 40% worldwide and inhibition of these microbes often requires levels of preservatives that are near or greater than legal limits (Reut Hazan et.al., 2004). Among the chemical preservatives that are in use in the preservation of foods, Sulfites, Dehydroacetic acid, Sodium nitrite, Ethyl formate, propionic acid, sorbic acid and Benzoic acid are most common and these are deemed as GRAS (Generally Regarded as Safe) in the specified concentrations (Jay, J.M, 2002). Efforts to minimize the copra spoilage by A.flavus group and aflatoxin threat can further be reduced by treating the copra with chemical preservatives that are commercially available and also ensures as a next step in reducing the A.flavus and other mycoflora entry into the copra after Trichoderma spp antagonism in the soil against these common copra inhabitants. Compatibility studies between A.flavus and the commonly used preservatives in food industry gives an idea of the nature of chemical preservatives to be used. Preservatives that have negative impact on the survival, growth and multiplication of

A.flavus in soil and on copra have to be used in order to combat the aflatoxin problem in copra. However, the preservatives that are to be recommended should not hinder the multiplication of the native Trichoderma spp which are antagonistic to A.flavus especially when applied to soil.

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