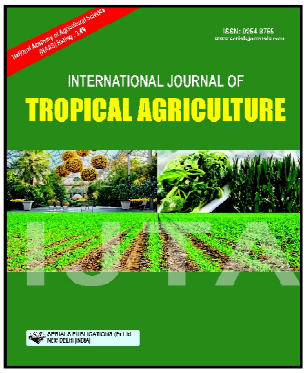

INTERNATIONAL JOURNAL OF TROPICAL AGRICULTURE

ISSN : 0254-8755

available at http: www.serialsjournals.com

Volume 36 • Number 1 • 2018

Evaluation of Spice oils for Antifungal Activity Against Food Borne Toxigenic Fungi

Shadanaika*, A. K. Vijayan, K. Dhanapal and A. B. Remashree

Indian Cardamom Research Institute, Spices Board, Myladumpara, Kailasanadu P.O., Idukki District, Kerala-685553
**Corresponding author: shadaicri@gmail.com*

Abstract: In the present investigation four known toxigenic fungi such as *Aspergillus flavus* (ATCC 46283), *Aspergillus parasiticus* (CFR 223), *Aspergillus ochraceus* (CFR 221) and *Fusarium sporotrichoides* (MTCC 1894) belonging to the genus *Aspergillus* and *Fusarium* were selected and as many as ten spice oils were evaluated for their antifungal potentiality against toxigenic fungi. The result revealed that the growth of *A.flavus* (ATCC 46283), *A.parasiticus* (CFR 223), *A.ochraceus* (CFR 221) and *F.sporotrichoides* (MTCC 1894) was inhibited by ajowan oil even at the lowest concentration of 0.001 % when incorporated into the liquid medium. Cinnamon oil could completely inhibit most of them at 0.002 % level, while cumin oil came next only as it could completely inhibit mycotoxigenic fungi at 0.002-0.004 % concentration. *F.sporotrichoides* being sensitive amongst other test fungi was completely inhibited by oils of ajowan, cinnamon, clove, celery, cardamom, turmeric, ginger, nutmeg and oleoresin of chilli. Progressive inhibition on growth was recorded for 2-96 % at 12 - 96 h time period by ajowan oil (0.001 %). Aflatoxin B_1 was inhibited maximum by 36 % at 84 h and >50 % inhibition of aflatoxin B_2, G_1 and G_2 at 72 h time period. *A.flavus* was inhibited up to 80 % at 96 h with >50 % inhibition of aflatoxin B_1 and B_2 by 60 h. At the same concentration oils of ajowan inhibited the growth of *A.ochraceus* by 90 % and ochratoxin A production by 100 % at 60 h and 48 h respectively. Ajowan oil showed more growth inhibition of *A.ochraceus* than *A.flavus* and *A.parasiticus*. Effective growth control of mycotoxigenic fungi at the concentration ranging from 0.001 - 0.004 % in liquid medium showed spice oils potentiality and promise for use in future as natural antifungal agents.

Keywords: *Aspergillus*, *Fusarium*, ajowan, mycotoxigenic, antifungal, inhibition.

1. INTRODUCTION

Recognition of the problems of mycotoxins in various countries led to the development of appropriate programmes for their prevention and control. These programmes formed part of the strategies to minimize the problems of mycotoxin contamination and included not only the prevention of mycotoxin formation in agricultural commodities but also their removal through detoxification or decontamination. Routine surveillance, regulatory measures to control the occurrence of mycotoxin in foods both at national and international trade as well as information, education and communication drive formed part of these programmes. **Several approaches both preharvest and post harvest are currently being tried to reduce or to eliminate mycotoxins from the food chain. T**he search for antifungal agents especially spice oils which could safely be used as substitutes for fungicides is extensively tried and there is a renewed interest in plant extracts / essential oils originated either from spices or other plants for their antimicrobial agent. Spice essential oils are the volatile aromatic extracts prepared by steam distillation of ground spices (Pruthi 1993, Annamali 1994). India accounts for about 35% of the global trade. Out of these pepper, ginger, clove, cinnamon, cassia, mace (nutmeg), pimento (allspice) and cardamom alone contribute 90% of the total world trade. About 80-85% of spices are sold as whole, ungrounded state and the rest are marketed as grounded spices or in mixes and as spices essential oils and oleoresins (Husain 1996). (Ayres *et al.* 1980, Prakash 1990, Mathews 1992, Pruthi 1993, Mehta et al. 1995). Therefore an attempt has been made to evaluate the various spice oils for their potential as antifungal agents.

2. MATERIALS AND METHODS

2.1. Spice oils

The essential oils such as large cardamom, small cardamom, celery, clove, cumin, cinnamon, pepper, turmeric, ajowan, ginger, nutmeg and chilli oleoresin used in this study were procured locally and from the department of PPSFT, CFTRI, Mysore. Known concentration of spice oils at 0.001, 0.002, 0.004, 0.006, 0.008, 0.01 and 0.02% were emulsified with 0.002% of tween 20 before adding to the media.

2.2. Indicator organism

Toxigenic strains of *Aspergillus flavus* (ATCC 46283), *A.parasiticus* (CFR 223), *A.ochraceus* (CFR 221) and *Fusarium sporotrichoides* (MTCC 1894) were grown on a potato-dextrose-agar slants for 5 days at 28 ± 2^0 C. Spores were harvested and suspension was prepared in 0.5% saline with 0.001% tween 20 as emulsifier and the spore concentration was adjusted to contain approximately 10^6 spores/ ml. The count of the spores in the inoculum was determined using haemocytometer.

2.3. Evaluation of essential oils for antifungal activity

Yeast extract sucrose (YES) was used as basal medium for the growth and toxin production by *Aspergillus* species (Davis *et al.,* 1966) and glucose yeast extract peptone (GYEP) for *Fusarium* species. The pH of the media were adjusted to 6.5 before sterilization and liquid media (50 ml) was dispensed in 100ml conical flasks, plugged and sterilized at 121 °C under 15 psi for 30 min. Spore suspension in 1 ml quantities was added to 50 ml YES medium. Essential oils were dispensed to give concentrations of 0.001, 0.002, 0.004, 0.006, 0.008, 0.01 and 0.02 % in the inoculated culture broth. The culture broth without the essential oils served as the controls. After addition of each essential oil, the culture flasks containing *A.flavus, A.parasiticus* and *A.ochraceus* individually were incubated for 7 days and *Fusarium sporotrichoidies were incubated for 25 days at 28 ± 2^0C.*

2.4. Estimation of fungal growth

The mycelial mat from the culture flasks were harvested after inactivating spores by methyl alcohol

(10ml/ 50ml media). Dry weight of mycelia was determined by collecting the mat on pre weighed Whatman no. 1 filter paper and washing the mat with distilled water. The mat with filter paper was dried at 80 + 2 ^{0}C for 12 h to constant weight.

2.5. Estimation of mycotoxins

Culture filtrate after separating the mycelial mat was extracted twice with chloroform (Scott *et al*, 1970) and the combined extract was dried through anhydrous sodium sulphate and the solvent evaporated to dryness on water bath. Aflatoxins and ochratoxin A were separated on TLC and quantified by dilution to extinction method (AOAC, 2000). The residue was dissolved in known quantities of chloroform applied on pre - activated TLC plate along with the standard aflatoxins and ochratoxin A. The plate was developed in solvent system containing benzene – acetic acid – methanol (90:5:5) in a TLC developing chamber. Aflatoxins, B_1, B_2, G_1, G_2 and Ochratoxin A were visualized by viewing under UV light (365 nm) (Scott *et al.* 1971). Aflatoxins, B_1 and G_1 were confirmed by treating them with strong acid like Trifloroacetic acid (TFA) as per the standard method. T-2 toxin and Diacetoxiscerpinol (DAS) which were extracted in chloroform were detected by HPLC using fluorescence detector at excitation 292 nm and emission 425 nm. HPLC column (C-18 supelco) was used with acetonitrile: water: acetic acid (65: 35: 0.7%) as mobile phase. The toxins were quantified by calculating resolution peak areas obtained on the graph in comparison with standard toxin peaks.

3. RESULTS AND DISCUSSION

3.1. Effect of spice oil on *A.parasiticus*

The strain of *A.parasiticus* (CFR 223) which produces all the four aflatoxins viz, B_1, B_2, G_1 and G_2, was used to evaluate various spice oils for their antifungal potential. The effect of celery, chilli oleoresin, ginger, large cardamom, nutmeg, pepper and turmeric oil on mycelial growth and aflatoxin production by *A.parasiticus* (CFR 223) at different concentrations is presented in the table 1. The sample untreated with spice oil served as control and results of various treatments at different concentrations (0.002 – 0.02%) are presented in comparison with control. The oil of celery was able to inhibit mycelial growth between 9.0 - 59.0% at the range tested. It had an inhibitory effect on production of aflatoxin B series (B_1 17.0 - 68.00; B_2 89 – 100.0%) and aflatoxin G (G_1 & G_2) series was inhibited completely. The percent inhibition of mycelial growth by chilli oleoresin was 10.0 - 23.0, production of aflatoxin B_1 (5.0 - 20.0), B_2 (22.0 - 60.0), G_1 (6.0 – 26.0) and G_2 (20.0 - 100.0). Ginger oil inhibited maximum of mycelial growth by 38.0%, B_1 and G_1 production by 23.0 and 40% respectively. Aflatoxins B_2 and G_2 were completely inhibited. Large cardamom inhibited mycelial growth at 9.0 - 92.0%, toxin production B_1 at 17.0 - 100.00% and other aflatoxins B_2, G_1 and G_2 were inhibited at > 50 to 100.0%. Nutmeg oil had the least inhibitory effect as it could inhibit growth only up to 1.0% at the concentration of 0.02 %. Pepper oil inhibited mycelial growth by 12.0 - 78.0 % and could completely inhibit aflatoxins. Turmeric oil inhibited mycelial growth by 18.0 - 84.0% and B_1 > 45.0%, B_2 > 88% and G_1 and G_2 were completely inhibited. Inhibitory effect of chilli oleoresin, ginger and nutmeg were comparatively less than that of celery oil (Table 1). At highest concentration of 0.02%, the fungal inhibition was only 23.0, 38.0 and 1.0 in chilli oleoresin, ginger and nutmeg respectively. The oils of clove and cinnamon which gave complete inhibition of mycelial growth at 0.002%, were further evaluated at lower concentration of 0.001%. The cinnamon and clove oil inhibited mycelial growth by 92 and 60% respectively. Cinnamon oil completely inhibited the formation of all four aflatoxins, while clove oil gave 96% inhibition of aflatoxin B_1 and complete inhibition of B_2, G_1 and G_2.

The figure 1 reveals the effect of cumin oil at the concentrations mentioned above on mycelial growth and aflatoxin viz, B_1, B_2, G_1 and G_2, production by toxigenic strain of *Aspergillus parasiticus* (CFR 223). Minimum inhibition of mycelial growth by 23.0 % was observed at the 0.001% concentration of oil. The aflatoxins B_1 and B_2 were inhibited by 53.0 and 90.0% respectively at the concentration of 0.001%. However, the oil completely inhibited mycelial growth and production aflatoxins B_1, B_2, G_1 and G_2 wwe at concentration oil 0.004%.

The small cardamom oil inhibited mycelial growth by 40.0 - 80.00% at the concentrations of oil ranging between 0.001 - 0.02%. Complete inhibition of aflatoxin B_1 wwe was observed at concentration of 0.01%. Aflatoxins B_2, G_1 and G_2 were completely inhibited at the concentration of 0.001%.

3.2. Effect of spice oil on *A.flavus*

The effect of various oils on the growth and aflatoxin B_1 and B_2 production by *Aspergillus flavus* (ATCC 46283) is presented in the table 2. It reveals the results on the effect of celery, chilli oleoresin, ginger, large cardamom, nutmeg, pepper and turmeric oil on mycelial growth and aflatoxin B_1 and B_2 production. Celery oil at 0.02% was found to inhibit maximum mycelial growth up to 70.0%, production of B_1 up to 87.0 % and completely inhibited B_2. Chilli oleoresin inhibited maximum mycelial growth, B_1 and B_2 up to 22.0, 17.0 and 34.0% respectively at the same concentration 0.02%. The cinnamon oil inhibited maximum of mycelial growth up to 74.0% and completely inhibited aflatoxin at the same concentration of 0.02%. Clove as well as cumin oil gave maximum inhibition of mycelial growth, B_1 and B_2 by 100.0% at the concentration level of 0.004%. The ginger oil inhibited maximum of mycelial growth up to 33.0%, B_1 90.0% by inhibiting B_2 completely at the concentration of 0.02%. Large cardamom oil was able to inhibit maximum of mycelial growth (61.0), B_1 (61.0) and B_2 (100.0)%, at the concentration 0.02%. Nutmeg oil inhibited maximum of mycelial growth (17.0), B_1 (37.0) and B_2 completely at the concentration 0.02%. Pepper oil inhibited mycelial growth (80.0), B_1 and B_2 completely at the concentration 0.02%. Small cardamom oil was able to inhibit maximum of mycelial growth (35.0), B_1 (67.0) and B_2 (100.0) % at the concentration 0.02 %. The oil of turmeric inhibited maximum of mycelial growth at 80.0% and completely inhibited B_1 and B_2 at the concentration 0.02%.

3.3. Effect of spice oil on *A.ochraceus*

Similar trend was noticed when spice oils were tested against *Aspergillus ochraceus* (CFR 221) for their effect on mycelial growth and ochratoxin A production and the results is presented in table 3. Oils of celery, Chilli oleoresin, ginger, large cardamom and nutmeg, inhibited maximum of mycelial growth by 66.0, 25.0, 23.0, 90.0 and 12.0% respectively at highest concentration of oil. On the other hand the inhibitory effect was more drastic on the production of ochratoxin A showing complete inhibition with celery oil, 80.0% inhibition with ginger and large cardamom and 27% with nutmeg. The oils of cinnamon, clove, cumin, pepper, small cardamom and turmeric oil inhibited both mycelial growth and ochratoxin A completely at highest concentration of oil.

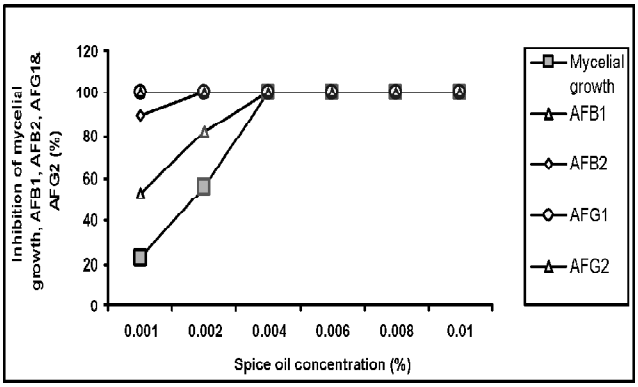

Figure 1: Effect of cum in oil on mycelial growth & aflatoxin production by *A. parasiticus*

Table 1
Effect of spice oil on *Aspergillus parasiticus* (CFR 223)

Name of spice oil		*Spice oil concentration (%)*					
		0.002	*0.004*	*0.006*	*0.008*	*0.01*	*0.02*
1		9.0	22.0	26.0	43.0	52.0	59.0
2		17.0	33.0	50.0	50.0	67.0	68.0
3	Celery	89.0	96.0	100	100	100	100
4		100	100	100	100	100	100
5		100	100	100	100	100	100
1		0.0	0.0	10.0	12.0	16.0	23.0
2		0.0	0.0	5.0	8.0	10.0	20.0
3	Chilli oleoresin	0.0	0.0	22.0	26.0	32.0	60.0
4		0.0	0.0	6.0	10.0	23.0	26.0
5		0.0	0.0	20.0	30.0	60.0	100
1		0.0	0.0	2.0	14.0	20.0	38.0
2		0.0	0.0	0.0	12.0	18.0	23.0
3	Ginger	0.0	0.0	0.0	80.0	90.0	100
4		0.0	0.0	0.0	10.0	26.0	40.0
5		2.0	0.0	0.0	86.0	100	100
1		9.0	13.0	35.0	43.0	78.0	92.0
2		17.0	17.0	17.0	33.0	80.0	100
3	Large cardamom	67.0	80.0	96.0	100	100	100
4		67.0	67.0	80.0	100	100	100
5		100	100	100	100	100	100
1		0.0	0.0	0.20	0.40	1.0	1.0
2		0.0	2.0	5.0	5.0	6.0	6.0
3	Nutmeg	0.0	20.0	20.0	23.0	33.0	50.0
4		0.0	1.0	1.0	6.0	8.0	20.0
5		0.0	1.0	6.0	12.0	18.0	20.0
1		12.0	17.50	22.0	39.0	57.0	78.0
2		6.0	18.0	36.0	80.0	100	100
3	Pepper	23.0	67.0	81.0	88.8	100	100
4		16.0	10.0	18.0	80.0	100	100
5		25.0	85.0	90.0	98.0	100	100
1		18.0	30.0	39.0	47.0	48.0	84.0
2		45.0	62.0	67.0	83.0	91.0	100
3	Turmeric	88.0	89.0	90.0	100	100	100
4		100	100	100	100	100	100
5		100	100	100	100	100	100

Note: 1 = % inhibition of growth of *A.parasiticus*,
2 = % inhibition of B_1 production,
3 = % inhibition of B_2 production,
4 = % inhibition of G_1 production,
5 = % inhibition of G_2 production

3.4. Effect of spice oil on *Fusarium sporotrichoides*

The strain of *Fusarium sporotrichoides* (MTCC1894) which produces T-2 toxin and Diacetoxiscerpinol (DAS) was used to evaluate the effect of various spice oils against mycelial growth and toxin production. The results of the study is presented in the table - 4. Mycelial growth inhibition in the range of 64 to 72 % occurred with oils of cinnamon, ginger, pepper and turmeric at 0.02 % concentration. At the same concentration oils of celery, chilli oleoresin, cumin, large cardamom, nutmeg and small cardamom could inhibit mycelial growth at ≤ 50.0%. Nutmeg had least inhibitory activity (< 50.0%) towards both the toxins. Oils of ginger and pepper could inhibit T-2 toxin at 86.0 and 91.0% and was more inhibitory towards DAS production. Whereas, other oils such as celery, chilli oleoresin, cinnamon, cumin, large cardamom, small cardamom and turmeric completely inhibited T-2 toxin and DAS production at 0.02% concentration. Clove oil completely inhibited mycelial growth itself at as low as 0.008% concentration of oil.

3.5. Effect of ajowan oil on mycotoxigenic fungi

Ajowan oil at the lowest concentration of 0.001% was evaluated for its efficacy towards inhibition of mycelial growth and toxin production by known mycotoxigenic fungi. The growth of fungi such as *Aspergillus flavus* (ATCC 46283), *A.parasiticus* (CFR 223), *A.ochraceus* (CFR 221) and *Fusarium sporotrichoides* (MTCC 1894) were found inhibited completely by ajowan oil at 0.001% concentration. The oils of cumin, clove and cinnamon were also found to be effective in inhibiting mycelial growth as well as toxin production next only to ajowan. The growth inhibition of *Aspergillus flavus* due to ajowan oil at various concentrations is presented in the figure 1. The effect of ajowan oil at concentration of 0.001 % was tested on mycelial growth and production of aflatoxin B_1, B_2, G_1 and G_2 by toxigenic strain of *Aspergillus parasiticus* (CFR 223) at different time interval is presented in the figure 2. The ajowan oil showed a progressive inhibitory effect on the mycelial growth showing 2.0 to 96.0% inhibition at 12 to 96 h. The production of aflatoxin B_1 was inhibited maximum by 36.0% at 84 h and > 50% inhibition of B_2, G_1 and G_2 production at 72 h time period (Fig. 2). Similarly an attempt was made to assess the effect of ajowan oil at 0.001% on mycelial growth and Aflatoxin B_1 and B_2 production by *Aspergillus flavus* (ATCC 46283) at different time interval 12, 24, 36, 48, 60, 72, 84 and 96 h. The figure 3 revealed the effect of ajowan oil at selected concentration of 0.001%. The oil inhibited mycelial growth by 19.0 to 79.0% at the interval of 12 – 96 h. Aflatoxins (B_1 and B_2) inhibition of > 50% was recorded at 60 h time period.

The effect of ajowan oil at concentration mentioned above was tried with *Aspergillus ochraceus* (CFR 221 to assess its effect on mycelial growth and ochratoxin A production by at various time interval between 12 – 96 h. The maximum mycelial growth inhibition of 90.0% was recorded at 60 h. The complete inhibition of ochratoxin A was recorded between 48 to 72 h time period (Fig. 4). All the 12 spice oils tested were able to inhibit growth of mycotoxigenic fungi comprising of *Aspergillus flavus* (ATCC 46283), *A.parasiticus* (CFR 223), *A.ochraceus* (CFR 221) and *Fusarium sporotrichoides* (MTCC 1894) completely at different concentrations when incorporated in culture medium. Amongst the spice oils, ajowan oil emerged as effective spice oil in inhibiting all the four toxigenic fungi belonging to two genera. Complete growth inhibition of *Aspergillus flavus*, *A.parasiticus* and *A.ochraceus* and *Fusarium sporotrichoides* was achieved by ajowan oil at the concentration of 0.001 %. Cinnamon oil was found effective in inhibiting *A.parasiticus, A.ochraceus* and *Fusarium sporotrichoides* at 0.002 %. The growth of *Aspergillus flavus* was also found inhibited completely by oils of cumin and ginger at 0.002 %. Nutmeg and clove oils completely inhibited the

growth of *Aspergillus flavus* (ATCC 46283) and *A.parasiticus* (CFR 223) at 0.006%. Celery, clove, ginger, small cardamom, chilli oleoresin and nutmeg oils completely inhibited *Fusarium sporotrichoides* (MTCC 1894) at 0.06% concentrations. Thymaldehyde a known constituent of thyme was found to inhibit *Fusarium* spp. at 500 ppm (Sumalan *et al.* 2013).

Table 2
Effect of spice oil on *Aspergillus flavus* (ATCC 46283)

Name of spice oil		*Spice oil concentration (%)*					
		0.002	*0.004*	*0.006*	*0.008*	*0.01*	*0.02*
1		30.0	30.0	32.00	40.00	50.0	70.00
2	Celery	67.0	67.0	75.0	75.0	75.0	87.0
3		87.0	100	100	100	100	100
1		0.0	8.00	8.0	10.0	16.0	22.0
2	Chilli oleoresin	4.0	5.0	12.0	14.0	17.0	17.0
3		0.0	0.0	17.0	30.0	33.0	33.0
1		5.0	5.0	8.0	15.0	32.00	74.0
2	Cinnamon	17.0	17.0	17.0	58.0	100	100
3		67.0	80.0	100	100	100	100
1		96.0	100	100	100	100	100
2	Clove	100	100	100	100	100	100
3		100	100	100	100	100	100
1		100	100	100	100	100	100
2	Cumin	100	100	100	100	100	100
3		100	100	100	100	100	100
1		0.0	2.0	8.0	19.0	29.0	33.0
2	Ginger	17.0	22.0	33.0	63.0	83.0	90.0
3		100	100	100	100	100	100
1		23.0	23.0	24.0	26.0	34.0	61.0
2	Large cardamom	17.0	17.0	17.0	33.0	33.0	61.0
3		65.0	86.0	90.0	100	100	100
1		0.0	0.0	0.0	8.0	17.0	17.0
2	Nutmeg	0.0	0.0	0.0	12.0	33.0	37.0
3		0.0	0.0	0.0	100	100	100
1		39.0	42.0	48.0	50.0	55.0	80.0
2	Pepper	17.0	17.0	17.0	58.0	92.0	100
3		33.0	68.0	87.0	93.0	97.0	100
1		20.0	20.0	20.0	23.0	31.0	35.0
2	Small cardamom	19.0	25.0	25.0	25.0	25.0	67.0
3		33.0	42.0	63.0	85.0	95.0	100
1		21.0	41.0	46.0	50.0	53.0	80.0
2	Turmeric	17.0	58.0	58.0	67.0	83.0	100
3		67.0	68.0	83.0	91.0	100	100

Note: 1 = % inhibition of growth of *A.flavus*,
2 = % inhibition of B_1 production,
3 = % inhibition of B_2 production.

Table 3
Effect of spice oil on *Aspergillus ochraceus* (CFR 221)

Name of spice oil		*Spice oil concentration (%)*					
		0.002	*0.004*	*0.006*	*0.008*	*0.01*	*0.02*
1		38.0	44.0	55.0	61.0	64.0	66.0
2	Celery	40.0	40.0	60.0	60.0	100	100
1		0.0	9.0	13.0	15.0	18.0	25.0
2	Chilli oleoresin	0.0	33.0	57.0	60.0	60.0	80.0
1		100	100	100	100	100	100
2	Cinnamon	100	100	100	100	100	100
1		45.0	67.0	82.0	89.0	97.0	100
2	Clove	60.0	83.0	100	100	100	100
1		36.0	61.0	76.0	81.0	92.0	100
2	Cumin	26.0	100	100	100	100	100
1		0.0	0.0	14.0	16.0	18.0	23.0
2	Ginger	0.0	0.0	33.0	62.0	70.0	80.0
1		56.0	62.0	71.0	84.0	90.0	90.0
2	Large cardamom	10.0	16.0	32.0	41.0	60.0	81.0
1		0.0	0.0	0.20	2.0	6.0	12.0
2	Nutmeg	0.0	0.0	2.0	10.0	18.0	27.0
1		20.0	36.0	52.0	61.0	81.0	100
2	Pepper	8.0	22.0	60.0	88.0	96.0	100
1		80.0	88.0	90.0	91.0	96.0	100
2	Small cardamom	40.0	50.0	60.0	64.0	80.0	100
1		30.0	45.0	50.0	52.0	54.0	100
2	Turmeric	60.0	62.0	62.0	80.0	80.0	100

Note: 1 = % inhibition of growth of *A.ochraceus*,
2 = % inhibition of OTA production,

A.flavus and *A.ochraceus* were found inhibited completely by oils of clove as well as celery at 0.100 %. *A.ochraceus* was completely inhibited by cinnamon and cumin at 0.002 %. *Fusarium sporotrichoides* was found inhibited completely by oil of turmeric at 0.004 %. Large cardamom and pepper gave complete inhibition of *Fusarium sporotrichoides* at 0.008 %. Spice oils such as cinnamon, followed by cumin and clove emerged as effective inhibitors of fungal growth next to ajowan oil (Table 5).

Many investigators used essential oils such as cinnamon, peppermint, basil and thyme to protect maize kernels against infection, without affecting germination and mold growth (Montes-Belmont and Carvajall, 1998). The inhibition of growth and aflatoxin production by *Aspergillus flavus* and *A.parasiticus* by spice oils and their active components has frequently been reported (Bullerman 1974; Hitokoto *et al.* 1980; Farag *et al.* 1989; Paster *et al.* 1990) with varied inhibitory levels of spice oils. Sometimes toxin production may be inhibited without fungal growth being affected (Bullerman 1974). Considerable interest has developed during recent years on the preservation of grains by the use of essential oils to effectively retard growth and mycotoxin production (Bullerman *et al.,* 1977). Anise and cinnamon oils inhibited growth of *Aspergillus parasiticus* and its aflatoxin B1 production completely

Table 4
Effect of spice oil on *Fusarium sporotrichoides* (MTCC1894)

Name of spice oil		*Spice oil concentration (%)*					
		0.002	*0.004*	*0.006*	*0.008*	*0.01*	*0.02*
1		14.0	29.0	29.0	29.0	43.0	43.0
2	Celery	14.0	23.0	27.0	87.0	100	100
3		27.0	77.0	100	100	100	100
1		8.0	11.0	15.0	16.0	26.0	36.0
2	Chilli oleoresin	62.0	82.0	88.0	98.0	100	100
3		79.0	91.0	100	100	100	100
1		20.0	40.0	48.0	50.0	66.0	72.0
2	Cinnamon	61.0	100	100	100	100	100
3		100	100	100	100	100	100
1		33.0	44.0	44.0	100	100	100
2	Clove	75.0	97.0	100	100	100	100
3		17.0	50.0	100	100	100	100
1		16.0	25.0	29.0	32.0	36.0	47.0
2	Cumin	95.0	98.0	98.0	100	100	100
3		65.0	95.0	99.0	100	100	100
1		5.5	14.0	30.0	37.0	46.0	66.0
2	Ginger	62.0	71.0	72.0	75.0	78.0	86.0
3		91.0	92.0	100	100	100	100
1		0.0	12.0	19.0	25.0	25.0	50.0
2	Large cardamom	61.0	64.0	69.0	72.0	98.0	100
3		45.0	56.0	100	100	100	100
1		0.3	0.3	1.0	2.0	3.0	9.0
2	Nutmeg	0.0	0.0	0.0	5.0	17.0	22.0
3		0.0	0.0	0.0	2.0	4.0	32.0
1		18.0	27.0	27.0	34.0	56.0	71.0
2	Pepper	96.0	99.0	99.0	100	100	100
3		86.0	88.0	95.0	96.0	97.0	98.0
1		11.0	13.0	22.00	26.0	33.0	50.0
2	Small cardamom	0.30	4.0	45.0	87.0	94.0	100
3		20.0	52.0	71.0	100	100	100
1		10.0	20.0	34.0	45.0	57.0	64.0
2	Turmeric	99.0	100	100	100	100	100
3		84.0	100	100	100	100	100

Note: 1 = % inhibition of growth of *F.sporotrichoides*,
2 = % inhibition of T-2 production,
3 = % inhibition of DAS production.

(Veerabhadra Rao *et al.* 1983). Cinnamon and clove oils inhibited *Aspergillus flavus* growth and subsequent Aflatoxin B1 production (Patkar *et al.* 1993). Extract of garlic and onion inhibited the growth of *Aspergillus flavus* (Mei-chin yin 1998). Oils of cinnamon, peppermint, basil, origanum, clove and thyme completely protected maize kernels from colonization of *Aspergillus flavus* (Montes-belmont 1998). Clove oil (eugenol) was most inhibitory to the growth of *A.parasiticus* and *F.moniliforme* followed by cinnamon (cinnamic aldehyde), oregano (thymol) and mace (myristin) oils (Juglal *et al.* 2002). Essential oils of thyme, cinnamon, anise and spearmint inhibited fungal growth and toxin production on wheat treated with oils (Soliman 2002). Garcinia spice extract inhibited growth of *Aspergillus flavus* and its toxin (Selvi *et al.* 2003). In a review (Qing Liu *et al.* 2017) on antifungal and antibacterial activities of spices and their essential oils, which has positive correlation with our results that many of the spice essential oils such ajowan, cinnamon, clove etc were effective in inhibiting growth of food borne toxigenic fungi. Our study also confirmed the similar trends of inhibition of mycelial growth as well as toxin production by spices oils at the least concentrations recommended by earlier researchers. Especially the oil of ajowan was found effective and emerged as the best antifungal agent of all the 12 spice oils evaluated against four mycotoxigenic fungi. *Trachyspermum ammi* at 50 mg / ml inhibited *S.paratyphii* (Dhrma Prasad *et al.* 2013). EOs in antifungal and antimycotoxigenic protection of food can be applied as surface protection (by smearing or applying in the form of biofilm (Kocic-Tanackov DS, Dimic RG. 2013). Extracts of *Cinnamomum zeylanicum* and *Euginia caryophyllata* were found to inhibit food pathogenic microorganisms (Sevil Toroglu 2011).

Table 5
Growth inhibition of mycotoxigenic fungi by spice oils

	Fungi *			
Spice oil (%)	*A.flavus (ATCC 46283)*	*A.parasiticus (CFR 223)*	*A.ochraceus (CFR 221)*	*F.sporotrichoides (MTCC 1894)*
0.001	Ajowan	Ajowan	Ajowan	Ajowan
0.020	Cumin Ginger	Cinnamon Clove	Cinnamon Cumin	Cinnamon
0.040	-	Cumin	-	Turmeric
0.060	Nutmeg	-	Clove Nutmeg	Celery, Clove, Ginger, Cardamom (small), Chilli-oleoresin, & Nutmeg
0.080	-	-	-	Cardamom (large), & Pepper
0.100	Clove	-	Celery	-

* = 100 % inhibition of fungal growth.

Figure 1: Culture flask with YES medium showing inhibition of *Aspergillus flavus* due to ajowan oil at various concentrations (no. 1 to 5 and 6.control)

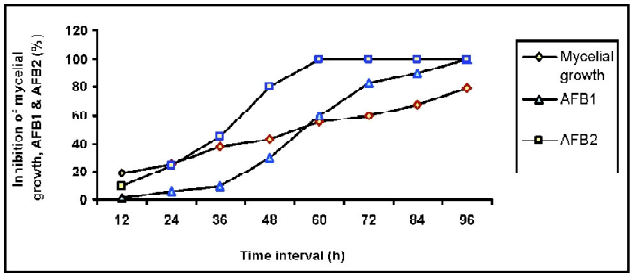

Figure 2: Effect of Ajowan oil on mycelial growth & aflatoxin production by *A. flavus* at various time interval

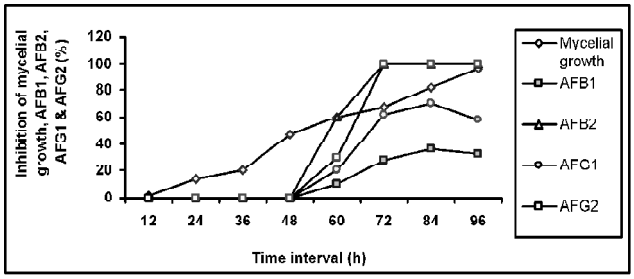

Figure 3: Effect of Ajowan oil on mycelial growth & aflatoxin production by *A. parasiticus* at various time interval

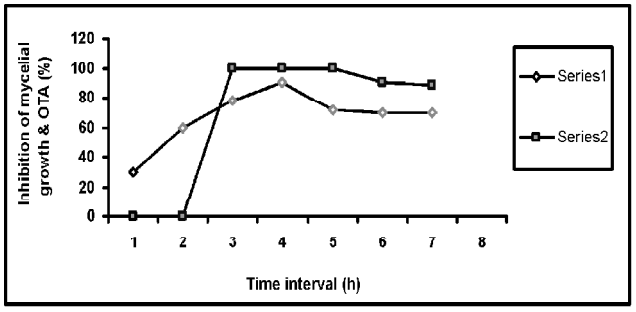

Figure 4: Effect of Ajowan oil on mycelial growth & ochratoxin A production by *A. ochraceus* at various time interval

ACKNOWLEDGMENTS

We thank Secretary (Chairman), Spices Board, Govt. of India, Cochin for having sanctioned study leave, and the Director, CFRTI, Mysore for having provided required facilities for this study. We also thank Dr. J.Puranaik, Scientist (Rtd), for having provided essential oils required for the study.

REFERENCES

AOAC, (2000). International Official Methods of Analysis. Gaithersburg, MD, USA. Official methods, 925.40.

Aziz, N.H., Farag, S.E., Mousa, L.A.A. and Abo-Zaid, M.A. (1998). Comparative antibacterial and antifungal effects of some phenolic compounds. *Microbios* **93:** 43–54.

Bullerman, L. B., Lieu, F. Y., and Seier, S. A. (1977). Inhibition of growth and aflatoxin production by cinnamon and 'clove oils, cinnamic aldehyde and eugenol. *J. Food Science.* 42:1107-1109.

Dhrma Prasad *et al.* (2013). Preliminary studies on some Nepalese kitchen spices for further antimicrobial activities. 12-JMMIHS ISSN:2091-1041 volume 1 / issue 2 /2013.

Farag, D.S., Daw, Z.Y., Hewedi, F.M. and El-Baroty, G.S. (1989). Antimicrobial activity of some Egyptian spice essential oils. *J. Food prot.* 52: 665-667.

Giridhar, P and Reddy, S.M. (1997). Inhibitory effect of black pepper on growth and toxin production of toxigenic fungi. *J. Indian bot Soc.* 76 : 43-45.

Hitokoko, H., Morozumi, S., Wauke, T., Sakai, S., and Kurata, H. (1980). Inhibitory effects of spices on growth and toxin production by toxigenic fungi. *Appl. Env. Microbiol.* 39:818-822.

http./www.indianspices. 2005.com/html/s1490uqa.htm. Spices Board, India Statistics. Spices market.

International Commission on Microbiological Specifications for Foods (ICMSF). 1996. Toxigenic Fungi: *Aspergillus*. pp 347-381. In: Micro-organisms in Foods. 5: Microbiological Specifications of Food Pathogens. Roberts, T A, Baird-Parker, A C and Tompkin, R B (eds). Blackie Academic & Professional, London, UK.

Jaspal, S. and Tripati, N.N. (1999). Inhibition of storage fungi of blackgram (*Vigna mungo* L.) by some essential oils. *Flavour Fragr. J.* 14: 1-4.

Juglal, S., Govinden, R. and Odhav, B. (2002). Spice oils for the control of co-occurring mycotoxins producing fungi. *J. Food Prot.* 65:683-687.

Kocic-Tanackov DS, Dimic RG. (2013). Fungi and mycotoxins - Food contaminants. Hem. Ind. DOI:10.2298/HEMIND120927108K.

LLewellyn, G.C., Burkitt, M. L., and Eadie, T. (1981). Potential mold growth, aflatoxin production and antimycotic activity of selected natural spices and herbs. JAOAC. 64:955-960.

Madhyastha, M.S. & Bhat, R.V., *Aspergillus parasiticus* growth and aflatoxin production on black and white pepper and the inhibitory action of their chemical constituents, *Appl. Environ. Microbiol.* 48: 1984, 376-379.

Martins, M. L. Martins, H. M. and Fernando Bernardo, F. (2001). Aflatoxins in spices market in Portugal. Food Additives and Contaminants. 18: 315-319.

Montes-belmont, R. and Carvajal, M. (1998). Control of *Aspergillus flavus* in maize with plant essential oils and their components. *J. Food Prot.* 61: 616-619.

Pafumi, J. (1986). Assessment of microbiological quality of spices and herbs. *J. Food Protect.* 49: 958-963.

Patkar, K.L., Usha, C.M., Shetty, H. S. Paster, N. and Lacey, J. (1993). Effect of spice essential oils on growth and aflatoxin B1 production by *Aspergillus flavus*. Letters in Applied Microbiology. 17: 49-51.

Paster, N., Menasherov, M., Ravid, U., and Juven, B. (1995). Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. *J. Food Protect.* 58: 81-85.

Prakash, V. (1996). Leaf Spices- Book Review. Indian Food Packer. Nov –Dec:157-158.

Prevention of Food Adulteration Act and Rules, (1998). Spices and Condiments, 191-211, G S R No. 179(E).

Prema, V. (2004). Mycotoxin Regulations for Foods: A Global Update. *J. Food Sci. Technol.* 41: 115-123.

Qing Liu, Xiao Meng, Ya Li, Cai-Ning Zhao, Guo-Yi Tang and Hua-Bin Li Review Antibacterial and Antifungal Activities of Spices. *Int. J. Mol. Sci.* 2017, 18, 1283; doi:10.3390/ijms18061283.

Reif K., W. Metzger. (1995). Determination of aflatoxins in medicinal herbs and plant extracts; *Journal of Chromatography A.* 692:131-136.

Holley, R, A and Patel, D. (2005). Improvement in self-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiology. 22: 4.

Santos, F. A., and Rao, V. S. (1997). Mast cell involvement in the rat paw oedema response to 1,8-cineole, the main constituent of eucalyptus and rosemary oils. *Eur. J. Pharmacol.* 331:253-258.

Selvi, T.A., Joseph, G.S. and Jayaprakasha, G.K. (2003). Inhibition of growth and aflatoxin production in *Aspergillus flavus* by *Garcinia indica* extract and its antioxidant activity. *Food Microbiol.* 20: 455-460.

Sethi,V. and Meena, M.R. (1997). Role of spices and their essential oils as preservatives and antimicrobial agents – A Review. Indian Food Packer. May-June: 25-43.

Sevil Toroglu. (2011). Studies on antimicrobial activities of solvent extracts of different spices. *J. Environ. Biol.* 32, 251-256 (2011) ISSN: 0254-8704.

Shantha, T., (1999). Fungal degradation of aflatoxin B_1. Natural Toxins. 7: 175-178.

Shantha, T. Sreenivasamurthy, V, Rati, E.R., and Prema, V. (1986). Detoxification of groundnut seeds by urea and Sunlight. *J. Food Safety.* **7**: 225 – 231.

Shantha, T.; Rati, E.R. and Murthy V.S. (1997). Nutritional evaluation of groundnut cake detoxified by in situ generated ammonia in albino rats. *J. Food Sci. Technol.* 34 : 405-407.

Shelef, L. A. (1983). Antimicrobial effects of spices. *J. Food Safety.* 6: 29-44.

Singh, P.L. (1983). Control of aflatoxin through Natural plant products. In Mycotoxin in food and feed. K.S. Bilgrami, T. Prasad and K.K.Sinha (Eds.) Allied Press, Bhagalpur, India, 307-315.

Soliman, K.M. and Badeaa, R.I. (2002). Effect of oil extracted from some medical plants on different mycotoxigenic fungi. *Food and Chemical Toxicology.* 40: 1669-1675.

Sumalan *et al.* (2013). Assessment of inhibitory potential of essential oils on natural mycoflora and Fusarium mycotoxins production in wheat. Chemistry Central Journal 2013, 7: 32.

Tantaoui-Elaraki A, Beraoud L. (1994). Inhibition of growth and aflatoxin production in *Aspergillus Parasiticus* by essential oils of selected plant materials. *J Environ Pathol Toxicol Oncol.* 13: 67-72.

Zaika, L. L. (1988). Spices and herbs: Their antimicrobial activity and its determination. *J. Food Safety.* 9: 97-118.