

## Evaluation of *Chenopodium ambrosioides* oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity

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Received 10 May 2006; received in revised form 11 October 2006; accepted 12 October 2006

### Abstract

Essential oil extracted from the leaves of *Chenopodium ambrosioides* Linn. (Chenopodiaceae) was tested against the aflatoxigenic strain of test fungus *Aspergillus flavus* Link. The oil completely inhibited the mycelial growth at 100 µg/ml. The oil exhibited broad fungitoxic spectrum against *Aspergillus niger*, *Aspergillus fumigatus*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Macrophomina phaseolina*, *Cladosporium cladosporioides*, *Helminthosporium oryzae* and *Pythium debaryanum* at 100 µg/ml. The oil showed significant efficacy in inhibiting the aflatoxin B1 production by the aflatoxigenic strain of *A. flavus*. During *in vivo* investigation it protected stored wheat from different storage fungi for one year. *Chenopodium* oil also exhibited potent antioxidant activity when tested by ABTS method. All these observations suggest the possible exploitation of the *Chenopodium* oil as potential botanical fungitoxicant in ecofriendly control of post harvest biodeterioration of food commodities from storage fungi.

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**Keywords:** ABTS; *A. flavus*; Aflatoxin; Antioxidant; Antifungal; *C. ambrosioides*; Shelf life

### 1. Introduction

Fungi are significant destroyers of foodstuffs during storage, rendering them unfit for human consumption by retarding their nutritive value and sometimes by producing mycotoxins. Approximately 25–40% of cereals world-wide are contaminated with mycotoxins produced by different storage fungi (Pittet, 1998). Aflatoxins are the most dangerous mycotoxins produced by *Aspergillus flavus*. About 4.5 billion people in developing countries are chronically exposed to the uncontrolled amounts of aflatoxin (Williams et al., 2004). Lipid peroxidation is also one of the major causes of deterioration of food products during processing and storage (Donnelly and Robinson, 1995). Products of lipid peroxidation influence other food constituents, alter taste and aroma and cause undesirable effects on the human health (Benzie, 1996). The use of synthetic chemicals as

antimicrobials has greatly contributed to management of such losses, but indiscriminate application of chemicals has led to a number of ecological and medical problems due to residual toxicity, carcinogenicity, teratogenicity, hormonal imbalance, spermatotoxicity, etc. (Bajaj and Ghosh, 1975; Omura et al., 1995; Pandey, 2003). Synthetic antioxidants, such as, butylated hydroxyanisole (BHA) and *tert*-butylhydroquinone (TBHQ), have also restricted use in foods due to carcinogenicity (Madhavi and Salunkhe, 1995). The recent reports on large scale use of different higher plant products viz. azadirachtin from *Azadirachta indica* (Devkumar and Sukhdev, 1993), carvone from *Carum carvi* (Hartmans et al., 1995) and allyl isothiocyanate from mustard and horseradish oil (Ward et al., 1998) have attracted the attention of microbiologists to search some newer plant chemicals for their exploitation as antimicrobials. Such products of higher plants would be biodegradable, renewable in nature and safe to human health (Varma and Dubey, 1999). Different crude extracts of spices, herbs and other plant materials rich in polyphenolics are becoming increasingly important in food industries because of their antifungal, antiaflatoxigenic and antioxidant activity. Hence,

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such plant chemicals can improve shelf life, quality and nutritional value of stored food commodities (Mishra and Dubey, 1994; Tripathi and Upadhyay, 2002; González et al., 2003; Tripathi and Dubey, 2004).

*Chenopodium ambrosioides* Linn. (Chenopodiaceae) is widely distributed throughout India. It is an erect much branched aromatic herb with a camphoreaceous odour. Leaves are oblong, lanceolate, sinuate dentate with obscure petiolate. Flowers are greenish, some turning into purplish in small clusters, simple or panniculate spikes, utricle membranous. Leaves are useful in the cure of influenza, pneumonia, typhoid and also as vermicide (Cheryl et al., 2006; Mishra, 2002). *Chenopodium* oil is a mixture ascaridole (55.3%), *p*-cymene (16.2%), alpha-terpinene (9.7%), isoascaridole (4.3%) and limonene (3.8%) (Cavalli et al., 2004). The main objective of the present study was to find out the efficacy of *Chenopodium* oil as a botanical fungitoxicant and an antioxidant for protecting stored wheat from fungal deterioration and enhancing their shelf lives. In the present investigation, antifungal activity of the essential oil of *C. ambrosioides* against the aflatoxigenic strains of the dominant storage fungus *A. flavus* Link. has been evaluated. Moreover, the potential of the oil in checking aflatoxin B1 production and also its antioxidant activity have been assessed.

## 2. Materials and methods

Fresh parts of 18 aromatic plants were collected at flowering stage from the campus of Banaras Hindu University. 250 g of fresh parts of each plant species was cut separately into small pieces and thoroughly washed with sterilized water. The volatile fractions (essential oils) were isolated through hydrodistillation by Clevenger apparatus. The isolated fractions of plant parts exhibited two distinct layers—an upper oily layer and the lower aqueous layer. Both the layers were separated and the essential oils were stored in clean glass vials after removing water traces with the help of capillary tubes and anhydrous sodium sulphate (Tripathi et al., 2004).

### 2.1. Antifungal analysis

The fungitoxic activity of the essential oils against the two aflatoxigenic strains of *A. flavus* viz., Navjot 4NSt and Saktiman 3NSt (procured from Mycotoxin Laboratory, University of Bhagalpur, India) was tested at 500 µg/ml by the poisoned food technique (Mishra and Dubey, 1994) using Czapek's medium. A requisite amount of essential oil was dissolved separately in 0.5 ml acetone and then mixed with 9.5 ml of autoclaved Czapek's medium (NaNO<sub>3</sub>, 2.0 g; K<sub>2</sub>HPO<sub>4</sub>, 1.0 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g; KCl, 0.5 g; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.01 g; Sucrose 30.0 g; Agar 15.0 g; distilled water 1 L; pH 6.8±2) in Petriplates (9.0 cm diameter). The control sets were prepared subsequently using sterilized distilled water in place of the oil. The prepared plates were inoculated aseptically with assay disc (5 mm) of the aflatoxigenic strains of the test fungus. The plates were then incubated at 28±2 °C for seven days. The inhibition of the radial growth of the test fungus by the oils was calculated following Pandey et al. (1982).

### 2.2. Minimum inhibitory concentration and nature of toxicity of essential oil of *C. ambrosioides*

To find out the minimum inhibitory concentration (MIC) at which the oil showed absolute fungitoxicity, experiments were carried out by the above mentioned poisoned food technique using graded concentrations of the *Chenopodium* oil with Czapek's medium, viz., 10, 50, 100, 500, 800 and 1000 µg/ml. The nature of toxicity (fungistatic/fungicidal) of the oil was determined following the method of Thompson (1989). The inhibited fungal discs of the oil treated sets were reinoculated into the fresh medium and revival of their growth was observed.

### 2.3. Fungitoxic properties of the essential oil of *C. ambrosioides* and comparison of efficacy with some synthetic fungicides

The mycotoxic spectrum of the *Chenopodium* oil was evaluated by poisoned food technique at 100 µg/ml against nine fungi, viz. *Aspergillus fumigatus*, *A. niger*, *Botryodiplodia theobromae*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Helminthosporium oryzae*, *Macrophomina phaseolina*, *Phythium debaryanum* and *Sclerotium rolfsii* (procured from the Division of Mycology and Plant Pathology, IARI, New Delhi, India). The efficacy of the oil was compared with some synthetic fungicides procured from Sigma (St. Louis, USA), viz., benzimidazole (Benomyl), diphenylamine (DPA), phenylmercuric acetate (Ceresan) and zinc dimethyl dithiocarbamate (Ziram) by the poisoned food technique.

### 2.4. Efficacy of the *Chenopodium* oil in checking aflatoxin production

SMKY medium (Diener and Davis, 1966) was used to check the efficacy of oil against aflatoxin production. 25 ml of SMKY medium was taken in each Erlenmeyer flask, to which a requisite amount of the *Chenopodium* oil was added so as to get 10, 100, 250 and 500 µg/ml concentrations. The flasks were aseptically inoculated with 7 day old culture of the aflatoxigenic strain Saktiman 3NSt of *A. flavus*. The control sets comprised the medium without the oil. The flasks were incubated for 10 days at 28±2 °C. After incubation, the content of each flask was filtered through Whatman filter paper No. 1. The mycelia were allowed to dry at 100 °C for 12 h. The mycelium dry weight (biomass) was used to compare fungal growth in treated and control sets. The filtrate was extracted with 20 ml chloroform in a separating funnel. The chloroform extract was passed through anhydrous sodium sulphate kept on a Whatman No. 42 filter paper. The extract was evaporated to dryness on water bath and the residue was dissolved in 1 ml chloroform. The amount of aflatoxin B1 (AFB1) produced in the treatment and control sets was determined by the TLC technique following Nabney and Nesbitt (1965). 50 µl of the chloroform extract spotted on TLC plate along with the standard of aflatoxin, was run in toluene:isoamyl alcohol:methanol (90:32:2 v/v). The developed plates were air dried and observed under longwave of UV light. Initial identification of different components of aflatoxins was made on visual basis by comparing the colour and intensity of

fluorescence of the sample and standard spots. The quantity of aflatoxin was estimated by a spectrophotometer (AOAC, 1984). The amount of aflatoxin B1 present in the sample was calculated according to the following formula:

$$\frac{D \times M}{E \times l} \times 1000$$

where,

<i>D</i>	absorbance,
<i>M</i>	molecular weight of aflatoxin (312),
<i>E</i>	molar extinction coefficient of aflatoxin B1 (21,800), and
<i>l</i>	path length (1 cm cell was used).

### 2.5. Antioxidant activity of *Chenopodium* oil

The antioxidant activity of essential oil was evaluated by the ABTS (2, 2-azino-bis 3-ethyl benzothiazoline-6-sulfuric acid) assay following Re et al. (1999) and Tiwari and Tripathi (2007). ABTS radical cation (ABTS<sup>•+</sup>) was freshly prepared by adding 5 ml of a 4.9 mM potassium persulphate solution to 5 ml of a 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with distilled water to yield an absorbance of 0.70 at 734 nm and the same was used for antioxidant assay. Stock solution of the *Chenopodium* oil was prepared in 1% methyl alcohol. The requisite amount of oil and ABTS<sup>•+</sup> solution was taken to make 1 ml of the final reaction mixture so as to get 500, 1000, 1500, 2000, 2500 and 3000 µg/ml concentrations. The reaction mixture was vortexed for 10 s and after 6 min absorbance was recorded at 734 nm by using an ELICO (SL150) UV–Vis Spectrophotometer and was compared with the control ABTS<sup>•+</sup> solution. Ascorbic acid was used as reference antioxidant compound. Appropriate solvent blanks were run in each assay. The total antioxidant activity (TAA) was calculated as percent inhibition of the ABTS<sup>•+</sup> quenching in relation to the aqueous control by the equation:

$$\text{Inhibition of } A_{734}(\%) = \frac{1-A_f}{A_0} \times 100$$

where,

<i>A</i> <sub>0</sub>	absorbance of uninhibited radical cation, and
<i>A</i> <sub>f</sub>	absorbance measured after 6 min upon addition of antioxidant samples.

### 2.6. In vivo efficacy of *Chenopodium* oil in the management of stored wheat samples against fungal deterioration

To find out the practical applicability of *C. ambrosioides* oil as fumigants for protection of stored wheat grain from bio-deterioration caused by fungi, the freshly harvested wheat samples (var Malviya 234) were fumigated with the oil by the method adapted by Shaaya et al. (1997) and Varma and Dubey (2001). The fumigation of wheat samples with the oil was made

in two sets. In one set (uninoculated treatment) requisite amounts of the oil was introduced into separate plastic containers (10 cm diameter × 16) containing 500 g of wheat by soaking in a piece of cotton so as to procure 100 µg/ml (v/v). In another set (inoculated treatment), the wheat samples, prior to treatment with the oil, were inoculated with a standard spore suspension of *A. flavus*. After 3 days of inoculation, the samples, similar to the first set, were separately treated by the *Chenopodium* oil. The control set also contained two sets—the uninoculated control and the inoculated control. In the uninoculated control set, the wheat was stored as such in plastic containers. In the inoculated control, the wheat sample was inoculated with a spore suspension of *A. flavus* and stored similarly. The inoculation of wheat seeds was done by preparing a standard spore suspension of *A. flavus*. Seven days old culture of *A. flavus* was used to inoculate in wheat sample. Spore suspension of the fungal strain was prepared in 0.1% Tween-80 and the spore density was adjusted to 285 × 10<sup>4</sup> spore/ml. The seeds were dipped in the normal spore suspension for 10 min and then dried in an oven at 45 °C for 12 h. The moisture content of the dried wheat was 11.13%. Three replicates were taken for each individual set.

After 12 months of storage at laboratory conditions (temperature 10–46 °C and RH 30–80%) wheat samples of both treatment and control sets were analysed for fungi using the blotter technique of Varma and Dubey (2001). The isolates were then examined and identified following Funder (1968) and Moore and Jaciow (1979). After the analysis of fungi associated with wheat samples of treatment and control sets, the percent occurrence of each fungus among the samples was analysed and the percent protection of wheat samples in the uninoculated and inoculated treatments were observed by following formula

Percent occurrence of fungus

$$= \frac{\text{No. of colonies of the fungus}}{\text{Total no. of colonies of all the fungal species}} \times 100$$

Percent protection of wheat samples

$$= \frac{D_c - D_t}{D_c} \times 100$$

Where,

<i>D</i> <sub>c</sub>	percent occurrence of total fungi in wheat samples of control set and
<i>D</i> <sub>t</sub>	percent occurrence of total fungi of wheat samples in treatment set.

### 2.7. Statistical analysis

All the measurements were replicated three times for each treatment and the data are reported as mean ± SE. The statistical analysis was performed by one way analysis of variance (ANOVA) using statistical software (SPSS, 10.0; Chicago, Ill, USA). The data also subjected to Students *t* test to analysed the effect of *Chenopodium* oil on protection of stored wheat with control and the linear regression analysis was done between

Table 1  
Screening of essential oils against two aflatoxigenic strains of *Aspergillus flavus*

Essential oils	Families	Percent inhibition of growth of the <i>A. flavus</i> at 500 µg/ml	
		Navjot 4NSt	Saktiman 3NSt
<i>Aegle marmelos</i> Linn. (leaf)	Rutaceae	8.6±0.5	8.1±1.1
<i>Artemisia nilagirica</i> Clarke (leaf)	Asteraceae	49.6±1.0	46.8±1.0
<i>Carum carvi</i> Linn. (seed)	Apiaceae	55.3±0.9	43.6±2.3
<i>Cymbopogon flexuosus</i> (Steud.) Wats. (leaf)	Poaceae	47.1±0.1	32.8±1.9
<i>Cymbopogon martinii</i> (Roxb.) Wats. in Atkins (leaf)	Poaceae	78.5±1.2	71.8±1.7
<i>Chenopodium ambrosioides</i> Linn. (leaf)	Chenopodiaceae	100.0±0.0	100.0±0.0
<i>Eupatorium cannabinum</i> Linn. (leaf)	Asteraceae	9.1±1.2	7.0±0.7
<i>Lavandula officinalis</i> Chaix (leaf)	Lamiaceae	24.1±2.7	13.2±1.3
<i>Lippia alba</i> Mill N.Br (leaf)	Verbenaceae	23.3±1.8	17.6±0.8
<i>Mentha arvensis</i> Linn. (leaf)	Lamiaceae	100.0±0.0	100.0±0.0
<i>Melaleuca leucadendron</i> Linn. Mart. (leaf)	Myrtaceae	31.7±1.8	40.2±1.8
<i>Ocimum canum</i> Sims (leaf)	Lamiaceae	65.1±1.4	5.3±0.6
<i>Pelargonium graveolens</i> Linn. ex Ait (leaf)	Geraniaceae	67.7±2.5	4.7±1.3
<i>Pogostemon cablin</i> Benth. (leaf)	Lamiaceae	13.2±1.8	12.4±50.8
<i>Santalum album</i> Linn. (wood)	Santalaceae	7.4±1.5	20.6±1.2
<i>Thymus vulgaris</i> Linn. (leaf)	Lamiaceae	78.5±1.2	71.8±2.5
<i>Vetiveria zizanioides</i> Linn. (rhizome)	Poaceae	1.4±3.2	6.3±0.6
<i>Zingiber officinale</i> Rosc. (rhizome)	Zingiberaceae	21.2±4.8	13.0±1.4

concentration and percent inhibition of free radical using the software Origin 6.0 (Northampton, MA, USA).

### 3. Results

#### 3.1. Fungitoxic activity of *Chenopodium* oil

During antifungal screening of essential oils isolated from 18 plant species against two aflatoxigenic strains of *A. flavus* at 500 µg/ml, the oils of *C. ambrosioides* and *M. arvensis* exhibited 100% fungitoxicity against both strains of *A. flavus* (Table 1). The oils of *Cymbopogon martinii* and *Thymus vulgaris* exhibited more than 70% fungitoxicity against both strains while those of *Aegle marmelos*, *Eupatorium cannabinum* and *Vetiveria zizanioides* were ineffective, showing less than 10% activity. *C.*

Table 2  
Fungitoxic spectrum of *C. ambrosioides* oil at 100 µg/ml

Fungi tested	Percent inhibition growth
<i>Aspergillus fumigatus</i> Fres.	100.0±0.0
<i>Aspergillus niger</i> Van Teigh	84.9±0.5
<i>Botryodiplodia theobromae</i> Pat.	100.0±0.0
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	51.5±0.4
<i>Fusarium oxysporum</i> Schlecht ex Fr.	100.0±0.0
<i>Helminthosporium oryzae</i> Breda de Hann.	75.5±1.0
<i>Macrophomina phaseolina</i> (Maubl.) Ashby	68.2±1.5
<i>Phythium debaryanum</i> Hesse.	100.0±0.0
<i>Sclerotium rolfsii</i> Sacc.	100.0±0.0

Data are significantly different with each other at  $P<0.005$ .

Table 3  
Effect of *C. ambrosioides* oil on mycelial biomass (g) and AFB1 production (µg/kg) against aflatoxigenic strain of *A. flavus*

Parameters	Concentration of <i>C. ambrosioides</i> oil (µg/ml)				
	Control	10	100	250	500
Mycelial biomass (g)	0.25±0.01	0.21±0.00	0.21±0.00	0.19±0.01	0.00±0.00
AFB1 (µg/kg)	447.48±12.39	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Data are significantly different with each other at  $P<0.005$ .

*ambrosioides* Linn. was selected for further studies because of its luxuriant growth as weed all over India.

*C. ambrosioides* oil inhibited the mycelial growth of the both strains of test fungi at 100 µg/ml. Therefore, the minimum inhibitory concentration (MIC) of oil was assigned to be 100 µg/ml. The oil of *C. ambrosioides* showed fungistatic nature of toxicity against both strains of *A. flavus*. *Chenopodium* oil was found to be more effective than the synthetic fungicides. The MICs of synthetic fungicides benzimidazole, zinc dimethyldithiocarbamate and phenylmercuric acetate against both strains were >5000, >5000 and 100 µg/ml, respectively. MIC of diphenylamine was 2000 µg/ml against Navjot 4NSt, but it was 3000 µg/ml against Saktiman 3NSt. The *Chenopodium* oil exhibited broad fungitoxic spectrum against all the tested fungi and it absolutely inhibited the growth of *A. fumigatus*, *B. theobromae*, *F. oxysporum*, *P. debaryanum* and *S. rolfsii* (Table 2).

#### 3.2. Antiaflatoxigenic property of *Chenopodium* oil

Effect of *C. ambrosioides* oil on production of aflatoxin B1 by the aflatoxigenic strain Saktiman 3NSt of *A. flavus* in SMKY medium is presented in Table 3. The oil completely inhibited the aflatoxin B1 production at 10 µg/ml. Mycelial growth was recorded up to 250 µg/ml in *Chenopodium* treated sets, but aflatoxin B1 production was completely inhibited. Thus, *C.*

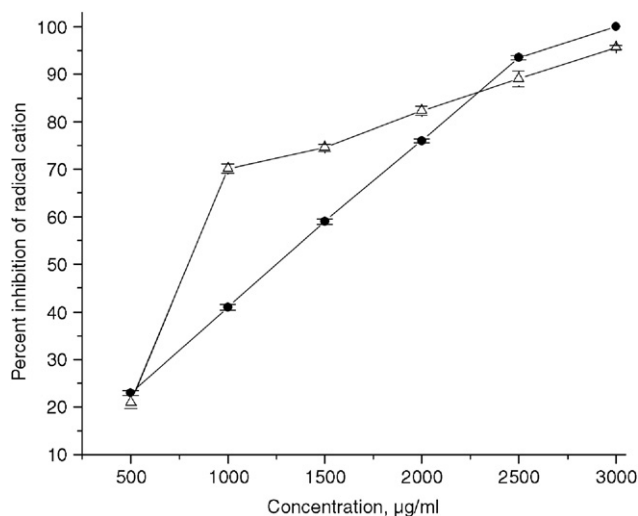


Fig. 1. Effect of *Chenopodium* oil and ascorbic acid on radical scavenging activity of ABTS radical cation; *Chenopodium* oil (—△—) and ascorbic acid (—●—). There was a significant relationship between the concentration and percent inhibition of radical cation ( $R=0.88$ ;  $P<0.05$ ).

Table 4  
Mycoflora of stored wheat samples treated with *Chenopodium* oil after 12 month of storage at 100 µg/ml

Fungi isolated	Percent occurrence of fungi			
	Uninoculated		Inoculated	
	Control	Treatment	Control	Treatment
<i>Aspergillus candidus</i>	3.7±0.2 <sup>a</sup>	0.0±0.0 <sup>b</sup>	4.3±0.3 <sup>a</sup>	0.0±0.0 <sup>b</sup>
<i>A. flavus</i>	46.7±1.7 <sup>a</sup>	0.0±0.0 <sup>b</sup>	89.0±2.7 <sup>a</sup>	8.3±0.8 <sup>b</sup>
<i>A. niger</i>	14.3±1.2 <sup>a</sup>	0.0±0.0 <sup>b</sup>	0.0±0.0	0.0±0.0
<i>A. parasiticus</i>	9.7±0.3 <sup>a</sup>	0.0±0.0 <sup>b</sup>	0.0±0.0	0.0±0.0
<i>A. terreus</i>	1.3±0.3 <sup>a</sup>	0.0±0.0 <sup>a</sup>	0.0±0.0	0.0±0.0
<i>Penicillium citrinum</i>	42.7±1.8 <sup>a</sup>	0.0±0.0 <sup>b</sup>	0.0±0.0	0.0±0.0

Within individual row values with different superscripts are significantly different with each other of control and treatment of uninoculated and inoculated sets respectively ( $P < 0.05$ , Student's *t* test).

*ambrosioides* oil was found effective in checking aflatoxin B1 production at a very low concentration.

### 3.3. Antioxidant activity of *Chenopodium* oil

ABTS<sup>++</sup> scavenging activity was enhanced with oil concentration, exhibited antioxidant activity of *C. ambrosioides* (Fig. 1). At 500, 1000, 1500, 2000, 2500 and 3000 µg/ml, the scavenging activity increased to 20.00, 70.00, 74.56, 82.30, 89.03 and 95.66%, respectively. The oil showed highest antioxidant activity at 3000 µg/ml concentrations.

### 3.4. In vivo fumigant efficacy of *Chenopodium* oil against fungal deterioration in stored wheat

The *Chenopodium* oil showed its efficacy in control of fungal association of wheat samples when it was tested as fumigant. The wheat samples of the uninoculated control set showed the presence of six fungi. *A. flavus* was found to be the dominant fungus, followed by *Penicillium citrinum*, *A. niger* and *A. parasiticus*. The *C. ambrosioides* oil checked the appearance of all the fungi appearing in uninoculated sets. In the case of inoculated control, two fungi were isolated. The oil completely checked the appearance of *A. candidus* while it significantly protected the wheat samples from *A. flavus* in the treatment set. The results indicate that there was 99.42% protection of wheat samples from storage fungi in uninoculated sets while 91.17% in inoculated sets (Table 4).

## 4. Discussion

The present findings indicate the possible exploitation of *Chenopodium* oil in treatment of food commodities against fungal infestations as well as lipid peroxidation. A fungitoxicant possessing broad fungitoxic spectrum may be employed successfully in controlling fungal infestations of stored food commodities incited by different fungi. Hence, the broad fungitoxic spectrum of *Chenopodium* oil would enhance its market value. The MIC of *Chenopodium* oil was comparatively lower than that of the synthetic fungicides tested, thereby, its potency as antifungal product of higher plant origin would be economical. A

perusal of literature shows that the MIC of *Chenopodium* oil (100 µg/ml) was also lower than other oils i.e. *Ocimum gratissimum*, *Thymus vulgaris*, *Cymbopogon citrates*, *Zingiber officinale* and *Monodora myristia* (Nguefack et al., 2004), *Alpinia galanga* (Tripathi et al., 1983), *Cinnamomum camphora* (Mishra et al., 1991) tested against *A. flavus*.

Some plant products have earlier been reported for their different biological properties mostly based on preliminary investigations. However, this is the first report on *Chenopodium* oil showing three virtues viz. in checking fungal deterioration of food commodities, inhibitory effect on aflatoxin synthesis and as antioxidant. Correlation in antifungal and antioxidant activity has been expressed in the case of some synthetic compounds (Thompson, 1991) and this has also been made in the present investigation with *Chenopodium* oil. The shelf life and quality of food commodities are affected by microbial infestation as well as the release of free radicals causing auto oxidation of unsaturated lipids (Kaur and Perkins, 1991). The *Chenopodium* oil was inhibitory to the growth of *A. flavus* as well as it checked the production of aflatoxin B1. The findings of the present study are relevant in enhancing shelf life of commodities by controlling micro organisms as well as free radical scavenging.

Lysis of the mycelia and spores of the toxigenic fungi is one of the characteristics of aflatoxin deactivation process which may proliferate under favourable conditions (Namazi et al., 2002). The results of the present investigation comply with the above mentioned characteristics. Food products are usually held at refrigerator temperature during their manufacture, storage and distribution. However, this provides opportunity for growth of psychrotolerant micro organisms. Essential oil components have been recommended in vegetable based food systems to explore their value as secondary preservatives (Shelef, 1984).

As *C. ambrosioides* widely grows in India as wasteland weed, its essential oil may be recommended as an easily available and renewal source of fungitoxicant and antioxidant in place of synthetic chemicals used for this purpose. *C. ambrosioides* has been used for a long time in the Indian system of medicine as vermicide (Cheryl et al., 2006; Mishra, 2002). Its oil may be thus recommended in food protection and food processing. In general concern for residues of essential oil on food commodities may be mitigated by the growing body of evidence that some essential oil constituents acquired through the diet are actually beneficial to human health (Huang et al., 1994). However, some experiments are further required to standardise for its safety limit and organoleptic tests with *Chenopodium* oil.

## Acknowledgement

The authors are thankful to the Department of Science and Technology, New Delhi, India for financial assistance.

## References

- AOAC, 1984. Natural poisons. Official Methods of Analysis of the Association of Official Analytical Chemists. 447–484.
- Bajaj, B.S., Ghosh, A.K., 1975. Antifungal antibiotics in perspective. In: Chaudhary, S.P., Varma, A., Bhargava, K.S., Mehrotra, B.S. (Eds.), Advances in Mycology and Plant Pathology. Sagar Printers, New Delhi, pp. 297–309.

- Benzie, I.F.F., 1996. Lipid peroxidation: a review of causes, consequences, measurement and dietary influences. *International Journal of Food Sciences and Nutrition* 47, 233–261.
- Cavalli, J.F., Tomi, F., Bernardini, A.F., Casanova, J., 2004. Combined analysis of the essential oil of *Chenopodium ambrosioides* by GC, GC–MS and 13C-NMR spectroscopy: quantitative determination of ascaridole, a heat-sensitive compound. *Phytochemical Analysis* 15, 275–279.
- Cheryl, L., Nancy, T., Gerhard, B., Grant, L., Karla, G., 2006. Ethnoveterinary medicines used for horses in Trinidad and in British Columbia, Canada. *Journal of Ethnobiology and Ethnomedicine* 31. doi:10.1186/1746-4269-2-31.
- Devkumar, C., Sukhdev, 1993. In: Radhwa, N.S., Parmar, B.S. (Eds.), *Neem Research and Development*. Society of Pesticide Science, New Delhi, pp. 63–96.
- Diener, U.L., Davis, N.D., 1966. Aflatoxin production by isolates of *Aspergillus flavus*. *Phytopathology* 56, 1390–1393.
- Donnelly, J.K., Robinson, D.S., 1995. Free radicals in foods. *Free Radical Research* 22, 147–176.
- Funder, S., 1968. *Practical Mycology: Manual for Identification of Fungi*. Hafner Publishing, New York.
- González, E., Felicio, J.D., Pinto, M.M., Rossi, M.H., Medina, C., Fernandes, M.J.B., Simoni, I.C., 2003. Inhibition of aflatoxin production by *Polymnia sonchifolia* and its *in vitro* cytotoxicity. *Arquivos do Instituto Biológico* 70, 139–143.
- Hartmans, K.J., Diepenhorst, P., Bakker, W., Gorris, L.G.M., 1995. The use of carvone in agriculture, sprout suppression of potatoes and antifungal activity against potato tuber and other plant diseases. *Industrial Crops and Products* 4, 3–13.
- Huang, M.T., Ferraro, T., Ho, C.T., 1994. Cancer chemoprevention by phytochemicals in fruits and vegetables. *American Chemical Society Symposium Series* 546, 2–15.
- Kaur, H., Perkins, J., 1991. The free radical chemistry of food additives. In: Aruoma, O.I., Holliwel, B. (Eds.), *Free Radicals and Food Additives*. Taylor and Francis, London, pp. 17–35.
- Madhavi, D.L., Salunkhe, D.K., 1995. Toxicological aspects of food antioxidants. In: Madhavi, D.L., Deshpande, S.S., Salunkhe, D.K. (Eds.), *Food Antioxidants*. Marcel Dekker, New York, p. 267.
- Mishra, A., 2002. Evaluation of some higher plant products for their pesticidal activity against some storage fungi and insects. Ph D thesis. Banaras Hindu University, Varanasi, India.
- Mishra, A.K., Dubey, N.K., 1994. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied and Environmental Microbiology* 60, 1101–1105.
- Mishra, A.K., Dwivedi, S.K., Kishore, N., Dubey, N.K., 1991. Fungistatic properties of essential oil of *Cinnamomum camphora*. *International Journal of Pharmacognosy* 29, 259–262.
- Moore, G.S., Jaciow, D.M., 1979. *Mycology for the Clinical Laboratory*. Reston Publishing Company, Virginia.
- Nabney, J., Nesbitt, B.F., 1965. A spectrophotometric method for determining the aflatoxins. *Analyst* 90, 155–160.
- Namazi, M., Allameh, A., Aminshahidi, M., Nohee, A., Malekzadeh, F., 2002. Inhibitory effects of ammonia solution on growth and aflatoxin production by *Aspergillus parasiticus* NRRL-2999. *Acta Poloniae Toxicologica* 10, 65–72.
- Nguefack, J., Leth, V., Amvam Zollo, P.H., Mathur, S.B., 2004. Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. *International Journal of Food Microbiology* 94, 329–334.
- Omura, et al., 1995. Comparative testicular toxicities of 2 isomers of dichloropropanol, 2,3-dichloro-1-propanol, and 1,3-dichloro-2-propanol, and their metabolites alpha-chlorohydrin and epichlorohydrin, and the potent testicular toxicant 1,2-dibromo-3-chloropropane. *Bulletin of Environmental Contamination and Toxicology* 55, 1–7.
- Pandey, R., 2003. Pesticides and sterility. *Everyman's Science XXXVIII*, 84–86.
- Pandey, D.K., Chandra, H., Tripathi, N.N., 1982. Volatile fungitoxic activity in higher plants with special reference to that of *Callistemon lanceolatus* D.C. *Phytopathologische Zeitschrift* 105, 175–182.
- Pittet, A., 1998. Natural occurrence of mycotoxins in foods and feeds — an update review. *Revue de Medecine Veterinaire* 149, 479–492.
- Re, R., Pellegrini, N., Progegente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine* 26, 1231–1237.
- Shaaya, E., Kostjukovski, M., Eilberg, J., Sukprakarn, C., 1997. Pant oils as fumigant and contact insecticides for control of stored product insects. *Journal of Stored Products Research* 33, 7–15.
- Shelef, L.A., 1984. Antimicrobial effects of spices. *Journal of Food Safety* 6, 29–44.
- Thompson, D.P., 1989. Fungitoxic activity of essential oil components on food storage fungi. *Mycologia* 81, 151–153.
- Thompson, D.P., 1991. Effect of butylated hydroxyanisole on conidial germination of toxigenic species of *Aspergillus flavus* and *Aspergillus parasiticus*. *Mycologia* 54, 375–377.
- Tiwari, O.P., Tripathi, Y.B., 2007. Antioxidant properties of different fractions of *Vitex negundo* Linn. *Food Chemistry* 100, 1170–1176.
- Tripathi, P., Dubey, N.K., 2004. Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. *Postharvest Biology and Technology* 32, 235–245.
- Tripathi, Y.B., Upadhyay, A.K., 2002. Effect of the alcohol extract of the seeds of *Mucuna pruriens* on free radicals and oxidative stress in albino rats. *Phytotherapy Research* 16, 534–538.
- Tripathi, N.N., Dubey, N.K., Dikshit, A., Tripathi, R.D., Dixit, S.N., 1983. Fungitoxic properties of *Alpinia galanga* oil. *Tropical Plant Science Research* 1, 49–52.
- Tripathi, P., Dubey, N.K., Banarji, R., Chansouria, J.P.N., 2004. Evaluation of some essential oils as botanical fungitoxicants in management of post harvest rotting of citrus fruits. *World Journal of Microbiology and Biotechnology* 20, 317–321.
- Varma, J., Dubey, N.K., 1999. Perspective of botanical and microbial products as pesticides of tomorrow. *Current Science* 76, 172–179.
- Varma, J., Dubey, N.K., 2001. Efficacy of essential oils of *Caesulia axillaris* and *Mentha arvensis* against some storage pests causing biodeterioration of food commodities. *International Journal of Food Microbiology* 68, 207–210.
- Ward, S.M., Delaquis, P.J., Holley, R.A., Mazza, G., 1998. Inhibition of spoilage and pathogenic bacteria on agar and pre-cooked roasted beef by volatile horse radish distillates. *Food Research International* 31, 19–26.
- Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M., Aggarwal, D., 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *American Journal of Clinical Nutrition* 80, 1106–1122.