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EFFECT OF VARIABLE PHYSICAL FACTORS ON THE TOXICITY OF PLANT VOLATILES AGAINST SOME *ASPERGILLUS* SPP.

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A B S T R A C T

The objective of the present study is to investigate the effect of physical factors on mycotoxic potency of *Chenopodium ambrosioides* Linn. and *Clausena pentaphylla* (Roxb.) DC oils against four species of *Aspergilli* such as *A. flavus* Link, *A. niger* van Tieghem, *A. ochraceus* Wilhelm, *A. terreus* Thom causing post-harvest deterioration of pigeon pea seeds. During experimentation it was observed that the potency of both oils remained unchanged even against heavy inoculum dose (maximum number of 10 fungal discs and diameter of 25 mm). Furthermore, the toxicity of both the oils did not alter even up to 120 ºC of temperature and 12 months of storage by exhibiting 100% mycelial inhibition of test fungi. The toxicity of both the oils was decreased at alkaline pH (7, 8). Physicochemical characterization of oils revealed that *C. ambrosioides* oil was pale yellow in colour, lighter than water, laevorotatory, acidic in nature and showed positive test for phenols. While the oil of *C. pentaphylla* had light pale color, dextrorotatory, slightly acidic and showed presence of phenols. Both the oils showed good solubility in various organic solvents.

Keywords: *Aspergillus spp*., *Chenopodium ambrosioides*, *Cluasena pentaphylla*, Essential oils, Physical factors, Physico-chemical properties.

INTRODUCTION

Essential oils keep stored food commodities free from pathogenic microorganisms, because they entail special promise for use as fumigants. These being lipophilic, can easily penetrate deeper through living tissue unbarred by selective permeability of cell membrane; hence they are of interest in the management of deep seated seed borne fungi (Lalitha and Raveesha, 2006). The essential oil of *Chenopodium ambrosioides* had earlier been reported to possess strong antifungal activity (Kumar *et al*., 2007) but mycotoxic reports on *Clausena pentaphylla* has not been studied. In previous studies we have screened essential oils against four dominant species of *Aspergillius* of stored pigeon pea seeds during which *Chenopodium* and *Clausena* oils were reported to be caused absolute toxicity, inhibiting mycelial growth of all test fungi at their MICs of 0.07 µl/ml. The present study was undertaken to investigate the changes in potency of *Chenopodium* and *Clausena* oils (at their MICs) under the influence of different physical factors. Further physico-chemical properties of both the oils were also assessed for their standardization.

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MATERIALS AND METHODS

Freshly collected twigs and leaves of plant species viz., *Chenopodium ambrosioides* and *Clausena pentaphylla* respectively were subjected to hydrodistilation in Clevenger type apparatus for isolation of essential oils separately. The essential oils were dried over anhydrous sodium sulphate and were stored at 4±1 ºC in dark places. The effect of different physical parameters viz., inoculum density, storage, temperature and autoclaving on fungal toxicity of the oils was studied by Inverted Petri plate method following Bocher (1938) at MIC of 0.07µl/ml. The test fungi were *Aspergillus flavus, A. niger, A. ochraceus* and *A. terreus*. A control set was maintained without essential oils. All the experiments were carried out in triplicates. The results were recorded in terms of per cent mycelial inhibition.

The effect of pH on potency of oils was done by Inverted Petri plate (Bocher, 1938) as well as poison food method (Grover and Moore, 1962). The pH of oils was determined by Elico pH meter. During Inverted Petri plate method, pH of oil was changed following the method of Shahi *et al.* (1999) with slight modification. Requisite amount of oil was mixed in PEG (Polyethylene glycol) and pH was adjusted at 5, 7 and 8 (using 1N NaOH and HCl) for *Chenopodium* (original pH 6) and 6, 7 and 8 for *Clausena* oil (original pH 5). However, during poison food method pH of medium was amended using citrate phosphate buffer following Dixit *et al.* (1982). The results were recorded in terms of per cent mycelial inhibition. Assays were maintained in triplicates. During each experiment a control set was maintained without additive.

Both the oils were standardized by determining their various physico-chemical properties viz. specific gravity, specific rotation, refractive index, acid number, saponification number, ester number, test for presence of phenolic content and solubility in various organic solvents following Langenau (1948).

RESULTS AND DISCUSSION

During experiments it was observed that both oils inhibited the growth of all ten discs as well as the growth of single mycelial disc of 25 mm diam of test fungi except *Aspergillus terreus* (Table 1). Both the oils remained effective after one year of storage exhibiting long shelf life (Table 2). The oil retained their fungitoxicity after exposure up to 120^{0}C indicating their thermostable nature (Table 3). Autoclaving (15 lb/inch^2 pressure for 15 min.) had no adverse effect on the toxicity of the oils against the test fungi. According to Wellman (1967), a pesticide must be stable to extreme of temperature and long shelf life. However the effects of storage and temperature on pesticidal activity of the oils have received little attention. Shukla (2009) reported that oil of *Cymbopogon pendulus* was thermostable up to 80ºC and toxic for 36 months. Similarly, in present investigation the toxicity of both oils was thermostable upto 120ºC and persisted for 12 months of storage.

The original pH of *C. ambrosioides* and *C. pentaphylla* oil was 6 and 5 respectively. Both the oils exhibited maximum toxicity against all the test fungi at pH 5, 6 and 7 when assessed by Inverted Petri plate method (Table 4). However during poisoned food method both the oils at the pH of 5 and 6 exhibited maximum toxicity towards *A. flavus*, *A. niger* and *A. ochraceus* while only pH 6 exhibited absolute toxicity for *A. terreus* (Table 5). The pH of the media has long been known to be a major influence on the growth of bacteria and fungi with most microorganisms having optimum growth in the range of pH 6-8 (Hood et al., 2004). In previous studies pH was found to have profound influence on the fungitoxicity of the oils (Pandey *et al*., 1982). Dixit et al. (1981) demonstrated that after amending the pH of test media, the fungitoxicity of *Cedrus* oil increased 6 times at pH 9 while that of *Mentha* oil 4 times at pH 9. Shahi et al. (1999) reported that efficacy of several *Eucalyptus* spp. oils against six dermatophytes increases when pH of test media is either increased or decreased. In both studies author adjusted the pH of the media prior to addition of essential oil and hence it is unknown what affect the essential oil had on media pH. In our study pH of media as well as oils was changed. The present work shows that toxicity of both the oils decreased at alkaline pH. Thus during fungitoxic preparations related to oils the activity of the oil can be enhanced by addition of acidic adjuvants.

Table 1. Effect of increased fungal inoculum on toxicity of essential oils.

No./Diam. of inoculated disc	Appearance of growth present (+)/absent (-)*							
	Chenopodium ambrosioides				*Clausena pentaphylla*			
	AF	*AN*	*AO*	*AT*	*AF*	*AN*	*AO*	*AT*
(a) No. of inoculum disc of 5 mm.								
2	-	-	-	+	-	-	-	+
4	-	-	-	+	-	-	-	+
6	-	-	-	+	-	-	-	+
8	-	-	-	+	-	-	-	+
10	-	-	-	+	-	-	-	+
(b) Diameter of inoculum disc in mm.								
10	-	-	-	+	-	-	-	+
15	-	-	-	+	-	-	-	+
20	-	-	-	+	-	-	-	+
25	-	-	-	+	-	-	-	+

AF-*Aspergillus flavus*, AN- *A. niger*, AO- *A. ochraceus*, AT- *A. terreus,* * Oil concentration 0.07µl/ml.

Table 2. Fungal toxicity of oils stored for different periods.

Storage period (in months)$	Per cent mycelial inhibition*							
	Chenopodium ambrosioides				*Clausena pentaphylla*			
	AF	*AN*	*AO*	*AT*	*AF*	*AN*	*AO*	*AT*
3	100	100	100	100	100	100	100	100
6	100	100	100	100	100	100	100	100
9	100	100	100	100	100	100	100	100
12	100	100	100	100	100	100	100	100

$ Duration of storage- November 2008- October 2009

Table 3. Fungal toxicity of oils treated to different temperatures.

Temperature (in ^{0}C)	Per cent mycelial inhibition*							
	Chenopodium ambrosioides				*Clausena pentaphylla*			
	AF	*AN*	*AO*	*AT*	*AF*	*AN*	*AO*	*AT*
40	100	100	100	100	100	100	100	100
60	100	100	100	100	100	100	100	100
80	100	100	100	100	100	100	100	100
100	100	100	100	100	100	100	100	100
120	100	100	100	100	100	100	100	100

Table 4. Effect of pH on toxicity of oils by Inverted Petri plate method.

pH levels	Per cent mycelial inhibition*							
	Chenopodium ambrosioides				*Clausena pentaphylla*			
	AF	*AN*	*AO*	*AT*	*AF*	*AN*	*AO*	*AT*
5	100	100	100	100	100	100	100	100
6	100	100	100	100	100	100	100	100
7	93.27	100	96.22	100	100	91.48	89.80	100
8	97.10	100	95.76	100	100	100	100	88.23

Table 5. Effect of pH on toxicity of oils by poison food method.

pH levels	Per cent mycelial inhibition*							
	Chenopodium ambrosioides				*Clausena pentaphylla*			
	AF	*AN*	*AO*	*AT*	*AF*	*AN*	*AO*	*AT*
5	100	100	100	97.86	100	100	100	98.8
6	100	100	100	100	100	100	100	100
7	93.27	90.40	96.22	94.98	93.34	91.48	89.80	92.50
8	97.10	79.54	95.76	90.67	83.45	80.67	82.54	78.03

Essential oils are mixture of organic substances of heterogeneous groups, the level of which may vary with plants growing in different ecological situations. Hence the quality of biologically active essential oils must be standardized in order to get the reproducible results (Rehman *et al*., 2007).

In present study therefore quality of fungitoxic oils was standardized by their various physicochemical properties. Table 6 reveals the physico-chemical characterization of *Chenopodium* and *Clausena* oils as an ideal fungitoxicants. Oil of *C. ambrosioides* was pale yellow in colour, lighter than water (specific gravity 0.8199 at 25ºC), laevorotatory (specific rotation -6º2' at 25ºC), acidic in nature and showed positive test for phenols. Oil was immiscible in water, while exhibited good solubility in various organic solvents.

Table 6. Physico-chemical properties of essential oils.

Parameters	Values	
	Chenopodium ambrosioides	*Clausena pentaphylla*
Colour	Yellow	Light yellow
Specific gravity	0.8199 at 25ºC	1.2415 at 25ºC
Specific rotation	-6º2' at 25ºC	+0.12º28' at 22ºC
Refractive index	1.436 at 29ºC	1.529 at 26ºC
Acid value	16.35	2.992
Saponification number	164.55	52.36
Ester number	181.30	49.37
Phenolic content	Present	Present
Solubility	1: 1 Soluble in 90% alcohol, 1:2 in Acetone and Ethyl acetate but insoluble in water	1: 1 Soluble in 90% alcohol, in Acetone and Ethyl acetate but insoluble in water

The oil of *C. pentaphylla* had specific gravity 1.2415, oil was dextrorotatory (+0.12º28' at 22ºC), slightly acidic, showed presence of phenols and good solubility in various organic solvents but insoluble in water.

In conclusion the findings suggest that *Chenopodium* and *Clausena* oils can be exploited as an ideal fungitoxicant against storage fungi due to their long shelf life, persistent toxicity at high temperature, activity at heavy inoculum doses and at variable ranges of pH.

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