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Allelopathic Impact of Volatile Components from *Eucalyptus* on Crop Plants

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Abstract. The effect of crude volatile oils from the leaves of *Eucalyptus globulus* and *E. citriodora* and the pure terpenes – cineole and limonene from these oils, (in vapour form) was studied on *Phaseolus aureus, Lens esculentum, Hordeum vulgare* and *Avena sativa*. The parameters like germination of seeds, seedling growth, values of cell survival, and content of water and chlorophyll of the crops formed the system of bioefficacy study. The allelopathic impact of the oil vapours from the eucalypt tree becomes evident from the negative response of the parameters studied. The impact of the *E. citriodora* oil vapours compared to that of *E. globulus* oil or the pure terpenes was seen to be relatively greater in almost all parameters under investigation. A strong reciprocal correlation that exist between the concentration and the seedling growth or the water content of the crops under study supports the dose linked allelopathic phenomenon. It is suggested that oil vapours of *Eucalyptus* exert their effect through impairing the respiratory as well as photosynthetic ability of the target plants.

Eucalyptus is alleged to discourage the growth of other plants under its canopy, in spite of enough light, space, nutrients and soil humidity (Del Moral 1970). Relatively very low density and diversity of vegetation under 7 year old *Eucalyptus tereticornis* plantation compared to that of *Grevillea robusta* has been demonstrated by Bhaskar and Dasappa (1986). Al-Mousawi and Al-Naib (1975) reported paucity of herbaceous vegetation under *E. microtheca*. α-pinene, β-pinene, camphene, and cineole (Al-Mousawi and Al-Naib 1976) and phenolic acids (Al-Naib and Al-Mousawi 1976) have been proposed to be the substances responsible for the inhibitory action. The allelochemic caused inhibition of vegetational composition, and density is well documented by Muller (1966).

The leaves of *Eucalyptus* are intensely aromatic releasing a number of volatile terpenes into the environment (Muller and Muller 1964, Del Moral and Muller 1970). Baker (1966) evidenced the inhibition of the growth of radicles of *Cucumis* sp. by volatile oils of *E. globulus*. Essential oils from *Eucalyptus* have been shown to affect the diffusibility and transpiration of leaves of other plants (Polová and Vicherková 1986, Vicherková and Polová 1986). The volatiles from

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E. camaldulensis have also been shown to be possible allelochemics for undercanopy plants (Del Moral and Muller 1970). The high vapour density of the oils from *Eucalyptus* leaves enable them to penetrate into soil, affecting adversely the undergrowing plants. The percentage of oil, and the nature and amounts of various components of the oils is different in different species of *Eucalyptus*. Huge annual loss because of allelopathic influence of plants on crop productivity has been proposed by Putnam (1988). The early growth of wheat is drastically affected by the aromatic substances released by *Salvia reflexa* (Lovett 1982). The impact increases if the leaves of *S. reflexa* are made wet (Lovett 1985).

Our study deals with the impact of crude oils from *E. globulus* Labill. and *E. citriodora* Hook. apart from the pure components, limonene and cineole purified from *E. globulus* on *Phaseolus aureus, Lens esculentum, Avena sativa* and *Hordeum vulgare*.

MATERIAL AND METHODS

Germination Trials

Pure line healthy, uniform, fresh viable seeds of *Phaseolus aureus* Roxb. (= *Vigna radiata* (L.) R. Wilczek) var. ML-267, *Lens esculentum* L. var. AL-76, *Avena sativa* L. var. Kent and *Hordeum vulgare* L. were procured from Seed Technology Unit, Punjab Agricultural University, Ludhiana.

The lower parts of 65 Petri dishes (11 cm diameter) were covered with Whatman No. 40 filter paper discs upon a thin layer of absorbent cotton wad. It was moistened with 20 ml distilled water. Such a set of 65 Petri dishes was maintained for each seed species under test.

Thirteen groups, each of 100 uniform size seeds, were made for each plant species under study. Each group was divided into 5 sub groups of 20 seeds each. Seeds of each of the four plant species were arranged equidistantly in concentric circles on top of the wet filter paper discs. The uncovered Petri dishes were placed in environmentally controlled chambers (3 m^3) programmed as per International Seed Testing Association (ISTA 1976) rules.

The seed germinators were used for dual purposes: first, as environmental-control chambers and second, as fumatoria. They were prefumigated (under 126.2 m^3 h^{-1} air displacement pressure) with the proper concentration of the requisite component vapours. Concentrations of 10, 20, 30 nl cm^3 of the space were maintained for each of the volatile components under study. Ordinary air in a chamber served as control.

Visual observations with the aid of a hand lens were made daily. Protrusion of radicle (to serve as an index of germination) on the twenty seeds of one of the dishes representative of each concentration of each treatment or the control was checked. Such observations on germination counts of the seeds were made daily, till for a week no more seed germinated. At the time of termination, the

numbers of seeds that had germinated in all the Petri dishes of a set were counted. Lengths of radicle and plumule of the twenty representative seeds that had been inspected daily were also measured. The vigour was calculated as described by Kumari *et al.* (1985) following the ISTA (1976) rules.

Treatment of Plants

One-month-old plants of *P. aureus* and *L. esculentum* grown in glazed plastic pots under normal condition were transferred to fumatoria (3 m^3) maintained at 28 $\pm$ 2 °C, 16 h photoperiod, 270 $\mu W\ cm^{-2}$ energy. The fumatoria were flushed with vapours (13.5 $nl\ cm^{-3}$) of the respective oil under test. On alternate days, the old vapours were replaced by a fresh lot. After 10 d of the vapour treatment, the plants were removed and examined for cell survival, chlorophyll content and water content.

Cell Survival Test

Fresh leaf discs (100 mg each) of the control and each of the treated samples were tested for formazan formation with 2,3,5-triphenyltetrazolium chloride by the method of Steponkus and Lanphear (1967). The values of absorbance read on Spectronic 1201 spectrophotometer were calculated in terms of dry mass unit equivalents and have been expressed as per cent of control.

Estimation of Chlorophyll Content

The total chlorophyll content from leaf discs (80 mg fresh mass) was extracted into dimethyl sulphoxide (DMSO) and estimated by the Hiscox and Israelstam (1979) method.

The calculations of the absorbance values were made on the basis of dry mass equivalents instead of fresh mass basis (as given in the original method). For use in calculating the dry mass, 80 mg of the fresh leaf discs were oven dried. This was considered essential for two reasons. 1. Treated leaves lose more water and thereby, provide more amount of the sample (in terms of fresh mass) compared to control. 2. Water content of fresh leaves varies in different seasons. Calculations in terms of dry mass equivalents becomes necessary for precision and reproducibility of data. The values have, therefore, been expressed as milligram per gram dry mass.

Determination of Water Content

The water content of leaves or seeds/seedlings was measured directly on Dean and Stark apparatus (see Trease and Evans 1983). This method was considered better than the fresh and dry mass method because of two defects of the latter. 1. Volatile components, if any, in the material get removed along with water on drying in the oven; this adds to the loss of mass. 2. There may be gain of

mass due to rehydration of dried samples from humid air during the period between removal of samples from the oven and bringing them to room temperature for weighing.

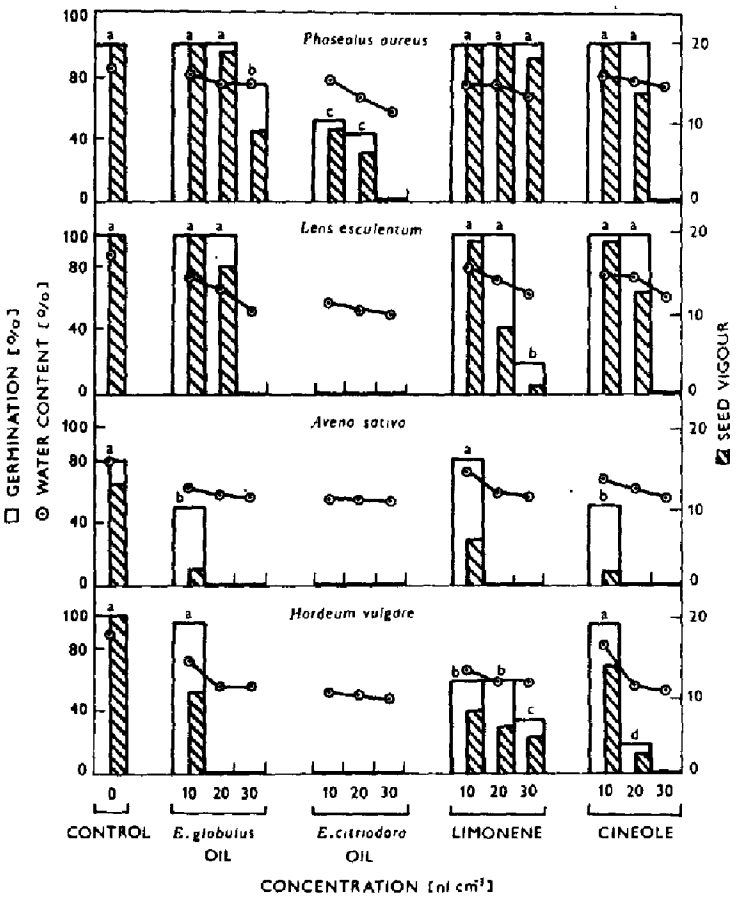

Fig. 1 Effect of some volatile oils and components from *Eucalyptus* leaves on per cent germination, seed vigour and water content of a few crop plants.*

* Means of per cent germination of respective plants are shown in the graph; those having same superscript symbols are insignificatnly different at 5 % level according to DMRT (Duncan 1955).

RESULTS

Germination Behaviour

In water treated controls, all the seeds of *P. aureus, L. esculentum* and *H. vulgare* germinated. However, only 80 % of the untreated control *A. sativa* seeds could germinate. No difference from the germination percentage of control was noticed in seeds of *P. aureus* and *L. esculentum* subjected to germination under the lower two concentrations (10 or 20 nl cm $^{-3}$) of *E. globulus* oil, limonene

TABLE I

Correlation coefficents of per cent water content and concentration of different oil vapours applied

Treatment	Correlation coefficient			
	P. aureus	*L. esculentum*	*A. sativa*	*H. vulgare*
E. globulus oil	−0.978	−0.995	−0.897	−0.940
E. citriodora oil	−0.992	−0.879	−0.815	−0.833
Limonene	−0.871	−0.997	−0.978	−0.899
Cineole	−0.987	−0.959	−0.988	−0.959

or cineole. Higher concentration of these three oil vapours treated seeds of these two plants showed decreased germination. Exception was, however noticed in case of limonene, where all seeds of *P. aureus* compared to 20 % for those of *L. esculentum* germinated. None of the seeds of these two plants under the treatment with cineole germinated.

Seventy-five per cent of *P. aureus* seeds and none of those of *L. esculentum* germinated in response to 30 nl cm^{-3} concentration of *E. globulus* oil. None of the seeds of the four plants except *P. aureus* were seen to germinate in chambers fumigated with any concentration of the *E. citriodora* oil. In contrast, 52 and 45 % of seeds of *P. aureus* treated with 10 or 20 nl cm^{-3} respectively, of *E. citriodora* oil vapour, germinated. Seeds of *A. sativa* treated with *E. globulus* oil or limonene or cineole (20 or 30 nl cm^{-3}) vapours showed no germination at all. However, those treated with 10 nl cm^{-3} concentration of *E. globulus* oil or cineole vapours permitted nearly 50 % germination compared to that of control or the limonene treated seeds. Seeds of *H. vulgare* treated with 10 nl cm^{-3} of *E. globulus* oil or cineole vapours did not show any significant difference in germination compared to control, whereas limonene vapour treated seeds showed about 60 % germination, which was significant difference compared to control. However, those treated with 20 nl cm^{-3} of limonene, cineole, or *E. globulus* oil vapours showed, compared to that of control, 60, 20 and 0 % germination, respectively. In response to 30 nl cm^{-3} of limonene 35 % of *H. vulgare* seeds germinated in contrast to no germination what-so-ever in those treated with *E. globulus* oil or cineole vapours (Fig. 1).

As is apparent from the seed vigour data all the untreated control seeds of *P. aureus, L. esculentum* and *H. vulgare* germinated on the very first day. *A. sativa,* however, showed lesser seed vigour. In response to any volatile oil under test, a trend towards decrease in seed vigour with increasing concentration of oil vapours could be noticed. However, *P. aureus* seeds treated with limonene vapour or the lower two concentrations of *E. globulus* oil vapours constituted exceptions; for these, seed vigour was not found to change significantly with

respect to the control. Though 100 % seeds of *L. esculentum* germinated in limonene vapour (20 nl cm^{-3}), the seed vigour was drastically low.

The water content of treated seeds or their seedlings compared to the respective seedlings in all the four plants under study decreased gradually with increasing concentrations of all four oil vapours; the correlation coefficients (r) between these two variables were seen to be strong, direct and negative in all cases (Table 1).

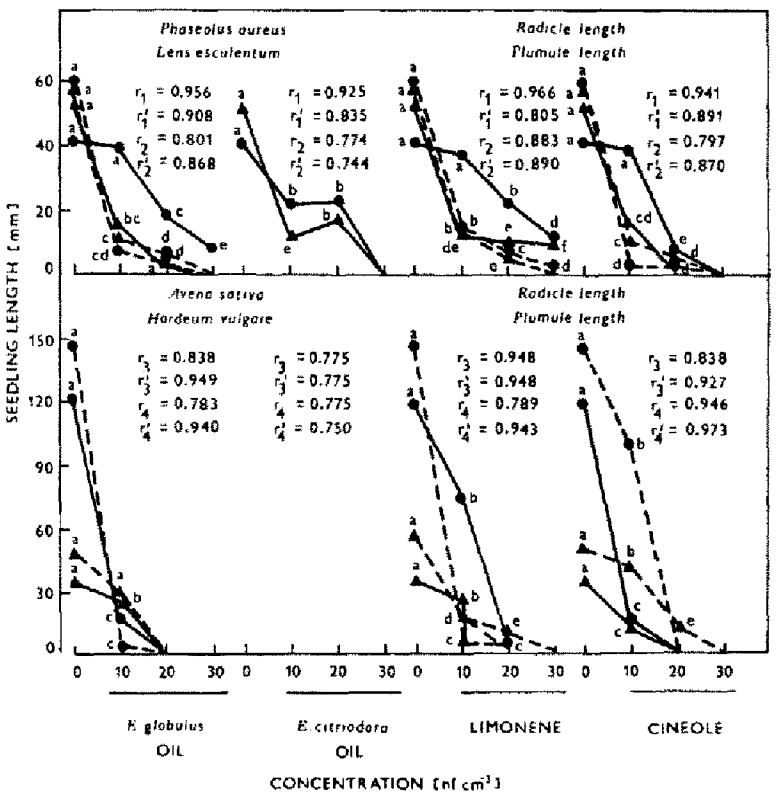

Fig. 2 Effect of different oil vapours on seedling growth (plumule and radicle) of a few crop plants.*, **

* Means of different parameters of respective plants are shown in the graph; those having same superscript symbols are insignificantly different at 5 % level according to DMRT (Duncan 1955).

** r: correlation coefficient between concentration and lengths of radicle (r_1, r_2, r_3, r_4) and plumule (r'_1, r'_2, r'_3, r'_4) of *P. aureus*, *L. esculentum*, *A. sativa* and *H. vulgare*, respectively.

Trends of the impact of oil vapours on the germinating seeds as regards lengths of radicle and plumule of seeds under test were almost the same. With increasing concentration of vapours, the seedling length decreased in all cases where seed germination had occured (Fig. 2). The values of correlation coefficients evidence a strong, negative, direct correlation between increasing

TABLE 2

Effect of *E. globulus* oil and *E. citriodora* oil on chlorophyll content, cell survival and water content of *P. aureus* var, ML-267 and *L. esculentum* var. AL-76*.

Plant	Treatment	Cell survival [%]	Total chlorophyll [g mg^{-1} dry mass]	water content [%]
P. aureus	Control	100.00[a]	13.55[a]	87.90[a]
	E. globulus oil	40.81[b]	5.05[b]	67.00[b]
	E. citriodora oil	36.11[b]	3.95[b]	40.00[c]
L. esculentum	Control	100.00[a]	10.96[a]	81.90[a]
	E. globulus oil	36.22[b]	4.36[b]	80.00[a]
	E. citriodora oil	17.12[b]	2.88[b]	39.70[b]

* Means are given in the columns. The observed parameters of the respective plant having the same letter in the same column are insignificantly different from each other according to DUNCAN's multiple range test (Duncan 1955) at 5 % level of significance.

concentration of oil vapours applied and the lengths of the radicle or plumule of the four plants under study.

Chlorophyll Content and Cell Survival

Leaves from plants of *P. aureus* and *L. esculentum* exposed to the vapours of *E. globulus* and *E. citriodora* oils (13.5 nl cm^{-3}) showed cell survival value and total chlorophyll content to be drastically less than those of the respective controls. The values for the two oil vapours, in both the plants, differ insignificantly. Those of *E. citriodora* oil vapours tended to be lower than those of *E. globulus* at 5 % level of significance (Table 2). However, at 10 % level of significance (data not shown) the difference became significant.

Compared to the control, more than 50 % of water was seen to have lost from leaves of *P. aureus* and *L. esculentum* plants exposed to the vapours (13.5 nl cm^{-3}) of *E. citriodora* oil. *E. globulus* oil vapours in the same concentration caused *P. aureus* leaves to show a loss of 23.7 %, while *L. esculentum* so treated showed no significant change in water content compared to that of its respective control.

DISCUSSION

The impact of *E. citriodora* oil vapours on seed vigour, seedling length (or growth), water content, chlorophyll content, and cell survival value of the plants studied was greater than that of other vapours tried. A dose-response relationship, a characteristic feature of chemically controlled physiological response

between vapours, and all the parameters studied is apparent. It is, however, important to point out that where 100 % seed germinated in volatile oil vapours, significant decrease in seed vigour was noticed. Even when there was no significant difference in seed vigour, seedling growth was drastically decreased. The negative strong correlation between concentration and seedling length clearly shows that with increasing concentration of oil vapours, the seedling length decreased drastically. The increased loss of water from seeds or leaves treated with oil vapours reveals that volatile allelochemicals exert their influence through desiccating the cells of the target plant. Since, the crude *E. globulus* and *E. citriodora* oils had relatively strong effects on germination than the pure compounds (limonene and cineole), these were further used on fully grown plants of *P. aureus* and *L. esculentum*.

The vapours from *E. globulus* and *E. citriodora* oils lowered the chlorophyll content and the cell survival value of *P. aureus* and *L. esculentum*. The decrease in chlorophyll content could be the result of enhancement of metabolic function responsible for active degradation of already synthesized chlorophyll pigments, or conversely due to inhibition of metabolic (enzymatic) system(s) responsible for the synthesis of new chlorophyll. It could be ascertained through further work on the enzymatic system involved in chlorophyll synthesis and breakdown. Nevertheless, the chlorophyll loss is bound to impair the photosynthetic process.

The decreased value of cell survival or increase in cellular killing represented by the amount of red formazan formed through tetrazolium reduction suggests that oil vapours exert their impact through the electron-transport system of the cell. The amount of red formazan represents the rate and extent at which protons (hydrogen ions) released through the respiratory electron-transport chain of the target cells are trapped by the tetrazolium salt. It shows that the vapours exert their impact through impairing the respiratory, apart from the photosynthetic activity, the two important physiological functions of the plants tissue. This is in contrast to the possibility of specificity of action of *Eucalyptus globulus* allelochemics extracted by use of different solvent system, in which one set depresses the respiratory while the other the photosynthetic system (Kohli 1987, Kohli *et al.* 1987).

Among the vapours tested, that of *E. citriodora* oil represents the most effective and potent allelochemic(s) responsible for inhibitory function in crops tested.

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