EXPERIMENT 2

Objective

To study the effect of monoterpenes namely citronellol, linalool, eugenol and limonene on growth and development of some weeds.

Hypothesis

Since the monoterpenes were found to adversely affect the germination of the test weeds, it was hypothesized that they may also affect the subsequent growth and development. For example, the seeds in the environment of aromatic plants like *Eucalyptus* may though germinate, but their seedlings fail to establish since they are in direct contact with the vapours of monoterpenes. Keeing this in mind, the present experiment was planned to assess the effect of monoterpenes on the growth and physiological processes of the test weeds.

Parameters studied

Measurement of seedling length and dry weight and determination of the amount of total chlorophyll and percent cellular respiration of the test weeds namely, *Amaranthus viridis*, *Bidens pilosa*, *Cassia occidentalis* and *Parthenium hysterophorus* in response to the monoterpenes citronellol, linalool, eugenol and limonene formed the parameters of study.
Experimental design

Collection of material

Seeds of each of the four weed species were divided into 108 groups (27 groups for each monoterpane) and were imbibed in water for 16-18h (details given in the chapter, "Materials and Methods"). After 8 days, seedling length (in cm) in each Petri dish was measured except *P. hysterophorus* where it was measured after 15 days. Dry weight of seedlings was measured over an electronic balance after oven drying.

Estimation of total chlorophyll content

The chlorophyll from leaf discs was extracted in dimethyl sulphoxide (DMSO) following the method of Hiscox and Israelstam (1979). Total chlorophyll content was calculated from extinction values following the equation of Arnon (1949). The content was expressed in terms of dry weight (μg / mg dry weight) as suggested by Rani and Kohli (1991).

Estimation of cell respiration

The cell survival (respiration) was evaluated following the method of Steponkus and Lanphear (1967) using 2,3,5-Triphenyl tetrazolium chloride salt. Values were calculated in terms of unit dry weight equivalents and expressed as percent with respect to control as suggested by Daizy (1990).

Statistical analysis

All data were expressed as means of respective parameters and the significance of treatment i.e. each concentration of each monoterpane was tested with respect to control applying ANOVA (Analysis of variance) followed by Duncan's multiple range test using the statistical package of SPSS version 10. Besides, values of correlation coefficient (r) were also calculated between the parameter and concentration of monoterpenes and were presented along each curve.
Observations and Results

1. Seedling length

The seedling length of all the test weeds was significantly reduced over that of control in samples treated with different concentrations i.e. 0.2, 1, 1.5, 2, 5, 10 and 15 nl / cc of citronellol (Fig. 2.1a).

*Citronellol*

In water treated control, the seedling length of *A. viridis* was measured to be 5.09 ± 0.16 cm. In samples exposed to the concentration of 0.2 nl / cc of citronellol, only 10% reduction in seedling length compared to control was observed. However, with the treatment of 1.0 and 1.5 nl / cc, the reduction in seedling length was about 32% and 47%, respectively compared to control. In case of treatment with 2 nl / cc, the reduction in seedling length was 68%. At concentration 5 nl / cc and beyond since complete inhibition of seed germination was observed, seedling length was not recorded (Fig. 2.1a). The correlation between different concentrations of citronellol and seedling length of *A. viridis* was not very strong with r = -0.776.

In *B. pilosa*, seedling length in control was recorded to be nearly 6.59 ± 0.45 cm. In response to the treatment of citronellol, the seedling length was nearly 93, 82, 77 and 70% compared to control with the treatment concentrations of 0.2, 1, 1.5 and 2 nl / cc of citronellol, thus exhibiting significant differences from that of control (Fig. 2.1a). Seedling length was measured to be still lesser compared to control at higher treatment concentrations and with increase in concentration, continuous decrease in seedling length was observed. In response to the concentration of 15 nl / cc and beyond, complete inhibition of germination was observed. The value of correlation coefficient (r) was high, reciprocal and close to one.

At lower concentrations i.e. 0.2 and 1 nl / cc, seedling length of *C. occidentalis* was recorded to be around 72% of control, thus exhibiting a reduction of
about 28% over control whereas at higher concentrations of 1.5 and 2 nl / cc, seedling length of *C. occidentalis* was nearly 67% of control. A reduction of about 40% was observed with the treatment of 5 nl / cc of citronellol. At 10 nl / cc however, complete inhibition of germination of *C. occidentalis* was observed (Fig. 2.1a). There was a strong correlation between citronellol concentrations and seedling length of *C. occidentalis* with $r = -0.919$.

In *P. hysterophorus*, the seedling length was measured to be $2.74 \pm 0.34$ cm in control. In case of the treatment of citronellol, the seedling length of *P. hysterophorus* was reduced appreciably even at lowest concentration of 0.2 nl / cc. Here the reduction in seedling length was nearly 13% compared to control and it was statistically significant. With the treatment of higher concentrations of citronellol, the seedling length was further reduced and at 2 nl / cc or beyond, the germination was completely inhibited (Fig. 2.1a). Here, the value of correlation coefficient ($r$) was calculated to be $-0.680$.

**Linalool**

Particularly at the higher concentrations of linalool also, a decrease in seedling length of all the test weeds was observed (Fig. 2.1b). In *A. viridis*, the decrease in seedling length was significant to that of control at all the concentrations. At 5 nl / cc, complete inhibition of seed germination was observed and thus seedling length data could not be recorded (Fig. 2.1b). The correlation between seedling length and different concentrations of linalool was calculated to be $r = -0.612$.

In *B. pilosa*, the seedling length in control was measured to be $6.43 \pm 0.55$ cm while in seeds treated with 0.2 and 1 nl / cc concentrations of linalool, it was recorded to be $6.18 \pm 0.06$ and $5.33 \pm 0.05$ cm, respectively. At 1.5 and 2 nl / cc decrease in seedling length was recorded to be nearly 35 and 49% and it was statistically significant compared to control. With the treatment of 5 nl / cc of linalool, the seedling length of *B. pilosa* was recorded to be $1.74$ cm exhibiting a reduction of about 73% compared to control (Fig. 2.1b). At still
higher concentrations the seedling growth was completely inhibited. The value of correlation coefficient obtained was –0.878.

Fig. 2.1: Effect of different concentrations of (a) citronellol (b) linalool (c) eugenol (d) limonene on the seedling length of the test weeds.
In *C. occidentalis*, when treatment of different concentrations of linalool was given, a decrease in seedling length was noticed at all the concentrations used. At lower concentrations of 0.2 and 1 nl / cc, a significant decrease in seedling length was noticed which was measured to be 10.4 and 9.5 cm, respectively compared to 11.7 cm in control, exhibiting a reduction of about 11 and 19%, respectively (Fig. 2.1b). At higher concentrations, a steady decrease in seedling length was noticed and at 20 nl / cc and above, seedling length could not be measured, thus indicating complete inhibition (Fig. 2.1b).

In *P. hysterophorus*, the seedling length in control was 3.3 ± 0.26 cm. At any of the treatment concentrations, a statistically significant decrease in seedling length compared to control was noticed. Nearly 39% reduction in seedling length was seen when the treatment of 0.2 nl / cc of linalool was given (Fig. 2.1b). The reduction in seedling length continued with the treatment of higher concentrations and at the concentration 10 nl / cc, seedlings did not emerge, indicating complete inhibition. The curves drawn between different concentrations of linalool and seedling length of *P. hysterophorus* indicate a dose-response relationship with significant value of correlation coefficient i.e. $r = -0.797$.

**Eugenol**

In *A. viridis*, mean seedling length in control was measured to be 6.78 ± 0.52 cm. A decrease of 26% compared to control was observed with the treatment of 0.2 nl / cc. With further increase in concentration, the decrease in seedling length continued and at highest concentration i.e. at 5 nl / cc, the seedling length was recorded to be 0.49 ± 0.02 cm which is 7.23% of control. At all concentrations the decrease in seedling length was statistically significant compared to control. The value of correlation coefficient obtained between different concentrations of eugenol and seedling length was calculated to be $-0.764$ (Fig. 2.1c).
In *B. pilosa*, compared to control, the decrease in seedling length was statistically significant in response to any of the concentrations. At lower concentrations of 0.2 and 1 nl/cc, a decrease of about 7 and 15%, respectively was noticed, while there was a further reduction of 25, 71, 81 and 89% compared to control with the treatments of 1.5, 2, 5 and 10 nl/cc, respectively. The \( r \) value in this case was calculated to be \(-0.844\) (Fig. 2.1c).

A similar trend was noticed in case of *C. occidentalis*, where reduction of around 19 and 32% were recorded with the treatment of even the lower concentrations of 0.2 and 1 nl/cc. At higher concentrations of eugenol, seedling length was further reduced compared to control (Fig. 2.1c) and at 5 nl/cc or beyond, seedling length was completely suppressed. There was a strong correlation between different concentrations of eugenol and seedling length of *C. occidentalis* and the value of \( r \) was -0.876.

The mean seedling length of control in *P. hysterophorus* was recorded to be 3.86 ± 0.24 cm. With the treatment of different concentrations of eugenol, a decrease in seedling length was observed. However, at 5 nl/cc, none of the seeds of *P. hysterophorus* germinated, thus seedling growth was completely suppressed (Fig. 2.1c). The correlation between different concentrations of eugenol and seedling length was not very strong with \( r = -0.765\).

**Limonene**

In *A. viridis*, the seedling length in control was recorded to be 5.34 ± 0.73 cm and it decreased with increasing concentrations of limonene. At a lower concentration of 50 nl/cc, the seedling length was measured to be 3.89 ± 1.79 cm, thus exhibiting a reduction of 27% over control. At higher concentrations of 100, 200, 300 and 400 nl/cc, seedling length was further noticed to be reduced. Likewise at 450 nl/cc, a reduction of 77% compared to control was noticed. Beyond this concentration, a complete inhibition in germination was noticed (Fig. 2.1d). The decrease in seedling length with
different concentrations of limonene was statistically significant with \((r)\) value close to -1.

In \textit{B. pilosa} also, the seedling length decreased with increasing concentrations of limonene. At 100 nl/cc, it was about 50% compared to control. With the treatment of higher concentrations of limonene, a further decrease in seedling length was seen (Fig. 2.1d). A similar trend was observed in case of \textit{C. occidentalis} where seedling length was measured to be significantly shorter compared to control at each treatment concentration. At the highest treatment concentration of limonene, i.e. at 450 nl/cc, there was reduction of nearly 90% over that of control (Fig. 2.1d).

In \textit{P. hysterophorus} also, a decreasing trend in seedling length was recorded with increasing concentrations of limonene used (Fig. 2.1d). With the treatment of 100 nl/cc, about 50% reduction in seedling length was seen and it was statistically significant over control. At 450 nl/cc, a reduction of about 90% in seedling length was recorded after which a complete inhibition of germination was observed.

There was strong negative correlation between different concentrations of limonene and seedling length of all the test weeds. Further, the values of correlation coefficient \((r)\) were more than -0.9 in case of all the four weeds.

2. Seedling dry weight

\textit{Citronellol}

The dry weight of all the weed species was reduced with the treatment of different concentrations of citronellol i.e. 0.2, 1, 1.5, 2, 5, 10 and 15 nl/cc (Fig. 2.2a). At lower concentrations i.e. 0.2 and 1 nl/cc, the seedling dry weight of \textit{A. viridis} was measured to be around 90% compared to control. At still higher concentrations, a further decrease in seedling length was observed (Fig. 2.2a). The correlation coefficient \((r)\) was calculated to be -0.833.
In *B. pilosa*, there was slight reduction in seedling dry weight at lower concentrations of citronellol i.e. 0.2, 1 and 1.5 nl / cc (where a reduction of less than 5% was seen) and the reduction at these concentrations was statistically insignificant. At higher concentrations i.e. 2, 5 and 10 nl / cc, a further decrease in dry weight was observed (Fig. 2.2a). A strong negative correlation between seedling dry weight and different concentrations of citronellol was observed, i.e. the value of *r* was calculated to be -0.962.

Dry weight of seedlings of *C. occidentalis* in case of control was measured to be 11.3 ± 0.04 mg. With increasing concentrations of citronellol, the dry weight of *C. occidentalis* decreased and at 5 nl / cc, it was measured to be 7.72 ± 0.09 mg, thus exhibiting a reduction of about 32% over control. The value of *r* was calculated to be high (-0.936), indicating a strong correlation (Fig. 2.2a). Seedling dry weight in *P. hysterophorus* also showed a decreasing trend like in other weeds. At the highest concentration i.e. at 1.5 nl / cc, the dry weight of *P. hysterophorus* was measured to be 0.16 ± 0.04 mg, thus indicating a reduction of about 49%. The value of correlation coefficient was however, calculated to be only -0.684.

**Linalool**

With the treatment of linalool also, a decrease in seedling dry weight of all the test weeds was observed, particularly at higher concentrations (Fig. 2.2b). In *A. viridis*, the dry weight continued to decrease with increasing concentrations of linalool and at 2 nl / cc, dry weight was measured to be 0.35 ± 0.02 mg, thus indicating a reduction of 22%. There was a strong correlation between different concentrations of linalool and dry weight of *A. viridis* with *r* = -0.839 (Fig 2.2b).
Fig. 2.2: Effect of different concentrations of (a) citronellol (b) linalool (c) eugenol (d) limonene on the seedling dry weight of the test weeds.

(a) Citronellol (nl / cc)

(b) Linalool (nl / cc)

(c) Eugenol (nl / cc)

(d) Limonene (nl / cc)

Similar symbols along each curve in each figure represent insignificant difference among each other at P < 0.05 applying DMRT. r represents value of correlation coefficient.

In B. pilosa, C. occidentalis and P. hysterophorus also, a similar trend of decrease in dry weight was observed. In these cases, maximum reduction in dry weight of B. pilosa and P. hysterophorus was observed at 5 nl / cc concentration while in C. occidentalis, similar situation could be observed in
response to 15 nl / cc. The correlation coefficients (r) were measured to be -0.927, -0.921 and -0.922 in case of B. pilosa, C. occidentalis and P. hysterophorus, respectively (Fig. 2.2b).

Eugenol

In A. viridis, 0.45 ± 0.07 mg seedling dry weight was recorded in control (without the treatment of monoterpane). With the treatment of 0.2 and 1 nl / cc of eugenol, there was a reduction of about 15 and 27% compared to control. A significantly high value of correlation coefficient (-0.903) was obtained (Fig 2.2c). In B. pilosa, at lower concentrations of 0.2 and 1 nl / cc, the reduction in seedling dry weight was not much and was statistically insignificant compared to control. At 1.5 nl / cc, a reduction of about 10% was seen. In this case also, a high value of r (-0.964) was obtained (Fig. 2.2c).

In C. occidentalis, mean seedling dry weight was measured to be 9.75 ± 0.14 mg in control. A decrease of 7-8% in dry weight was noticed with the treatments of 0.2, 1 and 1.5 nl / cc concentrations of eugenol while at 2 and 5 nl / cc, a reduction of about 15% in seedling dry weight was noticed (Fig. 2.2c). Here also, there was a strong correlation between different concentrations of eugenol and dry weight of C. occidentalis with r = -0.929. In P. hysterophorus, a reduction in seedling dry weight was noticed at all the concentrations of eugenol used compared to control. With the treatments of 0.2 nl / cc, a reduction of less than 4% was noticed in seedling dry weight (Fig. 2.2c). At 2 nl / cc concentration of eugenol, seedling dry weight was seen to be nearly 69% of control showing a reduction of about 31%. The r value in this case was calculated to be -0.839.

Limonene

Seedling dry weight of A. viridis was considerably reduced in response to any of the concentrations of limonene used. The difference in values over control
was statistically significant. With the treatment of 50 nl / cc, seedling dry weight was measured to be 0.47 ± 0.04 mg compared to 0.54 ± 0.05 mg in case of water-treated control. With the treatment of 100 nl / cc, it was about 76%. With the treatment of 450 nl / cc concentration of limonene, a reduction of about 75% was noticed compared to control (Fig. 2.2d). The value of correlation coefficient (r) was high, reciprocal and close to -1.

In *B. pilosa*, a steady decrease in seedling dry weight was noticed with the treatment of different concentrations of limonene. With the treatments of 50 and 100 nl / cc, the seedling dry weight was recorded to be 0.47 ± 0.13 and 0.43 ± 0.04 mg, respectively compared to 0.52 ± 0.08 mg in control and the decrease was statistically significant. At higher concentrations, a further reduction in dry weight of the seedlings was noticed. At 450 nl / cc concentration of limonene, a reduction of about 54% compared to control was noticed (Fig. 2.2d). A strong correlation with r = -0.906 between seedling dry weight and different concentrations of limonene was calculated.

In *C. occidentalis*, a decrease in seedling dry weight was noticed at each of the concentrations of limonene used, though the decrease was less than 5% with the treatments of 50, 100 and 200 nl / cc. The differences were statistically insignificant with respect to control. With the treatment of 300 nl / cc, a reduction of about 15% was noticed. At still higher concentrations i.e. 400 and 450 nl / cc, dry weight of seedlings was measured to be 0.15 ± 0.03 and 0.07 ± 0.02 mg, respectively compared to 8.67 ± 0.52 mg in control, indicating a reduction of 98 and 99%, respectively. The value of r was calculated to be high (-0.905) in this case (Fig. 2.2d).

Seedling dry weight of *P. hysterophorus* was also reduced considerably with different concentrations of limonene (Fig. 2.2d). With the treatment of 50 and 100 nl / cc concentrations of limonene, around 42 and 47% reduction in dry weight was noticed. At higher concentrations, there was a further decrease in seedling dry weight. With the treatment of 450 nl / cc concentration of
3. Total chlorophyll content

A decrease in the content of total chlorophyll was noticed with different concentrations of any of the four monoterpenes used. The chlorophyll content decreased with increasing concentrations of the monoterpenes.

**Citronellool**

Chlorophyll content in water-treated control was estimated to be 8.66 ± 0.08 µg/mg in case of *A. viridis*. With increasing concentrations of citronellool, there was a continuous decrease in the content of chlorophyll and the decrease was statistically significant in response to any concentrations compared to control. With the treatment of 0.2 nl/cc, a reduction of less than 4% compared to control was noticed. At higher concentrations i.e. 1, 1.5 and 2 nl/cc, reduction in chlorophyll content, however, was 24-26% and the decrease was statistically significant compared to control at all the concentrations (Fig. 2.3a).

In *B. pilosa*, less than 5% reduction in total chlorophyll content over that of control was measured when treated with 0.2, 1 and 1.5 nl/cc concentrations of citronellool. However, this decrease was statistically insignificant compared to control. It further decreased with the treatments of higher concentrations especially 2, 5 and 10 nl/cc of citronellool (Fig. 2.3 a).
In *C. occidentalis* also, a steady decrease in total chlorophyll content was noticed in samples treated with different concentrations of citronellol. The decrease was statistically significant when compared to control. Chlorophyll content was about 34% of that of control with the treatment of 5 nl/cc concentration of citronellol (Fig. 2.3a). Likewise, in *P. hysterophorus* also, chlorophyll content decreased with treatments of citronellol. It was about 69% at 0.2 nl/cc treatment. At 1 and 1.5 nl/cc concentration, the amount of chlorophyll was measured to be around 4 μg/mg exhibiting a decrease by about 60% compared to control in both the cases.

The data (Fig. 2.3a) was put to regression analysis and a strong negative correlation between the concentrations of citronellol used and the content of chlorophyll was seen.

**Linalool**

The content of total chlorophyll in the *A. viridis* seedlings treated with linalool was measured to be less compared to control. This decrease was statistically significant compared to control at all treatment concentrations i.e. 0.2, 1, 1.5 and 2 nl/cc. At the highest concentration, i.e. 2 nl/cc the content of chlorophyll was measured to be 0.13 ± 0.04 μg/mg compared to 8.66 ± 0.92 μg/mg, thus depicting a decrease of about 98%. In *B. pilosa* also, a steady decrease in chlorophyll content was noticed at all the concentrations of linalool used. With 0.2 and 1.0 nl/cc treatment, it was reduced to about 65% of control (Fig. 2.3b). A reduction of about 80% was noticed with 5 nl/cc treatment. The decrease at all the treatment concentrations was statistically significant with respect to control.
Fig. 2.3: Effect of different concentrations of (a) citronellol (b) linalool (c) eugenol (d) limonene on the total chlorophyll content of the test weeds.

Similar symbols along each curve in each figure represent insignificant difference among each other at \( P < 0.05 \) applying DMRT. \( r \) represents value of correlation coefficient.

In *C. occidentalis* also, similar observation was made i.e. chlorophyll content decreased in the treated tissue compared to control. With the treatment of 0.2, 1 and 1.5 nl / cc, chlorophyll content was reduced to nearly 75% exhibiting a reduction of 25% over control. It was further measured to be 71, 62, 44 and 38% of control with the treatments of 2, 5, 10 and 15 nl / cc concentrations.
(Fig. 2.3b) and the decrease at all concentrations was statistically significant over control.

*P. hysterophorus* also showed a decrease in the content of chlorophyll in response to treatment with linalool. With 0.2 nl / cc treatment, it was measured to be $5.47 \pm 0.12 \mu g / mg$ compared to control where chlorophyll content of $8.93 \pm 0.09 \mu g / mg$ was measured (Fig. 2.3b). With 5 nl / cc linalool treatment, a reduction of about 89% in chlorophyll content was noticed (Fig. 2.3b).

**Eugenol**

With the treatment of eugenol, chlorophyll content decreased with increasing concentrations. In case of *A. viridis*, there was a steady decrease in the content of chlorophyll with increasing concentrations of eugenol. With the treatment of 0.2 nl / cc concentration of eugenol, the total chlorophyll content was measured to nearly 90% of that of control. There was a further reduction of 55 and 67% in chlorophyll content with the treatments of 2 and 5 nl / cc eugenol (Fig. 2.3 c) and the decrease at all the concentrations was statistically significant over control. In *B. pilosa*, chlorophyll content was measured to be $9.85 \pm 0.36 \mu g / mg$ and $7.32 \pm 0.32 \mu g / mg$, respectively in case of treatments with 1 and 1.5 nl / cc eugenol in contrast to control where it was $15.5 \pm 0.07 \mu g / mg$. It was further reduced to mere $0.68 \pm 0.08 \mu g / mg$, with the treatment of 10 nl / cc eugenol (Fig. 2.3c) and the decrease was statistically significant over control at all the concentrations.

A decrease in chlorophyll content upon treatment with eugenol was also noticed in *C. occidentalis*. It was measured to be 80 and 73% of control with the treatments of 0.2 and 1 nl / cc eugenol. There was a further decrease in chlorophyll content of about 49% and 65% with higher concentrations of eugenol, i.e. 2 and 5 nl / cc, respectively (Fig. 2.3 c) and it was statistically significant over control. Chlorophyll content in *P. hysterophorus* decreased
steadily with different concentrations of eugenol. At 1.5 and 2 nl / cc concentrations, there was a reduction of about 48 and 55%, respectively. The reduction in chlorophyll was statistically significant at all the treatment concentrations (Fig. 2.3c). The data was put to regression analysis and high correlation coefficient values were calculated in all the cases.

**Limonene**

Like in all other monoterpenes, the total chlorophyll content was measured to be less in limonene treated weed seedlings. In *A. viridis*, the chlorophyll content was nearly 44% with the treatment of 50 nl / cc concentration of limonene and decrease was statistically significant over control and there was further reduction with increasing concentrations of limonene. Around 77% reduction was noticed with 450 nl / cc limonene treatment (Fig. 2.3d). A similar trend was observed in case of *B. pilosa* also (Fig. 2.3d).

Total chlorophyll content in *C. occidentalis* was noticed to be 7.33 ± 0.03 µg / mg in water-treated control (Fig. 2.3d). There was a reduction of nearly 25% with 50 nl / cc limonene treatment. It was further reduced to 1.02 ± 0.03 µg / mg (a reduction of about 86%) with the treatment of 450 nl / cc limonene concentration and the decrease was statistically significant over control. A similar trend followed in *P. hysterophorus*, where reduction in chlorophyll was significant at all the concentrations used with respect to control and a reduction of almost 40% was seen with 50 nl / cc limonene treatment. With 450 nl / cc limonene treatment, the chlorophyll content was reduced by nearly 87% compared to control (Fig. 2.3d). It is also clear from Fig. 2.3d that there is a strong negative correlation between different concentrations of limonene used and the reduction in the content of chlorophyll i.e. the curves drawn between different concentrations of limonene and chlorophyll content indicated a dose-response relationship.
4. **Cell Survival (Percent cellular respiration)**

**Citronellol**

As in case of other parameters, cellular respiration (a measure of cell survival) also decreased in all the test weeds. A reduction in cellular respiration was observed at all the concentrations used in *A. viridis*, which was measured to be about 87% of control with the treatment of 0.2 nl / cc. A further reduction was noticed with the treatment of higher concentrations of citronellol such as 1, 1.5 and 2 nl / cc and the reduction was statistically significant at all the concentrations (Fig. 2.4a).

In *B. pilosa*, a reduction of about 79% and 81% in cellular respiration was observed in case of samples treated with 2 and 5 nl / cc. A reduction of about 90% was noticed with the treatment of 10 nl / cc citronellol (Fig. 2.4a) and it was statistically significant over control as well as with all other treatment concentrations. In case of *C. occidentalis*, there was a decline of about 56% and 64% in cell respiration with the treatments of 1 and 1.5 nl / cc citronellol (Fig. 2.4a). Cellular respiration was only 21% at 5 nl / cc citronellol treatment and this differed significantly from control as well as from other concentrations. In *P. hysterophorus* also, a statistically significant decrease in cellular respiration was observed at all concentrations compared to control. The data was also put to linear regression analysis, and correlation coefficient values were found to be significant (Fig. 2.4a).

**Linalool**

A reduction of about 14 and 51% in cellular respiration was observed in *A. viridis* with the treatments of 0.2 and 1 nl / cc (Fig. 2.4b). With 1.5 and 2 nl / cc linalool treatments, cellular respiration was reduced by about 54 and 67%, respectively, and it was statistically significant over control at all the concentrations. In *B. pilosa*, 50% reduction in cellular respiration occurred with the treatment of 1.5 nl / cc linalool. With 5 nl / cc linalool treatment, cell
respiration was reduced by nearly 96% compared to control (Fig. 2.4b) and the decrease was statistically significant.

In *C. occidentalis*, cellular respiration was measured to be 86.70 and 71.36% with 0.2 and 1 nl / cc linalool compared to control (Fig. 2.4b). Further reduction of nearly 75% and 84%, respectively was noticed with 10 and 15 nl / cc of linalool treatments and it was statistically significant over control. A decrease of nearly 32% in cellular respiration was observed at 0.2 nl / cc linalool treatment in *P. hysterophorus*. Further decrease of nearly 92% and 99% was observed with 2 and 5 nl / cc linalool treatments (Fig. 2.4b) and the decrease at all the treatment concentrations used was statistically significant over control.

**Eugenol**

With the treatment of eugenol also, a statistically significant decrease was noticed in all the test plants. In *A. viridis*, a reduction of 38 and 47% was seen with lower concentrations of 1 and 1.5 nl / cc, respectively (Fig. 2.4c). With further increase in concentration, the cellular respiration decreased in the treated tissues and this decrease was statistically significant compared to control as well as to other treatment concentrations (Fig. 2.4c). In *B. pilosa*, *C. occidentalis* and *P. hysterophorus* also, cellular respiration decreased with increasing concentration of eugenol and this decrease was statistically significant compared to control (Fig. 2.4c). It is also clear from Fig. 2.4c that the correlation coefficient values between percent cellular respiration and different concentrations of eugenol were significantly high thus showing a strong correlation.
Fig. 2.4: Effect of different concentrations of (a) citronellol (b) linalool (c) eugenol (d) limonene on the seedling length of the test weeds.

(a) (b)
Percent cellular respiration
Percent cellular respiration
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(c) (d)
Percent cellular respiration
Percent cellular respiration

Similar symbols along each curve in each figure represent insignificant difference among each other at P < 0.05 applying DMRT. $r$ represents value of correlation coefficient.

**Limonene**

With the treatment of limonene also, there was decrease in cellular respiration in each of the four weeds tested and the decrease continued with higher concentrations of limonene (Fig. 2.4d). In *A. viridis*, cellular respiration was measured to be $41.93 \pm 6.52\%$ at highest concentration i.e. at 450 nl / cc,
while in *B. pilosa*, *C. occidentalis* and *P. hysterophorus*, cellular respiration at the same concentration was measured to be $4.63 \pm 2.17$, $3.34 \pm 0.16$ and $9.14 \pm 0.27\%$, respectively compared to control. In all these cases, the decrease was statistically significant over control (Fig. 2.4d). The data on percent respiration in response to limonene was also put to linear regression analysis and a strong correlation was noticed between the two (Fig. 2.4d).

**Discussion**

It is clear from the results that the monoterpenes adversely affect the seedling growth of test weeds viz. *A. viridis*, *B. pilosa*, *C. occidentalis* and *P. hysterophorus*. Growth measured in terms of seedling length and dry weight of all the test weeds decreased with increasing concentrations of monoterpene treatments. A number of other workers have also reported the inhibitory effects of several volatile and non-volatile allelopathic agents (Kohli and Singh, 1991; Scrivanti *et al.*, 2003; Vokou *et al.*, 2003). Why monoterpenes lead to decrease in seedling growth is not known. However, based on literature survey, this could be due to inhibition of mitosis of the growing seedlings by the monoterpenes. For example, reports of Vaughn and Spencer (1993); Baum *et al.* (1998) and Romagni *et al.* (2000a) have indicated that cineoles, particularly 1, 8-cineole inhibit mitosis. Dayan *et al.* (1999b) have reported that artemisinin, an allelochemical from *Artemisia annua* may disrupt the formation of microtubule organizing centers which, in turn, affect mitosis. Similar observations were made in case of onion root cells treated with terbutol and sindone B (Lehnen *et al.*, 1990; Lehnen and Vaughn, 1992). Though mitosis was not studied in our study but the inhibition of early seedling length may be because of adverse effect of monoterpenes on mitosis.

Not only the seedling growth, but the chlorophyll content was also reduced in response to the monoterpenes. Similar observations were also made by Singh *et al.* (2002a,c) and Romagni *et al.* (2000a) in response to various
monoterpenes. This reduction in chlorophyll content could be either because of reduced synthesis of chlorophyll or enhanced activity of the enzyme chlorophyllase, which causes hydrolysis of the chlorophyll pigment. Recently, Yang et al. (2004a) have indicated that both enhanced degradation and inhibition of synthesis of chlorophyll may be responsible for the loss of chlorophyll in the plants treated with allelochemicals – phenolic acids.

In the present study, all four monoterpenes significantly affected the cellular respiration also, thus causing changes in cellular energy production. Probably, monoterpenes interfere with mitochondrial respiration that in turn, affect energy balance of the plants leading to observed decrease in seedling growth of test plants (Penuelas et al., 1996). Likewise, Abrahim et al. (2000) have also reported the effect of monoterpenes on mitochondrial respiration that in turn, affect the early plant growth. However, the solubility of monoterpenes in water is a crucial factor in determining the magnitude of inhibition of plant growth by monoterpenes.