EXPERIMENT 6

Objective

To study the effect of four test monoterpenes on the content of total chlorophyll of the test weeds.

Hypothesis

Since monoterpenes were observed to adversely affect the total chlorophyll content of the test weeds under controlled laboratory conditions, it was hypothesized that they may also affect the total chlorophyll content of these weeds grown in pots under experimental dome conditions.

Parameters studied

Estimation of the content of total chlorophyll of the weeds, *Ageratum conyzoides*, *Amaranthus viridis*, *Bidens pilosa*, *Cassia occidentalis* and *Parthenium hysterophorus* for four consecutive weeks, after spray with various concentrations of monoterpenes, citronellol, linalool, eugenol and limonene formed the parameters of study.

Experimental design

For each of the five weeds under test, 170 pots were filled with a mixture of soil, sand and manure (1:1:1, v/v) and 10-12 seeds of either of the weeds were sown in them. After 45 days of sowing, 5 healthy and apparently uniform young plants in each pot were retained while rest of the plants were removed. Ten pots were maintained for each weed species. The plants of each group
were exposed to each of the four concentrations (25, 50, 100 or 200 mM) of either of the four monoterpenes (citronellol, linalool, eugenol and limonene). The respective treatment solution was prepared in water with addition of surfactant tween-80. The young plants of one set of 10 pots sprayed with pure water (with a few drops of tween-80) instead of monoterpenes served as control.

The volume of treatment solution, however, varied with the types of weeds. Thus, for *A. conyzoides* and *C. occidentalis*, spray of 2 ml per plant, for *A. viridis*, 3 ml per plant, for *B. pilosa*, 1.5 ml per plant and for *P. hysterophorus*, spray of 5 ml per plant was given. This variable amount of spray was selected on the basis of area of the leaves and results of a pilot small-scale experiment for determining the dose of the treatment.

The content of total chlorophyll was extracted by the method of Hiscox and Israelstam (1979) using DMSO and calculated on the basis of dry weight as suggested by Rani and Kohli (1991).

**Statistical analysis**

All data were expressed as mean of the chlorophyll content. The significance of treatment with respect to control was tested applying ANOVA and DMRT using the statistical package of SPSS version 10. The coefficient of correlation (r) between the chlorophyll content and each monoterpene concentration was also calculated.

**Observations and Results**

*Citronellol*

1. First week

In the first week, i.e. a day after the spray of monoterpenes, irrespective of the type, in general, a decrease in the content of total chlorophyll was observed. In *A. conyzoides*, 19.84 µg / mg chlorophyll was measured in control (Table
However, at initial concentrations of 25 or 50 mM of citronellol, the chlorophyll content was measured to be 19.16 and 19.11 μg / mg, respectively and it was statistically insignificant from that of control. Plants treated with higher concentrations of 100 or 200 mM, possessed chlorophyll content 42 and 64% less than that of control and the decrease was statistically significant with respect to control as well as between these two concentrations (Fig. 6.1a).

Table 6.1: Chlorophyll content (μg/mg) measured in different weeds spray treated with water to serve as control

<table>
<thead>
<tr>
<th>Weed</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. conyzoides</td>
<td>19.84 ± 0.05</td>
<td>19.40 ± 0.06</td>
<td>19.40 ± 0.34</td>
<td>19.40 ± 0.42</td>
</tr>
<tr>
<td>A. viridis</td>
<td>11.66 ± 0.04</td>
<td>11.64 ± 0.10</td>
<td>11.66 ± 0.26</td>
<td>11.83 ± 0.11</td>
</tr>
<tr>
<td>B. pilosa</td>
<td>10.54 ± 0.10</td>
<td>7.75 ± 0.11</td>
<td>7.12 ± 0.16</td>
<td>7.44 ± 0.25</td>
</tr>
<tr>
<td>C. occidentalis</td>
<td>7.76 ± 0.06</td>
<td>6.81 ± 0.17</td>
<td>7.76 ± 0.26</td>
<td>8.45 ± 0.09</td>
</tr>
<tr>
<td>P. hysterophorus</td>
<td>10.83 ± 0.15</td>
<td>9.32 ± 0.12</td>
<td>9.32 ± 0.13</td>
<td>9.11 ± 0.67</td>
</tr>
</tbody>
</table>

In A. viridis, compared to 11.66 ± 0.04 μg / mg in control, the chlorophyll content was 9.22 ± 0.09 and 7.35 ± 0.09 μg / mg, respectively (indicating a decrease of about 21 and 37%) when treated with 25 or 50 mM concentrations of citronellol. With higher treatment concentrations of 100 and 200 mM, the chlorophyll content was measured to be 7.07 ± 0.12 and 6.81 ± 0.06 μg / mg, respectively. The decrease in chlorophyll content at all the treatment concentrations was statistically significant compared to control (Fig. 6.1a). In B. pilosa also, a decrease in chlorophyll content with every increase in concentration of citronellol was noticed (Fig. 6.1a). Upon the treatment with 200 mM citronellol, it was measured to be 1.68 ± 0.05 μg / mg (Fig. 6.1a).

The plants of C. occidentalis treated with water possessed 7.76 ± 0.06 μg / mg chlorophyll. However, when exposed to treatment of 25 mM of citronellol, the content decreased to 2.38 ± 0.06 μg / mg (decrease of about 70% of control).
Compared to this, on increasing the concentration to 50, 100 or 200 mM, no significant change in the content was noticed (Fig. 6.1a). Untreated control plants of *P. hysterophorus* had 10.83 ± 0.15 µg / mg of chlorophyll. In contrast, those spray treated with any of the concentrations of citronellol had low content of chlorophyll. With every increase in concentration of the monoterpene treatment, the content of chlorophyll was measured to decrease (Fig. 6.1a). High reciprocal values of *r* between chlorophyll content and
concentration of citronellol were calculated in case of *A. conyzoides*, *B. pilosa* and *P. hysterophorus*.

2. Second week

A similar trend of decrease in chlorophyll content of test weeds was noticed with an increase in treatment concentrations of the monoterpenes during the second week also. However, this decrease was more pronounced than that in the first week. In *A. conyzoides*, the chlorophyll content was measured to be around 73% of control with the treatment of 25 and 50 mM citronellol solutions, while it was only about 24% of control with the treatment of 200 mM citronellol (Fig. 6.1b).

In *A. viridis*, the chlorophyll content was about 37% of control even with lowest treatment concentration i.e. 25 mM of citronellol while with the treatment of 100 and 200 mM, it was only about 25% and the decrease was statistically significant over control (Fig. 6.1b). In *B. pilosa*, a reduction of about 38% was observed with the treatment of 25 mM citronellol spray while a further reduction of 98% was noticed with 50 mM citronellol treatment. At still higher concentrations i.e.100 and 200 mM, however, the chlorophyll content was almost negligible (Fig. 6.1b).

In *C. occidentalis*, the chlorophyll content was measured to be 2.04 ± 0.15 μg / mg with the treatment of 25 mM citronellol solution compared to 6.81 ± 0.17 μg / mg in control (Table 6.1). Thus a reduction of about 70% over control was observed. In rest of the treatment concentrations, however, chlorophyll content could not be measured as the plants were already dead (Fig. 6.1b). In *P. hysterophorus*, with the treatment of 25 mM citronellol solution, the chlorophyll content was noticed to be about 89% of control and the decrease was statistically significant over control while it was about 77, 30 and 16% of control with the treatments of 50, 100 and 200 mM, respectively and the decrease was statistically significant over control (Fig. 6.1b). The data on
decrease in chlorophyll content with different concentrations of citronellol was also put to linear regression analysis and a dose-response relationship was observed (Fig. 6.1b) with high value of correlation coefficient (r) in case of A. conyzoides and P. hysterophorus (where r was close to −1).

3. Third week

In the third week, the chlorophyll content of A. conyzoides was measured to be 93 and 91% of control, respectively with the treatments of 25 and 50 mM citronellol solutions and both the values were statistically significant with respect to control. With the treatments of higher concentrations i.e. 100 and 200 mM, the plants of A. conyzoides were completely killed and thus no chlorophyll could be assessed (Fig. 6.1c). In A. viridis, there was a reduction of about 82 and 86% with the treatments of 25 and 50 mM citronellol concentrations during third week of spray and the decrease was statistically significant with respect to control but statistically insignificant with each other (Fig. 6.1c). Similar pattern of decrease in chlorophyll content was observed in case of B. pilosa also (Fig. 6.1c).

In C. occidentalis, chlorophyll content was about 51% of control with 25 mM citronellol treatment but it could not be measured on account of complete killing of plants at concentrations of 50, 100 and 200 mM and the decrease was statistically significant over control (Fig. 6.1c). In P. hysterophorus, the chlorophyll content was measured to be about 62 and 33% of control with the treatments of 25 and 50 mM citronellol and beyond this, the plants were completely killed (Fig. 6.1c).

Here also, the correlation was strong between chlorophyll content and citronellol concentrations, particularly in case of A. conyzoides and P. hysterophorus (Fig. 6.1c).
4. Fourth week

With the treatments of 25 and 50 mM citronellol concentrations, the content of chlorophyll was calculated to be about 78 and 71% of control, respectively in case of *A. conyzoides* (Fig. 6.1d). At higher concentrations however, the plants were completely killed. All plants of *A. viridis* and *C. occidentalis* treated with even lowest concentration of citronellol i.e. 25 mM were completely killed (Fig. 6.1d) while those of *B. pilosa* treated with 50 mM and above concentrations of citronellol were completely killed. In *P. hysterophorus*, the chlorophyll content was 6.61 ± 0.43 at 25 mM concentration compared to 9.11 ± 0.67 μg / mg in control and the decrease was statistically significant over control. At rest of the concentrations, complete killing of the plants was noticed (Fig. 6.1d).

Linalool

1. First week

In *A. conyzoides*, the chlorophyll content in control was determined to be 19.84 ± 0.05 μg / mg (Table 6.1) and it was measured to be about 92, 86, 82 and 65% of control, respectively with the treatments of 25, 50, 100 and 200 mM linalool. All these values were statistically significant over control (Fig. 6.2a). In *A. viridis*, a reduction of about 17% was noticed with 25 mM linalool treatment while a reduction of about 25 – 27% was noticed with 50, 100 and 200 mM solutions. The reduction with all linalool treatments was statistically significant over control (Fig. 6.2a).

In *B. pilosa*, the chlorophyll content was measured to be 8.03 ± 0.18 and 5.48 ± 0.09 μg / mg with 25 and 50 mM linalool treatments, respectively compared to 10.54 ± 0.10 μg / mg in control (Table 6.1). With the treatments of 100 and 200 mM, the chlorophyll content was measured to be 4.80 ± 0.25 and 2.46 ± 0.16 μg / mg, respectively indicating a decrease of about 54 and 77%, respectively, compared to control (Fig. 6.2a). In *C. occidentalis*, the
chlorophyll content was measured to be $7.76 \pm 0.06 \mu g / mg$ in control (Table 6.1). Compared to this, a decrease of about 22 and 31% was noticed at 25 and 50 mM concentrations of linalool. The decrease at all the treatment concentrations was statistically significant (Fig. 6.2a). A similar trend of decrease in chlorophyll content was noticed in *P. hysterophorus* treated with different concentrations of linalool. Here also, the decrease was statistically significant at all concentrations.

**Fig. 6.2 :** Effect of different concentrations of linalool on the total chlorophyll content of test weeds measured at (a) first week (b) second week (c) third week (d) fourth week after the treatment

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.
2. Second week

In *A. conyzoides* and *A. viridis*, the chlorophyll content decreased significantly with increasing concentrations of linalool (Fig. 6.2b). In *B. pilosa*, the chlorophyll content was measured to be 3.25 ± 0.05 µg / mg in plants treated with 100 mM linalool while it was 0.51 ± 0.07 µg / mg at 200 mM linalool treatment compared to 7.75 ± 0.11 µg / mg in control. This decrease was statistically significant over control as well as with respect to other treatment concentrations (Fig. 6.2b). In *C. occidentalis*, the chlorophyll content was measured to be between 72 to 78% of control with 25, 50 and 100 mM linalool treatments while it was about 63% with 200 mM treatment, showing a reduction of 37% over control. All the reductions were statistically significant (Fig. 6.2b). In *P. hysterophorus* also, a similar trend of decrease in chlorophyll content with increasing concentrations of linalool was noticed (Fig. 6.2b).

It is clear from Fig. 6.2b that monoterpene concentration and chlorophyll content exhibited a dose-response relationship and a strong correlation was seen in case of all the weeds except *A. viridis* where r value was only -0.570.

3. Third week

In *A. conyzoides*, the chlorophyll content was calculated to be 17.82 ± 0.36 µg / mg with the treatment of 25 mM linalool while it was 4.69 ± 0.07 with 200 mM linalool treatment. Compared to this, the chlorophyll content in control was measured to be 19.4 ± 0.34 µg / mg. The decrease was statistically significant over control (Fig. 6.2c). In *A. viridis*, the chlorophyll content was about 13% of control with the treatments of 25 and 50 mM linalool and it was statistically significant with respect to control (Fig. 6.2c) while at 100 and 200 mM concentrations, it could not be assessed as the plants were completely killed. In *B. pilosa*, *C. occidentalis* and *P. hysterophorus* also, a similar trend followed with various treatment concentrations of linalool (Fig. 6.2c).
The above data was analysed and the values of correlation coefficients obtained were high (-0.954 and -0.985 ) for \textit{P. hysterophorus} and \textit{A. conyzoides}, respectively while it was low for \textit{A. viridis}, thus showing a strong correlation in the former and a weak correlation in the latter case (Fig. 6.2c).

4. Fourth week

During the fourth week, chlorophyll content was about 79, 70 and 67\% of control with the treatments of 25, 50 and 100 mM linalool solutions in case of \textit{A. conyzoides}. It, however, could not be assessed at 200 mM linalool solution on account of complete wilting of the plants. All the values were statistically significant over control (Fig. 6.2d). In \textit{A. viridis}, the chlorophyll content could not be calculated at any of the treatment concentrations as the plants were completely killed (Fig. 6.2d). In \textit{B. pilosa} and \textit{C. occidentalis} also, the content of chlorophyll decreased with the increasing concentrations of linalool and at 200 mM linalool, the chlorophyll content was about 71.91 and 74.79\% of the respective controls of \textit{B. pilosa} and \textit{C. occidentalis} (Fig. 6.2d). In \textit{P. hysterophorus}, the chlorophyll content was about 84\% and 57\% of control with 25 and 50 mM linalool treatments and could not be calculated at higher concentrations i.e. 100 and 200 mM as the plants were completely killed and the decrease was statistically significant over control (Fig. 6.2d).

A strong correlation was seen between different treatment concentrations of linalool and the content of chlorophyll (Fig. 6.2d) and a high value of $r$ was obtained in case of all the weeds except \textit{A. viridis}, where a weak correlation with a low value of $r$, i.e. 0.530 was noticed.

\textit{Eugenol}

1. First week

With the treatments of different concentrations of eugenol also, a decrease in chlorophyll content was noticed in all the test weeds. In \textit{A. conyzoides}, the decrease in chlorophyll was 52\% at highest concentration used i.e. 200 mM.
In other weeds also, a significant loss of chlorophyll was observed. In general, greater reduction was observed at highest concentration. Maximum reduction was observed in *B. pilosa* and least in *A. viridis* at 200 mM concentration of eugenol. In all cases, the values of $r$ between eugenol concentrations and chlorophyll content were significant (Fig. 6.3a).

**Fig. 6.3 :** Effect of different concentrations of eugenol on the total chlorophyll content of test weeds measured at (a) first week (b) second week (c) third week (d) fourth week after the treatment.

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.
2. Second week

Chlorophyll content in leaves of *A. conyzoides* and *B. pilosa* was measured to be significantly less compared to control at all treatments, and at 200 mM chlorophyll could not be measured on account of complete killing of plants (Fig. 6.3b). In *P. hysterophorus*, the content of chlorophyll decreased with increasing concentrations of eugenol and it decreased by nearly 60% at 200 mM eugenol treatment (Fig. 6.3b).

From the values of *r*, a strong correlation between different concentrations of eugenol and the content of chlorophyll of all the five weeds was apparent, especially in case of *A. conyzoides*, *B. pilosa* and *P. hysterophorus* (Fig. 6.3b).

3. Third week

Upon treatment with eugenol, chlorophyll content in *A. conyzoides* was calculated to be 98% of control at 25 mM concentration and thus statistically insignificant with respect to control. It was 95, 82 and 0% of control with 50, 100 and 200 mM concentrations, respectively and the decrease was statistically significant over control (Fig. 6.3c). In *A. viridis*, the reduction in chlorophyll content was about 72 and 78% with 25 and 50 mM while its content could not be measured at 100 and 200 mM eugenol treatments as there was complete wilting of the plants and the decrease at all the concentrations was statistically significant with respect to control (Fig. 6.3c).

In *B. pilosa*, there was a reduction of about 17, 44 and 44% with the treatments of 25, 50 and 100 mM eugenol concentrations, respectively and all the reductions were statistically significant with respect to control (Fig. 6.3c). At 200 mM concentration plants of *B. pilosa* were completely killed. In *C. occidentalis*, the chlorophyll content was measured to be 92 and 77% with 25 and 50 mM eugenol concentrations, respectively. There was complete killing of the plants at higher (100 and 200 mM) concentrations. The decrease in all
these cases was statistically significant with respect to control (Fig. 6.3c). There was a reduction of 12 and 44% with the treatment of 25 and 50 mM eugenol in case of \( P. \) hysterophorus while plants were completely killed at 100 and 200 mM treatments (Fig. 6.3c).

After putting the data to linear regression analysis, it was found that the correlation between various eugenol concentrations and the content of chlorophyll was strong in case of all the weeds (Fig. 6.3c).

4. Fourth week

With the treatment of 25 and 50 mM concentrations of eugenol on \( A. \) conyzoides, the chlorophyll content was calculated to be about 73% of control while complete killing was noticed at 200 mM concentration during the fourth week and the decrease was statistically significant with respect to control (Fig. 6.3d). In \( A. \) viridis, the chlorophyll content was about 86 and 77% of control with 25 and 50 mM eugenol concentrations. At higher concentrations of 100 and 200 mM, the plants were completely killed (Fig. 6.3d). In \( B. \) pilosa, \( C. \) occidentalis and \( P. \) hysterophorus also, a similar trend was noticed and the plants were completely wilted at 200 mM concentration in case of \( B. \) pilosa, at 100 and 200 mM in \( C. \) occidentalis and at 50, 100 and 200 mM in \( P. \) hysterophorus (Fig. 6.3d).

**Limonene**

1. First week

With the treatment of different concentrations of limonene, though a reduction in chlorophyll content was measured, yet it was lesser compared to other monoterpenes.
In *A. conyzoides*, the reduction in chlorophyll content was negligible with the treatment of 25 mM limonene (about 2% over control) and was statistically insignificant. With the treatments of 50 and 100 mM limonene solutions, the chlorophyll was measured to be about 86 and 83% of control, respectively. Further, it decreased to about 79% of control with 200 mM treatment and the decrease was statistically significant over control (Fig. 6.4a). In *A. viridis*,

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**Fig. 6.4:** Effect of different concentrations of limonene on the total chlorophyll content of test weeds measured at (a) first week (b) second week (c) third week (d) fourth week after the treatment.
there was a reduction of about 26% with the treatment of 200 mM limonene treatment and the reduction was statistically significant over control (Fig. 6.4a).

In *B. pilosa*, a decrease of about 5% was observed at 25 mM treatment which was statistically insignificant compared to control. With increase in concentration, more reduction in chlorophyll was observed and at 200 mM, the reduction in chlorophyll was about 44% that was statistically significant (Fig. 6.4a). In *C. occidentalis* also, a similar trend was observed. Here, only a marginal loss (of about 1%) was observed at 25 mM concentration. However, at 100 and 200 mM concentrations, nearly 23% loss of chlorophyll was observed that was statistically significant (Fig. 6.4a). In *P. hysterophorus*, the chlorophyll content was measured to be 94, 89, 86 and 50% of control with the treatments of 25, 50, 100 and 200 mM concentrations of limonene and all the values were statistically significant over control (Fig. 6.4a).

A strong correlation was observed between the chlorophyll content and different concentrations of limonene, especially in case of *B. pilosa* and *P. hysterophorus* where $r$ was close to -1 ((Fig. 6.4a).

2. Second week

At 25 and 50 mM limonene treatment, the chlorophyll content of *A. conyzoides* leaves was measured to be around 16 µg / mg while it was about 12 µg / mg with 100 and 200 mM limonene spray, compared to 19.4 µg / mg of chlorophyll content in case of control. The decrease at all the treatment concentrations was statistically significant with respect to control (Fig. 6.4b). The chlorophyll content of *A. viridis* leaves sprayed with 25 mM limonene showed a reduction of about 14% over control. With 50 and 100 mM treatments, the reduction was about 19% while with 200 mM limonene spray, there was about 22% reduction and it was statistically significant with respect to control (Fig. 6.4b). In *B. pilosa*, there was a reduction of about 45% with 200 mM treatment of limonene and it was statistically significant over control.
(Fig. 6.4b). In *C. occidentalis* and *P. hysterophorus* also, a similar trend of decreasing chlorophyll content was noticed with different limonene concentrations (Fig. 6.4b).

The data were put to linear regression analysis and a high value of correlation coefficient i.e. $r$ was calculated, especially in case of *B. pilosa* and *C. occidentalis* were $r$ was calculated to be -0.974 and -0.981, respectively thus showing a strong correlation (Fig. 6.4b).

3. **Third week**

The decrease was to a lesser extent in case of spray with limonene on *A. conyzoides* plants. The reduction was about 1 to 3% with 25 and 50 mM limonene spray and it was statistically insignificant with respect to control while it was 16-17% with 100 and 200 mM limonene spray which was statistically significant with respect to control but insignificant with respect to each other (Fig. 6.4c). In *A. viridis*, there was a consistent decrease with various treatment concentrations of limonene during the third week and the decrease was statistically significant with respect to control at all the four treatment concentrations (Fig. 6.4c). The trend continued to be the same for other three weeds also (Fig. 6.4c).

It is clear from Fig. 6.4c that a strong correlation exists between the content of chlorophyll and different limonene concentrations in case of *A. conyzoides*, *B. pilosa* and *P. hysterophorus* while a weak one exists in case of *A. viridis* and *C. occidentalis*.

4. **Fourth week**

During fourth week after the spray with limonene, there was an increase in the content of chlorophyll compared to that recorded during the third week at all the concentrations and in all the weeks, except *A. conyzoides* and *P. hysterophorus* where instead, a decrease in chlorophyll content was noticed during this duration (Fig. 6.4d).
A comparison of the percent chlorophyll content of the test weeds in response to different concentrations of each of the four monoterpenes was also done for all the four weeks. The content of chlorophyll decreased not only with increasing concentrations of each of the four monoterpenes but also with the passage of time with a few exceptions.
Fig. 6.6: Effect of different concentrations of four monoterpenes on the chlorophyll content (percent of control) of *A. viridis* during (a) first week (b) second week (c) third week (d) fourth week

In *A. viridis*, the chlorophyll content decreased with increasing concentrations of the monoterpenes and also with increasing time period from first to fourth week except, lower concentrations of eugenol and limonene where chlorophyll content was more on the fourth week (Fig. 6.6).
In *B. pilosa* also, the chlorophyll content decreased with increasing concentrations of the four monoterpenes. However, during fourth week, there was complete killing of the plants at 200 mM eugenol and 50 mM and above concentrations of citronellol (Fig. 6.7) while revival of the plants was noticed at other concentrations.
Fig. 6.8: Effect of different concentrations of four monoterpenes on the chlorophyll content (percent of control) of C. occidentalis during (a) first week (b) second week (c) third week (d) fourth week

In C. occidentalis, the effect of all concentrations of cironellol and 100 and 200 mM of eugenol increased with the passage of time and complete killing of the plants was observed at these concentrations during fourth week. At other concentrations, the chlorophyll content increased during the fourth week, showing regeneration of the plants.
Fig. 6.9: Effect of different concentrations of four monoterpenes on the chlorophyll content (percent of control) of *P. hysterophorus* during (a) first week (b) second week (c) third week (d) fourth week.

Similar symbols along each bar in each figure represent insignificant difference among each other at $P < 0.05$ applying DMRT.

Similarly, a comparison of the chlorophyll content at each of the concentrations (a) 25 mM (b) 50 mM (c) 100 mM (d) 200 mM of each of the four monoterpenes was done for the test weeds *A. conyzoides* (Fig. 6.10), *A. viridis* (Fig. 6.11), *B. pilosa* (Fig. 6.12), *C. occidentalis* (Fig. 6.13) and *P. hysterophorus* (Fig. 6.14).
Fig. 6.10: Percent chlorophyll content of *A. conyzoides* measured at weekly intervals in response to (a) 25 mM (b) 50 mM (c) 100 mM (d) 200 mM concentrations of citronellol, linalool, eugenol and limonene.

Similar symbols along each bar in each figure represent insignificant difference among each other at $P<0.05$ applying DMRT.

In *A. conyzoides*, there was a decrease in the content of chlorophyll with increasing concentrations of all the four monoterpenes. The effect was observed to be maximum during third and fourth weeks in general (Fig. 6.10).
In *A. viridis* also, a significant decrease in chlorophyll content was observed even at lower concentrations i.e. 25 and 50 mM of citronellol, linalool and eugenol while the decrease was even more at higher concentrations of 100 and 200 mM (Fig. 6.11).
In *B. pilosa*, the effect of monoterpenes was quite less with 25 mM concentration of the monoterpenes. It increased with the treatments of 50 and 100 mM while the effect of all the monoterpenes was maximum for all the four weeks at 200 mM (Fig. 6.12).
In *C. occidentalis*, the effect of citronellol was quite high even at 25 mM concentration, where complete killing of the plants was observed during the fourth week. The effect of eugenol, though not very conspicuous at 25 mM was significantly high at rest of the concentrations (Fig. 6.13).
Fig. 6.14: Percent chlorophyll content of *P. hysterophorus* measured at weekly intervals in response to (a) 25 mM (b) 50 mM (c) 100 mM (d) 200 mM concentrations of citronellol, linalool, eugenol and limonene.

Similar symbols along each bar in each figure represent insignificant difference among each other at *P*<0.05 applying DMRT.

In *P. hysterophorus*, plants treated with 200 mM concentration of citronellol, linalool and eugenol were completely killed during third week, while those treated with 200 mM limonene showed a decrease of about 40% in chlorophyll content compared to control (Fig. 6.14).
Discussion

It is clear from the results that the content of chlorophyll of all the test weeds decreased with the spray of monoterpenes. The decrease in chlorophyll content was more at higher concentrations than at lower concentrations and also, the effect increased with increasing time period after treatment except in some cases where the plants recovered during the fourth week. Further, the effect also varied with the type of monoterpane and weed species. In general, citronellol was most effective as it caused maximum reduction in chlorophyll content of the weed species while limonene was the least. *A. viridis* was observed to be the most sensitive weed while *A. conyzoides* was the most resistant. This reduction in the content of chlorophyll indicates an effect on the photosynthetic activity of the target plants. A number of other workers have also reported that allelochemicals reduce the chlorophyll content of target plants and thus affect photosynthetic machinery. Artemisinin and its synthetic derivatives were reported to cause a reduction in the chlorophyll content to *Lactuca sativa* seedlings (Dayan et al., 1999b), parthenin, a sesquiterpene lactone from *P. hysterophorus* caused appreciable reduction in the content of chlorophyll in the leaves of *A. conyzoides* spray-treated with parthenin at 200 μM concentration (Singh et al., 2002a). Among monoterpenes, 1,4- and 1,8-cineole were reported to significantly reduce the chlorophyll content and also the photosynthetic efficiency of *Ehinochloa crus-galli* and *Cassia obtusifolia* (Romangi et al., 2000a). Further, Singh et al. (2002c) reported a reduction in the content of chlorophyll of *C. occidentalis* in response to some monoterpenes. This indicates that allelochemicals have a potential to reduce the chlorophyll content. However, it is not known whether the reduction in chlorophyll content is because of the inhibition of its synthesis or due to its enhanced degradation. A recent report has 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 (Yang et al., 2004a). In our study, however, this remains to be seen. There are reports that volatile essential oils cause changes in leaf diffusibility,
rate of transpiration and stomatal opening and closing in *Helianthus annuus* and *Secale cereale* plants (Polova and Vicherkova, 1986; Vicherkova and Polova, 1986). Rai *et al.* (2003) also reported the volatile essential oils from *Prinsepia utilis* L. to inhibit stomatal opening in *Vicia faba* L. These changes caused by essential oils may also affect the chlorophyll content observed in the present study.