EXPERIMENT 4

Objective

To study the effect of four test monoterpenes on the possible leakage of ions from the cotyledonary leaf and radicle tissues of Cassia occidentalis with a view to determine their effect on membrane permeability.

Hypothesis

The monoterpenes when come in contact with tissues may affect membrane permeability of treated tissues. If so, resultantly, excessive ions would leak out of such membranes. This can be reflected by rapid increase in the conductivity of liquid medium in which such tissues are placed.

Parameter studied

Measurement of conductivity in μS (micro Seimen) of bathing medium (aqueous medium, in which cotyledonary leaf or radicle tissue of C. occidentalis is placed) at different time intervals after the treatments of four monoterpenes viz. citronellol, linalool, eugenol and limonene formed the parameter of study.

Experimental design

For studying the ion leakage from tissues treated with different concentrations of monoterpenes under test, method of Galindo et al. (1999) was followed. For this, 100 seeds of C. occidentalis were sown in each of the four enamel trays and studies were conducted on 6-day-old seedlings raised therein. All the seedlings selected were of uniform size (5.5 ± 0.28 cm). Ion leakage was
measured from both cotyledonary leaves and radicles (100 mg each) dipped in a medium of MES (2-N-morpholino ethanesulfonic acid sodium salt) buffer (30 ml) containing 1% sucrose supplemented with 0.5 and 1 mM concentrations of each of the four monoterpenes. Two drops of tween-80 were added to each of these solutions, which served as an emulsifier. Three replicates were maintained for each treatment. A control, which consisted of 30 ml of MES buffer and 2 drops of tween-80 but without any supplement of the monoterpenes was also maintained. The conductivity of bathing medium was checked with the help of La Motte conductivity meter at different time intervals i.e. at 0, 1, 2, 4, 8, 12, 16 and 20 h under dark conditions and 22, 24, 26 and 28 h under light conditions. Besides, maximum conductivity of tissues was also checked by boiling the medium for 10 min.

Statistical analysis

All data were expressed as means of conductivity and the significance of treatment i.e. each concentration of each monoterpane was tested with respect to control applying ANOVA followed by DMRT using the statistical package of SPSS version 10. The values of correlation coefficient (r) were also calculated between conductivity and time.

Observations and Results

a) Ion leakage in cotyledonary leaves

The conductivity of boiled medium containing cotyledonary leaves (maximum conductivity) was 87 µS.

In case of cotyledonary leaves dipped in 0.5 mM citronellol solution, the conductivity of the solution was 10 µS at 0 h (immediately after immersing cotyledonary leaves into citronellol containing MES buffer solution), compared to 8 µS in control. There was a consistent increase in conductivity of bathing medium supplemented with 0.5 mM citronellol with time. After 16 h, the conductivity was measured to be 18 µS, compared to 11 in control, indicating
an increase of about 64% over control. This was statistically significant over control. The conductivity further increased with the passage of time and after 28 h, it was measured to be 37 in 0.5 mM citronellol solution compared to 13.67 μS in control, indicating an increase of 171% over control. The correlation between time and conductivity was strong, positive and close to one (Fig. 4.1a).

In cotyledonary leaves dipped in 1mM citronellol solution, there was more increase in conductivity of the solution. The conductivity was measured to be 11.67 μS after 1 h, compared to 9.67 in control at that time and was statistically insignificant with respect to control. It continued to increase with time. After 20 h, conductivity was 36.33 compared to only 11.33 μS in control, thus indicating an increase of 221% over control and it was statistically significant (Fig. 4.1a). After 20 h also, the increase in conductivity was steady i.e. it continued to increase with the same magnitude, irrespective of light or dark conditions. Conductivity measured at 28 h was 44.67 compared to 87 μS upon boiling which indicates maximum conductivity (Fig. 4.1a). The correlation between conductivity and time was strong with high value of correlation coefficient (close to one).

There was a steady increase in conductivity of the 0.5 mM linalool solution over time and it was statistically significant at all the time intervals with respect to control (Fig. 4.1 b). It was measured to be 18 μS at the end of the dark period (i.e. after 20 h) compared to 11.33 in control and it was statistically significant. The conductivity was measured to be 27.33 μS at the end of the light period i.e. at 28 h. It was, however, very less compared to maximum conductivity of 87 μS obtained on boiling. In case of cotyledonary leaves dipped in 1 mM linalool solution also, a similar trend of increase in conductivity with time was noticed (Fig. 4.1b).

In case of cotyledonary leaves dipped in 0.5 mM and 1 mM eugenol solutions, a similar trend of increase in conductivity of solutions with time was noticed.
However, the conductivity in these cases was higher compared to all other cases. In these, the conductivity at 28 h was measured to be 45 and 52 μS in 0.5 and 1 mM eugenol solutions, respectively. This indicated an increase of 229 and 280%, respectively over control and was statistically significant compared to control at all the time intervals (Fig. 4.1c) and these represented about 52 and 60% of maximum conductivity, respectively.

Fig. 4.1: Effect of different concentrations of (a) citronellol (b) linalool (c) eugenol (d) limonene on the conductivity of cotyledonary leaves of *C. occidentalis*. 

Similar symbols among different treatments (presented as three curves) represent insignificant difference among each other at \( P < 0.05 \) applying DMRT.
In cotyledonary leaves of *C. occidentalis* dipped in 0.5 mM and 1 mM solutions of limonene, a similar trend of increasing conductivity of the medium over time was noticed (Fig. 4.1d). In these cases, at 28 h, the conductivity was measured to be 25.67 and 32 μS, respectively compared to 13.67 in control and 87 on boiling and the increase was statistically significant over control.

**Fig. 4.2:** Comparative effect of four monoterpenes (at the highest concentration i.e. 1 mM) on the conductivity of leaf tissue of *C. occidentalis*.  

[Graph showing conductivity vs. hours for different treatments with symbols indicating significant difference.]

In general, compared to almost no increase in conductivity in control, the samples exposed to 0.5 or 1 mM of any of the four monoterpenes showed a steady increase in conductivity. The effect of each of the four monoterpenes i.e. citronellol, linalool, eugenol and limonene at the highest concentration i.e. 1 mM on the conductivity of the medium containing cotyledonary leaves was compared (Fig. 4.2). It was observed that eugenol caused maximum increase in conductivity of the medium followed by citronellol and limonene. Linalool was least effective where conductivity was lesser at all the time periods compared to other 3 monoterpenes (Fig. 4.2).
b) Ion leakage in radicles of C. occidentalis

Maximum conductivity of the medium containing radicle tissue of C. occidentalis was measured to be 73.5 μS, obtained on boiling.

In 0.5 mM and 1 mM citronellol solutions containing radicles, there was an increase in conductivity with time. The conductivity, however, increased more in case of radicles dipped in 1 mM citronellol solution than those in 0.5 mM. This was true for the values of conductivity at all the time intervals. After 1 h, the conductivity was measured to be 21.33 and 26 μS in 0.5 and 1 mM citronellol solutions (Fig. 4.3a) compared to 20 μS in control and this increase of 0.5 mM solution was statistically insignificant over control but of 1 mM was statistically significant over control. The increase in conductivity of radicles dipped in 0.5 mM citronellol solution was negligible over control at all time intervals or in other words, there was very little difference in the values of conductivity of radicles kept in control solution and 0.5 mM citronellol solution and the increase was statistically insignificant at all time intervals. At 28 h, the conductivity was measured to be 30 and 35 μS in 0.5 and 1 mM citronellol solutions compared to 28.67 μS in control, indicating an increase of 4.64 and 22%, respectively over control. The former increase was statistically insignificant compared to control while the latter was significant at 28 h. These values were about 41 and 48%, respectively of maximum conductivity. The coefficient of correlation between conductivity and time in case of 0.5 mM and 1 mM citronellol solutions was measured to be 0.726 and 0.715, respectively (Fig. 4.3a).

A similar trend of increase in conductivity with time was observed in linalool solution. In 0.5 mM linalool solution, there was a consistent increase in conductivity till 12 h when conductivity was measured to be 31 μS. After this, there was no increase in conductivity till 22 h. After 24 h and beyond, there was again a static phase i.e. there was no change in conductivity which was measured to be 32 μS (Fig. 4.3b). In the 1 mM linalool solution, conductivity increased till 8 h where it was 36 μS and it was statistically significant compared to control at all these time intervals. It remained unchanged i.e. 36
µS till 28 h i.e. no increase in conductivity was noticed from 8 to 28 h. The correlation coefficient between conductivity and time was calculated to be 0.751 and 0.667, respectively in 0.5 and 1 mM linalool solutions (Fig. 4.3b). At almost all the time intervals, increase between 1 mM and 0.5 mM and between 0.5 mM and control was statistically insignificant but between control and 1 mM treatment solution was statistically significant.

Fig. 4.3: Effect of different concentrations of (a) citronellol (b) linalool (c) eugenol (d) limonene on the conductivity of radicles C. occidentalis

Similar symbols among different treatments (presented as three curves) represent insignificant difference among each other at P < 0.05 applying DMRT.
In case of eugenol also, the conductivity increased with time (Fig. 4.3c). After 2 h, the conductivity was measured to be 28.33 and 32.67 μS, respectively in the solutions of 0.5 mM and 1 mM eugenol concentrations compared to 22 μS in control. At this time, increase in the conductivity of 1 mM solution was statistically significant with respect to control while increase in 0.5 mM solution was statistically insignificant with respect to control. The increase between 1 and 0.5 mM was also statistically insignificant. There was a steady increase in conductivity with time in the two solutions. After 28 h, conductivity was measured to be 46 and 51 μS in 0.5 and 1 mM eugenol solutions, respectively compared to 28.67 μS in control, thus depicting an increase of 60.45 and 77.89% over control in solutions of 0.5 and 1 mM eugenol solutions, respectively and the increase was statistically significant compared to control at all the time intervals. These values were about 63 and 69%, respectively of maximum conductivity of boiled tissue. This meant that they caused great loss of membrane integrity. The data was also put to linear regression analysis and the values of correlation coefficients were 0.751 and 0.667 in the solutions of 0.5 and 1 mM concentrations of eugenol, respectively (Fig. 4.3c).

In a similar manner, there was also an increase in the conductivity of limonene solutions containing C. occidentalis radicles. In this case, at the end of the dark period i.e. after 20 h, the conductivity was measured to be 36 and 39 μS in 0.5 and 1 mM limonene solutions, respectively compared to 28 μS in control, indicating an increase of 28.57 and 39.29%, respectively and the increase in both 0.5 and 1 mM limonene solutions was statistically significant over control at all the time intervals. At the end of the light period, i.e. after 28 h, the conductivity was measured to be 37 and 40.67 μS compared to 28.67 μS in control, indicating an increase of 29.05 and 41.86%, respectively over control and the increase was statistically significant (Fig. 4.3d).

The increase in conductivity of the liquid medium containing radicles of C. occidentalis and treated with 1 mM of each of the 4 monoterpenes was
evaluated (Fig. 4.4). The increase in conductivity was maximum at all the time intervals in the medium supplemented with eugenol. This was followed by limonene, closely followed by linalool and citronellol. Thus, citronellol was responsible for the least effect on membrane conductivity of the radicle tissue (Fig. 4.4).

Fig. 4.4: Comparative effect of four monoterpenes (at the highest concentration i.e. 1 mM) on the conductivity of radicle tissue of C. occidentalis.

![Graph showing conductivity of radicle tissue with different treatments](image)

Similar symbols among different treatments (presented as five curves) represent insignificant difference among each other at P < 0.05 applying DMRT.

**Discussion**

Plant cell membranes or plasma membranes consist of proteins and lipids and cells maintain an internal environment conducive to the biochemical reactions that sustain life. For this, the molecules of energy (fuels) and biosyntheses (building blocks) are transported from the exterior, while at the same time, toxic or secretory products are excreted outside. All this is possible because of the selectively permeable nature of the plasma membrane which allows only some of the solutes / ions to pass through while prohibits others. There are reports available in literature, which indicate that there is an increase in the leakage of ions from the plant cell membranes due
to the treatment of some allelochemicals, including terpenoids or monoterpenes to the plant tissues. Tworkoski (2002) reported an increase in the leakage of electrolytes from leaves of *Taraxacum officinale* Weber when treated with eugenol and isoeugenol. In our study also, there was an increase in the conductivity of the solutions when treated with various concentrations of monoterpenes. Eugenol was observed to cause maximum increase in conductivity or maximum leakage of ions into the solution, which lead to maximum loss of membrane integrity. Also, the magnitude of increase in conductivity was not affected when solutions were transferred from dark to light conditions in the present study. Dayan *et al.* (2000) have compared the electrolyte leakage caused by compounds acting in light-independent and light-dependent manner and have opined that light-independent electrolyte leakage (which is noticed in the present study also) may be associated with compounds that affect cellular respiration or cause oxidative stresses.

However, the increase in membrane permeability was very less in linalool and limonene solutions (0.5 and 1mM) containing cotyledonary leaves and citronellol and linalool solutions (0.5 and 1mM) containing dipped radicles of *C. occidentalis* compared to the maximum conductivity of the boiled tissue. This shows that these monoterpenes had a lesser effect on conductivity. Similar results have been obtained by Dayan *et al.* (1999b) who reported that artemisinin and other sesquiterpene analogues did not cause any effect on the conductivity of leaf discs of *Cucumis sativus* L.