3. RESULTS
3.1 GROWTH PATTERN AND GENERATION TIME STUDIES

Log O.D. versus time gives the growth curve with three distinct phases - lag, log and stationary, indicative of dormant, dividing and resting state of cells respectively.

Comparative analysis of growth patterns of *A. vinelandii* cells grown in the presence of different concentrations of monocrotophos and ceresan is shown in Fig. 1. From control growth characteristics of the cells, generation time obtained was 2.7 h (Fig. 1a) and in the presence of 500 ppm monocrotophos the growth rate remained unchanged. However, in the presence of 1 ppm ceresan the growth curve deviated from that of the control on temporal scale and tended to merge with the control at late stationary phase. Nevertheless, lag phase remained unaltered (Fig. 1b). The generation time was 33 percent more than control value (control 2.7 h, 1 ppm ceresan 3.6 h). In the presence of 3 ppm ceresan, the lag phase of the growth curve prolonged up to 6 h (Fig. 1c) while it was 2 h for control. Early log phase, too, lasted longer followed by a steeper one where generation time (5.1 h) was found to be almost double that of the control value (2.7 h). Further, the curve did not attain any stationary phase up to 28 h. Thus, an inhibition in the metabolism and a consequent decrease in growth rate of bacteria was reflected by increasing the concentration of ceresan in the medium. Monocrotophos appeared to be neutral in these respects.

Results obtained from similar kind of studies on *A. chroococcum* are shown in Fig. 2, where the growth characteristics in the presence of 500 ppm monocrotophos remained unaltered as compared
Fig. 1  Growth patterns and generation times (g) of *A. vinelandii* grown in: (a) medium alone and 500 ppm monocrotophos (b) 1 ppm ceresan, and (c) 3 ppm ceresan.
Fig. 2 Growth patterns and generation times (g) of A.chroococcum grown in: (a) medium alone and 500 ppm monocrotophos (b) 1 ppm cresan, and (c) 3 ppm cresan.
to control data (Fig. 2a). Generation time for *A. chroococcum* was 1.9 h, which is less than the generation time of *A. vinelandii* (2.7 h). In the presence of 1 ppm and 3 ppm ceresan the generation times were 2.1 h and 3.0 h respectively; the lag phase continued upto 4 h and 20 h respectively; which for control was 2 h (Fig. 2 a,b,c). The stationary phase was attained at 22 h for control and 26 h in case of 1 ppm ceresan treatment. However, 3 ppm treatment of ceresan did not allow the onset of stationary phase upto 34 h.

Comparison of Fig. 1 and Fig. 2 shows that inhibitory effect of ceresan on growth pattern is more pronounced in log phase (lengthening of generation time) of *A. vinelandii* while in *A. chroococcum* the inhibition caused by ceresan was more pronounced in the lag phase of the growth.

3.2 SURVIVAL STUDIES

To record the response of microbial population to varying magnitude of environmental stresses, survival curves were obtained by plotting the surviving fractions on log scale against the doses used on linear scale. The survival curves thus obtained help a great deal in explaining the cellular damage and cell death caused due to different physical and chemical stresses.

3.2.1 Single treatment on *A. vinelandii*: UV-C and ceresan

Fig. 3 shows the inactivation patterns of *A. vinelandii* cells exposed to UV-C radiation (253.7 nm) and ceresan separately. A prominent shoulder is seen in Fig. 3a, at low doses (upto 25 Jm\(^{-2}\))
Fig. 3  Surviving fractions of _A. vinelandii_ treated with different doses of stresses: (a) UV-C radiation (b) ceresan.

- **LD$_{50}$** = 12 Jm$^{-2}$
- **K** = -0.060 Jm$^{-2}$

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**Surviving Fraction**

- **LD$_{50}$** = 1 ppm
- **K** = -1.460/ ppm
followed by a steep exponential linear decline in survival corresponding to the higher doses of UV-C radiation (25-85 Jm\(^{-2}\)). The value of \(LD_{50}\) (lethal dose for 50\% survival) and \(K'(\text{inactivation constant})\) was found to be 12 Jm\(^{-2}\) and -0.060/Jm\(^{-2}\) respectively. The negative sign of \(K'\) indicates reduction in survival levels with increase in the amount of dose used.

Fig.3b depicts the inactivation profile of \(A.\ vinelandii\) cells treated with various concentrations of ceresan and the pattern obtained remains similar to that obtained in case of UV-C radiation. However, the shoulder obtained with ceresan treatment is prolonged when compared to UV-C exposure (Fig.3a) and is followed by a very sharp decline at doses 4 ppm to 6 ppm. The \(LD_{50}\) and \(K'\) values were 1 ppm and -1.460/ppm respectively.

3.2.2 Combined stresses on \(A.\ vinelandii\): UV-C and ceresan

Fig.4a presents surviving pattern of \(A.\ vinelandii\) cells, which have already been treated with 2 ppm ceresan, against the various doses of UV-C radiation. The inactivation trend appears similar to that in case of UV-C irradiation (Fig.3a) showing a shoulder at low doses (upto 35 Jm\(^{-2}\)) followed by a log linear decline in survival at higher doses (40 Jm\(^{-2}\) to 80 Jm\(^{-2}\)). The span of the shoulder was seen to have broadened by about 10 Jm\(^{-2}\) (Fig.4a) compared to UV-C irradiation alone (Fig.3a). The measure of shoulder gives the cumulative cellular damage before the onset of cell death (Alper, 1979). Pretreatment with a low dose of ceresan (2ppm) seemed to augment damage bearing capacity of cells against UV-C irradiation. \(LD_{50}\) and \(K'\) values were calculated to be 13.6 Jm\(^{-2}\) and -0.052/Jm\(^{-2}\) respectively (Fig. 4a). On compar-
Surviving fractions of *A. vinelandii* exposed to combined stresses: (a) 2 ppm ceresan followed by different doses of UV-C radiation, (b) 17 Jm⁻² UV-C followed by different concentrations of ceresan.
ing with UV-C irradiation (Fig. 3a), LD$_{50}$ value was found to be higher while K' value was reduced marginally.

The trend was similar in case of preirradiation (UV-C, 17 Jm$^{-2}$) of *A. vinelandii* cells followed by the exposure to ceresan (Fig. 4b) to that obtained in ceresan pretreated (2 ppm) cells later exposed to UV-C irradiation (Fig. 4a). LD$_{50}$ and K' values were calculated to be 1.3 ppm and -0.597/ppm respectively (Fig. 4b).

A respective increase and decrease was noticed in LD$_{50}$ and K' values compared to corresponding values for ceresan treatment alone (Fig. 3b).

The response of a higher dose ceresan pretreated (4 ppm) *A. vinelandii* cells towards varying doses of UV-C is shown in Fig. 5a. This curve has following characteristic features, - (i) biphasic nature, showing two distinct portions of cell population based on their differential sensitivity towards UV-C, and (ii) absence of shoulder even at substantially low doses of UV-C.

About 4 percent of the population showed UV-C resistant nature (Fig. 5a). LD$_{50}$ and K' values for photosensitive fraction of population were found to be 5.1 Jm$^{-2}$ and -0.130/Jm$^{-2}$ respectively (Fig. 5a).

A well marked shoulder (upto 3.5 ppm) was obtained when high-dose-preirradiated (UV-C, 34 Jm$^{-2}$) cells were exposed to different doses of ceresan (Fig. 5b). The inactivation pattern in Fig. 5b remained similar to that in Fig. 4b, though the pretreatment dose of ceresan (4 ppm) in earlier was double of that of later (2 ppm). The shoulder part was as usual followed by exponentially increasing lethality with increasing doses of
Fig. 5  Surviving fractions of *A. vinelandii* exposed to combined stresses: (a) 4 ppm ceresan followed by different doses of UV-C radiation, (b) 34 Jm$^{-2}$ UV-C followed by different concentrations of ceresan.
ceresan (Fig.5b). The values of LD$_{50}$ and K' were determined to be 0.4 ppm and -1.604/ppm respectively (Fig.5b).

3.2.3 Single treatments on *A. chroococcum*: UV-C and ceresan

Fig.6a and Fig.6b demonstrate the varying lethality brought about by UV-C and ceresan treatments on *A. chroococcum* cells. Interestingly, none of the inactivation curves revealed the presence of any shoulder thereby indicating sensitive nature of these cells towards the stresses used. The absence of a shoulder points to the fact that *A. chroococcum* cells behave as a single target/single hit system. Also, biphasic shape of the curve in case of UV-C radiation (Fig. 6a) shows that the bacterial culture contains a mixed population, which is partly sensitive and partly resistant towards UV-C radiation. About 4 percent of the whole population represented the radiation resistant portion of the bacteria (Fig.6a). LD$_{50}$ and K' values for major fraction (resistant, 90%) of bacterial population was found to be 5.1 Jm$^{-2}$ and -0.134/Jm$^{-2}$ respectively (Fig.6a).

Fig.6b depicts an exponential linear decrease in cell survival with increasing concentrations of ceresan used, i.e. 1 ppm to 8 ppm. Any resistant fraction of cell population was absent in this case. The values for LD$_{50}$ and K' were obtained to be 2.2 ppm and -0.317/ppm respectively (Fig.6b). LD$_{50}$ value with UV-C for *A. vinelandii* was more than two folds higher than that obtained for *A. chroococcum* while in case of ceresan treatment it was found to be reversed.
Fig. 6 Surviving fractions of A.chroococcum treated with different doses of stresses: (a) UV-C radiation, (b) ceresan.
3.2.4 Combined stresses on *A. chroococcum*: UV-C and ceresan

Ceresan pretreated (2 ppm) *A. chroococcum* cells irradiated with different doses of UV-C showed the same trend of inactivation pattern as was obtained in case of UV-C irradiation alone (Fig. 7a). About 8 percent of the bacterial population was seen to be UV-C resistant. The LD$_{50}$ value, which was found to be 11 Jm$^{-2}$ (Fig. 7a), is more than double compared to the value obtained from UV-C irradiation alone. Thus pretreatment of 2 ppm ceresan appeared to render the cells more resistant towards UV-C irradiation. K' value was equal to $-0.062$/Jm$^{-2}$ (Fig. 7a).

The inactivation profile of preirradiated (UV-C, 17 Jm$^{-2}$) *A. chroococcum* cells with ceresan is depicted in Fig. 7b. The curve shows the biphasic nature of bacteria towards ceresan treatment too. About 3 percent of the bacterial population was comprised of a ceresan resistant fraction. LD$_{50}$ and K' values obtained were 0.9 ppm and $-0.767$/ppm respectively (Fig. 7b). Thus LD$_{50}$ value is almost half the value of ceresan treatment alone.

The inactivation profile of higher dose of ceresan pretreated (4 ppm) *A. chroococcum* cells exposed to UV-C irradiation is shown in Fig. 8a. There is a very sharp decline in log surviving fractions at relatively low doses of UV-C (upto 10 Jm$^{-2}$) followed by an exponential parabolic decrease in cell survival corresponding to higher doses of UV-C (20 Jm$^{-2}$ to 85 Jm$^{-2}$). This parabolic part of the curve is again represented by the UV-C resistant fraction of bacteria which was found to be equal to 5 percent, a value which is slightly higher than the resistant population in Fig. 6a (4%). The LD$_{50}$ in this treatment regimen, which was obtained to
Surviving fractions of *A. chroococcum* exposed to combined stresses: (a) 2 ppm ceresan followed by different doses of UV-C radiation, (b) 17 J m\(^{-2}\) UV-C followed by different concentrations of ceresan.
Fig. 8  Surviving fractions of *A. chroococcum* exposed to combined stresses: (a) 4 ppm ceresan followed by different doses of UV-C radiation, (b) 34 Jm⁻² UV-C followed by different concentrations of ceresan.
be 4.0 Jm$^{-2}$, is a little lower compared to that obtained for UV-C irradiation alone (Fig.6a). The $K'$ value was computed to be $-0.148$/Jm$^{-2}$.

UV-C preirradiated (34 Jm$^{-2}$) A. chroococcum cells too showed similar exponential parabolic decrease in survival at higher doses of ceresan (4 ppm to 8 ppm) preceded by a very sharp log linear decrease at low doses of ceresan i.e. upto 4 ppm (Fig.8b). The LD$_{50}$ value was obtained to be 0.7 ppm which is as small as one third of the value computed for ceresan treatment alone (2.2 ppm). The value for $K'$ was calculated to be $-0.954$/ppm.

3.2.5 Inactivation of Azotobacter as a function of UV-wavelength

Fig.9a describes the oscillatory pattern of A. chroococcum mortality against 17.6 Jm$^{-2}$ dose of various wavelengths of UV used. At 260 nm, 280 nm, 300 nm, 320 nm and 340 nm the percent killing observed, was 45, 48, 71, 17 and 6.5 respectively. The maximum inactivation was noticed at 280 nm followed by sharp decline in killing at 300 nm. At 320 nm again a small peak was seen followed by a fall at 340 nm (Fig.9a).

The inactivation profile of A. vinelandii cells against 52.8 Jm$^{-2}$ dose of various wavelength-UV is shown in Fig.9b. The maximum killing was repeated at 280 nm similar to that of A. chroococcum. The percent mortality at 260 nm, 280 nm, 300 nm, 320 nm and 340 nm was noticed to be 61, 66, 8, 8 and 9.5 respectively. The additional small peak for killing was not obtained as shown in case of A. chroococcum. Based on these data 280 nm UV-B was further employed in other experiments. It is obvious from Fig.9 that A. chroococcum was much more prone to UV over the range used as com-
Fig. 9  Inactivation profiles of Azotobacter species as a function of wavelength of UV radiation: (a) A. chroococcum (dose = 17.6 Jm$^{-2}$) (b) A. vinelandii (dose = 52.8 Jm$^{-2}$).
pared to *A. vinelandii* under similar conditions.

3.2.6 **Single treatments on *A. vinelandii* and *A. chroococcum*: UV-B**

Fig. 10 describes the inactivation profiles of *A. chroococcum* and *A. vinelandii* against different doses of UV-B (280 nm) radiation. Both the strains of bacterium showed the exponential linear decrease in survival with increasing quantum of UV-B (upto 70 Jm$^{-2}$). The presence of shoulder at even very low doses of UV-B (4 Jm$^{-2}$) was not observed thereby indicating that both the strains showing reacted in a similar manner towards UV-B, i.e. a single target/single hit system. The LD$_{50}$ values for *A. chroococcum* and *A. vinelandii* were obtained to be 18 Jm$^{-2}$ and 31.5 Jm$^{-2}$ respectively. This establishes that *A. chroococcum* is more prone than *A. vinelandii* to UV-B radiation by a factor of about one and half folds. About threefold elevation in the LD$_{50}$ value for both the strains was observed when compared to their respective values obtained with UV-C alone. This proves UV-C to be more lethal than UV-B for *Azotobacter* strains. The K' values for *A. chroococcum* and *A. vinelandii* were computed -0.040/Jm$^{-2}$ and -0.021/Jm$^{-2}$ respectively (Fig. 10). However, no significant inactivation of either strain was obtained against increasing concentrations of monocrotophos as seen in Table 1.

3.2.7 **Combined treatments on *A. vinelandii*: ceresan and UV-B**

The log survival of UV-B preirradiated (4.2 Jm$^{-2}$) *A. vinelandii* cells plotted against different concentrations of ceresan is shown in Fig. 11a. The characteristic of the curve is the presence of shoulder at lower doses of ceresan (upto 1.5 ppm) followed by a dose dependent exponential linear decrease in sur-
Fig. 10  Surviving fractions of Azotobacter species exposed to different doses of UV-B radiation: (b) A. chroococcum, (b) A. vinelandii.
Fig. 11 Surviving fractions of \textit{A. vinelandii} exposed to combined stresses: (a) 4.2 Jm\textsuperscript{-2} UV-B followed by different concentrations of ceresan, (b) 2 ppm ceresan followed by different doses of UV-B radiation.
viving fractions (2 ppm to 6 ppm). Also it indicates the failure of UV-B pretreatment to alter the trend of inactivation for *A. vinelandii* cells against ceresan as seen in Fig. 3b. The LD50 and K' values were found to be 0.7 ppm and -0.870/ppm respectively (Fig. 11a).

A dose dependent log linear decline in survival of ceresan pretreated (2 ppm) *A. vinelandii* cells was obtained with UV-B radiation at all doses used i.e. upto 70 Jm⁻² (Fig. 11b). LD50 value was calculated to be 24.5 Jm⁻², which is one and half times less than the value obtained for UV-B irradiation alone (Fig. 10b). The value obtained for K' was -0.028/Jm⁻².

3.2.8. Combined treatments on *A. chroococcum*: UV-B and ceresan

Fig. 12a gives the inactivation of ceresan pretreated (2 ppm) *A. chroococcum* cells versus UV-B radiation. Log linear decrease in survival of cells was dose dependent at all doses used i.e. upto 45 Jm⁻² (Fig. 12a). The LD50 value was determined to be 18 Jm⁻² showing no appreciable difference in the value indicated in UV-B irradiation alone (Fig. 10a). The value for K' was computed to be -0.039/Jm⁻².

Fig. 12b depicts an interesting result in the sense that UV-B pre-irradiated (4.2 Jm⁻²) *A. chroococcum* cells showed a very remarkable resistance towards ceresan. The K' value obtained, was -0.043/ppm, the lowest value observed in both the strains with ceresan alone as well as in UV-preirradiated ceresan treatments.
Fig. 12  Surviving fractions of *A. chroococcum* exposed to combined stresses: (a) 2 ppm ceresan followed by different doses of UV-B radiation, (b) 4.2 Jm\(^{-2}\) UV-B followed by different concentrations of ceresan.
**Effect of monocrotophos on the survival of *Azotobacter* species.**

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<th>% survival of <em>A. chroococcum</em></th>
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<td>93.1</td>
</tr>
</tbody>
</table>
3.3 DNA STUDIES

3.3.1 Characterisation of DNA

The UV-absorption of *A. chroococcum* DNA recorded at various wavelengths is illustrated in Fig. 13. It shows the minimum and maximum absorbances at 230 nm and 257 nm respectively. The absorbance peak (257 nm) is immediately followed by a linear decrease in absorbance with increasing wavelengths and showed almost no absorption above 300 nm. The ratio of absorbance values at 260 nm and 280 nm was 1.86 (Fig. 13). Similarly, ratio of absorptions at 260 nm and 230 nm was found to be 2.2. These data indicate the purity of DNA extracted from *A. chroococcum* cells in the laboratory.

The thermal denaturation profile of *A. chroococcum* DNA dissolved in dilute saline citrate, pH 7.0, is depicted in Fig. 14a. The melting curve thus obtained helps a great deal in understanding the structural integrity and stability of DNA molecules. The melting temperature (T<sub>m</sub>) value of native DNA was noted to be 82.4°C (Fig. 14a). The GC content of *A. chroococcum* DNA was calculated to be 71 percent employing the equation of Hill (1968). The onset of melting transition commenced at 76°C (T<sub>i</sub> value) and reached the point of saturation at 88°C (T<sub>f</sub> value) giving rise to a value for melting transition (ΔT) of 12°C.

3.3.2. Melting studies of pesticides treated DNA

Fig. 14b describes the melting transition of monocrotophos treated *A. chroococcum* DNA. Even at very high concentration of monocrotophos (D/P=4) the value of T<sub>m</sub> remained unchanged as compared to control.
**Fig. 13** UV-absorption spectrum of *A.chroococcum* DNA.

**Fig. 14** Thermal denaturation profiles and melting temperatures \(T_m\) of *A.chroococcum* DNA: (a) control, (b) monocrotophos treated \(r = 4\).
The thermal denaturation patterns of DNA treated at various D/P ratios (r) of ceresan are recorded in Fig.15. The value of Tₘ at D/P ratios of r=0.5, r=1, r=2 and r=4 were obtained as 83°C, 83.4°C, 84.4°C and 84.8°C respectively (Fig.15 a,b,c, d). An elevation in Tₘ value compared to that of control DNA was thus obtained with increasing D/P ratios of ceresan-DNA complex and at highest level of ceresan used (r=4), was found higher by 2.4°C. The Tᵢ value, at all D/P ratios used, was lower than that of control by 1°C. The Tᵥ values were seen to be 89°C, 89°C, 91°C and 92°C respectively for D/P ratios r=0.5, r=1, r=2 and r=4. The ΔT values for corresponding r values were 14°C, 14°C, 16°C and 17°C respectively. Thus an increase was observed in ΔT value as compared to ΔT of 12°C for control DNA.

3.3.3 Melting studies of DNA irradiated with UV-C
255 Jm⁻², 510 Jm⁻², 1020 Jm⁻², and 1530 Jm⁻² were the doses of UV-C used to irradiate the DNA and the corresponding Tₘ values were computed to be 82.4°C, 82.0°C, 82.0°C and 81.8°C (Fig.16 a,b,c,d). The melting transition started at 74°C (Tᵢ value) in all UV-C treated DNA samples. Also, Tᵥ value of DNA remained constant (88°C) at all doses of UV-C used. ΔT value was obtained to be 14°C for all UV-C treated regimens of DNA. Thus ΔT value was raised by 2°C as compared to control value of 12°C. On comparison of Tₘ values for various UV-C doses with that of control, 255 Jm⁻² of UV-C showed no alteration while 510 Jm⁻² and 1020 Jm⁻² and 1530 Jm⁻² of UV-C reduced the Tₘ value, though marginally (0.4°C - 0.6°C).
Fig. 15  Thermal denaturation profiles and melting temperatures ($T_m$) of *A. chroococcum* DNA treated with various concentrations of ceresan: (a) $r = 0.5$, (b) $r = 1$, (c) $r = 2$, and (d) $r = 4$. 
Fig. 16 Thermal denaturation profiles and melting temperatures ($T_m$) of *A. chroococcum* DNA irradiated with various UV-C (253.7nm) doses: (a) 255 Jm$^{-2}$, (b) 510 Jm$^{-2}$, (c) 1020 Jm$^{-2}$, and (d) 1530 Jm$^{-2}$. 
3.3.4 Melting studies of DNA treated with combined stresses: UV-C and ceresan

Absorbance (260 nm) versus temperature plots of UV-C preirradiated (1020 Jm⁻²) DNA treated with various concentrations of ceresan are presented in Fig.17. A decrease in \( T_m \) value was observed with increase in concentration of ceresan. At D/P ratios of \( r=0.5, r=1, r=2 \) and \( r=4 \), the \( T_m \) values were 82.2°C, 81.5°C, 80.5°C and 76.2°C respectively (Fig.17 a,b,c,d) against 82.4°C for control DNA. However, treatment of DNA with ceresan alone had shown increase in \( T_m \) values with respect to control DNA (Fig.15). But the trend of change in \( T_m \) value of ceresan treated DNA was completely reversed on preirradiation with UV-C (1020 Jm⁻²). Preirradiated DNA ceresan complex at D/P ratios of \( r=0.5, r=1, r=2 \) and \( r=4 \) gave the \( T_i \) values as 72°C, 72°C, 73°C and 70°C respectively against 76°C for control DNA. \( T_f \) values for D/P ratios - \( r=2 \) and \( r=4 \) reduced by 2°C and 4°C respectively when compared to the control value (88°C). \( \Delta T \) value varies from 13°C to 16°C for various D/P ratios of UV-C preirradiated DNA-ceresan complex. Also the sigmoid (S) shape of the melting curve gradually changed to almost a straight line at D/P ratio of \( r=4 \) (Fig.17d).

Fig.18 depicts the melting profiles of ceresan pretreated (\( r=1 \)) DNA exposed to different doses of UV-C radiation. At doses 255 Jm⁻², 510 Jm⁻², 1020 Jm⁻², and 1530 Jm⁻², the \( T_m \) values were 84.2°C, 84.0°C, 84.8°C and 83.6°C respectively (Fig.18 a,b,c,d). \( T_i \) value was 75°C for all doses except 255 Jm⁻² UV-C where the \( T_i \) value was observed to be 74°C. \( T_f \) value for all doses of UV-C
Fig. 17  Thermal denaturation profiles and melting temperatures ($T_m$) of preirradiated (UV-C, 1020 Jm$^{-2}$) A. chroococcum DNA treated with various concentrations of ceresan: (a) $r=0.5$, (b) $r=1$, (c) $r=2$ and (d) $r=4$. 

$T_m=82.2^\circ C$

$T_m=81.5^\circ C$

$T_m=80.5^\circ C$

$T_m=76.2^\circ C$
Fig. 18 Thermal denaturation profiles and melting temperatures ($T_m$) of pretreated (cresan, $r=1$) A. chroococcum DNA irradiated with various doses of UV-C: (a) 255 Jm$^{-2}$, (b) 510 Jm$^{-2}$, (c) 1020 Jm$^{-2}$, and (d) 1530 Jm$^{-2}$. 
used was 89°C. $\Delta T$ value was equal to 14°C for all doses used except for 255 Jm$^{-2}$ UV-C ($\Delta T = 15°C$). Further, the $T_m$ value of ceresan pretreated (r=1) DNA at different doses of UV-C was retained higher than ceresan treatment alone ($T_m=83.4°C$, at r=1).

3.3.5 Melting studies of DNA exposed to gamma Absorbance (260 nm) versus temperature plots of DNA exposed to various doses of gamma radiation are illustrated in Fig.19. The $T_m$ values of DNA irradiated at 152.5 Gy, 305 Gy, 610 Gy and 915 Gy obtained, were 82.0°C, 81.0°C, 79.8°C and 78.6°C respectively (Fig.19 a,b,c,d). The decrease in $T_m$ values of gamma-irradiated DNA with respect to control (82.4°C) was considerably more than the decrease caused by UV-C radiation alone. The $T_i$ values of DNA irradiated at doses, 152.5 Gy, 305 Gy, 610 Gy and 915 Gy were 74°C, 73°C, 72°C and 71°C respectively, showing a continuous fall with respect to control value (76°C). $T_f$ and $\Delta T$ values varied from 86°C to 88°C and 14°C to 15°C respectively (at doses 152.5' Gy to 915 Gy).

3.3.6 Melting studies of DNA treated with combined stresses: gamma and ceresan

The thermal denaturation patterns of ceresan pretreated (r=1) DNA at various doses of gamma radiation are exhibited in Fig.20. A continuous decline in $T_m$ values of ceresan pretreated (r=1) DNA was observed with increasing doses of gamma irradiation. The $T_m$ values obatained, for ceresan pretreated DNA at doses, 152.5 Gy, 305 Gy, 610 Gy and 915 Gy, were 81.6°C, 80.8°C, 79.6°C and 79°C respectively (Fig.20 a,b,c,d). $\Delta T$ value was observed to be 16°C for all doses of gamma-radiation used. Pretreatment of ceresan
Fig. 19  Thermal denaturation profiles and melting temperatures ($T_m$) of *A.chroococcum* DNA exposed to various doses of gamma radiation: (a) 152.2 Gy, (b) 305 Gy, (c) 610 Gy, and (d) 915 Gy.
Fig. 20  Thermal denaturation profiles and melting temperatures ($T_m$) of pretreated (ceresan, $r=1$) A.chroococcum DNA irradiated with various doses of gamma: (a) 152.5 Gy, (b) 305 Gy, (c) 610 Gy, and (d) 915 Gy.
with DNA, thus remained almost ineffective to bring any qualitative or quantitative change against the doses of gamma radiation used when compared to single treatment of gamma. A rapid decline in $T_m$ values of gamma preirradiated DNA with increasing concentrations of ceresan is seen in Fig.21, at D/P ratios, $r=0.5$, $r=1$, $r=2$ and $r=4$. The $T_m$ values for gamma preirradiated DNA were 81.6°C, 81.0°C, 79.4°C and 76.0°C respectively (Fig.21 a,b,c,d). The $T_i$ and $T_f$ values were decreased from 71°C to 66°C and 88°C to 85°C respectively over the D/P ratios used ($r=0.5$ to $r=4$). The $\Delta T$ value varied between 17°C and 19°C over the D/P ratios used i.e. $r=0.5$ to $r=4$. Also, it is noticed that with increasing D/P ratio the melting transition deviates from characteristic sigmoid shape and becomes almost a straight line at D/P ratio $r=4$ (Fig.21 d). Further, from a comparative study of Fig.21 and Fig.19 it is observed that behaviour of DNA towards ceresan is reversed qualitatively as well as quantitatively due to gamma preexposure of DNA.

Fig.22 depicts a comparative analysis of changes in melting temperature ($\Delta T_m$) of *A. chroococcum* DNA, as a function of dose in case of various single as well as combined treatments of UV-C radiation and ceresan. In single treatments of ceresan at all D/P ratios ($r=0.5$ to $r=4$) and of UV-C at doses 510 Jm$^{-2}$ - 1530 Jm$^{-2}$ the sign of $\Delta T_m$ value for the former is positive while it is negative for the latter (Fig.22 a,b). This suggests that interaction of DNA with ceresan enhances its stabilising forces while UV-C radiation reduces these. Low dose of UV-C (255 Jm$^{-2}$) increases the $T_m$ value of ceresan pretreated ($r=1$) DNA with sub-
Fig. 21 Thermal denaturation profiles and melting temperatures ($T_m$) of preirradiated (gama, 305 Gy) *A. chroococcum* DNA treated with various concentrations of ceresan: (a) $r=0.5$, (b) $r=1$, (c) $r=2$, and (d) $r=4$. 
Fig. 22 Variations in $T_m$ values of *A. chroococcum* DNA exposed to different doses of stresses: (a) DNA treated with various concentrations of cereasan alone, (b) DNA irradiated with different doses of UV-C alone, (c) cereasan (r=1) pretreated DNA followed by irradiation with different doses of UV-C, and (d) UV-C (1020 Jm$^{-2}$) preirradiated DNA followed by treatment with various concentrations of cereasan.
sequent decrease in $T_m$ values at higher doses of UV-C (510 Jm$^{-2}$ - 1530 Jm$^{-2}$) (Fig.22c), nonetheless, the value of $T_m$ is retained higher than the value for ceresan treatment (r=1) alone. Similarly, at low D/P ratio of UV-C preirradiated (1020 Jm$^{-2}$) DNA, $T_m$ is slightly increased followed by a drastic reduction in $T_m$ value (by 0.9°C - 6.2°C) at higher D/P ratios (r=1 to r=4) (Fig.22d).

To have a comparative analysis of changes in $T_m$ of *A. chroococcum* DNA exposed to other agents such as gamma radiation, $\Delta T_m$ was plotted against various doses of ceresan and gamma radiation, used separately as well as in combination (Fig.23). With the increasing doses of gamma radiation, a continuous decrease in $T_m$ was observed and at the highest dose used i.e. 915 Gy, the $T_m$ was reduced by 3.8°C (Fig.23b). Gamma preirradiated (305 Gy) DNA showed a slight increase in $T_m$ (by 0.6°C) at low D/P ratio (r=1) followed by remarkable decrease in $T_m$ value with increase in D/P ratio (at r=4, $\Delta T_m = -6.4^\circ$C) (Fig.23d). Ceresan pretreated (r=1) DNA revealed the continuous decrease in $T_m$ value with increasing doses of gamma radiations (Fig.23c). It is interesting to note that pretreatment of DNA with either of the stresses changes its interaction pattern with the second stress.

3.3.7 Detection of DNA damage under electron microscope

The electron micrographs of *A. chroococcum* DNA as well as DNA treated with various stress agents separately, are given in Fig.24 and Fig.25. The electron micrograph (Fig.24a) shows the long, undistorted strands of native DNA, indicating the integrity of isolated DNA. DNA treated with a dose of 1530 Jm$^{-2}$
Fig. 23 Variations in $T_m$ values of *A. chroococcum* DNA exposed to different doses of stresses: (a) DNA treated with various concentrations of cereasan alone, (b) DNA irradiated with different doses of gamma, alone (c) cereasan ($r=1$) pretreated DNA followed by irradiation with different doses of gamma, and (d) Gamma (305 Gy) preirradiated DNA followed by treatment with various concentrations of cereasan.
Fig. 24 Electron micrographs of *Azotobacter chroococcum* DNA: (a) control and (b) exposed to UVC light (1530 J m$^{-2}$). Magnification 18200X.
Fig. 25 Electron micrographs of A. chroococcum DNA: (a) treated with cereasan (r =2) and (b) exposed to gamma rays (915 Gy). Magnification 18200X.
UV-C or ceresan (r=2) has not shown any appreciable damage and deformation (Fig.24b and Fig.25a) as compared to control DNA (Fig.24a). Nevertheless, at the same magnitude of the above mentioned stresses a well marked change in the thermal stability of the DNA duplex was obtained from thermal transition studies. DNA exposed to gamma radiation (915 Gy) seemed to have an effect, chopping the DNA strands into small fragments (Fig. 25b).

3.4 LEUCINE TRANSPORT ACROSS THE MEMBRANE

3.4.1 Km determination: In order to evaluate the $K_m$ value, (defined as half of the concentration of the substrate/solute at which the maximum transport velocity is attained) inverse of the leucine uptake scored by one mg total cellular protein in 30 sec was plotted against inverse of various leucine concentrations at which cells in reaction mixtures were incubated. The curves thus obtained in Fig.25 follow a straight line equation (for details, see 2.8.1). Taking the points of intersection on abscissa, $K_m$ values for L-leucine were obtained as 333 µM and 250 µM for _A. chroococcum_ and _A. vinelandii_ respectively (Fig.26 a,b). Two things are obvious from the $K_m$ values of L-leucine for both the strains: firstly, cell membrane of _A. vinelandii_ has got more affinity towards L-leucine than the cell membrane of _A. chroococcum_, since the latter exhibits a higher $K_m$ than the former, and secondly, uptake of leucine takes place by active transport and not merely through passive diffusion.
Fig. 26  Lineweaver – Burk plot for L-leucine in Azoto bacter species: (a) A. chroococcum, (b) A. vinelandii.
3.4.2 Leucine uptake in *A. vinelandii* cells exposed to environmental stresses

Fig. 27a elucidates the leucine uptake activity in control as well as in cells exposed to 51 Jm\(^{-2}\) of UV-B and UV-C separately. It is evident from the curves that uptake is very fast in the beginning of the reaction (upto 60 sec) followed by a slow leucine accumulation with increasing time periods (beyond 60 sec). Control cells showed leucine accumulation of 15 n moles/mg protein at 15 sec which attained the level of 40 n moles/mg protein at 150 sec. UV-B irradiated (51 Jm\(^{-2}\)) cells demonstrated a slightly higher uptake than control (8% increase) at 60 sec reaching a steady state level at longer time periods (105-195 sec) which is 20-30 percent less as compared to control (Table II). UV-C treated (51 Jm\(^{-2}\)) cells kept a lower profile of leucine accumulation at all time periods of observation compared to control cells (Fig. 27a, Table II) and followed a pattern qualitatively similar to that of the control.

Inhibition in leucine uptake by cells grown in presence of both 500 ppm monocrotophos and 1 ppm ceresan separately, was observed as compared to cells grown in the control medium alone (Fig. 28a, Table II). Reduction in leucine accumulation imparted by 1 ppm ceresan was upto 48 percent while in case of 500 ppm monocrotophos it was observed to be upto 32 percent at time periods selected for observation (upto 195 sec) with respect to control (Table II). This alteration in leucine transport is not instantaneous but is the reflection of change in intrinsic
Fig. 27  L-leucine uptake in *A. vinelandii* cells, exposed to single as well as combined stresses, as a function of time: (a) cells grown in medium alone, and exposed to UV-B (51 J m\(^{-2}\)) and UV-C (51 J m\(^{-2}\)) separately, (b) cells grown in presence of 500 ppm monocrotophos, and exposed to UV-B (51 J m\(^{-2}\)) and UV-C (51 J m\(^{-2}\)) separately.
Fig. 28 L-leucine uptake in A. vinelandii cells, exposed to single as well as combined stresses, as a function of time: (a) cells grown in medium alone, and in presence of 500 ppm monocrotophos and 1 ppm ceresan separately, (b) cells grown in presence of 1 ppm ceresan and exposed to UV-B (51 J m\(^{-2}\)) and UV-C (51 J m\(^{-2}\)) separately.
Percent change in leucine uptake of *A. vinelandii* cells due to single as well as combined stresses at different times with respect to control (cells grown in medium alone).

<table>
<thead>
<tr>
<th>Stress(es)/Time</th>
<th>15 sec</th>
<th>60 sec</th>
<th>105 sec</th>
<th>150 sec</th>
<th>195 sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>51 Jm⁻² UV-B</td>
<td>0</td>
<td>+7.8</td>
<td>-10.6</td>
<td>-21.7</td>
<td>-31.1</td>
</tr>
<tr>
<td>51 Jm⁻² UV-C</td>
<td>-10.3</td>
<td>-7.8</td>
<td>-15.1</td>
<td>-19.2</td>
<td>-23.3</td>
</tr>
<tr>
<td>500 ppm mono-crotophos</td>
<td>-13.3</td>
<td>-32.6</td>
<td>-32.3</td>
<td>-31.2</td>
<td>-30.0</td>
</tr>
<tr>
<td>1 ppm ceresan</td>
<td>0</td>
<td>-30.7</td>
<td>-41.1</td>
<td>-48.7</td>
<td>-48.0</td>
</tr>
<tr>
<td>500 ppm mono-</td>
<td>-13.3</td>
<td>-35.2</td>
<td>-30.3</td>
<td>-43.5</td>
<td>-47.7</td>
</tr>
<tr>
<td>crotophos +</td>
<td>51 Jm⁻² UV-B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 ppm mono-crotophos +</td>
<td>-3.3</td>
<td>-35.2</td>
<td>-21.4</td>
<td>-37.1</td>
<td>-41.1</td>
</tr>
<tr>
<td>51 Jm⁻² UV-C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ppm ceresan +</td>
<td>-6.6</td>
<td>-21.5</td>
<td>-17.8</td>
<td>-35.8</td>
<td>-42.2</td>
</tr>
<tr>
<td>51 Jm⁻² UV-B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ppm ceresan +</td>
<td>0</td>
<td>-17.6</td>
<td>-10.7</td>
<td>-26.9</td>
<td>-30.0</td>
</tr>
<tr>
<td>51 Jm⁻² UV-C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ ve sign indicate more uptake in comparison with control.
- ve sign indicate less uptake in comparison with control.
properties of cell membrane, which may be associated with compositional constituents of membrane. However, change in transport activity induced by radiation stress is instantaneous and is most probably due to its effect on the carrier proteins.

When the cells were grown in presence of 500 ppm monocrotophos and treated with 51 Jm$^{-2}$ of UV- radiations the pattern of leucine uptake was seen not to be disturbed by UV-C as is clear from Fig. 27b. Nevertheless, UV-B did cause further depression in uptake activity for leucine despite it being less energetic compared to UV-C.

Fig. 28b presents the comparison of leucine uptake in cells exposed to single as well as combined stresses at various time periods of observation. It is interesting to note that cells grown in the presence of 1 ppm ceresan showed an appreciable increase in the leucine uptake on exposure to 51 Jm$^{-2}$ dose of UV-B or UV-C. This is an example of antagonistic effect brought about by a chemical (ceresan) and radiation (UV) on the membrane transport of A. vinelandii cells. The first stress (ceresan) given to cells decreased the membrane transport while the second stress (UV-rad) tends not only to restore it but also multiply the phenomenon to some extent. But the combined treatment of monocrotophos with radiation (UV-B or UV-C) showed only additive effect on leucine transport of A. vinelandii membrane (Fig.27b).

3.4.3 Leucine uptake in A. chroococcum cells exposed to environmental stresses

The response of leucine uptake in cells towards 51 Jm$^{-2}$ of UV-B and UV-C with respect to untreated cells, at various time periods
is recorded in Fig. 29a. The untreated cells manifested a typical, energy dependent transport profile, which is characteristic of biological membranes. Leucine accumulation in untreated cells varied from 6 nmoles/mg protein to 10 n moles/mg protein during the time period of observations (15 sec to 195 sec). Strikingly enough, when cells were exposed to 51 Jm$^{-2}$ of UV-radiations a sudden rise in leucine uptake was observed at the initial stages (15 to 60 sec), followed by a gradual decline in leucine uptake level corresponding to the time periods of 60 sec to 195 sec (Fig. 29a). However, similar trends were not observed in case of leucine uptake by *A. vinelandii* cells exposed to the same doses of UV-radiations (Fig. 27a). More than 25 percent increase in the leucine influx was demonstrated by the cells grown in presence of 500 ppm monocrotophos and 1 ppm ceresan separately, with respect to control cells at all time periods of observation i.e. 15 sec to 195 sec (Fig. 30a, Table III). Conversely, an inhibition by monocrotophos and ceresan was obtained in *A. vinelandii* cells (Fig. 28a). The increase in leucine uptake by ceresan treatment is more than that caused by monocrotophos. The cells grown in presence of 500 ppm monocrotophos and subsequently exposed to 51 Jm$^{-2}$ UV-B showed an increase in leucine accumulation as compared to accumulation by cells grown in 500 ppm monocrotophos but received no radiation treatment (Fig. 29b). The presence of monocrotophos during the growth of cells protected their leucine uptake against UV-B, while it was not the case against UV-C and a decrease in leucine accumulation.
Fig. 29  L-leucine uptake in *A. chroococcum* cells, exposed to single as well as combined stresses, as a function of time: (a) cells grown in medium alone, and exposed to UV-B (51 J m\(^{-2}\)) and UV-C (51 J m\(^{-2}\)) separately, (b) cells grown in presence of 500 ppm monocrotophos and exposed to UV-B (51 J m\(^{-2}\)) and UV-C (51 J m\(^{-2}\)) separately.
Fig. 30  L-leucine uptake in *A. chroococcum* cells, exposed to single as well as combined stresses, as a function of time: (a) Cells grown in medium alone, and in presence of 500 ppm monocrotophos and 1 ppm ceresan separately, (b) Cells grown in presence of 1 ppm ceresan, and exposed to UV-B (51 Jm\(^{-2}\)) and UV-C (51 Jm\(^{-2}\)) separately.
TABLE - III

Percent change in leucine uptake of *A. chroococcum* cells due to single as well as combined stresses at different times with respect to control (cells grown in Medium alone).

<table>
<thead>
<tr>
<th>Stress(es)</th>
<th>Time</th>
<th>15 sec</th>
<th>60 sec</th>
<th>105 sec</th>
<th>150 sec</th>
<th>195 sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>51 Jm⁻² UV-B</td>
<td>+50.0</td>
<td>+36.1</td>
<td>+4.8</td>
<td>-34.0</td>
<td>-58.8</td>
<td></td>
</tr>
<tr>
<td>51 Jm⁻² UV-C</td>
<td>+67.8</td>
<td>+47.2</td>
<td>+9.7</td>
<td>-36.1</td>
<td>-62.7</td>
<td></td>
</tr>
<tr>
<td>500 ppm mono-crotophos.</td>
<td>+39.2</td>
<td>+38.8</td>
<td>+36.5</td>
<td>+27.6</td>
<td>+25.4</td>
<td></td>
</tr>
<tr>
<td>1 ppm ceresan</td>
<td>+64.2</td>
<td>+55.5</td>
<td>+53.6</td>
<td>+44.6</td>
<td>+39.2</td>
<td></td>
</tr>
<tr>
<td>500 ppm mono-crotophos + 1 ppm ceresan</td>
<td>+75</td>
<td>+69.4</td>
<td>+58.5</td>
<td>+44.6</td>
<td>+37.2</td>
<td></td>
</tr>
<tr>
<td>500 ppm mono-crotophos + 51 Jm⁻² UV-B</td>
<td>+107</td>
<td>+97.2</td>
<td>+65.8</td>
<td>+14.8</td>
<td>-33.3</td>
<td></td>
</tr>
<tr>
<td>1 ppm ceresan + 51 Jm⁻² UV-B</td>
<td>+50</td>
<td>+25</td>
<td>+14.6</td>
<td>+2.1</td>
<td>-5.8</td>
<td></td>
</tr>
<tr>
<td>1 ppm ceresan + 51 Jm⁻² UV-C</td>
<td>+14.2</td>
<td>+33.3</td>
<td>+48.7</td>
<td>+55.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ ve sign indicates more uptake in comparison with control.
- ve sign indicates less uptake in comparison with control.
at longer periods (beyond 100 sec) in case of the latter is reflected in Fig.29b.

Fig.30b illustrates the leucine accumulation at various time periods in *A. chroococcum* cells treated with combined stresses. Membrane leakage (efflux of leucine) due to UV-irradiations following 1 ppm ceresan treatment is not observed. The scoring of leucine level in cells exposed to ceresan and UV-B in combination has been less than that by cells treated with ceresan alone. The leucine uptake by the cells grown in 1 ppm ceresan followed by UV-C treatment (51 Jm⁻²) is lower upto 100 sec and higher beyond this time period than uptake by cells which received 1 ppm ceresan treatment alone.

A comparative account of leucine uptake in *A. chroococcum* cells which can be drawn from Table III. It is obvious that an elevation in leucine uptake took place in all single as well as in combined treatments except due to membrane leakage by UV-radiation at relatively longer time periods, which was not the case observed in *A. vinelandii* uptake studies (Table II).