# INTERACTIVE EFFECTS OF AZROUBINE (E 122) AND VITAMIN C ON MITOTIC CELL DIVISION, NUCLEIC ACIDS CONTENT AND GENE EXPRESSION

**H. M. MAHFOZ[1], H. EL-ATROUSH[1] AND S. AL-SHAMMRANI[2]**

1. *Botany Department, Faculty of Science, Ain-Shams University, Cairo, Egypt*
2. *Botany Department, Girls College of Education, KSA*

Synthetic food additive colours, have been introduced in our food in order to give an attractive colours to it. It has been noticed, however, that most of the applied synthetic food colours have properties similar to the mutagenic chemicals. Evidences have accumulated in the last years to indicate that a large number of synthetic food additives are capable of inducing genetic diseases to human (Miller *et al*., 1996; Karen *et al.*, 2006; Martin, 2007).

Food additives are substances added to food in order to retain or improve desirable characteristics or quality. In the last years many more additives have been introduced, of either natural or artificial origin. Many of which can be the cause of certain health conditions, such as E102 that can cause asthma attacks and has links to thyroid tumors, E104 which can cause dermatitis, E110 side effects are (kidney tumours, nausea and vomiting), E122 can cause bad reactions in asthmatics and people allergic to aspirin, E124 carcinogenic in animals, E407 recently linked to cancer (Martin, 2007). Some Europeans countries prevented many synthetic food colours (E142, E131 and E123), as this colours led to the formation of cancer as descried by (Martin, 2007). Azroubine or the other name is carmoisine (E122). It is red colour, coal tar derivative and can produce bad reactions in asthmatic and people allergic to aspirin. E122 is banned in USA.

Several evidences have indicated that some food additive colours can induce genotoxic effects (Macioszek and Kononowicz, 2004; Makoto and Noriho, 2005; Jamal, 2006). Most of these studies have been oriented to demonstrate the antimitotic and chromotoxic activities of these food additives and pointed out their danger as carcinogens or mutagens. However, some of these studies deal with the effect of these food additives on protein banding pattern and on nucleic acids content (Shuji *et al*., 2001; Wei *et al*., 2005). Vitamins supplements are known to enhance the plant activities and did not have a toxic or mutagenic action (Khan and Sinha, 1993; Fawzia, 2002; Surendra *et al*., 2005; Sesso *et al*., 2008).

The present investigation was planned to study the interactive effects of different concentrations of the synthetic food additive azroubine or carmoisine (E122) and vitamin C on mitotic activity

and nucleic acids content in root tips of *Allium cepa*. Also, the capacity of synthetic food additive to induce chromosomal aberration and changes in seed protein electrophoretic profiles were investigated.

## MATERIALS AND METHODS

*Allium cepa* (var., Giza 6) was used in the present study. This variety was supplied by the Agricultural Research Center, Giza, Egypt. Synthetic food colour Azroubine (E122) is a synthetic red azodye. It has other names (carmoisine and food red 3). Chemical name of E122 is dialuminum salt of 2-(4-sulpho-1-napthylazo) -1-napthol-4-sulphonic acid.E122 was used in drugs and cosmetics (marzipan, Swiss roll, jams and preserves, sweets, brown sauce, flavoured yogurts and packet soups, jellies, breadcrumbs and cheese cake mixes. Vitamin C: (L. ascorbic acid) was purchased from Memphis Co. (100 mg/l).

### *1) Cytological procedure for mitotic studies*

The concentrations selected for the synthetic food colour E122 were ranging between 0.164 and 10.500gm/l. The dose of vitamin C which was used in the present study was 100 mg/L. It was administered concurrently with synthetic food colour after 24 hrs treatment.

Bulbs of *Allium cepa* (var., Giza 6) were germinated in tap water. After the roots were about 2-3 cm long, the bulbs were divided into two groups. The first group was treated with different concentrations of E122 for 3,6,12 and 24 hours. The second group was exposed to the tested doses of E122 for 24 hours and then vitamin C for 3 hours Table (1). After treatment, the roots were cut off and fixed in 3 absolute ethyl alcohol: 1 glacial acetic acid (v/v) for 24 hrs. For cytological study, the roots were hydrolyzed in 1N HCl at 60°C for 6-8 min, then stained and squashed with Feulgen squash technique .Permanent slides were made by mounting in canada balsam. 90 microscopical fields were completely analyzed for each concentration for the effect on the mitotic index (MI), frequency of mitotic stages and the frequency of different mitotic abnormalities. The data obtained from the different treatments were statistically analysed using the t-test.

### *2) Quantitative estimation of nucleic acids*

Another experiment was conducted, where a known fresh weight of root tips of *Allium cepa* was used for the extraction and determination of the amounts of nucleic acids DNA and RNA Table (3). The method used here was a modified method based on that of *Shibko et al*. (1967). RNA was estimated colourimetrically by the orcinol reaction as described by Ashwall (1957), while DNA was estimated by diphenylamine (DPA) colour reaction described by Burton (1968).

### *3) Protein electrophoresis*

Dry $M_2$ seeds of the treated *Allium cepa* parents, were decoated and milled to fine powder. Total proteins were then ex-

tracted overnight using 0.2 M Tris-Hcl buffer pH 6.8 containing 2.25 SDS. After centrifugation at 9000 rpm for 6 min, the supernatant was collected. SDS-polyacrylamide gel electrophoresis was performed in 12.5% acrylamide slab gels following the system of Laemmli (1970). Gel samples were run at a current of 15 mA for 30 minutes followed by 30 mA till the tracing dye silver nitrate reached the gel bottom (Sammons *et al*. 1981). Alterations in seed storage protein profiles among the seed samples of the treated *Allium cepa* plants were compared with that of the control to measure both the mutagenic potentiality of synthetic food colour E122 and to evaluate the antimutagenic potentiality of vitamin C.

## RESULTS AND DISCUSSION

One of the major effects of the food Table (1) additive colour E122 used in this work is its influence on the rate of mitotic division Table (1). The various treatments of E122 showed a slight reduction in the mitotic index in the root treated for 3 hrs, compared with control value. But when the time of the treatment increased it showed highly significant decrease in the mitotic index. MI reached 0.25 at highest concentration (1.313 gm/L) after treatment for 24 hrs., Table (1), but lower concentration (0.164 gm/l) had no significant effect. These results resemble those obtained by Liu *et al*. (2003) who reported that the mitotic index of *Vicia faba* root tips were successively decreased and even stopped with the increase of $cd^{+2}$ concentrations and duration of treatments. The inhibition of mitotic activity has been regarded as a common effect induced by numerous chemical compounds and has been reported by many investigators (El-Bayoumi *et al*., 1985; Fiskejo, 1988; Hassanein and Shehata, 2000; Chandra and Chauhan, 2004; Marcano *et al*., 2004; Sang and Li 2004).

The mitodepressive effect of E122 may be attributed to its inhibitory effect on the onest of mitosis, by lengthing the mitotic cycle and/or delaying of spindle formation (Adam and Rashad, 1984; Arias, 1996; Polit *et al*., 2003). Also the reduction of MI in treated *Alluim cepa* roots with E122 may be due to its ability on blocking the mitotic cycle during interphase which may result from a prolonged $G_1$, S, or $G_2$ periods Mohands and Grant, (1972) or to the inhibition of DNA synthesis (Van't Hof 1968, Kim *et al*., 1996, Yim *et al*., 1996; Mohanty *et al*., 2004) or inhibiting protein synthesis Kim and Bendixen (1987). In the present treatments the reduction in mitotic activity was accompanied with a depressive action on the amounts of nucleic acids (DNA and RNA), (Table 3). So the inhibition of mitosis may be due to an inhibitory effect of the E122 on nucleic acids content.

Variation in the mitotic phases showed that, all treatments with the different concentrations of the applied food additive colour induced an accumulation of cells at metaphase and ana-telophase stages on the expense of prophase, where decrease in their percentage were recorded, Table (1). The decrease in pro-

phase frequency may be due to the reduction in the cell number entering mitosis. The increase in metaphase and ana-telophase percentage could be due to the lengthing of their duration that leads to their accumulation. Similar result was obtained by Abdelsalam *et al.* (1997); Shehab *et al.* (2000) and Tawab *et al.* (2004).

Most of the treatments with E122 resulted in a significant increase in the percentage of abnormal mitosis. This percentage increased as the concentration of E122 and the time of treatment increased. Different types of chromosomal abnormalities and percentages were recorded in mitotic division after all treatments Tables (1 & 2).

Chromosomal stickiness was the dominant abnormality induced in mitotic divisions after treatment especially with higher concentrations. Patil and Bhat, (1992) suggested that, stickiness is a type of physical adhesion involving mainly the proteinacious matrix of chromatin material,

Chromosome and chromatin bridges represented the other most common types of mitotic abnormalities, Table (2). The percentage of bridges generally increased with the increase of the E122 concentration and time of treatment. The formation of bridges could be attributed either to the general stickiness of chromosomes (Abraham and Kosly, 1979) or to chromosome breakage and reunion (Tomkins and Grant, 1972; Kabarity *et al.*, 1974). Most of the observed bridges in this study were sticky bridges.

Lagging of some chromosomes at metaphase and ana-telophase was another of some chromosomal aberration observed after treatment with E122. The induction of laggards could be attributed to the failure of the spindle apparatus to organize and function in a normal way (Patil and Bhat, 1992). The lagging chromosomes may ultimatly result in the formation of micronuclei. Furthermore, the synthetic food colour E122 also induced micronuclei in interphase. Micronuclei are of a true mutagenic effect which may lead to loss of the genetic material (Wei *et al.*, 1997; El-Nahas, 2000). Similar results were observed by Monarca *et al.* (2003) who reported that the micronucleus test and chromatin aberrations in root cells of *Vicia faba* revealed genotoxcity of water disinfected with sodium hydrochlorite, chlorine dioxide and peracetic acid, also Rosa *et al.* (2003) and Liu *et al.* (2003) observed such type of abnormalities after treating *Vicia faba* root tips with cadmium.

The synthetic food colour used in the present study has highly affected the spindle fiber apparatus leading to the production of different types of metaphase abnormalities. The first class comprises those which are due to complete inhibition of spindle fiber giving diploid C-metephase configuration. The inhibition of spindle fiber may be due to the effect of this food colour on the microtubule formation, a loss of microtubules and/or the interference with tubluin or depolymeriza-

tion of microtubular subunits forming the spindle apparatus (Pickett-Heaps *et al*., 1982; Sudhaharan *et al.,* 1994). The second class includes those which are due to the partial effect of this food colour on the spindle leading to a slight disturbance in the orientation of the chromosome during their separation and migration to the opposite poles of the cell giving disturbed-anaphase. These disturbed configurations were not observed in roots treated with high concentration but were abundant in the moderate and low ones.

The third class comprises those which are due to the effect of the synthetic food colour on the organization of the spindle rather than their inhibition. Few star metaphase cells were noticed after treatment with E122. Star metaphase is considered as being a fore-step of the complete disturbance of the spindle (Amer, 1966).

The results show that vitamin C has successfully reduced the effect of E122 on the mitotic division rate and the percentage of chromosomal abnormalities i.e., in roots treated with vitamin C (post treatment) for 3 hours after treatment with E122 for 24 hours Table (1). These protective effects showed that our result were similar to the result obtained by other workers on the anti-genotoxcity of vitamins against genotoxic effects of chemicals (Hoda and Sinha 1992; Fawzia 2002; Sesso *et al*., 2008).

Vitamin C minimized the induced of both mitoinhibition and genotoxic effects. So this improvement in cell division may be due to the effect of vitamin C on the defense system of organisms Hoda *et al*. (1991), or to activation the defense system of organism against the harmful exo and endogenous factors (Odin, 1997). In the present study a gradual decrease in percentage of chromosomal aberrations was observed when vitamin C was added, vitamin C successfully reduced the clastogenic effects of many chemical agents in animals and plants (Fawzia, 2002; Mohamed, 2002).

The presented results indicate that E122 is genotoxic. Accordingly much more care should be taken in using E122 because regular use may lead to toxicological hazards.

The reduction of mitotic activity as a result of treatment with most concentrations of E122 was accompanied with a depressive action on the amounts of nucleic acids (DNA & RNA). This is a particular evident from a comparison on of the values of (DNA & RNA) in treated roots to their values in control roots, Tables (1&3). This phenomenon is compatible with the hypothesis that inhibition of mitoses may be due to an inhibitory effect of the synthetic food colour E122 on DNA and RNA synthesis.

It was suggested that E122 inhibits DNA replication by reducing the oxidative phosphorylation which results in lower ATP levels. This reduction in DNA content was accompanied with strong inhibitions of RNA synthesis Sundhaharan *et al.* (1994). In the present study, the reduction in DNA contents was remarkably associ-

ated with the inhibition of mitosis. In conclusion, nucleic acids content (DNA & RNA) decreased with the increasing of concentration and time of treatment. But after vitamin C administration, DNA contents increased while there is a fluctuation in RNA contents in comparison with 24 hours treatment Table (3).

The used of synthetic food colour E122 caused many changes in the M2 seed storage protein banding patterns of *Allium cepa* whose parents were previously sprayed with E122, Fig. (1) and Table (4). These include changes in bands intensity, relative mobility, bands subfractionation, appearance of new bands and disappearance of some bands. They are heritable changes since they were transferred to the next generations. This conclusion is in agreement with Hassan, (1996).

Table (4) and Fig. (1) demonstrate the effect of the treatments of E122 and vitamin C on the protein banding patterns of *Allium cepa* seeds. The total number of protein bands recorded was 19 bands. Nine of which were common in the control and treatments. These common bands have molecular weight of 74, 54, 47, 45, 38, 35, 29, 20 and 8 KD. The most visible changes in SDS-PAGE patterns were the appearance of few new bands like bands with molecular weight of 33 and 43 KD in the treatments and disappearance of some bands such that having molecular weight 25, 23 and 22 KD Table (4). The alterations in the electrophoretic profiles of seed proteins are indicative of the ability of both E122 & vitamin C to alter the gene expression in exposed cells.

Disappearance of some bands in this study could be traced back to the induction of cytological abnormalities like bridges and laggards that lead to the loss of some of the genetic material. Therefore, some electrophoretic bands could have disappeared due to the deletion of their corresponding genes El-Khallal and Mohamed (2004). On the other hand, appearance of new characteristic bands could be explained on the basis of mutational event at the regulatory system of unexpected gene(s) that activate it (Abedelsalam *et al*., 1993 & 1997; El-Nahas, 2000).

Changes in the band intensity could be interpreted as a result of certain mutational events that would have occurred in the regulator genes, which would lead to inhibition, attenuation or constitutive gene expression. Therefore, the corresponding bands become faint or become more intense. The recorded changes in band intensity could also be attributed to the cytological abnormalities induced by synthetic food colour E122. This conclusion is in accordance with Abdesalam *et al*. (1993). They concluded that the increase in band intensity could be due to gene duplication. Also, Gamal El-Din *et al*. (1988) noticed that an increase of band intensity is due to duplication of chromosomal complement in *Vicia faba.*

## SUMMARY

*Allium cepa* roots and seeds were used to study the interactive effects of

different concentrations of the synthetic food additive azroubine (carmoisine E 122) and vitamin C on mitotic indices, chromosomes behaviour, nucleic acids contents and protein synthesis. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used for the characterization of differential expression of proteins of all treatments with E 122.

The effect of this synthetic food colour on mitotic activity, induction of mitotic abnormalities, changes in DNA & RNA contents and changes in the $M_2$ seed storage protein banding patterns has been investigated using *Allium cepa* plant. The results were obtained indicated that E122 caused a reduction in mitotic index, relative changes in the frequency of mitotic phases and induced a wide range of chromosomal abnormalities in mitotic cell division. These changes were accompained by a great variation in nucleic acids content. Also the electrophoretic analysis showed that E122 has agreat ability to induce changes in the protein banding patterns in *Allium cepa* $M_2$ seed storage protein as compared with the control. Vitamin C is one of antimutagenic agents that could minimize the genotoxicity induced by E122.

From all the above mentioned results it may be concluded, that the genotoxicity of Azroubine is indicated by its capacity to produce chromosomal aberrations was confirmed by it's effect on nucleic acids content (DNA & RNA) and as well as on protein banding pattern. The results of the present investigation is recommended that the use of this food additive colour E122 should be limited in order to protect our health from its mutagenic effect and it is recommended for human to eat vegetables and fruits which contain a considerable amounts of vitamin C in order to be protected from food additive colours mutagenicity.

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Table (1): Total cells examined, total mitoses, percentage of mitotic phases, percentage of total abnormal mitotic phases, mean mitotic index after treating *Allium cepa* root tips for 3, 6, 12 and 24 hours with different concentrations of synthetic food additive colour E 122 and vitamin C administration for 3 hours.

| Time of treat. | Conc. gm/L | Total Cells exam. | Total mitoses | Prophase | | | | Metaphase | | | | Ana-telophase | | | | MI ± S.E |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Norm. | % of Normal Prophase | Abnor. | % of Abn. Prophase | Norm. | % of Normal Metaphase | Abnor. | % of No. Ab. Meta-phase | Norm. | % of Normal Ana- telo-phase | Abnor. | % of No. Ab. Ana-telophase | |
| 3 hrs. | Cont. | 11720 | 530 | 260 | 49.06 | - | - | 172 | 32.45 | 4 | 2.33 | 98 | 18.49 | 2 | 2.04 | 1.14 ± 0.05 |
| | 1.31 | 13105 | 482 | 182 | 37.76 | - | - | 195 | 40.46 | 41 | 21.03 | 105 | 21.78 | 10 | 9.52 | 10.93 ± 5.32 |
| | 2.63 | 13160 | 530 | 153 | 28.87 | - | - | 210 | 39.62 | 69 | 32.86 | 167 | 31.51 | 16 | 9.59 | 16.31 ± 1.30* |
| | 5.25 | 10750 | 340 | 100 | 29.41 | 10 | 10.00 | 160 | 47.06 | 152 | 95.00 | 80 | 23.53 | 25 | 31.25 | 55.64 ± 3.25** |
| | 10.50 | 9860 | 76 | 5 | 6.58 | 2 | 40.00 | 52 | 68.42 | 50 | 96.15 | 19 | 25.00 | 6 | 31.58 | 76.41 ± 2.93** |
| 6 hrs. | Cont. | 10206 | 560 | 296 | 52.86 | - | - | 126 | 22.50 | 5 | 3.97 | 138 | 24.64 | 3 | 2.17 | 1.43 ± 0.14 |
| | 0.66 | 10236 | 532 | 254 | 47.74 | - | - | 132 | 24.81 | 26 | 19.70 | 146 | 27.44 | 34 | 23.29 | 11.53 ± 1.09 |
| | 1.31 | 10215 | 367 | 128 | 34.88 | 2 | 1.56 | 128 | 34.88 | 63 | 49.22 | 120 | 32.70 | 36 | 30.00 | 27.52 ± 1.48* |
| | 2.63 | 9815 | 321 | 116 | 36.14 | 1 | 0.86 | 146 | 45.48 | 124 | 84.93 | 59 | 18.38 | 36 | 61.02 | 50.18 ± 0.82** |
| | 5.25 | 10027 | 32 | 4 | 12.50 | 3 | 75.00 | 18 | 56.25 | 18 | 100.00 | 10 | 31.25 | 8 | 80.00 | 90.63 ± 0.25** |
| 12 hrs. | Cont. | 10321 | 620 | 310 | 50.00 | - | - | 128 | 20.65 | 3 | 2.34 | 182 | 29.35 | 1 | 0.55 | 6.02 ± 0.27 |
| | 0.33 | 10036 | 403 | 172 | 42.68 | 4 | 2.33 | 92 | 22.83 | 21 | 22.83 | 139 | 34.49 | 18 | 12.95 | 4.08 ± 0.60 |
| | 0.66 | 10112 | 286 | 62 | 21.68 | 2 | 3.23 | 121 | 42.31 | 72 | 59.50 | 103 | 36.01 | 16 | 15.53 | 2.70 ± 0.02* |
| | 1.31 | 10760 | 305 | 63 | 20.66 | 3 | 4.76 | 137 | 44.92 | 123 | 89.78 | 105 | 34.43 | 39 | 37.14 | 2.84 ± 0.03* |
| | 2.625 | 10643 | 172 | 28 | 16.28 | 3 | 10.71 | 96 | 55.81 | 86 | 89.58 | 48 | 27.91 | 47 | 97.92 | 1.61 ± 0.03** |
| 24 hrs. | Cont. | 11235 | 632 | 285 | 45.09 | - | - | 165 | 26.11 | 4 | 2.42 | 182 | 28.80 | 2 | 1.10 | 5.63 ± 0.17 |
| | 0.164 | 10212 | 510 | 214 | 41.96 | 10 | 4.67 | 135 | 26.47 | 25 | 18.52 | 161 | 31.57 | 15 | 9.32 | 5.00 ± 0.19 |
| | 0.328 | 9975 | 305 | 43 | 14.10 | 9 | 20.93 | 154 | 50.49 | 46 | 29.87 | 108 | 35.41 | 27 | 25.00 | 3.05 ± 0.07** |
| | 0.656 | 9532 | 150 | 30 | 20.00 | 9 | 30.00 | 78 | 52.00 | 39 | 50.00 | 42 | 28.00 | 14 | 33.33 | 1.58 ± 0.01** |
| | 1.313 | 9415 | 23 | 1 | 4.35 | 1 | 100.00 | 20 | 86.96 | 20 | 100.00 | 2 | 8.70 | 2 | 100.00 | 0.25 ± 0.01** |
| 24 hrs. + 3 hrs. vitamin C. | Cont. | 10168 | 529 | 205 | 38.75 | - | - | 148 | 27.98 | 2 | 1.035 | 176 | 33.27 | - | - | 5.21 ± 0.12 |
| | 0.164 | 10215 | 517 | 181 | 35.01 | 5 | 2.76 | 162 | 31.33 | 29 | 14.81 | 174 | 33.66 | 6 | 3.45 | 5.06 ± 0.10 |
| | 0.328 | 9965 | 376 | 105 | 27.93 | 16 | 15.24 | 180 | 47.87 | 43 | 23.89 | 91 | 24.20 | 11 | 12.09 | 3.78 ± 0.03* |
| | 0.656 | 10215 | 254 | 57 | 22.44 | 37 | 64.91 | 137 | 53.94 | 51 | 37.23 | 60 | 23.62 | 9 | 15.00 | 2.49 ± 0.07** |
| | 1.313 | 9520 | 17 | - | - | - | - | 15 | 88.24 | 14 | 93.33 | 2 | 11.76 | 1 | 50.00 | 0.17 ± 0.08** |

* Significant from control at 0.05 level (t. test). ** Significant from control at 0.01 level (t. test).
Norm = Normal; Abnor = Abnormal; Cont. = Control; treat. = treatment; Conc. = Concentration; MI = mitotic index; SE = standard error.

Table (2): Frequencies of different types of metaphase and ana-telophase abnormalities and mean percentage of abnormal mitoses after treating *Allium cepa* root tips for 3, 6, 12 and 24 hours with different concentrations of synthetic food additive colour E 122 and vitamin C administration for 3 hours.

| Time of treatment | Conc. gm/L | % of metaphase abnormalities | | | | | % of ana-telophase abnormalities | | | | Mean % of abn ± SE mitoses |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Stick. | CM 2n | Star | Lag. | Dist. | Stick | Bridge | Lag. | Dist. | |
| 3 hrs. | Cont. | - | - | - | - | 2.33 | - | - | - | 2.04 | 1.14 ± 0.05 |
| | 1.313 | 4.10 | 4.10 | 0.52 | 2.05 | 10.26 | 1.90 | 5.72 | - | 1.90 | 10.93 ± 5.32 |
| | 2.625 | 17.14 | 12.38 | - | - | 2.86 | 4.79 | 1.80 | 0.60 | 2.40 | 16.31 ± 1.30* |
| | 5.250 | 80.00 | 15.00 | - | - | - | 12.50 | 15.00 | - | 3.75 | 55.64 ± 3.25** |
| | 10.500 | 92.30 | 3.85 | - | - | - | 26.32 | 5.26 | - | - | 76.41 ± 2.93** |
| 6 hrs. | Cont. | - | - | - | - | 3.97 | - | - | - | 2.17 | 1.43 ± 0.14 |
| | 0.656 | 1.52 | 3.03 | - | - | 15.15 | 2.75 | 10.27 | - | 10.27 | 11.53 ± 1.09 |
| | 1.313 | 7.81 | 21.88 | - | - | 19.53 | 2.50 | 13.33 | 0.83 | 13.33 | 27.52 ± 1.48* |
| | 2.625 | 71.92 | 6.85 | - | - | 6.16 | 42.37 | 8.47 | - | 10.18 | 50.18 ± 0.82** |
| | 5.250 | 88.89 | 11.11 | - | - | - | 60.00 | 20.00 | - | - | 90.63 ± 0.25** |
| 12 hrs. | Cont. | - | - | - | 2.34 | 2.34 | - | - | - | 0.55 | 0.87 ± 0.31 |
| | 0.328 | - | 6.52 | - | 22.83 | 16.31 | 1.44 | 1.44 | - | 10.07 | 11.69 ± 1.79 |
| | 0.656 | 18.18 | 20.66 | - | 59.50 | 20.66 | 5.82 | 9.71 | - | - | 31.54 ± 0.87** |
| | 1.313 | 71.53 | 10.95 | - | 89.78 | 7.30 | 28.57 | 7.62 | - | 0.95 | 54.11 ± 1.52** |
| | 2.625 | 68.74 | 10.42 | - | 89.58 | 10.42 | 72.92 | 20.83 | - | 4.17 | 79.44 ± 1.01** |
| 24 hrs. | Cont. | - | - | - | - | 2.42 | - | - | - | 1.10 | 0.92 ± 0.18 |
| | 0.164 | - | 5.19 | - | - | 13.33 | 1.86 | 2.48 | 0.64 | 4.34 | 10.03 ± 0.88* |
| | 0.328 | 7.14 | 9.74 | - | - | 12.99 | 8.33 | 9.26 | - | 7.41 | 28.80 ± 1.06** |
| | 0.656 | 35.90 | 12.82 | - | - | 1.28 | - | - | - | - | 44.67 ± 2.72** |
| | 1.313 | 90.00 | 10.00 | - | - | - | - | - | - | - | 100.00 ± 0.00** |
| 24 hrs. + 3 hrs. vitamin C. | Cont. | - | - | - | - | 0.69 | - | - | - | - | 0.30 ± 0.05 |
| | 0.164 | - | - | - | 0.51 | 17.35 | - | - | - | 0.87 | 6.76 ± 1.08 |
| | 0.328 | 1.72 | 2.59 | - | - | 32.76 | - | - | - | 4.88 | 17.41 ± 1.09* |
| | 0.656 | - | 7.23 | - | - | 32.34 | - | - | - | 2.13 | 14.78 ± 0.18** |
| | 1.313 | 54.27 | 25.21 | 0.58 | - | 24.36 | 9.70 | - | - | 15.15 | 64.54 ± 2.07** |

* Significant from control at 0.05 level (t. test). ** Significant from control at 0.01 level (t. test).
CM = Colchicine metaphase; Ana. = Anaphase; Lagg. = Laggard; Dist. = Disturbed; Cont = Control.

Table (3): Percentage of DNA and RNA content after treating *Allium cepa* root tips for 3, 6, 12 and 24 hours with different concentrations of food additive colour E 122 and vitamin C administration for 3 hours.

| Time | Treatment concentration in gm/L | DNA | | RNA | |
|---|---|---|---|---|---|
| | | mg/g | % Content | mg/g | % Content |
| 3 hrs. | Cont. | 3.38 | 100.00 | 12.57 | 100.00 |
| | 1.313 | 2.67 | 78.99 | 9.71 | 77.25 |
| | 2.625 | 1.96 | 57.99 | 7.43 | 59.11 |
| | 5.250 | 1.51 | 44.67 | 6.29 | 50.04 |
| | 10.500 | 1.48 | 43.79 | 6.25 | 49.72 |
| 6 hrs. | Cont. | 3.29 | 100.00 | 12.00 | 100.00 |
| | 0.656 | 2.31 | 70.22 | 10.29 | 85.75 |
| | 1.313 | 1.87 | 56.84 | 8.50 | 70.83 |
| | 2.625 | 1.60 | 48.63 | 5.71 | 47.58 |
| | 5.250 | 1.49 | 45.29 | 5.70 | 47.50 |
| 12 hrs. | Cont. | 3.02 | 100.00 | 10.29 | 100.00 |
| | 0.328 | 2.22 | 73.51 | 9.71 | 94.36 |
| | 0.656 | 1.96 | 64.90 | 7.43 | 72.21 |
| | 1.313 | 0.98 | 32.45 | 3.43 | 33.33 |
| | 2.625 | 1.02 | 33.77 | 3.37 | 32.75 |
| 24 hrs. | Cont. | 3.29 | 100.00 | 11.43 | 100.00 |
| | 0.164 | 2.22 | 67.48 | 10.29 | 90.03 |
| | 0.328 | 1.96 | 59.57 | 7.43 | 65.00 |
| | 0.656 | 1.24 | 37.69 | 4.00 | 35.00 |
| | 1.313 | 1.22 | 37.08 | 3.52 | 30.80 |
| 24 hrs. + 3 hrs. vitamin C | Cont. | 3.02 | 100.00 | 14.86 | 100.00 |
| | 0.164 | 2.40 | 79.47 | 9.71 | 65.34 |
| | 0.328 | 2.31 | 76.49 | 8.57 | 57.67 |
| | 0.656 | 1.33 | 44.04 | 5.71 | 38.43 |
| | 1.313 | 1.32 | 43.71 | 3.40 | 22.88 |

Table (4): Effects of the synthetic food additive colour E122 and vitamin C on the protein banding pattern of *Allium cepa* seeds using SDS - PAGE technique.

| Band No. | M.wt (KDa) | Band % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Marker | Control | E122 | | | | E122 + Vitamin C | | | |
| | | | | Lane 1 | Lane 2 | Lane 3 | Lane 4 | Lane 5 | Lane 6 | Lane 7 | Lane 8 |
| 1 | 75 | | 5.41 | 5.39 | 5.36 | 5.27 | 5.47 | | | | |
| 2 | 74 | 9.09 | 4.89 | 5.11 | 4.28 | 4.26 | 2.55 | 10.63 | 5.41 | 4.48 | 9.93 |
| 3 | 65 | | 2.40 | | | | | 4.88 | 4.04 | 4.13 | 4.94 |
| 4 | 57 | 8.73 | 2.91 | 4.10 | 3.87 | 4.24 | 3.08 | 4.68 | | | |
| 5 | 54 | | 9.37 | 10.32 | 9.93 | 10.98 | 11.52 | 11.18 | 10.03 | 9.83 | 9.90 |
| 6 | 47 | | 7.70 | 9.75 | 9.08 | 7.94 | 7.41 | 7.60 | 7.55 | 6.36 | 9.09 |
| 7 | 45 | 21.60 | 2.60 | 2.86 | 3.13 | 3.33 | 10.77 | 4.00 | 2.51 | 3.10 | 2.70 |
| 8 | 43 | 2.61 | | | | | | | | 2.51 | 2.41 |
| 9 | 38 | | 4.37 | 4.47 | 4.69 | 11.94 | 5.03 | 6.92 | 4.15 | 3.82 | 4.01 |
| 10 | 35 | | 6.55 | 7.12 | 7.77 | 5.67 | 7.97 | 5.01 | 6.78 | 8.43 | 5.73 |
| 11 | 33 | | | | | | | 3.94 | | | |
| 12 | 31 | | | | | | | | | | 3.31 |
| 13 | 29 | | 4.31 | 5.84 | 5.38 | 3.50 | 5.65 | 3.11 | 4.97 | 5.23 | 5.63 |
| 14 | 25 | 20.48 | 8.31 | | 2.75 | 8.70 | 0.97 | 9.29 | | | |
| 15 | 23 | | 8.81 | | | | | | | | |
| 16 | 22 | | 5.81 | 4.29 | | 1.00 | | | 4.73 | 5.19 | 6.32 |
| 17 | 20 | | 15.01 | 11.20 | 10.58 | 8.03 | 9.94 | 7.81 | 14.05 | 13.23 | 5.60 |
| 18 | 14 | | 5.21 | 5.09 | 5.34 | | 4.83 | 2.02 | 5.29 | 5.07 | 5.93 |
| 19 | 8 | | 19.43 | 12.65 | 17.09 | 14.66 | 17.00 | 8.06 | 17.13 | 14.93 | 15.38 |
| Total No. of Bands | | | 16 | 13 | 13 | 13 | 13 | 14 | 12 | 13 | 14 |

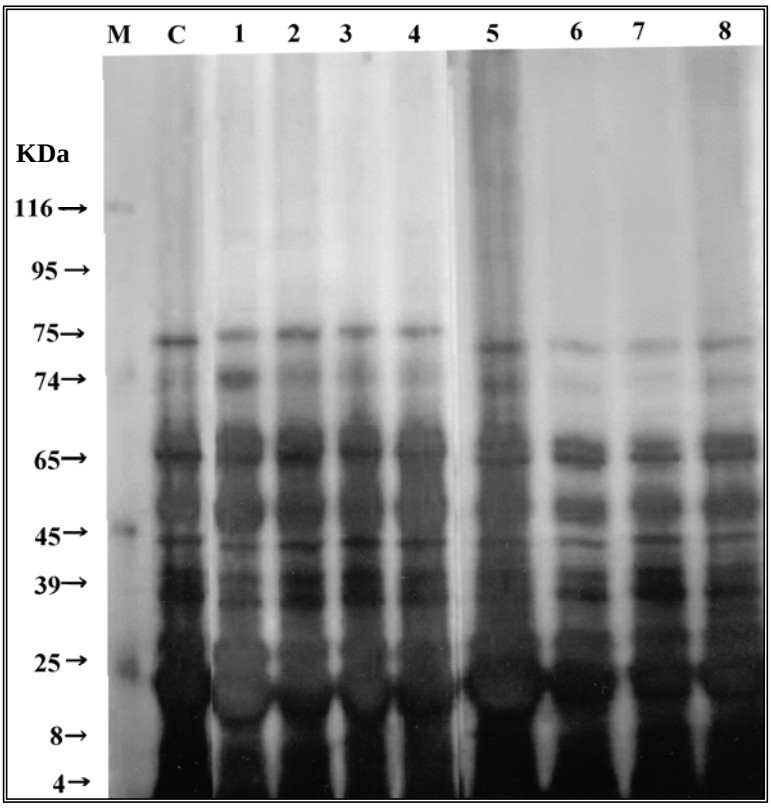


Fig (1): Electrophotograph produced by SDS-PAGE analysis of protein pattern of *Allium cepa* seed after treatment with E122 and vitamin C.

M = Marker
C = Control

Lane 1: Conc. 0.164 gm/L of E122 for 24hrs.
Lane 2: Conc.0.328 gm/L of E122 for 24hrs.
Lane 3: Conc.0.656 gm/L of E122 for 24hrs.
Lane 4: Conc.1.313 gm/L of E122 for 24hrs.
Lane 5: Conc.0.164 gm/L of E122 for 24hrs. + Treatment with 100 mg/L of vitamin C for 3 hrs.
Lane 6: Conc.0.328 gm/L of E122 for 24hrs + Treatment with 100 mg/L of vitamin C for 3 hrs.
Lane 7: Conc. 0.656 gm/L of E122 for 24hrs + Treatment with 100 mg/L of vitamin C for 3 hrs
Lane 8: Conc.1.313 gm/L of E122 for 24hrs + Treatment with 100 mg/L of vitamin C for 3 hrs.