

# **RESULTS**

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**I. Effect of short-term accelerated ageing (0,7,14 and 21 days) and long-term accelerated ageing (0 and 112 days) of gram, soybean, sunflower and safflower seeds, pretreated with Na-dikegulac on biochemical changes and TTC (2,3,5-triphenyl tetrazolium chloride) stainability of seeds.**

### **Effect of short-term accelerated ageing on seed metabolism**

A short-term accelerated ageing for three weeks was imposed on gram, soybean, sunflower and safflower seeds prior to seed pretreatment with 0, 1000, 2000 and 4000 µg/ml Na-dikegulac. Metabolic status of the seed lots was evaluated periodically by analysing a number of biochemical parameters which undergo changes along with the process of seed deterioration. Data represented in Tables 1 to 8. Visual observation showed that at least up to 21 days of seed ageing seed lots were not apparently attacked by microorganisms. Under microscopic observation, a few fungal species were found to appear on seed surface of sunflower and safflower seed lots only. However, in no case the incidence of fungal attack was at all severe.

**Effect on leaching of soluble carbohydrate (Table 1) :** From all the seed samples, soluble carbohydrate leached out and the magnitude of leaching showed positive correlation with the period of accelerated ageing. Data showed that at the 0-day after ageing Na-dikegulac at its highest concentration significantly increased leaching but during subsequent analyses the pretreating chemical, regardless of its concentrations, checked the higher leakage of sugars in all the cultivars. Results of final observation period showed marked inhibition of leaching of sugar from the chemical-pretreated seed samples over water-soaked ones.

**Effect on leaching of amino acids (Table 2) :** Accumulation of amino acids in seed leachates went on increasing in water-soaked seed samples of all the crop seeds with the advancement of ageing duration. The same trend was apparent in case of the chemical-

pretreated seed samples but here the rate of increase was considerably slowed down at all the concentrations. Like the changes of soluble carbohydrate, the chemical initially tended to increase the amino acid level at the 0-day after ageing treatment.

**Effect on protein content (Table 3) :** Unlike the changes of leakage products, protein content of seed kernels gradually declined keeping pace with the days of accelerated ageing both in water-soaked and Na-dikegulac pretreated seed samples of all the four cultivars. But the pretreating chemical at all its concentrations helped to maintain the protein level to a considerable extent at later observation periods. Data showed that out of the four sampling periods, initially the chemical reduced the protein level over control values; at the second sampling period the adverse effects were erased and during the two subsequent sampling periods the chemical partially averted the adverse effects as protein level was found higher in dikegulac-pres soaked samples than in control ones.

**Effect on RNA content (Table 4) :** Overall changing pattern of RNA level followed an identical trend with that of protein. However, the changes noted at 0-day after accelerated ageing were insignificant in all the seed samples. Na-dikegulac substantially alleviated the speed of fall of RNA level with the progress of ageing duration. After 21 days of accelerated ageing treatment, Na-dikegulac at all its concentrations showed higher levels of RNA and the effect was statistically significant in all the cases.

**Effect on internal soluble carbohydrate content (Table 5) :** With the progress of ageing, soluble carbohydrate level in the kernels of the four crop seeds increased up to 14 days after accelerated ageing which was followed by a decrease after 21 days. In the chemical-pretreated seeds the same trend was apparent except that it occurred at a much slower pace. Unlike the changes of protein and RNA levels, Na-dikegulac initially increased the level of internal soluble carbohydrate over control values in all the seed samples analysed.

**Effect on insoluble carbohydrate content (Table 6) :** So far the overall changes of insoluble carbohydrate level is concerned, a clear reverse picture was noted with the changes of soluble carbohydrate level. Thus, when observation was made after 14 and 21

days after accelerated ageing of seeds, a significant increase of insoluble carbohydrate level was recorded in the chemical pretreated seed samples. All the concentrations of Na-dikegulac were found almost equally efficient in averting the loss of insoluble carbohydrate content to a considerable extent.

**Effect on percent TTC-stained seeds (Table 7) :** Initial percentage of TTC-stained seeds of gram, soybean, sunflower and safflower was found 100, 90, 80 and 85 respectively. This started declining from 7 days after accelerated ageing in case of soybean, sunflower and safflower and from 14 days after ageing in case of gram seeds. At the final observation period of 21 days after seed ageing, percent TTC-stained seeds in water-soaked samples was reduced to 40, 40, 50 and 36 in case of gram, soybean, sunflower and safflower cultivars respectively. This reduction was substantially checked in seeds which were treated with Na-dikegulac. And the chemical-induced effect on the maintenance of TTC stainability of the seed species was found highly significant at the final observation period.

**Effect on total dehydrogenase activity (Table 8) :** Activity of dehydrogenase enzyme progressively declined with the advancement of accelerated ageing days both in control and in chemical pretreated seed samples regardless of cultivars. But the speed of fall of the enzyme activity was found to occur slowly in the latter cases. Although the changes noted at the initial observation period were statistically insignificant in all the crop seeds, Na-dikegulac was found to maintain relatively higher dehydrogenase activity beyond 7 days of seed ageing.

#### **Effect of long-term accelerated ageing on seed metabolism**

A few biochemical parameters viz., soluble and insoluble carbohydrates, free amino acids, protein and RNA contents and total dehydrogenase activity in conjunction with TTC-staining pattern of gram, soybean, sunflower and safflower seeds were analysed after 0 and 112 days of accelerated ageing. Results on the effect of forced ageing treatment on seed metabolism and its alteration after seed pretreatment with Na-dikegulac have been incorporated in Figures 2 to 9. Visual observation revealed that

unlike short-term accelerated ageing all the seed samples were attacked by storage fungi of different species and incidence of pathogenic attack was found severe in sunflower and safflower seed lots.

**Effect on leachable carbohydrate (Fig. 2) :** Leaching of soluble carbohydrates from the seeds of gram, soybean, sunflower and safflower increased from 6 to 8 times of the initial values when data were recorded after 112 days of accelerated ageing. However, this increase of sugar leakage was remarkably arrested in the seed samples which underwent presoaking with Na-dikegulac. Such relieving action was noted in all the cases irrespective of seed types and concentrations of the chemical used.

**Effect on leachable amino acid (Fig. 3) :** Concomitant with the rise of soluble carbohydrate level, amino acid contents increased to a great extent in the leachates of all the seed samples and this deleterious leakage was remarkably checked by seed pretreatment with Na-dikegulac. All the concentrations of the chemical were found more or less equally efficient in this regard. It was further noted that at the initial observation period of 0-day after seed ageing, the pretreating chemical tended to increase the leaching of amino acids. However, such effect seemed to be insignificant or least significant.

**Effect on protein content (Fig. 4) :** In contrast to the changes of soluble carbohydrate and amino acid, protein level in seed kernels of the four cultivars remarkably declined in control seed samples after 112 days of forced ageing treatment. The magnitude of fall of protein was, however, substantially arrested in the chemical pretreated seed samples, although at the initial period of analysis the chemical effect seemed to be adverse as evident from Na-dikegulac-induced significant reduction of protein level.

**Effect on RNA content (Fig. 5) :** As regards the changes of RNA level in seed kernels, almost an identical trend with that of protein was noted. Data clearly showed that RNA content markedly declined both in the chemical-pretreated and untreated seed samples after 112 days of accelerated ageing, but the declining drift seemed to be significantly slowed down in seed samples which were subjected to pretreatment with Na-dikegulac. Here also, immediate effect of the pretreating chemical seemed to be inhibitory.

**Effect on internal soluble carbohydrate content (Fig. 6) :** Internal soluble carbohydrate content in seed kernels was greatly augmented by 112 days of accelerated ageing period in all the crop seed types. Na-dikegulac at all its concentrations efficiently retarded the rapid rise of soluble carbohydrate, and the two higher concentrations seemed to be more promising in this retardation action as evident from lower level of soluble carbohydrate in seeds pretreated with 2000 and 4000 µg/ml Na-dikegulac.

**Effect on insoluble carbohydrate content (Fig. 7) :** As opposed to the changes of internal soluble carbohydrate level, a drastic fall of insoluble carbohydrate content was recorded when seeds experienced prolonged accelerated ageing for 112 days. Although the pretreating chemical, irrespective of its concentrations, initially lowered the carbohydrate levels over control values, it substantially maintained the level at all the concentrations of Na-dikegulac in all the seed species analysed after 112 days of forced ageing treatment.

**Effect on percentage TTC-stained seeds (Fig. 8) :** A drastic fall of TTC stainability was recorded in all the seed species when they experienced prolonged accelerated ageing for 112 days. In the chemical pretreated seed samples parallel reduction of TTC stainability was found but comparatively higher percentage of TTC-stained seeds was recorded than in the control samples.

**Effect on total dehydrogenase activity (Fig. 9) :** Activity of total dehydrogenases sharply declined after 112 days of ageing. But the extent of massive fall was relieved in dikegulac-pretreated seed samples regardless of the cultivars. The higher concentration of 2000 µg/ml seemed to be most successful in this regard at least in gram, soybean and sunflower seeds. The chemical, however, failed to show any immediate effect as the changes of the enzyme activity recorded at preageing analysis (0-day) were found insignificant.

## **II. Effect of accelerated ageing (10, 20, 30 and 40 days) of sunflower seeds, pretreated with Na-dikegulac on germination behaviour, seedling growth and biochemical changes in seeds.**

**Effect on seed germination and field emergence (Table 9) :** It is evident from Table 9 that the normal germination and field emergence per cent of the tested seed lots of sunflower were 90% and 85.6% respectively, and the values of both the parameters declined steadily with the progress of the days of accelerated ageing. This declining drift was found to be most pronounced in water-soaked seeds. Data showed that Na-dikegulac, irrespective of its concentrations, arrested the fall in germination and field emergence, particularly in case of seeds aged beyond 30 days.

**Effect on the speed of seed germination (Table 10) :** Data were recorded from the seed samples which had undergone accelerated ageing for 10, 20, 30 and 40 days but in this table the data of 0 and 30 days seed ageing samples were inserted. Speed of seed germination was found to reduce drastically in control seed samples which underwent accelerated ageing for 30 days. The same trend of reduction of germination percentage was recorded in Na-dikegulac pretreated seed samples but the magnitude of reduction was found much less than control samples. The effect of the pretreating chemical on partial alleviation of rapid fall of seed germination was found more significant at later observation periods. However, at the initial observation period of 24 hours after seed soaking the germination percentage was lower at all the concentrations of the pretreating chemical at 0-day of accelerated ageing and a reverse picture was found when data were recorded from accelerated aged seeds for 30 days.

**Effect on root length, shoot length and seedling dry weight (Table 11) :** Data showed that root length, shoot length and seedling dry weight decreased progressively in seedlings raised from water-soaked seeds with the advancement of ageing duration. Na-dikegulac, irrespective of its concentrations significantly increased root length, shoot-length as well as seedling dry weight. The effect of Na-dikegulac on alleviation of such deleterious effect was found more significant at later observation periods.

**Effect on DNA and RNA contents (Table 12) :** Both DNA and RNA levels in cotyledons of seeds declined keeping pace with the days of accelerated ageing in both treated and control samples. This ageing-induced declining rate was considerably slowed down in the chemical pretreated seed samples regardless of its concentrations used.

**Effect on soluble carbohydrate and amino acid (Table 13) :** Soluble carbohydrate content in the cotyledons of water-soaked seeds was found to increase steadily up to 30 days of accelerated ageing and after 40 days of seed ageing the level declined. In Na-dikegulac pretreated seed samples the same trend was recorded except that it occurred at a much slower pace. However, the pretreating chemical initially stimulated the increasing of the level of soluble sugar. Like the changes of soluble carbohydrate, amino acid level also increased with ageing duration but unlike the changes of soluble carbohydrate level this increase was found throughout the observation periods. Here also Na-dikegulac at all its concentrations checked the alarming increase of amino acids through seed membrane.

**Effect on dehydrogenase and amylase enzymes (Table 14) :** Activity of the enzyme dehydrogenase progressively declined with the advancement of accelerated ageing days both in the chemical pretreated and in distilled water treated seeds samples. However, the rate of decreasing in activity was found to occur slowly in seeds which received pretreatment with Na-dikegulac. On the other hand, amylase activity in the cotyledons of water-soaked seeds enhanced up to 30 days of accelerated ageing and then declined sharply. Na-dikegulac, regardless of its concentrations, was found to reduce such abrupt changes in the activity of this enzyme.

**Effect on catalase and protease enzymes (Table 15 ) :** Activity of catalase enzyme declined with the advancement of accelerated ageing duration both in control and in the chemical pretreated seed samples. Na-dikegulac was found to arrest the rapid loss in the activity of this enzyme. On the other hand, activity of the catabolic enzyme protease increased gradually with ageing duration and the chemical-induced amelioration of the enhanced increase in the activity of this enzyme was apparent at all the concentrations.

### **III. Effect of accelerated ageing (0 and 30 days) of sunflower seeds, pretreated with Na-dikegulac on changes in growth and metabolism of plants at three developmental stages and their impact on crop yield.**

**Effect on plant height and stem circumference (Table 16) :** Results showed that height was remarkably reduced in sunflower plants at all its developmental stages when the plants were raised from accelerated aged seeds for 30 days. Na-dikegulac, irrespective of its concentrations, checked the reduction of plant height when compared with the control plants. Concomitantly, the pretreating chemical also checked ageing-induced strong retardation of stem circumference and such effect of Na-dikegulac was found true for all the three developmental stages.

**Effect on leaf area and plant dry weight (Table 17) :** Accelerated ageing-induced decrease in leaf area and dry weight of sunflower plants was overcome to a significant extent in plants which were raised from the chemical pretreated seeds. The effect of the chemical was found to be more significant at later periods of observation.

**Effect on chlorophyll and protein levels (Table 18 ) :** A drastic reduction of chlorophyll and protein levels were found in leaves of plants developed from accelerated aged seeds for 30 days. The same trend of loss of chlorophyll and protein levels was recorded in the leaves of plants raised from accelerated aged seeds which underwent prior seed pretreatment with 1000,2000 and 4000 µg/ml Na-dikegulac. But the loss was not found as severe as in control sample. And this alleviation effect of Na-dikegulac on retardation of chlorophyll and protein levels was recorded at least up to head developmental stage.

**Effect on RNA and soluble carbohydrate levels (Table 19) :** The chemical was also found to partially retain the levels of RNA and soluble carbohydrates in leaves in spite of experiencing accelerated ageing treatment of seeds. And this is evident from the higher levels of the biochemical parameters in the plants, raised from the chemical pretreated seeds.

**Effect on the activities of catalase and superoxide dismutase enzymes (Table 20) :**

Both catalase and superoxide dismutase enzymes increased with the advancement of plant age and a remarkable and proportional reduction in the activities of these two enzymes was recorded in leaves of plants which were developed from accelerated aged seeds. Seed pretreatment with Na-dikegulac partially arrested the loss in the activities of both the enzymes.

**Effect on the activities of IAA-oxidase and protease enzymes (Table 21) :**

Unlike the accelerated ageing-induced impairment in the activities of catalase and superoxide dismutase enzymes, IAA-oxidase and protease enzymes increased in leaves of plants raised from accelerated aged control seeds. The pretreating chemical was found to retard efficiently the enhanced level of these two enzymes.

**Effect on days to attain various developmental stages (Table 22) :**

Results clearly showed that normal growth and development of sunflower plant was delayed when plants were raised from accelerated aged seeds thus delaying the life cycle as a whole. Delaying of all the important developmental stages of sunflower (viz., radicle emergence, leaf emergence, head initiation, ray floret opening, 50% floret opening, 100% floret opening, head yellowing and harvest) recorded in this investigation was relieved considerably by seed pretreatment with Na-dikegulac.

**Effect on yield attributes (Table 23) :**

Data revealed that yield attributes like head diameter, seed weight, filled seed percentage, and 1000 seed weight were adversely affected when plants were raised from accelerated aged seeds and these cumulatively resulted in strong impairment of crop yield. Pretreatment of seeds with Na-dikegulac caused partial alleviation of these adverse effects thus resulting in a substantial increase of crop yield in comparison to control samples. However, percentage seed oil was not affected either by accelerated ageing or by chemical treatments as the changes recorded failed to attain statistical significance.

#### **IV. Effect of accelerated ageing (0 and 30 days) of sunflower seeds, pretreated with Na-dikegulac and PEG-induced water stress treatment on germination behaviour, seedling growth and metabolism of plants.**

**Effect on percentage germination and T50 of germination (Table 24) :** Percentage seed germination was highly reduced in control sample as a result of accelerated ageing treatment and such reduction was further aggravated when accelerated aged seeds were imposed water deficit stress treatment using PEG 6000. The pretreating chemical, irrespective of its concentrations, was found to overcome the deleterious effects of accelerated ageing and water stress as evident from comparatively higher germination percentage in the chemical pretreated seed lots which underwent stress and/or accelerated ageing treatment. As regards T50 of seed germination it is clearly found that in both the stressed and nonstressed seed samples which experienced forced ageing treatment, 50% seed germination did not occur at all. In the chemical pretreated seed lots, although high T50 hours were recorded, 50% seed germination did occur and thus T50 values were obtained even after accelerated ageing and stress treatment.

**Effect on height and dry matter content of seedlings (Table 25) :** Na-dikegulac-induced beneficial effect on germination behaviour under stressful condition was reflected in seedling growth as evident from the comparatively higher stature of seedlings as well as increased level of dry matter of the seedlings which were raised from seeds, pretreated with Na-dikegulac. The ageing-as well as stress-induced strong adverse effects were at least partially ameliorated by the pretreating chemical irrespective of its concentrations.

**Effect on chlorophyll and protein levels (Table 26) :** The changes of germination behaviour and seedling growth were associated with a proportional shift in seedling metabolism. Accelerated ageing-induced impairment of chlorophyll and protein and stress-induced further loss of the macromolecules were found to overcome to a significant extent when sunflower seeds received pretreatment with Na-dikegulac prior to forced ageing and stress treatment.

**Effect on catalase and superoxide dismutase activities (Table 27) :** The pretreating chemical, irrespective of its concentrations, arrested the loss in the activities of both catalase and superoxide dismutase enzymes in leaves of seedlings as a result of forced ageing treatment on seeds and stress treatment on seedlings.