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Effect of some standard and prospective growth regulators on sunflower callus. II. Changes in protein, RNA, DNA and carbohydrate content

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ABSTRACT

An investigation of the effect of chlorinated β-phenyl-3-indolyl-propiophenone derivatives on the protein, nucleic acid and carbohydrate content of sunflower callus was carried out. Protein content of callus grown on the control medium and media 1 and 4 remained relatively constant during the incubation period of 90 days. However, protein content increased significantly with increasing time of culture in other media depending on the type of chemical compound used. The pattern of nucleic acid changes was similar to that of protein. Carbohydrate content decreased in the callus grown on all media.

It is concluded that the chemical compound β-(*P*-chlorophenyl)-β-(3-indolyl)-*P*-chloropropiophenone (compound H) may be used as a growth regulator to replace kinetin.

INTRODUCTION

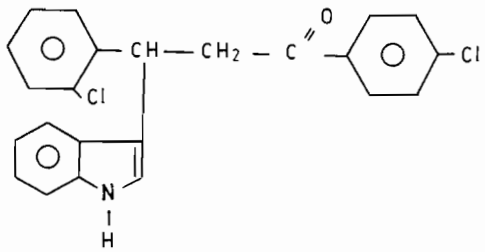
Callus growth is normally measured by the changes in fresh weight, dry weight, cell number and content of cellular protein and nucleic acid during the growth period (Street 1978). Growth regulators in general affect greatly protein and nucleic acid synthesis in tissue culture (Moore 1979). Yajima *et al.* (1980) showed that 2,4-D enhanced DNA and protein synthesis during callus induction in Jerusalem artichoke tuber tissue.

Mohammad & Hassan (1988) found that some chlorinated and/or methylated derivatives of β-phenyl-indolyl-propiophenone may replace auxin or kinetin in the initiation and growth of sunflower callus. Moreover, five of the tested chemical compounds were shown to affect callus initiation in sunflower plants in a manner similar to that of kinetin or 2,4-D. The main purpose of this paper is to provide further support for the use of these bioactive derivatives of β-phenyl-indolyl-propiophenone as new growth regulators. This is done by measuring their effects on protein, nucleic acid and carbohydrate content of sunflower callus in comparison with the responses to the standard growth regulators 2,4-D and kinetin.

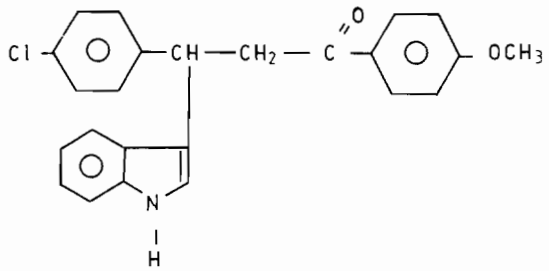
MATERIALS AND METHODS

Plant material and the methods relating to callus initiation and growth are the same as those described by Mohammad & Hassan (1988). Cultures were initiated and grown on Murashige & Skoog (1962) medium containing 10^{-3} M of 2,4-D + 10^{-7} M kinetin (control medium). The callus produced was compared with that on Murashige & Skoog medium containing 10^{-7} M of compound D (medium 1), compound F (medium 2), compound H (medium 3) and compound J (medium 4). The structures of these compounds are given in Fig. 1. The callus developed after 30, 60 and 90 days on each medium was used for protein, nucleic acid and carbohydrate content determination.

Protein was determined with Folin–phenol reagent (Lowry *et al.* 1951). The precipitated protein was solubilized in 1 N NaOH. A standard curve was obtained with bovine serum albumin. Nucleic acids (RNA and DNA) content was determined by a method based on the procedures used by Hutchinson & Munro (1961), Cherry (1962) and Walton & Soofi (1969) as developed by Mohammad & Al-Mashhadani (1976). DNA content was determined with diphenylamine reagent (Giles & Mayer 1965). RNA was determined by the absorption difference at 260 nm and 290 nm.



β-(*O*-phenyl)-β-(3-indolyl)-*P*-chloropropiophenone
(compound D)



β-(*P*-chlorophenyl)-β-(3-indolyl)-*P*-methoxypropiophenone
(compound F)

Fig. 1. Chemical structure of β-phenyl-3-indolyl-propiophenone derivatives. The IR and NMR spectra of these compounds are given in Mohammad & Hassan (1988).

Standard curves were obtained with yeast RNA and calf thymus DNA. Carbohydrates (soluble and insoluble) were extracted and quantitatively determined by the method used by Rutherford & Weston (1968). A standard curve was obtained with similarly treated 1:1 glucose and fructose mixture.

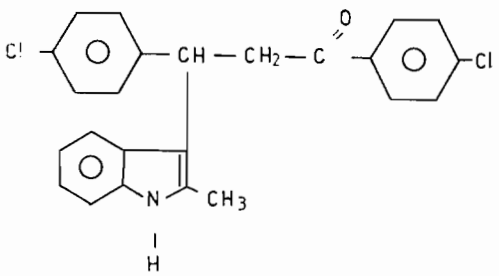
RESULTS

PROTEIN

Protein content of the callus grown on the control medium and media 1 and 4, respectively, remained constant over the period of 90 days (Fig. 2). In contrast, the protein content of callus grown on media 2 and 3 increased markedly during the same period.

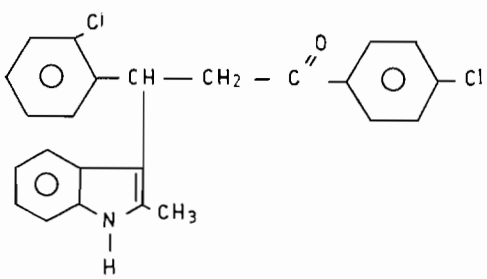
NUCLEIC ACIDS

RNA content in callus grown on the control medium and media 1, 2 and 4 showed no significant increase during the incubation period of 90 days (Fig. 3). However, RNA content of callus grown on medium 3 increased significantly during the same



β-(*P*-chlorophenyl)-β-(3-indolyl)-*P*-chloropropiophenone

(compound H)



β-chlorophenyl-β-(3-indolyl)-*P*-chloropropiophenone

(compound J)

Figure 1 (*continued*)

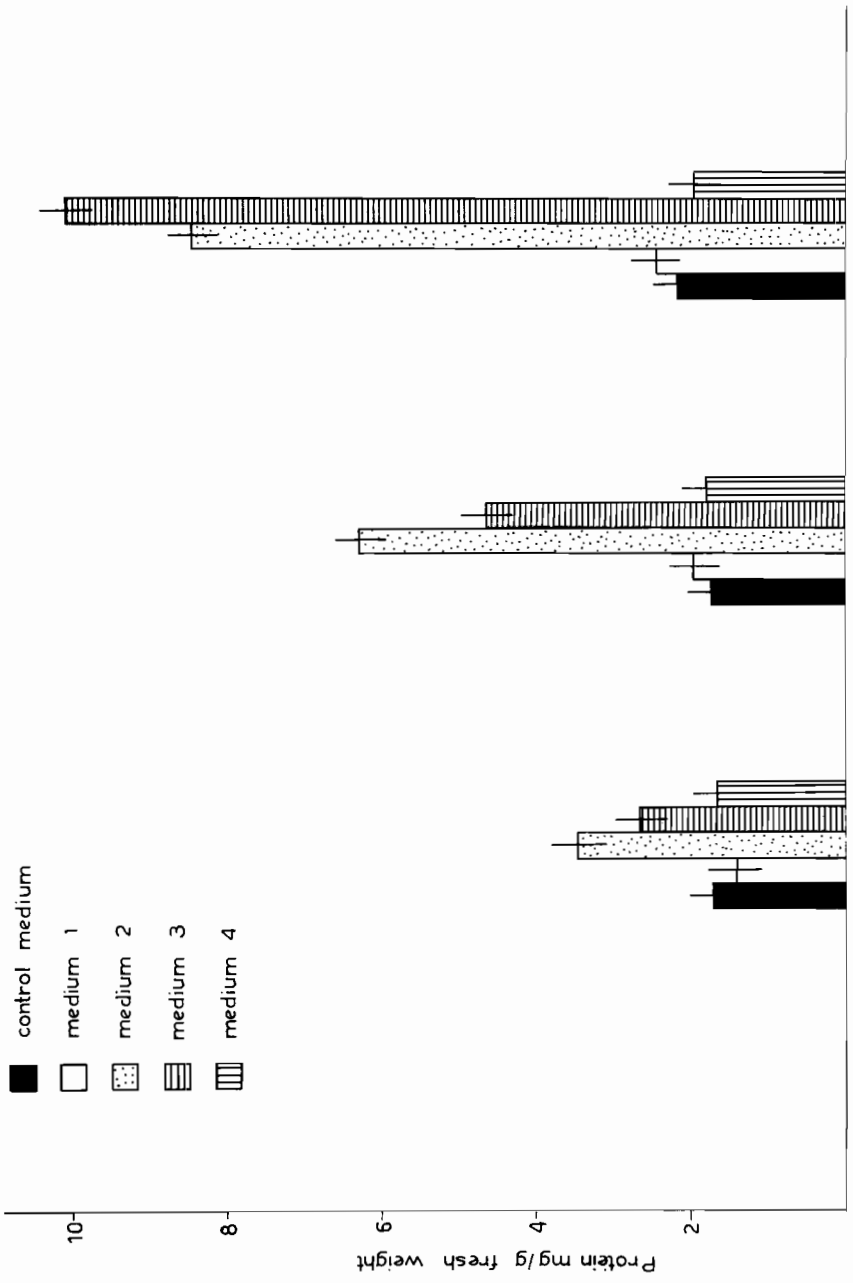


Fig. 2. Protein content of sunflower callus grown for 30, 60 and 90 days on different media. Each value represents the mean of 5 replicates. Vertical lines indicate the SE of the means. All media contain 10^{-3} M of 2,4-D, in addition to 10^{-7} M of kinetin control medium), compound D (medium 1), compound F (medium 2), compound H (medium 3) or compound J (medium 4).

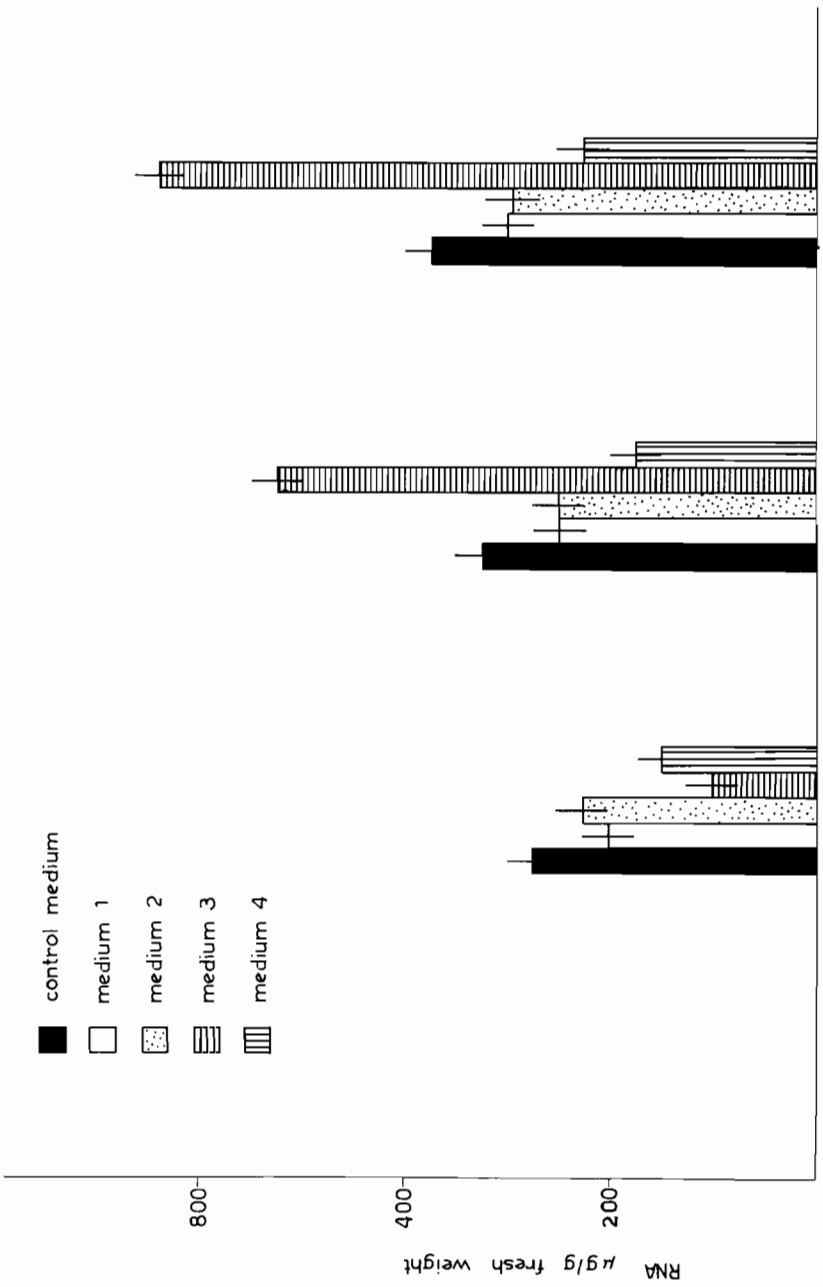


Fig. 3. Changes in RNA content of sunflower callus grown for 30, 60 and 90 days on different media. Each value represents the mean of 5 replicates. Vertical lines indicate the SE of the means. All media contain 10^{-3} M of 2,4-D, in addition to 10^{-7} M of kinetin (control medium), compound D (medium 1), compound F (medium 2), compound H (medium 3) or compound J (medium 4).

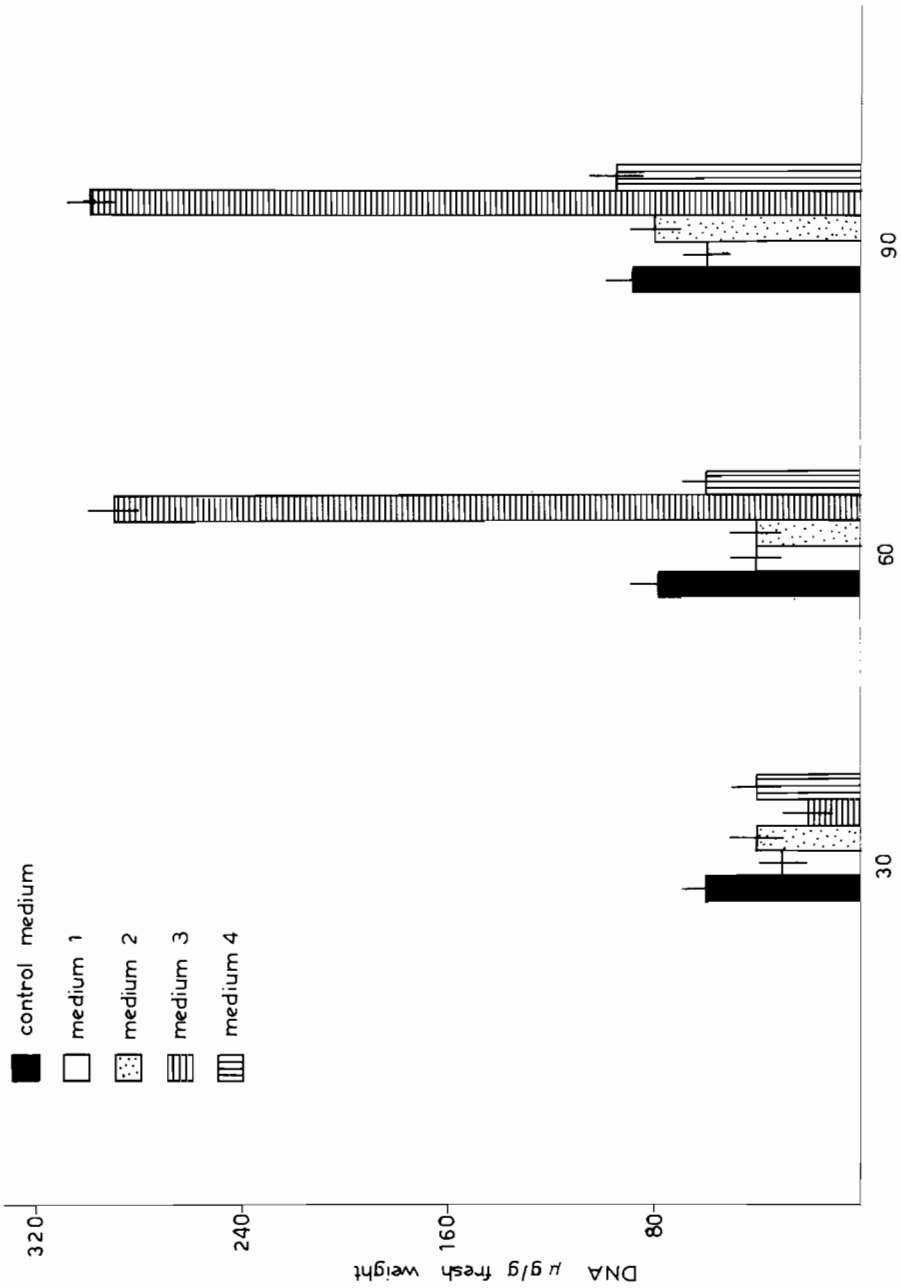


Fig. 4. Changes in DNA content of sunflower callus with time. Each value represents the mean of 5 replicates. Vertical lines indicate the SE of the mean. All media contain 10^{-3} M of 2,4-D, in addition to 10^{-7} M of kinetin (control medium), compound D (medium 1), compound F (medium 2), compound H (medium 3) or compound J (medium 4).

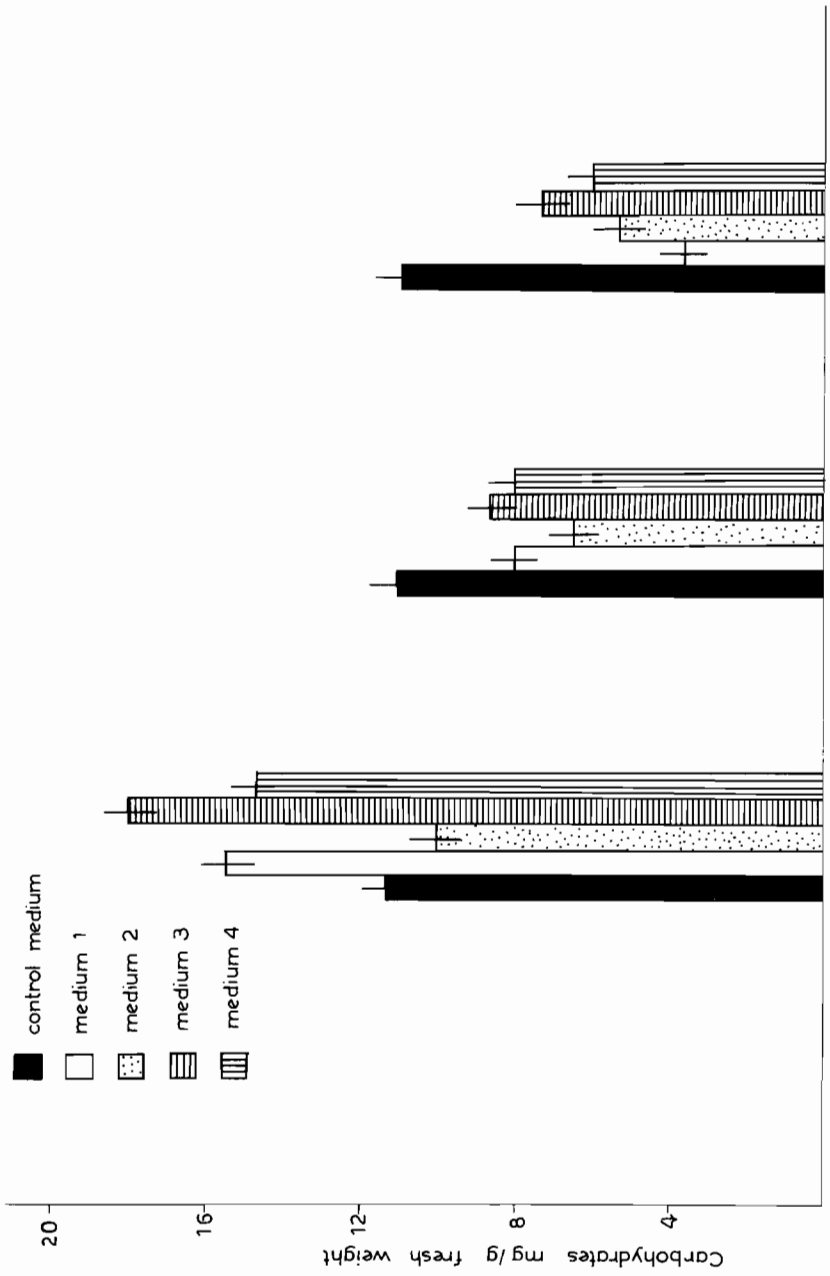


Fig. 5. Changes in soluble and insoluble carbohydrate content of sunflower callus on different media. Each value represents the mean of 5 replicates. Vertical lines indicate the SE of the mean. All media contain 10^{-3} M of 2,4-D, in addition to 10^{-7} M kinetin (control medium), compound D (medium 1), compound F (medium 2), compound H (medium 3) or compound J (medium 4).

period. Moreover, after 90 days the RNA content of callus grown on medium 3 was more than six times that produced after 30 days.

DNA content of callus grown on the control medium, media 1 and 2 increased insignificantly as the incubation period was lengthened (Fig. 4). However, the increase was gradual in medium 4. In contrast, DNA content of callus grown on medium 3 increased substantially between 30 and 60 days of incubation.

CARBOHYDRATES

The content of soluble and insoluble carbohydrates in callus grown on different media is given in Fig. 5. In the control medium, the carbohydrate content remained relatively constant during the incubation period of 90 days. In contrast, the carbohydrate content of the callus grown on the other media decreased significantly over the incubation period.

DISCUSSION

Growth and division of callus cells, in general, is accompanied by a marked increase in different cell constituents such as protein, carbohydrate and amino acids (Robertson 1966) as is the case of tissue cells of plant parts (Mohammad & Yousif 1980). The increase in fresh and dry weights of callus tissue (Mohammad & Hassan 1988) must be associated with an increase in the various constituents which leads to the division and differentiation of these cells (Mohammad & Al-Mashhadani 1976). Moreover, the synthesis of cell constituents requires energy which can be released by respiration and measured by the amount of oxygen uptake (Street 1978).

The results obtained in the present study reveal that there is an increase in the content of protein and nucleic acids in callus grown on medium 3 and to a certain extent on medium 2 (Figs 2–4). The fact that protein content on the control medium remained more or less unchanged indicate that the amount of protein synthesized is the same as that degraded as in the case of whole plant cells (Simon 1967). The increase in protein content was highly significant for callus grown on media 2 and 3 only. Such increase may indicate that callus is at the multiplication stage (Vajranabhaih & Mehta 1977). This could be due to the fact that compounds F and H may play an important and active role in increasing the synthetic ability of callus cells in relation to protein synthesis as is the case with kinetin and 2,4-D (Fosket & Tepfer 1978).

Carbohydrate content of the callus grown in media 1–4 decreased during the 90 days of growth (Fig. 5). This would suggest that carbohydrates may be used in certain pathways during the synthesis of protein as is the case with higher plant organs (Simon 1967).

The results show clearly that RNA content of the callus grown on the different media used followed the same pattern as protein. The same is also true for attached plant organs (Mohammad & Al-Mashhadani 1976; Mohammad & Yousif 1980) and for cultured explants (Fraser & Loening 1974). Moreover, RNA content is dependent on DNA. Therefore, any change in DNA content will affect the amount of RNA (Woolhouse 1967). The results reveal that the pattern of DNA change was generally similar to that of RNA. The increase in DNA content indicates that callus cells are able to divide and grow.

From the results obtained during the present investigation, it is concluded that the prospective growth regulators, particularly compound H (β-(*p*-chlorophenyl)-β-(3-indolyl)-*p*-propiophenone) can be used instead of kinetin, since that compound induces considerable callus growth when supplied with 2,4-D. At the same time, it is evident that compound H appears to modify the metabolism of the growing callus, since protein, nucleic acids and carbohydrate metabolism in medium 3 containing compound H appear to be fundamentally different from that in the control medium which contains 2,4-D + kinetin. The results also support the suggestion made previously (Mohammad & Hassan 1988) that compound H may replace kinetin as a new growth regulator.

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تأثير بعض منظمات النمو القياسية والمحتملة على كالس نبات عباد الشمس
الجزء الثاني ـ التغيرات في محتوى البروتين والحامض النووي الرايبوزي والديوكسي رايبوزي والكربوهيدرات

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خلاصة

شملت هذه الدراسة تأثير مشتقات المادة الكيمياوية β-phenyl-3-indolyl propiophenone على محتوى كالس نبات عباد الشمس من البروتينات والحوامض النووية والكربوهيدرات . إن المحتوى البروتيني للكالس النامي في الوسط القياسي والأوساط ١ و ٤ قد بقى ثابتا لحد ما خلال فترة ٩٠ يوما من النمو ، في حين أن محتوى البروتين ازداد بصورة معنوية في الأوساط الاخرى المستعملة . وقد تفاوت مقدار الزيادة وفقا لنوعية المادة المضافة لتلك الأوساط . واتخذت الحوامض النووية أنماطاً مشابهة للبروتينات . أما الكربوهيدرات فقد انخفض مستواها في الكالس النامي على جميع الأوساط الغذائية المستخدمة . وتشير الدراسة إلى أن المادة الكيمياوية
β-(*P*-chlorophenyl)-β-(-3-indolyl)-*P*-chloropropiophenone
(مادة *P*) يمكن أن تستعمل كمنظم للنمو بدلا من الكاينيتين .