American-Eurasian J. Agric. & Environ. Sci., 13 (11): 1516-1525, 2013
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2013.13.11.1993

Cinnamate-Kinetin Interaction-Effects on Metabolite Mobilization in Isolated Cucumber Cotyledons

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Abstract: Plant growth and development is governed by a mutual interaction among various plant growth regulators. Cinnamate is a ubiquitous plant phenol; being precursor for the synthesis of various phenylpropanoids and also di and polyphenols. The present study was undertaken to investigate the comparative effect/s of cinnamate individually as well as with various combinations of kinetin - an established plant growth regulator. The present study pertains the effects with regard to growth and metabolite mobilization using isolated cucumber cotyledons as bioassay. Surface sterilized seeds were germinated at 25°C temperature under constant illumination. The cotyledons were scored after 48 hr of germination and radical excision. These were then subjected to various different treatments by floating these in sterilized Petri plates lined with sterile chromatographic grade filter paper. Sampling was done at intervals of 24, 48, 72 and 96 hr and subjected to various metabolite fraction analyses. Observations after analysis seem to show that cinnamate acts more independently than having any interactive inhibition or synergism with kinetin as far as certain metabolite mobilization or dry matter accumulation in the isolated cotyledons of cucumber is concerned.

Key words: Cinnamic acid · Cotyledons · Metabolites · Kinetin · Phenols

INTRODUCTION

Despite ubiquitous presence of plant phenolics the understanding of their physiological role in plants is scanty. Some investigators believe that phenolics function as promoters as they have been reported to stimulate IAA, GA_3 and kinetin activity [1, 2], increase IAA oxidase, polyphenol oxidase, isoperoxidase, catalase and nitrate reductase activities [3-5], mobilize carbohydrates, proteins and total phenolics [6-8], regulate photoperiodism [9, 10], floral induction [11, 12], allelopathic substances [13, 14] and act as phytoalexins [15, 16]. However, others consider these compounds to be inhibitory to growth and developmental phenomena for example suppression of IAA biosynthesis and/or activation of IAA degradation [17], lowering of the growth stimulating activity of auxins, gibberllins or cytokinins [18], uncoupling of respiration and oxidative phosphorylation [19, 20], affect seed germination and dormancy [21-23]; induce both tap and adventitious rooting and growth [17]. Some groups of workers, however, argue against any basic role of phenolic constituents in plant growth regulation [24].

One of the most important phenolic acid that mimics the scent of cinnamon is the cinnamic acid. Cinnamate and its derivates generate precursors for mono, oligo and polyphenol synthesis such as tannin and lignin [25-34]. The biological activity of *cis*-cinnamic acid (*cis*-CA) was first reported in 1935 and was initially thought to promote growth in the pea split stem curvature test, the pea segment test and the *Avena* straight and curvature tests just as the auxins do [35, 36]. Later *cis*-CA vapors were found to act like ethylene. This was alluded to the presence of double bond (HC=CH) in its structure and also due to epinastic response induction in tomato plant [37]. By employing two mutants of tomato plant, Yang *et al.* [37] concluded that the *cis*-CA vapor acts independent of ethylene receptor dependent pathway and also that there are different action sites for *cis*-CA vapor and ethylene. Except a report for *Alpinia malaccensis*, that also in trace amounts, insufficient to have any physiological implications, *cis*-CA was hardly reported from any other natural plant source. This therefore, almost established it as a synthetic plant growth regulator for decades and prompted Zhiqui *et al*. [38] to infer that too little an effort was devoted to the study of production and

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function of this plant growth regulator in higher plants. *Trans*-CA is one of the sole precursor for the biosynthesis of all other phenylpropanoids [15]. Horbowicz *et al.* [39] reported growth inhibition of the primary root of buckwheat by *trans*-cinnamic acid. Keeping this in view the present work was carried out to study the effect of ubiquitously present phenol, cinnamate individually as well as in combination with already established plant growth regulator kinetin on with reference to certain storage metabolite mobilization in photosynthetic tissue of cucumber.

MATERIALS AND METHODS

Isolated cucumber (*Cucumis sativus* L.) cotyledons were used as a bioassay material. Cucumber seeds (*var*. Long Green) were procured from Agricultural station, Gwalior. The seeds were surface sterilized with 0.01% cetrimide solution and then thoroughly rinsed with sterile distilled water. Surface sterilized seeds were then transferred to 10cm dia. petri plates lined with sterile filter paper discs. Radicle emergence was treated as a parameter for germination. Cotyledons were however, scored after 48 hours by detaching these from the hypocotyls with sharp forceps. Scored cotyledons without injury were now floated overnight in distilled water under constant illumination to induce greening. 25 treatments of different concentrations of cinnamate ($1X10^{-4}$, $1X10^{-5}$, $5X10^{-4}$ and $5X10^{-5}$molar) and kinetin ($0.5X10^{-6}$, $1X10^{-6}$, $1.5X10^{-6}$ and $2X10^{-6}$molar) were prepared and 25 cotyledons of almost similar size were made to float in each petri plate. These petri plates were exposed to constant illumination (1000 lux) and temperature (30°C ± 1°C) in a culture rack.

Sampling done after 48, 72 and 96 hrs after treatments, were subjected to analysis for certain metabolite fractions, so as to assess their differences at tissue level and at different doses of cinnamate and kinetin both independently and in combinations. The metabolite fractions were those of total sugars, reducing sugars, total proteins and total phenolics. For fresh weight and dry matter assessment, 5 cotyledons were weighed and dried in an oven at 80°C for 24 hours cooled in a desecrator and reweighed. Difference in the weight constituted the moisture content. The same was then calculated as per cotyledon basis. Reducing sugars were estimated by Nelson Somogyi method [40]. Total carbohydrate fraction from the same pooled extract was estimated, using anthrone method of Hedge and Hofreiter [41].Total Protein content was estimated by the Standard Lowry's method [42]. Total phenols were estimated by the method described by Mallick and Singh [43].

RESULTS AND DISCUSSION

The seeds of *Cucumis sativus* L. (*var*. Long Green) after two hour soaking and surface sterility treatment showed a near 90 to 92% germination after 24 hours of floating on the filter paper discs with sufficient quantities of sterile distilled water. This percentage of germination under laboratory conditions overall can be taken as an assurance of the seed stock quality and its uniformity. The health of the seed can be visualized in Figs. 1 and 2 before and after germination within 24 hours, respectively. The bioassay left for greening under 1000 lux of fluorescent light intensity was observed to show uniform level of greening. The same has been presented in the Fig. 3. Cucumber seed, seedling or its isolated cotyledons or hypocotyls have been successfully employed by other workers also to ascertain physiological parameters in plant tissues [3, 44- 47]. In the present study also the use of isolated cotyledons of cucumber (*Cucumis sativus*) with some modifications in preparation for bioassay was justified.

Cinnamate is a plant phenol, predominantly being synthesized by the plants of almost all groups and also synthetically available [48]. Cinnamates and/or its derivatives have therefore, an ubiquitous presence in plants and have been extracted from every plant part such as roots, stem, leaves, flowers, fruits and seeds [49, 50]. Though salicylates have now almost been established for their having growth and developmental functions in plants and being recognized as plant growth regulators, the cinnamates despite their ubiquitous presence are yet to be identified as ones of any importance in plant growth regulation [24, 51].

In the presentation here a comparative study was undertaken to ascertain whether, even on preliminary basis, the cinnamate its natural *trans*- isomer can have some effect on a photosynthetic tissue *vis a vis* a known established growth promoting plant hormone kinetin. Therefore, the two were used alone or in combinations and at various concentrations to see their interactive effects if any, on the mobilization of certain tissue metabolites. For an overall presentation of the treatments at various combinations and at various time intervals to isolated cucumber cotyledons, the same are presented here in the figures 4, 5 and 6 corresponding to the physical condition of the cotyledons at 48, 72 and 96 hours of treatments respectively. From the figures 5 and 6 it is amply clear that higher cinnamate levels prove damaging, rather lethal at 96 hours treatment for the cotyledons. Therefore, the metabolite parameters have been limited to suboptimal cinnamate treatments only.

Fig. 1: Surface sterilized seeds of Cucumis sativus arranged for germination in petriplates over sterile filter papers.

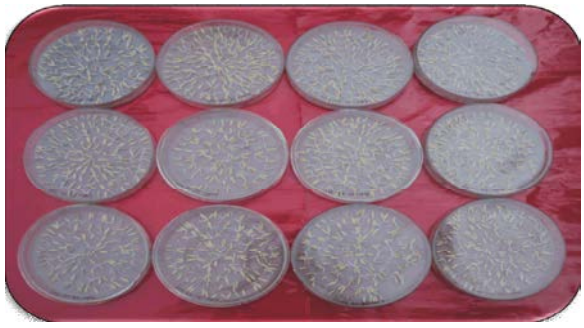

Fig. 2: Germinated seeds of Cucumis sativus after 24 hours of as shown in figure 3.1.

Fig. 3: Scored cotyledons of Cucumis sativus used as experimental bioassay after 48 hours of germination of seeds and detached from their hypocotyls and radicles after greening under constant illumination.

The cinnamate (CA) with or in combination with kinetin (KA) showed no increases in over all fresh weight content of cotyledons after 48 hours treatment. However, 72 hours after treatment the fresh weight of cotyledons increases appreciably with the increase in CA dose concentration with or without any of KA concentrations.

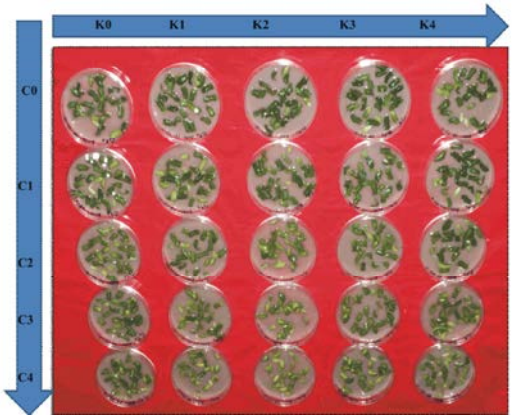

Fig. 4: Cotyledons of Cucumis sativus floating continuously on different concentrations of cinnamate and kinetin after 48 hours treatment. C0-C4 and K0-K4 represent different dose treatments of cinnamate and kinetin respectively. Dose concentrations are given in methods chapter.

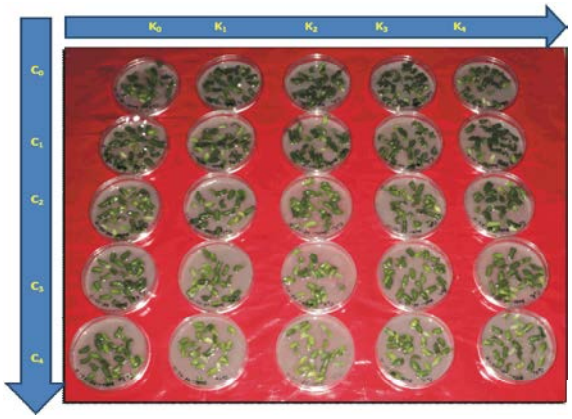

Fig. 5: Cotyledons of Cucumis sativus floating continuously on different concentrations of cinnamate and kinetin for 72 hours after treatment.

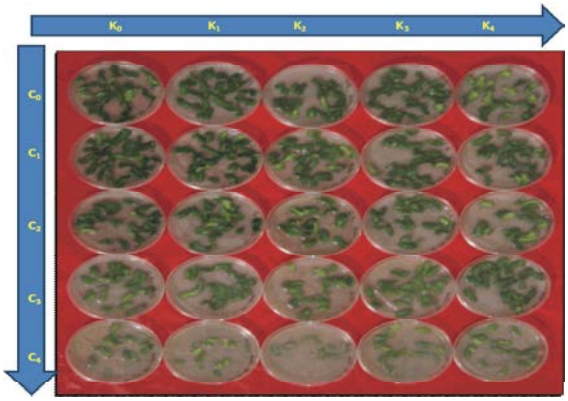

Fig. 6: Cotyledons of Cucumis sativus floating continuously on different Concentrations of cinnamate and kinetin for 96 hours after treatment.

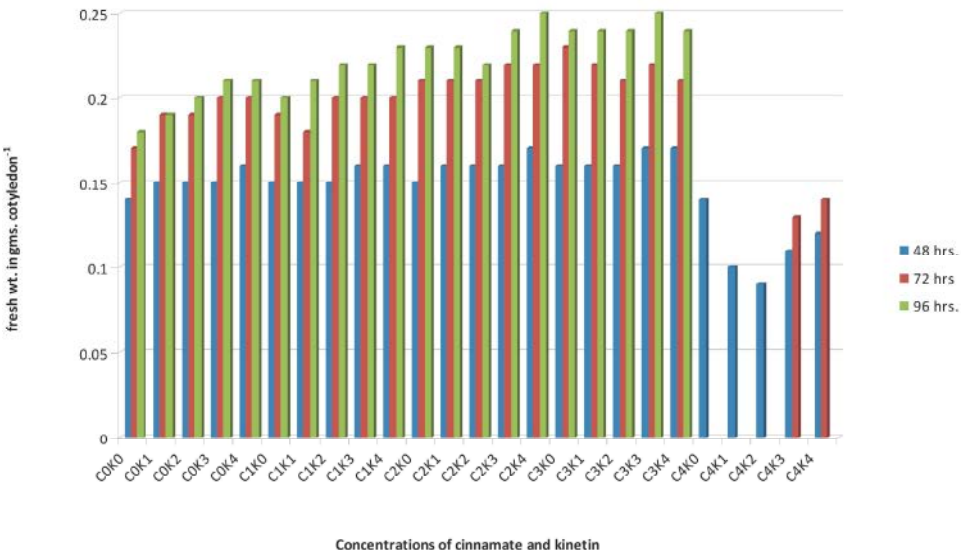

Fig. 7: Tissue fresh weight of Cucumis sativus cotyledons at different concentrations of cinnamate and kinetin after various durations of continuous floating. C4 treatment at that concentration proved lethal for the tissue, therefore erratic.

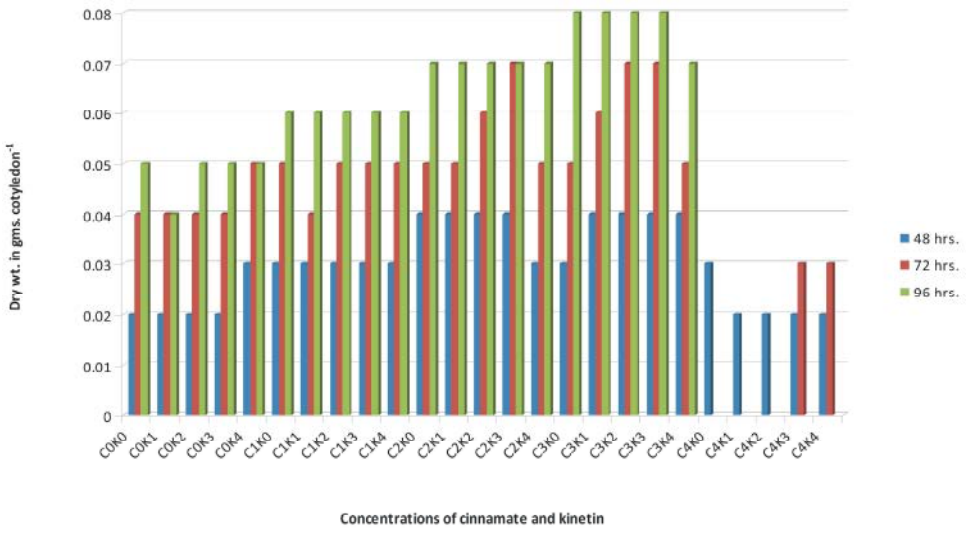

Fig. 8: Tissue dry weight content of Cucumis sativus cotyledons at different concentrations of cinnamate and kinetin.

This observation seems to persist even at 96 hours of treatment wherein the CA shows dose dependent fresh weight increase independent of KA. However, high Cinnamic acid concentrations proved to be degenerative for the cotyledons as the growth was inhibited followed by lethality. It is therefore, pertinent to observe in the present case that kinetin though known to independently effect fresh weight increase in cotyledons through cell expansion due to water uptake than any dry matter addition substantially [52] seems as if CA is taking over from KA in accumulating fresh weight despite the later being present (Figs. 7, 9). Dry matter content of cotyledonary tissue shows constant continuous significant increases with the increasing doses of CA and at every 24 hour interval (Figs. 8, 9). Interestingly the moisture percent content which defines the water levels of tissue seems to decrease with increasing CA doses. This therefore can lead to the conclusion that the increasing dry matter is accumulated due to CA and not due to KA since at every stage of treatment the levels of tissue dry matter do not change with added KA, rather remains the same as with CA alone treated cotyledons (Figs. 8, 9).

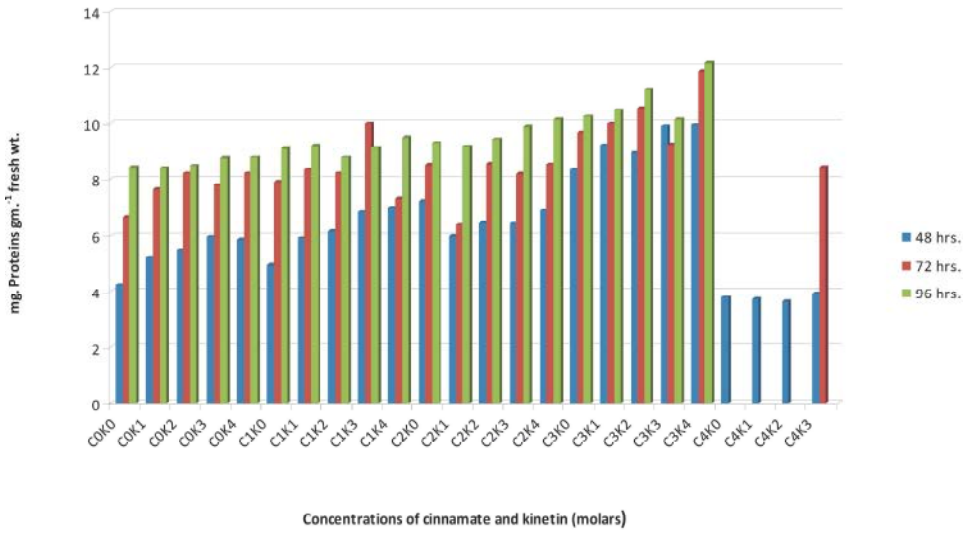

Fig. 9: Tissue Protein content of cotyledons at different treatments of cinnamate and kinetin.

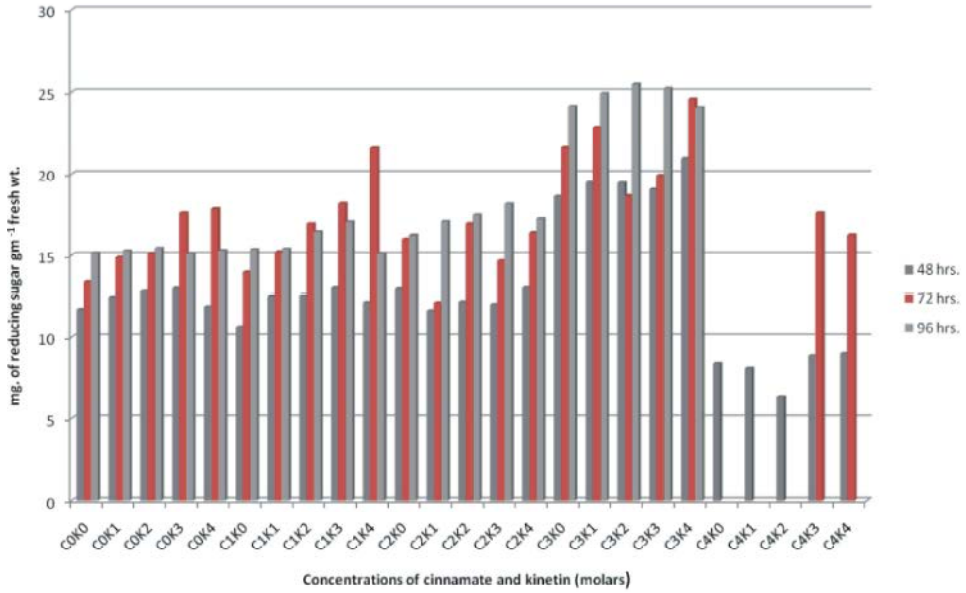

Fig. 10: Reducing sugar content of cotyledons at different treatments of cinnamate and kinetin at every 24 hour increment of time after floating on treatments.

Plant phenolic compounds have widely been reported to be substances stimulatory to plant growth and function as promoters [49, 51]. This, as is reported by various other workers, is done by the mobilization of metabolites like carbohydrates, proteins and total phenolics [7, 8, 10, 53,].

From the present study, an inference can be drawn, wherein the high levels of total and reducing sugars reduce considerably the non-structural sugar (carbohydrate) levels. The results showed that there is a constant, concomitant increase in the tissue reducing, total and therefore non-reducing sugar levels with the increasing doses of CA, irrespective of whether these are superimposed with KA or not; exception being that the CA at oligodynamic concentrations or otherwise was proving damaging to the soft tissue of cotyledons. Here again it seems that any dry matter accumulation is resulted by CA rather than by KA and don't seem to have any mutual inhibition or synergism in any of the parameters (Figs. 10, 11 and 12). As already discussed the mobilization of various metabolites like carbohydrates by phenolic compounds is already reported by Talaat and Balbaa [8] and He and Lin, [53]. Further kinetin and salicylic acid (SA), a phenolic substance has been reported to regulate plant growth and development by

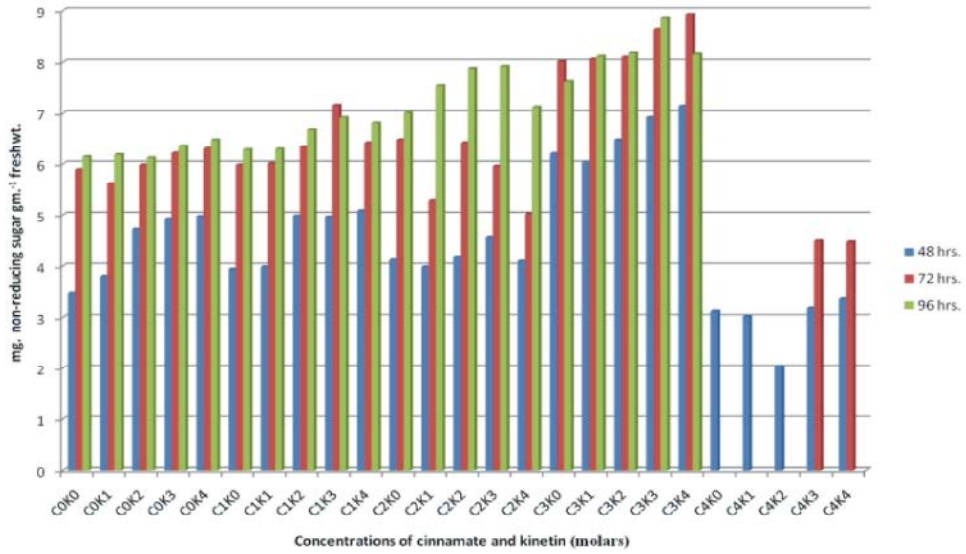

Fig. 11: Tissue non-reducing sugar content of cotyledons at different treatments of cinnamate and kinetin deduced after differences between total and reducing sugar contents at every 24 hour increase of treatments.

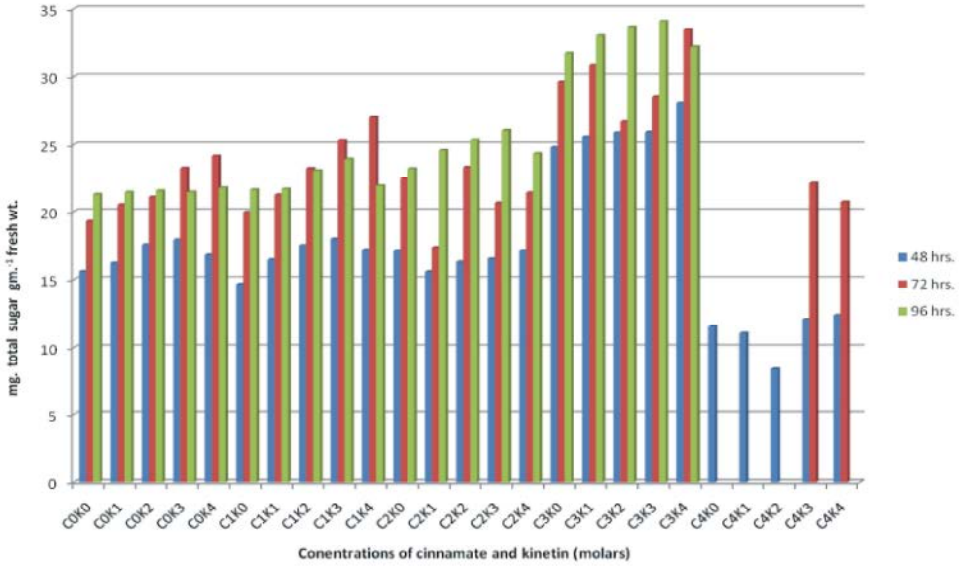

Fig. 12: Total sugar content of cotyledons at different treatments of cinnamate and kinetin at every 24 hour increment of time after treatment.

enhancing GA metabolism of plants[1]. The type and concentration of auxin and cytokinin, either alone or in combination has been known to strongly influence growth as well as the secondary metabolites in tissue culture [54].

The CA and KA in the present study do not seem to affect levels of proteins appreciably, though showing a small increment in combination or alone. With 48, 72 and 96 hours there is a time bound increase however, percentage wise this seems to be insignificant (Fig. 13). It is known that cytokinins alone can produce a large variety of physiological, metabolic, biochemical and developmental processes when applied to plants [55, 56]. Simultaneously however, CA along with its derivatives are also known to induce and play important role in plant development, protection against UV-B radiation and pathogen defense [57- 61]. The meager protein increase in the cotyledons in response to CA and KA therefore, seems to be a response of the metabolic channeling here something which is already known [62]. It seems that since the cotyledons are already provided with CA exogenously the endogenous synthesis of phenols and

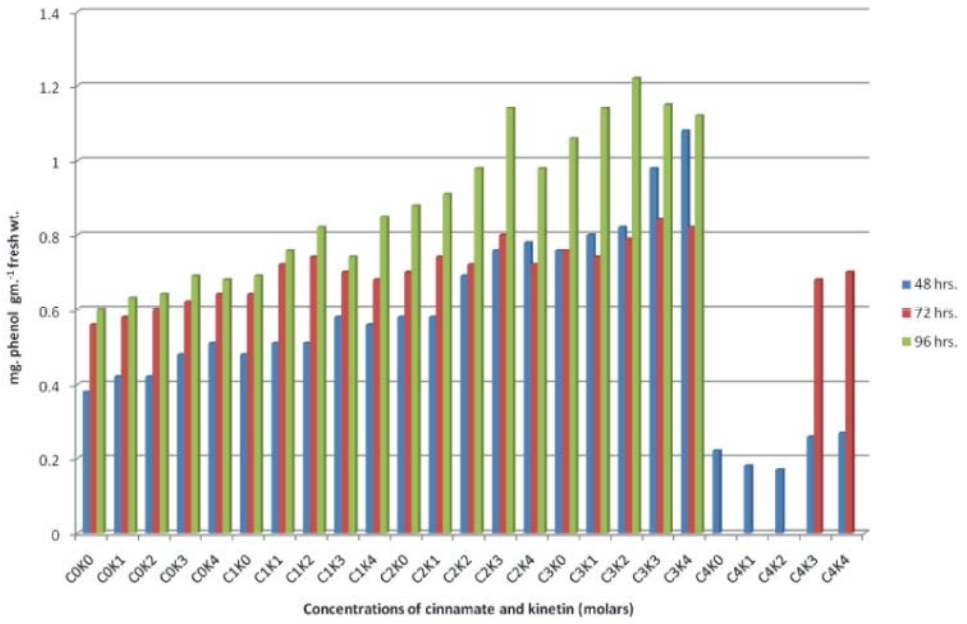

Fig. 13: Tissue total phenol content of cotyledons at different treatments of cinnamate and kinetin.

their concomitant enzymes and proteins are at low levels. Also phenolic substances sensed exogenously are known to be inhibitory for protein synthesis and therefore their levels. More so, cinnamate is an established allelopathic compound [14, 61, 63].

The tissue phenol levels keep marginally increasing with 24 hr intervals in the cotyledons. This may be a result of CA uptake exogenously; the basis for this inference being that the increase is gradual with increasing CA concentrations and exposure time showing the maximum at 96 hours treatment (Fig. 14). It seems that KA may not be interfering with the cinnamate uptake by the tissue since the total phenol increase seems irrespective of the presence of KA and whatever the dose. It has been shown that cinnamic acid has the ability to uncouple the energy transducing membrane and stimulates non-specific membrane permeability which allows the influx of the substances say for KA and CA itself across the cell wall and membrane [64]. This may explain a concomitant increases in total phenol level within the cotyledons.

CONCLUSION

From the analysis it therefore, seems that cinnamic acid in the present case acts independently rather than having any interactive inhibition or synergism with kinetin on the mobilization of certain metabolite fractions and dry matter accumulation in the isolated cotyledons of cucumber.

ACKNOWLEDGEMENT

The authors acknowledge the facilities provided by the Head, School of Studies in Botany, Jiwaji University, Gwalior. The grant by MPCST, Bhopal and UGC, New Delhi is gratefully acknowledged for carrying out this work.

REFERENCES

1. Mukharjee, D. and R. Kumar, 2007. Kinetin regulates plant growth and biochemical changes during maturation and senescence of leaves, flowers and pods of *Cajanus cajan* L. Biol. Plant. 50: 80-85.
2. Buer, C.S., N. Imin and M.A. Djordjevic, 2010. Flavonoids: new roles for old molecules. J. Integr. Plant Biol., 52: 98-111.
3. Singh, P.K., K.K. Koul, S.B. Tiwari and R.K. Koul, 1997. Effect of cinnamate on nitrate reductase activity in isolated cucumber cotyledons. Plant Growth Regulation. 21(3): 203-206.
4. Zhang, E., S. Zhang, W. Zhang, L. Li and T. Li, 2010. Effects of exogenic benzoic acid and cinnamic acid on the root oxidative damage of tomato seedlings. Journal of Horticulture and Forestry, 2(2): 022-029.
5. Singh, P.K., R. Singh and S. Singh, 2013. Cinnamic acid induced changes in reactive oxygen species scavenging enzymes and protein profile in maize (Zea mays L.) plants grown under salt stress. Physiol Mol. Biol. Plants, 19: 1 53-59.

6. Zeng, R.S., S.M. Luo and Y.H. Shi, 2001. Physiological and biochemical mechanism of allelopathy of secalonic acid on higher plants. Agron. J., 93: 72-79.
7. Talaat, M.I., 2005. Physiological effect of salicylic acid and tryptophan on Pelargonium graveolens. Egypt. J. Appl. Sci., 20: 751-760.
8. Talaat, M.I. and K.L. Balbaa 2010. Physiological Response of Sweet Basil Plants (*Ocimum basilicum* L.) to Putrescine and *Trans*-Cinnamic Acid. American-Eurasian J. Agric. and Environ. Sci., 8(4): 438-445.
9. Towers, G.H.N. and B. Abeysekera, 1984. Cell wall hydroxycinnamate esters as UV-A receptors in phototropic responses of higher plants-a new hypothesis Phytochemistry. 23(5): 951- 952.
10. Turner, L.B., I. Muller-Harvey and A.B. Mc Allan, 1993. Light-induced isomerization and dimerization of cinnamic acid derivatives in cell walls. Phytochemistry, 33(4): 791-796.
11. Ebrahimzadeh, H. and P. Abrishamchi, 2001. Changes in IAA, Phenolic Compounds, Peroxidase, IAA Oxidase and Polyphenol Oxidase in Relation to Flower Formation in Crocus sativus. Russian Journal of Plant Physiology. 48(2): 190-195.
12. Sheeja, T.E. and A.B. Mandal, 2003. In vitro flowering and fruiting in tomato (*Lycopersicon esculentum* Mill.). Asian Pacific Journal of Molecular Biology and Biotechnology. 11(1): 37-42.
13. Inderjit, D. and A.U. Malik, 2002. Chemical Ecology of Plants: Allelopathy in Aquatic and Terrestrial Ecosystems. Birkhauser Publishing Ltd. Basal.
14. Chen, S., B. Zhou, S. Lin, X. Li and X. Ye, 2011. Accumulation of cinnamic acid and vanillin in eggplant root exudates and the relationship with continuous cropping obstacle. African Journal of Biotechnology, 10(14): 2659-2665.
15. Dixon, R.A., 2001. Natural products and plant disease resistance. Nature. 411: 843-847.
16. Mazid, M., T.A. Khan and F. Mohammad, 2011. Role of secondary metabolites in defense mechanisms of plants. Biology and Medicine, 3(2) Special Issue: 232-249.
17. De Klerk, G., H. Guan, P. Huisman and S. Marinova, 2011. Effects of phenolic compounds on adventitious root formation and oxidative decarboxylation of applied indoleacetic acid in Malus 'Jork 9'. Plant Growth Regul., 63: 175-185.
18. Kefeli, V.I. and C.S. Kadyrov, 1971. Natural Growth Inhibitors, their Chemical and Physiological Properties. Annual Review of Plant Physiology. 22: 185-196
19. Iiyama, K., T.B.T. Lam and B.A. Stone, 1994. Review. Structural Characteristics of Cell Walls of Forage Grasses-Their Nutritional Evaluation For ruminants. Plant Physiol., 104: 315.
20. Hatfield, R.D., J. Ralph and J.H. Grabber, 1999. Cell wall cross-linking by ferulates and diferulates in grasses. Journal of the Science of Food and Agriculture, 79: 403-407.
21. Yao, J., 2007. Defect allelopathy of the processed tomato and research physiological specialty. Xinjiang Agricultural University.
22. Reigosa, M.J. and E. Pazos-Malvido, 2007. Phytotoxic effects of 21 plant secondary metabolites on Arabidopsis thaliana germination and root growth. Journal of Chemical Ecology, 33: 1456-1466.
23. Hussain, M.I., L. Gonzalez and M.J. Reigosa, 2008. Germination and growth response of four plant species towards different allelochemicals and herbicides. Allelopathy Journal. 22: 101-110.
24. Firn, R.D. and C.G. Jones, 2009. A Darwinian view of metabolism: Molecular properties determine fitness. J. Exp. Bot., 60: 719-726.
25. Santos, W.D., M.L.L. Ferrarese, A. Finger, A.C.N. Teixeira and F.O. Filho, 2004. Lignification and related enzymes in *Glycine max* root growth inhibition by ferulic acid. Journal of Chemical Ecology, 30: 1199-1208.
26. Ralph, J., 2006. What makes a good monolignol substitute? In: Hayashi, T. ed. The science and lore of the plant cell wall biosynthesis, structure and function. Boca Raton, FL, USA: Universal Publishers (Brown Walker Press), pp: 285-293.
27. Ralph, J., 2010. Hydroxycinnamates in lignification. Phytochemistry Reviews, 9: 65- 83.
28. Silva, C.G., E.C. Ramires and E. Frollini, 2006. Phenolic composites reinforced with celluloscis and lignocellulosic fibres. In: Proceedings World Polymer Congress, Macro 2006, 41st International Symposium on macromolecules, RJ, Brazil.
29. Kovacik, J., B. Klejdus, M. Backor and M. Repcak, 2007. Phenylalanine ammonia lyase activity and phenolic compounds accumulation in nitrogen-deficient Matricaria chamomilla leaf rosettes. Plant Sci., 172: 393-399.

30. Dos Santos, W.D., M.L.L. Ferrarese, C.V. Nakamura, K.S.M. Mourao, C.A. Mangolin and O. Ferrarese-Filho, 2008. Soybean (*Glycine max*) root lignification induced by ferulic acid. The possible mode of action. J. Chem. Ecol., 34: 1230-1241.
31. Zanardo, D.I.L., R.B. Lima, M.L.L. Ferrarese, G.A. Bubna and F.O. Filho, 2009. Soyabean root growth inhibition and lignification induced by *p*-coumaric acid. Environ Exp. Bot., 66: 25-30.
32. Vanholme, R., B. Demedts, K. Morreel, J. Ralph and W. Boerjan, 2010. Lignin biosynthesis and structure. Plant Physiology. 153: 895-905.
33. Vanholme, R., K. Morreel, C. Darrah, P. Oyarce, H. John, Grabber. J. Ralph and W. Boerjan, 2012a. Metabolic engineering of novel lignin in biomass crops. New Phytologist.
34. Vanholme, R., V. Storme, B. Vanholme, S. Sundin, J.H. Christensen, G. Goemine, C. Halpin, A. Rohde, K. Morreel and W. Boerjan, 2012b. A systems biology view of responses to lignin biosynthesis perturbations in Arabidopsis. Plant Cell., 112: 1025 74.
35. Hitchcock, A.E., 1935. Indole-3-n-propionic acid a growth hormone and quantitative measurement of plant response, Contributions from Boyce Thompson Institute. 7:87-85.
36. Haagen-Smit, S.A.J. and Went, F.W. 1935. A physiological analysis of the growth substance, Proceedings Koninklijke Akademie van Weten schappen te Amsterdam, 38: 852-857.
37. Yang, X.X., H.W. Choi, S.F. Yang and N. Li, 1999. A UV-light activated cinnamic acid isomer regulates plant growth and gravitropism via an ethylene receptor-independent pathway. Aust. J. Plant Physiol., 26: 325-335.
38. Zhiqui, Y.Q., W.S. Wong, W.C. Ye and N. Li, 2003. Biologically active *cis*- cinnamic acid occurs naturally in Brassica parachinensis. Chin. Sci. Bull., 48: 555-558.
39. Horbowicz, M., H. Mioduszewska, D. Koczkodaj and M. Saniewski, 2009. The effect of methyl jasmonate and phenolic acids on growth of seedlings and accumulation of anthocyanins in common buckwheat (*Fagopyrum esculentum* Moench). Acta Agrobot., 62: 49-56
40. Somogyi, M., 1952. Notes on sugar determination. Journal of Biological Chemistry. 195: 19-23.
41. Hedge, J.E. and B.T. Hofreiter, 1962. In: Carbohydrate Chemistry. 17 Ed. Whistler R L and BC Miller, J. N.) Academic Press New York.
42. Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem., 193: 265.
43. Malick, C.P. and M.B. Singh, 1980. In: Plant Enzymology and Histoenzymology Kalyani Publishers, New Delhi, pp: 286.
44. Sharma, Y.K. and M.P. Kaushik, 1982. Endogenous level of auxin and IAA- oxidase activity as affected by some phenolic acids in cucumber (Cucumis sativus). Acta Bot. India. 11: 32-35.
45. Daayf, F., R. Bel-Rhlid and R.R. Belanger, 1997. Methyl ester of *p*-coumaric acid: A phytoalexin-like compound from long English cucumber leaves. J. Chem. Ecol., 23: 1517-1526.
46. Ding, H., Y. Sun, C.L. Xiao, K. Shi, Y.H. Zhou and J.Q. Yu, 2007. Physiological basis of different allelopathic reactions of cucumber and fig leaf gourd plants to cinnamic acid. Journal of Experimental Botany. 58(13): 3765-3773.
47. Yu, J., Y. Sun, Y. Zhang, Y. Ding, X. Xia, C. Xiao, K. Shi and Y. Zhou, 2009. Selective *trans*-Cinnamic Acid Uptake Impairs $[Ca^{2+}]_{cyt}$ Homeostasis and Growth in *Cucumis sativus* L. J. Chem. Ecol., 35: 1471-1477.
48. Robbins, R.J., 2003. Phenolic acids in foods: an Phenolic acids in foods: An overview of analytical methodology. J. Agric. Food Chem., 51: 2866-2887.
49. Hegab, M.M., S.E.A. Khodary, O. Hammouda and H.R. Ghareib, 2008. Autotoxicity of chard and its allelopathic potentiality on germination and some metabolic activities associated with growth of wheat seedlings. Afr. J. Biotech. 7: 884-892.
50. Choi, O., C.Z. Wu, S.Y. Kang, J.S. Ahn, T.B. Uhm and Y.S. Hong, 2011. Biosynthesis of Plant-specific Phenylpropanoids by construction of an artificial Biosynthetic pathway in E. coli. J. Ind. Microbiol. Biotechnol., 38(10): 1657-65.
51. Ghareib, H.R., M.S. Abdelhamed and O.H. Ibrahim, 2010. Antioxidative effects of the acetone fraction and vanillic acid from *Chenopodium murale* on tomato plants. Weed. Biol. Manage., 10: 64-72.
52. Laloraya, M.M., 1986. Reversal of ABA induced stomatal closure by *trans*- cinnamic and *p*-coumaric acid. Plant Physiol., 81: 253- 258.
53. He, H.Q. and W.X. Lin, 2001. Studies on allelopathic physiobiochemical characteristics of rice. Chin. J. Eco.Agric., 9: 56-57.
54. Masoumian, M., A. Arbakariya, A. Syahida and M. Maziah, 2011. Flavonoids production in Hydrocotyle bonariensis callus tissues. Journal of Medicinal Plants Research. 5(9): 1564-1574.

55. Taiz, L. and E. Zeiger, 1991. Cytokinins. In: Taiz, L. Zeiger, E. (Eds.), Plant Physiology. Benjamin: Cummings, Redwood City, CA, pp: 452-472.
56. El-Shihaby, A.O., M.E.M. Younis, Z.M. El-Bastawisy and M.M. Nemat-Alla, 2002. Effect of kinetin on photosynthetic activity and carbohydrate content in waterlogged or seawater-treated *Vigna sinensis* and *Zea mays* plants. Plant Biosystems, 136(3): 277-290.
57. Hahlbrock, K. and D. Scheel, 1989. Physiology and molecular biology of phenylpropanoid metabolism. Annu Rev. Plant Physiol Plant. 40: 347-369.
58. Bais, H.P., R. Vepachedu, S. Gilroy, R.M. Callaway and J.M. Vivanco, 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. Science, 301: 1377.
59. Weir, T.L., S.W. Park and J.M. Vivanco, 2004. Biochemical and physiological mechanisms mediated by allelochemicals. Curr. Opin. Plant Biol., 7: 472.
60. Chon, S.U., H.G. Jang, D.K. Kim, Y.M. Kim, H.O. Boo and Y.J. Kim, 2005. Allopathic potential in lettuce (*Lactuca sativa* L.) plants. Sci. Hort., 106: 309-317.
61. Chobot, V., C. Huber, G. Trettenhahn and F. Hadacek, 2009. (±)-Catechin: chemical weapon, antioxidant, or stress regulator? J. Chem. Ecol., 35: 980-996.
62. Winkel, B.S.J., 2004. Metabolic channeling in plants. Annu. Rev. Plant Biol., 55: 85-107.
63. Fuzita, K. and I. Kubo, 2003. Synergism of polygodial and *trans*- cinnamic acid on inhibition of root elongation in lettuce seedling growth bioassays. J. Chem. Ecol., 29: 2253- 2262.
64. Chambel, A., C.A. Viegas and I. Sa-Correia, 1999. Effect of cinnamic acid on the growth and on plasma membrane H^+-ATPase activity of Saccharomyces cerevisiae. International Journal of Food Microbiology. 50: 173-179.