# Allelopathic Potential of Ferulic Acid on Tomato

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**ABSTRACT**

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This study deals with allelopathic effects of exogenous application of ferulic acid (FA) on biophysical and biochemical parameters of tomato (cv. Pusa ruby) seedlings grown in hydroponic culture. FA exhibited phytotoxic effects on tomato seedlings grown in a Hoagland solution. After 7 days of seedling growth, FA was added to the growth medium at concentrations of 0.1, 0.5, 1.0, and 1.5 mM. The Hoagland solution without FA was used for experimental units. The root and shoot length, fresh and dry weight of seedlings, chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll (chl t), carotenoids, protein and sugar content, nitrate reductase (NR) activity and antioxidant enzymes accumulation were examined in 25 days time period. FA exhibited significant inhibitory effects on some biophysical and biochemical traits. A gradual decrease in all studied traits in FA based-treatments was observed. The root and shoot length, the fresh and dry weight of the seedlings significantly decreased in a dose dependent manner under FA allelopathic potential. The chl a, chl b, chl t and carotenoids contents decreased with the increase of FA concentrations. A significant reduction in sugar and protein contents and in NR activity was recorded especially with the highest concentrations of FA used. Higher FA concentrations significantly enhanced the activities of antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX)] which appeared as a form of tolerance and/or a defense to allelochemicals used.

*Keywords*: Allelopathy, antioxidants, ferulic acid, Hoagland solution, hydroponic culture, tomato

Plants may affect the growth and productivity of neighboring ones directly through secondary metabolites (51). This phenomenon is known as allelopathy and involved secondary metabolites in the process are called allelochemicals (42) which are bioactive compounds that influence germination, growth and development of receptor plants. Allelochemicals are released through the leaching, evaporation and decomposing crop residues which have either inhibitory or stimulatory effects (46). Allelochemicals may affect physiological processes in plants, such as, stomatal closure (8), plant water balance (7), cell division (4), membrane permeability (24), nutrient uptake (6), photosynthesis (11) respiration (3) and many other metabolic processes.

Ferulic acid (FA) considered as allelopathic agent is one of the secondary metabolites which is a derivative of cinnamic acid (21). FA was extracted from soil with a range (0.01-0.1 mM) of concentrations (54, 55). Luthria *et al*. (37) found FA in tomato and this finding was also reported in other works (9, 25, 27, 48). Leaf leachates (2), root exudates (49, 50), plant debris (15, 18) and soil extracts (26, 43) were frequently investigated for

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allelopathic potential. Exposure to FA adversely affected various biophysical and biochemical processes in plants by reducing water utilization (30), foliar expansion and root elongation (14), nutrient uptake (12, 38), photosynthesis, leaf water potential, turgor and osmotic pressure and growth (23, 41). The inhibitory effects of FA on *Lemna minor* (20, 41), *Abutilon theophrasti* (40), soybean (19) and soybean and sorghum (22) were often reported in allelopathy studies.

FA was selected as allelochemical because it was frequently reported as an inhibitory substance to many plant species. This study was conducted to investigate the effect of FA on tomato growth and development.

## MATERIALS AND METHODS

**Plant material and treatments**.

Certified seeds of tomato (*Lycopersicon esculentum*, cv. Pusa ruby) were purchased from Seed Company of Allahabad, Uttar Pradesh, India. Seeds were sown in nursery beds (1 m × 1 m) at the experimental station, Allahabad University, Allahabad (24°47', 50°47' N latitude; 81°91', 82°21' E longitude; 78 m-asl). The seed bed was watered as required. After 15 days, seedlings were uprooted and washed with tap water followed by distilled water, then transferred at the rate of 10 seedlings per pot in transparent plastic pot [(23×17×9) =3519 $cm^3$] each containing 2 liters of Hoagland solution (29). FA at 0.1($T_1$), 0.5 ($T_2$), 1.0 ($T_3$) and 1.5 ($T_4$) mM concentrations were prepared in distilled water and used for treatment. After 1 week of establishment, the FA was added to Hoagland solution according to the treatment. The Hoagland solution free of FA was taken as control. Pots were covered with black papers to avoid the algal growth in the growth medium, then fitted with aerating tubes, and any pot opening was plugged with cotton to hold the seedlings in a vertical position. The seedlings growth experiment was conducted in a glass house, and replicated 3 times. Pots were continuously aerated, and data was collected after 72 h for biochemical and biophysical analyses.

**Measurements**.

Root and shoot lengths (RL, SL) of tomato seedlings were measured with a metric scale, as was the case for fresh and dry matters. Fresh plant materials were weighted (FW) and then oven dried at 70°C for 72 h to get the dry weight (DW).

**Estimation of chlorophyll, carotenoids and protein contents**.

Chlorophyll and carotenoids from plant materials were extracted with 80% acetone. The amount of photosynthetic pigments was determined by Lichtenthaler (34). A fresh leaf sample of 10 mg was homogenized in 10 ml of 80% acetone and then centrifuged. Supernatant was taken and optical density was measured at 663, 645 and 470 ηm.

Protein content was determined following the method of Lowry *et al*. (36). The amount of protein was calculated with reference to standard curve obtained from bovine serum albumin.

**Nitrate reductase activity**.

Nitrate reductase (NR) activity was assayed by modified procedure of Jaworski (31) based on incubation of fresh tissue (0.25 g) in 4.5 ml medium containing 100 mM phosphate buffer (pH = 7.5), 3% $KNO_3$ and 5% propanol. About 0.4 ml aliquot was treated with 0.3 ml 3% sulphanilamide in 3-N HCl and 0.3 ml 0.02% N-1-naphthyl ethylene diamine dihydrochloride (NEDD). The absorbance was measured at 540 ηm. NR

activity was calculated with a standard curve prepared from $NaNO_2$ and expressed in µmol $NO_2.g^{-1}FW.h^{-1}$.

**Sugar content**.

Sugar content was estimated following Hedge and Hofreiter method (28). About 0.25 g of the plant leaves were homogenized in 2.5 ml of 95% ethanol. After centrifugation, the sugar content was determined in 0.1 ml of the supernatant, mixed with 4 ml of anthrone reagent and heated in a boiling water bath for 8 min. Absorbance was taken at 620 ηm after rapid cooling. Standard curve was prepared with glucose.

**Antioxidant enzyme assay**.

Enzyme extract was prepared by homogenizing 500 mg of leaves tissue in 10 ml of 0.1 M sodium phosphate buffer (pH = 7.0). A homogenate was filtered and centrifuged at 15000 g at for 30 min and temperature was maintained at $4^oC$. The supernatant was collected and used for analysis of superoxide dismutase, catalase and peroxidase.

Superoxide dismutase (SOD) activity was determined by the nitroblue tetrazolium (NBT) photochemical assay method described by Beyer and Fridovich (13). The reaction mixture (4 ml) contained 63 µM NBT, 13 mM methionine, 0.1 mM ethylene diamintetra acetic acid (EDTA), 13 µM riboflavin, 0.5 M sodium carbonate and 0.5 ml clear supernatant. Test tubes were placed under fluorescent lamps for 30 min and absorbance was recorded at 560 ηm. One unit of enzyme was defined as the amount of enzyme which caused 50% inhibition of NBT reduction.

Catalase (CAT) activity was assayed following the method of Cakmak and Marschner (17). The reaction mixture (2 ml) contained 25 mM sodium phosphate buffer (pH = 7.0), 10 mM $H_2O_2$ and 0.2 ml enzyme extract. The activity was determined by measuring the rate of disappearance of $H_2O_2$ for 1 min at 240 ηm and estimated using extinction coefficient of 39.4 $mM^{-1}.cm^{-1}$ and expressed as enzyme unit/g fresh weight. One unit of CAT was defined as the amount of enzyme required to oxidize 1 µM $H_2O_2$/min.

Peroxidase (POX) activity was assayed following the method of Mc Cune and Galston (39). Reaction mixture contained 2 ml enzyme extract, 2 ml sodium phosphate buffer, 1 ml 0.1-N pyrogallol and 0.2 ml 0.02% $H_2O_2$ and determined spectrophotometrically at 430 ηm. One unit of enzyme activity was defined as the amount which produced an increase of 0.1 optical density per minute.

**Statistical analysis.**

Standard errors of means were calculated in triplicates. In addition, a one way ANOVA was carried out for all the data generated from this experiment test using GPIS package (GRAPHPAD, California, USA).

## RESULTS

The growth of tomato seedlings were measured in terms of RL, SL, FW and DW with respect to treated and untreated seedlings by FA. The growth was significantly affected for treated plants when compared to the control. The reduction in the RL, SL, FW and DW of seedlings was 35.43, 43.79, 40.43, and 67.76%, respectively, when compared with control. The maximum growth was recorded in the seedlings under control. Plant treated with 1.5 mM concentration exhibited the maximum reduction in growth (Table 1).

**Table 1.** Effects of ferulic acid (FA) applied at different concentrations on the shoot and root length and on the fresh and dry weight of tomato seedlings

| Treatment | Shoot length (cm) | Root length (cm) | Fresh weight (g/plant) | Dry weight (g/plant) |
|---|---|---|---|---|
| **C** | 15.3 ± 0.115 | 6.35 ± 0.028 | 13.85 ± 0.028 | 2.84 ± 0.051 |
| **$T_1$** | 12.4 ± 0.057 [a] | 5.9 ± 0.057 | 13.05 ± 0.086 [a] | 2.23 ± 0.049 [a] |
| **$T_2$** | 10.2 ± 0.057 [a] | 5.25 ± 0.202 [a] | 10.35 ± 0.259 [a] | 1.24 ± 0.008 [a] |
| **$T_3$** | 9.35 ± 0.202 [a] | 4.9 ± 0.346 [a] | 8.95 ± 0.202 [a] | 1.03 ± 0.026 [a] |
| **$T_4$** | 8.6 ± 0.635 [a] | 4.1 ± 0.230 [a] | 8.25 ± 0.028 [a] | 0.92 ± 0.019 [a] |

Data are means of 3 replicates ± SEM,
[a] Treatment significantly ($P < 0.001$) different to the control(C),
C: Control, $T_1$:0.1 mM, $T_2$: 0.5 mM, $T_3$,:1.0 mM, and $T_4$:1.5 mM concentrations of FA.

The chlorophyll content decreased under FA treatment. The maximum decrease in chlorophyll and carotenoids content were decreased by 50.95, 35.53, 45.81, and 52.08%, respectively, under the highest concentration of FA as compared with control (Table 2).

**Table 2.** Effects of ferulic acid (FA) applied at different concentrations on chlorophyll a, chlorophyll b, chlorophyll t and carotenoids contents of tomato seedlings

| Treatment | Chlorophyll a (mg/g FW) | Chlorophyll b (mg/g FW) | Total Chlorophyll (mg/g FW) | Carotenoids (mg/g FW) |
|---|---|---|---|---|
| **C** | 2.61 ± 0.040 | 1.21 ± 0.076 | 3.82 ± 0.036 | 2.40 ± 0.012 |
| **$T_1$** | 2.45 ± 0.002[b] | 1.16 ± 0.004 | 3.61 ± 0.007 [a] | 2.28 ± 0.005 |
| **$T_2$** | 2.13 ± 0.041[a] | 1.06 ± 0.033 | 3.20 ± 0.007 [a] | 1.87 ± 0.322 |
| **$T_3$** | 1.46 ± 0.040[a] | 0.92 ± 0.076[a] | 2.38 ± 0.036 [a] | 1.19 ± 0.501 [b] |
| **$T_4$** | 1.28 ± 0.005[a] | 0.78 ± 0.018[a] | 2.07 ± 0.012 [a] | 1.15 ± 0.001 [b] |

Data are means of 3 replicates ± SEM,
[a] Treatment significantly ($P < 0.001$) different to the control,
[b] Treatment significantly ($P < 0.01$) different to the control (C). C: Control, $T_1$: 0.1 mM, $T_2$: 0.5 mM, $T_3$:,1.0 mM, and $T_4$:1.5 mM concentrations of FA.

A significant reduction in protein content was also recorded under FA treatment. The maximum decrease (16.55%) in protein was related to 1.5 mM of FA as compared with control, while the other concentration of FA decreased significantly the protein content too. The inhibitory effect of FA showed a maximum 17.96% reduction in sugar content under the highest concentration of FA while the reduction was concentration dependent. The NR activity in the leaves of the treated tomato seedlings was adversely affected by FA treatment as compared with control. NR activity significantly decreased in a dose dependent manner. The maximum inhibition (44.63%) was recorded with 1.5 mM of FA as compared with control (Table 3).

**Table 3.** Effects of ferulic acid (FA) applied at different concentrations on protein and sugar content and nitrate reductase activity of tomato seedlings

| Treatment | Protein (mg/g FW) | Sugar (mg/g FW) | NR (µmol $NO_2$ $g^{-1}$ FW $h^{-1}$) |
|---|---|---|---|
| **C** | 61.91 ± 0.028 | 91.47 ± 0.583 | 20.23 ± 0.631 |
| **$T_1$** | 59.28 ± 0.072 [a] | 86.92 ± 0.291 [a] | 19.02 ± 0.288 [b] |
| **$T_2$** | 59.06 ± 0.028 [a] | 85.15 ± 0.145 [a] | 15.86 ± 0.126 [a] |
| **$T_3$** | 56.20 ± 0.028 [a] | 79.84 ± 0.291 [a] | 12.17 ± 0.090 [a] |
| **$T_4$** | 51.65 ± 0.202 [a] | 75.04 ± 0.729 [a] | 11.20 ± 0.072 [a] |

Data are means of 3 replicates ± SEM,
[a] Treatment significantly ($P < 0.001$) different to the control,
[b]. Treatment significantly ($P < 0.01$) different to the control. C: Control, $T_1$: 0.1 mM, $T_2$: 0.5 mM, $T_3$:1.0 mM, and $T_4$:1.5 mM concentrations of FA.

Activities of antioxidant enzymes (SOD, CAT, POX) increased significantly ($P < 0.001$) in response to FA. A maximum 60, 33 and 19% stimulation were recorded for SOD, CAT and POX activities with concentration of 1.5 mM treatment, respectively (Table 4).

**Table 4.** Effects of ferulic acid (FA) applied at different concentrations on antioxidant enzyme activities of tomato seedlings

| Treatment | SOD (Enzyme Unit/g FW) | CAT (Enzyme Unit/g FW) | POX (Enzyme Unit/g FW) |
|---|---|---|---|
| **C** | 22.31 ± 0.076 | 6.27 ± 0.816 | 70.35 ± 0.317 |
| **$T_1$** | 23.17 ± 0.038 | 7.15 ± 0.034 | 74.05 ± 0.692 [a] |
| **$T_2$** | 27.87 ± 0.903 [a] | 7.75 ± 0.035 [b] | 74.95 ± 0.115 [a] |
| **$T_3$** | 33.3 ± 0.653 [a] | 7.87 ± 0.035 [b] | 79.57 ± 0.158 [a] |
| **$T_4$** | 35.79 ± 0.057 [a] | 8.36 ± 0.035 [a] | 83.97 ± 0.793 [a] |

Data are means of 3 replicates ± SEM,
[a] Treatment significantly ($P < 0.001$) different to the control,
[b] Treatment significantly ($P < 0.01$) different to the control. C: Control, $T_1$: 0.1 mM, $T_2$: 0.5 mM, $T_3$,: 1.0 mM, and $T_4$:1.5 mM concentrations of FA.

## DISCUSSION

Allelochemicals released into the surrounding by evaporation, exudation, leaching, and residues decomposition affect the neighboring plants. Allelochemicals accumulate in the soil (47, 52) and affect the growth and metabolism of neighboring plants (47). Exposed plants to allelochemical undergo a drastic change in their biophysical and biochemical processes. Reduction in plant growth was common feature under allelopathic conditions as reported in literature (10, 44, 45, 46, 56). Decrease in RL and SL, FW and DW was the manifestation of impaired metabolic activities due to allelochemicals (41, 47).

The observed significant reduction in photosynthetic pigment contents (chl a, chl b, chl t) and carotenoids of tomato seedlings treated by FA are in complete agreement with the results reported by

Kanchan and Jayachandra (33) and Singh *et al*. (46). It is known that photosynthetic rate is dependent of pigment contents and the plant dry matter is closely related to chlorophyll content (16, 47).

Protein and sugar contents decreased significantly in response to FA application in to the growth medium of the tomato seedlings. This result is similar to what has been reported about the effect of allelochemicals with protein biosynthesis (47).

As it was the case for the protein content, the activity of NR decreased dependently of FA concentrations applied in the experiment. The reduced tomato plant growth and NR activity could be attributed to a decreased nitrate absorption by tomato plant roots as reported in previous researches (1, 47). It has been also reported that allelochemicals caused oxidative damage which induces antioxidant enzymes (5, 53). The prior findings are in support of the results relative to SOD, CAT and POX accumulation as form of tolerance to FA.

In the present study, FA has shown an inhibitory activity on tomato seedlings growth. Decreased pigment content, protein, sugar and NR activity indicated the allelopathic potential of FA. However, tomato seedlings induced antioxidative enzymes (SOD, CAT, POX) accumulation to mitigate the adverse effects of allelochemicals.

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**RESUME**

**Singh N.B. et Sunaina. 2014. Potentiel allélopathique de l'acide of férulique sur la tomate. Tunisian Journal of Plant Protection 9: 1-9.**

Cette étude porte sur les effets allélopathiques d'une application exogène d'acide férulique (FA) sur les paramètres biophysiques et biochimiques de plantules de tomate (cv. Pusa ruby) cultivées hydroponiquement. FA a montré des effets phytotoxiques sur les plantules de tomate cultivées dans une solution Hoagland. Après 7 jours de croissance, FA a été ajouté au milieu de culture à des concentrations de 0,1, 0,5, 1,0 et 1,5 mM. Une solution Hoagland sans FA a été utilisée pour les unités expérimentales. La longueur de la tige et des racines, les poids frais et sec des plantules, les teneurs en chlorophylle a (chl a), chlorophylle b (chl b), chlorophylle totale (chl t), caroténoïdes, protéines et sucre, l'activité nitrate réductase (NR) et l'accumulation d'enzymes antioxidantes ont été examinées en une période de temps de 25 jours. FA a montré des effets inhibiteurs significatifs sur certaines caractéristiques biophysiques et biochimiques. Une chute graduelle de toutes les caractéristiques étudiées a été observée avec les traitements à base de FA. La longueur des racines et de la tige ainsi que les poids frais et secs des plantules ont été significativement réduits d'une manière dépendante de la dose sous le potentiel allélopathique de FA. Les teneurs en chl a, chl b, chl t et caroténoïdes ont diminué avec l'augmentation des concentrations de FA. Une réduction significative des teneurs en sucre et en protéines et dans l'activité NR a été enregistrée surtout avec les concentrations de FA les plus élevées utilisées. Les concentrations de FA les plus élevées ont significativement augmenté les activités des enzymes antioxidantes [superoxide dismutase (SOD), catalase (CAT) et péroxidase (POX)] qui semble être une forme de tolérance et/ou de défense aux allélochimiques utilisés.

*Keywords*: Acide férulique, allélopathie, antioxidants, culture hydroponique, solution Hoagland, tomate.

## ملخص

**سينغ، نارسينغ باهادور وسونينة. 2014. القدرة على المجاهضة لحامض الفيروليك على الطماطم.**
**Tunisian Journal of Plant Protection 9: 1-9.**

تهتم هذه الدراسة بالتأثيرات المجاهضة لمعاملة خارجية لحامض الفيروليك (FA) على المقاييس البيوفيزيائية والبيوكيميائية لنبتات الطماطم (صنف Pusa ruby) المزروعة في الوسط المائي. لقد أظهر FA تأثيرات سمية على نبتات الطماطم المزروعة في محلول Hoagland. بعد 7 أيام من النمو، تمت إضافة FA إلى الوسط المائي بتركيزات 0.1 و 0.5 و 1.0 و 1.5 ميليمول. تم استعمال محلول Hoagland بدون إضافة FA في وحدات التجارب. وتم تتبع طول الساق والجذور والوزن الرطب والجاف للنبتات وكميات اليخضور أ (chl a) واليخضور ب (chl b) و اليخضور الكامل (chl t) والكاروتينات والبروتينات والسكر ونشاط اختزال النيترات وتراكم الأنزيمات المضادة للأكسدة وذلك لمدة 25 يوما. أظهر FA تأثيرات مثبطة ملحوظة على المقاييس البيوفيزيائية والبيوكيميائية. و لوحظ انخفاض متدرج في كل المقاييس تبعا لمعاملات المعالجة بـ FA. تقلص طول الجذور والساق والوزن الرطب والجاف للنبتات حسب التركيز تحت القدرة على المجاهضة لدى FA. وتقلص محتوى chl a وchl b وchl t والكاروتينات عند الزيادة في تركيزات FA. سُجل انخفاض ملحوظ في كميات السكر والبروتينات ونشاط اختزال النيترات مع التركيزات العالية لـ FA. وحفّزت التركيزات العالية بصفة ملحوظة نشاطات الأنزيمات المضادة للأكسدة [ superoxide dismutase (SOD) والكاتالاز (CAT) والبيروكسيداز (POX) ] التي تبدو كنوع من التحمل و/أو المقاومة لمواد المجاهضة المستعملة.



*كلمات مفتاحية*: حامض الفيروليك، المجاهضة، عناصر مضادة للأكسدة، زراعة في الوسط المائي، محلول Hoagland


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