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Callistemon Citrinus, Cymbopogon Citratus, and Oxalis Barrelieri Extracts Stimulate Defence of Tomato Against *Fusarium* Wilt

Dakole Daboy Charles[1, *], Dongmo Lekagne Joseph Blaise[1], Fonkoua Martin[1], Nguefack Julienne[1], Irénée Somda[2]

[1]Department of Biochemistry, Faculty of Science, University of Yaounde 1, Yaounde, Cameroon

[2]Department of Biology and Agronomy, Rural Institute of Development, Nazi Boni University of Bobo-Dioulasso, Bobo-Dioulasso, Burkina Faso

Email address:

cdakole@yahoo.fr (Dakole Daboy Charles)

[*]Corresponding author

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Abstract: In Cameroon, tomato yields remain low due to attacks by pathogens and insects. *Fusarium oxysporum* f.sp. *lycopersici* (FOL) is a fungus responsible for *Fusarium* wilt, a disease responsible for important economic losses. To contribute to the control of this pathogen, the stimulatory effect of the tomato defence system of extracts of plants in the tomato/FOL interaction was evaluated. Tomato plants were treated with the aqueous extracts (AE) of *Callistemon citrinus* (*C. citrinus*), *Cymbopogon citratus* (*C. citratus*), and *Oxalis barrelieri* (*O. barrelieri*) at 10% (W/V). After 4 days of spraying with the extracts, the plants were inoculated with a virulent strain of *Fusarium oxysporum* f.sp. *lycopersici* (FOL) in pots experiments. Tomato roots were used to determine the contents of phenols, proteins, carbohydrates, amino acids (AA) and proline. The activities of antioxidant enzymes were evaluated: ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (GPX) and superoxide dismutase (SOD). The results showed that treatment of tomato plants with extracts and their infection with FOL induced an increase in the contents of phenols, proteins, carbohydrates, lipids, amino acids and proline in tomato roots, an increase in APX, GPX, SOD activities and a reduction in CAT activity. Our results suggest that the increase and reduction of enzymatic activities, and the increase in the synthesis of some metabolites could mitigate the oxidative damage that takes place during the expansion of the pathogen. Aqueous extracts of *C. citrinus*, *C. citratus* and *O. barrelieri* could be used as natural products to stimulate the tomato defence system against FOL.

Keywords: Tomato, *Fusarium* Wilt, Plant Extracts, Defence System

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is a plant belonging to the Solanaceae family and is a vegetable cultivated in almost all countries of the world. Tomato has a high biological value due to its content in carotenes, vitamins C and E and lycopene and a balanced content of mineral elements [2]. Its global production in 2017 was estimated at 182.30 million tons with an average yield of 188 tons/ha. In Cameroon, tomato remains the most important vegetable in terms of production. In 2017, its production was estimated at 1.27 million tons with a yield of 12.14 tons/ha [18]. This yield is far from the world yield, this gap is mainly justified by many constraints faced by tomato cultivation. Among these, the most striking in Cameroon are adverse climatic conditions and pests, susceptibility to diseases (alternariosis, fusariosis, mildew...) [30]. Indeed, tomatoes are the target of many bacterial and fungal diseases. Phytosanitary analysis of crops and sampling carried out in tomato growing sites in Cameroon in the Center (Yaounde) and Western (Dschang) Regions revealed a recurrence of fungal diseases caused by

several fungi including *Fusarium oxysporum* f.sp. *lycopersici* that causes leaf yellowing, wilting and damping-off. This plant pathogenic fungus causes field losses ranging from 46 to 100%.

Nowadays, the control of most biotic plant diseases is mainly based on the very intensive use of synthetic chemical bactericides and fungicides [20]. However, despite the acceptable results, chemical control has several limitations, namely environmental pollution, high cost of synthetic chemicals, the risk of pathogen resistance to chemical substances, and human intoxication [37]. Thus, the use of natural substances (essential oils and solvent extracts) as plant disease control agents is receiving increasing attention. Plant extracts have several advantages, including a low negative impact on man and the environment, biodegradability, systemic action, simplicity of use and easy access to raw material [1].

Plant extracts contain compounds that not only have a direct antimicrobial effect on the pathogen, but also stimulate the plant's natural defences, making them one of the most promising alternatives among crop protection strategies [35]. Studies have shown that spraying rice plants with *Datura metel* leaf extracts can induce systemic resistance of this plant against *Rhizoctonia solani* and *Xanthomonas oryzea* pv. *oryzea* through the accumulation of pathogenicity-related proteins. Treatment of plants with aqueous neem extracts has enabled plants to resist pathogenic microorganisms and control several fungal diseases through metabolic changes (accumulation of phenolic compounds and antioxidant defence enzymes) [17].

The objective of this study was to evaluate the stimulating effect of the tomato defence system against *Fusarium oxysporum* f.sp. *lycopersici* of extracts of some tropical plants through the determination of the contents of some biomolecules phenols, proteins, carbohydrates, amino acids (AA) and proline in roots and the determination of the activities of the main antioxidant enzymes (ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (GPX), superoxide dismutase (SOD).

2. Materials and Methods

2.1. Plant Material

Three (3) plants from the traditional Cameroonian pharmacopoeia were used. The choice was made taking into account their ethnobotanical uses and knowledge of their antimicrobial potential [15]. These are *Callistemon citrinus* L. *(C. citrinus*), *Cymbopogon citratus* (DC), STAPF (*C. citratus*), and *Oxalis barrelieri* L. (*O. barrelieri*). These plant species were collected in Yaoundé in August 2020, and identified at the National Herbarium of Cameroon in Yaounde with the numbers 10356 SRF/Cam (YA), 18628 SRF/Cam (YA) and 19798 SRF/Cam (YA) respectively. The collected plants and plant parts were dried at room temperature (25-27°C) for 10 to 14 days. The dry plant material of each species was carefully crushed with a grinder. The powders were weighed and delipidated with pure hexane and stirred for 24 hours. The mixture was filtered on a mesh screen (diameter=150 µm) and the residue was collected and dried at room temperature until the hexane was completely evaporated. The dry residue thus delipidated was reintroduced into distilled water, followed by stirring and filtration with the grid cloth. The residue obtained was recovered and dried at room temperature. The filtrate was kept and the pellet redissolved in distilled water for a second period of 24 hours. The mixture was filtered again and the filtrate was retained. The filtrates obtained were then centrifuged using a centrifuge (Hettich universal 320) at 7000 rpm for 10 min. The supernatants were lyophilised using a lyophiliser (Millrock Technology Epic Series). The resulting powder was weighed and stored in labelled vials at -4°C.

Seeds of tomato variety "Roma VF" were bought at the Mfoundi market (Yaounde).

2.2. Fungal Material

Fusarium oxysporum f.sp. *lycopersici* Snyder & Hansen, the causal agent *Fusarium* wilt of tomato was isolated from field-harvested tomato roots, leaves and stems showing symptoms. Their isolation and identification were made at the Phytopathology Laboratories of the Agricultural Research Institute for Development (IRAD) in Nkolbisson (Yaounde) and the Institute of Rural Development (IDR) of the Polytechnic University of Bobo-Dioulasso (Burkina Faso). The identification was carried out according to Agarwal et al. [3] and Mathur and Kongsdal [26] and confirmed by the Phytopathology Laboratory of the Danish Seed Health Center (DSHC), Copenhagen (Denmark). The pathogenicity of these strains was proven on 20-day-old tomato plants from which the same pathogens were re-isolated. *Fusarium oxysporum* f.sp. *lycopersici* Snyder & Hansen was maintained on PDA (potato dextrose agar) medium in 90 mm diameter Petri dishes at 20 ± 2 °C in the dark. Fourteen-day cultures were used for inoculation of tomato plants.

2.3. Evaluation of the Defence System Stimulating Potential of Plant Extracts (C. Citrinus, C. Citratus, O. Barreleri) in the Tomato/Fol Interaction

2.3.1. Preparation of Plant Extracts, Salicylic Acid Solutions and Treatment of Tomato Seedlings with Plant Extracts

Aqueous macerates of *C. citrinus*, *C. citratus*, and *O. barrelieri* were prepared at 10% (W/V) and were used to spray tomato plants.

Salicylic acid, the natural plant defence stimulator was used as a positive control and prepared at 0.01% in methanol at 10% (V/V) as described by Mandal et al. [25].

The tomato seeds were sown in a nursery tray containing sterile sand/soil/clay (1/1/1) soil and fertilised with urea at 2 g/cm^3. The plants were left to grow with regular watering for 14 days, then transplanted into 5 litre pots and transferred to a controlled greenhouse with a relative humidity of 60-80%, and a temperature of 25°C/15°C (day/night). Ten (10) days

after transplanting, the tomato plants regularly watered were divided into 5 batches: the 1st batch consisted of control plants sprayed with distilled water (S); the 2nd batch consisted of plants treated with *C. citratus* (CYMB) aqueous extract at 10% (Tr Cymb); the 3rd batch consisted of plants treated with *O. barrelieri* (OX) aqueous extract at 10% (Tr Ox); the 4th batch consisted of plants treated with *C. citrinus* (CALL) aqueous extract at 10% (Tr Call) and the 5th batch consisted of plants treated with salicylic acid (Tr AS).

After 4 days of treatment (spraying), each batch was divided into 2 subgroups: Subgroup 1 consisted of tomato plants of each of the above treatments but not inoculated with FOL: S, Tr Cymb, Tr Ox, Tr Call, Tr AS; Subgroup 2 consisted of tomato plants from each of the above treatments inoculated with FOL: I (S having been inoculated), (Tr Cymb+I), (Tr Ox+I), (Tr Call+I), (Tr AS+I).

For each treatment, 5 replicates were conducted independently and the pots were arranged in the greenhouse in a completely randomised design.

2.3.2. Inoculation of the Pathogen

From a 14-day FOL culture on PDA medium, the fungal suspension was prepared and adjusted to 5×10^5 conidia/ml. Five (5) ml of the conidia suspension was homogenised and introduced into the soil at the base of each stem near the root zone of the plants. The inoculated plants were covered with black polyethylene bags for 48 h to create adequate moisture for pathogen growth and development. Uninoculated tomato plants were used as controls. For biochemical assays, roots were collected on the day of inoculation and at 2-day intervals for 10 days. These samples were washed with tap water, wrung out, weighed and frozen for further analysis.

2.3.3. Extraction and Determination of Total Phenols

The extraction of the phenolic compounds was carried out according to the method described by Mbouobda et al. [29]. Thus, 5 g of root were ground in a porcelain mortar containing sterile fine sand in a volume of 15 ml of 80% methanol. The grind was incubated for 30 min at 4° C and then centrifuged at 10000 g for 20 min at 4° C. The supernatant was recovered and represents the crude extract of phenolic compounds, stored at -20° C for subsequent analysis. The content of phenolic compounds was determined by the method described by Macheix et al. [23]. The results were expressed as mg chlorogenic acid equivalent. g^{-1} fresh matter.

2.3.4. Extraction and Determination of Total Soluble Proteins

The extraction of the total soluble proteins was carried out according to the method described by Mbouobda et al. [27]. Two (2) grams of roots were ground cold (4° C.) in 4 ml of TAMET buffer (Tris 0.5 M; 0.3M ascorbic acid; 2% permethylethanol; EDTA 0.01 M; Triton x100, 2%, pH 7) supplemented with the addition of polyvinyl pyrrolidone (PVP) and sterile fine sand. The grind was incubated for 30 min at 4° C and then centrifuged at 10,000 g for 20 min. The recovered supernatant constituting the total soluble protein extract was stored at -20° C. for subsequent analysis. The amount of protein in the crude extract was determined using the Bradford method (1976) with bovine serum albumin (BSA) as standard. The protein content was expressed in $mg.g^{-1}$ of fresh matter.

2.3.5. Determination of Carbohydrates

The plant samples were boiled in 80% ethanol for extraction. Test tubes containing 1 ml of ethanolic extract each were placed into the water bath for evaporation. The residue and 1 ml of distilled water were incubated at 49° C. for 30 min. The solution was then neutralised with 1N NaOH using methyl red as a colour indicator. 1 ml of Nelson's reagent was added to each tube. The tubes were heated for 20 min in a boiling water bath, were cooled and 1 ml of the arsenomolybdate was added. The solution was mixed well and diluted to obtain 25 ml and the OD was measured at 495 nm using a spectrophotometer. The concentration of reducing sugars was calculated from the glucose standard and expressed in $\mu g.g^{-1}$ of fresh matter [33].

2.3.6. Determination of Total Free Amino Acids and Proline Content

In test tubes, 1 ml of ethanol extract from the roots was taken up in 25 ml of distilled water and neutralised with 0.1N NaOH using methyl red as a colour indicator [31]. 1 ml of ninhydrin was added and the mixture was heated in a boiling water bath for 20 min, then 5 ml of a dilution solution (distilled water/n-propanol in equal volume) was added. This was followed by cooling and dilution to 25 ml. The absorbance was measured at 570 nm with a spectrophotometer. The standard line uses a leucine solution. The total amino acid content was expressed in $mg.g^{-1}$ of fresh matter.

The Proline Assay was carried out on a sample of 100 mg of fresh material taken and placed in a test tube to which 2 ml of 40% methanol was added. The sample was heated for 1 hour in a water bath at 85°C. After cooling, 1 ml of the extraction solution was added to 1 ml of the mixture (distilled water/acetic acid/ortho-phosphoric acid) (6/15/4) and 2 ml of ninhydrin. The mixture was brought to a boil at 100°C for 30 min in a water bath [6]. After cooling, 5 ml of toluene was added after vortexing. The OD reading was taken at 528 nm. The proline content was calculated from the proline standard and expressed as $\mu g.g^{-1}$ of fresh material.

2.3.7. Assessment of Ascorbate Peroxidase (APX) Activity

APX activity (EC 1.11.1) was assessed according to the method of Nakano and Asada [32]. Enzyme activity was determined by following the decrease in absorbance of ascorbate at 290 nm. The reaction medium consisted of: 50 mM phosphate buffer, pH 7; 0.5 mM ascorbic acid; H_2O_2 0.1 mM (5 / 2 /1) (V/V/V) in a final volume of 5 ml. To each test tube containing 5 ml of the reaction medium, the enzyme extract (10µl) was added. The optical density was read at 290 nm against a blank in which the enzyme extract is replaced by the extraction buffer. The molar extinction coefficient 2.8 $mM^{-1}.cm^{-1}$ was used to calculate the ascorbate peroxidase activity expressed as moles of H_2O_2 reduced. $min^{-1}.g^{-1}$ of

fresh material. One Unit of Ascorbate Activity is required for the reduction of 1 mole of H_2O_2 per minute at 25°C.

2.3.8. Assessment of Catalase (CAT) Activity

CAT activity (EC.1.11.1.6) was assessed by quantifying the disappearance of H_2O_2 according to the method described by [11]. The reaction medium (2 ml) consists of 25 mM phosphate buffer pH 7 and H_2O_2 10 mM. To this, 0.2 ml of the enzyme extract was added and the mixture was vortexed. The optical density was read at 290 nm. A CAT activity unit was defined as the change in optical density of 0.01 per minute per g of fresh plant material.

2.3.9. Evaluation of Guaiacol Peroxidase (GPX) Activity

GPX activity (EC.1.11.1.7) was assessed by determining the increase in absorbance at 470 nm due to the oxidation of guaiacol to tetraguaiacol according to the method described by Chance and Maehly [13]. The reaction mixture consisted of 20 mM guaiacol (0.5 ml); 0.1 mM acetate buffer pH 5 (2.1 ml); H_2O_2 40 mM (0.2 ml) and enzyme extract (0.2 ml) for a final volume of 3 ml. GPX activity was expressed in terms of Activity Unit per gram of fresh material and one Unit represents the amount of enzyme catalysing the oxidation of 1 µmol guaiacol per min per g of fresh material.

2.3.10. Assessment of Superoxide Dismutase (SOD) Activity

SOD activity (EC.1.15.1.1) was assessed using the method described by Beauchamp and Fridovich [7]. It measures the ability of the enzyme to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT). The reaction medium (3 ml) consists of: 50 mM phosphate buffer pH 7.8; 13mM L-methionine; NBT 75µM; EDTA 1 mM; 0.05M sodium carbonate; 20 µl of enzyme extract. Riboflavin 2µM was added after all reagents and the reaction was initiated by placing the test tube 30 cm below a 15W ampoule for 10 min at 25°C. The tube was then transferred into the dark for 10 min and the optical density read at 560 nm. One SOD Activity Unit was defined as the amount of enzyme required to inhibit the reduction of NBT by 50%. SOD activity was expressed as Units per minute per g of fresh plant material.

2.4. Statistical Analyses

The results obtained were subjected to statistical analysis for the calculation of means, standard deviations and the search for significant differences, using the SPSS 22.1 software. The one-way ANOVA test coupled with the Duncan tests was used to evaluate the Smallest Significant Difference (PPDS) at $P < 0.05$.

3. Results

3.1. Effect of Plant Extracts on Phenol Content

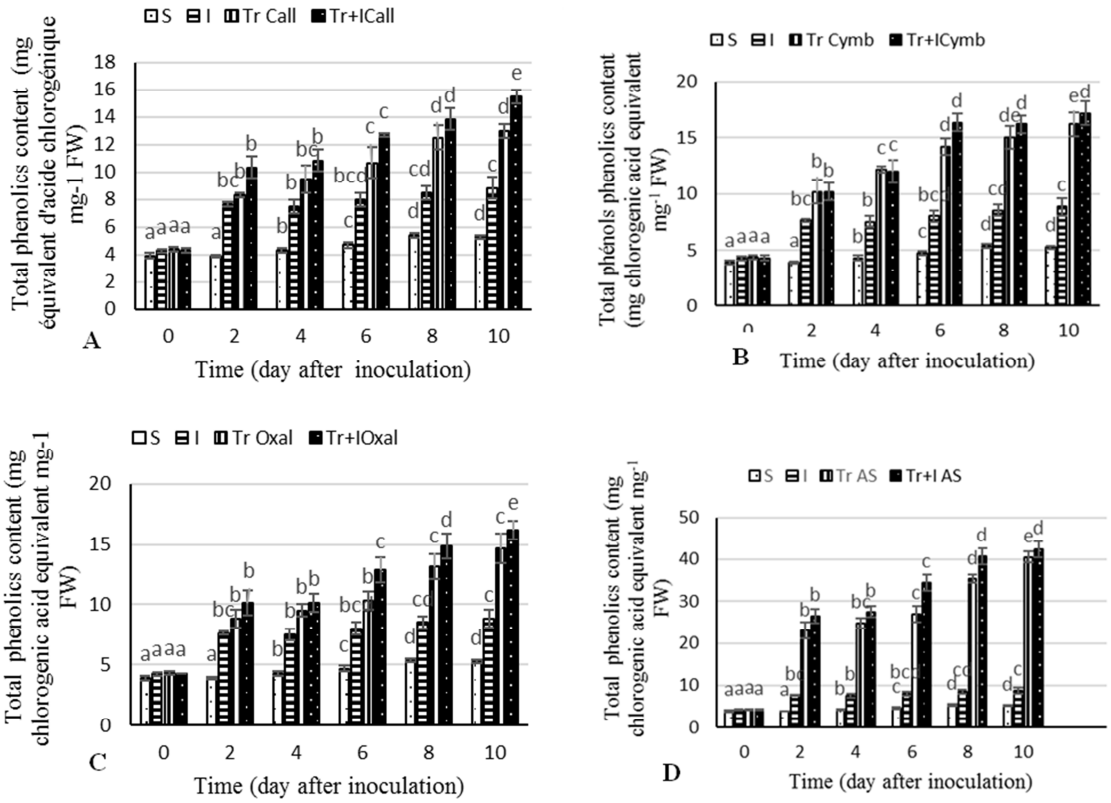

Figure 1. *Phenolics content (expressed in mg chlorogenic acid equivalent mg^{-1}) in roots of tomato plants on a time course after treatment with plant extracts and inoculation with FOL and in the control. The data presented are the means ± SD of five replicates. Different letters a, b, c, d, e, for the same treatment indicate a significant difference (P<0.05) between values, Duncan test.*

The phenol content was 3.90 ± 1.53 mg equivalent chlorogenic acid.mg^{-1} FW on day 0 in healthy plants (S). When plants are inoculated with FOL (I), there was a significant ($P < 0.05$) and progressive increase over time. On day 10, the phenol content increased by more than 106.79%. In the treated plants (Tr), the phenol content increased as early as day 2, this increase continued gradually and significantly over time. On day 10 the increase was 197.48%, 270.02%, 235.69% respectively with *C. citrinus*, *C. citratus*, and *O. barreleri*. In the treatment and inoculation condition (Tr+I), the phenol content increases significantly and gradually from day 2. This content increased gradually over time and reached the maximum at day 10 for all treatments. On day 8, increases ranged from 260.46% to 876.74%. The phenolic compounds content was higher in the treated and inoculated plants (Tr + I) than in simply treated plants (Figure 1).

The data presented are the means ± SD of five replicates. Different letters for the same treatment indicate a significant difference ($P<0.05$) between values, Duncan test. A: *C. citrinus*; B: *C. citratus*; C: *O. barrelieri*; D: Salicylic acid. S: control plants; I: plants inoculated with FOL; Tr C. *citrinus*, Tr *C. citratus*, Tr *O. barrelieri*, Tr Salicylic acid: plants treated respectively with *Callistemon citrinus*, *Cymbopogon citratus*, *Oxalis barreleri*, salicylic acid. Tr + I *C. citrinus*, Tr + I *C. citratus*, Tr + I *O. barrelieri*, Tr + I Salicylic acid: Plants treated with *Callistemon citrinus*, *Cymbopogon citratus*, *Oxalis barreleri*, salicylic acid respectively and inoculated with FOL.

3.2. Effect of Plant Extracts on Protein Content

In healthy plants, the protein content was 16.16 ± 1.80 mg.g^{-1} FW on day 0. It increased significantly ($P < 0.05$) from day 2 and remained stable until day 10. When the plants were inoculated (I), the protein content decreased on day 2 by 72.24%, remained low until day 6 and then increased on days 8 and 10. When the plants were treated with plant extracts (Tr), the protein content gradually and significantly increased over time by more than 100%. In general, inoculation after treatment with plant extracts (Tr + I) resulted in a significant and progressive increase in protein content over time. (Figure 2).

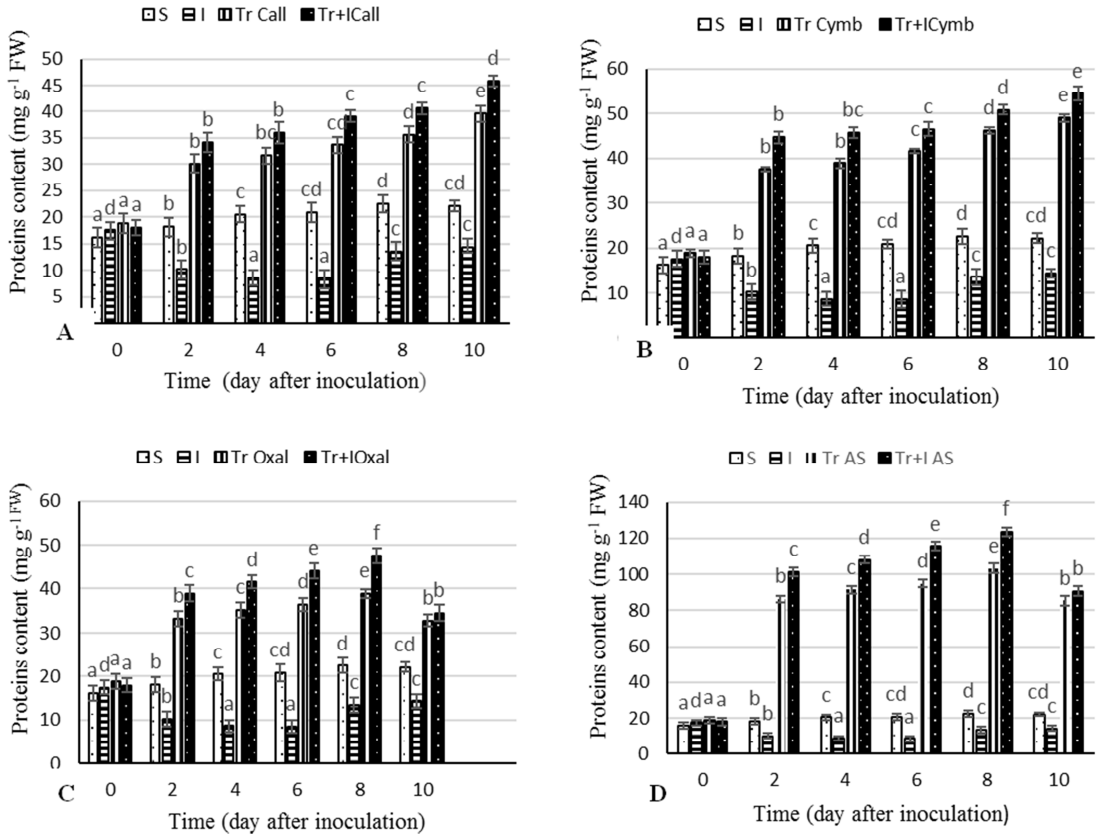

Figure 2. *Proteins content (expressed in mg.g^{-1} FW) in roots of tomato plants on a time course after treatment with plant extracts and inoculation with FOL and in the control. The data presented are the means ± SD of five replicates. Different letters a, b, c, d, e, for the same treatment indicate a significant difference (P<0.05) between values, Duncan test.*

3.3. Effect of Plant Extracts on Carbohydrates Content

In healthy plants (S), the carbohydrate content was 8.50 ± 1.32 mg g^{-1} FW on day 0, which did not vary significantly from day 2 to day 8. When the plants were inoculated (I), the carbohydrate content increased gradually and significantly ($P < 0.05$) with a peak on day 6. After treatment with plant extracts, this content increased by more than 100% from day

2 for all treatments and reached a maximum value on day 10. In the inoculated plants after treatment (Tr+I), there was a significant increase in the carbohydrate content from day 2. The percentage increases were 111.76%, 139.17% and 225.88% for *C. citrinus*, *C citratus*, *O. barrelieri* respectively. This content increased gradually over time. In general, the increase in this content was greater than in treated plants (Tr) (Figure 3).

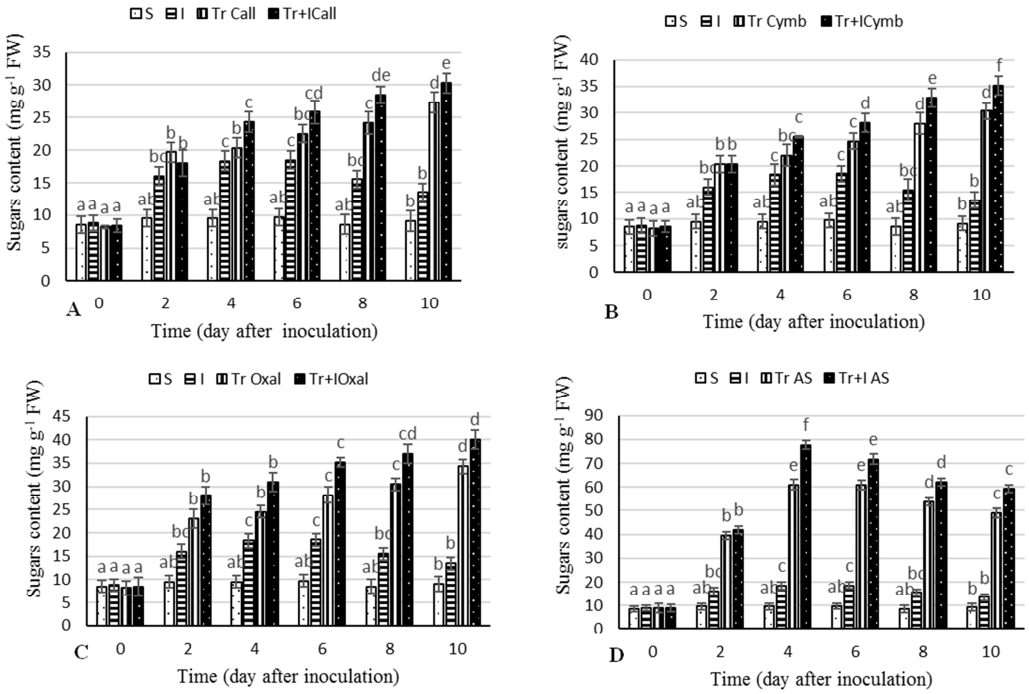

Figure 3. *Sugars content (expressed in mg.g^{-1} FW) in roots of tomato plants on a time course after treatment with plant extracts and inoculation with FOL and in the control. The data presented are the means ± SD of five replicates. Different letters a, b, c, d, e, f, for the same treatment indicate a significant difference (P<0.05) between values, Duncan test.*

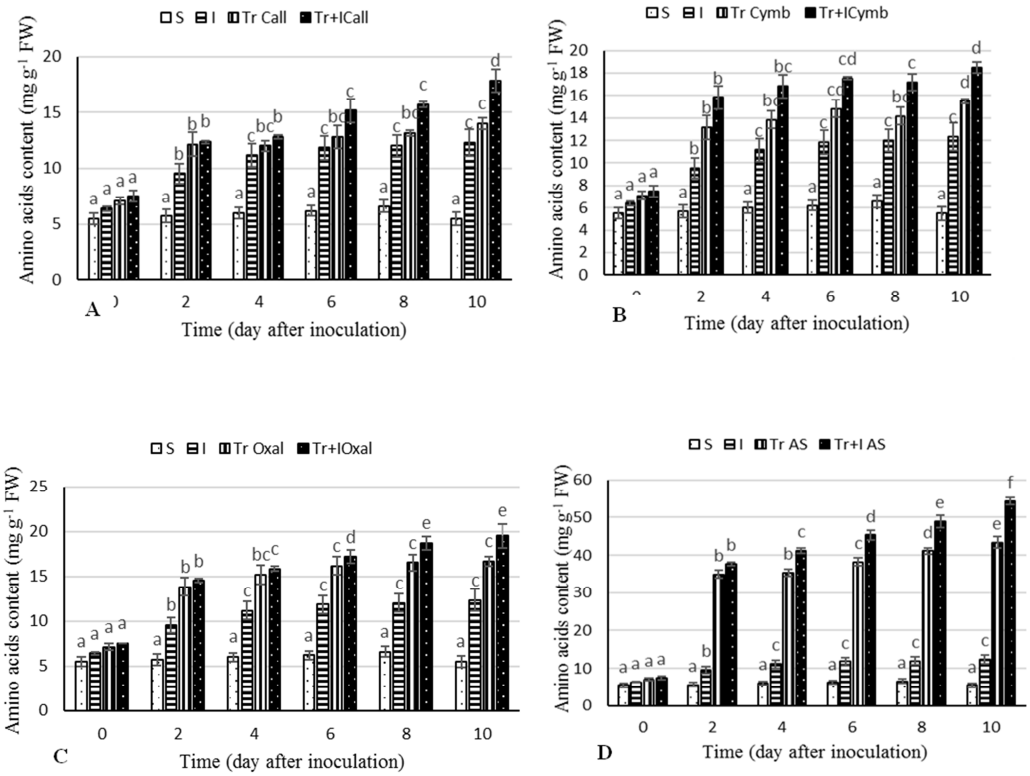

Figure 4. *Amino acid content (expressed in mg.g^{-1} FW) in roots of tomato plants on a time course after treatment with plant extracts and inoculation with FOL and in the control. The data presented are the means ± SD of five replicates. Different letters a, b, c, d, e, f, for the same treatment indicate a significant difference (P<0.05) between values, Duncan test..*

3.4. Effect of Plant Extracts on Amino Acid Content

In healthy plants (S), the amino acid content was 5.50 ± 0.50 mg.g^{-1} FW. When the plants were inoculated (I), the amino acid content increased gradually and significantly ($P < 0.05$) with a maximum content on day 10 with an increase of 92.65%. After treatment with plant extracts, there was a significant and gradual increase. From day 2, the percentage increase was 71.40%, 85.49%, and 94.78% respectively with *C. citrinus, C. citratus*, and *O. barrelieri*. On day 10, it was 2 to 6 times higher depending on the treatment. In plants inoculated after treatment (Tr + I), there was a significant and gradual increase in amino acid content from day 2. The percentages of increase on day 2 were 64.93%, 111.06%, and 93.33% respectively with treatments with *C. citrinus*, *C. citratus*, and *O. barrelieri*. In general, the amino acid content in treated and inoculated plants (Tr + I) was higher than treated plants (Tr) (Figure 4).

3.5. Effect of Plant Extracts on Proline Content

In healthy plants (S), the proline content on day 0 was 0.38 ± 0.02 µg.g^{-1} FW. When the plants were inoculated (I), there was a significant increase in the proline content ($P < 0.05$) from day 2 to 205.55%. Treatment of plants with plant extracts resulted in a gradual and significant increase in the proline content over time. A maximum value was obtained on day 10. The increase on day 2 was more than 100% for most treatments. In post-treatment inoculated plants (Tr + I), proline content increased gradually and significantly in the same way as in treated plants (Tr). The percentages of increase were more than 100% from Day 2 and on day 10, maximum values were obtained. In general, the proline content was greater in the treated and inoculated plants (Tr+I) than in treated plants (Tr) over time (Figure 5).

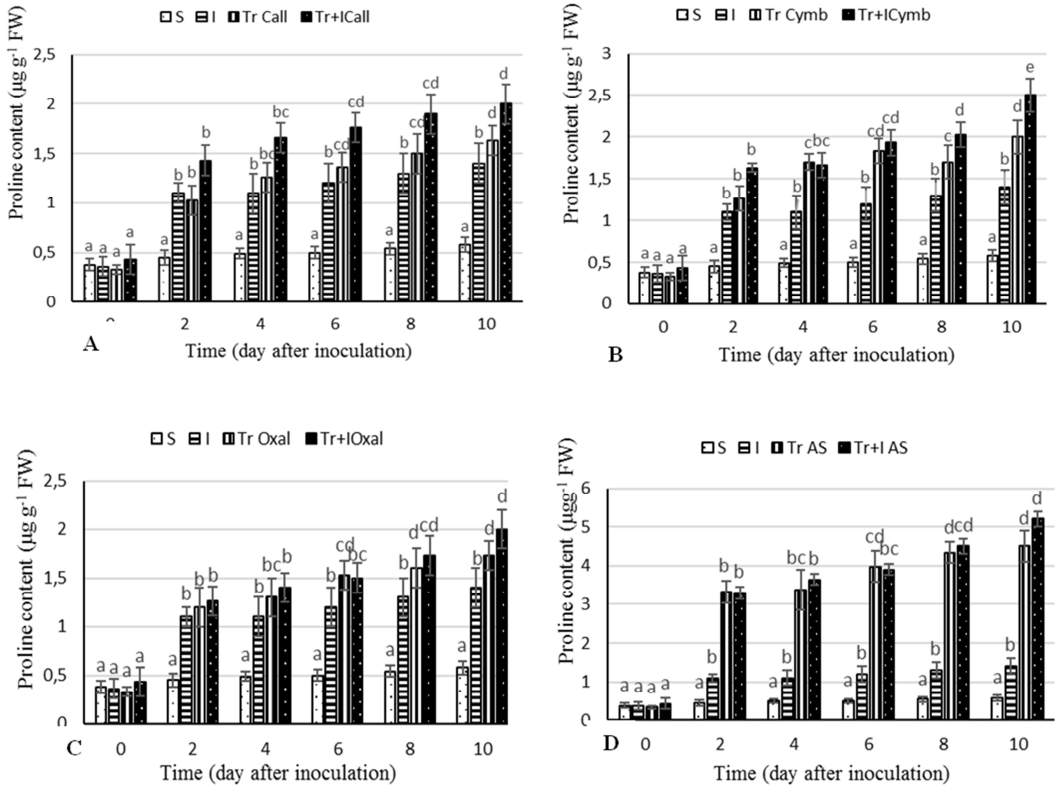

Figure 5. *Proline content (expressed in µg.g^{-1} FW) in roots of tomato plants on a time course after treatment with plant extracts and inoculation with FOL and in the control. The data presented are the means ± SD of five replicates. Different letters a, b, c, d, for the same treatment indicate a significant difference (P<0.05) between values, Duncan test.*

3.6. Ascorbate Peroxidase (APX) Activity

In healthy plants (S), ascorbate peroxidase (APX) activity ranged from 5.46 ± 0.25 U.g^{-1} FW to 4.66 ± 0.52 U.g^{-1} FW from day 0 to day 10. When plants were inoculated (I), APX activity increased significantly ($P < 0.05$) with a peak of activity on day 4. APX activity then gradually decreased until day 10 with values that remained higher than in healthy plants. Treatment of plants with plant extracts resulted in a significant increase in APX activity. With a value of 5.60 ± 0.20 U.g^{-1} FW at day 0, it increased with a peak on day 4 with values 2 to 5 times higher depending on the treatment. It then decreased progressively until day 10 but with values greater than healthy (S) and inoculated (I) plants. When plants were inoculated after treatment with plant extracts (Tr + I), APX activity increased in the same way as in treated plants but with higher values. (Figure 6).

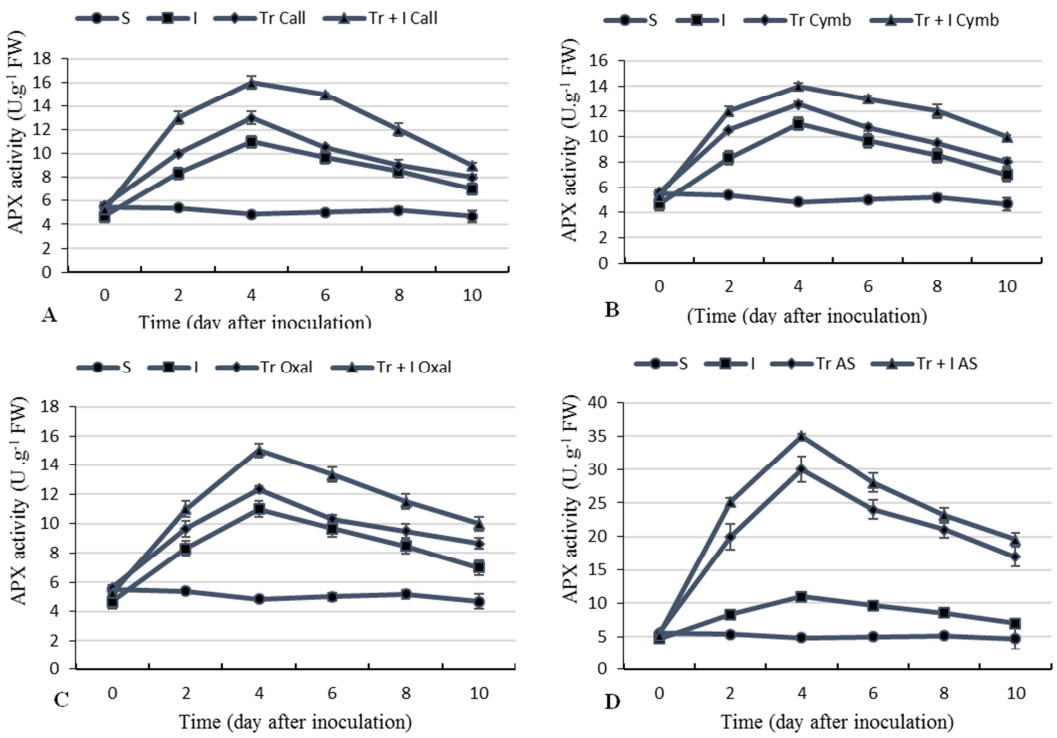

Figure 6. *APX Activity (Expressed in U. g^{-1} FW) in roots of Tomato Plants on a Time Course after Treatment with Plant Extracts and Inoculation with FOL and in the control. The Data Presented are the means ± SD of five Replicates. A: C. Citrinus; B: C. Citratus; C: O. barrelieri; D: Salicylic acid. S: Control Plants; I: plants Inoculated with FOL; Tr C. Citrinus, Tr C. Citratus, Tr O. Barrelieri, Tr Salicylic acid: plants treated respectively with Callistemon Citrinus, Cymbopogon Citratus, Oxalis barreleri, Salicylic Acid. Tr + I C. Citrinus, Tr + I C. Citratus, Tr + I O. barrelieri, Tr + I Salicylic acid: Plants Treated with Callistemon Citrinus, Cymbopogon Citratus, Oxalis Barreleri, Salicylic Acid respectively and inoculated with FOL.*

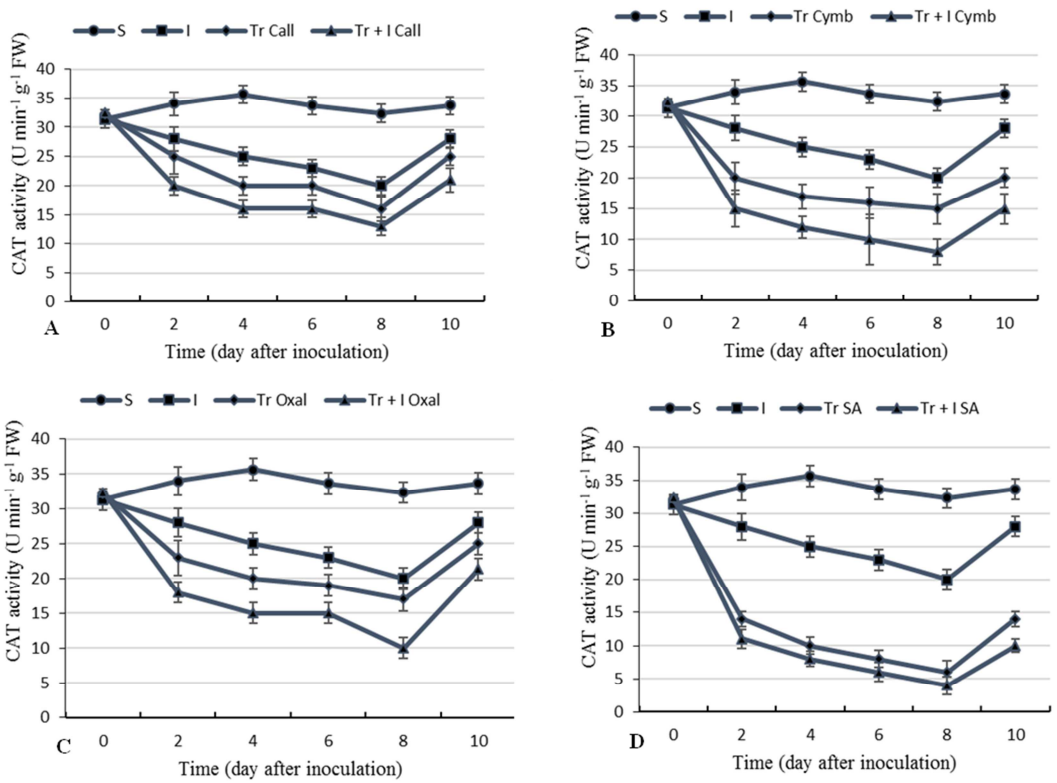

Figure 7. *CAT Activity (Expressed in U min^{-1} g^{-1} FW) in Roots of Tomato Plants on a Time Course After Treatment with Plant Extracts and Inoculation with FOL and in the Control. The Data Presented are the Means ± SD of Five Replicates. A: C. Citrinus; B: C. Citratus; C: O. barrelieri; D: Salicylic acid. S: Control Plants; I: plants Inoculated with FOL; Tr C. Citrinus, Tr C. Citratus, Tr O. Barrelieri, Tr Salicylic acid: plants treated respectively with Callistemon Citrinus, Cymbopogon Citratus, Oxalis barreleri, Salicylic Acid. Tr + I C. Citrinus, Tr + I C. Citratus, Tr + I O. barrelieri, Tr + I Salicylic acid: Plants Treated with Callistemon Citrinus, Cymbopogon Citratus, Oxalis Barreleri, Salicylic Acid respectively and inoculated with FOL.*

3.7. Catalase (CAT) Activity

Catalase activity (CAT) varied from 31.33 ± 1.52 $U.min^{-1}.g^{-1}$ FW to 35.66 ± 1.52 $U.min^{-1}.g^{-1}$ FW from day 0 to day 10. When the plants were inoculated (I), the activity decreased then increased significantly ($P < 0.05$) from day 2 and a peak was obtained on day 8 with a percentage of 56.65%. CAT activity then increased on day 10, but remained lower than in healthy (S) plants. The treatment of plants with extracts caused a decrease in catalase activity with values lower than those obtained with healthy (S) and inoculated (I) plants. A peak reduction was obtained on day 8 for all treatments with percentages of 101.62%, 115.06% and 89.76% respectively with *C. citrinus*, *C. citratus*, and *O. barreleri*. When plants were inoculated after treatment with plant extracts (Tr + I), the CAT activity decreased in the same way as in treated plants (Tr) but the decrease in activity was greater. (Figure 7).

3.8. Guaiacol Peroxidase (GPX) Activity

Guaiacol peroxidase (GPX) activity ranged from 27.33 ± 1.52 $U.g^{-1}$ FW to 34.33 ± 1.52 $U.g^{-1}$ FW in healthy plants. When the plants were inoculated (I), the GPX activity increases significantly ($P < 0.05$), with a peak of activity obtained on day 8 where it was 3 times higher. Treatment of the plants with plant extracts resulted in a significant increase in GPX activity. It was 5.60 ± 0.20 $U.g^{-1}$ FW on day 0 and a peak was obtained on day 8 with 3 to 7 times higher activity with the different treatments. When the plants were inoculated after treatment (Tr + I), then GPX activity increased in the same way as in treated plants (Tr) but with higher values. Thus peaks in activity were obtained on day 6 where the activity is 10 times higher. (Figure 8).

3.9. Superoxide Dismutase (SOD) Activity

In roots of healthy plants (S), superoxide dismutase (SOD) activity varied from 12.96 ± 0.15 $U.min^{-1}.g^{-1}$ FW to 14.30 ± 0.10 $U.min^{-1}.g^{-1}$ from day 0 to 10. When the plants were inoculated (I), the SOD activity increased by 136.26% on day 2. This activity increased gradually over time with a maximum value on day 8. After treatment of the plants (Tr), there was a significant increase ($P < 0.05$) in the activity with values 3 to 6 times higher on day 2 depending on the treatment. When plants were inoculated after treatment (Tr + I), the SOD activity was 4 to 10 times higher on day 2. The increase continued gradually and significantly reaching a maximum value on day 10 for all treatments (Figure 9).

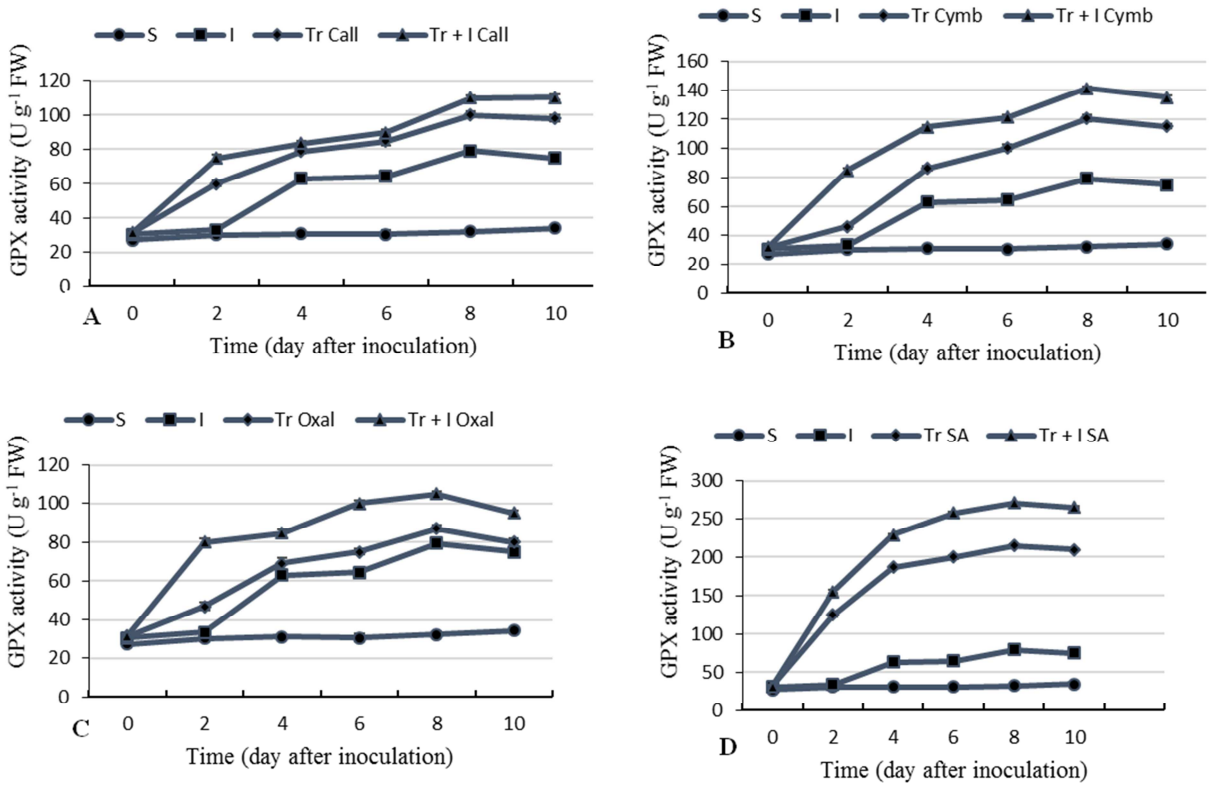

Figure 8. *GPX Activity (Expressed in $U.g^{-1}$ FW) in Roots of Tomato Plants on a time course after Treatment with Plant Extracts and Inoculation with FOL and in the Control. The Data Presented Are the Means ± SD of five Replicates. A: C. Citrinus; B: C. Citratus; C: O. barrelieri; D: Salicylic acid. S: Control Plants; I: plants Inoculated with FOL; Tr C. Citrinus, Tr C. Citratus, Tr O. Barrelieri, Tr Salicylic acid: plants treated respectively with Callistemon Citrinus, Cymbopogon Citratus, Oxalis barreleri, Salicylic Acid. Tr + I C. Citrinus, Tr + I C. Citratus, Tr + I O. barrelieri, Tr + I Salicylic acid: Plants Treated with Callistemon Citrinus, Cymbopogon Citratus, Oxalis Barreleri, Salicylic Acid respectively and inoculated with FOL.*

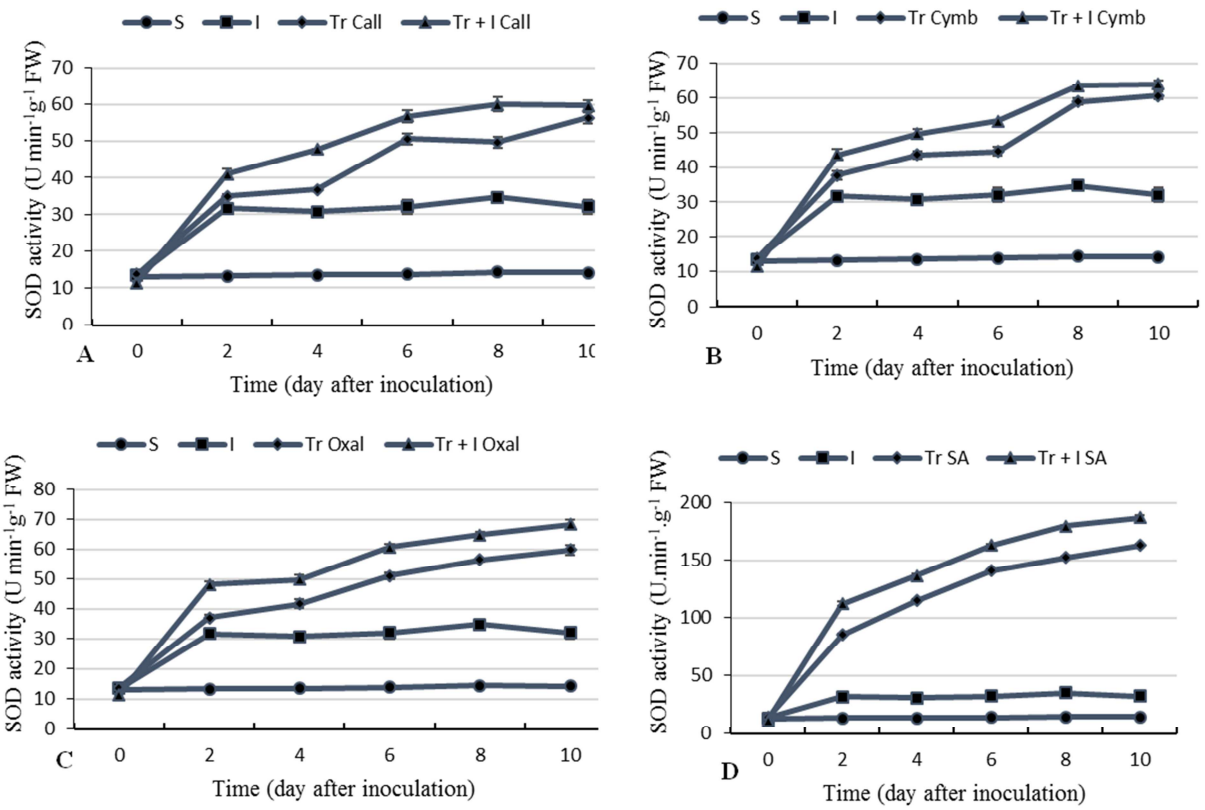

Figure 9. *SOD Activity (Expressed in U min^{-1} g^{-1} FW) in Roots of Tomato Plants on a time course after Treatment with Plant Extracts and Inoculation with FOL and in the Control. The Data Presented are the Means ± SD of five Replicates. A: C. Citrinus; B: C. Citratus; C: O. barrelieri; D: Salicylic acid. S: Control Plants; I: plants Inoculated with FOL; Tr C. Citrinus, Tr C. Citratus, Tr O. Barrelieri, Tr Salicylic acid: plants treated respectively with Callistemon Citrinus, Cymbopogon Citratus, Oxalis barreleri, Salicylic Acid. Tr + I C. Citrinus, Tr + I C. Citratus, Tr + I O. barrelieri, Tr + I Salicylic acid: Plants Treated with Callistemon Citrinus, Cymbopogon Citratus, Oxalis Barreleri, Salicylic Acid respectively and inoculated with FOL.*

4. Discussion

The effect of plant extracts on the tomato defence system against *Fusarium oxysporum* f.sp. *lycopersici* was evaluated by determining the content of biomolecules (phenols, proteins, carbohydrates, lipids, amino acids, proline) in the roots of the tomato and the evaluation of antioxidant enzymes activities (ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (GPX), Superoxide dismutase (SOD).

The phenolic content was high in treated plants. This accumulation of phenolic compounds in the tomato / *Fusarium oxysporum* interaction has been associated with plant infection by Mandal and Mitra [24]. For Behiry et al. [8], this accumulation could be explained by physiological differences between the plants associated with the treatments and could lead to the synthesis of new phenolic forms. Several phenolic compounds involved in the plant/pathogen interaction have been reported by several authors, notably apigenin and luteolin derivatives in the *Theobroma cacao* / *Phytophtora megakarya* interaction (Boudjeko et al. [9]; Djocgoue et al. [16]), and caffeoshikimic acid derivatives in the *Xanthosoma sagittifolium*/*Pythium myriotylum* interaction [28].

Protein content decreased in roots under inoculated conditions, but when tomato plants were treated with plant extracts, there was an increase in protein content. This result is in agreement with that obtained by Samir et al. [36] who showed that tomato plants inoculated with *Fusarium oxysporum* accumulate proteins. According to this author, the increase in protein content could be explained by an increased synthesis of the enzymes involved in detoxification reactions.

When tomato plants were inoculated or treated with plant extracts, there was an increase in total free sugar content. Indeed, plant extracts are at the origin of the physiological notification of tomatoes due to photosynthesis. The accelerating effect of the extracts on photosynthesis will allow an increased synthesis of carbohydrates. Furthermore, it has been shown that cell wall glycoproteins rich in glycine, hydroxyproline, and proline are involved in the response to plant stress [12]. Couée et al. [14] have shown that in some plant species, increased synthesis of sugar such as mannitol is linked to increased resistance to oxidative stress.

When tomato plants were inoculated or treated with plant extracts, there was an increase in the content of total amino acids and proline particularly. This result is in agreement with the one obtained by Omokolo and Boudjeko [34] in the *Xanthosoma sagittifolium*/*Pythium myriotylum* interaction. Indeed, the increase in amino acids content after treatment with plant extracts could serve in the synthesis of molecules

such as proteins, phenolic compounds and phytoalexins which are molecules involved in defence mechanisms. The particular increase in proline content suggests the preferential orientation of the metabolic pathways of amino acid synthesis pathways towards proline synthesis. Akladious et al. [4] showed that the treatment of tomato plants with salicylic acid under oxidative stress conditions was responsible for the accumulation of proline. Several other authors have shown that plants under various stresses accumulate proline and this accumulation is positively correlated with the tolerance of oxidative stress [5, 22].

When plants were inoculated with FOL, APX activity increased. Treatment of plants with extracts resulted in a significant increase in APX activity. When plants were inoculated after treatment with plant extracts, APX activity increased in the same way as in treated plants but with higher values. This result is in agreement with the one obtained by Mbouobda [21] in the roots of *Xanthosoma sagittifolium* treated with BTH and CTH and inoculated with *Xanthosoma sagittifolium*. Localized in peroxisomes and chloroplasts in plants, APX uses ascorbate as an electron donor and prevents H_2O_2 from leaving peroxisomes. In addition to being involved in various physiological processes such as photosynthesis, differentiation and growth, it is thought to be involved in stabilising plants under various biotic or abiotic stresses.

The treatment of plants with the extracts is at the origin of the decrease in catalase activity with values lower than those obtained with healthy and inoculated plants. The decrease in catalase activity in roots would be essential for the recovery of H_2O_2 in the peroxisomes and cytosol where it migrates from chloroplasts [4]. For Samir et al. [36], the decrease in this activity is a phenomenon linked to the accumulation of salicylic acid in oxidatively stressed plants. Furthermore, the inhibition of its activity may be due to an increase in the concentration H_2O_2 or that of reactive oxygen species arising during hypersensitive responses to pathogens.

Effective destruction of H_2O_2 requires SOD action. Our results indicate an increase in SOD activity in the roots of tomato plants treated with plant extracts, treated and inoculated. This increase in activity maintains a positive ROS balance in favour of H_2O_2 accumulation. This result is in agreement with the one obtained by Li et al. [21] suggesting an involvement of SOD in the apple defence mechanism at all maturity stages.

5. Conclusion

In conclusion, treatment of tomato with the aqueous extracts of *Callistemon citrinus* L., *Cymbopogon citratus* (DC) STAPF and Oxalis barrelieri L. stimulates its antioxidant defence system against *Fusarium oxysporum* f.sp. *lycopersici.* This stimulation is manifested in the roots of the tomato by the increase in the content of phenols, proteins, carbohydrates, amino acids, proline; increased activities of ascorbate peroxidase, guaiacol peroxidase, superoxide dismutase; reduction of catalase activity. Increasing metabolite synthesis and increasing and decreasing enzyme activities could mitigate oxidative damage that occurs during pathogen expansion. Aqueous extracts of *C. citrinus*, *C. citratus* and *O. barrelieri* could be used as natural products to stimulate the tomato defence system against FOL.

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