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# Plant Extracts Induced Resistance to *Fusarium oxysporum* f.sp. *lycopersici* in Tomato

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) is the most consumed vegetable in the world after potato. In Cameroon, the plant is cultivated in almost all agroecological areas, however, yields remain low due to attacks by various pathogens and insects. Among the pathogens, *Fusarium oxysporum* f.sp. *lycopersici* (FOL) is a phytopathogenic fungus responsible for Fusarium wilt, a disease responsible for enormous economic losses. To contribute to the control of this microbial pathogen, the stimulatory effect of the tomato defence system of extracts of some 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.*

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*barrelieri* ). After 4 days of spraying with the extracts, the plants were inoculated with a virulent strain of *Fusarium oxysporum* f.sp*. lycopersici* (FOL) under controlled conditions. Tomato roots were used to determine the contents of hydrogen peroxide ($H_2O_2$), phenols and malondialdehyde (MDA). The activities of the main antioxidant enzymes were also evaluated in tomato roots: catalase (CAT), phenylalanine ammonia lyase (PAL), peroxidase (POX) and superoxide dismutase (SOD). The results showed that treatment of tomato plants with plant extracts and their infection with FOL induced an increase in the contents of $H_2O_2$, phenols and MDA in tomato roots; an increase in PAL, POX, 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* at 10% (W/V) could be used as natural products to stimulate the tomato defence system against FOL. These results could contribute to the development of natural products to induce tomato resistance against FOL, thus improving productivity, quantity and quality of production.

*Keywords: Tomato; fungal diseases; plant extracts; induced resistance.*

## 1. INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) 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, lycopene and a balanced content of mineral elements [1]. 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 [2]. 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, pests and diseases (alternariosis, fusariosis, mildew...) [3]. 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 was reported to cause field losses ranging from 46 to 100% in Cameroon [4].

Nowadays, the control of most biotic plant diseases is mainly based on the very intensive use of synthetic chemical bactericides and fungicides [5]. 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, especially since analyses of vegetables and fruits in Cameroon have shown pesticide residues [6].

These limitations motivate and justify the search for effective and eco-friendly alternatives to control pathogenic microorganisms and crop pests. Thus, the use of natural substances (essential oils and solvent extracts) as plant disease control agents is receiving increasing attention. In Cameroon, local plant extracts have been reported as having properties in vitro and in vivo against various pathogens [3]. Botanicals with antifungal compounds have been identified and can be exploited for the management of plant diseases because they have low mammalian toxicity, target specificity, biodegradability and contain many active ingredients [7]. *Callistemon citrinus*, *Cymbopogon citratus* and *Oxalis barelierri* because of their antifungal activity notably against *Phytophthora infestans* and *Fusarium oxysporum* of tomato are used as a biocontrol agent [4]. 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 [8]. 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 extracts has enabled plants to resist pathogenic microorganisms and control several fungal diseases through metabolic changes (accumulation of phenolic compounds and antioxidant defence enzymes) [9].

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 (hydrogen peroxide ($H_2O_2$), phenols and malondialdehyde (MDA) in roots and the determination of the activities of the main antioxidant enzymes catalase (CAT), phenylalanine ammonia lyase (PAL), peroxidase (POX), 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 [4]. 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 numbers 10356 SRF / Cam (YA), 18622 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*., [10] and Mathur and Kongsdal [11] 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 the 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 and salicylic acid solutions

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., (2013).

#### 2.3.2 Treatment of tomato seedlings with plant extracts

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 were regularly watered and divided into 5 batches: the 1$^{st}$ batch consisted of control plants sprayed with distilled water (S); the 2$^{nd}$ batch consisted of plants treated with *C. citratus* (CYMB) aqueous extract at 10% (Tr Cymb); the 3$^{rd}$ 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 inoculated), (Tr Cymb+I), (Tr Ox+I), (Tr Call+I), (Tr AS+I). For each treatment, 3 replicates were conducted independently and the pots were arranged in the greenhouse in a completely randomised design.

### 2.3.3 Inoculation of the pathogen

From a 14-day FOL culture on PDA medium, the fungal suspension was prepared and adjusted to $5\times10^5$ 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.

### 2.3.4 Assessment of the incidence of the disease

The Disease incidence (DI) was assessed 2, 4, 6, 8, and 10 days after inoculation. For each of the 5 batches, 3 replicates were performed. Symptom assessment was done based on a symptom rating scale proposed by Weitang et al., [12] comprising 5 values:

0: no infection; 1: slight infection, 25% of leaves wilt, 1 or 3 leaves turn yellow; 2: moderate infection, 50% of leaves wilt, 2 or 3 leaves turn yellowing; 3. intense infection, 75% of leaves wilt, all leaves turn yellow, and growth is inhibited; 5: total infection, 100% of the leaves wilt, the whole plant turns yellows, and the plant dies. Based on these ratings, the disease incidence DI (%) was calculated according to the formula of Weitang et al., [12]:

DI (%) = Σ values x number of infected plants/ Highest value x total plants number x 100

The reduction in disease incidence RDI (%) was calculated as follows:

DI (%) = DI of infected control-DI of treated and inoculated plant/ DI of infected control x 100

### 2.3.5 Determination of the $H_2O_2$ content in roots during the *L. esculentum*/*F. oxysporum* interaction

The $H_2O_2$ content was determined according to the method described by Velikova [13]. The roots (1 g) of each sample were ground in a porcelain mortar with 2 ml of 0.1% trichloroacetic acid (TCA). After 30 min incubation, the mixture was centrifuged at 12000 rpm for 15 min at 4° C. The supernatant (0.5 ml) was mixed with a reaction medium consisting of 1 ml of a 1M potassium iodide (KI) solution and 0.5 ml of 10 mM phosphate buffer, pH 7.0. After 5 min of incubation, the optical density (OD) was read at 390 nm. The amount of $H_2O_2$ was determined using a calibration line obtained from known concentrations of $H_2O_2$ and expressed in $\mu mol.g^{-1}$ fresh matter.

### 2.3.6 Extraction and determination of total phenols

The extraction of the phenolic compounds was carried out according to the method described by Mbouobda et al., [14]. Thus, 5 g of the roots 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 rpm 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., [15]. The results were expressed as mg chlorogenic acid equivalent. $g^{-1}$ fresh matter.

### 2.3.7 Determination of the MDA (malondialdehyde) content

Lipid peroxidation was assessed in terms of malondialdehyde (MDA) content from the thiobarbituric acid (TBA) test, according to the method described by Heath and Packer [16]. Plant material (5 g of roots) was ground in a

porcelain mortar with 2 ml of 0.1% trichloroacetic acid (TCA). After 30 min incubation, the mixture was centrifuged at 12000 g for 15 min at 4° C. For the assay, 0.5 ml of the extract obtained was mixed with 2 ml of TBA at 5 g/l. The mixture was heated at 95° C for 30 min then cooled in melting ice for 10 min to stop the reaction. After centrifugation, at 10000 rpm for 15 min, the supernatant was collected and the absorbance was read at 532 and 600 nm. The non-specific absorbance value obtained at 600 nm was subtracted from the specific absorbance obtained at 532 nm. The concentration of MDA was determined using the Beer-Lambert law with the molar extinction coefficient ε = 155 $mM^{-1}.cm^{-1}$ and expressed as $\mu mol.g^{-1}$ fresh matter.

#### 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 Cakmak and Marschner [17]. 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 phenylalanine ammonia lyase (PAL) activity

PAL activity (EC.4.3.1.5) was assessed based on the rate of cinnamic acid production as described by Saunders and McClure [18]. Thus, 1 ml of 50 mM Tris-HCl buffer pH 8.8 containing β-mercaptoethanol, 0.5 ml of 10 mM L-phenylalanine, 0.4 ml of distilled water and 0.1 ml of enzyme extract were incubated at 37° C for 1 h. The reaction was stopped by adding 0.5 ml of 6M HCl and the product was extracted with 15 ml of ethyl acetate followed by evaporation of the extraction solvent using a rotary evaporator. The solid residue was taken up in 3 ml of 0.05 M NaOH and the concentration of cinnamic acid was quantified by reading the optical density at 290 nm. One PAL activity Unit is equal to 1 µmol of cinnamic acid produced per minute per g of fresh plant material.

#### 2.3.10 Evaluation of peroxidase activity (POX)

POX activity (EC.1.11.1.7) was assessed by the method described by Hammerschmidt et al., [19]. The reaction medium (3 ml) consisted of 10 mM sodium phosphate buffer pH 6 containing 10 mM $H_2O_2$ at 0.25% (V/V). A volume of 100 µl of enzyme extract was added to initiate the reaction. The enzyme activity was determined by calculating the ratio of optical Density at 470 nm per minute per 0.01 which was defined as the Activity Unit of peroxidase activity expressed in terms of Unit per g of fresh plant material.

#### 2.3.11 Assessment of superoxide dismutase (SOD) activity

SOD activity (EC.1.15.1.1) was assessed using the method described by Beauchamp and Fridovich [20]. 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.

#### 2.3.12 Statistical analyses

The results obtained were subjected to a 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 Evaluation of the Effect of *C. citrinus*, *C. citratus* and *O. barrelieri* on the Incidence of Fusarium Wilt

The disease incidence was 4.66 ± 0.26% on day 2 and significantly increased ($P < 0.05$) on day 4 (28.19 ± 1.96%) in inoculated plants. In treated and inoculated plants (Tr + I), the incidence of the disease was low compared to the inoculated control (I). On day 10, disease incidence was 3 to 6 times higher in inoculated plants (I) than in treated and inoculated (Tr + I) plants depending

on the treatment (Table 1). Treatment with plant extracts significantly reduced the incidence of the disease with percentages of reduction ranging from 51.13 to 84.32% on day 2 depending on the treatment. Among the plant extracts, the greatest reduction in disease incidence was obtained with *C. citratus,* followed by *C. citrinus* and *O. barrelieri*. (Fig. 1).

### 3.2 Effect of Plant Extracts on the $H_2O_2$ Content

In healthy plants (S), $H_2O_2$ content was 10.67 ± 1.76 µmole.g$^{-1}$ FW on day 0. When the plants were inoculated (I), this content increased over time and reached 61.32% on day 10. When the plants were treated (Tr) with plant extracts, a gradual increase was noted over time with a maximum content on day 10. In the plants inoculated after treatment (Tr + I), the $H_2O_2$ content increased significantly over time and on day 10, the increase was 239.80%, 317.47% and 323.98% respectively in the plants treated with *C. citrinus*, *C. citratus* and *O. barrelieri*. This increase was greater in treated and inoculated plants (Tr + I) than in inoculated (I) and treated (Tr) plants (Fig. 2).

### 3.3 Effect of Plant Extracts on Phenol Content

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% and 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 (Fig. 3).

### 3.4 Effect of Plant Extracts on MDA Content

The MDA content was 3.53 ± 0.25 µmole g$^{-1}$ FW on day 0. Inoculation resulted in a significant increase in MDA content with a peak at day 4 with an increase of 165.67%. When plants were treated (Tr), there was a significant increase ($P < 0.05$) and peaks were obtained on days 6 (*C. citrinus*) and 4 (*C. citratus*, *O. barrelieri*) with levels of 3 to 7 fold increases compared to day 0. Inoculation of the plants after treatment (Tr + I) also resulted in a significant increase in MDA content of more than 100% from day 2. This increase reached peaks on days 8 (*C. citrinus*), 6 (*C. citratus*) and 4 (*O. barrelieri*). It is noted that the content was higher in the treated and inoculated plants (Tr+I) than treated (Tr) and inoculated (I) plants (Fig. 4).

### 3.5 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, 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 (Fig. 5).

### 3.6 Phenylalanine Ammonia Lyase (PAL) Activity

Phenylalanine ammonia lyase (PAL) activity varied from 22.56 ± 0.15 µmole.min$^{-1}$.g$^{-1}$ FW to 27.63 ± 0.15 µmole.min$^{-1}$.g$^{-1}$ as a function of treatment in healthy plants. When plants were inoculated (I), PAL activity increased significantly ($P < 0.05$) on day 2 by 42.27%. Thereafter, this gradually activity increased over time with higher activity values than in healthy plants (S). After plant treatment (Tr), there is a significant increase in PAL activity. With a value of 23.13 ± 0.35 µmole.min$^{-1}$.g$^{-1}$ FW on day 0, it increased on day 2 by 68.61%, 72.93% and 64.28% in *C. citrinus*, *C. citratus*, and *O. barreleri* respectively. It then increased gradually and peaks of activity were obtained on day 8. When plants were inoculated after treatment (Tr+I), PAL activity

increased in the same way as in treated plants but with higher values. Peaks in activity were obtained on day 8 according to the different treatments and this activity remained higher than in healthy plants (S), inoculated (I) and treated (Tr) (Fig. 6).

### 3.7 Peroxidase (POX) Activity

In the roots of healthy plants (S), peroxidase activity (POX) varied from 2.40 ± 0.36 $U.g^{-1}$ FW to 3.20 ± 0.20 $U.g^{-1}$ FW. When plants were inoculated (I), the activity increased significantly ($P < 0.05$). From a value of 2.63 ± 0.15 $U.g^{-1}$ FW on day 0, it increased by 59.69% on day 2 and reached a peak on day 8. After treatment of the plants, there was a significant increase in POX activity. From a value of 2,76 ± 0,15 $U.g^{-1}$ FW on day 0, it was 2 to 4 times higher on day 2. PAL activity then increased gradually and peaked on day 8. When plants were inoculated after treatment (Tr + I), POX activity increased in the same way as in treated plants but with higher values. A peak in activity was obtained on day 8 with the different treatments (Fig. 7).

### 3.8 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 (Fig. 8).

## 4. DISCUSSION

In the current study, the effect of plant extracts on the tomato defence system against *Fusarium oxysporum* f.sp. *lycopersici* was evaluated by determining the content of biomolecules ($H_2O_2$, phenols, malondialdehyde) in the roots of the tomato and the evaluation of antioxidant enzymes activities: catalase (CAT), phenylalanine ammonia lyase (PAL), peroxidase (POX), Superoxide dismutase (SOD).

When plants were treated with plant extracts, there was a rapid and gradual increase in $H_2O_2$. Indeed, Li et *al*., [21] showed that various environmental stresses cause an accumulation of $H_2O_2$ in different tissues and the regulation of this rate is extremely important for plant metabolism. When plants are inoculated after

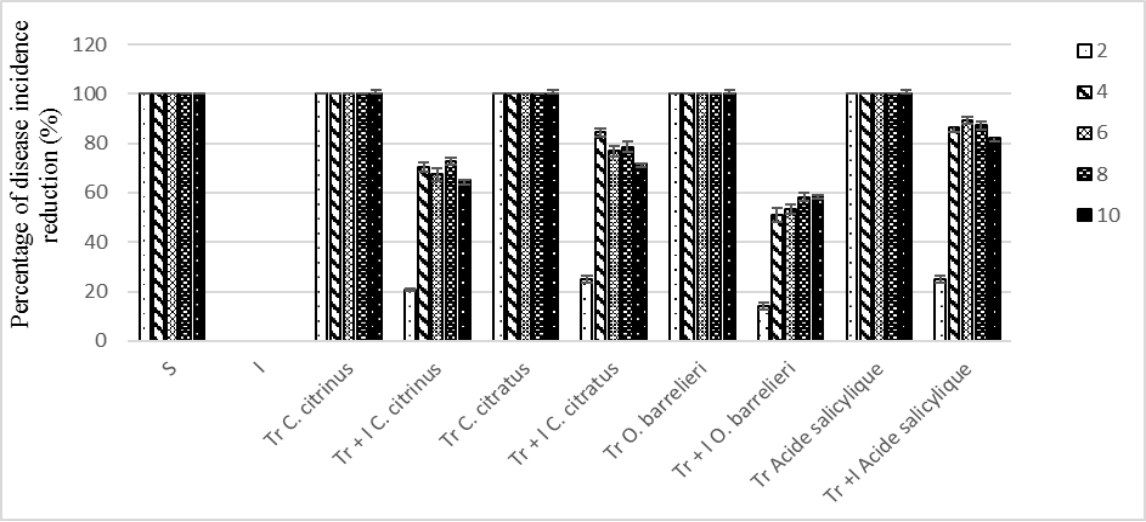


**Fig. 1. Percentage of tomato fusarium wilt incidence reduction after treatment with extracts and salicylic acid**

*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 and 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*

**Table 1. Incidence rate of tomato fusarium wilt after treatment by plant extracts and inoculation with FOL**

| DAI | Treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | S | I | *C. citrinus* | | *C. citratus* | | *O. barrelieri* | | Salicylic acid | |
| | | | Tr | Tr+I | Tr | Tr+I | Tr | Tr+I | Tr | Tr+I |
| 2 | 0.00$^{a}$ ± 0.00 | 4.66$^{a}$ ± 0.26 | 0.00$^{a}$ ± 0.00 | 3.70$^{a}$ ± 0.50 | 0.00$^{a}$ ± 0.00 | 3.50$^{a}$ ± 0.40 | 0.00$^{a}$ ± 0.00 | 4.00$^{a}$ ± 0.25 | 0.00$^{a}$ ± 0.00 | 3.50$^{a}$ ± 0.18 |
| 4 | 0.00$^{a}$ ± 0.00 | 28.19$^{b}$ ± 1.96 | 0.00$^{a}$ ± 0.00 | 8.35$^{b}$ ± 2.09 | 0.00$^{a}$ ± 0.00 | 4.42$^{ab}$ ± 1.56 | 0.00$^{a}$ ± 0.00 | 13.77$^{b}$ ± 0.35 | 0.00$^{a}$ ± 0.00 | 3.86$^{a}$ ± 0.24 |
| 6 | 0.00$^{a}$ ± 0.00 | 46.33$^{c}$ ± 2.18 | 0.00$^{a}$ ± 0.00 | 15.08$^{c}$ ± 1.66 | 0.00$^{a}$ ± 0.00 | 10.70$^{c}$ ± 2.04 | 0.00$^{a}$ ± 0.00 | 21.61$^{c}$ ± 1.60 | 0.00$^{a}$ ± 0.00 | 4.94$^{ab}$ ± 0.35 |
| 8 | 0.00$^{a}$ ± 0.00 | 62.44$^{d}$ ± 2.65 | 0.00$^{a}$ ± 0.00 | 17.01$^{cd}$ ± 1.56 | 0.00$^{a}$ ± 0.00 | 13.43$^{cd}$ ± 2.15 | 0.00$^{a}$ ± 0.00 | 26.07$^{d}$ ± 1.79 | 0.00$^{a}$ ± 0.00 | 8.01$^{b}$ ± 0.49 |
| 10 | 0.00$^{a}$ ± 0.00 | 73.99$^{e}$ ± 2.21 | 0.00$^{a}$ ± 0.00 | 25.85$^{e}$ ± 1.60 | 0.00$^{a}$ ± 0.00 | 20.81$^{e}$ ± 1.45 | 0.00$^{a}$ ± 0.00 | 30.30$^{e}$ ± 1.45 | 0.00$^{a}$ ± 0.00 | 13.01$^{c}$ ± 0.55 |

*Data are means ± SD of five replications. Different letters after the numbers in each column indicate a significant difference ($p<0.05$) between values, Duncan test*
*DAI: days after inoculation; S: control plants; I: plants inoculated with FOL; Tr: plants treated with extract or salicylic acid; Tr + I: plants treated and inoculated with FOL*

treatment with extracts, the $H_2O_2$ content is higher than in treated plants. The double stimulation due to the action of the extracts on the one hand and that of the fungus (FOL) on the other hand would explain this high level. Indeed, hydrogen peroxide is a stable molecule of reactive oxygen species and its synthesis increases after various biotic and abiotic stresses. Being a stable and diffusible molecule, $H_2O_2$ can constitute a signal molecule that inducees cellular stress [9].

The phenolic content is high in treated plants. This accumulation of phenolic compounds in the tomato / *Fusarium oxysporum* interaction has been associated with plant infection. Recently, Ramadan et *al*., [22] demonstrated that 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* [23,24], and caffeoshikimic acid derivatives in the *Xanthosoma sagittifolium*/*Pythium myriotylum* interaction [25].

The different stresses applied to tomatoes, namely FOL inoculation, the treatment with plant extracts and the treatment followed by inoculation, resulted in a significant increase in the MDA content, which is a product of the membrane lipids peroxidation. It is a marker of oxidative damage and its concentration indicates that induction of oxidative stress is effective [26]. The increase in MDA concentrations in plants could be explained by a higher peroxidation of lipids in root cells.

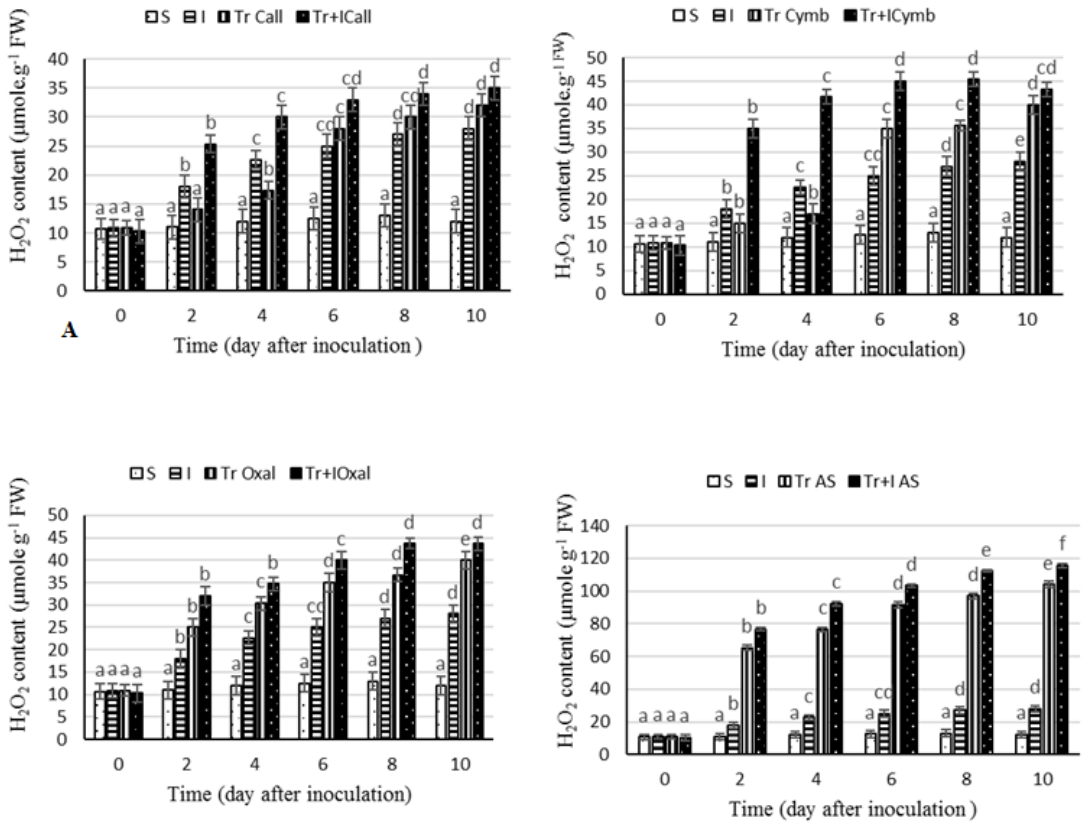


**Fig. 2. $H_2O_2$ content (expressed in µmole.g$^{-1}$ FW) in roots of tomato plants on a time course after treatment with plant extracts and inoculation with FOL and in the control**

*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*

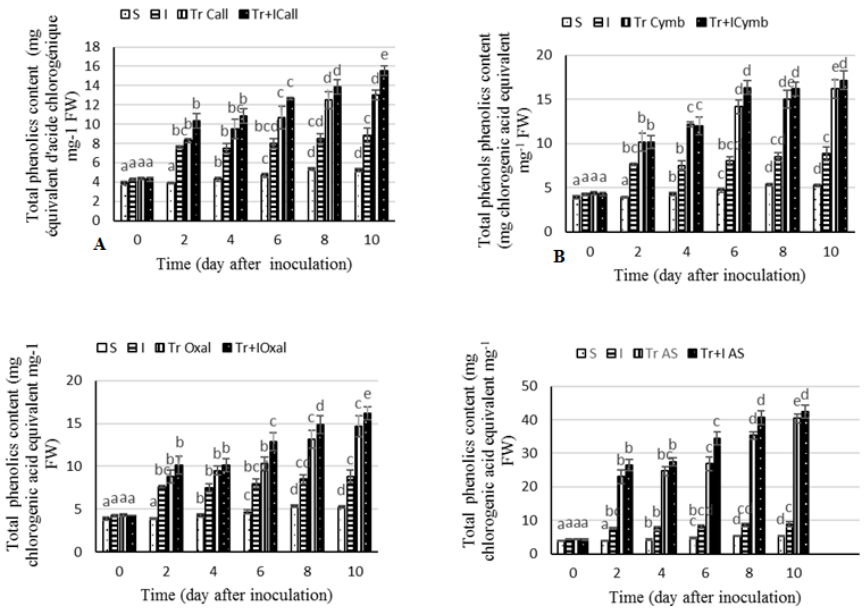


**Fig. 3. Phenolics content (expressed in mg chlorogenic acid equivalent $mg^{-1}$) in roots of tomato plants on a time course after treatment with plant extracts and inoculation with FOL and in the control**

*The data presented are the means ± SD of five replicates. Different letters for the same treatment indicate a significant difference (p<0.05) between values, Duncan test. 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*

**C**

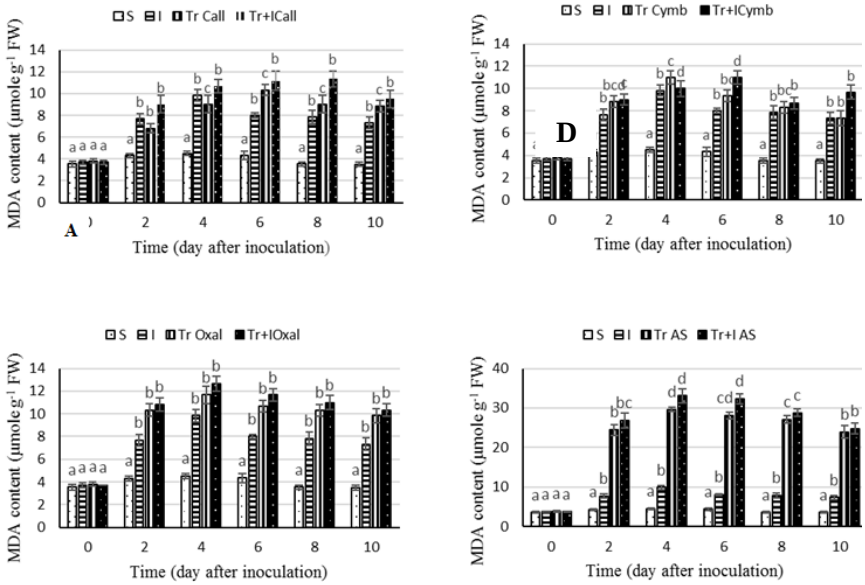


**Fig. 4. MDA content (expressed in µmole.$g^{-1}$ FW) in roots of tomato plants on a time course after treatment with plant extracts and inoculation with FOL and in the control**

*The data presented are the means ± SD of five replicates. Different letters for the same treatment indicate a significant difference (p<0.05) between values, Duncan test. 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*

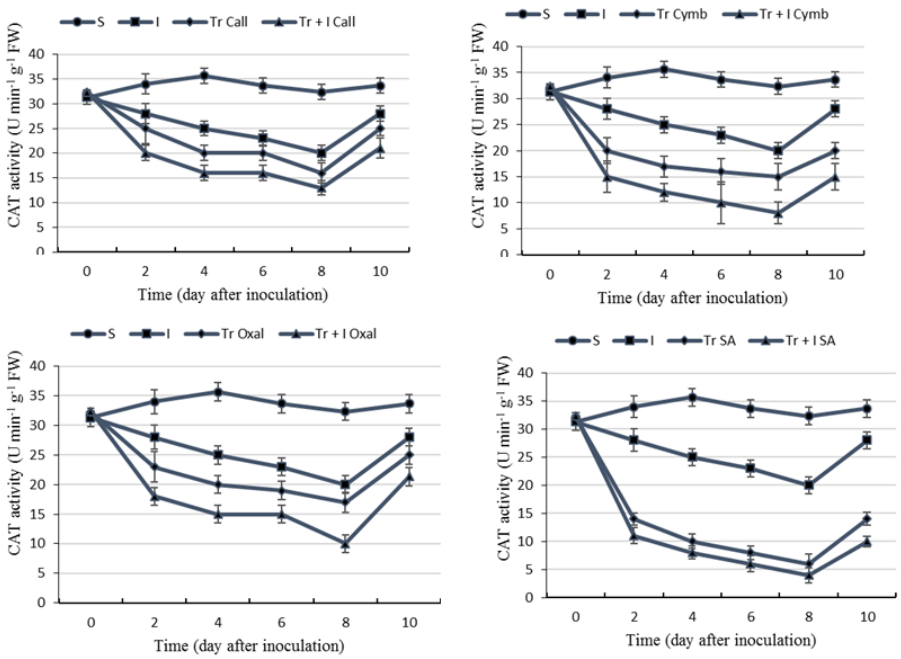


**Fig. 5. CAT activity (expressed in U min-1 g-1 FW) in roots of tomato plants on a time course after treatment with plant extracts and inoculation with FOL and in the control**

*The data presented are the means ± SD of five replicates. 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*

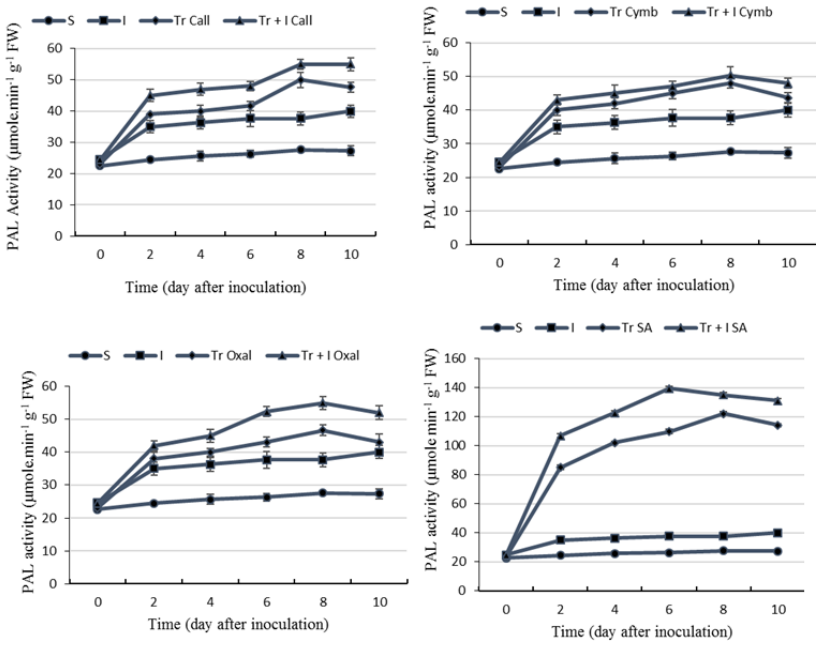


**Fig. 6. PAL activity (expressed in U.g$^{-1}$ FW) in roots of tomato plants on a time course after treatment with plant extracts and inoculation with FOL and in the control**

*The data presented are the means ± SD of five replicates. 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*

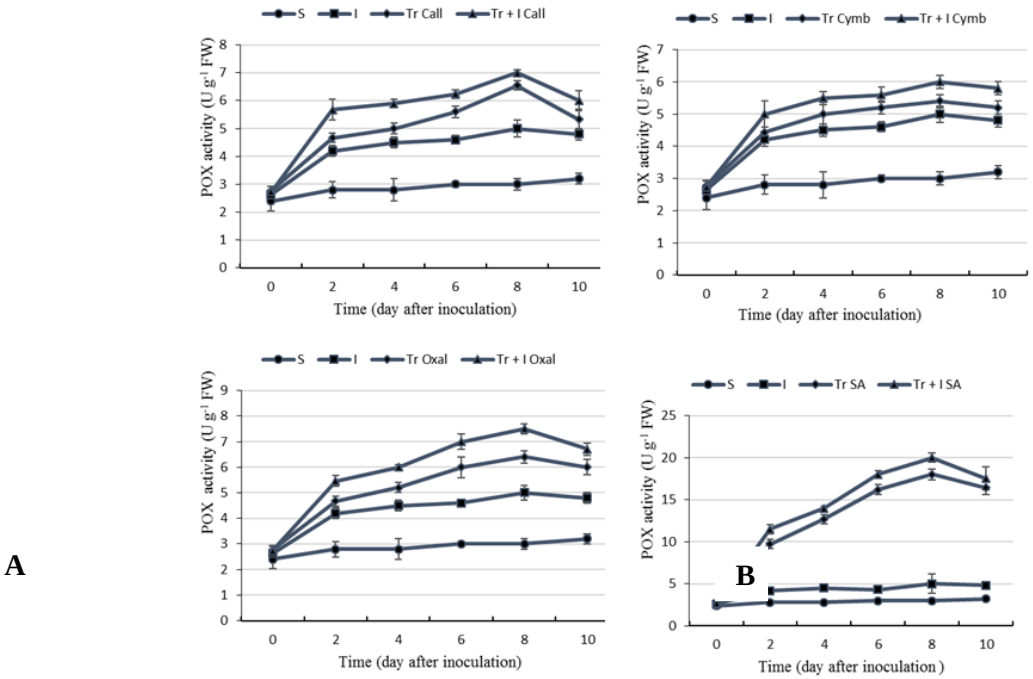


**Fig. 7. POX activity (expressed in U.g$^{-1}$ FW) in roots of tomato plants on a time course after treatment with plant extracts and inoculation with FOL and in the control**

*The data presented are the means ± SD of five replicates. 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*

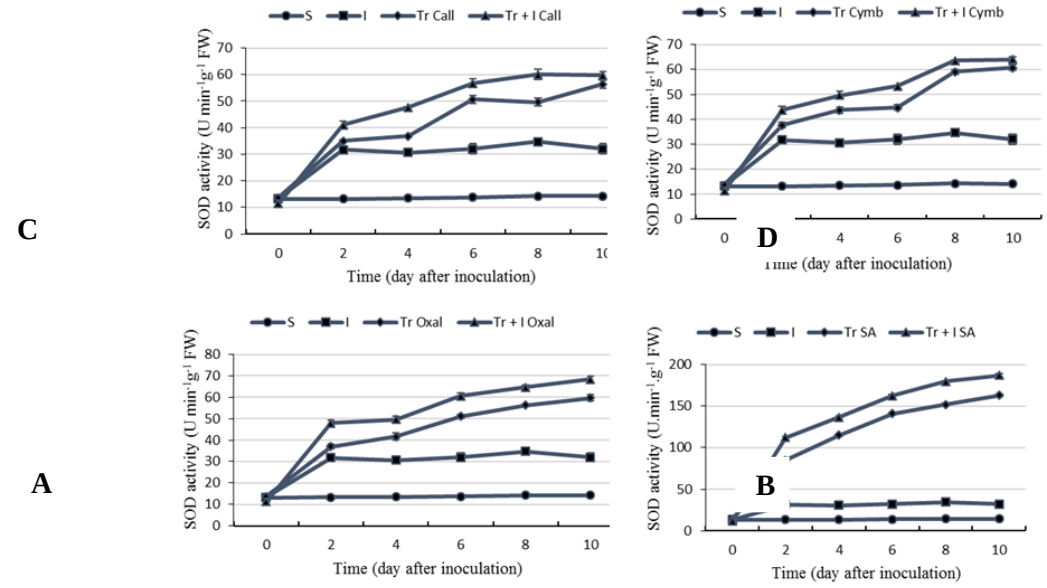


**Fig. 8. SOD activity (expressed in U min-$^{1}$ g$^{-1}$ FW) in roots of tomato plants on a time course after treatment with plant extracts and inoculation with FOL and in the control**

*The ata presented are the means ± SD of five replicates. 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*

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 [27]. For Shim et *al*., [28], 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 [26].

After inoculation of the tomato plants, the significant increase in PAL activity in the roots indicates the activation of this crossroads enzyme which would trigger the different defence mechanisms in the plant. When plants were treated with plant extracts and then inoculated with FOL, a significant increase in PAL activity is noted. Shadle et *al*., [29] have shown that PAL activity in tobacco increases after infection, which reduce the incidence of disease by stimulating defence reactions. This confirms the key role of PAL in the synthesis of phenylpropanoids which are molecules involved in the synthesis of secondary metabolites, mainly phytoalexins and salicylic acid which act in reducing the incidence of plant diseases through direct antifungal activity and stimulation of plant defence mechanisms [30].

POX activity was higher in plants treated with extracts than those not treated. This would be related to oxidative stress with the involvement of peroxidase activity as an antioxidant response against $H_2O_2$. For Samir et *al*., [31], this stress response involves a homeostatic response and control of the degradation process to maintain the integrity of vital cell functions. Mandal and Mitra, [32] have shown that treatment of *Lycopersicon esculentum* with chitosan induces a significant increase in POX activity, and an increase in the content of phenolic compounds, which protects them against parasitic attacks.

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 tomatoes 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 tomato by the increase in the content of $H_2O_2$, phenols, malondialdehyde; increased activities of phenylalanine ammonia lyase, 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* at 10% (W/V) could be used as natural products to stimulate the tomato defence system against FOL.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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