# *Nicotiana glauca*, a Key Plant for Tomato Growth Enhancement and for the Weed *Cynodon dactylon* Control

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**ABSTRACT**

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Worldwide, weeds are the costliest category of agricultural pests. They decrease yields and product quality, hence managing them is vital to successful crop cultivation which is the objective of the current study. The present work aims to evaluate the phytotoxicity of the vegetative part and the flowers of *Nicotiana glauca* on tomato and the weed *Cynodon dactylon.* Experiments were carried out under field conditions and a number of biochemical and physiological parameters were determined after harvest. The results showed that adding powdered dried flowers to potting soil (in amount of 1%) was the most effective treatment either to inhibit *C. dactylon* growth or to increase the tomato yield. The stimulations in shoot, root and fresh weight were respectively 35.25%, 328.97%, and 159.04%. It is also remarkable that aqueous extracts of the vegetative part and flowers spray and vegetative part incorporation into soil treatments were effective in stimulating the growth of tomato, but they were less effective in inhibiting the weed growth. In fact, the greatest inhibitions in shoot, rhizomes and fresh weight did not exceed 66.31%, 70.54% and 96.54% after adding powdered dried vegetative part (in amount of 0.6%). The defense strategy developed by lettuce to deal with allelopathic stress could explain the stimulation of tomato growth. Indeed, it increased the production of some metabolites such as polyphenols, flavanols, proanthocyanidins, flavonoids and tannins in addition to proline and carotenoids. An improvement of PAL and TAL activities with a stimulation of the antioxidant activity by increasing DPPH free radical-scavenging activity were also recorded. However, the respiration reduction and the membrane integrity perturbation (demonstrated by an increase in malondialdehyde content and electrolyte leakage) could explain the weed growth inhibition. These findings emphasize that the use of the powdered dried flowers of *N. glauca* are effective and easily approach to exploit its valuable secondary metabolites either to control *C. dactylon* or to improve the production of tomato.

*Keywords*: Allelochemicals, *Cynodon dactylon*, inhibition, *Nicotiana glauca*, stimulation, tomato

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Approaches to weed management is challenging, particularly when chemical herbicide application leads to a change in phytosociological composition of weeds, selection of biotypes resistant to herbicides as well as causing environmental and human health problems.

Actually, weed control mechanisms, including Integrated Pest

Management and Biological Control, are recommended to improve and complement the traditional control methods, particularly for problematic crop weed species management as in the case of Bermuda grass (*Cynodon dactylon*) which is a perennial stoloniferous and rhizomatous grass (Ngondya et al. 2019). According to Abdullahi (2002), *C. dactylon* is listed as one of the world's worst weeds because of its aggressive competitiveness and dynamism which cause substantial crop yield reductions. It is characterized by a rapid expansion forming a dense resilient turf. Additionally, it is prolific and has very strong competitive capacity with crops for plant resources (sunlight, water, nutrient, space, symbiotic organisms, gases (carbon dioxide and oxygen), etc.) (Juraimi et al. 2005).

Allelopathy is considered as an economical, effective and environmentally friendly weed management approach. It means that one plant produces allelochemicals to affect, positively or negatively, the development and the growth of other plants (Li et al. 2021). For sustainable agriculture, plant-based bioherbicides could be an effective alternative to current chemical herbicides (Hasan et al. 2021). In fact, certain plant-derived allelochemicals can control weeds, due to allelopathic effects (Li et al. 2021).

To date, numerous commercial herbicides have been successfully discovered from plant extracts or plants worldwide, showing the high research value and broad application prospects of plant-derived allelochemicals (Li et al. 2021). Allelochemicals are chemically diverse, e.g., alkaloids, phenols, terpenes, carbohydrates, glycosides and amino-acids, benzoic acid and cinnamic acid derivatives and carbohydrates. They can act through volatile emission, leaching from leaves and exudation from roots (Tuyen et al. 2018). According to Jmii et al (2020a), allelochemicals mode of action may include an inhibition of cell division and respiration, inhibition in root and/or seedling growth, inhibition in seed germination, decrease in photosynthesis, chlorophyll content, enzyme activities, and mineral uptake and disruption of cell membrane.

Tree tobacco (*Nicotiana glauca*) has received considerable attention due to its richness in natural products. *N. glauca*'s tissues contain nicotine, anatabine and anabasine (alkaloid compound similar to nicotine but it is more toxic) (Alghamdi 2021). In addition, since this plant is a harmful weed to the soil and the flora, it must be exploited as a source of useful secondary metabolites. It has been proven to contain many effective compounds with therapeutic properties or used as insecticides and biofungicides against some pathogenic fungi (Alghamdi 2021).

Thus, the aim of this work consists of investigate (i) the phytotoxicity of *N. glauca* vegetative part and flowers on tomato and the weed, *C. dactylon* (through pulverization of their aqueous extracts and adding their dried powder to potting soil, in pot experiments), (ii) to examinate the action mode of allelochemicals, via the measurement of certain biochemical and physiological parameters in lettuce (*Lactuca sativa*) which is a model plant known by its high sensitivity to allelochemicals).

## MATERIALS AND METHODS

### Plant material.

The seeds of lettuce and tomato were provided by the Technical Center of Organic Agriculture (Sousse, Tunisia), and the Institute of Arid Regions (Medenine, Tunisia), respectively. *C. dactylon* rhizomes were collected from an

uncultivated field (Chott Mariem-Sousse, Tunisia). The sampling of *N. glauca* was carried out on 24 May 2021, from a rocky plateau in the Sousse region. This location is situated in the central east of Tunisia and has the following geographic coordinates: latitude (35° 49' 31" N), longitude (10° 38' 13" E) and 24 m altitude above the sea level.

**Experimental equipment.**

Samples were sterilazed in an autoclave (Vision Scientific, VS 1221). For the centrifugation, a centrifuge machine (Sigma 113, Germany) was used. A digital conductivity meter (BCT-4308, Taiwan) was utilized to measure the electrical conductivity. The absorbance (nm) was measured using a spectrophotometer UV-Visible (Jenway 6305, Vietnam).

***Nicotiana glauca* vegetative part and flowers extraction.**

The collected *N. glauca* samples were separated into flowers and vegetative part, which were dried in an airy room in the darkness, ground to obtain a fine powder, packed in paper bags and stored in a cold dry chamber until use.

For the aqueous extract that will be used for pot trials, the powder of *N. glauca* vegetative part and flowers (0.750 kg) were soaked in 2.5 L of distilled water, in darkness, at room temperature, for 24 h as previously performed (Jmii et al. 2020a). The mixtures were filtered several times to remove debris, and the aqueous extracts were diluted with distilled water to give 10, 20 and 30%.

For the aqueous extracts that will be used for the hydroponic trial, they were prepared at concentration inducing 50% inhibition of root growth ($IC_{50}$). For this, *N. glauca* vegetative part and flowers powder (10 g) were soaked in 1 L of distilled water for 24 h, in darkness, and at room temperature. The mixtures were filtered with a paper Whattman No. 1, and then diluted with distilled water to give 2, 4, 6, 8 and 10 g/L. $IC_{50}$ was determined from the graph of the percentage of inhibition of lettuce root length against the different *N. glauca* vegetative part and flowers aqueous extract concentrations.

***Nicotiana glauca* application.**

Five pre-germinated seeds of tomato were sown in pots (1 seed per pot). Five pots contained *C. dactylon* rhizomes (3 cm), each one. Plastic pots (upper diameter of 16 cm and height of 13 cm) were used. They were filled with Potgrond-H Potting Soil-Klasmann (750 g/pot), consisting of dark peat moss for about 80% and light peat moss for about 20%. The pots were kept outdoor under field conditions at the Higher Institute of Agronomy of Chott Mariem, Tunisia, on 14 April 2022. They were kept moistened throughout the experimental period, by irrigation with tap water.

*N. glauca* was applied to tomato and *C. dactylon* in pots, by adding dried powder to potting soil or by pulverization of the aqueous extracts. Besides, *N. glauca* was applied to lettuce in hydroponic conditions.

***Pulverization.***

For control plants, five pre-germinated seeds of tomato were sown in pots (1 seed per pot). Five pots contained *C. dactylon* rhizomes (3 cm), each one. Same numbers were used for pulverized plants. The aqueous extracts of *N. glauca* vegetative part and flowers (10, 20 and 30%) were used for the treatment of tomato as well as *C. dactylon*, by pulverization. The treatment was applied on fifteen-day-old species (4 and 6 cm of shoots length for the tomato and the weed, respectively). After two weeks of the first treatment, a second pulverization was

applied on the same target species. For the control, the species were sprayed by tap water. A month after the second spray treatment, the fresh weight, and the length of shoots and roots/rhizomes of both species were determined as previously (Jmii et al. 2020a). The experiment was repeated twice.

***Dried powder.***

For the control plants, five pre-germinated seeds of tomato were sown in pots (1 seed per pot). Five pots contained *C. dactylon* rhizomes (3 cm), each one. Same numbers were used for treated plants. Powders of *N. glauca*, vegetative and flower parts, were spread separately, on the surface of pots, after sowing pre-germinated tomato seeds and *C. dactylon* rhizomes. The used doses were as follows: 0.2%, 0.6%, and 1% (w/w). The control pots consisted of powder-free soil. Two months after the treatment, the fresh weight, and the length of shoots and roots/rhizomes of both species were determined as previously (Jmii et al. 2020b). The experiment was repeated twice.

***Hydroponic conditions.***

In dark, and at room temperature, lettuce seeds were germinated in Petri dishes. They were irrigated with distilled water for a week. The seven-day old uniform seedlings were cultured in a greenhouse (at 20/17°C and 8 h darkness), in a hydroponic system. The system contained a complete Hoagland's medium (Hoagland et Arnon 1950). The culture media components were as follows: the macronutrients components including $MgSO_4$,$7H_2O$ (0.75 M), $KH_2PO_4$ (0.8 M), $KHPO_4$ (0.26 M), $KNO_3$ (1.5 M), $NH_4NO_3$ (1 M) and Ca $(NO_3)_2$, $4H_2O$ (1.75 M); the micronutrient components were $MnSO_4$, $4H_2O$ (8 mM), $CuSO_4$, $5H_2O$ (0.7 mM), $ZnSO_4$, $7H_2O$ (0.76 mM), $H_3BO_3$ (6.27 mM), $(NH_4)_6MO_7O_{27}$, $4H_2O$ (0.2 mM). Hoagland's medium contains also an iron solution (EDTA-K Fe) as a source of Fe (0.0028 mg/mL Fe). It was prepared following Jacobson's method (Jacobson 1951). A mass of 26.1 g of ethylenediaminetetra-acetic acid (EDTA) was dissolved in 268 mL of potassium hydroxide (KOH) (1 N) and 24.9 g of $FeSO_4$, $7H_2O$. The mixture was adjused to 950 mL with distilled water. The pH of the solution was adjusted to 5.5 with KOH (1 N). After that, and in order to stabilize the Fe-EDTA complex, the mixture was agitated for 24 h. The volume was adjusted to 1 L with distilled water. The solution was kept in the dark. Hoagland's medium was diluted eightfold before application to the plants. The culture media was renewed every three days.

After two months, the 150 plants were divided into three batches: 50 plants grown in aqueous extracts of *N. glauca* vegetative part, and 50 plants grown in aqueous extracts of *N. glauca* flowers (for three days, at concentration inducing 50% inhibition of roots growth ($IC_{50}$)); the third batch consisted of 50 plants cultured in the absence of aqueous extracts (control plants). Harvested plants were separated into leaves and roots.

**Evaluated parameters.**

***Pigments content.***

Carotenoid and chlorophyll (*chl a* and *b*) contents in leaves of lettuce were measured by a spectrophotometric analysis following the method described by Lichtenthaler and Wellburn (1983). A mass of 100 mg of leaves fresh weight was placed in 5 mL of acetone (80%). The absorbance was measured at 663, 645 and 440 nm. After filtration, the pigment contents were calculated from the following equations of Lichtenthaler and Wellburn (1983) and results were expressed as mg/g fresh weight:

Chlorophyll *a* (mg/g) = 12.7 $A_{663}$- 2.69 $A_{654}$; Chlorophyll *b* (mg/g) = 22.9 $A_{645}$- 4.68 $A_{663}$; Total chlorophyll (mg/g) = 20.2 $A_{645}$ + 8.02 $A_{663}$; and Carotenoids (mg/g) = (4.7 $A_{440}$ - (1.38 $A_{663}$ + 5.48 $A_{645}$)).

***Metabolic activity.***

The test of triphenyl tetrazolium chloride (TTC) was used to measure cell metabolic activity (Sampietro et al. 2006). After washing and drying between filter paper, fresh roots and leaves of lettuce (100 mg) were incubated in 5 mL of a TTC solution (0.2%, pH = 7) in the dark, for 4 h (at 37°C). To stop the reaction, 0.5 mL of sulfuric acid (1 M) was added to each sample. After that, they were washed another time with distilled water to be quickly dried between filter paper. Then, the different samples were ground in a mortar placed in ice and containing 3.5 mL of ethyl acetate. The volume was adjusted to 7 mL with ethyl acetate after filtration through paper Whattman No. 1. Absorbance was read at 485 nm. Formazan content was calculated as follows (Sampietro et al. 2006): Formazan content (%) = $DO_{485}$ treatment/$DO_{485}$ control.

***Tyrosine ammonia-lyase (TAL) and Phenylalanine ammonia-lyase (PAL) activities.***

For the extraction of PAL and TAL enzymes, 150 mg of fresh lettuce leaves and roots were ground in 20 mL of phosphate buffer (0.1 M) in ice. The phosphate buffer was prepared with pH = 9 and 8.7 for TAL and PAL activities, respectively. It was obtained from a mixture of $KH_2PO_4$ and $Na_2HPO_4$. The supernatant recovered (after homogenate filtration through Whatman paper N°1, and centrifugation at 15000 tr/min for 10 min at 5°C) constituted the crude enzyme extract. An initial optical density (DO1) of the reaction mixture (1 mL) containing 0.3 mL of supernatant and L-tyrosine 10 mM was read at 333 nm. However, the DO1 reaction mixture (1 mL) containing 0.2 mL of the crude enzyme extract and 50 mM L-phenylalanine was read at 270 nm. After incubation for 90 min (at 40°C) and cooling the tubes in ice (to stop the reaction), the staining intensity was measured at the same wavelength (DO2). The enzymatic activity was expressed in µmol/ min g fresh material (Higuchi and Kawamura. 1964).

***Membrane integrity.***

*Electrolyte leakage.* Electrolyte leakage ($E_L$) was estimated according to Lutts et al (1996). In dark, and at room temperature, the lettuce roots and leaves were homogenized in tubes containing 25 mL of distilled water. Using a digital conductivity meter, the initial electrical conductivity (L1) of the bathing solution where the samples were immersed, was measured after 24 h. After that, samples were autoclaved for 20 min at 121°C, in order to burst cell walls and liberate all electrolytes. Then, they were cooled down to 25°C, and incubated again in distilled water as indicated previously. A last conductivity reading (L2) was measured after 24 h. The electrolyte leakage ($E_L$) was determined as follows: $E_L$ (%) = (L1/L2) x 100.

*Lipid peroxidation.* In a mortar placed on ice, 250 mg samples were ground with 0.05 g PVP and 2.5 mL of 67 mM phosphate buffer (pH = 7). The supernatant obtained after extract centrifugation (at 2000 g for 15 min at 4°C) was used to determine the lipid peroxidation. A volume of 750 µL prepared extract was added to 3 mL 0.5% thiobarbituric acid (prepared in 20% trichloroacetic acid). The mixture was heated at 90°C for 10 min. After that, it was quickly cooled in an ice-bath. Samples were centrifuged. Supernatant absorbance

was read at 532 and 600 nm. The MDA concentration was calculated using the extinction coefficient of 155 m $M^{-1}$ $cm^{-1}$ (Doblinski et al. 2003).

***Proline content.***

A mass of 10 mg of dry lettuce roots and leaves was mixed with 1.5 mL 3% aqueous sulfosalicylic acid (w/v). The homogenate solutions were centrifuged at 14.000 tr/min for 10 min. Then, 1 mL ninhydrin reagent (prepared by warning 1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL $H_3PO_4$ (6 M)) and 1 mL of glacial acetic acid were reacted with 1 mL of the different supernatant from the different homogenates (for 1 h at 100°C). The tubes were cooled in an ice bath to stop the reaction. A volume of 2 mL of toluene was mixed vigorously with the reaction mixture for 20 s. The absorbance of the upper phase (pink-red) was read at 520 nm. Toluene was used for the blank (Bates et al. 1973). Proline concentration was calculated from a calibration curve obtained by proline solutions series (0-1 mg/mL).

***2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay.***

Lettuce leaves and roots samples were prepared by separating stock mixtures in microcrystalline cellulose at a concentration of 500 mg/g. Solid dilution series of stock mixtures were performed with cellulose to a final concentration of 15 mg/g for the DPPH scavenging assay.

Electron donation ability of obtained extracts was measured by bleaching the purple solution of DPPH, according to the method described by Hatano et al (1988). A mass of 10 mg of cellulose (for the control) or samples was mixed with 1 mL of DPPH methanolic solution and 0.5 mL of methanol. DPPH methanolic solution was prepared at a concentration of 30 mg/L and regulated at an optical density between 0.750 and 0.8 nm. After incubation in the dark for 2 h at room temperature, the optical density was measured at 517 nm. DPPH free radical percentage inhibition (PI) was calculated as follows: $PI = (A_{control}-A_{extract}/A_{control})\times100$; where PI = percentage of inhibition and A = Absorbance.

The study of antiradical activity variation as a function of the extract concentration allows the determination of the concentration which corresponds to 50% inhibition ($IC_{50}$). A low $IC_{50}$ value corresponds to a high efficiency of the extract.

***Secondary metabolite production in lettuce.***

For the dosage of secondary metabolites in lettuce leaves and roots, separate stock mixtures were prepared in microcrystalline cellulose at a concentration of 500 mg/g. Then, solid dilution series of stock mixtures were performed with cellulose to obtain a final concentration of 62 mg/g, which was used in the assays.

*Total phenolic content.* Total phenolic content was assayed using the Folin-Ciocalteu reagent, following the method described by Oktay et al (2003), and slightly modified by Dewanto et al (2002). The method is based on phosphowolframate-phosphomolybdate complex reduction by phenolics to blue reaction products. Diluted sample extracts (10 mg) were mixed with 125 µL Folin-Ciocalteu reagent and 500 µL distilled water. After agitation and rest for 5 min, 1250 µL $Na_2CO_3$ (7%) were added to the mixtures. The final volume was adjusted to 3 mL with distilled water. The absorbance was read at 760 nm versus the prepared blank, after incubation for 90 min in the dark. A standard curve was prepared using gallic acid solutions. Total phenolic

content was expressed as mg gallic acid equivalents/g dry weight (mg GAEs/g DW) using an equation obtained from the standard gallic acid graph ($R^2$ = 0.992).

*Total flavonoid content*. The flavonoid content was determined using the aluminum chloride colorimetric method (Djeridane et al. 2006). To 10 mg of each diluted extract, 75 µL $NaNO_2$ (5%) were added. A volume of 150 µL of a freshly prepared $AlCl_3$ solution (10%) was added to the mixtures, after incubation for 6 min at room temperature. Mixtures were allowed to stand for 5 min. Then, they were mixed with 500 µL of NaOH (1 M). The final volume was adjusted to 2500 µL with distilled water. The absorbance was measured at 510 nm. Total flavonoid content was expressed in mg quercetin equivalent/g dry weight (mg QEs/g DW) using quercetin calibration curve ($R^2$ = 0.996).

*Flavanol content.* Total flavanols content were estimated using the method reported by Adedapo et al (2008). To 10 mg of diluted extracts, 3 mL $NaNO_2$ (5%) and 0.2 mL $AlCl_3$ (2%) ethanol solution were added. After 2 h and 30 min incubation at 20°C, the absorbance was measured at 440 nm. Total flavanol content was expressed as mg quercetin equivalents/g dry weight (mg QEs/g DW) using quercetin calibration curve ($R^2$ = 0.995).

*Total tannin content.* Total tannins were estimated using the method reported by Hagerman and Butler (1978) on the basis of their precipitation by bovineserum albumin (protein). The method is based on obtaining $Fe^{2+}$ phenols colored complex, measured spectrophotometrically. A mass of 10 mg of each diluted extracts were mixed with 0.5 mL concentrated sulfuric acid and 3 mL vanillin (1%). Mixtures were allowed to stand for 15 min. The absorbance was read at 500 nm. The results were expressed as mg tannic acid equivalents/g dry weight (mg TAEs/g DW) using tannic acid calibration curve ($R^2$ = 0.988).

*Proanthocyanidin content.* Proanthocyanidin content was determined using butanol–hydrogen chloride assay (Maksimovic et al. 2005). A mass of 10 mg diluted extracts were mixed with 0.1 mL iron sulphate (2%) and 3 mL butanol-hydrogen chloride (95:5; v/v). The mixtures were vortexed. Then, they were incubated for 60 min at 90°C. The absorbance was measured at 530 nm. Proanthocyanidin content was expressed as mg catechin equivalents/g of dry weight (mg CEs/ g DW) using catechin calibration curve ($R^2$ = 0.998).

**Statistical analysis.**

The experiments were performed in complete randomized design. Data were reported as mean ± standard error (S.E) of five replicates. ANOVA and Duncan tests were performed with IBM SPSS Statistics version 20.00 (Duncan, 1955), for Windows program to analyze the differences between treatments. Differences were considered statistically significant at the 5 % level ($p < 0.05$).

## RESULTS

**Pot experiments.**

Adding powdered dried flowers to potting soil, in amount of 1%, was the most effective treatment either to inhibit *C. dactylon* growth or to increase the growth of tomato. In fact, the weed seedlings were completely burned in the presence of the dried powder from flower part. The stimulations in shoot, root and fresh weight of tomato were respectively 35.25%, 328.97% and 159.04% (Table 1).

It is eminent that despite aqueous extracts of vegetative part and flowers spray and vegetative part incorporation into soil treatments were effective in stimulating the growth of tomato, however they were less effective in inhibiting weed growth (Tables 1, 2). In fact, the greatest inhibitions in shoots, rhizomes and fresh weight did not exceed 66.31%, 70.54% and 96.54% after adding powdered dried vegetative part (in amount of 0.6%) (Table 1).

In the case of pulverization, even if the tomato growth was stimulated, this treatment showed lower phytotoxicity on *C. dactylon* than adding dried powder to soil. Flower aqueous extract spray (at 20%) showed the best inhibitions in shoots, rhizomes and fresh weight with 35.26%, 72.13% and 84.34%, respectively. These values did not exceed 32.47%, 51.44% and 82.89%, after vegetative part aqueous extract pulverization at 10% (Table 2).

**Table 1.** Biological parameters of tomato and the weed *Cynodon dactylon* measured after treatment with *Nicotiana glauca* powder

| Measured parameters | | Rate of added vegetative part (VP) and flowers (F) powder of *Nicotiana glauca* | | | |
|---|---|---|---|---|---|
| | | Control (0%) | 0.2% | 0.6% | 1% |
| | | Tomato | | | |
| Shoot length (cm) | F | $32.33^{a} \pm 1.8$ | $34.03^{b} \pm 1.69$ | $37.01^{c} \pm 2.41$ | $43.54^{d} \pm 2.8$ |
| | VP | $32.33^{a} \pm 1.8$ | $33.00^{b} \pm 1.3$ | $37.15^{c} \pm 1.27$ | $41.96^{d} \pm 2$ |
| Stimulation or Reduction (%) | F | - | +5.64 | +14.47 | +35.25 |
| | VP | - | +2.38 | +15.36 | +30.41 |
| Roots length (cm) | F | $3.58^{a} \pm 0.69$ | $12.10^{c} \pm 1.65$ | $8.08^{b} \pm 1.1$ | $15.04^{d} \pm 2.7$ |
| | VP | $3.58^{a} \pm 0.7$ | $6.17^{b} \pm 0.22$ | $8.09^{c} \pm 0.51$ | $12.09^{d} \pm 0.4$ |
| Stimulation or Reduction (%) | F | - | +244.65 | +131.62 | +328.97 |
| | VP | - | +77.31 | +132.35 | +246.87 |
| Fresh weight (g) | F | $5.59^{a} \pm 0.47$ | $10.94^{c} \pm 1.32$ | $7.56^{b} \pm 1.07$ | $14.49^{d} \pm 1.5$ |
| | VP | $5.59^{a} \pm 0.47$ | $6.77^{b} \pm 0.29$ | $12.11^{c} \pm 0.12$ | $18.32^{d} \pm 0.6$ |
| Stimulation or Reduction (%) | F | - | +95.41 | +34.75 | +159.04 |
| | VP | - | +21.44 | +117.65 | +228.96 |
| | | *Cynodon dactylon* | | | |
| Shoot length (cm) | F | $37.2^{c} \pm 1.43$ | $20.53^{b} \pm 1.4$ | $60^{d} \pm 2.38$ | $0^{a}$ |
| | VP | $37.2^{c} \pm 1.43$ | $20.09^{b} \pm 1.12$ | $12.54^{a} \pm 0.8$ | $50.14^{d} \pm 3.5$ |
| Stimulation or Reduction (%) | F | - | -44.84 | +61.3 | -100 |
| | VP | - | -46.00 | -66.31 | +34.75 |
| Rhizome length (cm) | F | $29.00^{c} \pm 1.14$ | $14.14^{b} \pm 1.60$ | $31.00^{d} \pm 1.4$ | $0^{a}$ |
| | VP | $29.00^{c} \pm 1.14$ | $24.04^{b} \pm 2.02$ | $8.53^{a} \pm 0.12$ | $41.07^{d} \pm 1.$ |
| Stimulation or Reduction (%) | F | - | -51.12 | +7.09 | -100 |
| | VP | - | -16.95 | -70.54 | +41.79 |
| Fresh weight | F | $13.53^{c} \pm 1.79$ | $1.61^{b} \pm 0.24$ | $19.52^{d} \pm 2.6$ | $0^{a}$ |
| | VP | $13.53^{c} \pm 1.79$ | $3.30^{b} \pm 0.21$ | $0.46^{a} \pm 0.01$ | $24.05^{d} \pm 1.4$ |
| Stimulation or Reduction (%) | F | - | -88.13 | +44.54 | -100 |
| | VP | - | -75.46 | -96.54 | +78.95 |

Values are means ± standard errors (n = 5). Values affected with the same letter are not significantly different at $p < 0.05$ (Duncan test).

**Table 2.** Biological parameters of tomato and the weed *Cynodon dactylon* measured after treatment with *Nicotiana glauca* aqueous extract

| Measured parameters | | Rate of added vegetative part (VP) and flowers (F) aqueous extract of *Nicotiana glauca* | | | |
|---|---|---|---|---|---|
| | | Control (0%) | 10% | 20% | 30% |
| | | Tomato | | | |
| Shoot length (cm) | F | $32.33^{a} \pm 1.8$ | $41.68^{b} \pm 1.29$ | $48.28^{d} \pm 1.43$ | $44.13^{c} \pm 1.6$ |
| | VP | $32.33^{a} \pm 1.8$ | $37.61^{b} \pm 1.3$ | $42.22^{c} \pm 2$ | $52.55^{d} \pm 2$ |
| Stimulation or Reduction (%) | F | - | +29.32 | +49.78 | +36.94 |
| | VP | - | +16.63 | +31.07 | +63.05 |
| Roots length (cm) | F | $3.58^{a} \pm 0.69$ | $12.08^{b} \pm 0.76$ | $17.85^{d} \pm 0.61$ | $14.28^{c} \pm 0.2$ |
| | VP | $3.58^{a} \pm 0.69$ | $10.03^{b} \pm 0.37$ | $13.11^{c} \pm 0.95$ | $15.3^{d} \pm 0.45$ |
| Stimulation or Reduction (%) | F | - | +246.97 | +413.75 | +310.81 |
| | VP | - | +189.22 | +275.33 | +340.1 |
| Fresh weight (g) | F | $5.59^{a} \pm 0.47$ | $13.81^{b} \pm 0.11$ | $23.22^{d} \pm 0.19$ | $15.1^{c} \pm 0.84$ |
| | VP | $5.59^{a} \pm 0.47$ | $12.09^{b} \pm 0.55$ | $17.9^{c} \pm 0.34$ | $21.2^{d} \pm 1.2$ |
| Stimulation or Reduction (%) | F | - | +148.24 | +317.28 | +170.54 |
| | VP | - | +116.91 | +222.05 | +281.82 |
| | | *Cynodon dactylon* | | | |
| Shoot length (cm) | F | $37.2^{c} \pm 1.43$ | $26.99^{b} \pm 1.57$ | $24.06^{a} \pm 0.23$ | $51.91^{d} \pm 1.8$ |
| | VP | $37.2^{b} \pm 1.43$ | $25.07^{a} \pm 1.04$ | $42.86^{c} \pm 1.2$ | $54.01^{d} \pm 1.5$ |
| Stimulation or Reduction (%) | F | - | -27.45 | -35.26 | +39.60 |
| | VP | - | -32.47 | +15.33 | +45.29 |
| Rhizome length (cm) | F | $29.00^{c} \pm 1.14$ | $21.31^{b} \pm 1.54$ | $8.07^{a} \pm 0.93$ | $44.19^{d} \pm 3.1$ |
| | VP | $29.00^{b} \pm 1.14$ | $14.1^{a} \pm 1.04$ | $41.1^{c} \pm 1.97$ | $43.01^{d} \pm 1.3$ |
| Stimulation or Reduction (%) | F | - | -26.36 | -72.13 | +52.58 |
| | VP | - | -51.44 | +41.66 | +43.01 |
| Fresh weight | F | $13.53^{c} \pm 1.79$ | $8.73^{b} \pm 0.33$ | $2.12^{a} \pm 0.23$ | $61.92^{d} \pm 0,8$ |
| | VP | $13.53^{b} \pm 1.79$ | $2.29^{a} \pm 0.11$ | $19.77^{c} \pm 1.26$ | $26.3^{d} \pm 1.04$ |
| Stimulation or Reduction (%) | F | - | -35.51 | -84.34 | +25.08 |
| | VP | - | -82.89 | +48.69 | +97.51 |

Values are means ± standard errors (n = 5). Values affected with the same letter are not significantly different at $p < 0.05$ (Duncan test).

## Allelochemicals mode of action.

### *Pigments content.*

Fig. 1 shows the effect of *N. glauca* flowers and vegetative part aqueous extracts on chlorophyll and carotenoid contents in lettuce leaves.

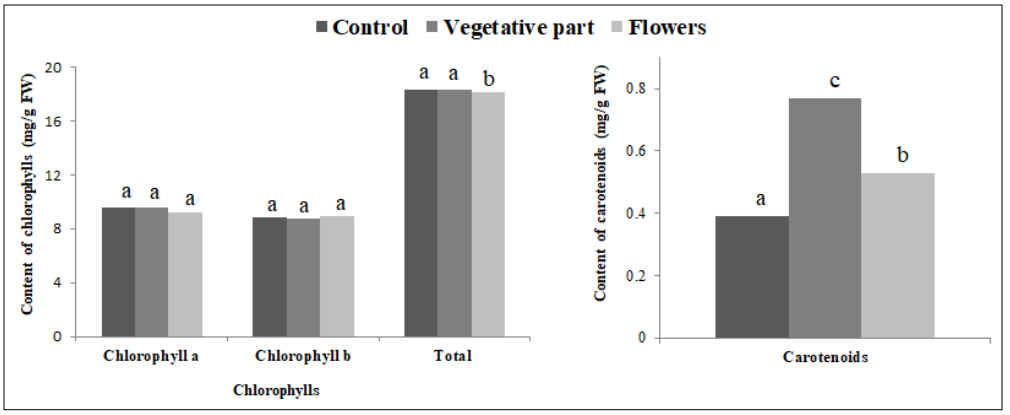


**Fig. 1.** Effect of *Nicotiana glauca* flower and vegetative part aqueous extracts on the contents of chlorophyll and carotenoids lettuce leaves. Values are means ± standard errors (n=5). Values affected by the same letter are not significantly different at $p < 0.05$ (Duncan test).

The carotenoid content increased significantly from 0.39 mg/g FW to 0.77 mg/g FW, in the presence of the vegetative extract, and to 0.53 mg/g FW in the presence of the flower extract. For chlorophylls *a*, *b* no significant effect was recorded, and the contents were similar to control, with 9.54 mg/g FW and 8.8 mg/g FW, respectively in control leaves against 9.6 mg/g FW and 8.73 mg/g FW in treated leaves with vegetative part extract, and against 9.21 mg/g FW and 8.96 mg/g FW in treated leaves with flower extract.

The treatment with the vegetative part extract did not also affect the total chlorophyll content. However, this content decreased slightly in the presence of the flower extract compared to control from 18.33 mg/g FW to 18.16 mg/g FW.

***Metabolic activity.***

Table 3 reports the formazan content, expressed in percent of control, of lettuce roots and leaves, from seedlings grown in the presence of *N. glauca* aqueous vegetative part and flower extracts (at concentration ($IC_{50}$) inducing a lettuce root growth 50% reduction). Results showed a decrease in the general mitochondria respiratory status evidenced by decrease in formazan production by a percentage of 19.45% and 17.52% respectively in roots and leaves treated with vegetative extract, and a reduction of 39.9% and 21.21% respectively in roots and leaves treated with flower extract.

**Table 3.** Effect of *Nicotiana glauca* vegetative part and flower aqueous extracts on the development of lettuce roots and leaves (expressed in Formazan content)

| Plant organs | Formazan content (% of control) | |
|---|---|---|
| | ***N. glauca* vegetative part extract** | ***N. glauca* Flower extract** |
| **Roots** | 80.55* ± 0.61 | 60.1 ± 0.19 |
| **Leaves** | 82.48* ± 0.89 | 79.79 ± 0.96 |

* = Significant difference at $p < 0.01$. Values are means ± standard errors (n = 5).

***Phenylalanine ammonia-lyase (PAL) and Tyrosine ammonia-lyase (TAL) activities.***

PAL activity was measured in lettuce leaves and roots grown in the absence and the presence of *N. glauca* aqueous flower and vegetative part extracts (Fig. 2A). In treated leaves, the PAL activity increased in a significant way, by 20% and 6% respectively in the presence of the extract of the vegetative part and that of the flowers, compared to the control. Similarly, in the case of roots treated with *N. glauca* vegetative part and flower aqueous extracts, this activity increased by 15% and 24% respectively, compared to the control, with values of $8.51\times10^{-8}$ µmol/min g FM and $9.21\times10^{-8}$ µmol/min g FM, against $7.43\times10^{-8}$ µmol/min g FM for the control.

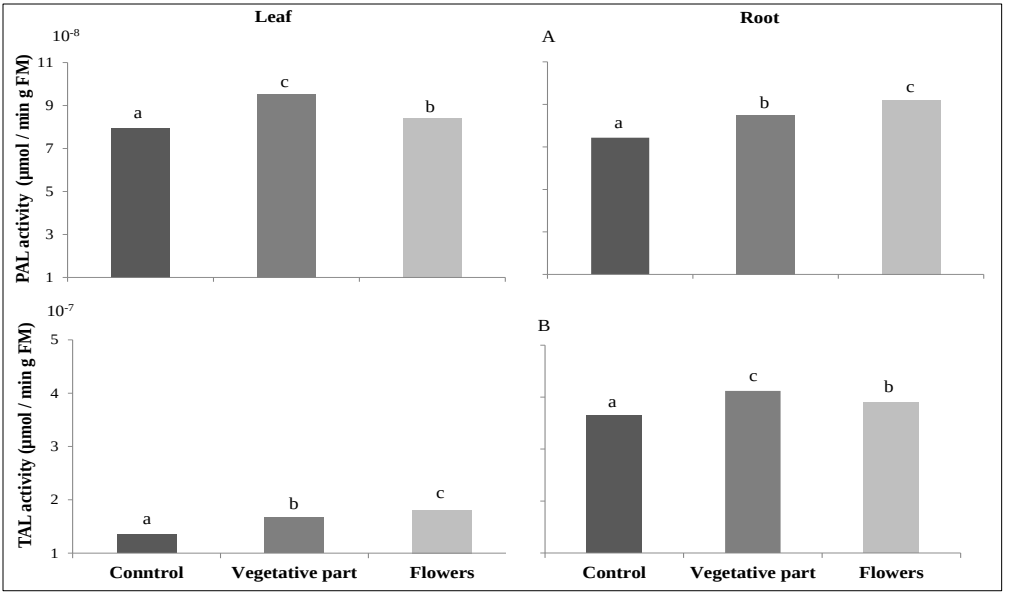


**Fig. 2.** Effect of *Nicotiana glauca* flower and vegetative part aqueous extracts on phenylalanine ammonia-lyase (PAL) (A) and tyrosine ammonia-lyase (TAL) (B) activity in lettuce leaves and roots. Values are means ± standard errors (SE) (n=5). Values affected by the same letter are not significantly different at $p<0.05$ (Duncan test).

For TAL activity, an increase of 24% in leaves treated with the vegetative part extract, and of 34% in those treated with flower extract, compared to the control ($1.35\times10^{-7}$ µmol/min g FM), was noted. Concerning roots, both extracts also significantly improved this activity, with stimulations of 13% and 7%, compared to the control ($3.65\times10^{-7}$ µmol/min g FM) (Fig. 2B).

***Electrolyte leakage and lipid peroxidation.***

Fig. 3 shows the variation of electrolyte leakage from lettuce roots and leaves treated with *N. glauca* vegetative part and flower aqueous extracts.

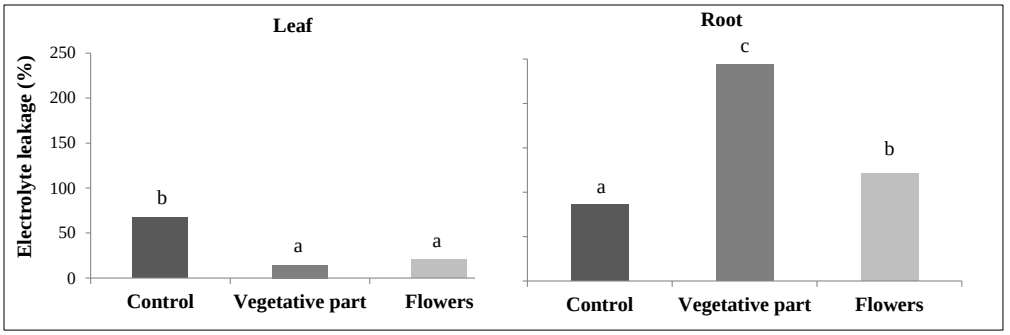


**Fig. 3.** Effect of *Nicotiana glauca* flower and vegetative part aqueous extracts on the electrolyte leakage (%) of lettuce roots and leaves. Values are means ± standard errors (SE) (n=5). Values affected by the same letter are not significantly different at $p<0.05$ (Duncan test).

Electrolyte leakage decreased significantly in leaves in the presence of both extracts, compared to control, from 67.58% to 14.42% in leaves treated with vegetative part extract, and to 20.48% in those treated with flower extract. This represents a considerable decrease exceeding 70% for both treatments. Similarly, a decrease in the amount of the major lipid peroxidation product, MDA, was observed in treated leaves (Fig. 4). The values were respectively 85.8 and 88.17 nmol MDA/g FW in treated leaves with vegetative part and flower aqueous extracts respectively, against 97.84 nmol MDA/g FW in control.

However, MDA content in treated roots increased significantly, compared to control, in the presence of both extracts. The increase was greater in roots treated with vegetative part extract, compared to those treated with flower one. The values were respectively 84.4 and 80.96 nmol MDA/g FW, against 59.67 nmol MDA/g FW in control (Fig. 4). Similarly for electrolyte leakage, which increased in a significant way, by 182% and 40% in the case of roots treated with vegetative part and flower extracts, respectively, compared to control.

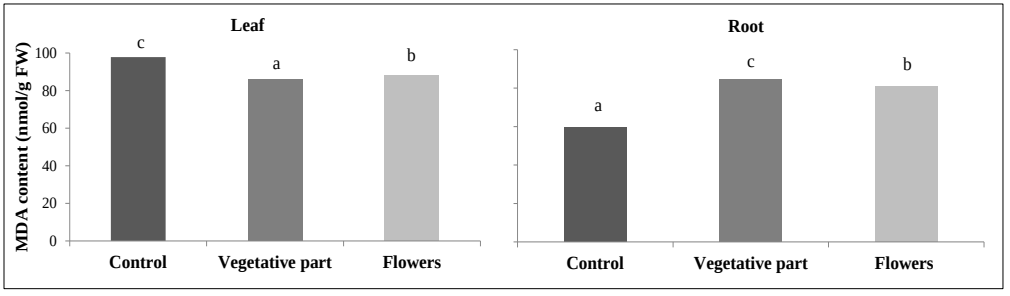


**Fig. 4.** Effect of *Nicotiana glauca* flower and vegetative part aqueous extracts on the content of malondialdehyde (MDA) lettuce roots and leaves. Values are means ± standard errors (SE) (n=5). Values affected by the same letter are not significantly different at $p<0.05$ (Duncan test).

***Proline content.***

Under the effect of *N. glauca* vegetative part extract, proline content increased significantly by 15.61%, in lettuce leaves. The proline content improvement in roots was higher than that in leaves. In fact, aqueous extract increased the root proline accumulation by 29.6% (Fig. 5).

Similarly, the proline content increased in the presence of flower extract, compared to control, by 33.83% and 11.12% in roots and leaves, respectively (Fig. 5).

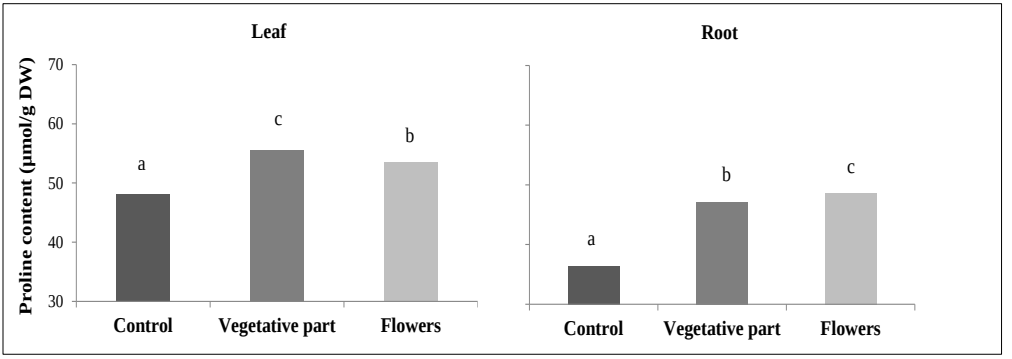


**Fig. 5.** Effect of *Nicotiana glauca* flower and vegetative part aqueous extracts on the content of proline lettuce leaves and roots. Values are means ± standard errors (SE) (n=5). Values affected by the same letter are not significantly different at $p<0.05$ (Duncan test).

***DPPH free radical-scavenging activity.***

Free radical scavenging was evaluated by the DPPH test. Table 4 illustrates the results from the radical scavenging assays for the studied samples. The best results were obtained with treated roots and leaves, with both extracts. Values were in the order of 300 µg/mL and 370 µg/mL from roots treated with vegetative part and flower extracts, and in the order of 290 µg/mL and 360 µg/mL from leaves treated with the same extracts. The values in untreated roots and leaves were 430 µg/mL and 400 µg/mL, respectively.

**Table 4.** Effect of *Nicotiana glauca* vegetative part and flower aqueous extracts on the DPPH free radical-scavenging activity of lettuce roots and leaves (expressed in µg dry weight/mL)

| Plant organs | DPPH (expressed in µg dry weight/mL) | | |
|---|---|---|---|
| | Control | Vegetative part | Flowers |
| **Roots** | $430^{c} \pm 0.05$ | $300^{a} \pm 0.07$ | $370^{b} \pm 0.09$ |
| **Leaves** | $400^{c} \pm 0.08$ | $290^{a} \pm 0.02$ | $360^{b} \pm 0.07$ |

* = Significant difference at $p < 0.01$. Values are means ± standard errors (n = 5).

***Secondary metabolites production.***

Secondary metabolite content in control and treated roots and leaves with *N. glauca* flower and vegetative part extract are represented in Table 5.

Results showed secondary metabolite stimulation in both types of organs, treated with both extracts, with significant differences with controls. This stimulation was higher in roots than in leaves, with stimulation percentages ranging from 23% to 205%, and from 10% to 38%, respectively. The most remarkable increase (205.35%) was in flavonoids, noted in treated roots with *N. glauca* flower extract.

**Table 5.** Effect of *Nicotiana glauca* vegetative part and flower aqueous extracts on secondary metabolites activity of lettuce roots and leaves

| Measured parameters | Total phenols mg GAEs/g DW | Flavonoids mg QEs/g DW | Flavanols mg QEs/g DW | Condensed tannins mg TAEs/g DW | Proanthocyanidin mg CEs/g DW |
|---|---|---|---|---|---|
| | **Leaves** | | | | |
| **Control** | $0.216^{a} \pm 0.002$ | $0.038^{a} \pm 0.001$ | $0.295^{a} \pm 0.001$ | $0.240^{a} \pm 0.001$ | $0.031^{a} \pm 0.001$ |
| **Vegetative part** | $0.237^{b} \pm 0.001$ | $0.044^{c} \pm 0.002$ | $0.347^{c} \pm 0.002$ | $0.331^{c} \pm 0.054$ | $0.037^{c} \pm 0.001$ |
| **Stimulation (%)** | 14.99 | 28.42 | 18.05 | 37.86 | 21.5 |
| **Flowers** | $0.258^{c} \pm 0.001$ | $0.041^{b} \pm 0.003$ | $0.337^{b} \pm 0.001$ | $0.293^{b} \pm 0.002$ | $0.034^{b} \pm 0.001$ |
| **Stimulation (%)** | 29.74 | 15.05 | 14.67 | 22.05 | 9.67 |
| | **Roots** | | | | |
| **Control** | $0.262^{a} \pm 0.023$ | $0.033^{a} \pm 0.002$ | $0.221^{a} \pm 0.013$ | $0.121^{a} \pm 0.002$ | $0.017^{a} \pm 0.001$ |
| **Vegetative part** | $0.406^{c} \pm 0.001$ | $0.053^{b} \pm 0.003$ | $0.270^{b} \pm 0.004$ | $0.203^{b} \pm 0.003$ | $0.026^{c} \pm 0.001$ |
| **Stimulation (%)** | 76.33 | 132.92 | 23.44 | 68.31 | 54.9 |
| **Flowers** | $0.361^{b} \pm 0.001$ | $0.064^{c} \pm 0.002$ | $0.305^{c} \pm 0.004$ | $0.215^{c} \pm 0.003$ | $0.021^{b} \pm 0.001$ |
| **Stimulation (%)** | 52.42 | 205.35 | 39.81 | 77.68 | 23.52 |

* = Significant difference at $p < 0.01$. Values are means ± standard errors (n = 5).

## DISCUSSION

To be effective, a judicious treatment against weeds must not damage the crop. In the present study, the effect of *N. glauca* was tested on a cultivated plant (tomato) and a vigorous weed (*C. dactylon*), through the pulverization of its flower and vegetative part aqueous extracts, and the incorporation of their powder to ground. Results showed that adding powdered dried flowers to soil surface was the most effective treatment either to inhibit *C. dactylon* growth or to increase the growth of tomato. These findings coincide with those of Alghamdi (2021) who reported that *N. glauca* contains varying amounts of flavonoids, alkaloids, tannins and steroids in leaves, stems, and flowers, but, only flowers contain saponins. So, the phytotoxicity of powdered dried flowers on the target weed could be attributed to saponins. The phytotoxicity attributed to saponins was evaluated by several studies that focused on an array of extracts containing these allelochemicals such as aqueous root/shoot extract from *Zea mays*, *Oenothera biennis*, and *Sapindus mukorossi*, among others. The saponins isolated from *Agave offoyana* have been reported to exhibit root growth inhibitory activity on lettuce. The tested saponins from *Microsechium helleri* on *Lolium perenne* showed significant radicle growth inhibition values (Durán et al. 2021).

However, the pulverization of flower aqueous extracts was less effective on *C. dactylon* control. Moreover, during pulverization treatment, there is direct contact of the target species with allelochemicals. These allelochemicals affect the target species directly.

Therefore, the environmental factors which could interfere with the phytotoxicity of allelochemicals (particularly in the soil), affecting their breakdown, and reducing or increasing their effectiveness, are absent (Jmii et al. 2020a). As a result, the inhibition of weed growth could be attributed to transformed forms of allelochemicals and not to their direct effect.

Additionally, adding powdered dried flowers to soil could confirm the interference of biotic (microorganisms, fungi, etc.) and abiotic (light, temperature, etc.) factors with the phytotoxic action of *N. glauca* secondary metabolites. It was reported by Scavo et al. (2019) that once released into the soil, allelochemicals interact with soil microorganisms. They are subjected to transformations by the complex of biological, chemical and physical characteristics of the soil environment. These interactions and transformations fix secondary metabolites phytotoxic levels and bioavailability (Scavo et al. 2019). It was shown also that the fresh weight of many weeds including palmer amaranth (*Amaranthus palmeri*), wonder berry (*Solanum nigrum*), and red root pigweed (*Amaranthus retroflexus*) were decreased significantly following the incorporation of canola crop residues into soil. Additionally, sorghum residue incorporation severely reduced weed number and stimulated the growth of broad bean (Ullah et al. 2022).

Generally, the incorporation of plant residues leads to a release of chemicals (from these residues) into soil, and weed suppression is observed (Ullah et al. 2022). The decomposition of residues into the soil not only provides allelochemicals, but also participates in nutrition for crop plants (such as nitrogen), which could explain tomato growth stimulation (Ullah et al. 2022). In addition, the improvement of soil properties, moisture retention, microbial activity, nutrient cycling due to the release of allelochemicals could explain this stimulation (Ullah et al. 2022).

For the mode of action of allelochemicals contained in the aqueous extract of *N. glauca*, the present study showed that they could affect the membrane integrity in lettuce roots. Indeed, an increase of MDA content and electrolyte leakage was observed. However, the decrease of these measured parameters in leaves could suggest that these organs had the ability to maintain membrane integrity. In fact, membranes are the main site of reactive oxygen species (ROS) attack, leading to severe lipid peroxidation, and protein polymerization and dimerization (Al-Zahrani et al. 2021, Jmii et al. 2020a). As a result, cell membranes could be altered (Al-Zahrani et al. 2021). MDA is an indicator metabolite of lipid peroxidation reactions (peroxidation of unsaturated fatty acids in membranes). The lipid peroxidation products are potential biomarkers for oxidative stress status (Vieira et al. 2018). Hence, changes in membrane integrity, permeability, and fluidity could be caused by ROS production and accumulation. These ROS can affect membranes, and result in DNA and protein damages, leading to cell death (Vieira et al. 2018). As a result, the increase of the antioxidant status responses, either in leaves or in roots, could be an alleviation of allelopathic-induced oxidative stress. These antioxidant status responses included an increase in DPPH radical-scavenging activity, and in carotenoid content. Indeed, the balance between ROS generation and ROS scavenging plays an important role to resist against the oxidative stress, and to build a tolerance to diverse environmental stress situations (Bano et al. 2021). ROS-induced damage can be alleviated using

lipophilic antioxidants, the carotenoids, known by their antioxidant property and their ability of detoxifying several kinds of ROS (Bano et al. 2021). However, even if roots enhanced antioxidative defense, these organs failed to maintain their membrane integrity. It was reported by Blokhina et al (2003) that the increase of antioxidative capacity does not correlate always positively with the protection degree. The antioxidant defense induce ability, compensation (and/or cooperation) between different antioxidant systems, antioxidant transport and synthesis, ROS formation compartmentalization and localization of antioxidant are the determinants of the antioxidant system competence (Blokhina et al. 2003).

In plant tissues, many secondary metabolites (including tannins, flavonoids, lignin, and hydroxycinnamate) are known for their antioxidant capabilities. They may work as ROS-scavenging compounds (Bano et al. 2021). Furthermore, Ahmed et al. (2017) revealed that the antioxidant power of flavonoids and polyphenols is due to the hydrogen-donating ability of their hydroxyl groups, as well as their ability to donate electrons (to arrest the production of free radicals). So, they may be regarded as a line of defense against reactive allelochemicals toxicants. Polyphenols have an aromatic ring with -OH or $OCH_3$ substituents that contribute to their antioxidant activity. They inhibit lipid peroxidation by scavenging lipid alkoxyl radicals (Bano et al. 2021). It was shown also that tannins (subclass of water-soluble phenolic compounds) have significant antioxidant properties. In order to minimize molecular damage of cells, they play an important role as free radical scavenger (Ahmed et al. 2017).

Otherwise, results showed a stimulation of proline production. In fact, it was demonstrated that during stress, proline is accumulated in huge concentrations in plants (Bano et al. 2021). This osmolyte is considered as a potent nonenzymatic antioxidant, employed to combat the detrimental effects of diverse ROS members (Bano et al. 2021).

Generally, the impact of allelochemicals on plant photosynthesis mainly involves damage and/or inhibition of the synthesis machinery, and acceleration of photosynthetic pigment decomposition (Hussain and Reigosa, 2011). Hence, allelochemicals could influence the performance of the three photosynthesis main processes: stomatal control of $CO_2$ supply, thylakoid electron transport (light reaction), and carbon reduction cycle (dark reaction), a conclusion recognized by Hussain and Reigosa (2011). In this study, no significant difference on total chlorophylls, chlorophyll *a*, *b* was recorded (except for the slight decrease in total chlorophylls, in the presence of the flowers extract). These results could be explained by protective roles of carotenoids, which protect chlorophyll from photo-oxidation (Khosravinejad et al. 2008). They protect the photosynthetic system by acting as an antioxidant. In fact, to avoid oxidative damage, they scavenge $^1O_2$. They quench excited chlorophyll (*Chl*) molecules and triplet sensitizer (*3Chl*) to prevent $^1O_2$ creation (Bano et al. 2021). The stimulation of proline biosynthesis in chloroplasts could also sustain the electron flow between photosynthetic excitation centers and stabilize the redoxe balance, leading to a decrease of photo-inhibition and photosynthetic apparatus damage. Additionally, the protection of subcellular structures and macromolecules by proline could explain the maintain of the green color in leaves (Jmii et al. 2020a). Flavonoids could also protect photosynthesizing cells by scavenging

superoxide radicals (Al-Zahrani et al. 2021).

PAL and TAL activities were improved in treated lettuce seedlings. In different plant species, these two enzyme stimulations are generally recognized as biotic and abiotic stresses marker (Al-Zahrani et al. 2021). The increase of their activities is a typical response against environmental stress in plants, which is concomitant with a stimulation of phenolic compounds production. TAL and PAL are important enzymes of phenolic compounds biosynthetic pathway (Al-Zahrani et al. 2021).

Otherwise, the respiration in lettuce roots and leaves decreased, shown by a decrease in formazan content. It was reported by Fakhry et al. (2015) that some allelochemicals impair directly the mitochondrial respiration by interacting with the mitochondrial membrane. In addition, allelochemicals interference in different stages of respiration could affect plant respiration, such as $CO_2$ generation by electron transport, oxidative phosphorylation, and the activity of ATP enzyme (Jmii et al. 2020a).

To conclude, the present study showed that the allelochemicals produced from the flowers and the vegetative parts of *N. glauca* could interact with the growth of *C. dactylon* and tomato. Adding flower powder to soil was the most effective treatment to inhibit *C. dactylon* growth and increase tomato growth as well. Although, the spraying of the aqueous extract of the vegetative part and flowers, and the addition of the vegetative part to soil through other treatments had stimulatory effect on tomato, however they did not inhibit the growth of *C. dactylon*.

These results could be potentially exploited for the development of natural herbicides in agricultural practices. In fact, this study showed that *N. glauca* is rich in allelochemicals and may be used to control the vigorous weed *C. dactylon* and to improve tomato growth. However, other target species should be evaluated to confirm the biostimulant and bioherbicide effect of *N. glauca* on cultivated crops and weeds, respectively.

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**RESUME**

**Jmii G., Sayari M., Mars M., Gharsallaoui S. et Haouala R. 2022. *Nicotiana glauca*, une plante clé pour l'amélioration de la croissance de la tomate et le contrôle de l'adventice *Cynodon dactylon*. Tunisian Journal of Plant Protection 17 (2): 77-96.**

Dans le monde entier, les adventices sont la catégorie la plus coûteuse des pestes agricoles. Elles diminuent les rendements et la qualité des produits. C'est pourquoi leur contrôle est vital pour une culture réussie, ce qui est l'objectif de cette étude. Le présent travail vise à évaluer la phytotoxicité de la partie végétative et des fleurs de *Nicotiana glauca* sur la tomate et l'adventice *Cynodon dactylon*. Des expériences ont été réalisées dans des conditions du champ et un certain nombre de paramètres biochimiques et physiologiques ont été mesurés après la récolte. Les résultats ont montré que l'ajout de fleurs séchées en poudre au terreau (en quantité de 1%) a été le traitement le plus efficace, soit pour inhiber la croissance de *C. dactylon*, soit pour améliorer le rendement de la tomate. La stimulation de la croissance des parties aériennes, des racines et du poids frais a été respectivement de 35.25%, 328.97% et 159.04%. Il est également remarquable que la pulvérisation des extraits aqueux de la partie végétative et des fleurs et l'incorporation de la partie végétative dans le sol ont été efficaces pour stimuler la croissance de la tomate, mais elles ont été moins efficaces pour inhiber la croissance de l'adventice. En effet, les inhibitions les plus importantes de la croissance des parties aériennes, des rhizomes et du poid frais n'ont pas dépassé 66.31%, 70.54% et 96.54% après l'incorporation de la poudre de la partie

végétative (en quantité de 0.6%). La stratégie de défense développée par la laitue pour faire face au stress allélopathique pourrait expliquer la stimulation de sa propre croissance. En effet, elle a augmenté la production de certains métabolites tels que les polyphénols, les flavanols, les proanthocyanidines les flavonoïdes et les tanins en plus de la proline et des carotenoïdes. Une amélioration des activités PAL et TAL avec une stimulation de l'activité antioxydante démontrée par l'augmentation de l'activité du piégeage des radicaux libres du DPPH a également été enregistrée. Cependant, la réduction de la respiration et la perturbation de l'intégrité membranaire (démontrée par une augmentation de la teneur en malondialdéhyde et de la fuite des électrolytes) pourraient expliquer l'inhibition de la croissance de l'adventice. Ces résultats soulignent que l'utilisation des fleurs séchées en poudre de *N. glauca* pourrait être une approche efficace et facile pour exploiter ses précieux métabolites secondaires, soit pour contrôler *C. dactylon*, soit pour améliorer la production de la tomate.

*Mots clés*: Allélochimiques, *Cynodon dactylon*, inhibition, *Nicotiana glauca*, stimulation, tomate

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**ملخص**

**جميعي، غفران ومروى سياري ومسعود مارس وسمير غرسلاوي وربيعة حوالة. 2022. التبغ الأزرق (*Nicotiana glauca*)، نبات أساسي لتحسين نمو الطماطم ومكافحة العشب الضار *Cynodon dactylon*.**
**Tunisian Journal of Plant Protection 17 (2): 77-96.**

في جميع أنحاء العالم تعتبر الأعشاب الضارة أكثر فئات الآفات الزراعية تكلفة. إنها تقلل من جودة المنتجات والمحاصيل، وبالتالي السيطرة عليها أمر حيوي لزراعة المحاصيل الناجحة وهو هدف الدراسة الحالية. يهدف هذا العمل إلى تقييم السمية النباتية للجزء الخضري وزهور *Nicotiana glauca* على الطماطم وعلى العشب الضار *Cynodon dactylon*. أجريت التجارب تحت الظروف الحقلية وتم تحديد عدد من العوامل البيوكيميائية والفسيولوجية بعد الحصاد. أظهرت النتائج أن إضافة الزهور المجففة إلى تربة التأصيص (بكمية 1%) هي العلاج الأكثر فعالية إما لتثبيط نمو *C. dactylon* أو لزيادة محصول الطماطم. كان تحفيز طول الأجزاء العلوية والجذور والوزن الطازج يساوي 35.25% و 328.97% و 159.04% على التوالي. من الملاحظ أيضا فعالية العلاجات عن طريق رش المستخلصات المائية للجزء الخضري والزهور وإضافة الجزء الخضري للتربة في تحفيز نمو الطماطم، إلا أنها كانت أقل فعالية في تثبيط نمو العشب الضار. في الواقع، لم يتجاوز تقليل طول الأجزاء العلوية والجذور والوزن الطازج مستويات 66.31% و70.54% و96.54% بعد إضافة الجزء الخضري المجفف (بنسبة 0.6%). يمكن أن تفسر إستراتيجية الدفاع التي طورها الخس للتعامل مع ضغط التبادل المرضي (الأليلوباثي) تحفيز نمو الطماطم. في الواقع، زاد إنتاج بعض المستقلبات مثل البوليفينول والفلافانول والبروأنثوسيانيدين والفلافونويد والعفص بالإضافة إلى البرولين والكاروتينويد. تم أيضا تسجيل تحفيز نشاط إنزيمات PAL و TAL مع تحفيز النشاط المضاد للأكسدة عن طريق زيادة نشاط الإزالة الراديكالية الحرة DPPH. لكن انخفاض التنفس واضطراب سلامة الغشاء (الذي يظهر من خلال زيادة مستويات malondialdehyde وتسرب الإلكتروليت) يمكن أن يفسر تثبيط نمو العشب الضار. تؤكد هذه النتائج على أن استخدام مسحوق الزهور المجففة لنبات *N. glauca* يمكن أن يكون طريقة فعالة وسهلة لاستغلال المستقلبات الثانوية القيمة إما للقضاء على *C. dactylon* أو لتحسين إنتاج الطماطم.

*كلمات مفتاحية*: تثبيط، تحفيز، طماطم، مركبات أليلوكيماوية، *Cynodon dactylon*، *Nicotiana glauca*

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