Tunisian Journal of Plant Protection

Volume 17 Number 2 December 2022

A Tunisian Half-Yearly Journal of Plant Health Sciences (TJPP)

http://www.tjpp.tn

*e*ISSN 2490-4368 *p*ISSN 1737-5436

Tunisian Journal of Plant Protection
http://www.tjpp.tn

https://doi.org/10.52543/tjpp

Volume 17, No 2, December 2022

Contents

Photo of the cover page: *Pratylenchus vulnus* (Courtesy *Noura Chihani-Hammas*)

Acknowledgement of Reviewers

Tunisian Journal of Plant Protection (TJPP) gratefully appreciates the volunteer help of reviewers which evaluate, with care and competence, papers proposed for publication in the 2 Issues of the 17th Volume, 2022. They are listed below in recognition of their contribution.

Abdellaoui, Khemais, ISAChM, Univ. Sousse, Chott-Mariem, Tunisia
Abu Irmaileh, Barakat, University of Jordan, Jordan
Aref Ali, AbdulSattar, Anbar University, Iraq
Asadi, Mohammad, FANR, Univ. Mohaghegh Ardabili, Ardabil, Iran
Aydi-Ben Abdallah, Rania, CRRHAB, Univ Sousse, Tunisia
Belkadhi, Mohamed Sadok, IRA, Univ. Gabes, Medenine, Tunisia
Chaabane-Boujnah, Hanène, INAT, Univ. Carthage, Tunis, Tunisia
Cheikh M'hamed, Hatem, INRAT, Univ. Carthage, Tunis, Tunisia
Chikh Rouhou, Hela, CRRHAB, Univ. Sousse, Tunisia
Ciancio, Aurelio, CNR IPSP, Bari, Italy
Dabaj, Khalifa H., FA, Univ. Tripoli, Libya
Elimem, Mohamed, ESAMo, Univ. Carthage, Tunisia
Hdidar, Chafik, INRAT, Univ. Carthage, Tunisia
Ilahy, Riadh, INRAT, Univ. Carthage, Tunisia
Khan, Muhamed Azim, Univ.Agriculture, Peshawar, Pakistan
Kumari, Safaa, ICARDA, Beirut, Lebanon
Mahgoob, Alaa M.M.A., FS, Univ.Cairo; Giza, Egypt
Mekki, Mounir, ISAChM, Univ. Sousse, Chott-Mariem, Tunisia
Royo-Esnal, Aritz, Univ. Lleida-Agrotecnio, Lleida, Spain

Special thanks go to (1) Dr. Bernie Dominiak, Adjunct Fellow Macquarie University, Orange, Australia, and (2) Dr. Saliou Niassy, International Center of Insect Physiology and Ecology, Senegal/Kenya, for writing for TJPP the Guest Editorials in respectively Issues No. 1 and No. 2 of the Volume 17 (2022).

Guest Editorial

Entomology at the Service of Africa and Humanity

Insects are the most diverse and abundant forms of life on Earth and affect the well-being of millions of people, especially in the tropics, positively or negatively. On the one hand, insect pests contribute to the poor performance of the agriculture and health sectors. This results in economic losses and food income and health problems. Invasive insect pests, for example, and the diseases they transmit, represent a significant challenge to the development of the agricultural sector and, thus, to food and nutrition security in Africa. Their global economic cost is estimated at US$ 1.4 trillion annually, while Africa faces the largest share. A recent estimate of the economic impacts of 5 major invasive insect pests, including Chilo partellus, Liriomyza *spp. and* Tuta absoluta, *on smallholder mixed maize production systems in six African countries indicated losses of US$ 0.9-1.1 billion; and future annual losses (next 5-10 years) of US$ 1.0-1.2 billion.*

The global health costs associated with invasive disease vectors, excluding malaria, which is largely endemic, are estimated at US$ 6.9 billion, with Africa having a considerable burden. The cost of invasive human diseases vectors such as Asian tiger mosquitoes, Aedes albopictus *and other epidemiologically important vectors of diseases such as yellow fever, dengue, chikungunya fever, Zika virus and filarial nematodes is also considerable. In the livestock sector, insects such as biting flies, tsetse flies and other vectors cause serious diseases and a marked decline in livestock productivity. Sixty million people are exposed to sleeping sickness, and annual livestock and crop losses due to tsetse are estimated at US$ 4,750 million.*

On the other hand, insects provide some essential ecosystem services, mainly to communities living in fragile and marginalized areas, through their use as food, feed and medicines, bioconversion agents, pollination, beekeeping and silk farming. Consumed as food or used as animal feed or high-value products, insects contribute significantly to food and nutrition security and poverty reduction in Africa. They provide environmental services through pollination and waste recycling. Africa has nearly 500 species of edible insects. Many contribute to several high-value commercial insect products such as insect protein, honey, beeswax, propolis, honey brood, silk, oils (fats) and dyes.

As the continent struggles to cope with the major challenges of modern *times, when it comes to insect pests, the emergency response of governments relies heavily on the application of broad-spectrum synthetic insecticides, which seriously compromises environmental health and negatively affects providers of beneficial ecosystem services such as natural enemies, pollinators, or other non-target organisms. The continent's vulnerability to ignorance of insect-related advantages and disadvantages is compounded by a weak innovation ecosystem. Given the importance of insect science, the diverse African continent must prioritize entomology to meet global challenges.*

Entomology (the Latin word in-sect-um *was modeled on the Greek word* ἔντομον, én-tom-on*, "cut up", namely into three parts: the head, thorax, and abdomen) is the branch of zoology whose object is the study of insects. This discipline was relegated to the background for a long time by focusing first on agriculture (mainly biological control and beekeeping), then taking a more scientific aspect. This discipline is still unknown because of its subtlety which requires specialized training. Because of the great diversity of insects of interest, African academicians and researchers have not been able to tame this discipline and contribute effectively to the well-being of populations. At a time of insect extinction, very few structures are dedicated to insect research. African universities that inherited colonial curricula certainly provide generalities, but only in theory, not enough in practical or relevant. They are still far behind due to the lack of technological infrastructure needed to study insects, apart from those of ICIPE, on the continent. Without reliable data, it is difficult to study insects and obtain benefits or solve the problems that may arise from them. High-tech equipment is therefore needed for research and development.*

Entomologists are rarely involved in the great debates and complex topics that challenge humanity about the future of the planet. Yet recent studies show a clear correlation between insect decline and human well-being. Therefore, entomology can provide or contribute to solving contemporary themes such as climate change, health, food, job creation, environment, and energy. Hence, governments, the African Union, and other bodies must work closely with African and international entomologists to promote this discipline.

While insects such as bees are useful for pollination and agricultural productivity, others, such as mosquitoes that transmit malaria, are disease vectors. Those that attack and destroy crops, leading to a decline in agricultural production, are pests. However, entomology goes beyond these purely materialistic considerations. Indeed, the effect of entomology applied to disciplines such as chemistry, mathematics and bioinformatics edifies us on many secrets of the universe and the functioning of the planet. This is an opportunity to salute the efforts of the African Association of Entomologists AAIS, ICIPE, and national entomological societies, among many other structures.

In conclusion, insects represent about 70% of the creatures on Earth, with about one million species already known. While entomology has seen significant advances through development projects, much remains to be done, especially to build capacity in medical and veterinary entomology, taxonomy, ecology, anatomy and physiology. Therefore, African institutions of higher education must develop entomology curricula to facilitate its teaching. Entomology must be integrated into education systems from primary schools to university training.

By Saliou Niassy
Researcher, Former President and Secretary of AAIS,
Editor-in-Chief of the International Journal of Tropical Insect Science

Phytochemical Evaluation and Nematicide Effect of a Dry Leaves Aqueous Extract of *Eucalyptus globulus* against *Pratylenchus vulnus* Infecting Apple

Noura Chihani-Hammas, UR13AGR04-Développement de la Protection Biologique et Intégrée au niveau de la parcelle en agriculture biologique, ISA Chott-Mariem, Université de Sousse, 4042, Sousse, Tunisia, **Lobna Hajji-Hedfi**, Centre Régional des Recherches Agricoles de Sidi Bouzid, BP 357, 9100, Sidi-Bouzid, Tunisia, **Asma Larayedh, Hajer Regaieg**, **and Najet Horrigue-Raouani**, UR13AGR04-Développement de la Protection Biologique et Intégrée au niveau de la parcelle en agriculture biologique, ISA Chott Mariem, Université de Sousse, 4042, Sousse, Tunisia. **(*Tunisia*)**
https://doi.org/10.52543/tjpp.17.2.1

ABSTRACT

Chihani-Hammas, N., Hajji-Hedfi, L., Larayedh, A., Regaieg, H., and Horrigue-Raouani, N. 2022. Phytochemical evaluation and nematicide effect of a leaf aqueous extract of *Eucalyptus globulus* against *Pratylenchus vulnus* infecting apple. Tunisian Journal of Plant Protection 17 (2): 43-54.

The nematicide effect of an aqueous extract from dried leaves of *Eucalyptus globulus* was evaluated against the root lesion nematode *Pratylenchus vulnus* in vitro and in vivo trials. In both experiments, the application of four concentrations of a dried leaf aqueous extract (30 g/100 mL: 100%, 20 g/100 mL :60%, 10 g/100 mL: 30%, 5 g/100 mL: 15%; w/v) significantly reduced the nematode number of females and males in roots of the apple rootstock MM106. In vitro tests showed that the highest mortality was recorded with the undiluted concentration (96%) after 72 h of exposure time. Results of in vivo experiment revealed also that the concentration 100% has significantly increased the reduction rates of the nematode population of females and males (84.43% and 91.40%, respectively) compared to the other concentrations. The chemical treatment with Oxamyl G has significantly reduced the female and male population by 98.30% and 100%, respectively. The chemical analysis of dried leaves of *Eucalyptus globulus* showed high levels of total phenol and total flavonoid contents and exhibited high antioxidant capacity. The obtained results suggest that aqueous extracts of *Eucalyptus globulus* leaves have a promising nematicide potential against *P. vulnus*.

Key words: Apple rootstock, bioactive metabolites, biological control, *Eucalyptus globulus*, *Pratylenchus vulnus*

Plant-parasitic nematodes are dangerous enemies of several agricultural and horticultural crops. The estimated annual yield loss due to plant parasitic nematodes on major crops of the world is 14% (Mesa-Valle et al. 2020). The

Corresponding author: Noura Chihani-Hammas
Email: noura.chihani@yahoo.com

Accepted for publication 13 December 2022

Pratylenchus species are economically important pests of many crops (Castillo and Volvas 2007). The root lesion nematode, *Pratylenchus vulnus* is recognized as a pathogen of apples and has been shown to be a serious nematode pest causing yield losses in apple orchards in warm Mediterranean environments (Pinochet et al. 1993) including Tunisia (Bouali et al., 2014; Chihani-Hammas et al. 2018).

The plant parasitic nematodes management is commonly based upon chemical treatment. Due to the harmful toxicity for the environment and risks to human health, the use of this type of material is discussed. Plant-derived nematicides could be promoting alternative to pesticides use and fit well to the principles of integrated pest management. Natural products obtained from plants have been used as pest management agents. They served also in some commercial biopesticides (Lima et al.2003; Cantrell et al. 2012).

Eucalyptus globulus (Myrtaceae) is an indigenous tree of Australia, widely applied in medicine (Bigendako, 2004). The leaves of several species of *Eucalyptus* have shown biological activities including anti-microbial (Ait-Ouazzout et al. 2011), fungicidal, insecticidal/insect repellent, herbicidal, acaricidal and nematicidal effects (Batish et al. 2008). The chemical profiles of *E. globulus* leaves, in particular the essential oil, have been widely studied (Adenike Fabiyi et al. 2020). The leaves of *E. globulus* contain 70% of eucalyptol (1,8-cinéole). A large number of monoterpenoids have been identified, mainly alpha-pinene, s-pinene, δ-limonene, para-cymene, camphene, α-phellandrene, α-fenchene, γ-terpinene. The aromadendrene and alloaromadendrene are the most found sesquiterpenoids. Monoterpen glycosides (globulisin, cypellocarpin, euglobulin), flavonoid (quercetin, rutin), polyophenols (catechol, cafeic acid, gallol, etc.) are other compounds most found in *E. globulus* leaves (Amakura et al. 2002; Wichtl and Anton 2003; Bruneton 2009). The nematicide effect of several plants is attributed to these chemical compounds (Osman and Viglierchio1988; Mahmood and Siddiqui1993; Yoshizawa et al. 1993; Nandakumar et al. 2017; Zaidat et al. 2020).

The present study aimed to evaluate the nematicide effect of aqueous extracts from dried leaves of *E. globulus* against *P. vulnus* in vitro and in vivo experiments. Furthermore, a chemical screening about total phenols and total flavonoids contents and antioxidant activity of the aqueous extracts was realized.

MATERIALS AND METHODS

Plant collection and extract preparation.

P. vulnus leaves were collected from wild trees in Oueslatia, Kairouan (center of Tunisia) and dried in the shade at room temperature (30 to 40°C) for fifteen days. A leaf powder was prepared by grinding the leaves using commercial mortar. To obtain the aqueous extract, 30 g of the leaf powder was added to 100 mL of sterilized distilled water in glass flasks. The flasks were placed under an orbital shaker for 48 h at room temperature (about 25°C). The obtained water suspension in the flasks was filtered through a Whatmann filter paper N°1 and the filtrate was used for in vitro and in vivo essays. The obtained filtrate was considered as a concentrated solution (100%) and other concentrations (60%, 30%, 15% w/v) were prepared, at the same time when the biotest and pot experiments were performed, by adding the required amount of sterilized distilled water.

Phytochemical analyses.

The preparation of samples and determination of total flavonoids, total phenols, antioxidant activity and HPLC-DAD for phenolic profile were determined as described by Hajji-Hedfi et al. (2019).

Sample Preparation. The 1.5 g of plant fine powder was extracted in absolute methanol by magnetic stirring for 30 min. The mixture was kept at 4°C for 24 h in the dark. The methanolic extract was filtered through Whatmann paper N°1 and stored at 4°C in a freezer till subsequent analysis (Dewanto et al. 2002).

Determination of the total flavonoids content. One mL of sample was added to 1 mL of freshly prepared aluminum chloride solution (AlCl3 .6H2O, 20%). The absorbance was determined at 430 nm after 40 min of incubation.

Determination of total phenol content. A 40 µL of methanolic extract was added to 200 µL of the Folin-Cicalteu reagent and 3.16 mL of bi-distilled water. At room temperature, the mixture was vortexed and incubated in the dark for 3 min. A volume of 600 µL of sodium carbonate (Na_2CO_3, 20%) was added to the mixture. The absorbance was determined at 765 nm after incubation for 30 min at 40°C.

Determination of antioxidant activity. To determine the antioxidant activity, the scavenging of the 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) stable free radical was measured. A 210 µL of plant extract was mixed with 1940 µL of DPPH solution. Then, the mixture was vortexed for 45 min in darkness. The absorbance was determined at 517 nm and compared with methanol alone (control).

Determination of HPLC-DAD for phenolic profile. The measured of phenolic compounds was determined by a Shimadzu UFLC XR system (Kyoto, Japan), equipped with a SIL-20AXR auto-sampler, a CTO-20 AC column oven, a LC-20ADXR binary pump and a quadripole 2020 detector system (in IRA Médnine).

In vitro nematicide assay.

P. vulnus specimens were extracted by double centrifugation method (De Grisse 1969) from apple roots of MM106 rootstock. The nematode population was reared monoxenically on carrot disks. After 2 months, all used inoculums were obtained by adding sterile water to carrot disks and collecting nematodes on the 38-µm sieve. Fifty adult of *P. vulnus* (10 males + 40 females) were placed in sterilized 5 cm Petri dishes; each dish contained 1 mL of a dried leaf aqueous extract (4 concentrations) with ten replicates for each treatment. Plates treated with only distilled water were used as a negative control (0%). The plates were maintained at 25°C in darkness. Then, the effect of the aqueous extracts on *P. vulnus* viability was checked after 24, 48 and 72 h. The dead nematodes were counted under a stereoscopic microscope. The nematodes that responded by moving after 24 h were considered alive, whereas those not responding were considered dead. The experiment was repeated twice.

In vivo experiment.

A pot trial was conducted under greenhouse conditions. A Total of 42 seedlings of the rootstock 'MM106' (one year old) originated from Oued Melliz in the governorate of Jendouba (north of Tunisia) were transplanted each one into

10 kg soil pots filled with a sterilized sandy loam soil (1:1 v/v). The initial average population is determined from all rootstock tree naturally infected by *P. vulnus* (1390 females and 123 males per 1 g of roots). Four concentrations of leaf aqueous extract of *E. globulus* were used (100%, 60%, 30% and 15%). Each pot was treated by injecting 10 mL of the corresponding concentration into two holes at 2-4 cm deep around root system. Additional pots were treated with 3 g of Oxamyl G and were used as positive control for comparison. Plants naturally infected and not treated are considered negative control. All treatments were arranged in a completely randomized design with seven replications.

After 2, 4, and 6 months from the treatments; a root sampling from each treatment was realized. In order to have a representative sample from each treatment, 10 g of roots from each replication were mixed. After extraction, the *P. vulnus* population was examined and counted under a stereoscopic microscope. At the end of experiment (after 6 months of the treatments), the following growth parameters were measured: foliage tree diameter, plant height, trunk diameter, branch number, and number of leaves per branch.

Statistical analysis.

The data were subjected to analysis of variance (ANOVA) using SPSS 20.0 for Windows. The bioassays data were analyzed by Probit regression to obtain the Lethal Dose (LD) values. Laboratory and pot experiments were repeated twice and the treatment means were compared by the Tukey's multiple range tests when the *F*-tests were statistically significant at $P < 0.05$.

RESULTS

Phytochemical analyses of dried leaf composition.

The results for flavonoid and total phenolic contents and antioxidant activities measured by DPPH method from aqueous extracts of dried leaves of *E. globules* showed that the total flavonoid and total phenol contents were 14183.28 µg QE/g D.W and 185.45 µg GAE/g D.W, respectively. It was also revealed that aqueous extracts have an important antioxidant activity going up to 62.19%.

The overall chemical composition of leaf aqueous extract from *E. globulus* proved the presence of a considerable number of phenolic acids such as quinic acid and gallic acid (Table 1), in addition to the quercetin, a main flavonoid constituent. Other phenolic acids were identified from aqueous extract like catechin (+), caffeic acid, 4-0-caffeoylquinic 3,4-di-O-caffeoyquinic acid, and 1,3-di-O-caffeoyquinic acid but with low concentrations (Table 1).

In vitro effect of *E. globulus* aqueous extract on the nematode.

The in vitro efficacy of *E. globulus* leaf aqueous extracts on *P. vulnus* mortality is presented in Table 2. The results showed that the four tested concentrations have significantly increased the *P. vulnus* mortality as compared with control. After 24 and 48 h, the undiluted extract C1 (100%) exhibited the highest mortality as compared with the diluted extracts at 60%, 30% and 15%. After 72 h of exposure, both treatment with C1 and C2 (60%) exhibited the greatest net mortality ($P < 0.05$).

The lethal doses of 50% and 90% (LD50 and LD90) of *E. globulus* aqueous extracts were 83.19 µg/mL and 189.12 µg/mL respectively.

In vivo efficacy of *E. globulus* aqueous extract on the nematode.

Nematode population development. The results in Table 3 obtained after 6 months of treatment revealed that the concentration C1 (100%) has significantly reduced ($P < 0.05$) *P. vulnus* population development as it corresponds to the highest total reduction rate comparing to other concentrations (C3 and C4).

The *P. vulnus* female populations were significantly reduced with the concentrations 100% and 60% but male populations were significantly reduced only under the concentration 100%.

The chemical treatment with Oxamyl G was related to the highest population reduction rate for both sex (98.30% for females and 100% for males).

In general, the invasion of the rootstock MM106 roots by *P. vulnus* has been significantly decreased by Oxamyl G followed by the concentrations 100%, 60%, 30% and 15% (Table 3).

Table 1: Phytochemical screening of phenolic constituents in dried leaves of *E. globulus*

Name	Concentration (ppm)
Quinic acid	5726.77
Rutin	973.74
Quercetin	309.38
Gallic acid	249.45
Quercetrin (quercetin-3-o-rhamonosid)	113.56
Chlorogenic acid	141.27
Syringic acid	96.03
Hyperoside (quercetin-3-o-galactoside)	63.35
4-O-Caffeoylquinic acid	55.35
Trans frulic acid	32.50
Cirsiliol	22.31
P-coumaric acid	16.01
Luteolin	5.98
Caffeic acid	4.80
Cirsilineol	4.19
Apegenin	2.85
Catechin (+)	2.81
1,3-di-O-caffeoyquinic acid	2.67
Kampherol	0.65
3,4-di-O-caffeoyquinic acid	0.40

Table 2: In vitro efficacy of different concentrations of the dried leaf aqueous extract of *Eucalyptus globulus* on the mortality of *Pratylenchus vulnus*

Treatment	Mortality/24 h (%)	Mortality/48 h (%)	Mortality/72 h (%)	Net Mortality (%)
***Eucalyptus globulus* (30 g/100 mL)**	62.00±10.32 c	85.00±5.27 d	96.00±5.16 c	95.78±5.46 b
***Eucalyptus globulus* (20 g/100 mL)**	45.00±7.15 b	73.00±6.74 c	92.00±6.32 c	91.39±7.50 b
***Eucalyptus globulus* (10 g/100 mL)**	41.00±9.94 b	58.00±4.21 b	78.00±7.88 b	77.17±7.66 a
***Eucalyptus globulus* (5 g/100 mL)**	43.00±8.23 b	59±3.16 b	78.00±4.21 b	77.05±4.05 a
Control	49.33±0 a	72.00±0 a	88.67±6.99 a	-

Values are means ± standard deviation of five replicates. Values in columns followed by the same letter are not significantly different at $P < 0.05$, according to Tukey's multiple-range test.

Table 3: In vivo efficacy of different concentrations of the dried leaf aqueous extract of *Eucalyptus globulus* on mortality of *Pratylenchus vulnus*

Treatment	Female reduction (%)	Male reduction (%)	Total reduction (%)	Sex ratio
***Eucalyptus globulus* (30 g/100 mL)**	84.43±4.45b	91.40±3.76 b	84.84±4.24b	1.08±0.06 b
***Eucalyptus globulus* (20 g/100 mL)**	77.97±4.32 b	82.84±5.87 a	78.30±4.15 b	0.97±0.22 b
***Eucalyptus globulus* (10 g/100 mL)**	64.95±9.66 a	81.05±5.71a	65.84±9.38 a	1.26±0.14 a
***Eucalyptus globulus* (5 g/100 mL)**	63.76±7.66 a	78.53±3.10 a	64.59±7.26 a	1.24±0.12 a
Oxamyl G (3g)	98.30±0.34 c	100±0 c	98.40±0.31c	1.01±0.03 c

Values are means ± standard deviation of four replicates. Values in columns followed by the same letter are not significantly different at $P < 0.05$, according to Tukey's multiple-range test.

.

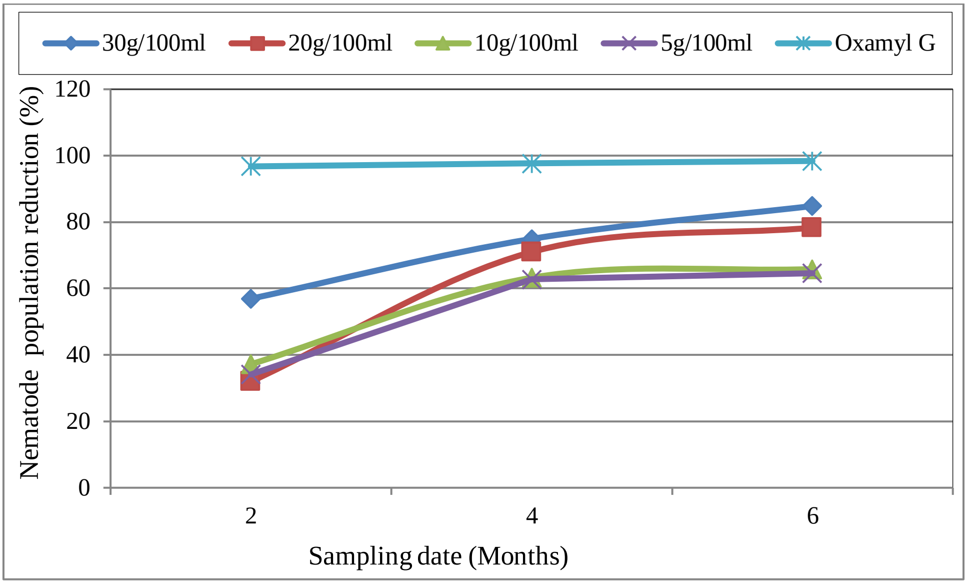

Fig. 1. Effect of different concentrations of *Eucalyptus globulus* aqueous extract on *Pratylenchus vulnus* total population development on apple rootstock roots.

According to results in Fig. 1, after 2 months, the undiluted aqueous extract reduced significantly (P < 0.05) female and male population rate by 56.23% and 60.13%, respectively, compared to other extract concentrations. Oxamyl G enhanced female and male population reduction rate respectively by 96.61% and 100%. After 4 months, the highest concentration showed significantly (P < 0.05) female and male population reduction rate (74.50% and 82.52%, respectively) compared to the chemical treatment reduction rate by 97.51% and 100%, respectively. At the end of experiments (after 6 months), the undiluted concentration revealed significantly (P < 0.05) female and male population reduction rate (84.43% and 91.4%, respectively).

Plant growth. The results showed in Table 4 indicate that Oxamyl G has significantly improved rootstock growth compared with the other treatments. The plants treated with the undiluted leaf aqueous extract showed maximum growth of branch number and number of leaves/branch as compared with both concentrations 60% and 30% (P < 0.05). Both chemical treatment and different concentrations of the leaf extract have significantly improved the number of leaves per branch in comparison with the negative control (Table 4).

Plants treated with Oxamyl G have been significantly distinguished by the highest foliage diameter, while there was no significant difference for this parameter between the negative control and the different concentrations of the leaf aqueous extract. For trunk diameter, no significant difference have been recorded between tested concentrations (Table 4).

Table 4: Efficacy of different concentrations of aqueous leaf extracts of *Eucalyptus globulus* on apple rootstock MM106 growth parameters

Treatments	Foliage tree diameter (cm)	Branch number	Trunk diameter (cm)	Plant height (cm)	Leaves per branch
***Eucalyptus globulus* (30 g/100 mL)**	10.29 a	6.86 b	2.71 ab	97.14 ab	10.43 bc
***Eucalyptus globulus* (20 g/100 mL)**	9.57 a	6.00 ab	2.57 ab	89.29 ab	8.57 ab
***Eucalyptus globulus* (10 g/100 mL)**	8.43 a	5.86 ab	2.36 ab	90.00 ab	7.86 ab
***Eucalyptus globulus* (5 g/100 mL)**	8.21a	5.71 ab	2.36 ab	90.29 ab	7.85 ab
Oxamyl G (3g)	17.71 b	9.43 c	2.93 b	98.57 b	11.71 c
Control	7.57 a	4.86 a	2.21 a	80.00 a	5.57 a

Values are means ± standard deviation of four replicates. Values in columns followed by the same letter are not significantly different at $P < 0.05$, according to Tukey's multiple-range test.

DISCUSSION

Considering the results of total flavonoid and phenol contents and the antioxidant capacity, the dried leaves of the medicinal plant *E. globulus* contained high levels of phenols and flavonoids and showed a high level of the 1,1diphényl-2-picryl-hydrazyl (DPPH). *E. globulus* has been previously reported containing biologically active terpenoids, flavonoids, polyphenols and high antioxidant activity (Almeida et al. 2009; Boulekbache-Makhlouf et al. 2013). Several recent studies have shown that the biocidal activity was attributed to several bioactive molecules (Singh et al. 2009; Mickymaray 2019; Laquale et al. 2020; Zaidat et al. 2020). Mousa et al. (2011) revealed that the capacity of plant extracts in the prevention and control of plant diseases is due to the presence of some natural components such sterols, saponins, tanins, flavonoids and alcohols. The mortality of root knot nematodes (*M. javanica*) treated by *Foeniculum vulgare* and *Mentha spicata* extracts was basically due to tanins, saponsides, estragol, phenol (Oka et al. 2000). The phenols were reported to be involved in plant defense against pathogens (Dhakshinamoorthy et al. 2014). The tomato roots immerged in five solutions of phenols showed the decrease of *Radopholus reniformis* infection (Mahmood and Siddiqui 1993). The quercetin (flavonoid) inhibited the development of *Meloidogyne javanica* (Osman and Viglierchio 1988). The rotenone as flavonoid showed a nematicide activity against *Caenorhabditis elegans* (Botelho et al. 2007).

Furthermore, the aqueous extract of dried leaves used in our trial, showed a significant potential on reducing development of the root lesion nematode *P. vulnus* both in vitro and in vivo experiments. These findings provide scientific evidence to support the value of medicinal plants for pest management and reveal that *E. globulus* could be a promising source for potential biological control agent. Further work should be realized about the identification and the isolation of the specific components responsible for the high nematicidal action of the leaf extract.

A crude extract from fruits of *E. globulus* showed strong antimicrobial activities (Pereira et al. 2014). Our results about biocide activity are in line also with the finding of Damjanovic-Vratnica et al. (2011) who reported a high antimicrobial potential for the essential oil of *E. globulus* in comparison with some used antibiotics (ceftriaxone, amykacine and tetracycline). Additionally, a number of investigations have demonstrated an acaricide effect against *Boophilus microplus* (Chagas et al. 2002) and an insecticide effect against several insect genera (Lucia et al. 2008; Maciel et al. 2010; Pant et al. 2014).

The potential use of plant extract for *Pratylenchus* spp. control has been reported by several authors. Mello et al. (2006) reported the nematicide effect of *Chenopodium ambrosioides* against *Pratylenchus brachyurus*. The aqueous extract of *Melia azedarach* exhibited a nematicide effect against *Pratylenchus thornei* (Kepenekci et al. 2016). Furthermore, 75% of mortality among *P. vulnus* juveniles was recorded after 96 h of exposure to 15 µg/mL of essential oils extracted from *Rosmarinus officinallis* (Avato et al. 2017).

RESUME

Chihani-Hammas N., Hajji-Hedfi L., Larayedh A., Regaieg H. et Horrigue-Raouani N. 2022. Evaluation phytochimique et effet nématicide de l'extrait aqueux de feuilles sèches d'*Eucalyptus globulus* contre *Pratylenchus vulnus* infectant le pommier. Tunisian Journal of Plant Protection 17 (2): 43-54

L'activité nématicide de l'extrait aqueux de feuilles sèches d'*Eucalyptus globulus* a été évaluée vis-à-vis du nématode des lésions racinaires *Pratylenchus vulnus in vitro* et *in vivo*. Dans les deux expériences, l'application de quatre concentrations de l'extrait aqueux de feuilles (100%, 60%, 30%, 15% p/v) a réduit de manière significative le nombre de femelles et de mâles sur les racines du porte-greffe de pommier MM106. Le test *in vitro* a montré que la mortalité nette la plus élevée a été enregistrée avec la concentration de 100% (96%) après 72 h d'exposition. L'étude expérimentale *in vivo* a montré que la concentration de 100% augmentait significativement les taux de réduction des populations des femelles et des mâles (84.43% et 91.4%, respectivement) par rapport aux autres concentrations. Le traitement chimique à l'oxamyl G a réduit significativement les populations des femelles et des mâles de 98.3% et 100%, respectivement. L'analyse chimique des feuilles sèches d'*E. globulus* a montré un taux élevé des phénols totaux, des flavonoïdes totaux et une capacité antioxydante élevée. Les résultats suggèrent que les extraits aqueux de feuilles d'*E. globulus* ont un potentiel nématicide prometteur contre *P. vulnus.*

Mots clés: *Eucalyptus globulus*, lutte biologique, métabolites bioactifs, porte-greffe de pommier, *Pratylenchus vulnus*

ملخص

شيهاني-حمّاص، نورة ولبنى حاجي-هادفي وأسماء لعريض وهاجر رقيق ونجاة حريق-رواني. 2022. تقييم كيميائي نباتي والتأثير المبيدي النيماتودي للمستخلص المائي للأوراق الجافة لشجرة *Eucalyptus globulus* ضد النيماتودا *Pratylenchus vulnus* الذي يصيب التفاح. Tunisian Journal of Plant Protection 17 (2): 43-54.

تم تقييم نشاط المستخلصات المائية للأوراق الجافة لنبات *Eucalyptus globulus* ضد نيماتودا آفة الجذر *Pratylenchus. vulnus* في الوسط الزجاجي وفي الجسم الحي. في كلا التجربتين، أدى تطبيق أربعة تراكيز من المستخلص المائي للأوراق (100%، 60%، 30%، 15%) إلى تقليل عدد الإناث و الذكور على جذور حامل طعوم التفاح MM106 بشكل معنوي. أظهر الاختبار في الوسط الزجاجي أن أعلى معدل وفيات سجل مع تركيز100% (96%) بعد 72 ساعة من وقت التعرض للمعاملة. وأشارت الدراسات في الجسم الحي إلى أن التركيز 100% زاد بشكل معنوي في معدلات نقص أعداد الإناث والذكور (84.43% و 91.4%، على التوالي) مقارنة بالتركيزات الأخرى. وأدت المعاملة الكيمياوية بأوكسميل جي (Oxamyl G) إلى انخفاض في معدلات أعداد الإناث و الذكور بنسبة 98.3% و 100%، على التوالي. أظهر التحليل الكيميائي للأوراق الجافة مستويات عالية من إجمالي الفينولات وإجمالي الفلافونويد كما أظهر قدرة عالية ضد التأكسد. تشير هذه النتائج إلى أن المستخلصات المائية لأوراق *E. globulus* لها إمكانيات مبيدية واعدة ضد النماتودا *P. vulnus*.

كلمات مفتاحية: حامل طعوم التفاح، مستقلبات ناشطة بيولوجيا، مكافحة بيولوجية، *Pratylenchus vulnus*، *Eucalyptus globulus*

LITERATURE CITED

Adenike Fabiyi, O., Atolani, O., and Ademola Olatunji, G. 2020. Toxicity Effect of *Eucalyptus globulus* on *Pratylenchus* spp. of *Zea mays*. Sarh. J. Agric. 36 (4): 1244-1253.

Ait-Ouazzout, A., Loran, S., Bakkli, M., Laqlaoui, A., Rota, C., Herrera, A., Paqan, R., and Conchello, P. 2011. Chemical composition and antimicrobial activity of essential oils of *Thymus algeriensis*, *Eucalyptus globulus* and *Rosmarinus officinalis* from Morocco. J. Sci. Food Agric.91(14): 2643-2651.

Almeida, I.F., Fernandes, E., Lima, J.L.F.C., Valentâo, P., Andrade, P.B., Seabra, R.M., Costa, P.C., and Bahia, M.F. 2009. Oxygen and nitrogen reactive species are effectively scavenged by *Eucalyptus globulus* leaf water extract. J. Med. Food 12: 175-183.

Amakura, Y., Umino, Y., Tsuji, S., Ito Hatano, T., Yoshida, T., and Tonogai, Y. 2002. Constituents and their anti-oxidative effects in eucalyptus leaf extract used as a natural food additive. Food Chem. J. 77:47–56.

Avato, P., Laquale, S., Argentieri, M.P., Lamiri, A., Radicci, V., and D'Addabbo, T. 2017. Nematicidal activity of essential oils from aromatic plants of Morocco. J. Pest. Sci**.** 90: 711-722.

Batish, D.R., Singh, H.P., Kohli, R.K., and Kaur, S. 2008. *Eucalyptus* essential oil as a natural pesticide. Forest. Ecol. Manag. 256: 2166–2174.

Bigendako, M.J. 2004. Identification et zonage des *Eucalyptus globulus* au Rwanda. Chemonics International Inc. Projet ADAR, Rwanda, 31 pp. (https://pdf.usaid.gov/pdf_docs/PNADK916.pdf)

Botelho, M.A., Nogueira, N.A.P., Bastos, G.M., Fonseca, S.G.C. et al.2007. Antimicrobial activity of the essential oil from *Lippiasidoides*, carvacrol and thymol against oral pathogens. Braz. J. Med. Biol. Res.40: 349-356.

BouAli, N., Kallel, S., and Horrigue Raouani, N. 2014.Caractérisation de différentes espèces de *Pratylenchus* associées aux marcottières et aux plants greffés de MM106 en pépinières d'arbres à pépins en Tunisie. Ecologia Mediterranea 40 (1): 75-86.

Boulekbache-Makhlouf, L., Meudec, E., Mazauric, J.P., Madani, K., and Chenier, V. 2013. Qualitative and semi-quantitative analysis of phenolics in *Eucalyptus globulus* leaves by high-performance liquid chromatography coupled with diode array detection and electrospray ionisation mass spectrometry. Phytochem. Anal 24: 162-170.

Brand-Williams,W., Cuvelier, M.E., and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol.28: 25-30.

Bruneton, J. 2009. Pharmacognosie, Phytochimie, Plantes Médicinales. Tec et Doc / Lavoisier (4ème Ed.), France, 1270 pp. (https://books.google.dz/books?id=jDVXngEACAAJ&printsec=frontcover&hl=fr&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false)

Cantrell, C.L., Dayan, F.E., and Duke, S.O. 2012. Natural Products as sources of new pesticides. J. Nat. Prod.75: 1231-1242.

Castillo, P., and Volvas, N. 2007. *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, biology, pathogenicity and management. Series: Nematology Monographs and Perspectives, Vol. 6. Brill Ed., Netherlands, 530 pp.

Chagas, A.C.S., Passos, W.M., Prates, H.T., Leitem, R.C., Furlong, J., and Fortes, I.C.P. 2002. Acaricide effect of *Eucalyptus* spp. EOs and concentrated emulsion on *Boophilusmicroplus*. Braz. J. Vet. Res. An. Sci.39: 247-253.

Damjanovic-Vratnica, B., Dakov, T., Sukovic, D., and Damjanovic, J. 2011. Antimicrobial effect of essential oil isolated from *Eucalyptus globulus*Labill, from Montenegro. Czech J. Food Sci.3: 277–284.

De Grisse, A.T. 1969. Re-description ou modification de quelques techniques utilisées dans l'étude des nématodes phytoparasitaires. Medelingen Rijkskulten Landbouw Wetenschappen Gent, XXXIV, (2): 351-359.

Dewanto, V., Wu, X., Adom, K.K., and Liu, R.H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem. 50: 3010-3014.

Dhakshinamoorthy, S., Mariama, K., Elsen, A. and De Waele, D. 2014. Phenols and lignin are involved in the defence response of banana (Musa) plants to Radopholussimilis infection.Nematology 16: 565-576.

Hajji-Hedfi, L., Larayedh, A., Chihani-Hammas, N., Regaieg, H. and Horrigue-Raouani, N. 2019. Biological activities and chemical composition of *Pistacialentiscus* in controlling *Fusarium wilt* and root-knotnematode disease complex on tomato. European Journal of Plant Pathology.155 (1): 281-291.

Kepenekci, I., Toktay, H., Saglam, H.D., Erdogus, D., and Imren, M. 2016. Effects of some indigenous plants extracts on mortality of the root lesion nematode, *Pratylenchusthornei* Sher & Allen. Egyptian Journal of Biological Pest Control 26 (1): 119-121.

Laquale, S., Avato, P., Argentieri, M.P. et al. 2020. Nematicidal activity of *Echinacea* species on the root-knot nematode *Meloidogyne incognita*. J. Pest Sci. 93: 1397-1410.

Lima, L.S., Barbosa, L.C.A., Alvarenga, E.S., Demuner, A.J., and Silva, A.A. 2003. Synthesis and Phytotoxicity Evaluation of Substituted para-benzoquinones. Aust. J. Chem.36: 625-630.

Lucia, A., Licastro, S., Zerba, E., and Masuh, H. 2008. Yield, chemical composition, and bioactivity of EOs from 12 species of *Eucalyptus* on *Aedes aegypti* larvae. Entomol. Exp. Appl.129: 107-114.

Maciel, M.V., Morais, S.M., Bevilaqua, C.M.L., Silva, R.A., Barros, R.S., Sousa, R.N., Sousa, L.C., Brito, E.S., and Souza-Neto, M.A. 2010. Chemical composition of *Eucalyptus* spp. EOs and their insecticidal effects on *Lutzomyrialongipalpis*. Vet. Parasitol.167: 1-7.

Mahmood, I., and Siddiqui, Z.A. 1993. Effect of phenolics on the growth of tomato and reproduction of *Rotylenchulusreniformis*. NematologiaMediterranea, 21: 97-98.

Mello, A.F.S., Machado, A., and Inomoto, M.M. 2006. Potential control of *Pratylenchusbrachyurus*by *Chenopodium ambrosioides*. FitopatologiaBrasileira 31(5): 513-516.

Mesa-Valle, C. M., Garrido-Cardenas, J .A., Cebrian-Carmona, J., Talavera, M. and Manzano-Agugliaro, F. 2020. Global Research on Plant Nematodes. Agronomy 10, 1148.

Mickymaray, S. 2019. Efficacy and Mechanism of Traditional Medicinal Plants and Bioactive Compounds against Clinically Important Pathogens. Antibiotics. 8(4): 257.

Mousa, E.M., Mahdy, M.E., and Younis Dalia, M. 2011. Evaluation of some plant extracts to control root-knot nematode *Meloidogyne* spp. on tomato plants. Egyptian Journal of Agronematology10 (1): 1-4.

Nandakumar, A., Vaganan, M.M., Sundararaju, P., and Udayakumar, R. 2017. Phytochemical Analysis and Nematicidal Activity of Ethanolic Leaf Extracts of *Datura metel*, *Datura innoxia* and *Brugmansiasuaveolens* against *Meloidogyne incognita*. Asian Journal of Biology 2(4): 1-11.

Oka, Y., Nacar, S., Putievsky, E., Ravid, U., Yaniv, Z., and Spiegel, Y. 2000. Nematicidal activity of essential oils and their components against the root-knot nematode. Phytopathology 90: 710-715.

Osman, A.A., and Viglierchio, D.R. 1988. Efficacy of biologically active agents as nontraditional nematicides for *Meloidogyne javanica*. Rev. Nematol.11:93-98.

Pant, M., Dubey, S., Patanjali, P.K., Naik, S.N., and Sharma, S. 2014. Insecticidal activity of *Eucalyptus* oil nano emulsion with karanja and jatropha aqueous filtrates. International Biodeterioration & Biodegradation 91: 119-127.

Pereira, V., Dias, C., Vasconcelos, M.C., Rosa, E., and Saavedra, M.J. 2014. Antibacterial activity and synergistic effects between *Eucalyptus globulus* leaf residues (essential oils and extracts) and antibiotics against several isolates of respiratory tract infections (*Pseudomonas aeruginosa*). Industrial Crops and Products 52: 1-7.

Pinochet, J., Camprubí, A., and Calvet, C. 1993. Effects of the root lesion nematode *Pratylenchusvulnus* and the mycorrhizal fungus *Glomus mosseae* on the growth of EMLA-26 apple rootstocks. Mycorrhiza4: 79-83.

Singh, H.P., Mittal, S., Kaur, S., Batish, D.R., and Kohli, R.K. 2009. Characterization and antioxidant activity of essential oils from fresh and decaying leaves of *Eucalyptus tereticornis*. J. Agric. Food Chem. 57: 6962-6966.

Singleton, V.L., and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphor molybdic phosphotungstic acid reagents. Am. J. Enol. Vitic. 16: 144-158.

Wichtl, M., and Anton, R. 2003. Plantes Thérapeutiques: tradition, pratique officinale, science et thérapeutique. Tec et Doc / Lavoisier (2ème Ed.), France, 692 pp. (https://www.lavoisier.fr/livre/sciences-de-la-vie/plantes-therapeutiques-2-ed/wichtl/descrip tif -9782743006310)

Yoshizawa, Y., Kawaii, S., Kanauchi, M., Chida, M., and Mizutani, J. 1993. Chavicol and related compounds as nematocides. Biosci. Biotech. Biochem.57:1572-1574.

Zaidat, S.A.E., Mouhouche, F., Babaali, D., et al. 2020. Nematicidal activity of aqueous and organic extracts of local plants against *Meloidogyne incognita* (Kofoid and White) Chitwood in Algeria under laboratory and greenhouse conditions. Egypt. J. Biol. Pest. Control. 30 (46): 1-8.

Allelopathic Effect of Barley *(Hordeum vulgare*) and Rapeseed (*Brassica napus*) Crops on Early Growth of Acetolactate Synthase (ALS)-Resistant *Glebionis Coronaria*

Zeineb Hada, Houda Jenfaoui, Messaad Khammassi*, Ahlem Matmati, and Thouraya Souissi, LR / Bioagresseurs et Protection Intégrée en Agriculture (LR14AGR02), Institut national Agronomique de Tunisie, Université de Carthage, 43 Avenue Charles Nicolle, Tunis-Mahrajène 1082, Tunisia, * Institut National des Grandes Cultures, Bousalem, Jandouba, Tunisia **(*Tunisia*)**
https://doi.org/10.52543/tjpp.17.2.2

ABSTRACT

Hada, Z., Jenfaoui, H., Khammassi, M., Matmati, A., and Souissi, T. 2022. Allelopathic effect of barley *(Hordeum vulgare*) and rapeseed (*Brassica napus*) crops on early growth of acetolactate synthase (ALS)-resistant *Glebionis coronaria*. Tunisian Journal of Plant Protection 17 (2): 55-66.

Glebionis coronaria is a serious threat to cereal production in Northern Tunisia. Previous results showed that *G. coronaria* has developed resistance to acetolactate synthase (ALS)-inhibiting herbicides which limits their use in cereal cropping systems. The use of allelopathic crops has been reported as a potential alternative to herbicides to control resistant weed populations. The aim of this study is to evaluate the effect of five allelopathic crops on the early growth of *G. coronaria*. In vitro experiments using aqueous extracts of different crops and in pots experiments with increasing rates of barley (*Hordeum vulgare*) and rapeseed (*Brassica napus*) residues were performed for this purpose. All tested crops showed allelopathic effects on *G. coronaria* and were able to reduce its root length at the concentration of 50 g/L. Barley and rapeseed were the most effective crops, with 70% and 60% of root length reduction, respectively. An aqueous extract concentration study with five increasing concentrations of 12.5, 25, 50, 75, and 100 % was performed with these two crops, and root length was significantly reduced with increasing concentration, being up to 80% with the highest aqueous extract concentration of barley. The in vitro results were supported by the in pots experiment: Shortest roots length and highest dry mass reduction were obtained by the highest barley (0.64 g/kg) and rapeseed (8 g/kg) residue concentrations. This study confirmed the allelopathic potential of both barley and rapeseed in reducing the early growth of ALS-resistant *G. coronaria*, suggesting the effectiveness of these crops if integrated with other control measures for the management of resistant population of *G. coronaria* in cereal fields.

Keywords: Allelopathic crops, aqueous extract, crops residues, root length, weed management

---

Corresponding Author: Zeineb Hada
Email: zeineb.hada@gmail.com

Accepted for publication 2 September 2022

Allelopathy is defined as the direct or indirect harmful or beneficial effects of one plant on another through the release of chemical compounds into the environment (Rice 1984). Several allelochemical phytotoxic substances isolated from plant tissues and soils inhibit the germination and the growth of other plants in the surrounding environment (Mushtaq et al. 2020). These substances are usually secondary plant products or waste products of the main metabolic pathways of plants (Ashrafi et al. 2007; Chon and Kim 2002). The allelochemicals interfere with the growth of other plants after being released as residues, exudates and leaches from leaves, stem, roots, fruits and seeds (Asgharipour and Armin 2010).

Recently, allelopathy is recognized as an appropriate potential technology to control weeds using chemicals released from decomposed plant parts of various crop species (Naseem et al. 2009). Additionally, allelopathic crops integrated in weed management as cover crops, mulch, smother crops, intercrops or green manures, or grown in rotational sequences, build up soil fertility include organic matter, thereby reducing soil erosion, and improving crop yields (Khanh et al. 2005). The use of plant aqueous extracts alone or in combination with reduced doses of herbicides has been widely reported, to provide effective control of weeds and to reduce the overreliance on synthetic herbicides for weed management (Naby and Ali 2020).

In Tunisia, weed flora is highly diversified and composed of annual, perennial and parasitic species that cause important yield losses, which is estimated up to 60% in cereal crops (INGC 2013). Weed management methods commonly used to control weed species in crops are essentially cultural and chemical. However, intensive use of herbicides to control weeds has adversely affected the environment and water quality, and has resulted in the evolution of herbicide resistant biotypes in many weed species, such as *Glebionis coronaria*, which is recently confirmed as cross-resistant to acetolactate synthase (ALS) inhibiting herbicides (Hada et al. 2020). Such concern has triggered interest towards the development of weed management alternatives that minimize the use of herbicides while sustaining crop production.

This study aimed to assess (i) the allelopathic effect of the aqueous extracts from different crops on early growth of *G. coronaria* in laboratory bioassays (ii) the effect of increasing concentrations of aqueous extracts of barley and rapeseed on *G. coronaria* root length, and (iii) the effect of the incorporation of barely and rapeseed residues into the soil on the early growth of *G. coronaria* in pots.

MATERIALS AND METHODS

Plant material.

Seeds of a cross-resistant population of *G. coronaria* to ALS-inhibiting herbicides (R) were collected from a cereal field in Fritissa region located in Bizerte Governorate, Northern Tunisia (37°10’50.91.00’’N; 9°42’026’92. 00’’E). As well, five known allelopathic crops were harvested from the experimental field site of the National Institute of Agronomy of Tunisia - INAT (36°49’49.01’’N; 10°11’02.07’’E). The crops are *Sorghum bicolor* (var. Jawher), *Brassica napus* (var. Trapper), *Secale cereale* (var. Allawi), *Avena sativa* (var. Chapela) harvested at their flowering stage, and *Hordeum vulgare* (var. Rihane) harvested at its vegetative stage.

Preparation of aqueous extract from crops.

Based on the method of Ben Hammouda et al. (2002), plants were carefully washed with tap water and dried on towel paper. The whole plants were oven dried at 60 °C for 24 h. Dried samples were ground into fine powder and stored dry at 4 °C until their use. The aqueous extracts of each crop were prepared by adding 2.5 g of plant powder in 50 ml of distilled water. The extracts were shaken at 200 rpm at room temperature for 24 h, filtered using four (04) layers of cheesecloth to remove fiber debris and centrifuged twice for 40 min at 9,000 rpm at 8°C. The supernatant was filter-sterilized through a millipore filter 0.22 µm. The filtered solutions (stock solutions) were freshly prepared before each experiment.

Preparation of growth medium.

According to the same previous reference, the growth media for the in vitro bioassays was prepared by adding 12 g of agar to 1 L of distilled water. The mixture was then autoclaved for 20 min at 120 °C and left cooling below 50 °C. For each crop aqueous extract, 20 ml of the stock solution (20% w/v) were added to the water agar. Controls received 20 ml of sterile distilled water. Bioassays were conducted in Petri dishes containing each 40 ml of the growth media.

In vitro experiments.

Seeds of the herbicide-resistant population of *G. coronaria* were sterilized using 3% of commercial bleach Sodium hypochlorite (NaOCl) solution for 2 min, and subsequently washed with sterile distilled water. Seeds were then immersed in 70 % of ethanol solution for 30 s and washed again with sterile distilled water. After sterilisation, seeds were allowed to pre-germinate on a moistened filter paper. The photoperiod was set at 16h/8h day and night respectively, under 350 µmol m^{-2} s^{-1} photosynthetic photon-flux density. Germinated seeds with 1-2 mm of root lengths were used for the experiments.

First experiment. Crops were screened for their phytotoxic ability to reduce the root length of pre-germinated seeds of *G. coronaria.* The growth medium was prepared as mentioned above.

Four (04) Petri diches were used for each crop water extract with ten (10) pre-germinated weed seeds on the surface of the water-agar. All Petri dishes were kept in a growth incubator at a temperature of 22°C/20°C day/night with 16h/8h light/dark photoperiod for 7 days. The root lengths of *G. coronaria* were measured and compared to control seeds.

Second experiment. The effects of increasing concentrations of the aqueous extracts of *B. napus* and *H. vulgare* on the weed root length were investigated. Five concentrations of aqueous extract (12.5, 25, 50, 75, and 100 %) were used for this experiment by diluting the stock solution of both crops, with four (04) replications each. For each replication, ten (10) pre-germinated seeds were used and root length of *G. coronaria* was measured after 7 days of incubation in growth incubator at a temperature of 22°C/20°C day/night with 16h/8h light/dark photoperiod.

The rate of root growth inhibition was determined using the following equation:

$$RL_{in} = \left(\frac{RL_c - RL_e}{RL_c}\right) \times 100$$

where RL_{in} is the rate (%) of root length inhibition, RL_c is the root length of the control plants, and RL_e is the root length of the plants placed at the corresponding extract concentration.

Pot experiments.

Pot experiments were conducted to evaluate the effect of the incorporation of crop residues into the soil on the growth of *G. coronaria* seedlings. The amount of residues used in this experiment were determined based on previous estimations of the average crop residues/ha in Tunisian fields (Ben Jeddi, 2005). The residues are about 500 kg/ha and 2000 kg/ha respectively for barley and rapeseed. Three (03) concentrations of residues for each crop (Table 1) were mixed with 1 kg of sand-perlite mixture per pot (1:2 w/w) and allowed to decompose for one week before transplanting the weed seedlings. Each pot of 7 cm-diameter was filled by 100 g of residue-soil mixture. The seeds of *G. coronaria* were pre-germinated as described above, and transplanted into pots at two-cotyledon growth stage. The experiment was conducted as a complete randomized design with 10 replications per residue mixture and crop. Ten (10) pots without residue were used as control. The pots were kept under greenhouse conditions (25°C/20°C day/night with 16h/8h light/dark photoperiod) and regularly irrigated to keep the soil moisture at field capacity. Fourteen days after transplantation, all seedlings were gently uprooted and the lengths of shoots and roots were determined, the weed samples were oven-dried (for 48 h at 70 °C) and the dry weight of each part per plants was recorded.

Statistical analysis.

Prior to each analysis, the normal distribution and homogeneity of the data were checked. All data were subjected to analyses of variance (ANOVA), using the SPSS software (IBM SPSS statistics 20). The comparison of means was performed by the Duncan post-hoc pairwise test at 0.5% of probability.

RESULTS

Effect of the aqueous extracts of crops on the root length of *G. coronaria.*

Results showed a significant reduction in the root length of *G. coronaria* seedlings in the presence of aqueous extracts for all tested crops compared to the control. Significant differences were also observed between the crops. The root reduction arranged from 30 to 74 % (Fig. 1).

The most pronounced inhibitory effect on the root elongation of *G. coronaria* seedlings was observed with barley aqueous extract (around 70%), followed by rapeseed extract (around 60%).

Effect of increasing aqueous extract concentrations of rapeseed and barley on root length of *G. coronaria.*

Results of the root length of *G. coronaria* under increasing concentrations of aqueous extracts of barley and rapeseed showed that reduction of root length (%) increased by increasing the concentration of aqueous extract of crops.

For all tested aqueous extract concentrations, barley have the highest inhibitory effect on *G. coronaria* growth of root length (Fig. 2). At a concentration of 25%, the inhibitory effect of barley exceeded significantly the inhibitory effect of rapeseed. The root length inhibition reaches up to 80% with the highest aqueous extract concentration of barley.

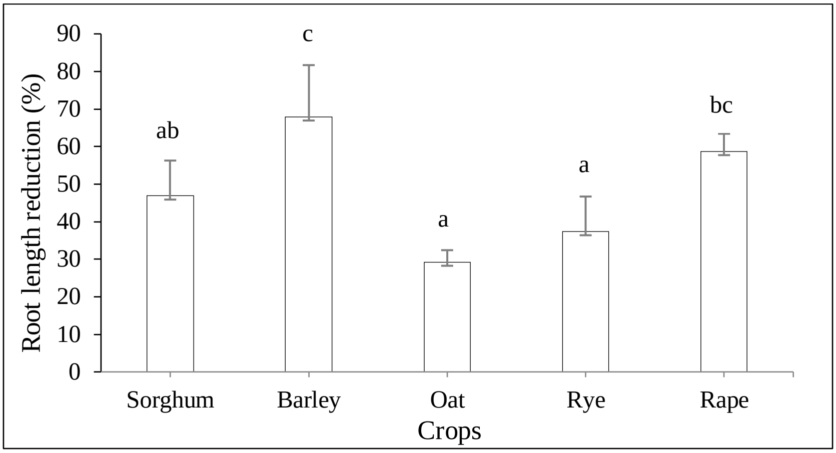

Fig. 1. The root length reduction (%) of *G. coronaria* seedling in the presence of crop aqueous extracts. Bars surmounted by the same letter are not significantly different at *P* = 0.05.

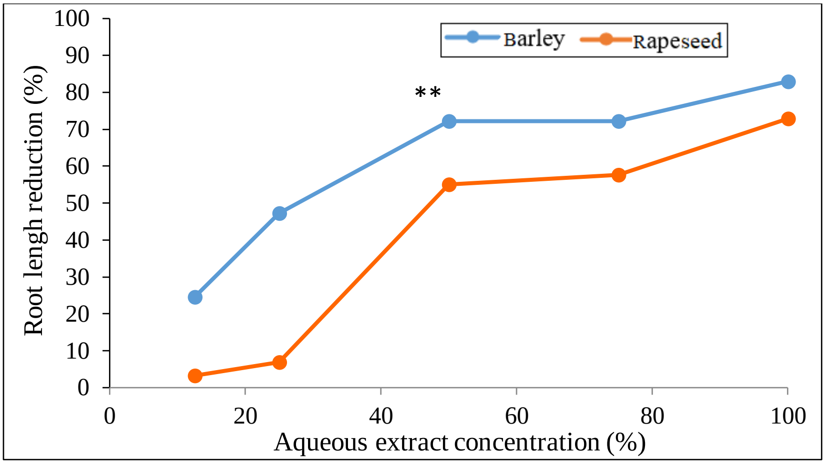

Fig. 2. Root length reduction (%) of *G. coronaria* seedlings at increasing concentrations of aqueous extract of barley and rapeseed. **: Difference highly significant (*P* < 0.01).

Effects of barley and rapeseed residues on *G. coronaria* growth.

Under controlled environment, the results of the experiment carried out in pots showed that the effects varied between crop residues and between the residue amounts. Barley and rapeseed residues caused significant reductions in root length of *G. coronaria* seedlings. However, significant stimulatory effects were observed on the shoot length. The root length was reduced compared to control by 84.5% and 73.2 % with the residues of barley and rapeseed at the concentrations of 0.64 g/kg (B3) and 8 g/kg (R3), respectively (Fig. 3).

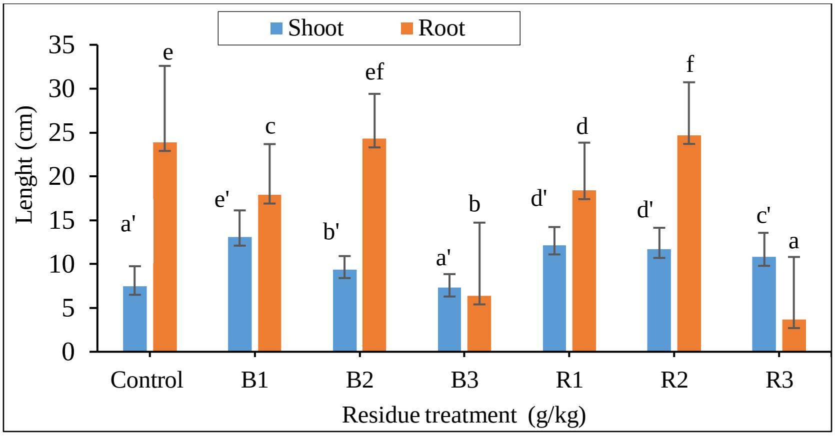

Fig. 3. The effects of increasing concentrations of barley and rapeseed residues on root and shoot length of *G. coronaria* seedlings. Bars surmounted by the same letter are not significantly different at P = 0.05. B1: 0.16 g/kg, B2: 0.32 g/kg, B3:0.64 g/kg; R1: 2 g/kg, R2: 4 g/kg, R3: 8 g/kg.

For barley, the lowest amount of residues incorporated in soil (B1 = 0.16 g/kg) caused a significant reduction of seedling root length with an average of 23%, whereas shoot length was significantly stimulated. With the B2 treatment (0.32 g/kg), root length was statistically similar to control and less stimulation was observed in shoot length, suggesting almost no effect of this treatment on *G. coronaria* seedling growth.

A comparative effect was observed between the different amounts of rapeseed residues. The treatment R1 (2 g/kg) inhibited the root length by 25.1% compared to control and stimulated the shoot length by 42.8%. A slight reduction was observed in root length with the treatment R2 (4 g/kg), and shoot length was stimulated by 20.2% with the same treatment.

Results of the dry matter confirm the results of root and shoot length recorded. Indeed, significant differences were found between different amounts of crop residues (Table 2). Both treatments R3 and B3 showed the highest effect on dry matter accumulation of *G. coronaria* seedling. The dry matter reductions were 45.7% and 52.7% with R3 and B3, respectively. With B1, B2 and R2 treatments, the dry matter of *G. coronaria* were increased compared to control.

Table 2. *Glebionis coronaria* average dry matter reduction at 14 days after incorporation in the soil of barley and rapeseed residues at different concentrations

Crop	Treatment	Dry weight (g)	Variation of dry weight (%)
Barley	B1	0.13	144.4 b
	B2	0.16	177.8 a
	B3	0.05	56.6 d
Rapeseed	R1	0.08	88.9 c
	R2	0.15	166.7 a
	R3	0.06	66.7 cd
Control	-	0.09	-

DISCUSSION

Our results revealed that the root length of *G. coronaria* seedlings was affected by a wide range of crops commonly cultivated in Tunisia. Poaceae family are previously reported as one of the most botanical family with pronounced allelopathic potential to suppress weeds (Favaretto et al. 2018; Sánchez-Moreiras et al. 2003; Tantiado and Saylo 2012). Poaceae is an ecologically dominant plant family and it is economically important worldwide, known for its chemical diversity, which represents an important source to allelopathic molecules. In this study, *Sorghum bicolor* reduced *G. coronaria* root length up to 50%, most probably, due to sorgoleone, a lipid benzoquinone released from sorghum plant, that reduces weed growth through its action on the Photosystem II (Hejl and Koster 2004), in a similar way to herbicides of the triazine class (Gniazdowska and Bogatek 2005). *Secale cereal* is usually used as cover crop to compete weeds (Farooq et al. 2011; Nagabhushana 2001) or as a mulch to inhibit the emergence and the growth of weeds (Teasdale and Mohler 2000) by mean of hydroxamic acid (Pérez and Ormeno-Nunez1993), which reduces the need of herbicides (Bhadoria 2011). In the same way, the allelopathic potential of *Avena sativa* was largely explored in weed control. Previous studies reported the inhibiting effect of oat due to phenolics namely scopoletin, a bioactive coumarin known for its allelopathic effect that successfully controls weeds when released into the soil (Belz et al. 2005).

The results showed also that the highest growth inhibition of *G. coronaria* seedlings was caused by barley and rapeseed aqueous extracts and varied with the concentration of the extract.

These results are in agreement with previous research, where the activity of barley extracts was directly related to its concentrations (Ashrafi et al. 2009). The release of water-soluble allelochemicals from vegetative components is thought to be the primary factor contributing to the overall weed suppressive effect of crops (Kremer and Ben-Hammouda 2009). Knowledge on putative barley allelochemicals traces back to Liu and Lovett (1993). Both gramine (N,N-dimethyl1H-indole-3-methanamine) and hordenine (p-[2- (dimethylamino) ethyl] phenol) are the main alkaloids constitutively exuded from barley (Jabran 2017). Phenolic compounds are also among the important allelochemicals produced by barley (Oueslati et al. 2009) that inhibit seed germination and seedling growth of several weed species such as *Echinochloa crus-galli*, *Eclipta prostrata* and *Bromus dianrus* (Bouhaouel et al. 2016; Chon et al. 2004; Oueslati et al. 2009). On the other hand, the family of Brassicaceae receives great attention as source of allelochemicals, often used for weed suppression (Bialy et al. 1990). Most of the species of the Brassica genus, such as rapeseed (*Brassica napus*), produces allelochemicals that inhibits germination and growth of weed species such as *Sorghum halepense* (Uremis et al. 2009b). Brassica glucosinolates play a key role for weed suppression as they can be converted to the corresponding isothiocyanates by the enzyme myrosinase (Petersen et al. 2001). According to Uremis et al. (2009a), higher allelopathic potential could be attributed to the higher isothiocyanates content.

Results from experiment, carried out in pots under controlled environment, showed pronounced effects on roots rather than on shoots, suggesting that the inhibitory effect of barley and rapeseed are more relevant on the underground elongation of *G. coronaria* at this early stage of growth. These results are in line with the finding that water extracts of allelopathic plants generally have more pronounced effects on radicle/root, rather than hypocotyl or shoot growth (Ashrafi et al. 2009; Turk and Tawaha 2002). This may be attributable to the fact that allelochemicals are first in contact with radicle (Ashrafi et al. 2009). Besides the inhibition of root elongation, many of the extracts also altered root morphology, producing distorted and twisted radicles compared to normal seedlings (Bouhaouel et al. 2016; Jennings and Nelson 2002). In addition, results of the pot experiments showed that barley and rapeseed residues leached water soluble allelochemicals that inhibit the growth of *G. coronaria*. However, when incorporated into the soil at increasing rates, crops residues are effective only at first and third level of crop residues (B1, B2 and R1, R3). For both B2 and R2 crop residue levels, no significant effect on the root elongation was observed compared to the control. According to Narwal (1994), allelochemicals that inhibit the growth of some species at certain concentrations might, in fact, stimulate the growth of the same species at another concentration, and it is thus essential to identify the appropriate crop residue level at which growth inhibition occurs, before using allelopathic crops in weed management programs (Ashrafi et al. 2009). In general, the allelochemicals released in the soil affect the growth of weeds through changes in the cell structure, inhibition of cell elongation/division, disruption of membrane structures, and disruption of water and nutrient uptake and the process of photosynthesis (Jabran 2017).

Allelopathic crop residues are widely used for weed management (Tabaglio et al. 2008). For barley crop, the release of water-soluble allelochemicals from vegetative components is thought to be the primary factor contributing to the overall weed suppressive effect of barley used as mulch (Kremer and Ben-Hammouda 2009). Barley residues added to the soil surface of field plots inhibited emergence of *Solanum ptycanthum* by 98% and *Setaria glauca* by 81% at 30 days after planting the weed species (Creamer et al., 1996). Subsequent studies confirmed the effectiveness of barley residues in reducing plant densities of the weeds *Portulaca oleracea* and *Digitaria ischaemum* 60 days after killing the barley with paraquat (Kremer and Ben-Hammouda, 2009). Rapeseed residues are generally incorporated into the soil after harvest in order to control weed. Previous study showed that perennial herbicide resistant *Sorghum halepense* control with over 90% was achieved by rapeseed residues 30 day after its incorporation (Uremis et al. 2009a). Weeds, especially small seeded weeds, could be controlled with the incorporation of rapeseed residues in the soil. Rapeseed residues were reported to inhibit the germination and growth of *Amaranthus retroflexus*, *Solanum nigrum*, *Portulaca oleracea* and *Echinochloa colonum* (Uremis et al. 2009b). For allelopathic residues, it was recommended the combined application of various allelopathic materials to increase the efficiency in weed management, due to the synergistic effect of diverse allelochemicals. The soil surface allelopathic residues could be integrated with no-tillage or reduced tillage (Teasdale and Mohler 2000).

General results from this study showed the allelopathic potential of both barley and rapeseed to reduce the seedling growth of a cross-resistant population of *G. coronaria*. Thus, suggesting the advantage to incorporate these allelopathic crops in resistance management programs. Both crops could be used as water extract, incorporated in the soil or as a part of a rotation program to control herbicide resistant population of *G. coronaria* in field. Further studies should be conduct to investigate the effect of barley and rapeseed in field conditions in order to manage the spreading of resistant population of *G. coronaria*.

RESUME

Hada Z., Jenfaoui H., Khammassi M., Matmati A., et Souissi T. 2022. Effet allélopathique des cultures d'orge (*Hordeum vulgare*) et de colza (*Brassica napus*) sur la croissance précoce de *Glebionis coronaria* résistante aux herbicides inhibiteurs de l'acétolactate synthase (ALS). Tunisian Journal of Plant Protection 17 (2): 55-66.

Glebionis coronaria est une menace sérieuse pour la production céréalière dans le nord de la Tunisie. Des résultats antérieurs ont montré que *G. coronaria* a développé une résistance aux herbicides inhibiteurs de l'acétolactate synthase ALS qui limite l'utilisation de ces herbicides dans les systèmes de production basés sur les céréales. L'utilisation de cultures allélopathiques a été reportée comme une alternative potentielle aux herbicides pour lutter contre les populations de mauvaises herbes résistantes. L'objectif de cette étude est d'évaluer l'effet de cinq cultures allélopathiques sur la croissance de *G. coronaria*. Des expériences *in vitro* avec des extraits aqueux des cultures et en pots avec des quantités croissantes de résidus d'orge (*Hordeum vulgare*) et de colza (*Brassica napus*) ont été réalisées à cette fin. Les résultats obtenus ont confirmé l'effet allélopathique de toutes les cultures testées sur la réduction de la longueur des racines de *G. coronaria* à une concentration de 50 g/L. Les plus fortes réductions de la longueur des racines ont été obtenues avec les extraits de l'orge et du colza avec des pourcentages de réduction de 70% et 60%, respectivement. L'étude de l'effet de cinq concentrations des extraits aqueux (12,5, 25, 50, 75 et 100 %) d'orge et de colza, a montré que la longueur des racines a été significativement réduite avec la concentration la plus élevée de l'extrait de l'orge (jusqu'à 80% de réduction). Les résultats *in vitro* ont été confirmés par l'essai en pot. Les réductions de la longueur racinaire et de la biomasse sèche les plus marquées ont été obtenues avec les plus fortes concentrations de résidus d'orge (0,64 g/kg) et de colza (8 g/kg). Cette étude a confirmé le potentiel allélopathique de l'orge et du colza dans la réduction de la croissance de *G. coronaria* résistante aux inhibiteurs de l'ALS, ce qui suggère l'efficacité de ces cultures si elles sont intégrées avec d'autres mesures dans la lutte contre les populations résistantes de *G. coronaria* dans les champs des céréales.

Mots-clés: Cultures allélopathiques, extrait aqueux, résidus de cultures, longueur des racines, gestion des mauvaises herbes

---

ملخص

حاده، زينب وهدى جنفاوي ومسعد خماسي وأحلام مططاطي وثريا السويسي. 2022. تاثير التبادل-المَرَضي للشعير (*Hordeum vulgare*) والسلجم (*Brassica napus*) على النمو المبكر للنبتة الضارة *Glebionis coronaria* المقاومة لمبيدات الأعشاب المثبّطة لأنزيم أسيتولاكتات سينثاز (Acetolactate synthase, ALS).
Tunisian Journal of Plant Protection17 (2): 55-66.

تشكّل النبتة الضارة *Glebionis coronaria* تهديدًا خطيرًا على انتاج الحبوب في الشمال التونسي، إذ أظهرت نتائج سابقة أنّ هذه النبتة قد طورت نوعا من المقاومة لمبيدات الأعشاب المثبّطة لأنزيم أسيتولاكتات سينثاز (Acetolactate synthase, ALS) ممّا يحدّ من استخدام هذه المبيدات للسيطرة على هذه النبتة في أنظمة زراعة الحبوب. ويُذكر أنّ استخدام الزراعات ذات التبادل-المَرَضي (Allelopathy) عُرف كبديل محتمل لمبيدات الأعشاب للسيطرة على مجموعات الأعشاب الضارة المقاومة. وتهدف هذه الدراسة إلى تقييم تأثير خمس زراعات ذات تبادل-مَرَضي فى التحكم في النمو المبكر لنبتة *G. coronaria*. لهذا الغرض، أُجريت تجارب في المختبر باستخدام المستخلصات المائية لهذه الزراعات، كما أجريت تجارب في الأصص باستعمال كميات تصاعدية من مخلفات الشعير(*Hordeum vulgare*) والسلجم (*Brassica napus*). وقد بيّنت المجموعة الأولى من التجارب تأثير جميع الزراعات المختبرة بتخفيض طول جذر نبتة *G. coronaria* عند استعمال التركيز 50 غ/ل، حيث بلغت أعلى معدلات انخفاض طول الجذر نسبة 70% و60% مع مستخلصات الشعير والسلجم، على التوالي. كما أظهرت هذه الدراسة تأثير خمسة تراكيز من المستخلصات المائية (12.5 و 25 و 50 و 75 و 100 %) للشعير والسلجم، أن طول الجذر انخفض بشكل ملحوظ مع أعلى تركيز لمستخلص الشعير (حيث بلغ الانخفاض 80%). وكانت نتائج تأثير مخلفات الشعير والسلجم على النمو المبكر لنبتة *G. coronaria* داعمة للنتائج المخبرية، حيث تمّ ارجاع انخفاض طول الجذور والكتلة الحيوية الجافة إلى أعلى تركيز لمخلفات الشعير(0.64 غ/كغ) والسلجم (8 غ/كغ). وتؤكد هذه الدراسة أنّ لكل من الشعير والسلجم إمكانيات تبادل-مَرَضي تخفّض النمو المبكر لنبتة *G. coronaria* المقاومة لمبيدات الأعشاب المثبطة لأنزيم ALS، ممّا يشير إلى فعالية هذه الزراعات إذا تمّ دمجها مع تدابير زراعية أخرى للسيطرة على مجموعات نبتة *G. coronaria* المقاومة للمبيد في حقول الحبوب.

كلمات مفتاحية: محاصيل ذات تبادل-مَرَضي، مستخلص مائي، بقايا المحاصيل، طول الجذر، إدارة الأعشاب الضارة

LITERATURE CITED

Asgharipour, M.R., and Armin, M. 2010. Inhibitory effects of *Sorghum halepens* root and leaf extracts on germination and early seedling growth of widely used medicinal plants. Advances in Environmental Biology 9: 316-325.

Ashrafi, Z.Y., Sadeghi, S., and Mashhadi, H.R. 2007. Allelopathic effects of barley (*Hordeum* *vulgare*) on germination and growth of wild barley. Weed Science Research 13 (1-2): 99-112.

Ashrafi, Z.Y., Sadeghi, S., and Mashhadi, H.R. 2009. Inhibitive effects of barley (*Hordeum vulgare*) on germination and growth of seedling quack grass (*Agropyrum repens*). Icelandic Agricultural Sciences 22: 37-43.

Belz, R.G., Hurle, K., and Duke, S.O. 2005. Dose-response: a challenge for allelopathy? Nonlinearity in Biology, Toxicology, and Medicine 3 (2): 173-211.

Ben-Hammouda, M., Ghorbal, H., Kremer, R.J., and Oueslati, O. (2002). Autotoxicity of barley. Journal of Plant Nutrition 25(6): 1155-1161.

Ben Jeddi, F. 2005. *Hedysarum coronarium,* L.: Genetic Variation, Varietal Creation and Place in Tunisian Rotations. Doctorate Thesis in Applied Biological Sciences, Faculty of Science in Bioengineering, University of Gent Belgium (French), 216 pp.

Bhadoria, P.B. S. 2011. Allelopathy: a natural way towards weed management. Journal of Experimental Agriculture International 1 (1): 7-20.

Bialy, Z., Oleszek, W., Lewis, J., and Fenwick, G.R. 1990. Allelopathic potential of glucosinolates (mustard oil glycosides) and their degradation products against wheat. Plant and Soil 129 (2): 277-281.

Bouhaouel, I., Gfeller, A., Fauconnier, M.L., Delory, B., Amara, H.S., and du Jardin, P. 2016. Evaluation of the allelopathic potential of water-soluble compounds of barley (*Hordeum vulgare* L. subsp. *vulgare*) and great brome (*Bromus diandrus* Roth.) using a modified bioassay. Biotechnology, Agronomy, Society and Environment 20 (4): 482-494.

Chon, S.U., and Kim, J.D. 2002. Biological activity and quantification of suspected allelochemicals from alfalfa plant parts. Journal of Agronomy and Crop Science 188(4): 281-285.

Chon, S.U., and Kim, Y.M. 2004. Herbicidal potential and quantification of suspected allelochemicals from four grass crop extracts. Journal of Agronomy and Crop Science 190 (2): 145-150.

Creamer, N.G., Bennett, M.A., Stinner, B.R., Cardina, J., and Regnier, E.E. 1996. Mechanisms of weed suppression in cover crop-based production systems. HortScience 31(3): 410-413.

Farooq, M., Jabran, K., Cheema, Z.A., Wahid, A., and Siddique, K.H. 2011. The role of allelopathy in agricultural pest management. Pest Management Science 67 (5): 493-506.

Favaretto, A., Scheffer-Basso, S.M., and Perez, N.B. 2018. Allelopathy in Poaceae species present in Brazil. A review. Agronomy for Sustainable Development 38 (2):1-12.

Hada, Z., Khammassi, M., Jenfaoui, H., Menchari, Y., Torra, J., and Souissi, T. 2022. Approach to Demography of ALS-Resistant Glebionis coronaria as Influenced by Management Factors: Tillage, Allelopathic Crops and Herbicides. Agronomy 12 (5):1083-1098

Gniazdowska, A., and Bogatek, R. 2005. Allelopathic interactions between plants. Multi-site action of allelochemicals. Acta Physiologiae Plantarum 27 (3): 395-407.

Hejl, A.M., and Koster, K.L. 2004. The allelochemical sorgoleone inhibits root H+-ATPase and water uptake. Journal of chemical ecology 30 (11): 2181-2191.

INGC. 2013. Technical report of The National Institute of Field Crops. 310 pp.

Jabran, K. 2017. Barley allelopathy for weed control. Pages: 57-63. In: Manipulation of Allelopathic Crops for Weed Control. K. Jabran. Ed. Springer Cham, Suisse.

Jennings, J.A., and Nelson, C.J. 2002. Zone of autotoxic influence around established alfalfa plants. Agronomy Journal 94 (5): 1104-1111.

Khanh, T.D., Chung, M.I., Xuan, T.D., and Tawata, S. 2005. The exploitation of crop allelopathy in sustainable agricultural production. Journal of Agronomy and Crop Science 191 (3): 172-184.

Kremer, R.J., and Ben-Hammouda, M. 2009. Allelopathic Plants. 19. Barley (*Hordeum vulgare* L). Allelopathy Journal 24 (2): 225-242

Liu, D.L., and Lovett, J.V. 1993. Biologically active secondary metabolites of barley. II. Phytotoxicity of barley allelochemicals. Journal of Chemical Ecology 19 (10): 2231-2244.

Mushtaq, W., Siddiqui, M.B., and Hakeem, K.R. 2020. Allelopathy Potential of Important Crops. Pages 25-35. In: Allelopathy. W. Mushtaq, M.B. Siddiqui, and K.R. Hakeem. Ed. Springer Cham. Suisse.

Naby, K.Y., and Ali, K.A. 2020. Integrated weed management in wheat crops by applying sorghum aqueous extract and reduced herbicide dose. Plant Archives 20 (2): 3618-3623.

Nagabhushana, G.G. 2001. Allelopathic cover crops to reduce herbicide use in sustainable agricultural systems. Allelopathy Journal 8: 133-146.

Narwal, S.S. 1994. Allelopathy in Crop Production. Scientific Publishers, Jodhpur, India, 249 pp.
Naseem, M., Aslam, M., Ansar, M., and Azhar, M. 2009. Allelopathic effects of sunflower water extract on weed control and wheat productivity. Pakistan Journal of Weed Science Research 15 (1): 107-116.
Oueslati, O., Ben-Hammoudam, H., Ghorbel, M., El Gazzeh, M., and Kremer, R.J. 2009. Role of phenolic acids in expression of barley (*Hordeum vulgare*) autotoxicity. Allelopathy Journal, 23 (1): 157-166.
Pérez, F.J., and Ormeno-Nunez, J. 1993. Weed growth interference from temperate cereals: the effect of a hydroxamic-acids-exuding rye (*Secale cereale* L.) cultivar. Weed Research 33 (2): 115-119.
Petersen, J., Belz, R., Walker, F., and Hurle, K. 2001. Weed suppression by release of isothiocyanates from turnip-rape mulch. Agronomy Journal 93 (1): 37-43.
Rice, E.L. 1984. Allelopathy. 2nd Ed. Academic Press, New York, USA, 422 pp.
Sánchez-Moreiras, A.M., Weiss, O.A., and Reigosa-Roger, M.J. 2003. Allelopathic evidence in the Poaceae. Botanical Review 69 (3): 300-319.
Tabaglio, V., Gavazzi, C., Schulz, M., and Marocco, A. 2008. Alternative weed control using the allelopathic effect of natural benzoxazinoids from rye mulch. Agronomy for sustainable development 28 (3): 397-401.
Tantiado, R.G., and Saylo, M.C. 2012. Allelopathic potential of selected grasses (Family Poaceae) on the germination of Lettuce seeds (*Lactuca sativa*). International Journal of Bio-Science and Bio-Technology 4 (2): 27-34.
Teasdale, J.R., and Mohler, C.L. 2000. The quantitative relationship between weed emergence and the physical properties of mulches. Weed Science 48 (3): 385-392
Turk, M.A., and Tawaha, A.M. 2002. Inhibitory effects of aqueous extracts of barley on germination and growth of lentil. Pakistan Journal of Agricultural Sciences 1: 28-30.
Uremis, I., Arslan, M., Sangun, M.K., Uygur, V., and Isler, N. 2009b. Allelopathic potential of rapeseed cultivars on germination and seedling growth of weeds. Asian Journal of Chemistry 21 (3): 2170-2184.
Uremis, I., Arslan, M., Uludag, A., and Sangun, M. 2009a. Allelopathic potentials of residues of 6 brassica species on johnsongrass [*Sorghum halepense* (L.) Pers.]. African Journal of Biotechnology 8 (15): 3497-3501.

Interpreting Morphology and Yield Response of Okra (*Abelmoschus esculentus*) to Weed Variables Using Regression Analysis

Olatunde Philip Ayodele, Department of Agronomy, Adekunle Ajasin University Akungba Akoko, Ondo State, Nigeria (*Nigeria*)
https://doi.org/10.52543/tjpp.17.2.3

ABSTRACT

Ayodele, Olatunde Philip. 2022. Interpreting morphology and yield response of okra (*Abelmoschus esculentus*) to weed variables using regression analysis. Tunisian Journal of Plant Protection 17 (2): 67-76.

The cohabitation of weeds with crops may not always pose problems. Weeds are plant pests that negatively impact crop yield. However, it is essential to determine when pest status is reached in crops for weed management decisions in crop production. Field experiments were conducted during the rainy seasons of 2017 and 2018 at the research farm of the Institute of Agricultural Research and Training Ibadan (7°38' N 3° 84' E), located in the rainforest-savanna transition agroecology of southwestern Nigeria. The experiments consisted of nine weed management practices and a weedy check arranged in Randomised Complete Block Design with three replications. Weed density and weight, along with okra's morphological attributes such as the number of leaves, leaf area, plant height and stem girth, were recorded 9 weeks after sowing (WAS). At commercial maturity, unlignified okra pods were harvested from 9-12 WAS. Simple linear correlation and regression analysis between weed parameters and okra traits was performed. The result showed a negative relationship between weed growth and okra yield in both trials. The negative impact of weeds on some morphological traits of okra probably led to the reduction in okra yield. However, plant height and number of nodes did not significantly ($P < 0.05$) influence okra yield. Weeds attained pest status for okra at a weed density of about 14.17 plants/m^2 with weed dry and fresh weights of 10.05 g/m^2 and 54.86 g/m^2, respectively. Hence, weed management is necessary for okra production to reduce yield loss.

Keywords: Okra, pest status, weed, yield

Okra (*Abelmoschus esculentus* L. Moench) is a commercial vegetable crop in the family *Malvaceae*. It originated from tropical Africa and is widely cultivated in many parts of the world, especially in tropical and sub-tropical countries (Hayati 2020). Okra is an annual crop mainly valued for its immature fruits, consumed boiled or fried (Odey 2022). The mucilage produced from its pod is used to prepare stew or soup, which combines well with starchy foods cooked to a dense paste popularly called 'swallow' in Nigeria. Okra fruit has a proximate composition of 2.01% protein, 88.29% moisture content, 1.96% fat, 1.66% ash, 3.67% crude fibre, and 4.72% carbohydrate (Aladesanwa 2005). It is also rich in carotene, vitamin A, vitamin C and

Corresponding author: Olatunde Philip Ayodele
Email: olatunde.ayodele@aaua.edu.ng

Accepted for publication 31 December 2022

trace elements such as Fe and Zn (Liu et al. 2021). The production of okra has increased recently due to its nutritional value (Odey 2022). However, weeds negatively impact okra yield (Opadokun and Olorunmaiye 2019).

Farmers are targeting sustainable high okra yield. Therefore, it is essential to identify the roles of the agroecosystem components in achieving this objective. Studies have shown that biotic factors such as weeds may impact crop yield (Ayodele 2021; Ayodele et al. 2021). Weeds are wild plants which may hamper cultivated crops from growing properly. In rare cases, leguminous weeds promote crop growth due to their nitrogen-fixing ability (Bhandari and Sen 1979). Though the detrimental effect of weeds on crops may eventually result in yield loss, the benefits accruing from weeds, such as erosion control (Moreau et al. 2020) and pollinator attractants (Kleiman et al. 2021), make weed eradication indefensible.

The cohabitation of weeds with crops may not always pose yield problems. An earlier study revealed that a milkweed plant (*Euphorbia heterophylla*) cohabiting with okra at a distance of 20 cm or 30 cm had a comparable pod yield with weed-free (Opadokun and Olorunmaiye 2019). At 5% crop yield loss due to weeds, pest status is consequently attained (Khan et al. 2014). Weed management aims only to permit the minimal harmful effect of weeds. Hence, crop and weed growth monitoring is essential to avoid low yields. Morphological features of crops are reliable yield indicators (Shu et al. 2021). Parts of plant serving as yield differ in different crops. Hence, it is essential to study the relationship between yield and other morphological attributes of crops. The same morphological attribute may reflect different yield levels in different crops. For instance, fiber yield in cotton (*Gossypium hirsutum*) correlates positively with plant height (Rehman et al. 2020). Contrarily, roselle (*Hibiscus sabdariffa*), in the same *Malvaceae* family, has calyx yield which negatively correlates with plant height (Sanoussi et al. 2011).

Generally, plant morphological attributes such as plant height, plant girth, number of leaves and leaf area have been associated with the yield in many agricultural crops (Aladesanwa and Ayodele 2011; Ayodele 2021). A study by Norman et al. (2020) showed that okra pod yield positively correlates with plant height and leaf area. Also, these morphological traits positively correlate with the number of leaves and plant girth. However, weeds impact morphological characteristics in different ways. For instance, weed pressure reduces the plant height of maize (Begna et al. 2001) and increases soybean height (Braz 2021). Hence, this study seeks to investigate the empirical relationship between weeds and the morphological characteristics of okra as well as its pod yield.

MATERIALS AND METHODS

Experimental site.

Field experiments were conducted at the research farm of the Institute of Agricultural Research and Training (IAR&T) Ibadan, Nigeria (7º38' N 3º 84' E) during the 2017 and 2018 rainy seasons. The experimental site had clay loam soil with soluble organic carbon of 18.0 g kg^{-1}, N 1.5 g kg^{-1}, P 11.39 mg kg^{-1}, K 0.46 cmol kg^{-1}, Ca 1.85 cmol kg^{-1}, Mg 1.31 cmol kg^{-1} and pH of 4.9 at 5 cm soil depth. The experimental site was partitioned into 2 m × 2 m plots with an alley of 0.5 m and 1 m between plots and blocks, respectively. Okra seeds (v 35) were sown at 40 cm × 50 cm on the flat with three seeds per 1 cm depth hole. At 2 weeks after sowing (WAS), seedlings were later thinned to one plant per stand.

Experimental treatment and design.

The study consisted of ten experimental treatments, viz., cassava effluent (CE) of 24 g cyanide (CN)/ha applied at 3 WAS, pendimethalin of 1.2 kg a.i/ha applied at sowing (P), hoe-weeding (1 hoe-weeding) at 3 WAS, CE of 24 g CN/ha applied at 3 WAS and hoe-weeding at 5 WAS (CE + 1 hoe-weeding), hoe-weeding at 3 and 5 WAS (2 hoe-weeding), CE of 24 g CN/ha applied at 3 and 5 WAS (CE + CE), pendimethalin of 1.2 kg a.i/ha applied at sowing and CE of 24 g CN/ha applied at 5 WAS (P + CE), pendimethalin of 1.2 kg a.i/ha applied at sowing and hoe-weeding at 5 WAS (P + 1 hoe weeding), weedy check and weed-free. The trials were laid out in a randomized complete block design with three replications.

Data collection and analysis.

Weed density and weights were assessed at 9 WAS using 0.25 m^2 quadrats randomly fixed at two spots along the diagonals of the plots. Weeds collected from each plot were piled, weighed, oven-dried at 80°C for 48 h, and reweighed. The weed control efficiency (WCE) was mathematically derived as follows:

$$\mathrm{WCE} = \frac{(x-y)}{x} \times 100,$$

where x = weed dry weight in weedy check, y = weed dry weight in the treatment.

The morphological characteristics of okra, such as plant height, stem girth, number of leaves, and leaf area, were assessed at 9 WAS from six okra plants tagged within each plot. The plant heights and stem diameters were measured using the meter rule and vernier calliper. Unlignified okra pods, ranging from 3-5 cm in length, were harvested from the tagged plants at three-day intervals starting from 9-12 WAS. Simple linear correlation and regression analysis between weed and okra variables was performed using Microsoft Excel®.

RESULTS

Effect of weeds on the growth of okra.

The prominent weeds in the experimental field were *Brachiaria deflexa*, *Euphorbia heterophylla*, *Senna obtusifolia*, *Tridax procumbens*, *Mitracapus villosus*, *Cyperus rotundus*, and *Oldenlandia corymbosa* The descriptive statistics of okra and weed components that resulted from the 2017 and 2018 trials at 9 WAS are presented in Table 1.

The regressions of weed weight and okra growth parameters such as plant height, number of nodes and stem girth were not significant (P < 0.05) in both trials (Tables 2, 3). However, the regressions of weed weight and number of okra leaves were significant (P < 0.05). The correlation coefficient was 0.79 in 2017 and 0.7 in 2018. The weed dry and fresh weight showed similar relationships with okra growth. There were negative relationships between weed weight (X) and okra growth parameters (Y) such as the number of leaves, plant stem girth, leaf area, plant fresh weight, and plant dry weight in the 2017 and 2018 trials (Tables 2, 3). Contrarily, a positive correlation existed between weed weight and plant height of okra in both trials. The relationship between weed weight and number of nodes on okra plant differed in both trials. The number of nodes on okra plants correlated positively with weed weight in 2017 trial, whereas the same variables were negatively correlated in 2018 trial.

The regressions of weed density and okra growth parameters such as plant height and number of nodes were not significant (P < 0.05) in the trials, whereas leaf area and plant weight of okra had significant (P < 0.05) regressions with weed density. The regressions of weed density with stem girth and number of leaves of okra were significant (P < 0.05) in 2017 and 2018 trials, respectively. Weed density negatively correlated with the number of leaves, stem girth, leaf area, number of nodes, plant fresh weight and plant dry weight of okra in the trials (Table 4). However, the directions of correlation coefficients between weed density and plant height were inconsistent in both trials. It was negative in 2017 trial and positive in 2018 trial.

Table 1. Characteristics of okra and weeds grown under field conditions in 2017 and 2018 trials at 9 weeks after sowing

Statistics	Okra				Weeds			
	Plant height (cm)	No of leaves	Stem girth (mm)	Leaf area (cm^2)	Density (plant/m^2)	Fresh weight (g/m^2)	Dry weight (g/m^2)	WCE (%)
	2017 Trial							
Max	28.2	8.1	8.1	183.4	312	1027.2	180.9	100
Min	17.9	2.9	4.2	63	0	0	0	-48.4
Average	22.01	6.03	6.11	115.12	98.4	446.4	84.55	30.61
SV	3.67	1.73	1.22	34.36	89.94	326.33	61.67	50.61
	2018 Trial							
Max	40.2	13.73	17.7	240.2	245.3	1883.2	243.7	100
Min	24.6	4.8	5.7	75.2	0	0	0	0
Average	33.96	7.92	9.33	145.71	120.54	700.62	92.64	61.99
SV	4.99	2.74	3.65	52.88	91.39	629.67	83.11	34.1

WCE = Weed control efficiency; SV = Standard deviation.

Table 2. Linear correlation and regression at 9 weeks after sowing between growth parameters of okra (Y) and weed dry weight (X) (n = 10)

Growth Parameter	2017 Trail		2018 Trail	
	Correlation coefficient (R)	Regression equation	Correlation coefficient (R)	Regression equation
Plant height (cm)	0.15	Y=21.23+0.009x	0.31	Y=32.21+0.02x
No of leaves	-0.79	Y=7.89-0.02x*	-0.7	Y=10.05-0.02x*
Stem girth (mm)	-0.52	Y=6.94-0.01x	-0.33	Y=10.67-0.01x
Leaf area (cm^2)	-0.41	Y=134.39-0.23x	-0.71	Y=187.42-0.45x*
Number of nodes	0.1	Y=7.20+0.002x	-0.17	Y = 9.33-0.002x
Plant fresh weight (g)	-0.25	Y=31.95-0.04x	-0.7	Y=103.01-0.34x*
Plant dry weight (g)	-0.24	Y=6.21-0.007x	-0.58	Y= 22.02-0.06x

* = Significant at $P < 0.05$.

Table 3. Linear correlation and regression at 9 weeks after sowing between growth parameters of okra (Y) and weed fresh weight (X) (n = 10)

Growth Parameter	2017 Trial		2018 Trail	
	Correlation coefficient (R)	Regression equation	Correlation coefficient (R)	Regression equation
Plant height (cm)	0.16	Y=21.21+0.002x	0.32	Y=32.2+0.003x
No of leaves	-0.81	Y=7.95-0.004x*	-0.68	Y=9.99-0.003x*
Stem girth (mm)	-0.51	Y=6.96-0.002x	-0.31	Y=10.6-0.002x
Leaf area (cm^2)	-0.38	Y=133.01-0.04x	-0.7	Y=186.89-0.059x*
Number of nodes	0.07	Y=7.24+0.0002x	-0.39	Y=9.53-0.0005x
Plant fresh weight (g)	-0.24	Y=31.82-0.007x	-0.68	Y=102.16-0.043x*
Plant dry weight (g)	-0.23	Y=6.2-0.001x	-0.69	Y=23.12-0.01x*

* = Significant at $P < 0.05$.

Table 4. Linear correlation and regression at nine (9) weeks after sowing between growth parameters of okra (Y) and weed density (X) (n = 10)

Growth Parameter	2017 Trial		2018 Trial	
	Correlation coefficient (R)	Regression equation	Correlation coefficient (R)	Regression equation
Plant height (cm)	-0.4	Y=23.61-0.016x	0.19	Y=32.7+0.01x
No of leaves	-0.55	Y=7.06-0.01x	-0.69	Y=10.42-0.021x*
Stem girth (mm)	-0.81	Y=7.19-0.011x*	-0.32	Y=10.85-0.013x
Leaf area (cm^2)	-0.8	Y=145.14-0.305x*	-0.71	Y=195.38-0.412x*
Number of nodes	-0.44	Y=7.83-0.005x	-0.48	Y=9.69-0.004x
Plant fresh weight (g)	-0.66	Y=35.53-0.07x*	-0.71	Y=109.34-0.312x*
Plant dry weight (g)	-0.67	Y=6.97-0.014x*	-0.7	Y=24.56-0.069x*

* = Significant at $P < 0.05$; Weed density = Number of weeds/m^2.

Effect of weeds and okra growth parameters on yield.

The weed control efficiency (WCE) of the treatments had a positive relationship with okra pod yield in both years (0.78, 0.54 at $P < 0.05$, respectively), and the prediction equation was significant in 2017 (Table 5). The trials showed negative relationships between okra pod yield and weed growth. Regressing okra pod weight per unit area (*Y*) against weed parameters (*X*) indicated negative relationships for weed dry weight, weed fresh weight, and density in the trials with significant ($P < 0.05$) prediction equations in 2017. It was deduced from the study that to prevent weeds from reaching pest status on the okra field, weed dry weight and fresh weight should be less than 10.05 g/m^2 and 54.86 g/m^2, respectively. Also, weed density should be less than 14.17 $plants/m^2$.

The correlation between okra yield and okra morphological characteristics such as plant height, number of leaves, leaf area, stem girth, number of nodes and plant weight indicated positive relationships in the trials. However, the regression equations were not significant ($P < 0.05$) for plant height and number of nodes.

Table 5. Linear correlation and regression between okra yield per unit area (Y) and growth parameters of weeds and okra (X) at 9 weeks after sowing (n=10)

Growth Parameters	2017		2018	
	Correlation Coefficient (R)	Regression Equation	Correlation Coefficient (R)	Regression Equation
	Weed Parameters			
Weed dry weight (g/m^2)	-0.78	Y=369.71-1.84x*	-0.54	Y=289.80-1.04x
Weed fresh weight (g/m^2)	-0.74	Y=362.09-0.33x*	-0.54	Y=288.98-0.14x
Weed density (plant/ m^2)	-0.72	Y=328.78-1.16x*	-0.55	Y=309.68-0.96x
WCE (%)	0.78	Y=145.63-2.24x*	0.54	Y=37.37+2.52x
	Okra Parameters			
Plant Height (cm)	0.39	Y= -129.06+15.60x	0.51	Y= -359.07 + 16.29x
No of Leaves	0.75	Y= -168.24+63.41x*	0.9	Y= -221.63 + 52.46x*
Stem girth (mm)	0.88	Y= -425.54+104.84x*	0.7	Y= -90.20 + 30.46x*
Leaf Area (cm^2)	0.83	Y= -191.56 +3.53x*	0.92	Y= -209.07 +2.77x*
Number of Nodes	0.38	Y= -177.42+53.37x	0.56	Y= -844.06 +133.15x
Plant fresh weight (g)	0.75	Y= -108.48 +11.29x*	0.87	Y= -53.66 + 3.45x*
Plant dry weight (g)	0.74	Y= -110.83+58.06x*	0.86	Y= -56.37 +15.36x*

* = Significant at $P < 0.05$; WCE = Weed control efficiency; for WCE $n = 9$; Y is expressed in g/m^2.

DISCUSSION

This study agrees with Law-Ogbomo et al. (2013) that okra morphology, such as number of leaves, leaf area and stem girth, reduces as weed weight increases. However, it differs based on the evidence that okra plant height increased with increasing weed weight in the trials. Similarly, Moody (2009) and Agahiu (2020) reported an increase in the height of crops in the presence of weeds. The increase in plant height of okra with decreasing stem girth could be attributed to etiolation (de Oliveira et al. 2017). This phenomenon reflects intense competition between okra and weeds for sunlight.

The consistent significant correlation between weed weight and number of leaves suggests that the weed weight could be used in predicting the number of leaves on okra plants growing in a weedy environment. Similarly, on the same premise, it is opined that weed density could be reliably used in predicting okra leaf area and plant weight. The significant negative correlation between weed density and okra leaf area may be attributed to the shading effect of weeds (Shadbolt and Holm 1956), resulting in reduced photo-assimilates and manifesting in reduced weight of okra plant.

The negative correlation between weed density and growth components of okra corroborates previous reports that increasing weed density reduces crop growth (Ayodele 2021; Bajwa et al. 2020). Also, Okayi et al. (2017) reported a reduction in plant height, number of leaves and plant weight of okra with increasing density of tick weed (*Cleome viscosa*). Similarly, Opadokun and Olorunmaiye (2019) reported that increasing density of milkweed (*Euphorbia heterophylla*) reduced the plant height, number of leaves, stem diameter, and pod yield of okra.

The positive correlation between okra pod yield and okra morphological characteristics, such as plant height, number of leaves, leaf area, stem girth, number of nodes and plant weight, agrees with the findings of Mahmoud et al. (2020) that good vegetative growth is necessary to attain high okra yield. However, the positive and negligible effect of plant height on okra pod yield in this study corroborates Mohammed et al. (2020) who reported the same.

The negative relationship between weed growth parameters and okra pod yield in both trials corroborates the reports of previous studies that weeds may reduce crop yield (Ibrahim and Hamma 2012; Rajasree et al. 2017; Santos et al. 2017). Also, the positive correlation between weed control efficiency and okra pod yield implies that superior weed control may result in higher yield. This could be due to reduced competition for nutrients, light and water. Hence, the prediction equations of weed growth and okra yield provide an empirical basis for determining when weed management actions are required in okra production.

The negative impact of weeds on some morphological characteristics of okra probably led to the reduction in okra yield since these were positively correlated. However, the effect of weeds on the height and the number of nodes of the okra plant is inconsequential as it did not substantially impact okra yield in the trials. The negligible positive relationship between okra yield and growth parameters such as plant height and number of nodes suggests a different viewpoint to the reports of Echezona et al. (2010) that taller plants and more nodes give a better chance for more okra pods. Generally, morphological attributes such as leaf area and the number of leaves directly correlate with the plant photosynthetic capacity, which determines the yield (Li et al. 2022; Yang et al. 2019). Also, stem girth could be a reliable growth indicator since the plant stem is a major sink organ for carbon assimilates from the source leaf (Kanai et al. 2011).

RESUME

Ayodele, Olatunde Philip. 2022. Interprétation de la réponse de la morphologie et du rendement du gombo (*Abelmoschus esculentus*) aux variables des adventices à l'aide d'une analyse de régression. Tunisian Journal of Plant Protection 17 (2): 67-76.

La cohabitation des adventices avec les cultures ne pose pas toujours des problèmes. Les adventices sont des plantes pestes qui ont un impact négatif sur le rendement des cultures. Cependant, il est essentiel de déterminer quand le statut de peste est atteint dans les cultures pour les décisions de gestion des adventices dans la production agricole. Des expériences au champ ont été menées pendant les saisons des pluies de 2017 et 2018 à la ferme de recherche de l'*Institute of Agricultural Research and Training Ibadan* (7º38' N 3º84' E), situé dans la forêt tropicale-savane agroécologique de transition du sud-ouest du Nigeria. Les expériences consistaient en 9 pratiques de gestion des adventices et un test de contrôle des adventices, réalisé avec le dispositif expérimental des blocs aléatoires complets, avec trois répétitions. La densité et le poids des adventices, ainsi que les traits morphologiques du gombo tels que le nombre de feuilles, la surface foliaire, la hauteur de la plante et la circonférence de la tige, ont été enregistrés 9 semaines après le semis (SAS). A maturité commerciale, les gousses du gombo non lignifiées ont été récoltées après 9-12 SAS. Une simple corrélation linéaire et une analyse de régression entre les paramètres des adventices et les caractéristiques du gombo ont été réalisées. Le résultat a montré une relation négative entre la croissance des adventices et le rendement du gombo dans les deux essais. L'impact négatif des adventices sur certains traits morphologiques du gombo a probablement conduit à la réduction de son rendement. Mais la hauteur de la plante et le nombre de nœuds n'ont pas influencé significativement (P < 0,05) le rendement du gombo. Les adventices ont atteint le statut de peste pour le gombo à une densité d'environ 14,17 plants/m^2 avec des poids secs et frais de 10,05 g/m^2 et 54,86 g/m^2, respectivement. Par conséquent, la gestion des adventices est nécessaire pour la production du gombo afin de réduire les pertes de rendement.

Mots clés: Adventices, rendement, okra, statut de peste

ملخص

أيوديلي، أولاتاندي فيليب. 2022. تفسير تجاوب مرفولوجيا وإنتاجية البامية (*Abelmoschus esculentus*) مع متغيرات الأعشاب الضارة باستخدام تحليل الانحدار.Tunisian Journal of Plant Protection 17 (2): 67-76

قد لا يسبب تعايش الأعشاب الضارة مع المحاصيل الزراعية مشاكل دائمًا. الأعشاب الضارة هي آفات نباتية تؤثر سلبًا على غلة المحاصيل. ومع ذلك، من الضروري تحديد متى يتم الوصول إلى مستوى الآفة في المحاصيل لاتخاذ قرارات إدارة الأعشاب الضارة في إنتاج المحاصيل. أجريت تجارب ميدانية خلال مواسم الأمطار لسنتيْ 2017 و 2018 في مزرعة الأبحاث التابعة لمعهد البحوث الزراعية والتدريب بإبادان (7º38' N 3º84' E)، الواقعة في البيئة الزراعية الانتقالية للغابات المطيرة والسافانا في جنوب غرب نيجيريا. شملت التجارب 9 ممارسات للتعامل مع الأعشاب الضارة وفحصها مرتبة في تصميم القطاعات العشوائية الكاملة بثلاثة مكررات. تم تسجيل كثافة الأعشاب الضارة ووزنها مع الصفات المورفولوجية للبامية مثل عدد الأوراق ومساحة الورقة وارتفاع النبات ومحيط الساق بعد 9 أسابيع من تاريخ البذر. عند النضج التجاري، تم جني قرون البامية غير المتخشبة بعد 9-12 أسبوع من تاريخ البذر. تم حساب ارتباط خطي بسيط وتحليل انحداري بين معاملات الأعشاب الضارة وصفات البامية. أظهرت النتائج وجود علاقة سلبية بين نمو الأعشاب الضارة ومحصول البامية في كلا التجربتيْن. يمكن أن يكون التأثير السلبي للأعشاب الضارة على بعض الصفات المرفولوجية للبامية قد أدى إلى انخفاض محصولها. لكن ارتفاع البامية وعدد عقدها لم يؤثر معنويا (P < 0.05) على محصولها. بلغت الأعشاب الضارة مستوى آفة للبامية عند وصولها إلى كثافة تبلغ حوالي 14.17 نبتة/م^2 بأوزان جافة وطازجة بلغت 10.05 غم/م^2 و 54.86 غم/م^2، على التوالي. لهذا، فإن إدارة الأعشاب الضارة ضرورية لإنتاج البامية والتقليل خسارة غلتها.

كلمات مفتاحية: آفة، أعشاب ضارة، بامية، محصول

LITERATURE CITED

Agahiu A.E. 2020. Weed control in soybean (*Glycine max* (L.) Merrill) using acifluorfen and bentazon herbicides in north-central zone, Nigeria. GSC Biological and Pharmaceutical Sciences 11 (2): 226-232. https://doi.org/10.30574/gscbps.2020.11.2.0139

Aladesanwa, R. D. 2005. Screenhouse evaluation of atrazine for soil residual activity on growth, development and nutritional quality of okra

(*Abelmoschus esculentus* Moench) in southwestern Nigeria. Crop Protection 24 (10): 927-931. https://doi.org/10.1016/j.cropro.2005.01.022

Ayodele, O. P. 2021. Growth, yield and nutritional quality of Lagos spinach (Celosia argentea L.) as influenced by the density of goat weed (Ageratum conyzoides L.). Journal of Plant Protection Research 61 (1): 20-27. https://doi.org/10.24425/ jppr.2021.136265

Ayodele, O. P., Aluko, O. A., and Adegbaju, O. D. 2021. Effects of catnip (*Nepeta cataria* L.) and Mexican sunflower (*Tithonia diversifolia* L.) density on growth, yield, and proximate composition of jute mallow (*Corchorus olitorius L.*). Plant Varieties Studying and Protection 17 (2): 155-163. https://doi.org/10.21498/2518-1017.17.2.2021.236523

Bajwa, A., Nawaz, A., Farooq, M., Chauhan, B., and Adkins, S. 2020. Parthenium weed (Parthenium hysterophorus) competition with grain sorghum under arid conditions. Experimental Agriculture 56 (3): 387-396. https://doi.org/10.1017/ S0014479720000034

Begna, S.H., Hamilton, R.I., Dwyer, L.M., Stewart, D.W., Cloutier, D., Assemat, L., Foroutan-Pour, K., and Smith, D.L. 2001. Morphology and yield response to weed pressure by corn hybrids differing in canopy architecture. European Journal of Agronomy 14 (4): 293-302. https://doi.org/10.1016/S1161-0301(01)00092-2

Bhandari, D.C., and Sen, D.N. 1979. Agro-ecosystem analysis of the Indian arid zone I. Indigofera cordifolia heyne ex roth. as a weed. Agro-ecosystems 5 (3): 257-262. https://doi.org/10. 1016/ 0304- 3746(79)90005-2

Braz, G. B. P., Cruvinel, A. G., Caneppele, A. B., Takano, H. K., Da Silva, A. G., and De Oliveira R. S. 2021. Sourgrass interference on soybean grown in Brazilian Cerrado. Revista Caatinga 34:350-358. https://doi.org/10.1590/ 1983-21252021v34n211rc

de Oliveira, A. B., de Brito Neto, J. F., Cardoso, G. D., and do Vale, L. S. 2017. Growth and yield of castor bean (*Ricinus communis* L.) cv. 'BRS Energia'under different spacings. Tropical and Subtropical Agroecosystems 20 (2): 289 – 295.

Hayati, P. D. 2020. Evaluation of Agro-Morphological Traits of Some Introduced Okra [*Abelmoschus esculentus* (L) Moench] Varieties: Correlation, Variability and Heritability Studies. JERAMI Indonesian Journal of Crop Science 3 (1): 5-11. https://doi.org/10.25077/jijcs.3.1.5-11.2020

Ibrahim, U., and Hamma, I. 2012. Influence of farmyard manure and weeding regimes on growth and yield of okra (*Abelmoschus esculentus* L. Moench) in Zaria. World Journal of Agricultural Sciences 8 (5): 453-458. https://doi.org/10.5829/ idosi.wjas.2012.8.5.1660

Kanai, S., Moghaieb, R. E., El-Shemy, H. A., Panigrahi, R., Mohapatra, P. K., Ito, J., Nguyen, N.T., Saneoka, H., and Fujita, K. 2011. Potassium deficiency affects water status and photosynthetic rate of the vegetative sink in green house tomato prior to its effects on source activity. Plant Science 180 (2): 368-374. https://doi.org/10. 1016/j.plantsci.2010.10.011

Khan, F., Mazid, M., Khan, T. A., Patel, H. K., and Roychowdhury, R. 2014. Plant derived pesticides in control of lepidopteran insects: Dictum and directions. Research Journal of Biology 2 (1): 1-10.

Kleiman, B., Koptur, S., and Jayachandran, K., 2021. Beneficial interactions of weeds and pollinators to improve crop production. Journal of Research in Weed Science 4 (2): 151-164. https://doi.org/ 10. 26655/JRWEEDSCI.2021.2.2

Law-Ogbomo, K.E., Osaigbovo, A.U., and Ewansiha, S.U. 2013. Responses of okra (Abelmoschus esculenta) to various periods of weed interference in a humid tropical environment. International Journal of Agriculture and Rural Development 16 (1): 1368-1371.

Li, H., Liu, Z., Chen, Y., Zhang, X., and Chen, D. (2022). A positive correlation between seed cotton yield and high-efficiency leaf area index in directly seeded short-season cotton after wheat. Field Crops Research 285, 108594. https://doi.org/10.1016/j.fcr.2022.108594

Liu, Y., Qi, J., Luo, J., Qin, W., Luo, Q., Zhang, Q., Wu, D., Lin, D., Li, S., Dong, H., and Chen, H. 2021. Okra in food field: Nutritional value, health benefits and effects of processing methods on quality. Food Reviews International 37 (1): 67-90. https://doi.org/10.1080/87559129.2019. 1695833

Mahmoud, B. A., Hamma, I. L., Saidu, M. S., and Sadiq, I. A. 2020. Effect of farmyard manure and integrated weed management on correlation matrix of growth and yield traits of okra in Dadinkowa. Nigerian Journal of Agricultural Technology (NJAT) 17: 26-31.

Mohammed, J., Mohammed, W., and Shiferaw, E. 2020. Correlation and path coefficient analysis among agro-morphological and biochemical traits of okra [*Abelmoschus esculentus* (L.) Moench] genotypes in Ethiopia. Acta Agriculturae Slovenica 115 (2): 329-339. https://doi.org/10.14720/aas.2020.115.2.1411

Moody S.K.C. 2009. Effects of Weeds on yield and yield parameters of two grain legumes. Tropical Science Journal 4 (2): 25-30.

Moreau, D., Pointurier, O., Nicolardot, B., Villerd, J., and Colbach, N. 2020. In which cropping systems can residual weeds reduce nitrate leaching and soil erosion? European Journal of Agronomy 119, 126015. https://doi.org/10. 1016/j.eja.2020. 12601

Norman, J. E., Quee, D. D., Samura, A. E., and Fomba, S. N. 2020. Influence of mulch materials on flea beetles (*Podagrica uniforma* L.), weeds, growth and yield of okra (Abelmoschus esculentus L. Moench) in Njala, Southern Sierra Leone. Journal of Entomology and Zoology Studies 8 (2): 404-40.

Odey, S. O. 2022. Finite modelling of growth and yield of okra using different tillage systems in Obubra, Nigeria. Agricultural Engineering International: CIGR Journal 24 (1): 188-196.

Okayi, H. C., Claudius-Cole, A. O., & Awodoyin, R. O. 2017. Phenology of Tick weed (*Cleome viscosa* L.) and its interaction with okra (*Abelmoschus esculentus* (L.) Moench) and Melodoigyne incognita. Archives of Phytopathology and Plant Protection 50 (7-8): 389-397. https://doi.org/10.1080/03235408. 2017.1319032

Opadokun, W.O., and Olorunmaiye, K.S. 2019. Effects of weed density and distance on the growth and yield of two okra varieties. Notulae Scientia Biologicae 11 (3): 429-435. https://doi.org/10. 15835/nsb11310448

Rajasree, V., Sathiyamurthy, V., Shanmugasundaram, T., and Arumugam, T. 2017. Integrated weed management on growth, yield and economics in okra (*Abelmoschus esculentus* (L.) Moench) under Kharif. Madras Agricultural Journal 104 (1-3): 81-84.

Rehman, A., Mustafa, N., DU, X., and Azhar, M.T. 2020. Heritability and correlation analysis of morphological and yield traits in genetically modified cotton. Journal of Cotton Research 3 (1): 1-9. https://doi.org/10.1186/s42397-020-00067-z

Sanoussi, A., Hadiara, H. S., Yacoubou, B., Benoicirc, T. S., Issaka, L., and Mahamane, S. 2011. Yield character variability in Roselle (*Hibiscus sabdariffa* L.). African Journal of Agricultural Research 6 (6): 1371-1377.

Santos, R.N.V., Rodrigues, A.A.C., Silva, M.R.M., Correa, M.J.P., and Mesquita, M.L.R. 2017. Phytosociology and weed interference in okra under organic cropping system. African Journal of Agricultural Research 12 (4): 251-259. https://doi.org/10.5897/AJAR2016.11888

Shadbolt, C. A., and Holm, L. G. 1956. Some quantitative aspects of weed competition in vegetable crops. Weeds 4 (2): 111-123. https://doi.org/10.2307/4039983

Yang, Y., Chen, M., Tian, J., Xiao, F., Xu, S., Zuo, W., and Zhang, W. 2019. Improved photosynthetic capacity during the mid-and late reproductive stages contributed to increased cotton yield across four breeding eras in Xinjiang, China. Field Crops Research 240: 177-184. https://doi.org/10. 1016/ j.fcr.2018.11. 003

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

Ghofrane Jmii, Marwa Sayari, Messaoud Mars, Samir Gharsallaoui, and Rabiaa Haouala, LR/Agrobiodiversité et Ecotoxicologie (LR21AGRO2), Institut Supérieur Agronomique de Chott-Mariem, Université de Sousse, 4042, Chott-Mariem, Tunisia
https://doi.org/10.52543/tjpp.17.2.4 (*Tunisia*)

ABSTRACT

Jmii, G., Sayari, M., Mars, M., Gharsallaoui, S., and Haouala, R. 2022. *Nicotiana glauca*, a key plant for tomato growth enhancement and for the weed *Cynodon dactylon* control. Tunisian Journal of Plant Protection 17 (2): 77-96.

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

---

Corresponding author: Ghofrane Jmii
Email: jmii.ghofrane@yahoo.com

Accepted for publication 31 December 2022

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} ± 1.8	34.03^{b} ± 1.69	37.01^{c} ± 2.41	43.54^{d}± 2.8
	VP	32.33^{a} ± 1.8	33.00^{b} ± 1.3	37.15^{c} ± 1.27	41.96^{d} ± 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} ± 0.69	12.10^{c} ± 1.65	8.08^{b} ± 1.1	15.04^{d} ± 2.7
	VP	3.58^{a} ± 0.7	6.17^{b} ± 0.22	8.09^{c} ± 0.51	12.09^{d} ± 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} ± 0.47	10.94^{c} ± 1.32	7.56^{b} ± 1.07	14.49^{d} ± 1.5
	VP	5.59^{a}± 0.47	6.77^{b} ± 0.29	12.11^{c} ± 0.12	18.32^{d} ± 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} ± 1.43	20.53^{b} ± 1.4	60^{d} ± 2.38	0^{a}
	VP	37.2^{c} ± 1.43	20.09^{b} ± 1.12	12.54^{a} ± 0.8	50.14^{d} ± 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} ± 1.14	14.14^{b} ± 1.60	31.00^{d} ± 1.4	0^{a}
	VP	29.00^{c} ± 1.14	24.04^{b} ± 2.02	8.53^{a} ± 0.12	41.07^{d} ± 1.
Stimulation or Reduction (%)	**F**	-	-51.12	+7.09	-100
	VP	-	-16.95	-70.54	+41.79
Fresh weight	**F**	13.53^{c} ± 1.79	1.61^{b} ± 0.24	19.52^{d} ± 2.6	0^{a}
	VP	13.53^{c} ± 1.79	3.30^{b} ± 0.21	0.46^{a} ± 0.01	24.05^{d} ± 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} ± 1.8	41.68^{b} ± 1.29	48.28^{d} ± 1.43	44.13^{c} ± 1.6
	VP	32.33^{a} ± 1.8	37.61^{b} ± 1.3	42.22^{c} ± 2	52.55^{d} ± 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} ± 0.69	12.08^{b} ± 0.76	17.85^{d} ± 0.61	14.28^{c} ± 0.2
	VP	3.58^{a} ± 0.69	10.03^{b} ± 0.37	13.11^{c} ± 0.95	15.3^{d} ± 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} ± 0.47	13.81^{b} ± 0.11	23.22^{d} ± 0.19	15.1^{c} ± 0.84
	VP	5.59^{a}± 0.47	12.09^{b} ± 0.55	17.9^{c} ± 0.34	21.2^{d} ± 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} ± 1.43	26.99^{b} ± 1.57	24.06^{a} ±0.23	51.91^{d} ± 1.8
	VP	37.2^{b} ± 1.43	25.07^{a} ± 1.04	42.86^{c} ± 1.2	54.01^{d} ± 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} ± 1.14	21.31^{b} ± 1.54	8.07^{a} ± 0.93	44.19^{d} ± 3.1
	VP	29.00^{b} ± 1.14	14.1^{a} ± 1.04	41.1^{c} ± 1.97	43.01^{d} ± 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} ± 1.79	8.73^{b} ± 0.33	2.12^{a} ± 0.23	61.92^{d} ± 0,8
	VP	13.53^{b} ± 1.79	2.29^{a} ± 0.11	19.77^{c} ± 1.26	26.3^{d} ± 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.

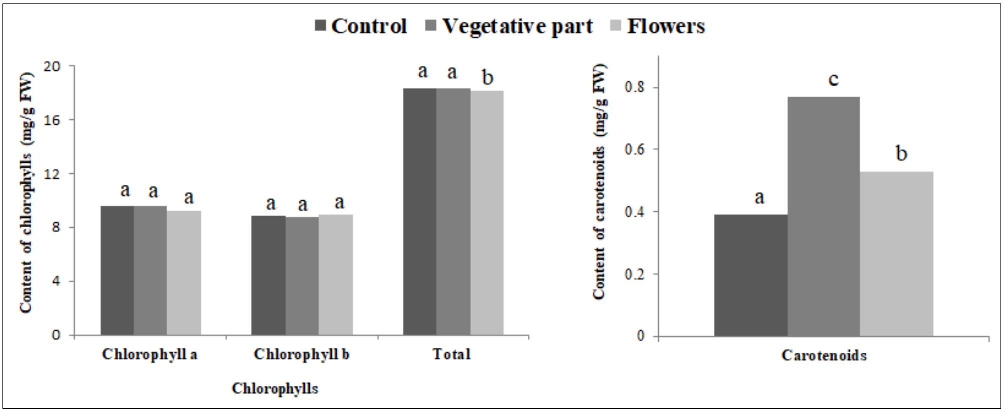

Fig. 1. Effect of *Nicotiana glauca* flower and vegetative part aqueous extracts on the contents of chlorophyll and carotenoids lettuce leaves. Values are means ± standard errors (n=5). Values affected by the same letter are not significantly different at $p < 0.05$ (Duncan test).

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	$80.55^{*} \pm 0.61$	60.1 ± 0.19
Leaves	$82.48^{*} \pm 0.89$	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 8.51×10^{-8} µmol/min g FM and 9.21×10^{-8} µmol/min g FM, against 7.43×10^{-8} µmol/min g FM for the control.

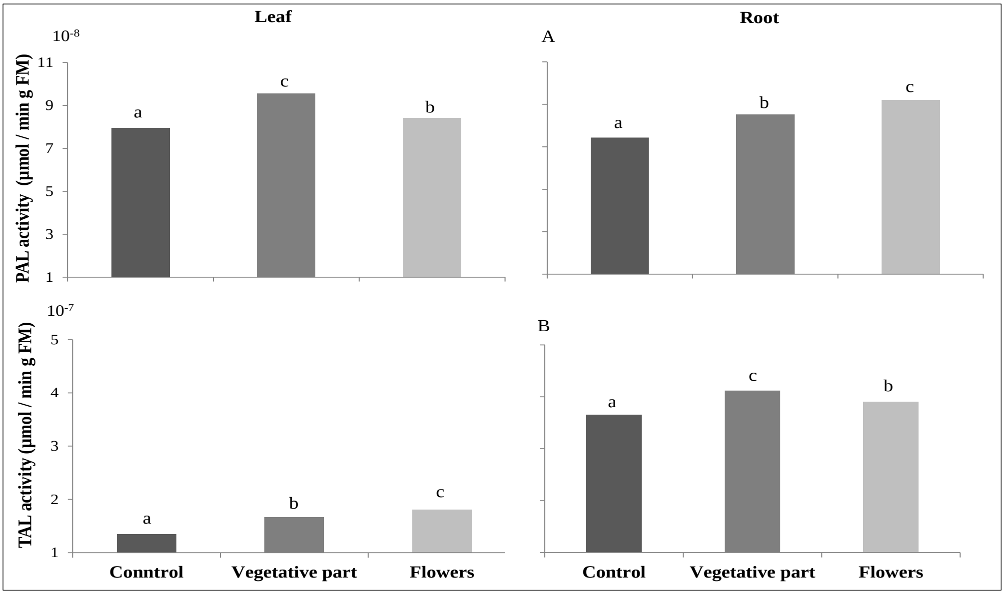

Fig. 2. Effect of *Nicotiana glauca* flower and vegetative part aqueous extracts on phenylalanine ammonia-lyase (PAL) (A) and tyrosine ammonia-lyase (TAL) (B) activity in lettuce leaves and roots. Values are means ± standard errors (SE) (n=5). Values affected by the same letter are not significantly 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 (1.35×10^{-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 (3.65×10^{-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.

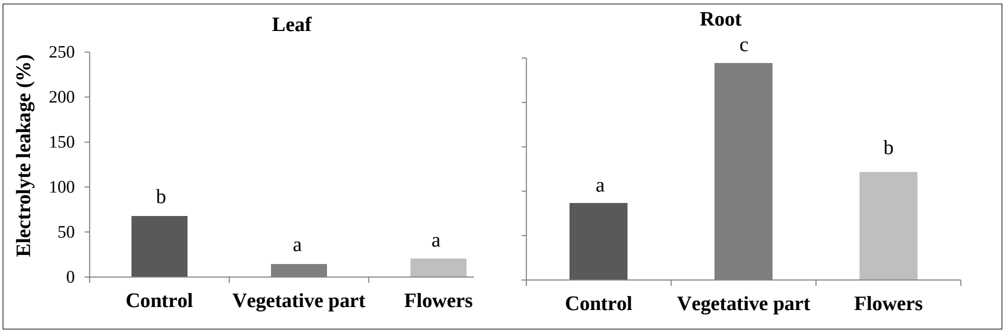

Fig. 3. Effect of *Nicotiana glauca* flower and vegetative part aqueous extracts on the electrolyte leakage (%) of lettuce roots and leaves. Values are means ± standard errors (SE) (n=5). Values affected by the same letter are not significantly different at $p<0.05$ (Duncan test).

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.

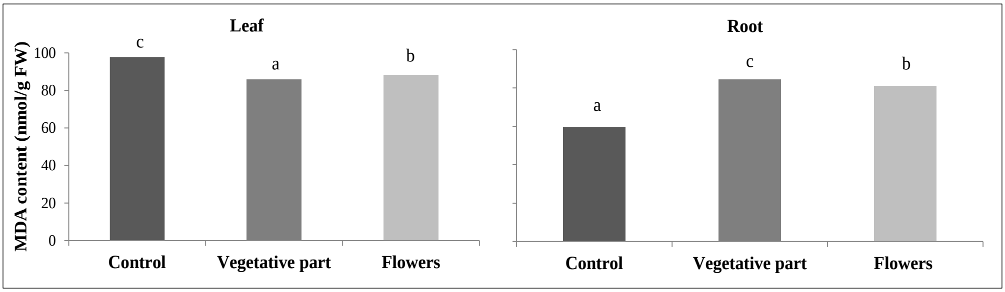

Fig. 4. Effect of *Nicotiana glauca* flower and vegetative part aqueous extracts on the content of malondialdehyde (MDA) lettuce roots and leaves. Values are means ± standard errors (SE) (n=5). Values affected by the same letter are not significantly different at $p<0.05$ (Duncan test).

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).

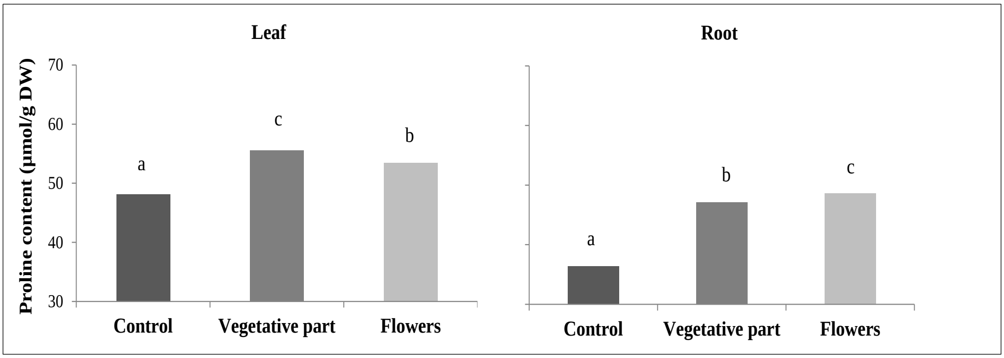

Fig. 5. Effect of *Nicotiana glauca* flower and vegetative part aqueous extracts on the content of proline lettuce leaves and roots. Values are means ± standard errors (SE) (n=5). Values affected by the same letter are not significantly different at $p<0.05$ (Duncan test).

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] ± 0.002	0.038[a] ± 0.001	0.295[a] ± 0.001	0.240[a] ± 0.001	0.031[a] ± 0.001
Vegetative part **Stimulation (%)**	0.237[b] ± 0.001 14.99	0.044[c] ± 0.002 28.42	0.347[c] ± 0.002 18.05	0.331[c] ± 0.054 37.86	0.037[c] ± 0.001 21.5
Flowers **Stimulation (%)**	0.258[c] ± 0.001 29.74	0.041[b] ± 0.003 15.05	0.337[b] ± 0.001 14.67	0.293[b] ± 0.002 22.05	0.034[b] ± 0.001 9.67
			Roots		
Control	0.262[a] ± 0.023	0.033[a] ± 0.002	0.221[a] ± 0.013	0.121[a] ± 0.002	0.017[a] ± 0.001
Vegetative part **Stimulation (%)**	0.406[c] ± 0.001 76.33	0.053[b] ± 0.003 132.92	0.270[b] ± 0.004 23.44	0.203[b] ± 0.003 68.31	0.026[c] ± 0.001 54.9
Flowers **Stimulation (%)**	0.361[b] ± 0.001 52.42	0.064[c] ± 0.002 205.35	0.305[c] ± 0.004 39.81	0.215[c] ± 0.003 77.68	0.021[b] ± 0.001 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.

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

---

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 dactylo*n. 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*

LITERATURE CITED

Abdullahi, A.E. 2002. *Cynodon dactylon* control with tillage and glyphosate. Crop Protection 21 (10): 1093-1100. https://doi.org/10.1016/S0261-2194(02)00062-5

Adedapo, A.A., Jimoh, F.O., Koduru, S., Afolayan, A.J., and Masika, P.J. 2008. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. BMC Complementary and Alternative Medicine 8, 53. http://dx.doi.org/10.1186/ 1472-6882-8-53

Ahmed, R., Tanvir, E.M., Hossen, M.S., Afroz, R., Ahmmed, I., Rumpa, N.E., Paul, S., Gan, S.H., Sulaiman, S.A., and Khalil, M.I. 2017. Antioxidant Properties and Cardioprotective Mechanism of Malaysian Propolis in Rats. Evidence-Based Complementary and Alternative Medicine 2017, 5370545. https://doi.org/10.1155/2017/5370545

Alghamdi, A.A. 2021. Phytoconstituents screening and antimicrobial activity of the invasive species *Nicotiana glauca* collected from Al-Baha region of Saudi Arabia. Saudi Journal of

Biological Sciences 28 (3): 1544-1547. https://doi.org/10.1016/j.sjbs.2020.12.034
Al-Zahrani, H.S., Alharby, H.F., Hakeem, K.R., and Rehman, R.U. 2021. Exogenous Application of Zinc to Mitigate the Salt Stress in *Vigna radiata* (L.) Wilczek-Evaluation of Physiological and Biochemical Processes. Plants 10 (5): 1005. https://doi.org/10.3390/plants10051005
Bano, A., Gupta, A., Rai, S., Fatima, T., Sharma, S., and Pathak, N. 2021. Mechanistic role of reactive oxygen species and its regulation *via* the antioxidant system under environmental stress. Pages 107-124. In: Plant Stress Physiology-Perspectives in Agriculture. H. Mirza., N. Kamran., and B. Tomasz, Ed. Editions IntechOpen, London, United Kingdom. http://dx.doi.org/10.5772/intechopen.101045
Bates, L.S., Waldren, R.P., and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39: 205-207. https://doi.org/10.1007/BF00018060
Blokhina, O., Virolainen, E., and Fagerstedt, K.V. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Annals of Botany 91(2):179-194. https://doi.org/10.1093/aob/mcf118
Dewanto, V., Wu, X., Adom, K.K., and Liu, R.H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agriculture and Food Chemistry 50 (10): 3010-3014. https://doi.org/10.1021/jf0115589
Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., and Vidal, N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry97:654-660. https://doi.org/10.1016/j.foodchem.2005.04.028
Doblinski, P.M.F., Ferrarese, M.L.L., Huber, D.A., Scapim, C.A., Braccini, A.L., and Ferrarese-Filho, O. 2003. Peroxidase and lipid peroxidation of soybean roots in response to p-coumaric and p-hydroxybenzoic acids. Brazilian Archives of Biology and Technology 46: 193-198. http://dx.doi.org/10.1590/S1516-89132003000200009
Duncan, D.B. 1955. Multiple range and multiple F test. Biometrics 11: 1-42. https://psycnet.apa.org/doi/10.2307/3001478
Durán, A.G., Benito, J., Macías, F.A., and Simonet, A.M. 2021. Agave Steroidal Saponins as Potential Bioherbicides. Agronomy 11 (12): 2404. https://doi.org/10.3390/agronomy11122404
Fakhry, A., El-Kenany, E., and Khattab, K. 2015. Allelopathic Potential of *Ruta graveolens* L. on Seed Germination and Seedling Growth of two weed species *Panicum turgidum* Forssk. and *Phalaris minor* Retz. Catrina: The International Journal of Environmental Sciences 13 (1): 17-24
Hagerman, A.E., and Butler, L.G. 1978. Protein precipitation method for the quantitative determination of tannins. Journal of Agriculture and Food Chemistry 26 (4): 809-812. https://doi.org/10.1021/jf60218a027
Hasan, M., Mokhtar, A. S., Rosli, A. M., Hamdan, H., Motmainna, M., and Ahmad-Hamdani, M.S. 2021. Weed control efficacy and crop-weed selectivity of a new bioherbicide WeedLock. Agronomy 11 (8): 1488. https://doi.org/10.3390/agronomy11081488
Hatano, T., Kagawa, H., Yasuhara, T., and Okuda, T. 1988. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chemical and Pharmaceutical Bulletin 36(6): 2090-2097. https://doi.org/10.1248/cpb.36.2090
Higuchi, T., and Kawamura, I. 1964. Enzymes of aromatic biosynthesis. Pages 260-289. In: Modern Methods of Plant Analysis-Moderne Methoden der Pflanzenanalyse. K. Paech., and M.V. Tracey, Ed. Editions Springer Verlag Berlin, Göttingen, Heidelberg, Germany. https://doi.org/10.1007/978-3-642-48141-3_7
Hoagland, D.R., and Arnon, D.I. 1950. The water-culture method for growing plants without soil. California agriculture experimental station Editions, Berkeley, USA, 32 pp.
Hussain, M.I., and Reigosa, M.J. 2011. Allelochemical stress inhibits growth, leaf water relations, PSII photochemistry, non-photochemical fluorescence quenching, and heat energy dissipation in three C_3 perennial species. Journal of Experimental Botany 62 (13):4533-4545. https://doi.org/10.1093/jxb/err161
Jacobson, L. 1951. Maintenance of iron supply in nutrient solutions by a single addition of ferric potassium ethylenediamine tetra-acetate. Plant Physiology26:411-413. https://doi.org/10.1104%2Fpp.26.2.411
Jmii, G., Khadhri, A., and Haouala, R. 2020a. *Thapsia garganica* allelopathic potentialities explored for lettuce growth enhancement and associated weed control. Scientia Horticulturae 262:109068. https://doi.org/10.1016/j.scienta.2019.109068
Jmii, G., Molinillo, J.M., Zorrilla, J.G., and Haouala, R. 2020b. Allelopathic activity of *Thapsia garganica* L. leaves on lettuce and weeds, and identification of the active principles. South African Journal of Botany 131: 188-194. https://doi.org/10.1016/j.sajb.2020.02.027
Juraimi, A.S., Drennan, S.D., and Anuar, N. 2005. Competitive effect of *Cynodon dactylon* (L.)

Pers. on four crop species, soybean [Glycine max (L.) Merr.], maize (*Zea mays*), spring wheat (*Triticum aestivum*) and faba bean [*Vicia faba* (L.)]. Asian Journal of Plant Sciences 4: 90-94. http://dx.doi.org/10.3923/ajps.2005.90.94

Khosravinejad, F., Heydari, R., and Farboodnia, T. 2008. Effects of salinity on photosynthetic pigments, respiration, and water content in two barley varieties. Pakistan Journal of Biological Sciences 11(20):2438-2442. https://doi.org/10.3923/pjbs.2008.2438.2442

Li, J., Chen, L., Chen, Q., Miao, Y., Peng, Z., Huang, B., Guo, L., Liu, D., and Du, H. 2021. Allelopathic effect of *Artemisia argyi* on the germination and growth of various weeds. Scientific reports 11 (1): 1-15. https://doi.org/10.1038/s41598-021-83752-6

Lichtenthaler, H., and Wellburn, A.R. 1983. Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions 11: 591-592. https://doi.org/10.1042/BST0110591

Lutts, S., Kinet, J.M., and Bouharmont, J. 1996. NaCl-induced senescence in leaves of rice (Oryza sativa L.) cultivars differing in salinity resistance. Annals of Botany 78: 389-398. https://doi.org/10.1006/anbo.1996.0134

Maksimovic, Z., Malencic, D., and Kovacevic, N. 2005. Polyphenol contents and antioxidant activity of *Maydis stigma* extracts. Bioresource Technology 96 (8): 873–877. https://doi.org/10.1016/j.biortech.2004.09.006

Ngondya, I.B., Treydte, A.C., Ndakidemi, P.A., and Munishi, L.K. 2019. Can *Cynodon dactylon* suppress the growth and development of the invasive weeds *Tagetes minuta* and *Gutenbergia cordifolia*? Plants 8 (12): 576. https://doi.org/10.3390/plants8120576

Oktay, M., Gulcin, I., and Kufrevioglu, O.I. 2003. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT-Food Science and Technology 36:263-271. https://doi.org/10.1016/S0023-6438 (02) 00226-8

Sampietro, D.A., Vattuone, M.A., and Isla, M.I. 2006. Plant growth inhibitors isolated from sugarcane (*Saccharum officinarum*) Straw. Journal of Plant Physiology 163: 837-846. https://doi.org/10.1016/j.jplph.2005.08.002

Scavo, A., Abbate, C., and Mauromicale, G. 2019. Plant allelochemicals: agronomic, nutritional and ecological relevance in the soil system. Plant and Soil 442 (1): 23-48. https://link.springer.com/article/10.1007/s11104-019-04190-y

Tuyen, P., Xuan, T., Tu Anh, T., Mai Van, T., Ahmad, A., Elzaawely, and A., Khanh, T. 2018. Weed suppressing potential and isolation of potent plant growth inhibitors from *Castanea crenata* Sieb. et Zucc. Molecules 23 (2): 345. https://doi.org/10.3390/molecules23020345

Ullah, R., Aslam, Z., Attia, H., Sultan, K., Alamer, K.H., Mansha, M.Z., Althobaiti, A.T., Al Kashgry, N.AT., Algethami, B., and Zaman, Q.U. 2022. Sorghum Allelopathy: Alternative Weed Management Strategy and Its Impact on Mung Bean Productivity and Soil Rhizosphere Properties. Life 12(9):1359. https://doi.org/10.3390/life12091359

Vieira, L.R., Silva, E.R.D., Soares, G.L.G., Fior, C.S., Ethur, E.M., Hoehne, L., and Freitas, E.M.D. 2018. Phytotoxic effects of *Morus nigra* aqueous extract on germination and seedling growth of *Lactuca sativa*. Rodriguésia 69: 2153-2161. http://dx.doi.org/10.1590/2175-7860201869443

Plant Protection Events

Report

on

The 13th Arab Congress of Plant Protection (ACPP)

Hammamet, Tunisia, 16-21 October, 2022

تونس - ACPP 2022 - Tunisia

PLANT HEALTH

For Secure and Safe Food

The 13th Arab Congress of Plant Protection (ACPP 2022) has been held, for the first time in Tunisia, during the period 16-21 October, 2022.

The Organizing Committee is extremely happy with the great success of this scientific event organized by the Arab Society for Plant Protection (ASPP) and the Tunisian Ministry of Agriculture represented by the National Institute of Agronomic Research of Tunisia (INRAT).

The congress was attended by more than 270 participants from Arab and non-Arab countries.

The Congress started on Monday 17th October with an opening ceremony under the patronage of Prof. Mahmoud Elyes Hamza, Minister of Agriculture, Water Resources and Fisheries of Tunisia,followed by a keynote address by Dr. Sophien Kamoun (Norwich Research Park, UK) entitled "Plant Health Vision for the 21st Century".

The scientific program included four plenary symposia covering the following themes:

1. Plant health for food security and safety.
2. Advances in molecular plant protection and its applications in pest management.
3. Research and innovation for sustainable crop protection.
4. Application of behavioral control tools as a safe and effective alternative in pest management.

The symposia plenary sessions included presentations by eminent invited speakers from well-known Research Centers or Universities (Italy, Germany, France, *United Kingdom, and Switzerland) or from International Organizations (FAO, ICARDA, ICIPE, CIMMYT, EPPO, CIHEAM, AOAD, and IOFS). These sessions included presentations on important plant protection topics such as:*

- *Mycotoxins as a hidden threat for food and feed safety.*
- *Importance of phytosanitary regulations and international standards for plant health to enhance food security.*
- *Conservation and use global plant genetic resources for enhancing insect pest and disease resistance: major foliar diseases of barley as an example.*
- *"Tomato plant*-Trichoderma-Phytophthora nicotianae*", a complex of interaction system for understanding plant defense.*
- *Parasitoid pre-adaptation improves biological control of symbiont-protected aphids.*
- *Integrated modern systematics and applications for mite biodiversity characterization.*
- *Challenges of automatic counting and identification of insect threats using smart technology.*
- *How to cope with resistance to insecticides to improve pest management.*
- *Role of pheromone applications in sustainable crop protection.*
- *Manipulation of plant pests host-finding and acceptance behavior for practical applications in integrated pest management.*

In addition to the symposia, around 40 concurrent oral sessions were organized in different conference rooms, focusing on specialized topics within the plant protection field such as:

- *Soil-borne pathogens,*
- *Red palm weevil,*
- *Fungal diseases,*
- *Bacterial diseases,*
- *Virus diseases and phytoplasma,*
- *Biological control,*
- *Economic entomology,*
- *Plant extracts,*
- *Food security and plant protection,*
- *Chemical pesticides,*
- *Climate change and plant protection,*
- *Transboundary pests,*
- *Nematodes*
- *Diseases of olives,*
- Tuta absoluta,
- *Research coordination in plant health,*
- *Beneficial insects.*

Moreover, along with this rich and diversified program, more than 100 posters on the same above mentioned topics were presented.

Additionally, the ACPP 2022 organized a touristic and agricultural one day trip during which participants visited two private companies specialized in nursey plant propagation materials production, followed by a visit to some historical sites close to Tunis city.

During the congress, a new ASPP Executive Committee for the 2023-2025 period was elected and is composed as follows:

- *Dr. Safaa Kumari (Syria):* ***President***
- *Dr. Ahmad M. Katbeh-Bader (Jordan):* ***Vice President,***
- *Dr. Zinette Melhem Moussa (Lebanon):* ***Secretary-Treasure,***
- *Dr. Emad M. Ghalib Al-Maaroof (Iraq):* ***Member & Chairman of Translation Committee,***
- *Dr. Houda Boureghda (Algeria):* ***Member & Chairman of Publication Committee,***
- *Dr. Hassan Dahi (Egypt):* ***Member & Chairman of Membership Committee,***
- *Dr Asma Najar (Tunisia):* ***Member & Chairman of Honour and Awards Committee,***
- *Dr. Ibrahim Al-Jboory (Iraq):* ***Member & Editor-in-Chief, ANEPPB,***
- *Dr. Khaled Makkouk (Lebanon):* ***Member & Editor-in-Chief, AJPP.***

Acknowledgements: *The Organizing Committee would like to appreciate the financial support of all the sponsors that contributed toward the success of the ACPP 2022. Support was offered by international organizations, governmental and non-governmental institutions, and private companies.*

Prof. Asma Najar
INRAT, University of Carthage, Tunis, Tunisia
Chairperson of the Organizing Committee of the 13th ACPP 2022

Report

on

The training organized by the International Plant Protection Convention (IPPC) for facilitators in Phytosanitary Capacity Evaluation (PCE)

Castel Gandolfo-Roma, Italy, 28 Nov-8 Dec, 2022

Context and objective.

The ***Phytosanitary Capacity Evaluation (PCE)*** *is a fully comprehensive NPPO (National Plant Protection Organization)-led, facilitator-enabled, International Plant Protection Convention (IPPC) Secretariat supported process of multiple phases, with a wide range of benefits, to help countries evaluate their phytosanitary capacities.*

The training was organized by the IPPC Secretariat in the framework of the project "Strengthening capacity and governance in food and plant health control in Africa (GCP/GLO/949/EC)" funded by the EU. Among the objectives of this project is to conduct phytosanitary capacity evaluations (PCEs) in 9 countries of the Common Market for Eastern and Southern Africa (COMESA) during 2023. The goal of this training was therefore to prepare PCE facilitators in order to respond to the demands of COMESA countries. Indeed the PCE of a NPPO is a voluntary and flexible process implemented by the IPPC-FAO on country request, which aims to assess the national phytosanitary capacity in order to identify its weaknesses and strengths. At the end a consensus on the areas that need to be improved in priority should be reached. Based on this diagnosis, the PCE facilitator guides the NPPO staff to develop a strategy to improve the fulfillment of its prerogatives. An important element to point out is that the PCE is a participatory approach involving the NPPO and its key stakeholders for a maximum ownership by the country. The PCE process uses various analytical tools of the result-based management (problem analysis, SWOT analysis, Logical Framework for project designing) to establish a good diagnosis of an initial situation and to design a project to correct the weaknesses identified. This effort to improve phytosanitary capacities of NPPOs is done in accordance with the international plant protection framework and trade requirements i.e the provisions of the IPPC and the International Standards for Phytosanitary Measures (ISPMs) and the WTO SPS agreement.

The participants to the training myself included, came from 8 countries of Africa, and were selected following a call for applications issued by the IPPC in September 2022. Prior to the face-to-face training held in Castel Gandolfo-Roma, Italy, 6 mandatory modules related to the IPPC and phytosanitary systems have been taken online and validated by the participants during October and November. Other participants from several countries working for the IPPC-FAO took the course also and that was a *great opportunity to exchange experiences. The contacts established and the interactions between the whole group, trainers and participants from the different countries (Jamaica, Belize, Morocco, France, Madagascar, South Africa, Gabon, Zambia, Kenya, Ghana, Zimbabwe, Sudan, Columbia, Japan, Mexico, Pakistan and Tunisia) were very enriching and promising for future collaboration.*

Group of trainees and instructors in the PCE training for facilitators

The e-learning courses which were newly developed by the IPPC (https://www.ippc.int/fr/) are freely accessible to everyone on the following links:

1- Introduction to IPPC and International Standards for Phytosanitary Measures (ISPMs): https://www.ippc.int/fr/publications/90624/

2- Monitoring and national reporting obligations: https://www.ippc.int/fr/publications/91282/

3- Pest Risk Analysis (PRA): https://www.ippc.int/fr/publications/90642/

4- Phytosanitary export certification system: https: //elearning.fao.org/course/view.php?id=860

5- Phytosanitary import inspection: https://www.ippc.int/en/e-learning/

6- Results-based management (RBM).

Only the RBM course that focuses on the "Logical Framework" and "SWOT Analysis" tools for project design, was delivered by CIDT (Center for International Development and Training, University of Wolverhampton, UK) and access was provided specifically to IPPC training participants.

At the end of the training, participants acquired technical knowledge and strategic planning skills that make them able to evaluate and guide strengthening phytosanitary systems. The next step for the qualified trainees after the in person-course is to successfully deliver *a PCE process in a country, that should be validated by a certified facilitator.*

Training program.

The face-to-face training was conducted by 4 experienced IPPC PCE facilitators, led by Ms. Sarah Brunel, Head of the IPPC Facilitation of Implementation Unit (*IFU*)*. Other experts from the IPPC-FAO, including lawyers and specialists in phytosanitary legislation, also contributed to the course. A focus on the gender issue was part of the course and a detailed and instructive virtual presentation was given to participants from Geneva.*

The 10 day-training was very intensive, but very fruitful. The facilitators (*Ms. Fitzroy White, Ms. Francisco Gutierrez and Ms. Mekki Chouibani*) *introduced the participants to the different steps in conducting a PCE as well as the technical aspects covered by the 13 PCE modules and the strategic planning process. Each session contained theoretical presentations, practical exercises and at the end a wrap-up of the day to summarize the new elements introduced. To facilitate learning, interactive activities in groups, brainstorming sessions and role-playing were organized while working on a fictitious country. Participants benefited greatly from the facilitators' commitment, experiences and availability.*

At the end of the training during which each participant was evaluated for its potential to be a future PCE facilitator, each one received a certificate of participation to the course.

Group discussion addressing a concrete situation of conducting a PCE of a fictitious country

Conclusion.

The training, although very intensive (two and a half months of hard work for both steps: online and face-to-face) was 100% successful. It made me more aware of the essential role of a NPPO in this era of increased international trade, which is to protect the country (or region) against the introduction and spread of quarantine pests. Indeed, there is a tendency to focus more on domestic pests and how to manage them and we often forget that it is fundamental for the food security of a country to protect itself from new intrusions that can be devastating for crops and biodiversity.

This training was also a valuable opportunity to improve my knowledge (as a researcher) on the international phytosanitary context, the ISPMs and the international treaties such as the SPS and the TBT agreements, and to rise my awareness about including new modules in the curriculum of the students in agricultural universities. Indeed, Pest Risk Analysis (PRA) for example, which is a discipline in its own right, is currently the basis for establishing phytosanitary measures against a regulated pest. The PRA is required to establish the import requirements of a country for a given commodity. Many European legislations and developed countries conduct PRAs as scientific basis for establishing their phytosanitary measures. The Tunisian phytosanitary legislation should gradually align with this requirement. In this regard, the training of future engineers with this competency is necessary.

Prof. Synda Boulahia-Kheder
National Agronomic Institute of Tunisia (INAT)
University of Carthage, Tunis, Tunisia

TUNISIAN JOURNAL
OF PLANT PROTECTION

Vol. 17 No. 2 DECEMBER 2022

CONTENTS

NEMATOLOGY

WEED SCIENCE

Photo of the cover page: *Pratylenchus vulnus* (Courtesy *Noura Chihani-Hammas*)

A Tunisian Half-Yearly Journal of Plant Health Sciences (TJPP)

Plantae Sanae in Terra Sana