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Contents

Photo of the cover page: *Aeolothrips* sp. (Courtesy *Abdelhak Khallou*)

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Guest Editorial

Electronic Traps for the Mediterranean Fruit Fly, Ceratitis capitata *Monitoring and Control, with Focus on the Project FruitFlyNet-ii*

Precision agriculture uses technologies at the field level to obtain accurate and instantaneous parameters - such as climatic data, infestation levels, and pest population dynamics - allowing better precision compared to conventional methods.

The Mediterranean fruit fly, Ceratitis capitata (*MedFly*), *is the most damaging pest of Mediterranean soft fruits, causing significant economic losses annually. Current control methods rely on monitoring adult flies using various types of sexual and/or food traps (Delta, McPhail, Tephri, and others), as well as assessing fruit veraison, BBCH stages, and fruit infestation to make decisions on spraying and/or adult mass trapping.*

Without gathering information on pest dynamics and related ecological factors, it is nearly impossible to implement effective pest control at the right location and time. Traditional pest control methods - including manual trap inspection - to obtain reliable data and inform control decisions can be challenging. Identifying captured insects requires specialists, and data acquisition and analysis often face delays that may take weeks before insecticide sprays are applied. Additionally, recording traps can be costly, and transportation poses logistical difficulties.

Recent technological advances have focused on developing electronic insect trap systems that automate pest detection, counting, and environmental data collection. These systems enhance Integrated Pest Management (*IPM*) *by promoting sustainability and precision control.*

Monitoring pests with automatic electronic traps (*e-traps*) *provides more accurate information about targeted insect abundance. When geo-located, this data enables a better understanding of the spatial and temporal distribution of the pest.*

Within the framework of the ENI CBC Mediterranean Sea Basin Programme's FruitFlyNet-ii Project, in which the first author participated, a Location Aware System (LAS) for MedFly ground spraying control was developed. This system includes electronic traps and a digital decision support service (DSS) tested in peach orchards.

The system features a new model of 3D-printed Delta e-trap equipped with: (*1*) *a solar panel mounted on one side providing continuous power via a rechargeable battery, charging during daylight hours to run sensors, cameras, and communication modules at night or on cloudy days,* (*2*) *a camera integrated inside the trap allowing early insect detection on the sticky surface, capturing photos twice daily and* (*3*) *a cellular 3G/4G dongle for real-time data transmission to a central station.*

The traps are baited with either a food attractant for females (three compounds, Biolure) or the parapheromone trimedlure, which attracts males.

Captured images are analyzed by specially designed software using image recognition algorithms. Identification is based on morphological characteristics such as wing spots, color, size, and ovipositor shape, enabling recognition of insect type and sex through algorithms developed by computer scientists. This allows early detection, continuous monitoring, and automated identification.

The captured data - including insect counts and environmental parameters (temperature, relative humidity, wind speed) provided by an automatic weather station - is transmitted wirelessly to a centralized platform accessible via web or smartphone applications. These data feed into sophisticated e-services supporting IPM decision-making, including spraying track maps, risk assessments, and e-guides for precise pest management.

This system enables farmers to detect the insect presence early and apply insecticides only where and when necessary, using spatial maps of population hotspots. This minimizes environmental contamination and reduces production costs. The innovation drastically cuts labor costs and error rates associated with manual trap monitoring while providing real-time pest surveillance at fine spatial and temporal scales.

Despite the clear advantages of using e-traps for monitoring and controlling MedFly, some limitations hinder large-scale adoption: (1) the initial cost to assemble the e-trap, (2) electronic components require regular maintenance, including battery replacement, cleaning, and occasional repairs, and (3) the system requires good internet coverage, which may be lacking in remote rural areas.

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Exogenous Spermidine Enhances Salt Tolerance in *Nicotiana rustica*: Physiological Mechanisms and Implications for Phytoremediation

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ABSTRACT

Essid, I., M'rah, S., and Chaffei-Haouari, Ch. 2025. Exogenous spermidine enhances salt tolerance in *Nicotiana rustica*: Physiological mechanisms and implications for phytoremediation. Tunisian Journal of Plant Protection 20 (2): 29-41.

Salt stress disrupts plant physiology by impairing photosynthesis, osmotic balance, and ion homeostasis. This study evaluated the effect of exogenous spermidine (Spd, 1 mM) on *Nicotiana rustica* exposed to moderate (100 mM NaCl) and severe (200 mM NaCl) salinity. Salt stress significantly reduced chlorophyll a, carotenoids, protein contents, and biomass, while inducing proline and soluble sugar accumulation and disturbing K^+/Na^+ and NO_3^-/Cl^- balances. Spd application markedly alleviated these effects under moderate salinity, restoring chlorophyll a and protein levels, sustaining biomass production, limiting excessive proline and sugar accumulation, and reducing Na^+ and Cl^- toxicity while maintaining K^+ and NO_3^- uptake. However, its protective effects were largely absent under severe stress, with only marginal improvements in biomass and osmolyte regulation. These findings indicate that spermidine confers substantial tolerance to moderate but not severe salinity, basically through stabilization of photosynthesis, preservation of protein metabolism, and regulation of ion homeostasis.

Keywords: Ion homeostasis, *Nicotiana rustica*, osmolytes, phytoremediation, polyamines, salt stress, spermidine

Soil salinization is a major abiotic constraint threatening agricultural productivity in Tunisia, where nearly 30% of irrigated lands are affected (Munns and Tester 2008). In arid and semi-arid regions such as Sfax and Gabes, groundwater salinity frequently exceeds 5 g/L, necessitating the adoption of salt-tolerant crops and biostimulants. *Nicotiana rustica*, which is well-adapted to marginal soils, represents a promising crop for both phytoremediation and biomass production (Kabir et al. 2024).

Polyamines, such as spermidine (Spd), play essential roles in plant stress responses by stabilizing membranes, scavenging reactive oxygen species (ROS), and regulating ion transport (Groppa and Benavides 2008). Despite growing interest, the application of polyamines in Tunisian agriculture remains largely unexplored. We hypothesize that exogenous spermidine can enhance salt tolerance in *N. rustica*,

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with its efficacy being dependent on the severity of the stress.

Salt stress disrupts plant physiology through osmotic imbalance, ion toxicity (especially Na^+ and Cl^-), nutrient deficiencies, and oxidative stress, which collectively inhibit growth and reduce yield (Zhu 2016). In response, plants activate adaptive mechanisms, such as the accumulation of osmoprotectants (e.g. proline, glycine betaine, and soluble sugars) and the regulation of ion transport systems to maintain cellular homeostasis (Flowers and Colmer 2008). Among the biochemical mediators of salt tolerance, polyamines, including putrescine, spermidine, and spermine, have garnered attention for their role in stress mitigation (Gill and Tuteja 2010). Specifically, spermidine functions as both a signaling molecule and an antioxidant, contributing to membrane stability, ROS scavenging, and ion channel regulation (Groppa and Benavides 2008). Previous studies have demonstrated that exogenous spermidine application enhances salt tolerance in various crops by improving photosynthetic performance, reducing oxidative damage, and optimizing ion homeostasis (ElSayed et al. 2018, Raziq et al. 2022).

Despite advances in understanding polyamine mediated stress tolerance, the mechanisms by which spermidine mitigates salt stress in *N. rustica* remain unclear. This species is recognized for its phytoremediation potential due to its vigorous growth and metal accumulation capacity. This study investigates the physiological and biochemical responses of *N. rustica* to salt stress under hydroponic conditions, with and without spermidine supplementation. Plants were exposed to two concentrations of NaCl (100 and 200 mM). Parameters including growth, ion homeostasis (K^+, Na^+, Cl^-, NO_3^-), and metabolic adjustments (proline and soluble sugars) were measured. By elucidating spermidine's protective role, this research aims to contribute to sustainable strategies for enhancing crop salt tolerance and to promote the use of *N. rustica* in phytoremediation and saline agriculture (Zhou et al. 2005).

MATERIALS AND METHODS

Plant cultivation.

Seedlings were initially cultivated in a ¼-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Following leaf emergence, they were transferred to 2-liter containers, with six seedlings per container. After 8-9 days, once the cotyledons were fully developed, the seedlings were irrigated with a ½-strength Hoagland's solution for three days before being transferred to a full-strength solution.

The hydroponic growth was conducted under controlled environmental conditions with an artificial light intensity of 150 µmol m^{-2} s^{-1}, a 16/8-hour light/dark photoperiod, a daytime temperature of 25 °C, and relative humidity of 70% (day) and 90% (night). The nutrient solutions were continuously aerated using air pumps and replaced every three days (Epstein and Bloom 2005). After 30 days of growth, salt stress was induced by adding NaCl at two concentrations (100 mM and 200 mM). These salt treatments were applied either alone or in combination with 1 mM spermidine. All treatments were maintained for 12 days.

Biomass assessment.

Plants were harvested and separated into shoots (leaf blades) and roots. Roots were rinsed three times with distilled water and blotted dry using filter paper. Fresh weight (FW) was recorded immediately, after which samples were then oven-dried at 60 °C for 72 hours to determine dry weight (DW) (Jones 2001).

Chlorophyll content.

Chlorophyll pigments were extracted from 100 mg of fresh leaf tissue by homogenization in 80% (v/v) acetone, following the method of Lichtenthaler and Wellburn (1983). The samples were incubated for 7 days at 4°C and then centrifuged at 1500 × g for 10 min. The absorbance (Abs) of the supernatant was measured at 663 nm, 645 nm, and 460 nm using a spectrophotometer. Chlorophyll a (Chl a) and carotenoid (Car) concentrations were calculated according to the following equations:

* Chl a = (12.7 × Abs 663) - (2.69 × Abs 645),
* Car = [5 × Abs 663) - ((Chl a × 3.19) + (Chl b × 130.3))]/20,

and results are expressed in µg/g FW.

Mineral ion contents (Na^+, K^+, and Cl^-).

Ion content analysis was performed on 25 mg of dried, ground leaf tissue. The tissue was digested in 25 mL of 0.5 N sulfuric acid. Sodium (Na^+) and potassium (K^+) concentrations in the digest were measured using a flame photometer (Corning). Chloride (Cl^-) content was determined by argentometric titration according to the Buchler-Cotlove method (Yemm and Willis 1954, Chapman and Pratt, 1961). All results are expressed in $\mu mol \cdot g^{-1}$ DW.

Nitrate content.

Nitrate was extracted in cold 0.1 N sulfuric acid and quantified by colorimetric analysis according to the method of Henriksen and Selmer-Olsen (1970).

Total soluble proteins.

Soluble protein content was determined by the Bradford assay (Bradford, 1976). A 25 µL aliquot of extract was mixed with 975 µL of 5 × diluted Bradford reagent. After color development, absorbance was read at 595 nm. Results are expressed in mg. g^{-1} FW.

Soluble sugars.

Soluble sugars were extracted from 25 mg of dried tissue using 5 mL of 80% ethanol and incubated at 70°C for 30 min (McCready et al. 1950). After centrifugation (6000 rpm, 15 min), 25 µL of the supernatant was mixed with 5 mL of anthrone reagent and boiled at 100°C for 10 min. After cooling on ice, absorbance was measured at 640 nm. Quantification was done using a glucose standard curve (0.1 g/L) (Staub 1963).

Proline content.

Proline content was estimated according to Bates et al. (1973). Fresh tissue (100 mg) was homogenized in 1.5 mL of 3% sulfosalicylic acid at 4°C and centrifuged at 14,000 rpm for 20 min. The supernatant (500 µL) was mixed with 500 µL of 3% SSA, 1 mL of acid ninhydrin, and 1 mL of glacial acetic acid. The mixture was incubated at 100°C for 20 minutes and cooled to 4°C. The chromophore was extracted using 2 mL of toluene. After mixing and resting for 1 hour, absorbance was read at 520 nm. Results are expressed as µmol/g FW.

Statistical analysis.

A two-way analysis of variance (ANOVA) was performed using XLSTAT software (version 2015) to evaluate the main effects of NaCl concentration (0, 100, and 200 mM) and spermidine treatment (0 or 1 mM), as well as their interaction, on all measured physiological parameters. When the ANOVA indicated significant differences ($p < 0.05$), post-hoc comparisons of means were conducted using Tukey's Honest Significant Difference (HSD) test at a 5% significance level.

RESULTS

Effects of salt stress and spermidine on photosynthetic pigments.

A two-way ANOVA revealed highly significant effects of both salt stress and spermidine treatment on photosynthetic pigment concentrations in *N. rustica* ($p < 0.001$). Chlorophyll a (Chl a) content in control plants was 0.85 µg·g^{-1} FW. Exposure to 100 mM NaCl caused a significant 41% reduction in Chl a to 0.50 µg·g^{-1} FW ($p < 0.001$). This decline was significantly alleviated by treatment with 1 mM spermidine, which restored Chl a content to 0.64 µg·g^{-1} FW ($p < 0.05$ compared to 100 mM NaCl), statistically indistinguishable from the control ($p > 0.05$). Under severe salt stress (200 mM NaCl), Chl a content further decreased to 0.48 µg·g^{-1} FW, with spermidine application failing to exert a significant protective effect (0.29 µg·g^{-1} FW; $p > 0.05$ vs 200 mM NaCl alone). Carotenoid concentrations exhibited a similar pattern, declining from 0.26 µg·g^{-1} FW in controls to 0.22 µg·g^{-1} FW under 100 mM NaCl; spermidine treatment restored carotenoids to 0.28 µg·g^{-1} FW ($p < 0.05$ vs 100 mM NaCl). At 200 mM NaCl, carotenoid levels were 0.205 µg·g^{-1} FW and spermidine had no significant effect (0.215 µg·g^{-1} FW; $p > 0.05$) (Fig. 1)

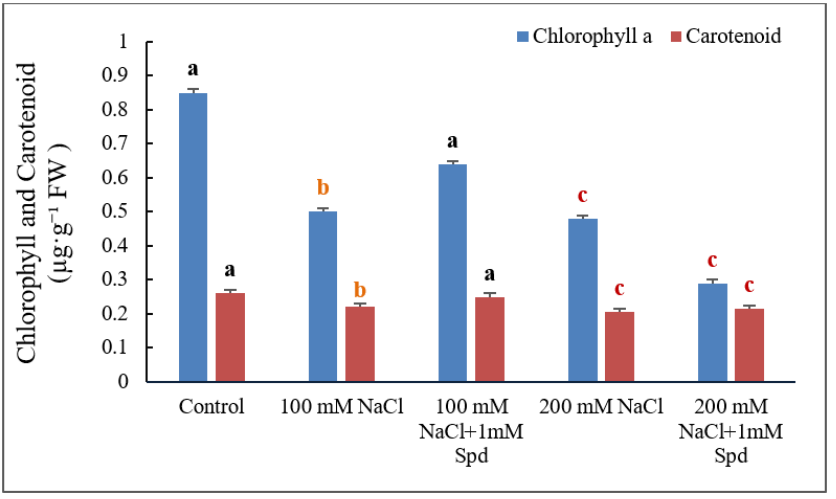

Fig. 1. Effect of 1 mM spermidine on chlorophyll a and carotenoid contents of *Nicotiana rustica* under NaCl stress. Values represent means ± SD (n = 3). Different letters labelling each bar (with same color) indicate significant differences according to one-way ANOVA followed by Tukey's test ($p < 0.05$).

Effects of salt stress and spermidine on biomass composition.

Salinity stress significantly decreased the biomass of *N. rustica* (Two-way ANOVA, $p < 0.001$). Exposure to 100 mM NaCl reduced leaf and root fresh weight by 39% and 48%, respectively, compared to the control ($p < 0.001$). The application of 1 mM spermidine (Spd) significantly mitigated this reduction ($p < 0.01$ vs. 100 mM NaCl), restoring leaf and root fresh weights to 93% and 98% of control values, respectively. A similar protective effect was observed for dry matter accumulation; Spd application significantly improved leaf and root dry weights to approximately 91% and 98% of the control ($p < 0.05$).

In contrast, under severe salt stress (200 mM NaCl), the protective effect of Spd was limited and not statistically significant ($p > 0.05$): leaf fresh weight increased modestly from 44% to 52% of control, and root fresh weight from 37% to 55% (Fig. 2).

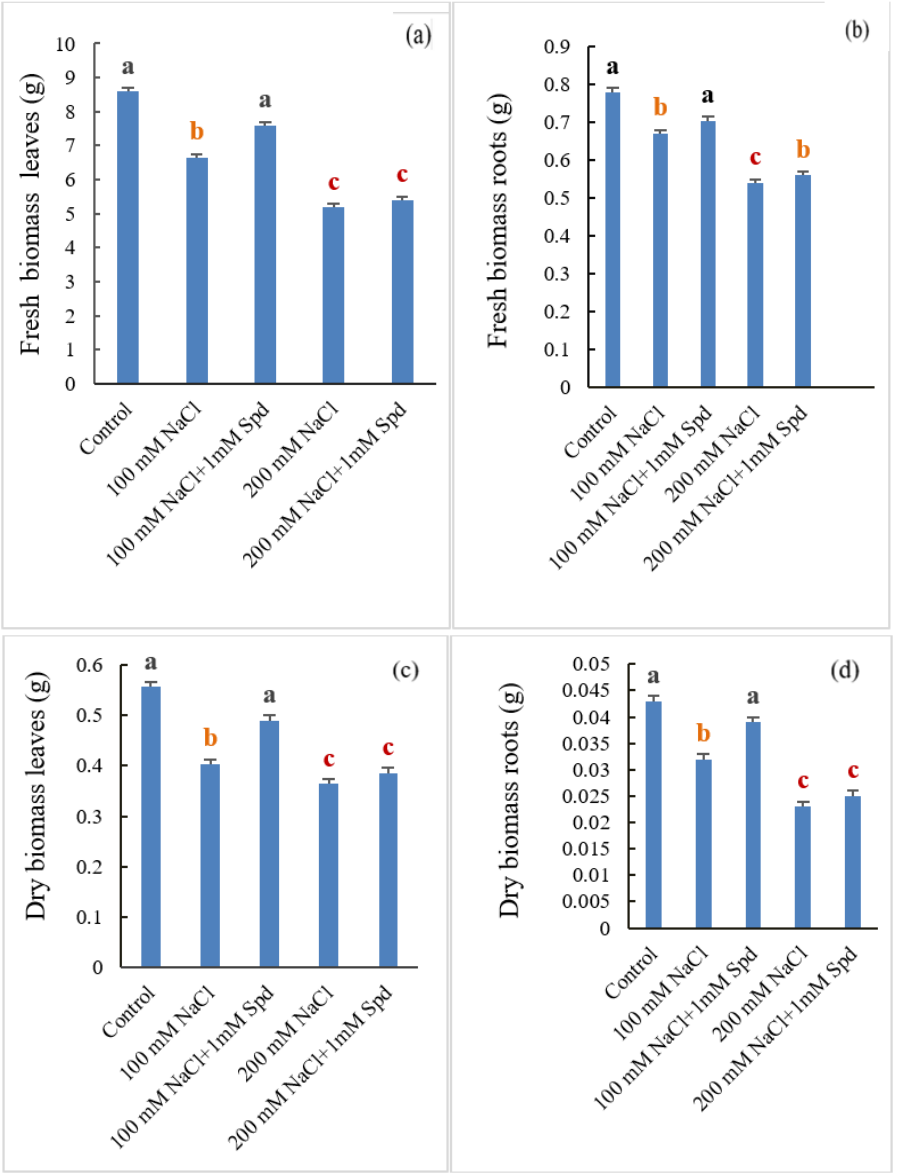

Fig. 2. Effect of 1 mM spermidine on fresh (a, b) and dry biomass (c, d) production in leaves and roots of *Nicotiana rustica* under NaCl stress. Values represent means ± SD (n = 3).

Effect of spermidine and salt stress on proline content.

Proline content showed a highly significant response to salt stress and spermidine treatment with tissue-specific differences (Two-way ANOVA, $p < 0.001$). In leaves, 100 mM NaCl induced a 13-fold proline accumulation, rising from 0.07 mg·g^{-1} FW in controls to 0.982 mg·g^{-1} FW ($p < 0.001$). Spermidine application at this salinity reduced leaf proline by 8% to 0.906 mg·g^{-1} FW ($p < 0.05$ vs. 100 mM NaCl). In contrast, root proline decreased by 87% under 100 mM NaCl to 0.099 mg·g^{-1} FW ($p < 0.001$ vs. control), with spermidine having no significant effect (0.09 mg·g^{-1} FW; $p > 0.05$). At 200 mM NaCl, leaf proline remained elevated (0.22 mg·g^{-1} FW, a 3.1-fold increase; $p < 0.001$), and spermidine treatment caused a non-significant decrease to 0.21 mg·g^{-1} FW ($p > 0.05$). Root proline at 200 mM NaCl returned to control levels (0.22 mg·g^{-1} FW; $p > 0.05$), unaffected by spermidine (Fig. 3)

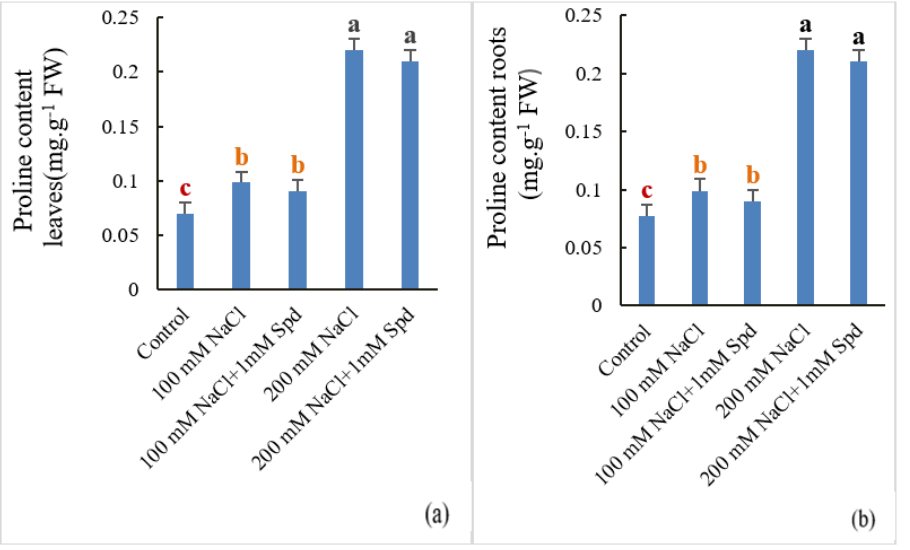

Fig. 3. Effect of 1 mM spermidine on proline content in leaves and roots of *Nicotiana rustica* under NaCl stress. Values represent means ± SD (n = 3).

Effect of spermidine and salt stress on soluble sugar content in leaves and roots.

Salt stress significantly elevated soluble sugar accumulation in *N. rustica* ($p < 0.05$). In leaves, sugar content increased from 84 µmol·g^{-1} FW in controls to 93 µmol·g^{-1} FW under 100 mM NaCl (not statistically significant, $p > 0.05$) and further to 139 µmol·g^{-1} FW under 200 mM NaCl ($p < 0.01$). Roots exhibited a more pronounced response, with sugar concentration rising from 12 µmol·g^{-1} FW in controls to 135 and 156 µmol·g^{-1} FW under 100 and 200 mM NaCl, respectively ($p < 0.001$ for both). Application of 1 mM spermidine substantially mitigated sugar accumulation at moderate salinity (100 mM NaCl), reducing sugar levels to 81 µmol·g^{-1} FW in leaves (not significantly

different from controls, $p > 0.05$) and 106 µmol·g⁻¹ FW in roots ($p < 0.05$ compared to 100 mM NaCl alone). Conversely, under severe salinity (200 mM NaCl), spermidine displayed limited efficacy, only slightly reducing sugar content to 135 µmol·g⁻¹ FW in leaves and 151 µmol·g⁻¹ FW in roots, with no significant difference relative to untreated stressed plants ($p > 0.05$) (Fig. 4)

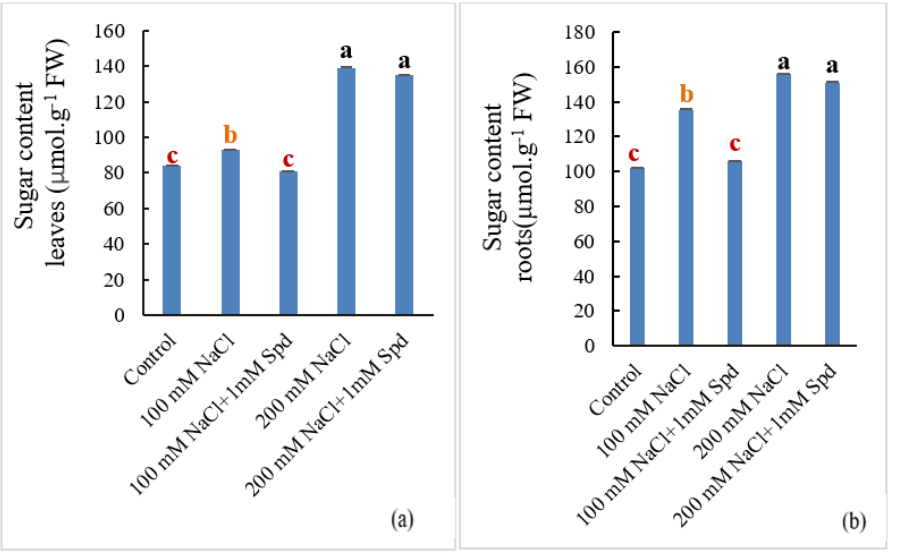

Fig. 4. Effect of spermidine (1 mM) on soluble sugar content in leaves and roots of *Nicotiana rustica* under NaCl stress. Values represent means ± SD (n = 3).

Effect of spermidine and salt stress on soluble protein content in leaves and roots.

Salt stress induced a pronounced and statistically significant reduction in total soluble protein content in *N. rustica* ($p < 0.001$). In leaves, protein concentration decreased markedly from 2.82 mg·g⁻¹ FW in control plants to 0.85 mg·g⁻¹ FW and 0.36 mg·g⁻¹ FW under 100 mM and 200 mM NaCl treatments, respectively ($p < 0.001$). A similar decline was observed in roots, where protein content dropped from 2.42 mg·g⁻¹ FW in controls to 0.74 mg·g⁻¹ FW and 0.35 mg·g⁻¹ FW under moderate and severe salinity stress, respectively ($p < 0.001$). Application of 1 mM spermidine significantly alleviated protein degradation at 100 mM NaCl, restoring leaf protein levels to 2.60 mg·g⁻¹ FW and root protein to 2.49 mg·g⁻¹ FW, both statistically comparable to controls ($p > 0.05$). However, under severe salt stress (200 mM NaCl), spermidine's protective effect was minimal, with protein contents remaining low at 0.53 mg·g⁻¹ FW in leaves and 0.52 mg·g⁻¹ FW in roots, showing no significant improvement compared to untreated stressed plants ($p > 0.05$). (Fig. 5).

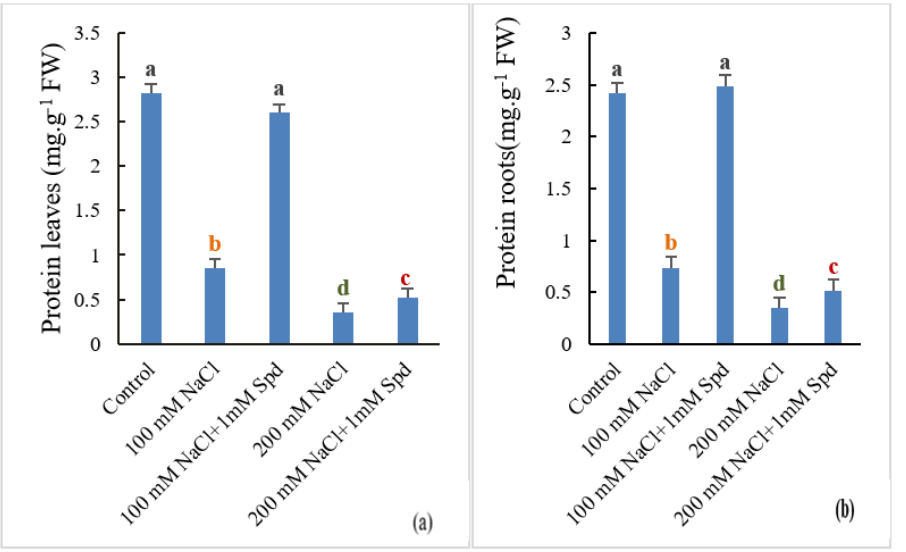

Fig. 5. Effect of spermidine (1 mM) on protein content in leaves and roots of *Nicotiana rustica* under NaCl stress. Values represent means Values represent means ± SD (n = 3).

Effect of spermidine and salt stress on ion content (Na^+, K^+, Cl^-, NO_3^-) in leaves and roots.

Salt stress significantly perturbed ion homeostasis in *N. rustica* ($p < 0.05$), while exogenous spermidine (Spd) application exerted a salinity-dependent protective effect. Under moderate salt stress (100 mM NaCl), Spd markedly alleviated ionic imbalances. In particular, Spd reduced the accumulation of toxic ions in leaves, lowering Na^+ content from 8598 to 693 µmol·g^{-1} DW and Cl^- content from 8425 to 825 µmol·g^{-1} DW ($p < 0.05$). Moreover, Spd contributed to the maintenance of essential ions: root K^+ content was preserved at levels comparable to the control (5763 vs. 6215 µmol·g^{-1} DW), and leaf NO_3^- concentration was fully restored to 301.23 µmol·g^{-1} DW, which was statistically indistinguishable from the control (329.35 µmol·g^{-1} DW; $p > 0.05$).

Conversely, under severe salt stress (200 mM NaCl), the protective effect of Spd was negligible. Na^+ and Cl^- levels remained elevated (e.g., root Na^+: 14068 µmol·g^{-1} DW with Spd vs. 14060 µmol·g^{-1} DW without Spd; $p > 0.05$), while K^+ and NO_3^- concentrations exhibited no significant improvement ($p > 0.05$) (Table 1).

Table 1. Effect of spermidine (Spd) application on ion contents (Na^+, Cl^-, K^+, and NO_3^-, expressed in µmol g^{-1} DW) in leaves and roots of *Nicotiana rustica* under salt stress

Ion	Plant organs	Control	100 mM NaCl	200 mM NaCl	100 mM NaCl + 1 mM Spd	200 mM NaCl + 1 mM Spd
K^+	**Leaves**	9654±36,769**a**	5864±170,41**c**	4213±63,639**d**	8954±72,831**b**	4542±219,203**d**
	Roots	3214±0,707**c**	2305±205,768**d**	6215±137,17**a**	5763±24,416**b**	3423±50,028**c**
Na^+	**Leaves**	−	8598±1,41**b**	16179±26,21**a**	693± 27,18**c**	16695±87,45**a**
	Roots	−	6541±3,54**b**	14060±36,68 **a**	795±22,32**c**	14068±45
NO_3^-	**Leaves**	329,35±6,84**a**	195,695±11,79**b**	132,54±0,70**c**	301,23±11,79**a**	139,32±21,27**c**
	Roots	520,32±10,60**a**	386,36±12,72**b**	253,24±21,92**c**	510,65±11,31**a**	235,36±6,15**c**
Cl^-	**Leaves**	−	8425±18,38**b**	12956±493,560**a**	825±21,920 **c**	11672±309,00**a**
	Roots	−	5324±86,974**b**	8654±14,007**a**	562±17,483**c**	8745±29,813**a**

Values represent means ± SD (n = 3). Different letters within each row indicate significant differences according to one-way ANOVA followed by Tukey's test ($p < 0.05$).

DISCUSSION

This study demonstrates that spermidine (Spd) significantly alleviates the adverse effects of salt stress in *N. rustica*, albeit dependent on stress severity. At moderate salinity (100 mM NaCl), Spd enhanced physiological performance, while at higher salinity (200 mM NaCl), its protective effects were largely ineffective. This aligns with the established role of polyamines as modulators of plant stress responses, with efficacy influenced by stress intensity and duration (ElSayed et al. 2022, Saha et al. 2015).

Salt stress notably reduced chlorophyll a and carotenoid contents, consistent with oxidative damage to thylakoid membranes caused by ionic toxicity (Wang et al. 2024). Spd's partial restoration of pigment levels under moderate stress indicates its role in stabilizing chloroplast membranes and photosystem II complexes, potentially through binding to negatively charged phospholipids and protecting chlorophyll-binding proteins from oxidative degradation (Wang et al. 2024, Yaakoubi et al. 2014). This stabilization likely supported increased photosynthetic efficiency and near-complete recovery of biomass at 100 mM NaCl, consistent with reports in cucumber and tomato under similar conditions (ElSayed et al., 2022). Under severe stress (200 mM NaCl), pigments declined drastically despite Spd application, reflecting irreversible chloroplast damage and impaired pigment biosynthesis pathways (Wang et al., 2024) (Figs. 1, 2).

Notably, the recovery of dry weight consistently exceeded that of fresh weight (e.g., root dry weight improved by 18%), suggesting Spd enhanced carbon assimilation and biomass formation more than water retention under high salinity. This is consistent with findings in other species, where polyamines were shown to significantly improve photosynthetic carbon fixation, chlorophyll biosynthesis, and dry matter accumulation under saline conditions (Hossain, et al., 2025).

Osmotic adjustment, a critical tolerance mechanism, involves proline and soluble sugars accumulation to maintain

cellular turgor (Hmidi et al. 2018, Khare et al. 2022). In *N. rustica*, proline levels increased in leaves but decreased in roots under moderate salt stress, indicating tissue-specific osmotic responses. Spd marginally reduced leaf proline accumulation, implying alleviation of osmotic and oxidative stress and thus reduced osmolyte demand. Roots accumulated soluble sugars under stress, with Spd suppressing excessive sugar buildup, suggesting improved carbon allocation toward growth rather than osmolyte storage (ElSayed et al. 2022, Khare et al. 2018). These findings suggest that spermidine contributes to osmotic adjustment by modulating soluble sugar accumulation effectively under moderate salt stress but has reduced protective capacity at higher salinity levels. However, Spd's modulation of osmolytes failed under severe salt stress, demonstrating compromised osmotic adjustment capacity (Hmidi et al. 2018) (Figs. 3, 4)

A significant protective effect of Spd was observed on soluble protein content under moderate salinity. Salt stress degraded proteins by impairing synthesis and increasing proteolysis, but Spd restored protein levels close to controls, likely by enhancing ribosome stability and reducing protease activity (ElSayed et al. 2022, Hossain et al. 2025). These findings indicate that spermidine effectively preserves protein synthesis and stability under moderate salinity but loses efficacy under severe ionic stress. This protein preservation explains better dry matter recovery compared to fresh weight, marking improved metabolic stability. Under severe salinity, Spd could not prevent protein loss, indicating irreversible damage to translational mechanism (ElSayed et al. 2022) (Fig. 5)

Ionic homeostasis was also better maintained with Spd at moderate salt stress, through reduced Na^+ and Cl^- accumulation and sustained K^+ and NO_3^- uptake (Pottosin et al. 2014, Saha et al. 2015 ;). Polyamines regulate ion transport by modulating H^+-ATPase and Na^+/H^+ antiporter activities, reducing toxic ion influx and promoting selective ion uptake (Saha et al. 2015). This preservation of favorable K^+/Na^+ ratios and nutrient uptake support photosynthetic and protein synthesis processes. Under severe stress, Spd failed to mitigate ion imbalances, reflecting limited protective capacity against ionic toxicity (Pottosin et al. 2014). All Spd effects are summarized in Fig. 6.

The results in table 1 demonstrate that Spd effectively modulates ion transport and alleviates ionic toxicity under moderate salinity; however, its protective mechanisms are insufficient to counteract the ionic stress imposed by severe salt conditions (Saha et al., 2015).

This study conclusively demonstrates that exogenous spermidine enhances salt tolerance in *N. rustica* under moderate salinity conditions (100 mM NaCl), a concentration relevant to real-world irrigation practices such as those in Tunisian coastal aquifers. The protective effects of spermidine arise from a complex interplay of mechanisms, including the preservation of photosynthetic efficiency via antioxidant activity, improved carbon assimilation and biomass allocation, restoration of osmotic balance by reducing stress-induced osmolyte accumulation, and critical maintenance of selective ion homeostasis that minimizes Na^+ and Cl^- toxicity while sustaining essential nutrient uptake. However, this protective capacity significantly declines at higher salinity levels (200 mM NaCl) due to the disruption of ionic regulation, establishing a threshold beyond which spermidine alone is insufficient. These findings underscore the potential of spermidine as a priming agent for crops facing moderate

salinity stress and point to the need for future research into combinatory treatments involving compatible solutes, phytohormones, or nutrient supplements to extend tolerance to more severe salt stress conditions. Such integrative approaches could enhance the practical application of spermidine in agricultural systems affected by salinity.

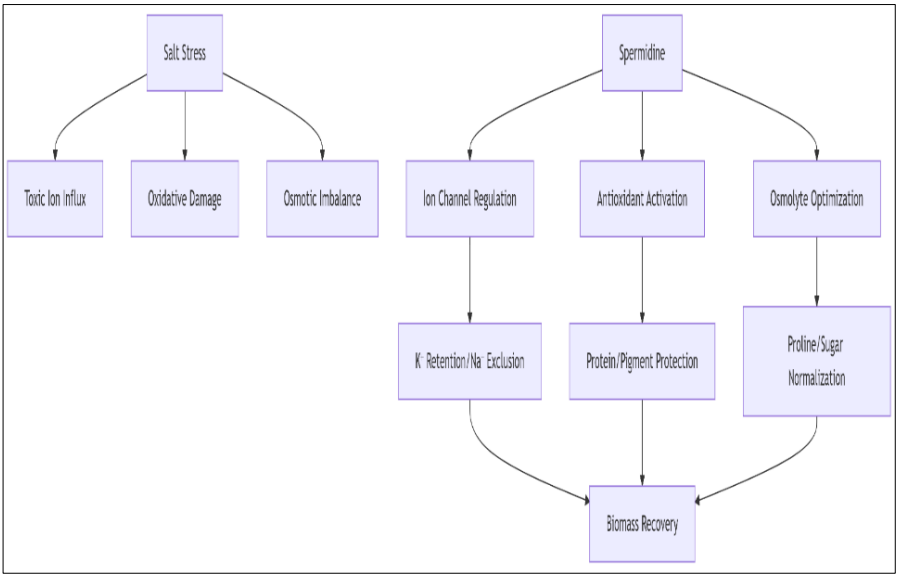

Fig. 6. Mechanisms of spermidine-mediated salt stress tolerance in *Nicotiana rustica*: From ion homeostasis to physiological recovery.

RESUME

Essid I. M'rah S. et Chaffei-Haouari Ch. 2025. La spermidine exogène améliore la tolérance au sel chez *Nicotiana rustica*: Mécanismes physiologiques et implications pour la phytoremédiation. Tunisian Journal of Plant Protection 20 (2): 29-41.

Le stress salin constitue l'un des principaux facteurs abiotiques limitant la croissance et la productivité des plantes, en perturbant la photosynthèse, le métabolisme des protéines, l'équilibre osmotique et l'homéostasie ionique. Cette étude a évalué l'effet de l'application exogène de spermine (Spd, 1 mM) sur *Nicotiana rustica* soumis à une salinité modérée (100 mM NaCl) et sévère (200 mM NaCl). Le stress salin a entraîné une réduction significative de la chlorophylle a, des caroténoïdes, de la biomasse et des protéines solubles, tout en induisant une accumulation de proline et de sucres solubles de manière spécifique aux tissus, ainsi qu'une perturbation des rapports K^+/Na^+ et NO_3^-/Cl^-. L'application de Spd sous salinité modérée a significativement atténué ces effets, en restaurant les niveaux de la chlorophylle a et des protéines, en maintenant la production de biomasse, en modulant l'accumulation des osmolytes et en réduisant la toxicité du Na^+ et du Cl^- tout en préservant l'absorption de K^+ et de NO_3^-. En revanche, sous salinité sévère, l'effet protecteur de Spd s'est révélé limité et largement inefficace. Ces résultats suggèrent que la spermidine améliore la tolérance au stress salin modéré principalement en stabilisant la photosynthèse, en préservant le métabolisme des protéines et en régulant l'homéostasie ionique, mais demeure insuffisante pour contrer les dommages induits par une salinité extrême.

Mots clés: Homéostasie ionique, *Nicotiana rustica,* osmolytes, phytoremédiation, polyamines, spermidine, stress salin

الملخص

الصيد، إشراق وصباح مراح وشراز الشافعي-الهواري. 2025. تحسين تحمل نبات *Nicotiana rustica* للملوحة بواسطة السبيرميدين الخارجي :الآليات الفسيولوجية وانعكاساتها على المعالجة النباتية.

Tunisian Journal of Plant Protection 20 (2): 29-41.

تُعد ملوحة التربة من أهم القيود البيئية التي تحد من إنتاجية المحاصيل، خاصة في المناطق الجافة وشبه الجافة. ويُعد الإجهاد الملحي أحد أهم العوامل اللاأحيائية التي تحد من نمو النباتات وإنتاجيتها، حيث يؤدي ذلك إلى اختلال عملية التمثيل الضوئي، واضطراب أيض البروتينات، واختلال التوازن الأسموزي والأيوني .هدفت هذه الدراسة إلى تقييم تأثير المعاملة الخارجية لمادة السبرمدين بتركيز 1 مليمول على نبات *Nicotiana rustica* المعرض لملوحة معتدلة (100 ميليمول NaCl) وملوحة شديدة (200 ميليمول NaCl). أظهر الإجهاد الملحي انخفاضاً ملحوظاً في الكلوروفيل a والكاروتينات والكتلة الحيوية والبروتينات الذائبة، بالتوازي مع زيادة تراكم البرولين والسكريات الذائبة بصور خاصة في الأنسجة، بالإضافة إلى اضطراب في نسبة توازن K^+/Na^+ و NO_3^-/Cl^-. ساعدت المعاملة بالسبرمدين تحت الملوحة المعتدلة على التخفيف من هذه التأثيرات، حيث استعاد النبات مستويات الكلوروفيل a والبروتينات، وحافظ على النمو والكتلة الحيوية، وعدّل تراكم المواد الأسموزية، وخفّض من سمية Na^+ و Cl^- مع الحفاظ على امتصاص K^+ و NO_3^-. في المقابل، كان تأثيره محدوداً وغير فعّال تحت الملوحة الشديدة. تشير هذه النتائج إلى أن السبرمدين يعزز من قدرة النبات على تحمل الملوحة المعتدلة عبر تثبيت عملية التمثيل الضوئي والحفاظ على أيض البروتين وتنظيم التوازن الأيوني، إلا أنه غير كافٍ للتغلب على الضرر الناجم عن الملوحة القصوى.

كلمات مفتاحية: إجهاد ملحي، بوليأمينات، توازن الأيونات، سبرميدين، معالجة نباتية، مواد أسموزية، *Nicotiana rustica*

LITERATURE CITED

Bates, L.S., Waldren, R.P., and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil 39: 205-207.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.

Chapman, H.D., and Pratt, P.F. 1961. Methods of Analysis for Soils, Plants and Waters. University of California, Division of Agricultural Sciences, California, USA, 309 pp.

ElSayed, A.I., Rafudeen, M.S., and Golldack, D. 2022. Polyamines: Bioactive molecules with stress-protective roles in plants under salinity stress. Environmental and Experimental Botany 205: 105106. DOI: 10.1016/j.sjbs.2022.02.053

Epstein, E., and Bloom, A.J. 2005. Mineral Nutrition of Plants: Principles and Perspectives, 2nd Edition. Sinauer Associates, Massachusetts, USA, 400 pp.

Flowers, T.J., and Colmer, T.D. 2008. Salinity tolerance in halophytes. New Phytologist 179: 945-963. https://doi.org/10.1111/j.1469-8137.2008.02531.x

Gill, S.S., and Tuteja, N. 2010. Polyamines and abiotic stress tolerance in plants. Plant Signaling and Behavior 5: 26-33. https://doi.org/10.4161/psb.5.1.10291

Groppa, M.D., and Benavides, M.P. 2008. Polyamines and abiotic stress: Recent advances. Amino Acids 34: 35-45. https://doi.org/10.1007/s00726-007-0501-8

Henriksen, A., and Selmer-Olsen, A.R. 1970. Automatic methods for determining nitrate and nitrite in water and soil extracts. Analyst 95: 514-518 https://doi.org/10.1039/AN9709500514

Hmidi, D., Ben Amar, R., and Bouslama, M. 2018. Effect of salinity on osmotic adjustment, proline and soluble sugar contents in plants. Plant Science Today 5: 20-29.

Hoagland, D.R., and Arnon, D.I. 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular 347, University of California, Berkeley, California, USA, 32 pp.

Hossain, M.M., Kordrostami, M., Goto, F., and Rahimi, M. 2025. Spermine treatment improves salinity tolerance in *Plantago major* by altering growth parameters, biochemical profiles and gene expression. Scientific Reports 15:

25762. https://doi.org/10.1038/s41598-025-11903-0
Jones, J.B. 2001. Laboratory Guide for Conducting Soil Tests and Plant Analysis. CRC Press, Boca Raton, Florida, USA, 363 pp.
Kabir, M.A., Hossain, M.K., Hossain, M.A., Molla, M.O.F., Khatun, M.S., and Mostofa Jamal, M.A.H. 2024. Impact of water and soil salinity on coastal agriculture in Bangladesh: Insights and mitigation strategies. American Journal of Multidisciplinary Research and Innovation 3: 36-48.
Khare, T., Srivastav, A., Shaikh, S., and Kumar, V. 2018. Polyamines and their metabolic engineering for plant salinity stress tolerance. Pages 339-358. In: Salinity Responses and Tolerance in Plants, Volume 1: Targeting Sensory, Transport and Signaling Mechanisms. Springer International Publishing, Cham.
Lichtenthaler, H.K., and Wellburn, A.R. 1983. Determinations 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
McCready, R.M., Guggolz, J., Silviera, V., and Owens, H.S. 1950. Determination of starch and amylose in vegetables. Analytical Chemistry 22: 1156-1158.
Munns, R., and Tester, M. 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology 59: 651-681. https://doi.org/10.1146/annurev.arplant.59.032607.092911.
Pottosin, I., and Shabala, S. 2014. Polyamines control of cation transport across plant membranes: Implications for ion homeostasis and abiotic stress signaling. Frontiers in Plant Science 5: 154. https://doi.org/10.3389/fpls.2014.00154
Raziq, A., Mohi Ud Din, A., Anwar, S., Wang, Y., Jahan, M.S., He, M., Ling, C.G., Sun, J., Shu, S., and Guo, S. 2022. Exogenous spermidine modulates polyamine metabolism and improves stress responsive mechanisms to protect tomato seedlings against salt stress. Plant Physiology and Biochemistry 187: 1-10.
Saha, J., Brauer, E.K., Sengupta, A., Popescu, S.C., Gupta, K., and Gupta, B. 2015. Polyamines as redox homeostasis regulators during salt stress in plants. Frontiers in Environmental Science 3: 21. https://doi.org/10.3389/fenvs.2015.00021
Shabala, S., and Cuin, T.A. 2008. Potassium transport and plant salt tolerance. Physiologia Plantarum 133: 651-669. https://doi.org/10.1111/j.1399-3054.2007.01008.x
Staub, A.M. 1963. Extraction and estimation of plant sugars. Pages 51-54. In: Methods in Enzymology, Vol. 6. S.P. Colowick and N.O. Kaplan, Eds. Academic Press, New York, USA.
Wang, X., An, T., Hu, X., and Yu, Z. 2024. Sensitivity and responses of chloroplasts to salt stress: oxidative and ionic stress effects. Frontiers in Plant Science 15: 1374086. https://doi.org/10.3389/fpls.2024.1374086
Yaakoubi, K., Da Silva, D., and Amiar, Z. 2014. Protective action of spermine and spermidine against oxidative damage in plant photosystems. PLoS ONE 9 (11): e112893. https://doi.org/10.1371/journal.pone.0112893
Yemm, E.W., and Willis, A.J. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochemical Journal 57: 508-514. https://doi.org/10.1042/bj0570508
Zhou, F.G., Chen, B., and Yang, Z.M. 2005. Polyamines regulate H^+-ATPase activity under salt stress. Plant Physiology 144: 1064-1074.
Zhu, J.K. 2016. Abiotic stress signaling and responses in plants. Cell 167: 313-324. https://doi.org/10.1016/j.cell.2016.08.029

Reduction of Ammonium Toxicity by Potassium in Hydroponically Cultivated Alfalfa (*Medicago sativa*): Growth and Physiological Responses

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https://dx.doi.org/10.4314/tjpp.v20i2.2 (***Tunisia***)

ABSTRACT

Rhimi, F., Essid, I., Chaffei-Haouari, Ch., and M'rah, S. 2025. Reduction of ammonium toxicity by potassium in hydroponically cultivated alfalfa (*Medicago sativa*): Growth and physiological responses. Tunisian Journal of Plant Protection 20 (2): 43-55.

Alfalfa (*Medicago sativa*) typically thrives on nitrate nutrition, but the environmental impact of nitrate overuse necessitates alternative nitrogen sources. While ammonium (NH_4^+) is a promising candidate, it often induces toxicity in plants. This study investigates the potential of potassium (K^+) supplementation to mitigate NH_4^+ toxicity in hydroponically grown alfalfa over a 17-day period. Plants were subjected to varying nitrogen regimes: a nitrate control (3 mM NO_3^-), and two NH_4^+ concentrations (3 and 6 mM) combined with three K^+ levels (0.2, 1.2, and 3 mM). A combination of 6 mM NH_4^+ and 3 mM K^+ proved lethal, causing complete plant mortality. In contrast, the specific regimen of 3 mM NH_4^+ with 3 mM K^+ significantly alleviated toxicity, restoring plant biomass to approximately 50% of the nitrate control. This mitigation was linked to a significant reduction in toxic NH_4^+ accumulation in shoots and a 2.3-fold increase in K^+ uptake compared to low-K^+ treatments. Physiologically, this optimal treatment maintained chlorophyll content, soluble protein, and polyphenol levels comparable to control plants, while also boosting antioxidant activity by 35%. These results demonstrate that a balanced NH_4^+/K^+ nutrition, specifically at a 1:1 ratio (3 mM each), can effectively mitigate ammonium toxicity by regulating its uptake and translocation, preserving photosynthetic function, and enhancing antioxidant capacity. This strategy offers a sustainable approach for incorporating ammonium-based fertilizers in alfalfa crop.

Keywords: Alfalfa, ammonium toxicity, hydroponics, *Medicago sativa*, nitrogen nutrition, oxidative stress, phenolic compounds, potassium interaction

Alfalfa (*Medicago sativa*) is a cornerstone species within the Fabaceae family, renowned for its exceptional agricultural value. As a high-yielding perennial legume, it serves dual roles as both a premium fodder crop (Zaidi 2021) and a natural soil enricher through nitrogen fixation via Rhizobium symbiosis (Mauriés 2003). The plant's nutritional profile, characterized by 18-22% protein content and rich carotenoid concentrations (Hadidi et al. 2023), along with its documented medicinal properties (Khairy et al. 2025), underscores its economic and ecological importance in Mediterranean agroecosystems.

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Although alfalfa naturally acquires nitrogen through symbiosis, mineral nitrogen fertilization is often used in hydroponic systems or soils where nodulation is ineffective. Conventional agriculture relies heavily on nitrate (NO_3^-) fertilizers, but their environmental impact is increasingly problematic. Nitrate's high solubility leads to substantial leaching losses, contributing to groundwater contamination (Smith et al. 2021). Furthermore, microbial denitrification of excess nitrate generates nitrogen oxides, potent greenhouse gases that contribute to stratospheric ozone depletion. These environmental impacts have driven the search for alternative nitrogen management strategies in forage production systems.

Ammonium (NH_4^+) presents a potentially sustainable alternative due to its positive charge, which enhances soil retention compared to nitrate. However, NH_4^+ nutrition poses significant physiological challenges for alfalfa, including rhizosphere acidification, disruption of cation balance, and accumulation of reactive nitrogen species (Liu et al. 2017). Recent studies have identified several mitigation strategies for NH_4^+ toxicity, including enhanced assimilation pathway activity (Liu and Wirén 2017) and application of organic osmolytes like γ-aminobutyric acid (Ma et al. 2016). Among these approaches, potassium (K^+) supplementation has emerged as particularly promising due to its dual role as an essential macronutrient and NH_4^+ antagonist.

The competitive interaction between K^+ and NH_4^+ at transport sites (Szczerba et al. 2008) suggests that optimized K^+ nutrition could maintain alfalfa productivity under NH_4^+-based regimes. This study hypothesizes that K^+ alleviates NH_4^+ toxicity by reducing ammonium uptake and accumulation, maintaining ionic homeostasis, and protecting physiological processes. The study specifically examines: (1) the concentration-dependent effects of K^+ on NH_4^+ toxicity alleviation, and (2) the physiological mechanisms underlying this mitigation, including ion homeostasis, photosynthetic performance, and oxidative stress responses. Through controlled hydroponic experiments employing two NH_4^+ levels (3 and 6 mM) combined with three K^+ concentrations (0.2, 1.2, and 3 mM), we aim to establish practical guidelines for implementing ammonium-potassium fertilization systems in alfalfa crop.

MATERIALS AND METHODS

Plant cultivation and experimental design.

Seed sterilization and germination. Alfalfa seeds were surface-sterilized in 20% (v/v) sodium hypochlorite solution for 10 min, followed by five rinses with distilled water. Sterilized seeds were germinated on moist filter paper in Petri dishes under ambient laboratory conditions (25 ± 2°C).

Hydroponic system establishment. After 7 days, uniformly germinated seedlings were transferred to 2 L crystallizing dishes containing a modified Hoagland and Arnon (1940) nutrient solution. Plants were maintained in a growth chamber under controlled environmental conditions: a 16/8 h light/dark photoperiod, a photosynthetic photon flux density of 150 μmol m^{-2} s^{-1}, day/night temperatures of 25/18°C, and 65% relative humidity.

Experimental treatments. At 19 days' post-germination, seedlings were divided into seven treatment groups (n = 7 per group). The NH_4^+ concentrations (3 and 6 mM) were selected to represent a moderate and a high, potentially toxic dose. The K^+ levels (0.2, 1.2, and 3 mM)

represent deficient, sufficient, and high luxury consumption ranges, respectively. The control (M1) contained 0.2 mM K^+ to provide a baseline for comparison with the ammonium treatments at the same low K^+ level.

- M1: 3 mM NO_3^- + 0.2 mM K^+ (Control)
- M2: 3 mM NH_4^+ + 0.2 mM K^+
- M3: 3 mM NH_4^+ + 1.2 mM K^+
- M4: 3 mM NH_4^+ + 3.0 mM K^+
- M5: 6 mM NH_4^+ + 0.2 mM K^+
- M6: 6 mM NH_4^+ + 1.2 mM K^+
- M7: 6 mM NH_4^+ + 3.0 mM K^+

Nutrient solutions were renewed every 3 days to maintain stable ion concentrations. Plants were harvested for physiological and biochemical analyses after 17 days of treatment exposure. The complete nutrient solution composition for all treatments is detailed in Table 1.

Table 1. Composition of experimental growth media (M1–M7)

Salts	Media						
	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**
KNO_3	1 mM	-	-	-	-	-	-_
KH_2PO_4	0.2 mM	0.2 mM	1.2 mM	3 mM	0.2 mM	1.2 mM	3 mM
$Ca(NO_3)_2$	1 mM	-	-	-	-	-	-_
$MgSO_4$	0.4 mM	0.4 mM	0.4 mM	0.4 mM	0.4 mM	0.4 mM	0.4 mM
NH_4Cl	-	3 mM	3 mM	3 mM	6 mM	6 mM	6 mM
$CaCl_2$	-	1 mM	1 mM	1 mM	1 mM	1 mM	1 mM

Plant growth analysis and water content determination.

Following treatment, plants were carefully separated into aerial (shoot) and root components. Each tissue fraction was gently blotted dry using filter paper to remove surface moisture before immediate weighing to determine fresh weight (FW) using an analytical balance (precision ±0.1 mg). For dry weight (DW) measurements, samples were placed in pre-weighed aluminum foil pouches and dried to constant mass in a forced-air oven at 60°C for 72 h.

Water content was calculated using the following equation:

$$\text{Water Content (\%)} = [(FW - DW)/FW] \times 100.$$

Mineral element analysis.

Potassium ion (K^+) quantification was performed following acid digestion of plant tissues in 0.5% (v/v) nitric acid (HNO_3). K^+ concentrations were determined using flame emission spectrophotometry (Jenway PFP7 Flame Photometer) with appropriate blank corrections. A five-point standard calibration curve (0-50 ppm K^+) was generated using analytical grade KCl for quantitative analysis (Jones 2001).

Ammonium (NH_4^+) content was measured according to Weatherburn's (1967) phenol-hypochlorite method. Briefly, tissue extracts were reacted with alkaline phenol and sodium hypochlorite, forming indophenol blue whose absorbance was measured at 625 nm. NH_4^+ concentrations were calculated against an ammonium sulfate standard curve (0-10 μg mL^{-1} range).

Chlorophyll quantification.

Total chlorophyll extraction was performed using fresh leaf tissue homogenized in 80% (v/v) ice-cold acetone under dim light conditions. After

centrifugation at 3,000 × g for 10 min at 4°C, the supernatant absorbance was measured at 645 nm and 663 nm using a Beckman DU 640 UV-Vis spectrophotometer (Beckman Coulter, USA). Total chlorophyll content was calculated according to Torrecillas et al. (1984) using the following equations:

Chlorophyll a (µg/mL) = 12.7(A663) - 2.69(A645),
Chlorophyll b (µg/mL) = 22.9(A645) - 4.68(A663),
Total Chlorophyll = Chl a + Chl b.

Results were normalized to fresh weight and expressed as µg chlorophyll per mg fresh tissue (µg mg^{-1} FW).

Protein extraction and quantification.

Fresh leaf tissue samples were immediately flash-frozen in liquid nitrogen and homogenized to a fine powder using a pre-chilled mortar and pestle. The frozen powder was suspended in ice-cold extraction buffer (50 mM phosphate buffer, pH 7.2, containing 5% (v/v) glycerol, 1 mM dithiothreitol (DTT), 1 mM EDTA, and 5% (w/v) polyvinylpyrrolidone (PVP)) to prevent protein degradation and phenolic compound interference. The homogenate was centrifuged at 12,000 × g for 20 min at 4°C to pellet cellular debris.

The resulting supernatant was used for soluble protein quantification via the Bradford assay (Bradford 1976). Briefly, 100 µL of protein extract was mixed with 1 mL Bradford reagent (Coomassie Brilliant Blue G-250) and incubated for 10 min at room temperature. Absorbance was measured at 595 nm using a spectrophotometer (Beckman DU 640). Protein concentrations were determined from a standard curve generated using bovine serum albumin (BSA) standards (0-50 µg mL^{-1}). All measurements were performed in triplicate, with results expressed as µg protein per mg fresh weight (µg mg^{-1} FW).

Quantification of total polyphenolic compounds.

The total polyphenol content was determined using a modified Folin-Ciocalteu assay (Dewanto et al. 2002). Briefly, 100 µL of plant extract was mixed with 500 µL of 10% (v/v) Folin-Ciocalteu reagent and allowed to react for 5 min at room temperature. Subsequently, 400 µL of 7.5% (w/v) sodium carbonate solution was added, and the mixture was incubated for 60 min in the dark at 25°C. The absorbance of the resulting blue complex was measured at 760 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800).

A standard calibration curve was prepared using gallic acid solutions (0-500 mg L^{-1}) with the same treatment conditions. The total polyphenol content was calculated from the standard curve and expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE g^{-1} DM).

Assessment of total antioxidant capacity.

The total antioxidant activity was evaluated using the phosphomolybdenum reduction assay according to Prieto et al. (1999) with modifications. Briefly, 100 µL of plant extract was combined with 1 mL of freshly prepared reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate in distilled water. The reaction mixture was incubated at 90°C for 95 min in a temperature-controlled water bath.

After cooling to room temperature, the absorbance of the green phosphomolybdenum complex was measured at 695 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). A standard curve was generated using gallic acid solutions (0-500 µg mL^{-1}) subjected to identical treatment conditions. The total antioxidant capacity was calculated from the standard curve and

expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE g^{-1} DM).

Statistical analysis.

All experimental data were derived from seven biological replicates per treatment (n = 7), consistent with the experimental design. The study included a total of seven distinct nutrient treatments (M1-M7, with M1 serving as the nitrate-based control). However, due to complete plant mortality in the M7 treatment, no data were available for this group. Data are presented as mean values ± standard deviation (SD). Statistical comparisons were performed using one-way analysis of variance (ANOVA) implemented in Costat software (version 6.451, Cohort Software). When ANOVA indicated significant differences ($p < 0.05$), Dunnett's post-hoc test was applied to compare all treatment means against the control group (M1). The assumption of homogeneity of variance was verified using Levene's test prior to ANOVA. All statistical tests were conducted at a 95% confidence level ($\alpha = 0.05$).

RESULTS

Impact of ammonium-potassium interactions on alfalfa growth performance.

Fig. 1 presents the biomass accumulation patterns. Dry matter production in both root and shoot tissues exhibited significant variation ($p < 0.05$) across the six viable treatments. Plants grown under nitrate nutrition (M1) demonstrated maximal biomass accumulation. Among ammonium-grown plants, those receiving 3 mM NH_4^+ combined with 3 mM K^+ (M4) showed the most favorable growth response, achieving approximately 50% of control biomass levels. This contrasted sharply with other ammonium treatments (M2, M3, M5, M6), where biomass reductions were more severe. The combination of 6 mM NH_4^+ with 3 mM K^+ (M7) proved lethal, resulting in complete plant mortality; therefore, no biomass data were available for this treatment.

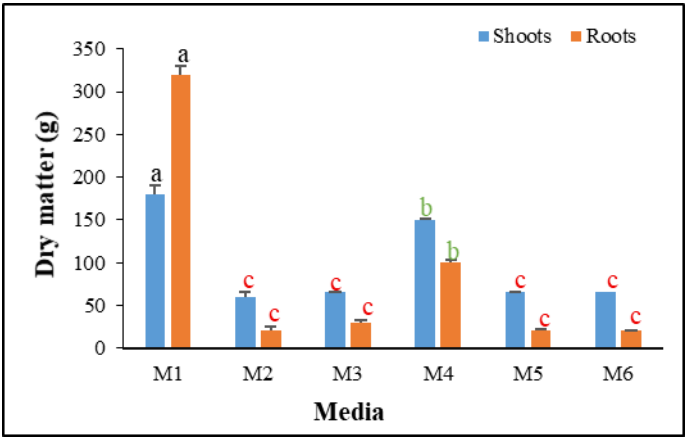

Fig. 1. Dry matter biomass partitioning in alfalfa under different nitrogen-potassium regimes after 17 days of treatment exposure. Treatments: M1 (3 mM NO_3^- + 0.2 mM K^+, control); M2 (3 mM NH_4^+ + 0.2 mM K^+); M3 (3 mM NH_4^+ + 1.2 mM K^+); M4 (3 mM NH_4^+ + 3 mM K^+); M5 (6 mM NH_4^+ + 0.2 mM K^+); M6 (6 mM NH_4^+ + 1.2 mM K^+).

Vegetative growth parameters followed similar response patterns (Fig. 2). Plants in the nitrate control (M1) developed the most extensive root systems and tallest shoots. Among the ammonium treatments, M4 plants (3 mM NH_4^+ / 3 mM K^+) showed a significant mitigation of growth inhibition, achieving intermediate values for both shoot height and root length. While all ammonium treatments significantly ($p < 0.05$) reduced these parameters relative to the M1 control, the M4 treatment consistently resulted in less severe reductions, representing a significant improvement over the other ammonium treatments (M2, M3, M5, M6).

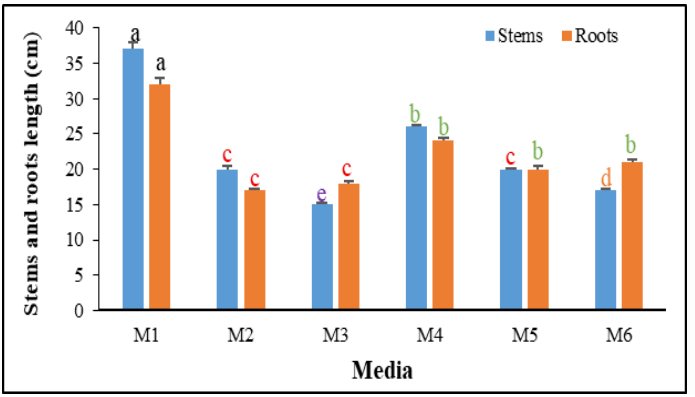

Fig. 2. Comparative stem height and primary root length measurements of alfalfa after 17-day exposure. Treatment codes: M1 (3 mM NO_3^- + 0.2 mM K^+); M2 (3 mM NH_4^+ + 0.2 mM K^+); M3 (3 mM NH_4^+ + 1.2 mM K^+); M4 (3 mM NH_4^+ + 3 mM K^+); M5 (6 mM NH_4^+ + 0.2 mM K^+); M6 (6 mM NH_4^+ + 1.2 mM K^+).

Tissue-specific mineral accumulation patterns.

The analysis of tissue mineral content revealed distinct allocation patterns for potassium and ammonium (Fig. 3). A strong positive correlation was observed between potassium concentration in the growth medium and K^+ content in both shoots and roots, with foliar tissues consistently maintaining higher concentrations than root systems (Fig. 3A). Conversely, ammonium accumulation exhibited an inverse relationship with potassium availability (Fig. 3B). Plants in low-K^+ treatments (M2, M5) showed the highest NH_4^+ accumulation, particularly in shoots. The M4 treatment (3 mM NH_4^+ / 3 mM K^+) demonstrated a significant mitigating effect, reducing shoot NH_4^+ concentration and resulting in a greater proportion of the absorbed ammonium being sequestered in the roots.

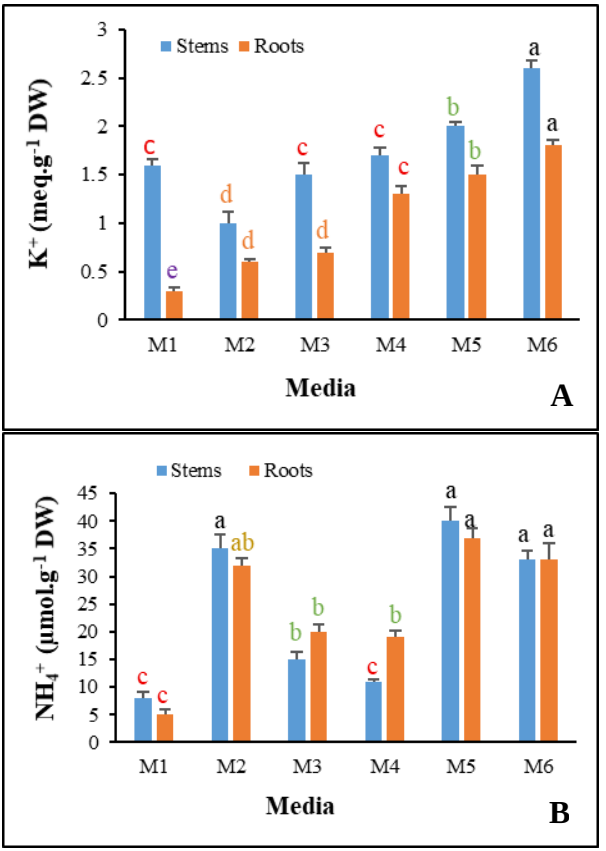

Fig. 3. K^+ (A) and $NH4^+$ (B) contents of stem and root of alfalfa after 17 days of cultivation on M1 (3mM NO^{3-} ; 0, 2 mM K^+) ; M2 (3 mM $NH4^+$; 0, 2 mM K^+) ; M3 (3 mM NH_4^+ ; 1, 2 mM K^+) ; M4 (3 mM NH4+ ; 3 mM K+) ; M5 (6 mM NH_4^+ ; 0,2 mM K^+) ; M6 (6 mM NH_4^+ ; 1,2 mM K^+).

Effects of ammonium/potassium concentration on chlorophyll and protein contents of alfalfa.

The results of the protein contents of the aerial parts and roots of alfalfa, depending on the treatments applied, are shown in Fig. 4A These concentrations reach a maximum on a strictly nitrate medium. In the presence of ammonium 3 mM and in the presence of the lowest potassium concentration, the content decreases significantly compared to the M1 medium. On the other hand, in the presence of 3 mM NH_4^+ and 3 mM K^+, the content reaches a value which is approximately equal to that obtained in the organs of plants in the M1 medium.

In the presence of 6 mM ammonium and regardless the concentration of potassium, the protein content decreases significantly. They are still highest in the aerial parts.

Fig. 4B shows the chlorophyll levels after 17 days of treatment on nitric medium or media containing different concentrations of ammonium and

potassium. Chlorophyll content was significantly altered by the growth media. Compared to the M1 control (1.00 mg g^{-1} FW), content was 25% higher in M4 but 51% lower in M6. The M2, M3, and M5 treatments showed intermediate reductions, ranging from 20% to 35% lower than the control.

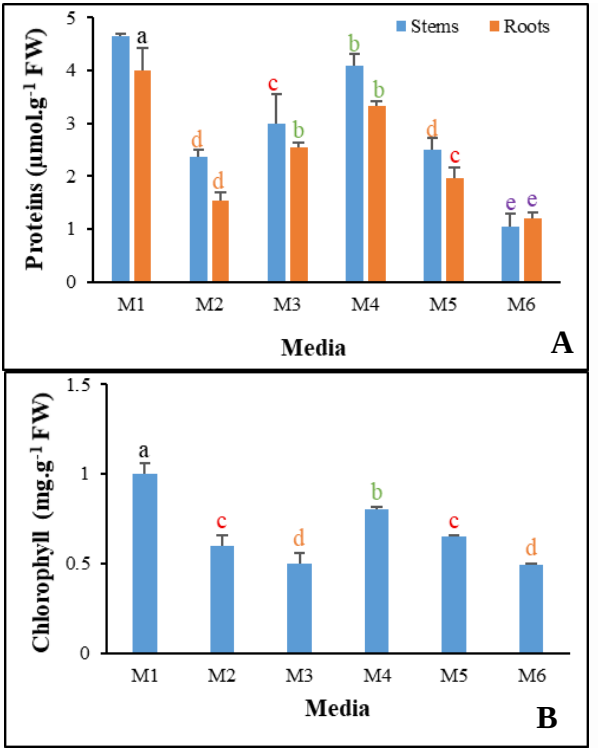

Fig. 4. Biochemical responses of alfalfa to nitrogen-potassium treatments: (A) Soluble protein content and (B) total chlorophyll concentration in aerial tissue after 17 days of cultivation on M1 (3mM NO^{3-} ; 0, 2 mM K^+) ; M2 (3 mM $NH4^+$; 0, 2 mM K^+) ; M3 (3 mM NH_4^+ ; 1, 2 mM K^+) ; M4 (3 mM NH4+ ; 3 mM K+) ; M5 (6 mM NH_4^+ ; 0,2 mM K^+) ; M6 (6 mM NH_4^+ ; 1,2 mM K^+).

Effects of ammonium/potassium concentration on antioxidant and polyphenol level.

The effects of ammonium and potassium concentrations on their accumulation were investigated alongside changes in antioxidant activity and polyphenol levels in alfalfa. As shown in Fig. 5A, total antioxidant activity reached its peak in the M4 treatment, after which it declined in M5 and M6. The lowest activity was recorded in M3 for stems and M6 for roots.

Polyphenol accumulation showed a biphasic response, with peaks at the M1 and M4 treatments (Fig. 5B). The

M4 medium yielded the highest polyphenol concentration in aerial parts, while M1 resulted in the highest concentration in roots. In contrast, plants in M2, M3, M5, and M6 exhibited substantially lower polyphenol levels, with the M6 treatment showing the least accumulation in both tissues.

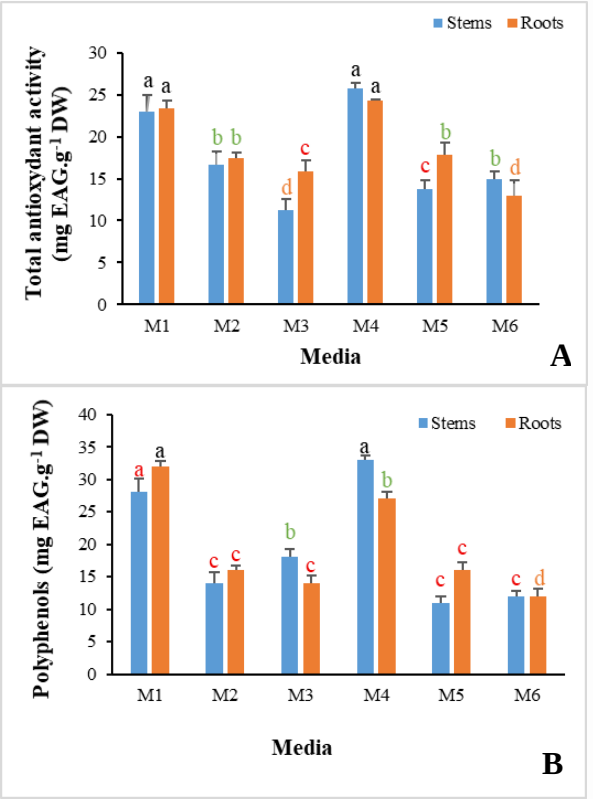

Fig. 5. Oxidative stress markers in *Medicago sativa* under varying nitrogen regimes: (A) Total antioxidant capacity (A) and (B) polyphenolic compound accumulation in shoot and root systems. of *M. sativa* after 17 days of cultivation on M1 (3mM NO^{3-} ; 0, 2 mM K^+); M2 (3 mM $NH4^+$; 0, 2 mM K^+); M3 (3 mM NH_4^+; 1, 2 mM K^+); M4 (3 mM $NH4^+$; 3 mM K+); M5 (6 mM NH_4^+; 0,2 mM K^+); M6 (6 mM NH_4^+; 1,2 mM K^+).

DISCUSSION

Nitrogen stands as an essential element for plant growth and development. While nitrate represents the preferred nitrogen source for many species, ammonium can induce toxicity symptoms when supplied as the sole nitrogen form (M'rah et al. 2010, Esteban et al. 2016).

In the present study, we specifically examined the role of potassium in alleviating ammonium toxicity in alfalfa. The results revealed complete mortality in plants grown in the

M7 medium (6 mM NH_4^+/3 mM K^+). Among the ammonium treatments, the M4 medium (3 mM NH_4^+/3 mM K^+) supported the most favorable growth outcomes. (Miller and Cramer 2004).

The beneficial effects observed in the M4 treatment can be attributed to several key mechanisms supported by potassium availability. First, these plants accumulated significantly less ammonium in their aerial tissues, aligning with findings that adequate potassium competes with NH_4^+ for uptake via transporters such as AKT1 and HAK5 (Coskun et al. 2017), thereby reducing tissue ammonium concentrations. Second, the maintained potassium levels in M4 plants support critical physiological processes including stomatal regulation and enzyme activation. Furthermore, sufficient K^+ is crucial for maintaining cytosolic pH homeostasis, which is often disrupted under NH_4^+ stress, and for facilitating the assimilation of NH_4^+ into amino acids via the GS-GOGAT cycle (Szczerba et al. 2008, Masclaux-Daubresse et al. 2020).

Notably, M4 plants preserved higher chlorophyll and protein content compared to other ammonium treatments, indicating maintained photosynthetic capacity and more efficient nitrogen assimilation. This contrasts with the photosynthetic inhibition typically associated with ammonium nutrition.

Our investigation of secondary metabolites revealed that M4 plants maintained relatively higher polyphenol and antioxidant levels. The enhanced antioxidant activity suggests that K^+ sufficiency empowers the plant's defense systems, potentially by reducing ROS generation through improved photosynthetic efficiency and by interacting with Ca^{2+} signaling pathways (Anschütz et al.,2014). This finding may have important implication for plant stress adaptation and nutritional quality.

While this study was conducted hydroponically, the implications extend to soil conditions. In the field, maintaining an adequate K^+ status is essential when using ammonium-based fertilizers to prevent toxicity, as soil cation exchange and microbial nitrification can modulate NH_4^+ availability. A balanced NH_4^+/K^+ nutrition strategy can enhance nitrogen use efficiency and sustainability in alfalfa crop (Sitienei et al. 2013).

In conclusion, our results demonstrate that a balanced 1:1 molar ratio of NH_4^+ to K^+ (3 mM each) provides optimal conditions for alfalfa to withstand ammonium toxicity, specifically under a moderate 3 mM NH_4^+ regime. Potassium exerts its protective effects through multiple interconnected mechanisms, as evidenced by our data: it competitively limits ammonium uptake and its accumulation in shoots, maintains ionic homeostasis and essential potassium-dependent physiological functions, preserves the integrity of the photosynthetic apparatus as seen in sustained chlorophyll and protein levels, and supports the production of defensive secondary metabolites, notably enhancing antioxidant capacity. These findings underscore the critical importance of maintaining adequate potassium levels in ammonium-based fertilization systems. This strategy not only improves our understanding of nutrient interactions in plant physiology but also offers a practical framework for enhancing nitrogen use efficiency and crop performance in sustainable agricultural practices that utilize ammonium fertilizers.

Acknowledgements

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Rhimi F., Essid I., Chaffei-Haouari Ch. et M'rah S. 2025. Reduction de la toxicité de l'ammonium par le potassium chez la luzerne (*Medicago sativa*) cultivée en hydroponie: Réponses de la croissance et physiologiques. Tunisian Journal of Plant Protection 20 (2): 43-55.

La luzerne (Medicago sativa) se développe généralement grâce à la nutrition azotée, mais l'impact environnemental de la surutilisation des nitrates impose la recherche de sources d'azote alternatives. Bien que l'ammonium (NH_4^+) soit une option prometteuse, il induit souvent une toxicité chez les plantes. Cette étude examine le potentiel d'une supplémentation en potassium (K^+) pour atténuer la toxicité du NH_4^+ chez la luzerne cultivée en hydroponie sur une période de 17 jours. Les plantes ont été soumises à différents régimes azotés: un témoin nitrate (3 mM NO_3^-) et deux concentrations de NH_4^+ (3 et 6 mM) combinées à trois niveaux de K^+ (0,2, 1,2 et 3 mM). Une combinaison de 6 mM de NH_4^+ et de 3 mM de K^+ s'est avérée létale, entraînant la mortalité totale des plantes. En revanche, le traitement spécifique à 3 mM de NH_4^+ et 3 mM de K^+ a considérablement atténué la toxicité, restaurant la biomasse végétale à environ 50% du témoin (nitrate). Cette atténuation était liée à une réduction significative de l'accumulation toxique de NH_4^+ dans les parties aériennes et à une augmentation de 2,3 fois de l'absorption de K^+ par rapport aux traitements à faible concentration de K^+. Sur le plan physiologique, ce traitement optimal a maintenu les teneurs en chlorophylle, en protéines solubles et en polyphénols à des niveaux comparables à ceux des plantes témoins, tout en augmentant l'activité antioxydante de 35%. Ces résultats démontrent qu'une nutrition équilibrée NH_4^+/K^+, en particulier à un ratio de 1:1 (3 mM chacun), peut atténuer efficacement la toxicité de l'ammonium en régulant son absorption et sa translocation, en préservant la fonction photosynthétique et en renforçant la capacité antioxydante. Cette stratégie offre une approche durable pour l'incorporation d'engrais à base d'ammonium dans la culture de la luzerne.

Mots-clés: Composés phénoliques, culture hydroponique, interaction avec le potassium, luzerne, *Medicago sativa*, nutrition azotée, stress oxydatif, toxicité de l'ammonium

ملخص

رحيمي، فاتن وإشراق الصيد وشيراز شافعي-هواريو صباح مراح .2025. تخفيض سُمِّية الأمونيوم بواسطة البوتاسيوم في نبات الفصة/البرسيم (*Medicago sativa*) في الزراعة المائية: استجابات النمو والفيسيولوجيا.
Tunisian Journal of Plant Protection 20 (2): 43-55.

تزدهر الفصة/البرسيم (Medicago sativa) عادةً بالتغذية بالنترات، إلا أن الأثر البيئي بالإفراط في استخدامها يستدعي البحث عن مصادر بديلة للنيتروجين. ورغم أن الأمونيوم (NH_4^+) يُعدّ خيارًا واعدًا، إلا أنه غالبًا ما يُسبب تسممًا للنباتات. تبحث هذه الدراسة في إمكانية استخدام البوتاسيوم (K^+) كمكمّل غذائي للتخفيف من سمية الأمونيوم في الفصة المزروعة مائيًا على مدى 17 يومًا. خضعت النباتات لأنظمة نيتروجينية مختلفة: نظام شاهد بالنترات (3 ميليمول NO_3^-)، وتركيزان من الأمونيوم (3 و6 ميليمول/لتر) مع ثلاثة مستويات من البوتاسيوم (0.2، 1.2، و3 ميليمول). وقد ثبت أن مزيج 6 ميليمول من الأمونيوم و3 ميليمول من البوتاسيوم قاتل، مما أدى إلى موت النبات بالكامل. على النقيض من ذلك، أدى النظام الغذائي المحدد بتركيز 3 ميليمول من الأمونيوم مع 3 ميليمول من البوتاسيوم إلى تخفيف السمية بشكل ملحوظ، حيث استعادت الكتلة الحيوية للنباتات إلى حوالي 50% من كتلة مجموعة الشاهد المعالجة بالنترات. وارتبط هذا التخفيف بانخفاض كبير في تراكم الأمونيوم السام في الأجزاء الهوائية، وزيادة امتصاص البوتاسيوم بمقدار 2.3 ضعف مقارنةً بالمعالجات منخفضة البوتاسيوم. وعلى المستوى الفيزيولوجي، حافظت هذه المعالجة المثلى على محتوى الكلوروفيل والبروتين الذائب ومستويات البوليفينول مماثلة لنباتات الشاهد، مع تعزيز النشاط المضاد للأكسدة بنسبة 35%. تُظهر هذه النتائج أن التغذية المتوازنة بالأمونيوم والبوتاسيوم، وتحديدًا بنسبة 1:1 (3 ميليمول لكل منهما)، يمكن أن تخفف بشكل فعال من سمية الأمونيوم عن طريق تنظيم امتصاصه وانتقاله، والحفاظ على وظيفة التمثيل الضوئي، وتعزيز القدرة المضادة للأكسدة. توفر هذه الاستراتيجية نهجًا مستدامًا لدمج الأسمدة القائمة على الأمونيوم في محصول البرسيم.

LITERATURE CITED

Anschütz, U., Becker, D., and Shabala, S. 2014. Going beyond nutrition: Regulation of potassium homoeostasis as a common denominator of plant adaptive responses to environment. Journal of Plant Physiology 171: 670-687. https://doi.org/10.1016/j.jplph.2014.01.009

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.

Coskun, D., Britto, D.T., Shi, W., and Kronzucker, H.J. 2017. Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. Nature Plants 3: 1-10. https://doi.org/10.1038/nplants.2017.74

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 Agricultural and Food Chemistry 50: 3010-3014. https://doi.org/10.1021/jf0115589

Esteban, R., Ariz, I., Cruz, C., and Moran, J.F. 2016. Mechanisms of ammonium toxicity and the quest for tolerance. Plant Science 248: 92-101. https://doi.org/10.1016/j.plantsci.2016.04.008

Hadidi, M., Orellana Palacios, J.C., McClements, D.J., Mahfouzi, M., and Moreno, A. 2023. Alfalfa as a sustainable source of plant-based food proteins. Trends in Food Science and Technology 135: 202-214.

Hoagland, D.R., and Arnon, D.I. 1940. The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular 347: 1-39.

Jones, J.B. 2001. Laboratory Guide for Conducting Soil Tests and Plant Analysis. CRC Press, Boca Raton, Florida, USA, 363 pp.

Khairy, A., Hammoda, H.M., Metwally, A.M., Zaatout, H.H., and Ibrahim, R.S. 2025. Therapeutic potential of Medicago sativa (alfalfa): A comprehensive review of its bioactive compounds and pharmacological properties. Journal of Advanced Pharmaceutical Sciences 2 (2): Article 1043.

Liu, G., Du, Q., and Li, J. 2017. Interactive effects of nitrate-ammonium ratios and temperatures on growth, photosynthesis, and nitrogen metabolism of tomato seedlings. Scientia Horticulturae 214: 41-50.

Liu, Y., and von Wirén, N. 2017. Ammonium as a signal for physiological and morphological responses in plants. Journal of Experimental Botany 68: 2581-2592. https://doi.org/10.1093/jxb/erx086

M'rah Helali, S., Nebli, H., Kaddour, R., Mahmoudi, H., Lachaâl, M., and Ouerghi, Z. 2010. Influence of nitrate–ammonium ratio on growth and nutrition of Arabidopsis thaliana. Plant and Soil 336: 65-74. https://doi.org/10.1007/s11104-010-0445-8

Ma, X., Zhu, C., Yang, N., Gan, L., and Xia, K. 2016. γ-Aminobutyric acid addition alleviates ammonium toxicity by limiting ammonium accumulation in rice (Oryza sativa) seedlings. Physiologia Plantarum 158: 389-401. https://doi.org/10.1111/ppl.12473

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., and Suzuki, A. 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Annals of Botany 105: 1141-1157. https://doi.org/10.1093/aob/mcq028

Mauriès, M. 2003. Luzerne: culture, récolte, conservation, utilisation. 2e édition. Editions France Agricole, Paris, France, 310 pp.

Miller, A.J., and Cramer, M.D. 2004. Root nitrogen acquisition and assimilation. Plant and Soil 274: 1-36. https://doi.org/10.1007/s11104-004-0965-1

Prieto, P., Pineda, M., and Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry 269: 337-341. https://doi.org/10.1006/abio.1999.4019.

Sitienei, K., Home, P.G., Kamau, D.M., and Wanyoko, J.K. 2013. The influence of fertilizer type and application rates in tea cultivation on nitrogen and potassium efficiencies. African Journal of Agricultural Research 8: 3770-3777. https://doi.org/10.5897/AJAR2013.7337

Smith, P. 2021. How to measure, report and verify soil carbon change. Pages GC41D-01. In: AGU Fall Meeting Abstracts, Vol. 2021.

Szczerba, M.W., Britto, D.T., Ali, S.A., Balkos, K.D., and Kronzucker, H.J. 2008. NH_4^+-stimulated and -inhibited components of K^+ transport in rice (Oryza sativa L.). Journal of Experimental Botany 59: 3415-3423. https://doi.org/10.1093/jxb/ern190

Weatherburn, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Analytical Chemistry 39: 971-974.

Zaidi, N., Cheima, J., Nourhene, M., Khawla, R., Bilel, K., Naceur, D., Ndiko, L., and Badri Mounawer, B. 2021. Variation of Medicago sativa varieties tolerance to Phoma medicaginis infection. Journal of Oasis Agriculture and Sustainable Development 3: 56-63. https://doi.org/10.56027/JOASD.spiss092021

Influence of Biopesticides on the Nutrient Content of Stored Cowpea (*Vigna unguiculata*) in Yola, Adamawa State, Nigeria

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ABSTRACT

Tame, V.T., Gungula, D.T., and Zira, D.K. 2025. Influence of biopesticides on the nutrient content of stored cowpea (*Vigna unguiculata*) in Yola, Adamawa State, Nigeria. Tunisian Journal of Plant Protection 20 (2): 57-67.

The influence of biopesticides on the nutrient content of stored cowpea grains was investigated to determine the effect of these plant extracts on the proximate composition. The treatments consisted of cowpea grains treated with pure and mixtures of castor oil, cashew oil, lemon balm, and scent leaf powders applied at different rates. The experiment was laid out in a completely randomized design (CRD) with 18 treatments replicated three times and stored for 120 days. The result showed a highly significant effect of the botanicals on the proximate content of the grains. Cowpeas treated with 10 g scent leaf powder (SLP) had the highest moisture content (12.11%), 15 g SLP-treated grains also recorded the highest ash and crude protein content (7.22 and 39.39%, respectively) and cowpea treated with 20 g SLP showed the highest crude fat (6.80%). In terms of crude fibers, samples treated 15 g lemon balm powder (LBP) reported the highest content (5.43%) and grains treated with 20 g LBP sample produced the highest dry matter content (90.22%). Both pure and mixtures of castor and cashew oil treated samples recorded the highest carbohydrates (66.98-66.79%) whereas the highest calorific value occurred with samples subjected to 2 ml of castor oil (372.31kcal/g).

Keywords: Biopesticides, *Callosobruchus maculatus*, cashew oil, castor oil, cowpea, lemon balm powder, scent leaf powder, *Vigna unguiculata*

Cowpea (*Vigna unguiculata* (L.) Walp) is the most produced legume in West Africa and other tropical countries. The crop is cultivated intentionally for food, fodder, and green manure; its production has expanded worldwide over the past few decades (Gusmao et al. 2013, Mkenda and Ndakidemi 2014, Sanon et al. 2018). In 2017, over 87% of the crop was produced in Africa. Cowpea is of vital importance to the livelihoods of millions of people in the semi-arid regions of West and Central Africa, and is a major source of plant proteins. It is the most valuable grain legume crop in sub-Saharan Africa and Brazil (Gusmao et al. 2013, Langyintou et al. 2003, Swamy et al. 2020). Due to its high protein and low-fat content, it is grown mainly for human consumption (Gerrano et al. 2017), it also aids in the prevention of diverse metabolic

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and cardiovascular diseases. The whole above-ground cowpea plant is a multipurpose crop being consumed for its leaves, green pods, green beans, and matured beans, or processed into paste or flour and used as food ingredients (Xu and Chang 2012). The cowpea is also utilized as fodder, fertilizer, and as a quick-growing cover crop and plays a particularly critical role in erosion control and feeding animals during the dry season.

One of the major challenges facing the production of cowpea is the pest attack by cowpea weevil, which infests cowpea pods from the field to the store, causing highly significant postharvest losses (Sanon et al. 2018). According to Mkenda and Ndakidemi (2014), 30-80% of cowpea, equivalent to US$300 million is lost to bruchid (*Callosobruchus maculatus*) attacks annually in Africa. Cowpea weevils include weight, nutritional, economic, sensory, and seed viability losses (Gusmao et al. 2013, Mkenda and Ndakidemi 2014, Sanon et al. 2018).

Botanical pesticides have recently attracted more attention due to the drawbacks related to chemical pesticides and widespread concern for human health, food safety, and environmental aspects such as environmental pollution, killing of beneficial and non-target fauna and flora, pest resistance, pest resurgence, and human toxicity due to chemical residue accumulation in the treated grains (Anukiruthika et al. 2021, El-Sharkawy et al. 2023, Gusmao et al. 2013, Mkenda and Ndakidemi 2014, Sanon et al. 2018, Swamy et al. 2020).

These botanical pesticides contain active compounds like monoterpenes, diterpenes, sesquiterpenes, myrcene, carvone, piperitenone, linalool, alkaloids, saponins, tannins low molecular weight aromatic compounds, volatile compounds, secondary metabolites, flavioboids, phenols, rotenoids, etc (Gusmao et al. 2013, Mkenda and Ndakidemi 2014, Sanon et al. 2018) whose effect on nutrient content of the processed products is not yet ascertained. There is an urgent need to understand the effect of these compounds on the nutrient content of treated grains for a wider application in the postharvest management of cowpea weevil. There is also a dearth of information on the effect of castor, cashew oil, lemon balm, and scent leaf powders on the effect of these products on proximate composition of cowpea. It is against this backdrop that this research is designed to investigate the influence of these botanical pesticides on the proximate composition of the cowpea grains after storage.

MATERIALS AND METHODS

Experimental site.

The experiments were conducted in the laboratory (Yola, Nigeria), in 2020 to test the effect of plant products on the sensory quality of the cowpea grains after storage. The study was conducted from March to June 2020. The daily storage temperature and relative humidity were 34-35ºC and 30 34%, respectively.

Materials used.

The materials used for this study are: cowpea (*V. unguiculata*) grains, castor (*Ricinus communis*) oil, cashew (*Anacardium occidentale*) oil, scent leaf (*Ocimum gratissimum*) powder, and lemon balm (*Melissa officinalis*) powder, 10-micrometer mesh muslin cloth, sieve, digital weighing balance, hand lens, conical flask, glass jar, Petri dishes, digestive flask, electric oven, heat mantle, and crucible plate. The cowpea sample (black-eyed peas) used for the study was purchased directly from a farmer in Mubi Local Government Area of Adamawa State. Cashew oil was extracted using a local method of extraction. Castor oil was

purchased from an authorized Pharmacy dealer in Jimeta, while Cashew oil was extracted manually using the traditional method of oil extraction. Scent leaves were obtained directly from the vegetable garden in Girei Local Government Area of Adamawa State, while lemon balm was obtained from fields within Modibbo Adama University, Yola.

Sample preparation.

Cowpea grains were disinfested using the electric oven at 60 °C for 3 hours and packed in a 12 kg air-tight bag to prevent cross-contamination by other insect pests and stored in the laboratory for subsequent use. Then the cowpea grains were thoroughly cleaned, sieved, and placed in round-bottom flasks for treatment.

Both scent leaf and lemon balm powders were prepared by shade drying, grinding, and sieving until a fine powder was obtained.

Application of test materials.

The botanicals (oils and leaf powders) were poured into a conical flask containing the cowpea grains and mixed vigorously to obtain uniform coverage and then transferred into the jars according to the method of Ilesanmi and Gungula (2010), and Shikaan and Uvah (2002).

rearing procedure for experimental insects (*C. maculatus*).

The bruchid insects (*Callosobruchus maculatus* (Fabricius)) used were obtained from infested cowpea grains from the produce market in Jimeta, Yola, placed inside a plastic container containing 1 kg of uninfested cowpea grains and covered with 10 µm mesh muslin cloth to allow free air circulation and also to prevent the insects from escaping. The plastic containers were kept in the laboratory for five weeks at ambient conditions. After rearing, both adult and dead bruchids were removed from the plastic containers to allow for successful hatching of the eggs. Newly hatched bruchids (1 to 3 days old) were then collected and used to infest the experimental cowpea grains.

Treatments and experimental design.

The cowpea grains were treated with (i) Castor oil, (ii) Cashew oil, (iii) Scent leaf powder, and (iv) Lemon balm powder at different concentrations. Two hundred grams of cowpea were weighed into nineteen (19) glass jars. Treatments T1-T6 consisted of cowpea grains treated with three different concentrations of castor and cashew oils (1, 2, and 3 ml/200 g) each. The mixture of castor and cashew oils in the ratio of 1:1, 1:2, and 2:1 were used to treat cowpea grains labelled as treatments T7-T9. Cowpea grains treated with three different concentrations of scent leaf and lemon balm (10, 15, and 20 g/200 g) each were placed under treatments T10-T15. Similarly, scent leaf and lemon balm powder ratios of 1:1, 1:2, and 2:1 were used to treat cowpea grains under treatment T16-T18. The control was left untreated.

Each glass jar housed 20 unsexed 1-3 days old weevils together with cowpea grains and covered with 10 µm mesh muslin cloth. Each treatment was replicated three times and laid out in a Completely Randomised Design (CRD). All the treated cowpea grains were kept at the ambient condition in the laboratory. At the end of the storage periods (120 days), data were collected on the proximate composition of the cowpea grains.

Laboratory analysis.

Determination of proximate composition. Proximate composition was determined according to the method of the AOAC (2020) protocol reported by

Ahmed et al. (2023) to determine crude fat, moisture, ash crude fibers, proteins, and carbohydrates of both treated and untreated cowpeas.

Determination of moisture content. Five grams from each sample were weighed into a pre-weighed aluminum drying dish. The samples were dried to a constant weight in an oven at 105ºC for four (4) hours. The moisture content was then determined using equation 1:

$$Moisture\ content = \frac{M1 - M2}{M1 - M0}\ x\ 100,$$

where M_o = Weight of aluminum dish, M_1 = Weight of free sample + dish, and M_2 = Weight of dry sample dish.

Determination of dry matter. Dry matter was determined by using equation 2:

$$\text{Dry matter} = 100 - \text{moisture content.}$$

Determination of ash content. Five grams of each sample were weighed into a porcelain crucible. Organic matter was charred by igniting the material on a hot plate in the fume cupboard. The crucible was then placed in the muffle furnace (ECF3 Chesterfield, UK) and maintained at 550ºC for 6 h. It was then cooled in a desiccator and was weighed out on a digital weighing balance. Rate ash was determined using equation 3:

$$\text{Ash (\%)} = \frac{(\text{weight of crucible with sample}) - (\text{weight of empty crucible})}{\text{Sample weight}}\ x\ 100.$$

Determination of crude protein content. For the determination of crude proteins, a Kjedahl nitrogen method was used. One gram of the sample from each treatment was introduced into an 800 mL digestion flask. Kjedahl catalyst (5 selenium tablets) was added to the sample. Twenty milliliters of concentrated sulfuric acid was added to each sample and fixed to the digestion flask until a clear solution was obtained.

The cooling digest was transferred into a 100 ml volumetric flask and was made up to the mark with distilled water. The distillation apparatus was set up and rinsed for 10 min. After boiling, 20 ml of 4% boric acid was pipetted into conical flasks, drops of methyl red were added to each flask as an indicator, and the digest was diluted with 75 ml of distilled water. Ten milliliters of the digest were made alkaline with 20 ml of 20% NaOH and distilled. The steam exit of the distillatory was closed and the color of the boric acid solution changes to green. The mixture was distilled for 15 min and boric acid along with distillate was then titrated against 0.1 N hydrochloric acid. The percentage total nitrogen was calculated as indicated in equation 4:

$$\text{Total Nitrogen (\%)} = [(\text{Titer x Normality x } 0.014) / \text{ Weight of sample}] \text{ x } 100,$$

where Normality = 0.1N, Crude proteins (%) = Total nitrogen x 6.25 (%) [6.25 is a constant].

Determination of fat content. Five grams of the sample were weighed in a thimble and plugged with cotton wool. The thimble was then inserted into a Soxhlet system. A previously weighed clean, dried 250 ml flask was filled with 200 ml of petroleum ether (boiling point 40-60°C). The Soxhlet apparatus was assembled and allowed to reflux for about 6 h. At the end of 6 h, the solvent was recovered and the flask with the extract was dried in the oven (DHG-9023A, England) at 105°C for 30 min. It was then cooled in the desiccator and weighed and determined using equation 5:

$$fat\ (\%) = \frac{W3 - W2}{W1}\ x\ 100,$$

where, W_1 = weight of sample, W_2 = weight of empty flask, W_3 = weight of flask extracted oil.

Determination of crude fiber content. The defatted sample (2 g) was placed in a 500 ml conical flask, and 150 ml boiling 1.25 % sulfuric acid solution was added. The sample was digested for 30 min, and then the acid was drained out and the sample was washed with boiling distilled water. After this, 1.25 % sodium hydroxide solution (150 ml) was added. The sample was then digested for 30 min; thereafter, the sodium hydroxide solution was drained out, and the sample was then washed with boiling distilled water. Finally, the sample was placed in a dried crucible and oven-dried at 110°C overnight. The sample was allowed to cool in a desiccator and then weighed (W_1). The sample was ashed at 550°C in a muffle furnace for 2 ho, cooled in a desiccator, and reweighed (W_2). The extracted fiber content was expressed as a percentage of the original understated sample and calculated by equation 6:

$$Crude\ fibre(\%) = \frac{\mathrm{Wi} - \mathrm{W2}}{\mathrm{Wo}} x100,$$

where W_1 = Digested sample, W_2 = Ashed sample, W_0 = Weight of sample.

Determination of carbohydrate content. Carbohydrates were calculated by subtracting the sum of the percentage of moisture from 100 as described by Egan et al. (1981) in equation 7:

$$\text{Carbohydrates} = 100 - [\text{moisture (\%)} + \text{proteins (\%)} + \text{ash (\%)} + \text{fat (\%)} + \text{crude fibers (\%)}].$$

Energy content. The energy content was estimated following the method employed by Emmanuel and Folasade (2011) by applying equation 8:

$$\text{Energy (Kcal/100 g)} = (\text{Crude lipid } x\ 8) + (\text{Crude proteins } x\ 2) + (\text{Carbohydrates } x\ 4).$$

Data analysis.

All the data collected were subjected to Analysis of Variance (ANOVA) appropriate for Completely Randomized Design (CRD), using Genstat statistical software Discovery Edition, and the means were separated using the Duncan Multiple Range Test (DMRT) at a 5% level of probability.

RESULTS

Moisture content.

There were highly significant differences ($p \leq 0.01$) among the various treatments (Table 1). The highest moisture content was observed in samples treated with 10 g Scent Leaf Powder (SLP), with a mean value of 12.11% and the lowest moisture content was observed in samples treated with 20 g Lemon Balm Powder (LBP), with a mean value of 9.78%.

Dry matter content. Samples treated with 20 g LBP had the highest dry matter content (90.22%) due to the highly significant differences ($p \leq 0.01$) caused by the treatments as depicted in Table 1, This is followed by 2:1 SLP / LBP, 1:1 castor (CT)/cashew (CS) oils and 1:1 SLP/LBP with their respective mean values of 89.78%, 89.56% and 89.55%. The least was observed in 10 g SLP samples with a mean value of 87.56%.

Ash content. The result of the ash content showed highly significant differences ($p \leq 0.01$) between the treated and untreated samples (Table 1). The highest ash content was recorded on samples treated with 10 and 15 g SLP, which had mean values of 7.55% and 7.22% respectively, followed by 1:1 SLP/LBP (7.00%), and the least mean values were observed in castor and cashew oils as presented in Table 1. There were no significant differences between 2 ml castor oil, 3 ml cashew oil, and 2:1 CT/CS oils as they had the same mean values of 2.33% each.

Crude proteins. The result showed a highly significant effect ($p \leq$

0.01) of the biopesticides on cowpea grain's crude protein content (Table 1). Cowpea grains treated with 15 g LBP were observed to have the highest mean crude proteins (39.39%). Followed by control with a mean value of 34.14% and then 10 g scent leaf powder samples with a mean value of 33.55%, the least was observed in the sample treated with 3 ml cashew oil, with a mean value of 15.47% and 1 ml castor oil with the value of 15.04%.

Fat content. The results in Table 1 showed that there were highly significant differences ($p \leq 0.01$) in fat content among the various treatments. Cowpea treated with 20 g scent leaf powder sample recorded the highest fat content with a mean value of 6.66% while 1 ml and 1:2 castor/cashew oils recorded the lowest fat content with a mean value of 2.86% and 2.86%, respectively.

Crude fibers. The results of the effect of biopesticides on crude fibers were highly significant ($p \leq 0.01$) as presented in Table 1. Cowpea treated with 15 g LBP was presented to have the highest crude fibers as compared with the other treated and untreated samples, with a mean value of 5.43% while 2 ml CT and 3 ml CS oils recorded the lowest fiber content with the mean value of 2.13% and 1.917%, respectively.

Percentage carbohydrate content. The percentage carbohydrate content of cowpea samples was influenced in a highly significant ($p \leq 0.01$) way, as presented in Table 1. The results showed that there were differences among the various treatments. It was observed that 2 ml CT oil-treated samples had the highest percentage carbohydrate content with a mean value of 66.79% followed by 3 ml CS oil-treated samples with a mean value of 65.77%, and the least was observed in 15 g LBP-treated samples with a mean value of 34.18%.

Calorific value. The energy value of the cowpea sample presented in Table 1 showed that there were highly significant differences ($p \leq 0.01$) among the various treatments in terms of calorific value. The results showed that 2 ml CT oil had the highest energy value with a mean value of 372.31 Kcal/g, followed by 3 ml CS oil and 2:1 CT/CS oils with their respective mean value of 370.50 and 368.03 Kcal/g. Whereas the least was observed in the 10 g LBP sample with a mean value of 344.03 and the 1:1 SLP/LBP sample with a mean value of 348.35 Kcal/g.

DISCUSSION

Treatment of cowpea grains with SLP increases moisture content due to the absorption of moisture from the storage atmosphere caused by increased insect population and metabolism. The moisture content of these analyzed samples is within the United States Storage Recommendation Standard of 8 to 12% recommended for long-term storage. The moisture content recorded in this study is in tandem with that of other researchers (Davis et al. 1991, Illesami and Gungula 2016, Singh 2014, Therese et al. 2019).

The application of 20 g LBP assisted in conserving dry matter content after storage and the values reported in this work agreed with those of Adino et al. (2018) and Agele et al. (2017). They observed that the variations in moisture content can be the sources of differences in the dry matter production and partitioning as a result of the effect of some biopesticides.

Table 1. Proximate composition and calorific value of cowpea treated with biopesticides

Treatment	Moist. Cont.	Dry Mat.	Ash Cont.	Crude Prot.	Fat Cont.	Crude Fibers	Carbohy.	Energy Kcal/g
1 ml CT	10.44^{cd}	89.56^{ab}	3.333^{c}	18.82^{fg}	3.787^{efg}	2.597^{gh}	61.02^{a}	366.89^{abc}
2 ml CT	10.33^{cd}	89.67^{ab}	2.333^{c}	15.04^{g}	3.243^{fg}	1.917^{h}	66.79^{a}	372.31^{a}
3 ml CT	10.22^{cd}	89.78^{ab}	2.667^{c}	19.76^{fg}	3.453^{fg}	2.923^{efg}	60.98^{a}	359.88^{ad}
1 ml CS	11.33^{abc}	88.67^{bcd}	5.110^{b}	24.15^{de}	4.343^{def}	3.330^{def}	52.14^{b}	358.67^{bcd}
2 ml CS	10.45^{bcd}	89.30^{ab}	2.667^{c}	20.26^{ef}	2.863^{f}	2.790^{fgh}	60.89^{a}	364.16^{abc}
3 ml CS	10.67^{bcd}	89.33^{abc}	2.333^{c}	15.47^{g}	3.633^{og}	2.130^{h}	65.77^{a}	370.50^{ab}
10 g SLP	12.11^{a}	87.56^{d}	5.777^{ab}	25.38^{de}	5.413^{bcd}	3.450^{de}	47.46^{bcd}	354.78^{cde}
15 g SLP	11.78^{ab}	88.22^{cd}	7.223^{a}	26.83^{cd}	6.073^{ab}	3.697^{cd}	44.40^{cde}	354.64^{cde}
20 g SLP	11.22^{abc}	88.78^{bcd}	6.330^{ab}	24.38^{de}	6.797^{a}	3.347^{def}	47.92^{bc}	365.38^{bc}
10 g LBP	11.11^{abc}	88.89^{bc}	7.553^{a}	33.55^{b}	4.780^{cde}	4.633^{b}	37.71^{fg}	344.03^{e}
15 g LBP	10.33^{cd}	89.67^{ab}	5.890^{ab}	39.39^{a}	4.777^{cde}	5.430^{a}	34.18^{g}	354.66^{cde}
20 g LBP	9.78^{d}	90.22^{a}	6.663^{ab}	32.09^{b}	5.827^{abc}	4.407^{b}	41.24^{def}	326.07^{abc}
1:1CT/CS	10.44^{cd}	89.56^{ab}	2.887^{c}	19.59^{fg}	3.300^{fg}	2.657^{gh}	61.12^{a}	366.03^{abc}
1:2CT/CS	11.00^{abc}	89.00^{abc}	2.777^{c}	17.80^{fg}	2.857^{g}	2.470^{gh}	63.10^{a}	362.23^{abc}
2:1CT/CS	11.11^{abc}	88.89^{bc}	2.333^{c}	18.09^{fg}	3.683^{efg}	2.480^{gh}	62.30^{a}	368.03^{ab}
1:1SL/LB	10.45^{cd}	89.55^{ab}	7.000^{ab}	30.19^{bc}	3.893^{efg}	4.160^{bc}	44.31^{cde}	348.35^{de}
1:2SL/LB	11.33^{abc}	88.67^{bcd}	5.777^{ab}	26.84^{cd}	5.443^{bcd}	3.687^{cd}	47.20^{bcd}	359.59^{ad}
2:1SL/LB	10.22^{cd}	89.78^{ab}	6.447^{ab}	34.13^{b}	5.283^{bcd}	4.700^{b}	39.22^{efg}	358.83^{bcd}
Control	10.56^{cd}	89.44^{ab}	6.667^{ab}	34.14^{b}	6.663^{a}	4.710^{b}	37.26^{fg}	362.51^{abc}
Pr. of F	0.0044	0.0047	<.0001	<.0001	<.0001	<.0001	<.0001	0.0004
S E ±	0.623	0.673	1.014	2.513	0.679	0.358	3.508	6.549

Values are means of three replications. Mean values in the same column with different letters differ significantly ($p \leq 0.05$) according to DMRT. CT = Castor oil, CS = Cashew oil, SLP = Scent Leaf Powder, LBP = Lemon Balm Powder.

This work showed that leaf powders biopesticides increase ash content of cowpea after storage, which could be due to infestation by *C. maculatus* that eats up the endosperm, leaving the seed coat, which is rich in ash as noted by Gerrano et al. (2015). The ranges of values recorded in this study were slightly higher than the ranges of values reported by Ajeigbe et al. (2008) and Ilesami and Gungula (2016). The import of the high ash content is an indication that the cowpea could be an important source of minerals and energy, as noted by Babarinde et al. (2016).

The leaf powder used could not give a long-term desirable protection against cowpea weevils due to a short persistence level, as such infestation by insects destroyed the cowpea grains, and subsequently the eggs, larvae, life, and death weevils present in the infested grains contributed to the higher protein content. However, samples treated with oils recorded the lowest values of proteins due to the low level of infestation by the weevils, which gave effective control against *C. maculatus* infestation. This study concurred with the finding of Gerrano et al. (2015), who reported that the significantly higher protein content could be due to the presence of many eggs and larvae of *C. maculatus* on the cowpea grains due to infestation. The protein content of this study was slightly higher than the range of values reported by Uduak (2018) on brown and white beans. This study also agrees with the findings of Ajeigbe et al. (2008), who reported that

protein content is positively correlated with ash and fat and negatively correlated with carbohydrate content. This finding is similar to that of Davis et al. (1991), who reported on the proximate composition of various infested cowpea samples, indicating a higher percentage increase in the protein content in infested cowpea when compared with uninfested ones.

The high fat content observed on untreated and powder-treated cowpea could also be due to infestation by *C. maculatus*. The powders used could not provide a desirable protection against cowpea weevils, and hence, infestation set in, which destroyed cowpea grains, leaving weevil eggs, larvae, and live and dead insects that increase the fat content. The low percentage of fat content in cowpea subjected to oil treatments could be caused by its toxicity, repellency, and ovicidal effects on *C. maculatus*. The findings of this work are congruent with those of Khalid et al. (2012), who found that oils from some plants help to preserve cowpeas against weevil attacks.

The variation between oils and powders treated cowpea on the percentage crude fiber content recorded in oils treatments was very low when compared with powders treated cowpea. The low crude fiber content indicated that castor and cashew oils used were effective in bruchid control, while the high fiber content observed on cowpea treated with leaf powders and control is an indication that the powders used were not effective in protecting the treated cowpea up to 120 days. Thus, infestation by *C. maculatus* hollowed out cowpea grains and left behind the seed coat, which has high fiber content. These findings align with the results of Davis et al. (1991) and Uduak et al. (2018), who reported on the proximate composition of various infested cowpea.

The highest carbohydrate content was observed in cowpeas treated with castor and cashew oils, which could be due to the endosperm and the embryo that was not destroyed by *C. maculatus* during storage, which helps to conserve the carbohydrate content of the cowpea. However, the low value recorded in leaf powder-treated cowpea is an indication that the powder used was not strong enough to give complete suppression of *C. maculatus*, as such a bulk of carbohydrate content in the endosperm portion of cowpea grains was devoured by the insects after 120 days. Davis et al. (1991) also reported that there is a higher percentage increase in carbohydrates of infested cowpea when compared with not infested cowpea samples. Carbohydrates have been reported to influence the water absorption capacity of foods (Adejuyitan 2009). The result of this study is congruent with that of Ajeigbe et al. (2008), Ilesemi and Gungula (2016), and Therese et al. (2019), who noted that infested cowpea had lower carbohydrate content during storage.

Since carbohydrate content is the main substrate for respiration that produces energy, the high carbohydrate content observed in cowpeas treated with 2 ml castor and 3 ml cashew oil could be the reason for the high calorific value recorded. The calorific value is directly proportional to the carbohydrate content, as it provides the fuel for metabolic activities that produce the required energy. The results of this study are in agreement with those of Uduak (2018), who reported that infested cowpeas have a higher calorific value than those not infested, because the pests have exhausted the carbohydrate content of the grain. In addition, the value obtained in this work is similar to that recorded by Uduak (2018), 326.52 Kcal/g for brown beans and 381.19 Kcal/g for white beans.

In conclusion, castor and cashew oils may be more effective in *C. maculatus* control than scent leaf and lemon balm

powders, but in terms of nutrient content conservation. The application of 10, 15, and 20 g of SLP could give the best moisture, dry matter, and ash content of the stored cowpea, while 15 g LBP had the best crude protein and fiber content. Similarly, cashew and castor oils at the concentration of 3 ml and mixed ratios of castor and cashew oils 1:1, 1:2, and 2:1 ml per 200 g of cowpea recorded the best carbohydrate content of the grains after storage.

ABSTRACT

Tame, V.T., Gungula, D.T., and Zira, D.K. 2025. Influence de biopesticides sur la teneur en nutriments du niébé (*Vigna unguiculata*) stocké à Yola, État d'Adamawa, Nigéria. Tunisian Journal of Plant Protection 20 (2): 57-67.

L'influence de biopesticides sur la teneur en nutriments des graines de niébé stockés a été étudiée afin de déterminer l'effet de ces extraits végétaux sur leur composition. Les traitements consistaient à traiter des graines de niébé avec de l'huile de ricin, de l'huile de noix de cajou, de la poudre de la mélisse et de la poudre de feuilles de basilic africain (ou encens), pures ou en mélange, appliquées à différentes doses. L'expérience a été menée selon un dispositif complètement randomisé (PCR) avec 18 traitements répétés trois fois et un stockage de 120 jours. Les résultats ont montré un effet hautement significatif des extraits végétaux sur la composition des graines. Les niébés traités avec 10 g de poudre de feuilles de basilic africain (PFA) présentaient la teneur en eau la plus élevée (12,11 %). Les graines traitées avec 15 g de PFA affichaient également les teneurs les plus élevées en cendres et en protéines brutes (7,22% et 39,39%, respectivement), tandis que ceux traités avec 20 g de PFA présentaient la teneur en matières grasses brutes la plus élevée (6,80%). Concernant les fibres brutes, les échantillons traités avec 15 g de poudre de mélisse (PM) présentaient la teneur la plus élevée (5,43%), tandis que les graines traitées avec 20 g de PM affichaient la teneur en matière sèche la plus importante (90,22%). Les échantillons traités avec de l'huile de ricin pure ou en mélange avec de l'huile de noix de cajou présentaient les teneurs en glucides les plus élevées (66,98 à 66,79 %), tandis que la valeur calorique la plus élevée a été observée pour les échantillons traités avec 2 ml d'huile de ricin (372,31 kcal/g).

Mots clés: Biopesticides, *Callosobruchus maculatus,* huile de noix de cajou, huile de ricin, niébé, poudre d'encens, poudre de mélisse, *Vigna unguiculata*

تلخيص

تام، فادلاي تيزهي ودانيال تيرّمون قونقولا ومحمد أحمد ودلما كوادا زيرا. 2025. تأثير المبيدات الحيوية على المحتوى الغذائي للوبيا المخزنة (*Vigna unguiculata*) في يولا، ولاية أداماوا، نيجيريا.

Tunisian Journal of Plant Protection 20 (2): 57-67.

دُرست تأثيرات المبيدات الحيوية على المحتوى الغذائي لحبوب اللوبيا المخزنة لتحديد تأثير هذه المستخلصات النباتية على تركيبها الكيميائي. شملت المعاملات معالجة حبوب اللوبيا بمسحوق نقي ومخاليط من زيت الخروَع، وزيت الكاجو، ومسحوق المليسة، ومسحوق أوراق الريحان، بتراكيز مختلفة. صُممت التجربة وفق تصميم عشوائي كامل (CRD) بـ 18 معاملة، كُررت ثلاث مرات، وخُزّنت الحبوب لمدة 120 يومًا. أظهرت النتائج تأثيرًا معنويًا للغاية للمستخلصات النباتية على المحتوى الكيميائي للحبوب. احتوت حبوب اللوبيا المعالجة بـ 10 غ من مسحوق أوراق الريحان على أعلى نسبة رطوبة (12.11%)، كما سجلت الحبوب المعالجة بـ 15 غ من مسحوق أوراق الريحان أعلى نسبة رماد وبروتين خام (7.22% و39.39% على التوالي)، بينما أظهرت حبوب اللوبيا المعالجة بـ 20 غ من مسحوق أوراق الريحان أعلى نسبة دهون خام (6.80%). فيما يتعلق بالألياف الخام، سجلت العينات المعالجة بـ 15 غ من مسحوق بلسم الليمون أعلى نسبة (5.43%)، بينما أنتجت الحبوب المعالجة بـ 20 غ من مسحوق بلسم الليمون أعلى نسبة من المادة الجافة (90.22%). وسجلت كل من العينات المعالجة بزيت الخرَوع النقي ومزيج زيت الكاجو أعلى نسبة من الكربوهيدرات (66.98-66.79%)، في حين سُجلت أعلى قيمة حرارية في العينات المعالجة بـ 2 مل من زيت الخروَع (372.31 كيلو كالوري/غ).

LITERATURE CITED

Adejuyitan, J.A., Olunotu, E.T., Akande, F.A., Bolarinwu I.F., and Oladokun, F.M. 2009. Some physicochemical properties of flour are obtained from the fermentation of tiger nuts (*Cyperus esculentus*). Food Science Journal 3: 51-55.

Ahmed, M., Aliyu, A.M., Tame, V.T., and Gungula, D.T. 2024. Effects of postharvest management on the nutrient content of potato (*Solanum tuberosum*) tubers during storage. Agricultural Sciences 16: 90-99.

Agele, S.O., I.K. Oyewusi, O.P. Aiyelari, and I.B. Famuwagun, 2017. Dry matter production, biomass partitioning and seed setting efficiencies in early- and late-rainy season cowpea in the Rainforest Agroecology of South-West Nigeria. International Journal of Botany 13: 115-125.

Adino, S., Wondifraw, Z, and Addis M. 2018. Replacement of soybean grain with cowpea grain (*Vigna unguiculata*) as protein supplement in Sasso x Rir crossbred chicks diet. Poultry Fish Wildlife Science 6: 188.

Ajeigbe, H.A., D. Ihedioha., and Chikoye, D. 2008. Variation in physio-chemical properties of the seed of selected improved varieties of cowpea as it related to industrial utilization of crop. Africa Journal of Biotechnology 7: 20-29.

Anukiruthika, T., Jian, F., and Jayas, D.S. 2021. Movement and behavioral response of stored product insects under stored grain environments - A review. J. Stored Prod. Res. 90: 1-14.

AOAC 2020. Association of Official Analytical Chemists. Official methods of analysis, 19th Edition. Washington, DC, USA.

Babarinde, S.A., Akinyemi, A.O, Odewole, A.F., Yisa, S., Oke O.O, and Adeyemo, A.O. 2016. Phytochemical composition and insecticidal properties of mechanically extracted castor, seed oil against cowpea seed bruchid (*C. maculatus* Fab.) infesting Bambara groundnut. Elixir International Journal 92: 39086-39092.

Davis, D.W., Oelke A., Oplinge E.S., Doll J.D., Hanson C.V., and Putnum, D.H. 1991. Cowpea alternative field crops manual. 1991. www.hort.purdue.edu. (05.02.2010).

El-Sharkawy, H.A., Abo-El-Wafa, S.A., Mostafa, N.A., and Yousef, A.M. 2023. Boosting biopesticides potential of *Trichoderma harzianum* for controlling the downy mildew and improving the growth and the productivity of King Ruby seedless grape. Egyptian Journal of Biological Pest Control 61: 1- 8

Gerrano, A.S., Jansen van Rensburg, W.S., and Adebola, P.O. 2015. "Genetic variability and heritability estimates of nutritional composition in the leaves of selected cowpea genotypes (*Vigna unguiculata* (L.)) Walp. Hort. Science, 50: 1435-1440.

Gerrano, A.S., Jansen van Rensburg, W.S., and Adebola, P.O. 2017. Preliminary evaluation of seed and germination traits in cowpea *Vigna unguiculata* (L.) Walp. Genotypes. South African Journal of Plant and Soil, 34: 399-402.

Gusmão, N.M.S., de Oliveira, J.V., Navarro, D.M.A.F., Dutra, K.M., da Silva, W.A., and Wanderley, M.J.A. 2013. Contact and fumigant toxicity and repellency of *Eucalyptus citriodora* Hook., *Eucalyptus staigeriana* F., *Cymbopogon winterianus* Jowitt and *Foeniculum vulgare* Mill. essential oils in the management of *Callosobruchus maculatus* (FABR.) (Coleoptera: Chrysomelidae, Bruchinae). Journal of Stored Products Research 54: 41-47

Langyintou, A.S., Lowenberg-DeBoer J., Faye M. Lambert, D. Ibro, G., and Moussa, B. 2003. Cowpea supply and demand in West and Central Africa. Field Crops Research 82:215-231.

Ilesanmi, J.O. and Gungula, D.T. 2010. Presentation of cowpea (*vigna unguiculata* [L.] walp) Grains against cowpea Bruchids (*C. maculatus*) using Neem and Moringa seed oil. International Journal of Agronomy Volume 2010, Article ID 235280, 8 pp.

Ilesanmi, J.O.Y., and Gungula, D.T. 2016. Proximate composition of cowpea (*Vigna unguiculata* (L.) Walp) grains preserved with mixtures of neem (*Azadirachta indica* A. Juss) and moringa (*Moringa oleifera*) seed oils. African Journal of Food Science and Technology 7:118-124.

Mkenda, A.T., and Ndakidemi, P.A. 2014. Pesticidal efficacy of four botanical pesticides on survival, oviposition, and progeny development of bruchid, *Callosobruchus maculatus* in stored cowpea (*Vigna unguiculata).* International Journal of Plant and Soil Science 3: 1504-1523.

Khalid, S.B. Elhardallou, and E.A. Elkhalifa, 2012. Composition and functional properties of

cowpea (*Vigna ungiculata* L. Walp) flour and protein isolates. American Journal of Food Technology 7: 113-122.

Sanon, A., Zakaria, I., Dabrie-Benso, C.L., Niango, B.M., and Nebie, R.C.H. 2018. Potential of botanicals to *Callosobruchus maculatus* (Col.: Chrysomelidae, Bruchinae), a major pest of stored cowpea in Burkina Faso: A Review. International Journal of Insect Science.10: 1-8.

Shikaan, T.O., and Uvah I.I, 2002. Effect of some materials on progeny development in *Callosobruchus maculatus* F. in Established Infestation on Cowpea Grain at Samaru, Nigeria. Journal of Entomology 19: 70-77.

Singh, B.B. 2014. Cowpea: The food legume of the 21st century. CSA News 59 (10): 4.

Swamy, S.V.S.G., Raja, D.S., and Wesley, B.J. 2020. Sustainability of stored chickpeas to bruchid infestation as influenced by physic-chemical traits of the grain. J. Stored Prod. Res. 87: 101583.

Therese, M.O., Emmanuel, O.A., Phumzile, M, and Maziya-Dixion, B. 2019. Cowpea (*Vigna unguiculata* (L) Walp) for food security; an elevation of end-user traits of improved varieties in Swaziland. Science Rep. 9: 15991.

Uduak, I. A. 2018. Comparison of the proximate and mineral composition of two cowpea varieties obtained from mile 12 market, Logos. Communication in physical sciences, 3: 43-48.

Xu, B.J., and Shang, S.K.C. 2012. Comparative of ant proliferation properties and cellular antioxidant activities of commonly consumed food legume against nine human cancer cells. Food chemistry 134: 1287-1296.

Evaluation of *Trichoderma* and *Bacillus* Species for the Management of Watermelon Charcoal Rot and Plant Growth Promotion

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ABSTRACT

Mannai, S., Ben Salem, I., and Boughalleb-M'Hamdi, N. 2025. Evaluation of *Trichoderma* and *Bacillus* species for the management of watermelon charcoal rot and plant growth promotion. Tunisian Journal of Plant Protection 20 (2): 69-83.

Macrophomina phaseolina responsible for watermelon charcoal rot, is a soilborne pathogen spread worldwide and in Tunisia. This study aimed to control this important disease using eco-friendly treatments. Two fungal and one bacterial antagonists were tested. Dual culture trials showed the efficacy of *Trichoderma harzianum*, *Trichoderma viride* and *Bacillus subtilis* to reduce *M. phaseolina* mycelial growth by 36.45% to 53.67% for MP4 and MP1 isolates confronted to *B. subtilis*. In the in vivo tests, the use of *T. harzianum* and *B. subtilis* and their combination showed that the preventive application is more effective than the simultaneous and the curative treatments. In fact, the preventive application of *T. harzianum* and *B. subtilis* and their combination reduced the disease severity index by 37.5%, 91.75% and 25%, respectively, compared to the inoculated control. Preventive application of *B. subtilis* significantly enhanced root volume, root length, and shoot length by 81.81%, 67.41%, and 73.07%, respectively, compared to the inoculated control. The application of *B. subtilis*, *T. harzianum* and their combination simultaneously with inoculation significantly increased the length of plants by 99%, 90.85% and 34.11%, respectively, compared to the inoculated control. Our findings indicate that *T. harzianum* and *B. subtilis* can be effectively employed as preventive soil treatments to suppress charcoal rot and promote watermelon growth.

Keywords: Bacillus subtilis, biological control, charcoal rot, *Macrophomina phaseolina*, *Trichoderma* spp., watermelon

Macrophomina phaseolina is a soilborne pathogen affecting several plants worldwide (Islam et al. 2012, Manici et al. 1995, Marquez et al. 2021). Diseases caused by *M. phaseolina* are frequently identified as charcoal rot due to the formation of black microsclerotia in the infected part of plants (Pratt et al. 1998). *M. phaseolina* induces the disease using different enzymes like amylases, phosphatidases, pectinases, hemicellulases and proteases, (Islam et al. 2012, Marquez

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et al. 2021), and toxic metabolites such as asperlin, phomenone, and phaseolinone (Abbas et al. 2020, Mahato et al. 1987, Marquez et al. 2021).

M. phaseolina has been reported as the causal agent of charcoal rot disease and declining in cucurbits grown worldwide (Boughalleb-M'Hamdi et al. 2017, Cohen et al. 2016 , Egel et al. 2020, Negreiros et al. 2019, Wu et al. 2022). On watermelon plants, this pathogen caused numerous small, black sclerotia on the roots, premature yellowing of the top leaves followed by leaf drop (Boughalleb-M'Hamdi et al. 2017). Furthermore, *M. phaseolina* is recognized as a primary etiological agent of root rot and vine decline, with the potential to cause yield losses up to 40% in watermelon cultivation (Alves et al. 2025, Cohen et al. 2016, Gomes-Silva et al. 2018, Porto et al. 2019, Wu et al. 2022).

Strategies to control charcoal rot disease are limited due to the large host range and the long survival of *M. phaseolina* microsclerotia in the soil. Several systemic fungicides like carbendazim, thiophanate methyl, hexaconazole, tebuconazole, difenoconazole, azoxystrobin and non-systemic fungicides like mancozeb, chlorothalonil, and captan were evaluated against *M. phaseolina* at different concentrations (Lokesh et al. 2020, Parmar et al. 2017). However, no chemical product has been registered to control the charcoal rot (Marquez et al. 2021).

Several studies have been accentuated on testing different antagonistic microorganisms for the management of this disease, which are ecologically safe solutions (Khare et al. 2010, Muthukumar et al. 2011). *Trichoderma* and *Bacillus* are the most used antagonists to control different plant diseases (Devi et al. 2022, Mannai and Boughalleb-M'Hamdi 2022, Mannai et al. 2018, Podbielska et al. 2020).

Trichoderma species isolated from different types of soils are efficient against several phytopathogens such as *M. phaseolina* (Bastakotiet al. 2017, Shahid et al. 2014). These microorganisms have different antagonistic mechanisms like competition for nutrients, production of antibiotics and mycoparasitism. Furthermore, some species are also able to increase the plant growth (Chowdappa et al. 2013, Martinez-Medina et al. 2016, Zaim et al. 2018).

Replacing agrochemicals with Plant Growth-Promoting Rhizobacteria (PGPR) represents a sustainable and safe strategy to secure crop production, as these microorganisms enhance both plant growth and health (Bhat et al. 2020). The genus *Bacillus* is frequently used as a biocontrol agent (Devi et al. 2022, Simonetti et al. 2015, Torres et al. 2016).

However, conventional methods for controlling plant diseases are limited by serious drawbacks, including risks to human health and environmental contamination from chemical inputs. Therefore, alternative eco-friendly approaches, such as biological control, are increasingly required (Rojo et al. 2007).

The objectives of the present study were (i) to assess the in vitro antifungal activity of *T. harzianum*, *T. viride*, and *B. subtilis* against *M. phaseolina* isolates associated with watermelon charcoal rot, and (ii) to investigate the efficacy of preventive, simultaneous, and curative applications of *T. harzianum* and *B. subtilis*, individually and in combination, in mitigating disease severity and enhancing the growth of watermelon seedlings.

MATERIALS AND METHODS

Pathogen culture and inoculum preparation.

Four *M. phaseolina* isolates (MP1, MP2, MP3, and MP4) obtained from watermelon seedlings exhibiting symptoms of charcoal rot disease and collected from Chott-Mariem region in Tunisia were used in this study. Isolates identification was achieved through morphological characterization, focusing on colony morphology and microsclerotia features, following the criteria described by Beas-Fernández et al. (2006) and Mayek-Pérez et al. (1997). To prepare the inoculum for the in vivo tests, mycelia of *M. Phaseolina* isolate MP1was harvested from five Petri plates containing one-week-old cultures grown on Potato-Dextrose-Agar (PDA) and subsequently homogenized in 0.5 liter of Sterile Distilled Water (SDW) using an electric mixer. The resulting pathogen inoculum was then used to inoculate watermelon seedlings by watering them with 50 ml/plant (Cohen et al. 2016).

Culture conditions and inoculum preparation of biocontrol agents for in vivo evaluation.

Two isolates of *Trichoderma (T. harzianum* and *T. viride*) and one isolate of *B. subtilis*, naturally associated to watermelon seedlings, were tested in this investigation.

The identification of *Trichoderma* isolates was conducted following a 7-day incubation period for each colony on PDA medium at a temperature of 28°C and based on macro- and micro-morphological traits of the colony, conidiophores, phialides and conidia (Shah and Afiya2019, Siddiquee 2017). The *B. subtilis* isolate was identified through both morphological, biochemical and genetic analyses, as detailed in Furuya et al. (2011).

For the in vivo assay, the concentration of conidial suspension obtained from *T. harzianum* cultures grown on PDA was adjusted to 10^7 CFU/mL by a Malassez slide. *B. subtilis* isolate was incubated at 25°C for two days on Nutrient Agar (NA). Then, cell suspension was prepared by scraping bacterial colony, adjusted to 10^6 CFU/ml by dilution in SDW. Then, 50 ml of each inoculum was used per plant for individual treatment. However, for the combination method (*T. harzianum* + *B. subtilis*), 25 ml of each inoculum was used (Boughalleb-M'Hamdi et al. 2018).

in vitro assessment of the antifungal activity of biocontrol agents against *Macrophomina phaseolina.*

The antifungal activities of the tested antagonists against *M. phaseolina* mycelial growth were determined according to Mannai and Boughalleb-M'Hamdi (2023) method. Agar plugs (6 mm in diameter) of the pathogen culture were positioned on the opposite side of the antagonists plugs. Before use, *B. subtilis* was grown on NA for 48 h and *T. harzianum* and *T. viride* were cultivated on PDA medium for 7 days, at 25°C. The antagonist plugs were replaced with agar plugs for the control plates. Six plates were used per individual treatment, and the experiment was repeated twice in time. All the plates were incubated at 25°C for four days. Then, *M. phaseolina* mycelial growth inhibition was determined according to following formula (Hmouni et al. 1996):

$$I\ (\%) = (1 - T/C) \times 100$$

where, I: mycelial growth inhibition, T: the radius of pathogen colony in treated plates, C: the radius of pathogen colony in control plates.

Effect of *T. harzianum* and *B. subtilis* on watermelon charcoal rot disease incidence.

Based on the in vitro results, *T. harzianum* proved to be a more effective antagonist against *M. phaselina* than *T. viride*, and was therefore selected, together with *B. subtilis*, for the in vivo trials, applied both individually and in combination. Thirty days old watermelon plants cv. Crimson seedlings were used for the in vivo trials. Theses ones were transplanted in plastic pots (17 cm diameter) containing a mixture of peat and vermiculite (v/v) used as soil substrate.

Four types of treatments were carried out for individual antagonists and their combination: preventive, simultaneous treatment and two curative treatments. The preventive treatment consists of watering the watermelon seedlings with 50 ml/plant of the suspension of each tested antagonist (10^7 CFU/mL for *T. harzianum* and 10^6 CFU/mL of *B. subtilis*) 15 days before inoculation. The simultaneous treatment consists of watering seedlings with 50 ml/plant of the spore/cell suspension of each antagonist, just after inoculation with pathogen (at the same day) also applied out by seedling watering. The first curative treatment consists of the plant inoculation and subsequently the treatment with antagonists (50 ml/plant) 15 days after inoculation. The treatment was carried out by watering seedlings around their collars. The second curative treatment consists of inoculating the plants with the pathogen followed by two treatments with the antagonists with an interval of 15 days one month after inoculation with *M. phaseolina*. This treatment was also carried out by watering the seedlings around their collars (Hibar et al. 2006). Inoculated untreated and uninoculated untreated controls were used. A completely randomized factorial design with two factors (antagonist and treatment types) was followed in the current assay. Each treatment was subjected to three replicates and the experiment was repeated twice.

Sixty days after the preventive treatment with antagonists, six parameters were noted: the charcoal rot index rated onto 0–6 scale according to Zanella et al. (2020) with some modifications (0 = symptomless; 1 = 1-3% yellowing of the basal leaves; 2 = 4-10% yellowing with slight discoloration of the roots; 3 = 11-25% yellowing and wilting of the apical part; 4 = 26-50% yellowing and necrosis of leaves and wilting and crown root rot; 5 = 51-75%: the leaves are dried out with blackening of the roots; 6 = 76-100%: complete drying out and rotting).

Based on this rating scale, the disease incidence was calculated using the formula of Wheeler (1969) in each treatment:

Disease incidence (%) = (S*100)/T × M

with S: Sum of all numerical ratings; T: Total observed plant; M: Maximum disease grade.

In this essay, the vegetative and root length, its fresh weight, dry weight, and the volume are also measured. The root volume (cm^3) was estimated by the immersion method as described by Musick et al. (1965).

Statistical analyses.

Statistical analyses (ANOVA) were conducted for both in vitro and in vivo assays using a completely randomized factorial design. For the in vitro assays, the fixed factors were the antagonists and pathogen isolates, whereas for the in vivo assays they were the antagonists and treatment timing. Each treatment was replicated six times in vitro and three times in vivo. Data were analyzed using SPSS version 23, and mean comparisons were performed with the

Student-Newman-Keuls (SNK) test at a least significance level of $p \leq 0.05$.

RESULTS

In vitro effect of antagonists on *M. phaseolina* growth.

Statistical analyses of the in vitro biocontrol essay (dual culture method) revealed a highly significant interaction between the two tested factors (antagonists and pathogen isolates; $p \leq 0.05$) as well as a highly significant main effect of the antagonists. Indeed, the antagonist reacted differently towards all the isolates after four days of incubation at 25°C (Table 1). Inhibition rates ranged from 36.45% to 53.67% for MP4 and MP1 isolates when challenged with *B. subtilis*. This antagonist exhibited the highest efficacy against MP1, but was less effective against MP3 and MP4. In contrast, *T. harzianum* and *T. viride* showed greater inhibitory activity than *B. subtilis*, with inhibition rates of 51.02% and 49.84% against MP3, and 51.58% and 47.80% against MP4, respectively (Table 1). Fig. 1 illustrates the comparative effects of the different antagonists on the four *M. phaseolina* isolates.

Table 1. Inhibition rate of *Macrophomina phaseolina* mycelial growth by the tested antagonists (%), recorded after four days of incubation at 25°C

Antagonists/Pathogen isolates	MP1	MP2	MP3	MP4	*p*-value
Trichoderma harzianum	48.38a±1.55^{aA}	48.97±0.56^{aA}	51.02±0.87^{aA}	51.58±3.43^{aA}	≥ 0.05
Trichoderma viride	47.80±1.29^{aA}	51.33±5.28^{aA}	49.84±2.19^{aA}	47.80±2.68^{aA}	≥ 0.05
Bacillus subtilis	53.67±5.21^{aA}	40.73±8.98^{bA}	38.76±2.69^{bB}	36.45±1.41^{bB}	≤ 0.05
***p*-value**	≥ 0.05	≥ 0.05	≤ 0.05	≤0.05	-

* Within each row, values followed by different lowercase letters differ significantly according to the SNK test at $p \leq 0.05$.
** Within each column, values followed by different uppercase letters differ significantly according to the SNK test at $p \leq 0.05$.
MP1, MP2, MP3, and MP4: *M. phaseolina* isolates used in this study.

In vivo effect of *T. harzianum* and *B. subtilis* on watermelon charcoal rot disease incidence.

Analysis of variance of the charcoal rot disease index calculated 45 days after inoculation of watermelon plants, revealed a highly significant interaction, between the two factors (treatment timing x used antagonists) as well as a highly significant effect for each factor analyzed alone. In fact, preventive treatment has been shown to be more effective than the other three treatments. The preventive application (15 days before inoculation) of *T. harzianum* and *B. subtilis* as well as their combination significantly reduced disease severity index by 37.5%, 91.75%, and 25%, respectively, compared to the inoculated control. However, no significant reduction in disease severity index was found for both antagonists and their combination when they were applied simultaneously, once or twice after plant inoculation (Fig. 2).

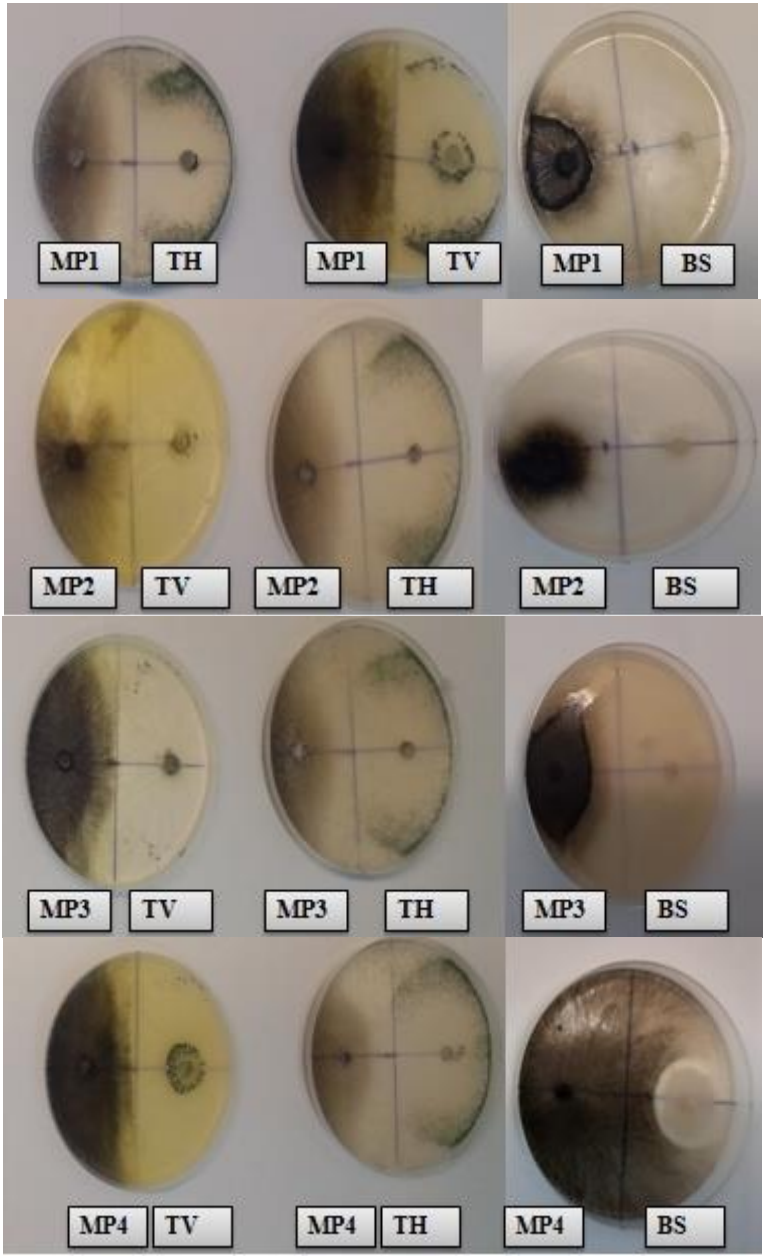

Fig. 1. Confrontations of four isolates of *Macrophomina phaseolina* isolates with the three antagonists after 4 days of incubation at 25°C.
TV: *T. viride*; TH: *T. harzianum*; BS: *B. subtilis*; MP1, MP2, MP3, and MP4: Isolates of *M. phaseolina*

The disease incidence ranged from 33.34% for plants treated preventively with *B. subtilis* to 100% for plants with only one curative treatment by *B. subtilis* and the combination between the antagonists. The timing treatment with *T. harzianum* did not affect this parameter (83.34%). However, the preventive application of *B. subtilis* is the most effective treatment followed by its simultaneous application (Fig. 2).

In vivo effect of antagonists on growth parameters.

Regarding vegetative growth, variance analysis revealed that both factors (treatment timing and used antagonists), considered independently and in interaction, have had a highly significant effect ($p \leq 0.05$). In fact, preventive and simultaneous treatments were found to be more effective than the curative treatments. The preventive application of *T. harzianum* and *B. subtilis* significantly improved this parameter by 37.82% and 73.07%, respectively. Their simultaneous application significantly increased the vegetative growth by 99% and 90.85%, respectively. *B. subtilis* was the most effective with lengths comparable to those of the healthy control. On the other hand, the curative applications were not effective (Fig. 3).

Variance analysis for the root length parameter revealed a highly significant interaction between treatment timing and antagonists, as well as significant individual effects of each factor ($p \leq 0.05$). Preventive application of *B. subtilis* (15 days before inoculation) was the only effective treatment, increasing root length by 67.41% compared to the inoculated control. In contrast, simultaneous or curative applications of the antagonists, alone or in combination, showed no significant effect on this parameter (Fig. 3).

Statistical analysis revealed a highly significant difference in the effectiveness of the antagonists ($p \leq 0.05$), as well as a significant interaction between the two studied factors (treatment timing and type of antagonist) on root volume. Notably, none of the treatments improved this parameter, except for the preventive application of *B. subtilis*, which resulted in an 81.81% increase compared to the inoculated control (Fig. 3).

A highly significant effect of the antagonists and of the timing of their application ($p \leq 0.05$), as well as a highly significant interaction between these two factors ($p \leq 0.05$), was observed on root fresh weight. The preventive treatment was the most effective. Specifically, the preventive and simultaneous applications of *B. subtilis* increased root fresh weight by 185.96% and 84.21%, respectively, compared to the inoculated control. Similarly, the preventive and both curative applications of the two antagonists significantly enhanced this parameter by 107% and 119.30%, respectively (Fig. 3)

A significant interaction between the two factors (treatment timing * antagonist type) and a highly significant effect ($p \leq 0.05$) of antagonists had been proved on the root dry weight. The preventive and simultaneous applications of *B. subtilis* improved this parameter by 109.52% and 142.85%, respectively. The curative applications of the antagonist's combination significantly improved the plants roots dry weight by 90.47% (Fig. 3).

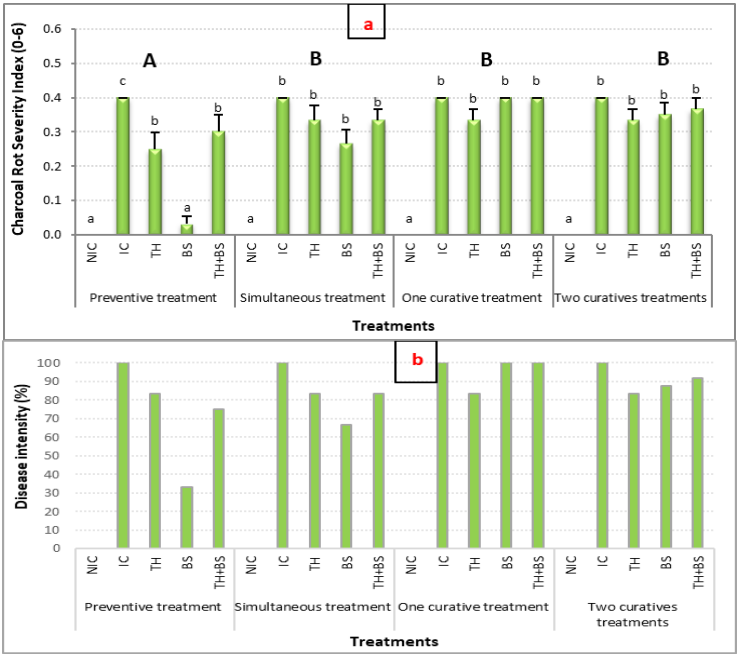

Fig. 2. Disease incidence on watermelon plants (cv. Crimson) treated with different antagonists compared to control, 45 days after inoculation with *Macrophomina phaseolina* isolate MP1. For each treatment type (preventive, simultaneous, curative treated once, curative treated twice), bars sharing the same lowercase letter do not differ significantly according to the SNK test ($p \leq 0.05$).

The capital letters (A, B and C) presented the statistical comparison between the treatment timings regardless of the used antagonist, according to the SNK test at $p \leq 0.05$. NIC: Uninoculated control, IC: Inoculated and untreated control, TH: *Trichoderma harzianum*, BS: *Bacillus subtilis*, TH+BS: Combination between *T. harzianum* and *B. subtilis* used.

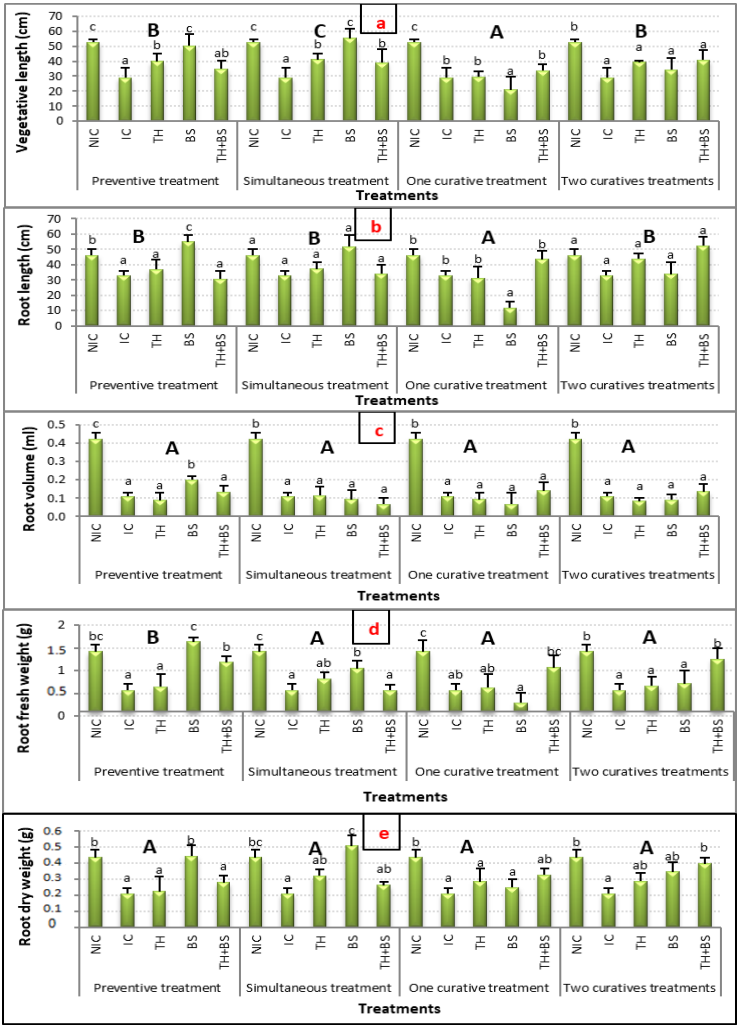

Fig. 3. Vegetative length (a), root length (b), root volume (c), root fresh weight (d), and root dry weight (e) of watermelon plants (cv. Crimson) treated with different antagonists compared to control, 45 days after inoculation with *Macrophomina phaseolina* isolate MP1. For each treatment type (preventive, simultaneous, curative treated once, curative treated twice), bars sharing the same lowercase letter do not differ significantly according to the SNK test ($p \leq 0.05$).

The capital letters (A, B and C) presented the statistical comparison between the treatment timings regardless of the used antagonist, according to the SNK test at $p \leq 0.05$. NIC: Uninoculated control, IC: Inoculated and untreated control, TH: *Trichoderma harzianum*, BS: *Bacillus subtilis*, TH+BS: Combination between *T. harzianum* and *B. subtilis* used.

DISCUSSION

Biocontrol agents like *B. subtilis*, *T. viride*, and *T. harzianum* are used in the management strategies to control cucurbits charcoal rot. Different products made from *Bacillus* spp. and *Trichoderma* spp. are known to suppress *M. phaseolina* growth under specific conditions (Gacitua et al. 2009).

The present study has shown the effectiveness of *B. subtilis*, *T. harzianum* and *T. viride* to reduce *M. phaseolina* mycelial growth. Several studies have focused on the effectiveness of these agents in limiting the development of several plant pathogens. In fact, Boughalleb-M'Hamdi et al. (2018) demonstrated the antagonistic efficacy of different *T. harzianum* and *T. viride* isolates against different soilborne phytopathogenic fungi infecting melon and watermelon (*Fusarium oxysporum* f. sp. *niveum*, *Fusarium solani* f. sp. *cucurbitae*, *F. oxysporum* f. sp. *melonis*, and *M. phaseolina*). In addition, the mycelial growth of five *M. phaseolina* isolates decreased in the presence of *T. harzianum* (between 38.74 and 52.42%) and *T. viride* (between 33.27 and 42.43%) (Boughalleb-M'Hamdi et al. 2018). Besides, the mycelial growth of 14 isolates of *M. phaseolina* was significantly reduced by *T. harzianum*, *T. viride* and *B. subtilis* isolated from rhizosphere of groundnut, with an inhibition rate (%) varing from 65.64 to 81.25, 63.33 to 81.62 and 38.46 to 62.50, respectively (Kumar et al. 2015). Several other studies have shown that *B. subtilis* has an important inhibitory activity against several plant pathogens such as *Macrophomina*, *Pythium*, *Phytopythium*, and *Fusarium* spp. (Devi et al. 2022, Mannai and Boughalleb- M'Hamdi 2022, 2023, Singh et al. 2008). In fact, *B. subtilis* cell suspensions produced an inhibitory activity greater than 50% against three strains of *M. phaseolina* (Torres et al. 2016). Besides, among five screened *B. subtilis* strains, the M-4 *B. subtilis* strain showed a high antagonistic activity in vitro against *M. phaseolina* (81.5%) (Chauhan et al. 2022). The strain *B. subtilis* BGS-10 showed higher mycelial inhibition (61%) against *M. phaseolina* associated to *Gloriosa superba* root rot (Dhanabalan et al. 2024).

T. harzianum has been shown to be effective in vivo to reduce the charcoal rot severity when used preventively. These results are similar to those of Boughalleb-M'Hamdi et al. (2018) who revealed that the preventive application of *T. harzianum* reduced the charcoal rot severity. Besides, *T. harzianum* is able to decrease the charcoal rot incidence by 37-74% on melon plants grown in fields infested with *M. phaseolina*. The *Trichoderma* genus has long been characterized by its ability to act as biological control agent against plant pathogens. The primary biological control mechanisms are food competition, mycoparasitism, and antibiosis (Ghildyal and Pandey 2008, Umamaheswari et al. 2009). In addition, *Trichoderma* species generate compounds that have antimicrobial properties and various enzymes, including pectinases, glucanases, proteases, and chitinases, which break down the cell walls of harmful fungal pathogens (Tchameni et al. 2020, Verma et al. 2007). The in vivo antagonism test in our study also demonstrated the efficiency of *B. subtilis* in reducing disease incidence when applied preventively. These findings are consistent with those of Singh et al. (2008), who reported the effectiveness of *B. subtilis* in suppressing root rot symptoms in *Pinus roxburghii* caused by *M. phaseolina*. Moreover, several studies have recognized *Bacillus* spp. as potent antagonists against *M. phaseolina* (Muhammad and Amusa 2003, Pal et al.

2001, Simonetti et al. 2015). The inhibitory effect of *B. subtilis* is partly explained by its ability to produce lytic enzymes, chitinase and b-1,3-glucanase, which degrade the mycelium and the cell wall component of *M. phaseolina* (Singh et al. 2008). On the other hand, the preventive application of the combined antagonists *T. harzianum* and *B. subtilis* also reduced disease incidence. However, the effectiveness of this combination was lower than that of *B. subtilis* applied alone and comparable to that of *T. harzianum* used individually. This reduced efficacy could be attributed to a possible incompatibility or antagonistic interaction between the *B. subtilis* strain and the *T. harzianum* isolate. This incompatibility was previously reported by Thilagavathi et al. (2007), who tested the biocontrol agents *T. viride* and *B. subtilis* individually and in combination against root rot of green gram caused by *M. phaseolina*. Their results showed that *T. viride* strains were not compatible with *B. subtilis*.

The present study showed also that the tested antagonists improved the plants growth. In fact, *T. Harzianum* and *B. subtilis* improved the growth (length of the vegetative part; root length and volume) following its preventive and simultaneous applications. The simultaneous treatment using the combination increased the length of the plants. These results are in agreement in part with those of Singh et al. (2008) who showed *B. subtilis* effectiveness in increasing the dry weights of *Pinus roxburghii* plant roots and vegetative parts. In addition, previous study proved that the treatment of melon seeds with *T. harzianum* improved the plant fruit yield by 61% compared to plants from non-treated seeds grown in soils naturally infested with *M. phaseolina* (Elad et al. 1986 in Rhouma et al. 2021).

Based on our results, *B. subtilis* could be employed as an individual preventive treatment to control the charcoal rot disease while promoting watermelon plant growth and development.

RESUME

Mannai S., Ben Salem I. et Boughalleb-M'Hamdi N. 2025. Evaluation d'espèces de *Trichoderma* et de *Bacillus* pour la gestion de la pourriture charbonneuse de la pastèque et la stimulation de la croissance des plantes. Tunisian Journal of Plant Protection 20 (2): 69-83.

Macrophomina phaseolina, responsable de la pourriture charbonneuse de la pastèque, est un pathogène tellurique répandu dans le monde et en Tunisie. La présente étude visait à contrôler cette importante maladie en utilisant des traitements respectueux de l'environnement. Deux antagonistes fongiques et un antagoniste bactérien ont été testés. Un essai de confrontation directe sur milieu de culture a montré l'efficacité de *Trichoderma harzianum*, *Trichoderma viride* et *Bacillus subtilis* à réduire la croissance mycélienne de *M. phaseolina* de 36,45% à 53,67% pour les isolats MP4 et MP1 confrontés avec *B. subtilis*. Le test in vivo moyennant *T. harzianum* et *B. subtilis* appliqué individuellement ou en combinaison a montré que le traitement préventif est plus efficace que celui appliqué simultanément ou curativement. En effet, l'application préventive de *T. harzianum* et *B. subtilis* et leur combinaison ont réduit l'indice de la maladie de 37,5%, 91,75% et 25%, respectivement. L'application préventive de *B. subtilis* a augmenté significativement le volume et la longueur des racines et la longueur de la partie végétative des plantes de 81,81%, 67,41% et 73,07% respectivement, par rapport au

témoin inoculé. En revanche, l'application de *B. subtilis*, *T. harzianum* et leur combinaison simultanément à l'inoculation a augmenté d'une manière significative la longueur des plantes de 99%, 90,85% et 34,11%, respectivement, par rapport au témoin inoculé. Sur la base de nos résultats, *T. harzianum*, *B. subtilis* et leur combinaison pourraient être utilisés comme traitement préventif du sol pour contrôler la pourriture charbonneuse et augmenter la croissance des plants de pastèque.

Mots clés: *Bacillus subtilis*, lutte biologique, *Macrophomina phaseolina*, pourriture charbonneuse, pastèque, *Trichoderma* spp.

ملخص

مناعي، صبرين وابتسام بن سالم ونعيمة بوغلاب-محمدي. 2025. تقييم أنواع من *Trichoderma* و *Bacillus* لإدارة التعفن الفحمي للبطيخ الأحمر و تعزيز نمو النباتات.

Tunisian Journal of Plant Protection 20 (2): 69-83.

الفطر *Macrophomina phaseolina* هو المسبب لمرض التعفن الفحمي للبطيخ الأحمر والذي ينتقل عن طريق التربة وهو منتشر في جميع أنحاء العالم وفي تونس. تهدف هذه الدراسة إلى مكافحة هذا المرض المهم باستخدام علاجات صديقة للبيئة. تم اختبار اثنين من المضادات الفطرية ومضاد واحد بكتيري. أظهرت التجارب المخبرية *فعالية Trichoderma harzianum* و *Trichoderma viride* و *Bacillus subtilis* في تثبيط نمو الفطر *M. phaseolina* بنسبة 36.45% إلى 53.67% للعزلات MP4 وMP1 عند المواجهة مع *B. subtilis* وقد أظهرت الاختبارات على النبتة أن استعمال *T. harzianum* أو *B. subtilis* أو مزيجهما، أن العلاج الوقائي كان أكثر فعالية من العلاجات المتزامنة او المتأخرة. في الواقع، أدى التطبيق الوقائي للفطر *T. harzianum* والبكتيريا *B. subtilis* ومزيجهما إلى تقليل مؤشر شدة الإصابة بنسبة ٪ و91.75% و25%، على التوالي. أدى التطبيق الوقائي للبكتيريا *B. subtilis* إلى زيادة في حجم وطول الجذور وطول الجزء الخضري من النباتات بنسبة 81.81% و67.41% و73.07%، على التوالي، مقارنة بالشاهد، في حين أدى استعمال الفطر *T. harzianum* والبكتيريا *B. subtilis* ومزيجهما في وقت واحد مع الإعداء إلى زيادة في طول النباتات بنسبة 99% و90.85% و34.11%، على التوالي، مقارنة بالشاهد. وبناءً على نتائجنا، يمكن استخدام الفطر *T. harzianum* والبكتيريا *B. subtilis* ومزيجهما كعلاج وقائي للتربة للسيطرة على التعفن الفحمي و زيادة نمو نبات البطيخ الأحمر.

كلمات مفتاحية: بطيخ الأحمر، تعفن فحمي، مكافحة بيولوجية، *Bacillus subtilis*، *Macrophomina phaseolina*، *Trichoderma* spp.

LITERATURE CITED

Abbas, H.K., Bellaloui, N., Butler, A.M., Nelson, J.L., Abou-Karam, M., and Shier, W.T. 2020. Phytotoxic responses of soybean (*Glycine max* L.) to botryodiplodin, a toxin produced by the charcoal rot disease fungus, *Macrophomina phaseolina*. Toxins 12 (1): 25. https://doi.org/10.3390/toxins12010025

Aegerter, B.J., Gordon, T.R., and Davis, R.M. 2000. Occurrence and pathogenicity of fungi associated with melon root rot and vine decline in California. Plant Disease 84(3): 224-230.

Alves, C.P.S.S., Negreiros, A.M.P., Cavalcante, A.L.A., de Barros, A.P.O., · Correia, K.C., Viana, D.M., Tavares, M.B., and Júnior, R.S. 2025. Pathogenicity of *Macrophomina* spp. associated with watermelon plants. Tropical Plant Pathology 50 :8. https://doi.org/10.1007/s40858-025-00699-z

Bastakoti, S., Belbase, S., Manandhar, S., and Arjyal, C. 2017. *Trichoderma* species as biocontrol agent against soil borne fungal pathogens. Nepal Journal of Biotechnology 5: 39-45. doi:10.3126/njb.v5i1.18492

Beas-Fernández, R., De Santiago-De Santiago, A., Hernandez-Delgado, S., and Mayek-Perez, N. (2006). Characterization of Mexican and non-Mexican isolates of *Macrophomina phaseolina* based on morphological characteristics, pathogenicity on bean seeds and endoglucanase genes. Journal of Plant Pathology 88 (1): 53-60.

Bhat, M.A., Kumar, V., Bhat, M.A., Wani, I.A., Dar, F.L., Farooq, I., Bhatti, F., Koser, R., Rahman, S., and Jan, A.T. 2020. Mechanistic insights of

the interaction of plant growth-promoting rhizobacteria (PGPR) with plant roots toward enhancing plant productivity by alleviating salinity stress. Frontiers in Microbiology 11: 1952. doi:10.3389/fmicb.2020.01952

Boughalleb-M'Hamdi, N., Rhouma, A., Ben Salem, I., and M'Hamdi, M. 2017. Screening and pathogenicity of soil-bornefungal communities in relationship with organically amended soils cultivated by watermelon in Tunisia. Journal of Phytopathology and Pest Management 4: 1-16.

Boughalleb-M'Hamdi, N., Salem, I.B., and M'Hamdi, M. 2018. Evaluation of the efficiency of *Trichoderma*, *Penicillium*, and *Aspergillus* species as biological control agents against four soilborne fungi of melon and watermelon. Egyptian Journal of Biological Pest Control 28: 25. DOI 10.1186/s41938-017-0010-3

Chauhan, P., Bhattacharya, A., Giri, V.P., Singh, S.P., Gupta, S.C., Verma, P.,Dwivedi, A.,Rajput, L.S., and Mishra, A. 2022. *Bacillus subtilis* suppresses the charcoal rot disease by inducing defence responses and physiological attributes in soybean. Archives of Microbiology 204 (5): 266. https://doi.org/10.1007/s00203-022-02876-z

Choudhary, K., Meena, A.K., Chand, K., Nain Y., and Maurya Sh. 2022. Impact of epidemiological factors on the incidence of charcoal rot of sesamum incited by *Macrophomina phaseolina* Biological Forum - An International Journal 14 (1): 264-268.

Chowdappa, P., Kumar, S.M., Lakshmi, M.J., and Upreti, K.K. 2013. Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. Biological Control 65 (1): 109-117. http://dx.doi.org/10.1016/j.biocontrol.2012.11.009

Cohen, R., Elkabetz, M., and Edelstein, M. 2016. Variation in the responses of melon and watermelon to *Macrophomina phaseolina*. Crop Protection 85: 46-51. https://doi.org/10.1016/j.cropro.2016.03.015

Devi, N.O., Tombisana Devi, R.K., Debbarma, M., Hajong, M., and Thokchom, S. 2022. Effect of endophytic *Bacillus* and arbuscular mycorrhiza fungi (AMF) against Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. Egyptian Journal of Biological Pest Control 32 (1): 1-14. https://doi.org/10.1186/s41938-021-00499-y

Dhanabalan, S., Muthusamy, K., Iruthayasamy, J., Kumaresan, P.V., Ravikumar, C., Kandasamy, R., Natesan, S., and Periyannan, S. 2024. Unleashing *Bacillus* species as versatile antagonists: Harnessing the biocontrol potentials of the plant growth-promoting rhizobacteria to combat *Macrophomina phaseolina* infection in *Gloriosa superba*. Microbiological Research 283: 127678. https://doi.org/10.1016/j.micres.2024.127678

Egel, D.S., Guan, W., Creswell, T., and Bonkowski, J. 2020. First report of *Macrophomina phaseolina* causing charcoal rot of cucumber in Indiana. Plant Disease 104: 2030. https://doi.org/10.1094/PDIS-11-19-2421-PDN

Elad, Y., Zvieli., and Chet, I. 1986. Biological control of *Macrophomina phaseolina* (Tassi) Goid by *Trichoderma harzianum*. Crop Protection 5: 288-292 (In Rhouma, A., Salih, Y.A., Abdullah, M.M., Khrieba, M.I., and Matrood, A.A.A. 2021. Technical Document on charcoal rot of cucurbits. Journalof Global Agriculture and Ecology 12 (4): 1-9.

Furuya, S., Mochizuki, M., Aoki, Y., Kobayashi, H., Takayanagi, T., Shimizu, M., and Suzuki, S. 2011. Isolation and characterization of *Bacillus subtilis* KS1for the biocontrol of grapevine fungal diseases. Biocontrol Science and Technology 21: 705-720. https:// doi. org/ 10. 1080/ 09583 157. 2011. 574208

Gacitua, S., Valiente, C., Diaz, K., Hernandez, J., Uribe, M., and Sanfuentes, E. 2009. Identification and biological characterization of isolates with activity inhibitive against *Macrophomina phaseolina* (Tassi) Goid. Chilean Journal of Agricultural Research 69: 526-533.

Ghildiyal, A., and Pandey, A. 2008. Isolation of cold tolerant antifungal strains of *Trichoderma* sp. from glacial sites of Indian Himalayan region. Research Journal of Microbiology 3(8): 559-564.

Gomes-Silva, F., Almeida, C.M.A., Silva, A.G., Leão, M.P.C., Silva, K.P., Oliveira, L.G., and Lima, V.L.M. 2018. Genetic diversity of isolates of *Macrophomina phaseolina* associated with cowpea from Brazil semi-arid region. Journal of Agricultural Science 9: 112-116. https://doi.org/10.5539/jas.v9n11p112

Hibar, K., Daami-Remadi, M., Hamada W., and El-Mahjoub M. 2006. Bio-fungicides as an alternative for tomato *Fusarium* crown and root rot control. Tunisian Journal of Plant Protection 1: 19-29.

Hmouni A., Hajlaoui M. R. and Mlaiki A. 1996. Resistance of *Botrytis cinerea* to benzimidazoles and dicarboximides in protected tomato crops in Tunisia. EPPO Bulletin 26: 697-705.

Islam, M., Haque, M., Islam, M., Emdad, E., Halim, A., Hossen, Q.M., Hossain, M.Z., Ahmed, B., Rahim, S., Rahman, M.S., Alam, M.M., Hou, S., Wan, X., Saito, J.A., and Alam, M. 2012.

Tools to kill: genome of one of the most destructive plant pathogenic fungi *Macrophomina phaseolina*. BMC Genomics 13: 493. doi:10.1186/1471-2164-13-493.

Khare, A., Singh, B.K., and Upadhyay, R.S. 2010. Biological control of *Pythium aphanidermatum* causing damping-off of mustard by mutants of *Trichoderma viride* 1433. Journal of Agricultural Science and Technology 6 (2): 231-243. http://www.ijat-rmutto.com/

Kumar, P., Gaur, V. K., and Rani, R. 2015. Evaluation of antagonists against *Macrophomina phaseolina* causing root rot of groundnut. African Journal of Microbiology Research 9 (3): 155-160.

Lokesh, R., Rakholiya, K.B., and Thesiya, M.R. 2020. Evaluation of different fungicides against *Macrophomina phaseolina* (Tassi) goid. causing dry root rot of chickpea (*Cicer arietinum* L.) in vitro. International Journal of Current Microbiology and Applied Sciences 9: 1-11. doi:10.20546/ijcmas.2020.907

Mahato, S.B., Siddiqui, K.A., Bhattacharya,G., Ghosal,T.,Miyahara,K.,Sholichin, M., and Kawasaki, T. 1987. Structure and stereochemistry of phaseolinic acid: A new acid from *Macrophomina phaseolina*. Journal of Natural Products 50 (2): 245-247.

Manici, L.M., Caputo, F., and Cerato, C. 1995. Temperature response of isolates of *Macrophomina phaseolina* from different climatic regions of sunflower production in Italy. Plant Disease 79: 834-838.

Mannai, S., and Boughalleb-M'Hamdi, N. 2022. *In vitro* and *in planta* potential effect of some indigenous antagonists against *Fusarium* and Pythiaceous species associated with peach seedlings decline. Egyptian Journal of Biological Pest Control 32: 60 .https://doi.org/10.1186/s41938-022-00540-8

Mannai, S., and Boughalleb-M'Hamdi, N. 2023. Evaluation of *Trichoderma harzianum*, *Bacillus subtilis* and *Aspergillus* species efficacy in controlling *Pythium ultimum* associated with apple seedlings decline in nurseries and their growth promotion effect. Egyptian Journal of Biological Pest Control 33: 59. https://doi.org/10.1186/s41938-023-00705-z

Mannai, S., Jabnoun-Khiareddine, H., Nasraoui, B., and Daami-Remadi, M. 2018. *Rhizoctonia* root rot of pepper (*Capsicum annuum*): Comparative pathogenicity of causal agent and biocontrol attempt using fungal and bacterial agents. Journal of Plant Pathology & Microbiology09: 431-36. DOI: 10.4172/2157-7471.1000431

Marquez, N., Giachero, M.L., Declerck, S., and Ducasse, D.A. 2021. *Macrophomina phaseolina*: General characteristics of pathogenicity and methods of control. Frontiers in Plant Science 12: 634397. doi:10.3389/fpls.2021.634397

Martinez-Medina, A., Pozo, M.J., Cammue, B.P.A., and Vos, C.M.F. 2016. Belowground defence strategies in plants: the plant-*Trichoderma* dialogue. Pages 301-327. In: Belowground Defence Strategies in Plants. Editedby C. M. F. Vos, and K. Kazan, ChamSpringer International Publishing. doi:10.1007/978-3-319-42319-7_13

Mayek-Pérez, N., López-Castañeda, C., and Acosta-Gallegos, J.A. 1997. Variación en características culturales in vitro de aislamientos de *Macrophomina phaseolina* y su virulencia en frijol. Agrociencia 31: 187-195.

Muhammad, S., and Amusa, N.A. 2003. In-vitro inhibition of growth of some seedling blight inducing pathogens by compost-inhabiting microbes. American Journal of Botany 2(6): 161-164.

Musick, G.L., Fairchild, M.L., Fergusson, V.L., and Zuber, M.S., 1965. A method of measuring root volume in corn (*Zea mays* L.). Crop Science 5: 601-602.

Muthukumar, A., Eswaran, A., and Sangeetha, G. 2011. Induction of systemic resistance by mixtures of fungal and endophytic bacterial isolates against *Pythium aphanidermatum*. Acta Physiologiae Plantarum 33: 1933-1944. DOI 10.1007/s11738-011-0742-8

Negreiros, A.M.P., Sales, J.R., León, M., Melo, N.J., Michereff, S.J., Ambrósio, M.M., Medeiros, H.L., and Armengol, J. 2019. Identification and pathogenicity of *Macrophomina* species collected from weeds in melon fields in Northeastern Brazil. Journal of Phytopathology 167: 326-337. DOI: 10.1111/jph.12801

Pal, K.K., Tilak, K.V.B.R., Saxcna, A.K., Dey, R., and Singh, C.S. 2001. Suppression of maize root diseases caused by *Macrophomina phaseolina, Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. Microbiological Research 156 (3): 209-223. http://www.urbanfischer.de/journals/microbiolres

Parmar, H.V., Kapadiya, H.J., and Bhaliya, C.M. 2017. Efficacy of different fungicides against *Macrophomina phaseolina* (Tassi) Goid causing castor root rot. International Journal of Chemical Studies 5:1807-1809.

Podbielska, M., Kus-Liśkiewicz, M., Jagusztyn, B., Piechowicz, B., Sadło, S., Słowik-Borowiec, M., Twaruzek,M., and Szpyrka, E. 2020. Influence of *Bacillus subtilis* and *Trichoderma harzianum* on penthiopyrad degradation under laboratory and field studies. Molecules 25 (6):

1421. https://doi.org/10.3390/molecules25061421

Porto, M.A.F., Ambrósio, M.M.Q., Nascimento, S.R.C., Cruz, B.L.S., and Torres, T.M. 2019. Interaction of *Fusarium solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* as root rot pathogens of *Cucumis melo*. Summa Phytopathologica 45: 355-360.

Pratt, R.G., McLaughlin, M.R., Pederson, G.A., and Rowe, D.E. 1998. Pathogenicity of *Macrophomina phaseolina* to mature plant tissue of alfalfa and white clover. Plant Disease 82: 1033-1038. https://doi.org/10.1094/PDIS.1998.82.9.1033

Rojo, F.G., Reynoso, M.M., Sofia, M.F., and Torres, A.M. 2007. Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. Crop Protection 26: 549-555. https://doi.org/10.1016/j.cropro.2006.05.006

Shah, M.M., and Afiya, H. 2019. Introductory chapter: identification and isolation of *Trichoderma* spp. Their significance in agriculture, human health, industrial and environmental application. Pages 3-14. In: *Trichoderma*-the most widely used fungicide. Edited by M.M. Shah, U. Sharif, and B.T. Rufai, Published in London, UK. https:// doi. org/ 10. 5772/ intec hopen.83528

Shahid, M., Srivastava, M., Singh, A., Kumar, V., Rastogi, S., Pathak, N., and Srivastava, A. 2014. Comparative study of biological agents, *Trichoderma harzianum* (Th-Azad) and *Trichoderma viride* (01PP) for controlling wilt disease in pigeon pea. Journal of Microbial and Biochemical Technology 6: 110-115. DOI: 10.4172/1948-5948.1000130

Siddiquee, S. 2017. Morphology-based characterization of *Trichoderma* species. Pages 41-73. In: Practical handbook of the biology and molecular diversity of *Trichoderma* species from tropical regions. Fungal Biology. Cham Springer International Publishing. https://doi.org/10.1007/978-3-319-64946-7_4

Simonetti, E., Viso, N.P., Montecchia, M., Zilli, C., Balestrasse, K., and Carmona, M. 2015. Evaluation of native bacteria and manganese phosphite for alternative control of charcoal root rot of soybean. Microbiological Research 180: 40-48.doi: 10.1016/j.micres.2015.07.004

Singh, N., Pandey, P., Dubey, R.C., and Maheshwari, D.K. 2008. Biological control of root rot fungus *Macrophomina phaseolina* and growth enhancement of *Pinus roxburghii* (Sarg.) by rhizosphere competent *Bacillus subtilis* BN1. World Journal of Microbiology and Biotechnology 24: 1669-1679. DOI 10.1007/s11274-008-9680-z

Tchameni, S.N., Cotârleț, M., Ghinea, I.O., Bedine, M.A.B., Sameza, M.L., Borda, D., Bahrim, G., and Dinică, R.M. 2020. Involvement of lytic enzymes and secondary metabolites produced by *Trichoderma* spp. in the biological control of *Pythium myriotylum*. International Microbiology 23: 179-188. https://doi.org/10.1007/s10123-019-00089-x

Thilagavathi, R., Saravanakumar, D., Ragupathi, N. and Samiyappan, R. 2007. A combination of biocontrol agents improves the management of dry root rot (*Macrophomina phaseolina*) in greengram. Phytopathologia Mediterranea 46 (2): 157-167.

Torres, M.J., Brandan, C.P., Petroselli, G., Erra-Balsells, R., and Audisio, M.C. 2016. Antagonistic effects of *Bacillus subtilis* subsp. *subtilis* and *B. amyloliquefaciens* against *Macrophomina phaseolina*: SEM study of fungal changes and UV-MALDI-TOF MS analysis of their bioactive compounds. Microbiological Research 182: 31-39. doi:10.1016/j.micres.2015.09.005

Umamaheswari, M., Asokkumar, K., Sivashanmugam, A.T., Remyaraju, A., Subhadradevi, V., and Ravi, T.K. 2009. In vitro xanthine oxidase inhibitory activity of the fractions of *Erythrina stricta* Roxb. Journal of Ethnopharmacology 124 (3): 646-648. https://doi.org/10.1016/j.jep.2009.05.018

Verma, M., Brar, S.K., Tyagi, R.D., Surampalli, R.N., and Valero, J.R. 2007. Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. Biochemical Engineering Journal 37 (1): 1-20. https://doi.org/10.1016/j.bej.2007.05.012

Wheeler, B.E.J. 1969. An introduction to plant disease. John Wiley Sons Ltd, London, UK, 374 pp.

Wu, H., Li, C., Chakraborti, P., Guo, Z., Peng, B., Gu, W., Kang, B., and Gu, Q. 2022. First Report of Watermelon Charcoal Rot (*Macrophomina phaseolina*) in China. Plant Disease 106: 1521. https://doi.org/10.1094/PDIS-07-21-1362-PDN

Zaim, S., Bekkar, A.A., and Belabid, L. 2018. Efficacy of *Bacillus subtilis* and *Trichoderma harzianum* combination on chickpea Fusarium wilt caused by *F. oxysporum* f. sp. *ciceris*. Archives of Phytopathology and Plant Protection 51: 217-226. https://doi.org/10.1080/03235408.2018.1447896

Zanella, E.J., Berghetti, J., Scheidt, B.T., Casa, R.T., Bogo, A., Gonçalves, M.J., and Martins, F.C. 2020. Charcoal rot severity and yield components of common bean cultivars inoculated with *Macrophomina phaseolina*. Summa Phytopathologica 46 (4): 299-304.

Morphological, Morphometrical, Molecular Characterization, and Phylogenetic Relationship of *Paratylenchus holdemani* from Kale (*Brassica oleracea* var. *acephala*) Cultivation Areas in Turkey

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ABSTRACT

Güvercin, B, Akyazı, F, and Yiğit, U. 2025. Morphological, morphometrical, molecular characterization, and phylogenetic relationship of *Paratylenchus holdemani* from kale (*Brassica oleracea* var. *acephala*) cultivation areas in Turkey. Tunisian Journal of Plant Protection 20 (2): 85-95.

Pin nematodes (*Paratylenchus* spp.) have been recorded in association with a wide range of economically significant crops worldwide, including various cereals, vegetables, and ornamental plants. In October 2021, soil samples were collected from kale (*Brassica oleracea* var. *acephala*) growing field in the Giresun province of Turkey. In this study nematodes were extracted from the soil using a modified baermann funnel method. Standard morphological characters were measured and compared to those reported in previous studies. For molecular characterization DNA was extracted from immature females and the D2-D3 expansion region of the 28S rRNA gene was amplified using primer pair D2A (5-ACA AGTACCGTGAGGGAAAGTTG-3) and D3B (5- TCGGAAGGAACCAGCTACTA-3). PCR product (750 bp) was sequenced and then compared with sequences of *Paratylenchus holdemani* available in the GenBank database. The NCBI BLAST analysis of the Turkish population sequences showed 100% similarity with *Paratylenchus holdemani* sequences registered in GenBank. The results obtained from morphological, morphometrical, molecular and phylogenetic relationship studies showed that the pin nematode population was *P. holdemani*.

Keywords: D2-D3, Kale, morphological characters, *Paratylenchus holdemani*, Phylogeny, Turkey

Nematodes, which cause significant damage to cultivated plants worldwide, constitute an important pest group that causes approximately 12% crop loss, and the financial value of this loss is estimated to be around 100 billion dollars (Sasser and Freckman 1987). Pin nematodes (*Paratylenchus* spp.) are obligate ectoparasites that attack many plant species including herbaceous plants, shrubs, and trees that are distributed worldwide (Singh et al. 2021). The genus *Paratylenchus* currently includes more than 100 plant parasitic species (Munawar et al. 2021). Some of those are *P. bukowinensis, P. hamatus, P. sarissus, P. alleni, P. holdemani, P. nainianus* and *P.*

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nanus. Particularly, *Paratylenchus holdemani* Raski, 1975 (Tylenchida: Tylenchulidae) is one of the crucial species from this genus (Singh et al. 2021). It has been reported in many countries such as Spain, El Salvador, Czech Republic, Belgium and Turkey (Clavero-Camacho et al. 2021). In Turkey, it has been reported for the first time on peach (Kepenekci 2001).

Kale crop (*Brassica oleracea* var. *acephala*) has an important place as a nutritional raw material in Turkey. It suffers from crop losses due to diseases, pests and weeds. In particular, nematodes such as *P. holdemani*, are among the important plant pests responsible for some of these losses. Accurate identification of those plant-parasitic nematodes is crucial for effective nematode management in agriculture (Devran and Söğüt 2009). However, the high intraspecific variability within this species presents challenges for identification based solely on morphological characteristics (Palomares-Rius et al. 2018). Consequently, molecular approaches are essential for precise identification (Palomares-Rius et al. 2021). In this study, morphological, morphometric, and molecular characteristics, along with phylogenetic analyses, were utilized to identify *Paratylenchus* populations collected from a kale field in Giresun Province, Turkey.

MATERIALS AND METHODS

Soil sampling and nematode extraction.

Soil samples were collected from kale field at the bases of the plants on October 2021, from Giresun province (40°57'27.0"N 38°48'18.0"E) in the Black Sea region of Turkey (Fig. 1).

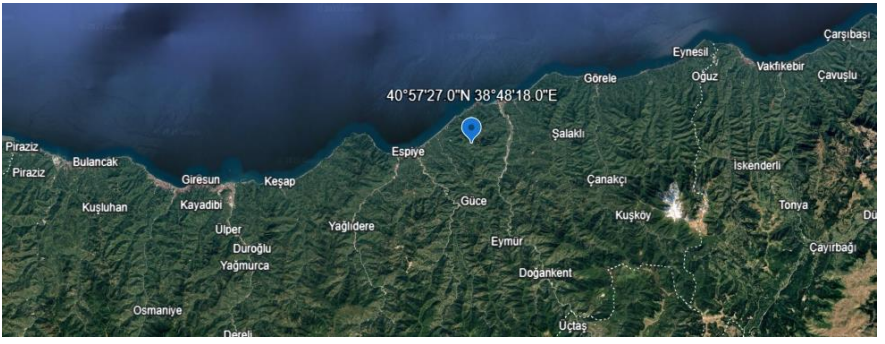

Fig. 1. A map of the location where kale are produced in Giresun

Soil samples were collected in polyethylene bags and transported to the laboratory for analysis. The samples were stored at 4°C until the nematode extraction process. Nematodes were isolated from 100 cm³ of soil using the centrifuge sugar water method (Jenkins 1964). Identification and enumeration of

nematodes were performed under an inverted microscope at 40× magnification.

Morphological characterization.
Standard morphological characters were studied on *P. holdemani*. For morphometric characters, 10 females were used. Nematodes were placed in a drop of pure water on a clean glass slide and killed by heat for 4-6 s. Then, the specimens were examined for morphological characters and morphometric measurements using a camera (AXIOCam 105) mounted on the ZEISS primo Vert microscope (ZEISS,) at a size of 400X. Microsoft Excel was used for the analysis of female morphometric measurements.

DNA extraction.
For molecular studies the genomic DNA were extracted as follows: Each single specimen was collected into 10 µl of extraction buffer (10 mM Tris-HCl, pH 8.8; 1 mM EDTA; 1% Triton X-100 (v/v); 20 mg/ml Proteinase K) in a 1.5 ml Eppendorf tube. Sample tubes were kept at -20°C for 1 night (Pagan et al. 2015). The samples were digested using a glass capillary tube and incubated at 56°C for 1 h, followed by an additional incubation at 95°C for 10 min. The resulting mixture was then utilized as a DNA template for PCR analysis.

PCR amplification.
For the amplification of the 28S rDNA (D2/D3 region of the large subunit, LSU), the primers D2A (5´-ACA AGT ACC GTG AGG GAA AGT TG-3´) and D3B (5´-TCG GAA GGA ACC AGC TAC TA-3´) were used (De Ley et al. 1999). PCR thermocycling was performed using a Mastercycler Veriti (Singapore). The thermocycling conditions included an initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 2 min, with a final extension at 72°C for 7 min.

Electrophoresis.
DNA fragments were separated by electrophoresis in a 1.5% agarose gel using 1X TAE buffer. The gel was stained with ethidium bromide, and visualization was performed using an ErBiyotek GEN-BOX imagERFx system, followed by documentation through photography. The PCR products were then sent to Metabion International AG (Germany) for sequencing analysis.

Phylogenetic analysis.
For the purpose of conducting the phylogenetic analysis, the acquired raw sequences were manually reviewed and edited with BioEDIT version 7.2.5 (Hall 1999). The consensus sequences that were generated were then compared with those stored in the GenBank database utilizing the BLAST search engine to assess sequence homology. The D2/D3 sequences collected in this research, along with those accessed from the GenBank databases (Table 1), were aligned using Clustal W, which facilitated multiple alignments for 31 nucleotide sequences employing MEGA 11.0 software (Kumar et al. 2016). Subsequently, the alignment was examined to determine the model of base substitution for these sequences, again using MEGA 11. Finally, a phylogenetic tree was constructed by applying the Maximum Likelihood Model (ML) with 1000 bootstrap replicates, executed in MEGA 11.0 (Kumar et al. 2016), as illustrated in Fig. 3.

RESULTS
The *Paratylenchus* populations from Turkey that were examined in this research were taxonomically classified and found to possess a phylogenetic

connection to *P. holdemani*. High densities of *P. holdemani* individuals, specifically measuring 130 nematodes per 100 cm³ of soil, were identified in the rhizosphere of kale. Additionally, the morphological, morphometrical, molecular characteristics and phylogenetic associations of the female specimens extracted from soil samples were scrutinized.

Table 1. Species of nematodes used in the phylogenetic analysis, including GenBank accession numbers and origin

Species	GenBank Accession No	Country
Paratylenchus holdemani	MW413636.1	Belgium
Paratylenchus holdemani	MW413639.1	Belgium
Paratylenchus holdemani	MW413637.1	Belgium
Paratylenchus holdemani	MW413634.1	Belgium
Paratylenchus holdemani	MW413640.1	Belgium
Paratylenchus holdemani	MW413642.1	Belgium
Paratylenchus holdemani	MW413641.1	Belgium
Paratylenchus holdemani	MW413635.1	Belgium
Paratylenchus holdemani	MW798300.1	Spain
Paratylenchus holdemani	MZ265107.1	Spain
Paratylenchus holdemani	MZ265106.1	Spain
Paratylenchus holdemani	OQ749960.1	USA
Paratylenchus sp.	ON873243.1	Spain
Paratylenchus sp.	MW413667.1	Belgium
Paratylenchus sp.	MW413665.1	Belgium
Paratylenchus sp.	MW413670.1	Belgium
Paratylenchus sp.	MW319821.1	Belgium
Paratylenchus sp.	OR177652.1	South Korea
Paratylenchus sp.	MN535544.1	Belgium
Paratylenchus tenuicaudatus	OL884401.1	Spain
Paratylenchus tenuicaudatus	OL884411.1	Spain
Paratylenchus tenuicaudatus	OL884403.1	Spain
Paratylenchus tenuicaudatus	OL884421.1	Spain
Paratylenchus tenuicaudatus	MW798307.1	Spain
Paratylenchus nanus	PQ859461.1	USA
Paratylenchus nanus	MH237651.1	USA
Paratylenchus nanus	KY468900.1	South Korea
Paratylenchus bukowinensis	MN088372.1	Iran
Paratylenchus bukowinensis	MN783703.1	Belgium
Paratylenchus caravaquenus	MW798272.1	Spain
Xiphinema simile	ON500623.1	Russia

Morphological and morphometrical characterization.

The nematodes exhibited a transformation from a C-shape when subjected to heat. The head rounded, the pharynx is well-developed, constituting approximately one-fourth of the total body length. The secretory-excretory pore is typically located between the mid-isthmus and the level of the end bulb. The vagina is oriented obliquely, extending to two-thirds of the body width. The tail measures between 25 to 30 µm in length and has a conical shape, characterized by a consistently fine terminal roundness that may occasionally appear blunt. Male is not found.

The female nematodes body length was characterised (387.9±14.4 µm) (Fig. 2A). Stylet length 26±0.9 µm, stylet knobs width 3.5±0.2 µm, stylet knobs height 1.8±0.2 µm on average (Fig. 2B). The maximum body diameter is 17±1.1 µm. The vulva is located close to the posterior parts of the body and its distance from the anterior part is 314±15 µm. The tail is 27.6±1.1 µm on average and the tail ends rounded (Fig. 2C). Table 2 showed comparison of the morphobiometric characteristics of *P. holdemani* females with previously recorded world populations.

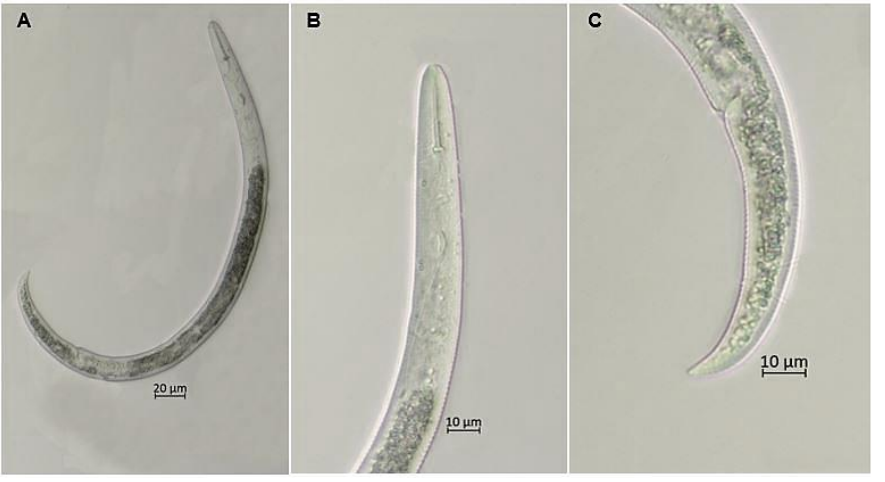

Fig. 2. Light micrographs of female *Paratylenchus holdemani* from Turkey: A: Whole body, B: Female anterior region, C: Tail region.

Table 2. Comparison of the morphobiometric characteristics of *Paratylenchus holdemani* females with previously recorded world populations (All measurements are in µm (except L in µm) and expressed as means ± standard deviation (Min-Max range).

Characters	Turkey Present Study	Belgium Singh et al. (2021)	Spain Clavero-Camacho et al. (2021)	USA Raski (1975)
n	**10**	**31**	**4**	**7**
L	**387.9**±14.4 (359.2-410.3)	**359**±47 (285-475)	**392.3**±30.2 (364-435)	**320** (290-350)
a	**22.9**±1.2 (20.8-24.1)	**20.9**±1.9 (16.4-25.2)	**24.9**±1.6 (23.5-27.2)	**22** (19-24)
b	**4.1**±0 (3.8-5.3)	4.1±0.7 (2.2-5.1)	**3.9**±0.3 (3.6-4.1)	**4** (3.7-4.7)
c	**14.1**±1 (13-15)	14.8±1.4 (12.4-17.7)	**13.3**±1 (12.2-14.6)	**17** (16-19)
c'	**3.1**±0.2 (2.6-3.3)	**2.5**±0.3 (2.1-3.2)	**3**±0.4 (2.7-3.5)	-
Maximum body width	**17**±1.1 (15.4-19.3)	**17.3**±3 (11.3-23.8)	**15.8**±0.6 (15-16.5)	-
Stylet length	**26**±0.9 (24.6-27.4)	**22.5**±2 (19-26.1)	**26.8**±0.6 (26-27.5)	**22** (21-23)
Stylet knobs height	**1.8**±0.2 (1.5-2.2)	-	-	-
Stylet knobs width	**3.5**±0.2 (3.2-3.9)	**3.3**±0.4 (2.9-4.2)	-	-
Pharynx length	**95.8**±8.9 (77.1-103.9)	**89.7**±21.5 (66.1-161)	**100.3**±5.1 (93-105)	-
Anterior end to excretory pore	**84.5**±4 (77.3-89.7)	**74.8**±9.1 (60.1-99)	**85.5**±5.2 (79.5-82)	**73** (67-77)
Anterior end to vulva	**314**±15 (274.9-330.2)	**303**±40.9 (238-391)	-	-
V%	**80.9**±1.6 (76.5-82.3)	**84.3**±1.8 (81.3-90.5)	**81**±0.8 (79.9-81.9)	**85** (84-86)
Body width at anus	**9**±0.6 (8-10)	**10**±1.3 (7.2-12.3)	**9.8**±0.3 (9.5-10)	-
Tail length	**27.6**±1.1 (26.1-29.6)	**25.2**±2.8 (20-29.5)	**29.6**±3.4 (26.5-33.5)	-

Abbreviations: n: number of nematodes measured; L: body length; a: body length/maximum body width; b = body length / pharyngeal length; c: body length/tail length; c': tail length/body width at anus; V%: distance of the vulva from anterior end expressed as a percentage of body length.

Sequence results

The amplification of the ribosomal region 28S rDNA (D2/D3 region of the large subunit, LSU) gene expansion segments produced a single 750 bp fragment, as confirmed by gel electrophoresis (Fig. 3). Comparing the sequences of the ribosomal region 28S rDNA (D2/D3 region of the large subunit, LSU) gene obtained from PCR products of

P. holdemani in Turkey with those present in the GenBank database revealed a whole similarity (100%) with the species *P. holdemani* (MW413637.1). The phylogenetic relationships of the Turkey population of *P. holdemani* with other populations using the 28S rDNA region are also depicted in Fig. 3.

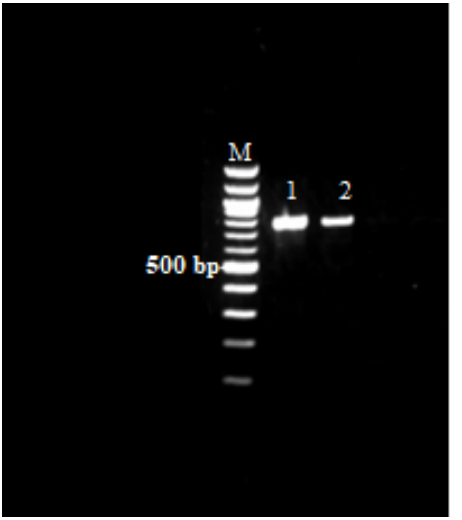

Fig. 3. PCR products of *Paratylenchus holdemani* species. (a) 28S rDNA (D2/D3 region of the large subunit, LSU) gene using D2A/D3B primer pair (Line1-2).

Phylogenetic analysis

Phylogenetic analysis was conducted to determine the evolutionary relationships of *P. holdemani*, as presented in Figure 4. The phylogenetic tree was reconstructed using 31 sequences, including 12 sequences from the *P. holdemani* group, 7 from the *Paratylenchus* spp. group, 5 from the *P. tenuicaudatus* group, 3 from *P. nanus*, 2 from *P. bukowinensis*, and 1 from *P. caravaquenus*, with *Xiphinema simile* used as the outgroup taxon. Alignment and phylogenetic analysis of the D2-D3 sequences revealed multiple clades, which were distinguished by varying bootstrap support (BS) values in the Maximum Likelihood (ML) analysis.

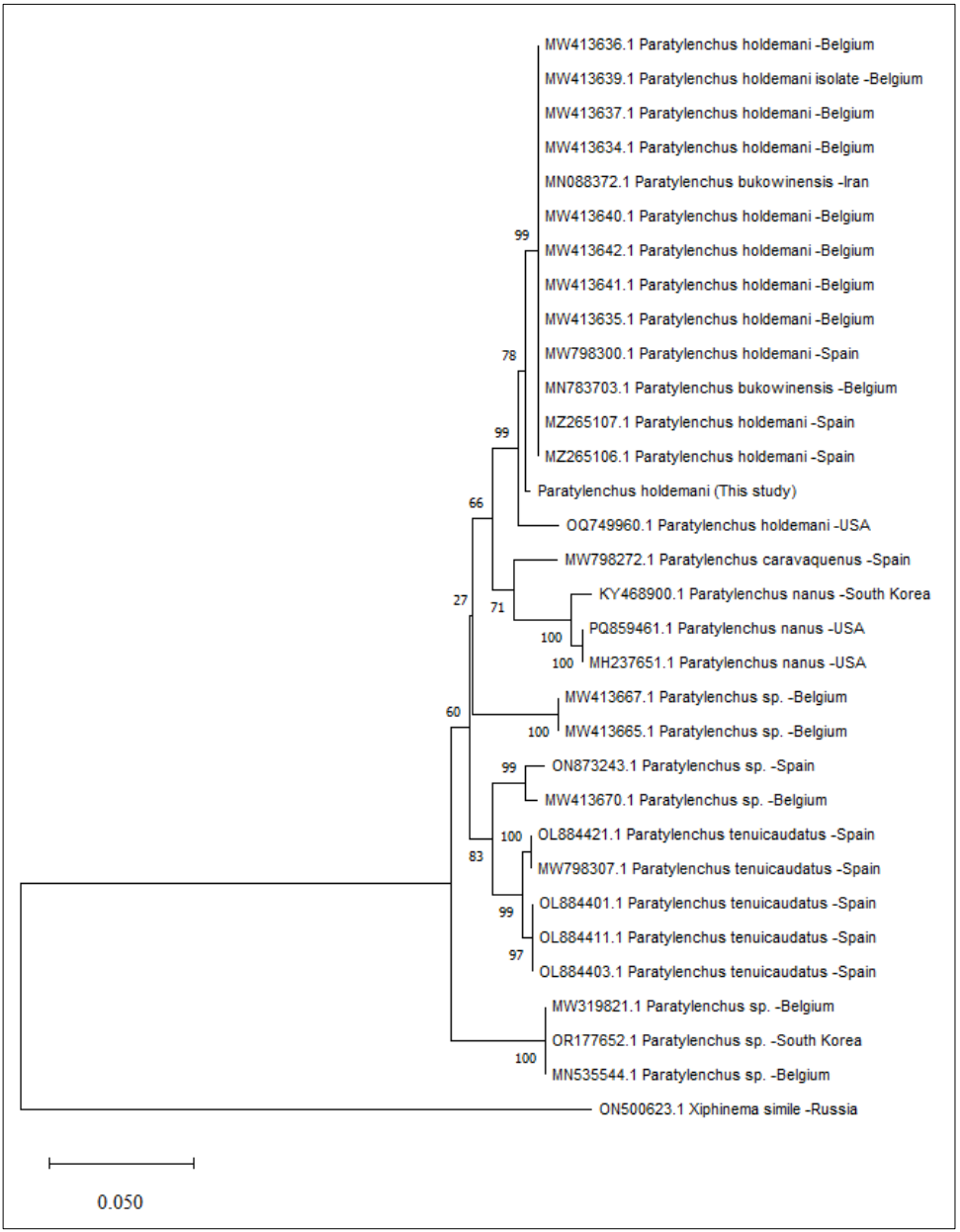

Fig. 4. Maximum likelihood (ML) phylogenetic tree of *Paratylenchus holdemani*, inferred from D2 expansion segment of LSU rDNA. The analysis was using 1000 bootstrap replicates. *P. holdemani* (Turkey) obtained from this study. *Xiphinema simile* sequences were used as out group for the construction of phylogram.

DISCUSSION

Although morphological identification methods are important in nematology, molecular methods are increasingly applied in nematode diagnostics due to their ability to provide precise identification. As a result, ribosomal DNA has emerged as the preferred marker for identifying nematodes. The ribosomal ITS regions of nematodes are highly variable, making them particularly useful for diagnostic purposes (Subbotin et al. 2011). Precise identification of plant-parasitic nematode species that adversely impact crop productivity is essential for the development of effective control strategies.

Previous molecular studies have reported identical D2–D3 expansion segment sequences for populations identified as *P. holdemani* from Belgium and Spain. The molecular results obtained in the present study are very similar to those reports. These findings support the view that the sequences belong to *P. holdemani* and confirm the usefulness of molecular markers for reliable species identification in different geographical regions (Singh et al. 2021).

P. holdemani has been reported on almonds in Spain, on coffee in El Salvador, on unidentified host in USA and in wild olive in the southern Iberian Peninsula (Clavero-Camacho et al. 2021, Álvarez-Ortega et al. 2023). Turkish population of *P. holdemani* has a slightly shorter mean body length than that of specimens from Spanish population (387 µm vs. 392.3). However, compared with that of the Belgium and USA (359 µm vs. 320 µm), it is slightly longer, respectively. The stylet of the Turkish population is longer than that of Belgium and USA specimens (24.6-27.4 µm vs. 19-26.1 µm and 21-23 µm) but compared with that of the Spain stylet (24.6-27.4 vs. 26-27.5 µm), it is slightly shorter. The stylet knobs width of the Turkish population are furthermore almost the same than that of Belgium (3.5 µm vs. 3.3 µm). The pharynx length of the Turkish population is furthermore longer than that of Belgium (95.8 µm vs. 89.7 µm) but compared with that of the Spain pharynx length (95.8 µm vs. 100.3 µm), it is shorter. The variations observed in the measurements are believed to be influenced by climatic and regional conditions.

In conclusion, *P. holdemani* was discovered in kale soil in the Giresun provinces in Turkey. The identification presents a significant opportunity for future nematological studies, such as the identification of different species. As a result, *P. holdemani* in kale fields offers valuable insights that can fuel research aimed at enhancing control measures for *Paratylenchus* species, ultimately leading to increased kale yield. We underscore the continued need for further investigation into the genus *Paratylenchus* to mitigate potential yield losses in kale production.

RESUME

Güvercin B., Akyazı F. et Yiğit U. 2025. Caractérisation morphologique, morphométrique et moléculaire, et relations phylogénétiques de *Paratylenchus holdemani* dans les zones de culture du chou frisé (*Brassica oleracea* var. *acephala*) en Turquie. Tunisian Journal of Plant Protection 20 (2): 85-95.

Les nématodes des pins (*Paratylenchus* spp.) sont fréquemment associés à une grande variété de cultures importantes à travers le monde, notamment diverses céréales, légumes et plantes ornementales. En Octobre 2021, des échantillons de sol ont été prélevés dans un champ de chou frisé (*Brassica oleracea* var. *acephala*) de la province de Giresun en Turquie. Dans cette étude, les nématodes ont été extraits du sol par la méthode de l'entonnoir de Baermann modifiée. Leurs caractères morphologiques standards ont été mesurés et comparés à ceux rapportés dans des études antérieures. Pour la caractérisation moléculaire, l'ADN a été extrait de femelles immatures et la région d'extension D2-D3 du gène de l'ARNr 28S a été amplifiée à l'aide des amorces D2A (5'-ACA AGTACCGTGAGGGAAAGTTG-3') et D3B (5'-TCGGAAGGAACCAGCTACTA-3'). Le produit de PCR (750 pb) a été séquencé puis comparé aux séquences de *Paratylenchus holdemani* disponibles dans la base de données GenBank. L'analyse BLAST du NCBI des séquences de la population turque a révélé une similarité de 100% avec les séquences de *P. holdemani* enregistrées dans GenBank. Les résultats des études morphologiques, morphométriques, moléculaires et phylogénétiques ont confirmé que la population de nématodes appartenait à l'espèce *P. holdemani*.

Mots-clés: D2-D3, caractères morphologiques, chou frisé, *Paratylenchus holdemani*, phylogénie, Turquie

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ملخص

الخصائص المورفولوجية والمورفومترية والجزيئية والعلاقة التطورية لنماتودا Paratylenchus holdemani في مناطق زراعة الكرنب الأجعد (*Brassica oleracea* var. *acephala*) في تركيا.

Tunisian Journal of Plant Protection 20 (2): 85-95.

تم تسجيل وجود النيماتودا الصنوبريات (*Paratylenchus* spp.) في مجموعة واسعة من المحاصيل ذات الأهمية الاقتصادية حول العالم، بما في ذلك أنواع مختلفة من الحبوب والخضراوات ونباتات الزينة. في أكتوبر 2021، جُمعت عينات من التربة من حقل زراعة الكرنب الأجعد (*Brassica oleracea* var. *acephala*) في محافظة غيرسون بتركيا. في هذه الدراسة، عُزل النيماتودا من التربة باستخدام طريقة قمع بايرمان (Baermann) المُعدلة. قِيست الصفات المورفولوجية القياسية وقورنت بتلك المذكورة في الدراسات السابقة. وللتحليل الجزيئي، استُخلص الحمض النووي من الإناث غير الناضجة، وجرى تضخيم منطقة التوسع D2-D3 من جين S rRNA28 باستخدام زوج البادئات D2A (5-ACA AGTACCGTGAGGGAAAGTTG-3) و D3B (5-TCGGAAGGAACCAGCTACTA-3). تم تنفيذ التسلسل الناتج تفاعل البوليميراز المتسلسل (750 زوجًا قاعديًا) ثم مقارنته بتسلسلات *Paratylenchus holdemani* المتوفرة في قاعدة بيانات GenBank. أظهر تحليل NCBI BLAST لتسلسلات العينة التركية تطابقًا بنسبة 100% مع تسلسلات *P. holdemani* المسجلة في GenBank. وأظهرت نتائج الدراسات المورفولوجية والمورفومترية والجزيئية ودراسات العلاقات التطورية أن مجموعة النيماتودا هي *P. holdemani*.

الكلمات المفتاحية: تركيا، صفات مورفولوجية، علم النشوء والتطور، كالي، D2-D3، *Paratylenchus holdemani*

LITERATURE CITED

Álvarez-Ortega, S., Subbotin, S.A., Wang, K.H., Stanley, J.D., Vau, S., Crow, W., and Inserra, R.N. 2023. Morphological and molecular diversity among pin nematodes of the genus *Paratylenchus* (Nematoda: Paratylenchidae) from Florida and other localities and molecular phylogeny of the genus. Plants 12: 2770. https://doi.org/10.3390/plants12152770

Clavero-Camacho, I., Palomares-Rius, J.E., Cantalapiedra-Navarrete, C., León-Ropero, G., Martín-Barbarroja, J., Archidona-Yuste, A., and Castillo, P. 2021. Integrative taxonomy reveals hidden cryptic diversity within pin nematodes of the genus *Paratylenchus* (Nematoda: Tylenchulidae). Plants 10: 1454. https://doi.org/10.3390/plants10071454

Cobb, N.A. 1923. Notes on *Paratylenchus*, a genus of nematodes. Journal of the Washington Academy of Science 13: 251-257.

De Ley, P., Félix, M.A., Frisse, L.M., Nadler, S.A., Sternberg, P.W., and Thomas, K.W. 1999. Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). Nematology 1: 591-612.

Devran, Z., and Söğüt, M.A. 2009. Distribution and identification of root-knot nematodes from Turkey. Journal of Nematology 41: 128.

Edward, J.C., and Mısra, S.L. 1963. *Paratylenchus nainianus* n. sp. (Nematoda: Criconematidae) from Uttar Pradesh, India. Nematologica 9: 215-217.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In Nucleic acids symposium series 41: 9598.

Jenkins, W.R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48: 692.

Kepenekci, İ. 2001. Plant parasitic nematodes of Tylenchida (Nematoda) associated with stone fruits (apricots and peaches) in Southern Turkey. Pakistan Journal of Nematology 19: 49-61.

Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870-1874. https://doi.org/10.1093/molbev/msw054

Micoletzky, H. 1922. Die Freilebenden Erd-Nematoden. Archiv für Naturgeschichte, Berlin A. 87: 1-650.

Munawar, M., Yevtushenko, D.P., Palomares-Rius, J.E., and Castillo, P. 2021. Species diversity of pin nematodes (*Paratylenchus* spp.) from potato growing regions of southern Alberta, Canada. Plants 10: 188. https://doi.org/10.3390/plants10020188

Pagan, C., Coyne, D., Carneiro, R., Kariuki, G., Luambano, N., Affokpon, A., and Williamson, V. M. 2015. Mitochondrial haplotype-based identification of ethanol-preserved root-knot nematodes from Africa. Phytopathology 105: 350-357. https://doi.org/10.1094/PHYTO-08-14-0225-R

Palomares-Rius, J.E., Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Azpilicueta, A.S., Saborido, A., Tzortzakakis, E.A., and Castillo, P. 2021. New distribution and molecular diversity of the reniform nematode *Rotylenchulus macrosoma* (Nematoda: Rotylenchulinae) in Europe. Phytopathology 111: 720-730. https://doi.org/10.1094/PHYTO-04-20-0148-R

Palomares-Rius, J.E., Cantalapiedra-Navarrete, C., Archidona-Yuste, A., Tzortzakakis, E.A., Birmpilis, I.G., Vovlas, N., and Castillo, P. 2018. Prevalence and molecular diversity of reniform nematodes of the genus Rotylenchulus (Nematoda: Rotylenchulinae) in the Mediterranean Basin. European Journal of Plant Pathology 150: 439-455. https://doi.org/10.1007/s10658-017-1292-8

Raski, D.J. 1975. Revision of the genus *Paratylenchus micoletzky*, 1922 and descriptions of new species. Part II of three parts. Journal of Nematology 7: 274.

Sasser, J.N., and Freckman, D.W., 1987. A world perspective on nematology: The role of the Society. Pages 7-14. In: Veech, J.A. and Dickson, D.W., Eds., Vistas on Nematology, Hyattsville, Maryland, USA.

Singh, P.R., Karssen, G., Couvreur, M., Subbotin, S.A., and Bert, W. 2021. Integrative taxonomy and molecular phylogeny of the plant-parasitic nematode genus *Paratylenchus* (Nematoda: Paratylenchinae): Linking species with molecular barcodes. Plants 10: 408. https://doi.org/10.3390/plants10020408

Subbotin, S.A., Deimi, A.M., Zheng, J., and Chizhov, V.N. 2011. Length variation and repetitive sequences of internal transcribed spacer of ribosomal RNA gene, diagnostics and relationships of populations of potato rot nematode, *Ditylenchus destructor* Thorne, 1945 (Tylenchida: Anguinidae). Nematology 13: 773-785.

Tarjan, A.C. 1960. A review of the genus *Paratylenchus micoletzky* 1922 (Paratylenchinae: Nematoda) with a description of two new species. Annals of the New York Academy of Sciences 84: 331-332.

Thorne, G., and Allen, M.W. 1950. *Paratylenchus hamatus* n. sp, and *Xiphinema index* n. sp., two nematodes associated with fig roots, with a note on *Paratylenchus anceps* Cobb. Proceedings of the Helminthological Society of Washington 17: 27-35.

Management of Thrips Species in Citrus Groves in the Marrakech Region: Exploring Biological Control Methods as Alternatives to Chemical Methods

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ABSTRACT

Khallou, A., Smaili, M.C., Mennani, M., Haddad, N., Kokiçi, H., And Boutaleb-Joutei, A. 2025. Management of thrips species in citrus groves in the Marrakech region: Exploring control methods as alternatives to chemical methods. Tunisian Journal of Plant Protection 20 (2): 97-116.

Thrips, which historically caused minimal damage to Moroccan citrus orchards, have become a significant concern since 2018. A study was conducted from March to July 2021 in a citrus orchard in the Marrakech region and aimed to identify thrips species and natural enemies, monitor population dynamics, and evaluate alternative control methods. Two trials were conducted on the effect of several products on thrips species in the citrus orchards; the first one (chemical trial) where Flonicamib, Spirotetramat, Formetanate, Acetamiprid, Cyantaraniliprole, and Abamectine were assessed, the second trial (biological control), Pyrethrum, Neem oil, *Beauveria bassiana*, a mixture of Azadirachtin and paraffinic mineral oil, a mixture of Pyrethrum and neem oil, and a mixture Pyrethrum and azadirachtin were evaluated. Six thrips species were recorded: *Frankliniella occidentalis*, *Thrips tabaci*, *Pezothrips kellyanus*, *Scirtothrips* sp., *Aeolothrips* sp., and *Haplothrips* sp. Population peaks varied by citrus variety, with adult thrips reaching their highest levels during petal fall for clementine Nules (4 ± 1 individuals/beating) and during summer shoot growth for mandarin Afourer (8.8 ± 1 individuals/beating), while larval populations peaked 10 days later on Nules but remained low on Afourer. Four predatory; *Coccinella septempunctata*, *Orius* sp., *Chrysoperla carnea*, and *Aeolothrips* sp. were found on citrus trees and their population peaked during petal fall. Formetanate showing the highest efficacy (88.7% after 3 days), while Spirotetramat was less effective to control thrips species. *Beauveria bassiana* and the mixture of Azadirachtin and paraffinic mineral oil were two treatments that showed the highest significant efficacy (75.9% and 78.82 %, respectively, after two weeks). These findings underscore the increasing threat of thrips species in commercial Moroccan citrus orchards and highlight the potential of

integrated pest management strategies that combine chemical and biological control for sustainable thrips management.

Keywords: Biopesticides, Citrus, Insecticides, Morocco, *Pezothrips kellyanus*, *Scirthothrips* sp.

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Thrips species (Thysanoptera: Thripidae) represent a diverse group of tiny insects, with nearly 6000 species described worldwide, many of which are associated with cultivated plants (Cloyd 2016, Morse, and Hoddle 2006, Mouden et al. 2017, Nault, and Shelton 2010, Reynaud 2010, Reitz et al. 2011, Reitz et al. 2020). While most species are innocuous (Mound et al. 2022), several have emerged as key agricultural pests due to their capacity to cause direct feeding damage, transmit plant viruses, and reduce the marketability of fruits and vegetables. Citrus crops are no exception, and numerous thrips species have been reported as economically significant pests in citrus orchards (Blank, and Gill 1997, Costa et al. 2006, Mueller et al. 2019, Navarro-Campos et al. 2013, Rao et al. 2019, Vassiliou 2007, 2008).

Several thrips species are known to infest citrus flowers, leaves, and fruits, resulting in scarring, silvering, and deformation that substantially reduce fruit quality and commercial value. *Scirtothrips citri* (Moulton) is considered a major pest of citrus in California (Flint e*t* al. 1991, Mueller et al. 2019), whereas *Pezothrips kellyanus* (Bagnall) has become a primary citrus pest in Australia (Mound, and Jackman 1998) and in several Mediterranean countries, including Spain (Navarro-Campos et al. 2011, 2013), Italy (Conti et al. 2001, Marullo 1998), Cyprus (Vassiliou 2007, 2008), Tunisia (Belaam-Kort, and Boulahia-Kheder 2017, Belaam-Kort et al. 2020a), and Morocco (Abbassi 2014). Other species, such as *Frankliniella occidentalis* (Pergande) and *Thrips tabaci* (Lindeman), are also frequently recorded in citrus groves and are among the most destructive and polyphagous pests worldwide (Belaam-Kort et al. 2021, Cloyd 2016, De Grazia, Marullo 2013, Donghwang et al. 2000, Makabe et al. 2014, Reitz 2009, Reitz et al. 2020, Tsuchiya, and Furuhashi 1993).

Thrips control has been based on chemical sprays, especially organophosphates (i.e., chlorpyrifos), carbamates (i.e., methomyl), neonicotinoids (i.e., imidacloprid and acetamiprid), and microbial pesticides (i.e., Spinosad) (Childers 1992, Colloff et al. 2003, Conti et al. 2001, Rao et al. 2019, Vassiliou 2007). Although these compounds provide effective short-term control, their repeated use has resulted in widespread resistance. Documented cases include resistance of *P. kellyanus* to chlorpyrifos (Baker et al. 2004, Purvis 2002), *Scirtothrips aurantii* (Faure) to pyrethroids such as cypermethrin, deltamethrin, and lambda-cyhalothrin (Rattan 1992), *S. citri* to dimethoate and formetanate (Immaraju et al. 1989, 1990), and *F. occidentalis* to a wide range of both chemical and biological pesticides (Gao et al. 2012). Beyond resistance concern, the use of broad-spectrum insecticides has severe ecological drawbacks, often disrupting beneficial arthropods including natural enemies such as predators, parasitoids, and pollinators that are crucial for citrus ecosystem resilience (Belaam-Kort et al. 2020b, Jacas et al. 2010, Mansour et al. 2018).

These limitations have prompted increasing attention to more sustainable, eco-friendly strategies. Biorational approaches, such as the release of predators (i.e., *Chrysoperla carnea* (Stephens), *Coccinella septempunctata* (Linnaeus)) and parasitoids, or the application of entomopathogenic fungi like *Beauveria bassiana*, are being explored as alternatives to conventional pesticides (Crisp, and Baker 2011, Baker 2016, Wu et al. 2016, Navarro-Campos et al. 2020). In Mediterranean citrus orchards, surveys revealed a diverse thrips fauna, but only a few species consistently reached pest status. For instance, in Tunisia, 21 thrips species were recorded in citrus, though *P. kellyanus*, *F. occidentalis*, and *Thrips major* (Uzel) were the most prevalent (Trabelsi, and Boulahia-Kheder 2009, Belaam-Kort, and Boulahia-Kheder 2017, Belaam-Kort et al. 2020a). In Morocco, recent studies reported the presence of *P. kellyanus* and *F. occidentalis* in major citrus-growing regions such as Souss Massa and Marrakech-Safi, where they have been associated with significant fruit scarring and economic losses (Abbassi 2014, Smaili et al. 2018, 2020). Recent studies by Belaam-Kort and Boulahia-Kheder (2019) reported that fruit scarring caused by thrips affected approximately 20% of fruits across all citrus species and orange varieties. Such levels of damage lead to significant downgrading of fruit quality and consequently substantial economic losses. Recently, emphasis has been placed on implementing safer and sustainable pest management strategies to control the thrips insects in Morocco. This requires a deeper understanding of thrips population dynamics, their relative abundance across citrus phenological stages, the role of native natural enemies, and the efficacy of alternative control measures. In this context, the present study aimed to (i) identify the thrips species present and their associated natural enemies, (ii) assess the seasonal abundance and crop damage caused by thrips populations, and (iii) evaluate the effectiveness of selected biological, botanical, and chemical insecticides under field conditions. By integrating ecological observations with control efficacy trials, this research provides insights that may guide the development of integrated pest management (IPM) programs tailored to Moroccan citrus systems.

MATERIALS AND METHODS

Study sites.

The study was conducted within a commercial citrus orchard with approximately 500 ha in the Marrakech-Safi region at El Bahja (Les Domaines Agricoles, Morocco; 31.608975° N, 8.123152° W). The orchard comprises a wide range of citrus species, including clementines, mandarins, and oranges. Two experimental plots were selected for thrips monitoring and control trials: i) Afourer plot for control trial (a Mandarine *Citrus riticulata* Blanco var. Afourer), planted on 25/06/2019, grafted on C35 rootstock, with a planting density of 6 m × 2 m and covering 2.26 ha, where chemical and biological applications were conducted; and ii) Nules (Clementine *Citrus clementina* var., Nules) with four plots for monitoring (P1, P2, P3, P4), planted on 30/07/2008, grafted on *Citrus volkameriana* rootstock, with a planting density of 6 m × 2 m and covering 2.3, 2.5, 3, and 5 ha. Throughout the experimental period, the plot designated for the trial was not subjected to any phytosanitary treatments, nor in the three months prior to the trial.

Thrips species identification and population monitoring.

Thrips adult specimens were collected from citrus trees between March and July 2021 using beating method with Fauvel funnel method (Fauvel et al., 1981). Thrips were sampled from all citrus varieties present in the orchard, including *Citrus sinensis* (var., Navel), *Citrus clementina* (var., Orogrande, var., Nules, var., Bruno, and var., Nour), and *Citrus reticulata* (var., Afourer). For each variety, thrips were collected by beating 60 citrus twigs obtained from 10 trees (i.e., six twigs per tree). Each twig was gently struck over a white tray to dislodge adult and larval thrips, which were then collected using a fine brush and preserved in 70% ethanol for identification.

For the ground vegetation, twelve weed samples were collected beneath the canopy of the same trees, mainly belonging to the genus *Sinapis*. Weed foliage was shaken over a tray using the same procedure to collect thrips individuals. All collected individuals were preserved in 70% ethanol for subsequent laboratory identification. Specimens were examined using an Olympus CX23 compound microscope and identified based on morphological keys provided in Mound, and Palmer (1981) and Navarro-Campos (2013).

Population monitoring of adults and larvae, however, was concentrated on the Nules variety, which exhibited the most significant thrips infestation. To ensure robust and representative observations, monitoring was carried out across several spatially separated four Nules plots within the same farm.

Monitoring focused on three key periods: flowering, fruit set, and 6-8 week post-petal fall. During flowering, 50 southeast-facing branches per plot (25 cm each) were tapped five times into plastic funnels to collect thrips and natural enemies. Predatory mites were sampled weekly from 100 leaves per plot. Observations for the Nules variety were conducted weekly from early April to mid-May. Natural enemies were identified using keys and illustration in Marullo (2003) and Déroulez et al. (2014).

Chemical trials.

The chemical control trial was conducted on the Afourer variety, as the thrips populations on other citrus varieties were insufficient to allow several replicates. Thrips sampling was conducted during a single trial in 2021, specifically during the fruit enlargement period, when thrips density typically reaches its peak. This timing was selected based on preliminary observations and because a notably high population was recorded on the Afourer mandarin variety, using a 16 L backpack sprayer with an adjustable nozzle to ensure uniform coverage of fine droplets capable of reaching larvae within fruit crevices. Each tree received 1.2 L of solution, and four young shoots per plot were observed, with buffer zones of one row of trees separating the plots to prevent cross-contamination. Seven chemical treatments were evaluated: T0 (untreated control), T1 (Flonicamid, 10 g/hl), T2 (Spirotetramat, 100 cc/hl), T3 (Formetanate, 50 g/hl), T4 (Acetamiprid, 30 g/hl), T5 (Cyantraniliprole, 100 cc/hl), and T6 (Abamectin, 15 cc/hl). A Completely Randomized Block Design with four replications was used within the Afourer plot (Fig. 1, Table 1). The trial was not repeated in the following year due to the low thrips population, which would not have allowed meaningful replication.

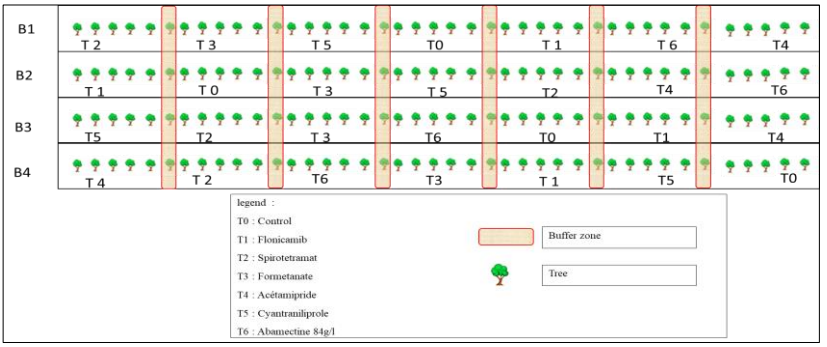

Fig. 1. Experimental set-up for the chemical control trial on the Afourer variety during 2021.

Biological trials.

The biological control trial was conducted on the Afourer variety due to insufficient thrips populations on other citrus varieties to allow meaningful replication. The trial evaluated the efficacy of several organic products, including Azadirachtin, Pyrethrum, Neem oil, and *Beauveria bassiana*, selected because of the lack of registered active ingredients for thrips control on citrus in the country. Seven modalities were assessed: T0 (untreated control), T1 (Pyrethrum, 1.5 l/ha with 1000 l/ha spray volume), T2 (Neem oil, 1 l/hl), T3 (*B. bassiana*, 40 cc/hl), T4 (Azadirachtin, 100 cc/hl + paraffinic mineral oil, 2 l/hl), T5 (Pyrethrum + neem oil), and T6 (Pyrethrum + azadirachtin, 100 cc/hl) (Fig. 2, Table 1).

The experiment was arranged as a randomized block design with four replications, including buffer zones between plots to minimize drift and mitigate the effect of the prevailing west-to-east wind gradient. Trees were evenly distributed across the plots to ensure consistent assessment of efficacy assessment. This trial was not repeated in the following year due to the low thrips population, which would not have allowed meaningful replication.

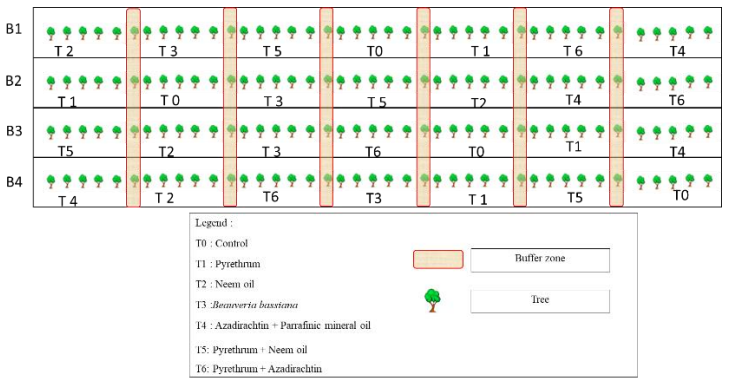

Fig. 2. Experimental set-up for the organic trial on the Afourer variety during 2021.

Table 1. Insecticide treatment used in field trials in 2021

Treatment	Active ingredient	Commercial name	Dose
Chemical	Flonicamid	TEPPEKI WG 500 WG	10 g/hl
	Spirotetramat	MOVENTO 100 SC	100 cc/hl
	Formetanate	DICARZOL 500 SG	50 g/hl
	Acetamiprid	MOSPILAN 20 SP	30 g/hl
	Cyantraniliprole	EXIREL TM	100 cc/hl
	Abamectin	AGRIMEC GOLD SC	15 cc/hl
Biological	Pyrethrum	PYRECRIS 20 EC	150cc/hl
	Neem oil	TRIACT 90 EC	1 L/hl
	Beauveria bassiana souche ATCC 74040	NATURALIS OD	40 cc/hl
	Azadirachtin	NEEMIX 4,5 EC	100 cc/hl
	Paraffinic mineral oil	INSECTICIDE 101 TOP	2%

Statistical analysis.

All statistical analyses were conducted using IBM SPSS Statistics (Version 21). Data obtained from the randomized complete block design were analyzed using one-way analysis of variance (ANOVA) to assess the effect of treatments on the number of adult thrips per beating. The effects on larval stages and beneficial organisms were not analyzed because their abundances were too low across the experimental units. When significant differences were detected, means were separated using the Student-Newman-Keuls (SNK) test at a significance level of $p \leq 0.05$, after verification of data normality. Treatment efficacy was evaluated using two complementary indices. The percentage reduction (Ri) was calculated as: Ri (%) = (1-Ti/T0i) × 100; where Ti is the mean of the studied variable (count) in treated plots, and T0i is the mean control plots (Abbott 1925). The treatment efficacy (Ei) was calculated according to Henderson, and Tilton (1955) as: Ei (%) = [(1- T0i befor treatment × Ti after treatment)/(T0i after treatment × Ti before treatment)] × 100; where T0i and Ti represent the mean of the studied variable in control and treated plots, respectively, before and after treatment (Henderson, and Tilton 1995).

RESULTS

Thrips species and natural enemies.

During this survey, six thrips species associated with citrus were identified; *F. occidentalis*, *T. tabaci*, *P. kellyanus*, *Haplothrips* sp., *Aeolothrips* sp., and *Scirtopthrips* sp. (Fig. 3, Table 2).

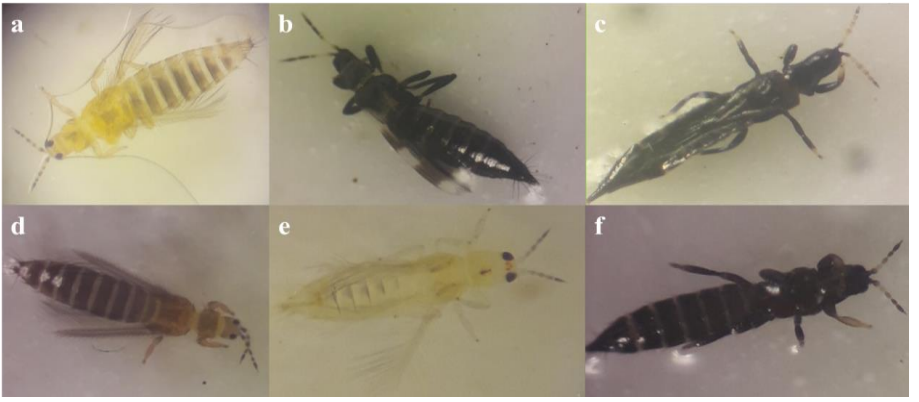

Fig. 3. Thrips species associated with citrus orchards in Marrakech during 2021: **A.** *Frankliniella occidentalis*, **B.** *Aeolothrips* sp., **C.** *Haplothrips* sp., **D.** *Thrips tabaci*, **E.** *Scirtopthrips* sp., and **F.** *Pezothrips kellyanus*.

Table 2. Species of thrips found on citrus crops in the Marrakech region in 2021

Suborder	Family	subfamily	Species
Terebrantia	Thripidae	Thripinae	*Frankliniella occidentalis* (Pergande)
			Pezothrips kellyanus (Bagnall)
			Sirtopthrips sp.
			Thrips tabaci (Lindeman)
Aeolothripidae	Aeolothripinae	-	*Aeolothrips* sp.
Phlaeothripidae	Phlaeothripinae	-	*Haplothrips* sp.

In parallel, only a few beneficial species were observed during the study period (Table 3). These included *C. carnea*, the seven-spotted lady beetle *Coccinella septempunctata*, the predatory thrips *Aeolothrips* sp., the minute pirate bug *Orius* sp., and the predatory mite *Euseius* sp. In addition, several unidentified spider species were also noted in the orchards.

Table 3. Natural enemies collected in citrus orchards in the Marrakech region during 2021

Order	Family	Species
Neuroptera	Chrysopidae	*Chrysoperla carnea* (Stephens)
Coleoptera	Coccinellidae	*Coccinella septempunctata* (Linnaeus)
Thysanoptera	Aeolothripidae	*Aeolothrips* sp.
Hemiptera	Anthocoridae	*Orius* sp.
Mesostigmata	Phytoseiidae	*Euseius* Stipulatus (Athias-henriot)
Araneae	-	Several species

Dynamics of thrips densities and naturel enemies on citrus fruit.

Population monitoring highlighted clear differences among thrips species. *T. tabaci* and *F. occidentalis* were consistently more abundant than the other recorded species. The population of *T. tabaci* increased rapidly from early April, reaching a maximum of approximately two individuals per strike in parcel P2, and then declined sharply, dropping below 0.2 individuals per beating by April 20. Similarly, *F. occidentalis* densities rose after early April, reaching a maximum approximately 12 days later, and subsequently declined to near-zero levels after May 10. In contrast, *P. kellyanus* remained scarce throughout the observation period, with mean densities below 0.1 individuals per strike during flowering, and disappeared completely thereafter (Fig. 4).

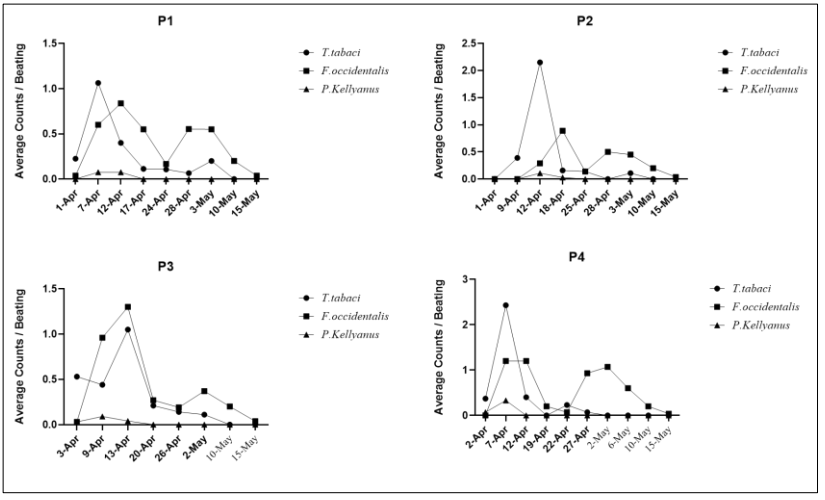

Fig. 4. Dynamics of thrips species on the Nules variety (P1-P4) in Marrakech during 2021.

At the larval stage, the first individuals were detected around April 7, with maximum densities observed on April 22. Adult populations increased rapidly across all four parcels from early April, reaching a maximum of 2.5 individuals per strike in parcel P2. This peak was followed by a pronounced decline by mid-April, coinciding with the end of petal fall. Thrips adult densities decreased sharply from the second week of May and reached undetectable levels across all parcels by May 15 (Fig. 5). The temporal overlap between adults (at the end of flowering) and larvae (during petal fall) highlights the close synchrony of thrips population dynamics with citrus phenology.

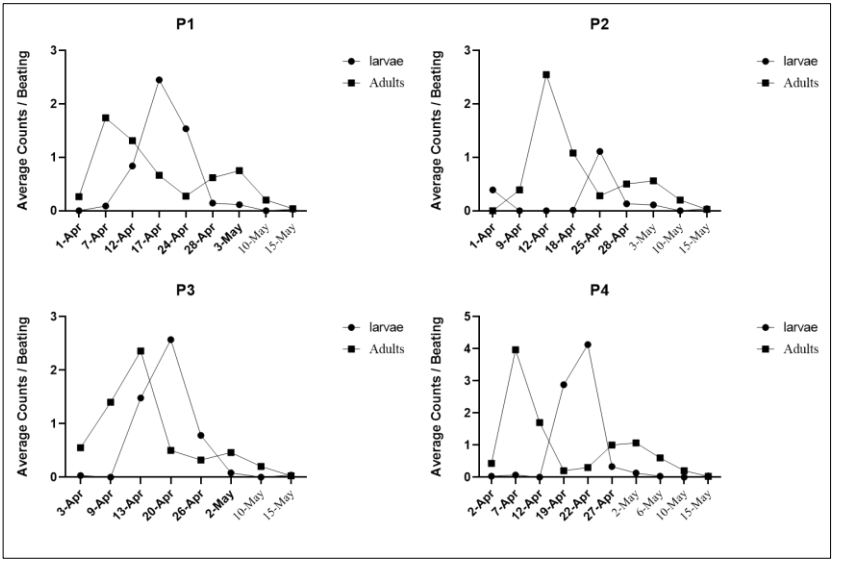

Fig. 5. Dynamics of thrips populations in Marrakech citrus orchards during 2021at four parcels (P1-P4).

Monitoring of natural enemies associated with thrips revealed distinct temporal dynamics across the four studied parcels (Fig. 6). Among the recorded predators, spiders were by far the most abundant, with densities peaking between late April and early May, reaching values above 0.8 individuals per beating in parcels P2 and P3. Other natural enemies such as *C. septempunctata*, *C. carnea*, *Orius* sp., and *Aeolothrips* sp. were detected only sporadically and in much lower numbers (≤ 0.2 individuals per beating). Their occurrence was generally restricted to the flowering and early fruit set stages.

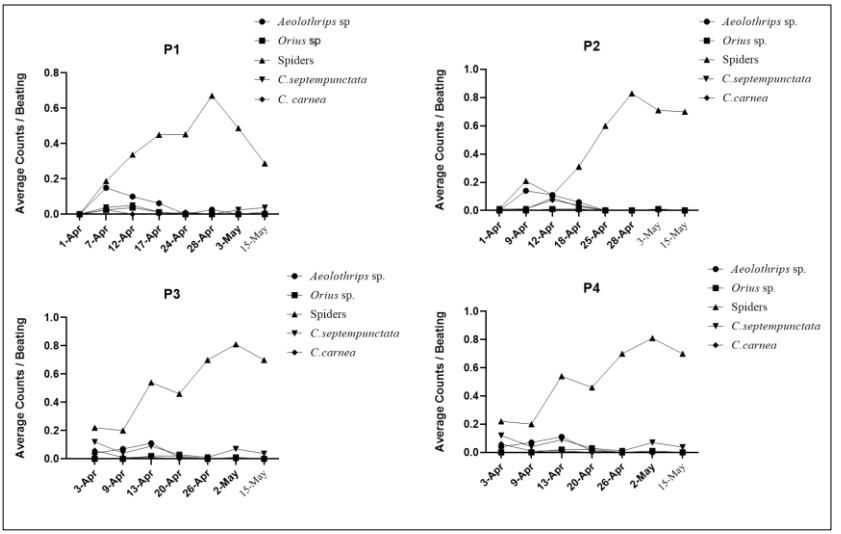

Fig. 6. Dynamics of natural enemy in citrus orchards of Marrakech during 2021 at four plots (P1-P4).

Chemical trial.

Field trials conducted in Marrakech region during 2021 revealed differences in efficacy between the insecticide formulations in suppressing thrips populations on citrus. Before treatment application, thrips counts did not differ significantly among treatments, including the untreated control ($F_{(6,18)}$ = 0.45; p = 0.83). One day after spraying, however, significant reductions were recorded in most treatments relative to the control ($F_{(6,18)}$ = 4.54; $p < 0.05$). The untreated control averaged 8.81±1.7 adults per tap, whereas Spirotetramat-treated plots still harbored relatively high densities (4.6±1.4 adults per tap). By day three, all treatments except Spirotetramat induced significant declines in thrips populations ($F_{(6,18)}$ = 6.6; $p < 0.01$). At seven and fourteen days post-application, all insecticides exhibited strong suppressive effects, with thrips densities reduced to near-zero levels ($F_{(6,18)}$ = 14.95, $p < 0.05$). By day 21, no significant differences were detected among treatments ($F_{(6,18)}$ = 1.3; p = 0.263), largely due to a natural decline in thrips abundance across all plots, including the control (1.5±0.15 adults beating method), similar to Abamectin-treated plots (0.4±0.12 adults/beating method per tap) (Table 4).

These results highlight the short-term effectiveness of most of the insecticides tested in reducing thrips infestations, while also highlighting the limited and inconsistent performance of Spirotetramat. The observed population collapse by the third week suggests that chemical interventions may only provide transient benefits and should be strategically integrated with other sustainable management approaches.

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Table 4. Average number of adults by beating method (% ± SE), ANOVA with One Factor used to compare the effect of chemical treatments on captured populations J-1 and DAT in Marrakech region during 2021

Active Ingredient	BFA	d+1	d+3	d+7	d+14	d+21
Control	10.19±1.33a	8.81±1.7b	5.94±0.87c	4.75±0.88b	1.94±0.23c	1.56±0.16a
Flonicamib	8.31±1.01a	1.25±0.39a	1.88±0.44a	1.19±0.29a	1.25±0.30abc	1.44±0.18a
Spirotetramat	8.06±1.20a	4.63±1.46a	6.06±1.43bc	0.75±0.23b	1.63±0.41bc	1.56±0.16a
Formentanat	6.63±0.85a	1.58±0.52a	0.44±0.22a	0.31±0.12a	0.38±0.13a	0.19±0.14a
Acetamiprid	8.50±0.99a	4.19±1.73a	2.13±0.85a	0.63±0.29a	0.38±0.13a	0.19±0.10a
Cyantaraniliprole	8.56±1.29a	2.31±0.75a	2.06±0.52a	1.06±0.27a	0.75±0.19ab	0.19±0.10a
Abamectin	8.69±1.01a	2.31±0.55a	3.13±0.74ab	1.25±0.27a	0.94±0.27ab	0.44±0.13a
F	F(6,18)=0.45	F(6,18)=4.54	F(6,18)=6.65	F(6,18)=14.95	F(6,18)=5.96	F(6,18)=1.30
p	0.833>0.05	0<0.05	0<0.05	0<0.05	0<0.05	0.263>0.05

*BFA: before foliar product application, d: day.

The efficacy and reduction rates of the tested insecticides against thrips are summarized in Table 5. One day after application, Flonicamid exhibited the strongest performance, with an efficacy of 82.60% and a reduction rate of 85.81%, while Spirotetramat remained the least effective, with efficacy not exceeding 33.56%. At three days post-treatment, all insecticides except Spirotetramat achieved efficacy levels above 50%, with Formentanat displaying the highest efficacy at 92.59% and a corresponding reduction rate of 88.62%. Spirotetramat, in contrast, showed no measurable efficacy or reduction at this stage.

By day seven, all products maintained high levels of efficacy, ranging from 69.14% for Abamectin to 89.97% for Formentanat. At day fourteen, efficacy values generally declined, with Flonicamid showing only 20.99% efficacy compared to 76.52% for Acetamiprid and 69.89% for Formentanat. Interestingly, the long-term effectiveness of certain compounds, such as Acetamiprid and Cyantraniliprole, remained relatively stable, achieving efficacy levels of 85.59% and 85.69%, respectively, by day 21. Formentanat also demonstrated consistent performance, maintaining an efficacy of 81.28% after 21 days (Table 5).

Overall, with the exception of Spirotetramat, all insecticides provided significant and sustained reductions in thrips populations, with Formentanat, Acetamiprid, and Cyantraniliprole emerging as the most reliable options under field conditions. These findings highlight the importance of selecting compounds with both immediate and persistent activity when developing integrated pest management strategies for thrips in citrus orchards.

Table 5. Reduction rate (R) and efficacy (E) of treatments against thrips populations assessed using the beating method ([j + n] = days after treatment) in Marrakech region during 2021

Treatment	d+1		d+3		d+7		d+14		d+21	
	R(%)	E(%)	R(%)	E(%)	R(%)	E(%)	R(%)	E(%)	R(%)	E(%)
Flonicamib	85.81	82.60	68.35	61.19	74.95	69.28	35.57	20.99	7.69	0.00
Spirotetramat	47.45	33.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Formentanat	82.07	72.44	92.59	88.62	93.47	89.97	80.41	69.89	87.82	81.28
Acétamipride	52.44	42.98	64.14	57.01	86.74	84.10	80.41	76.52	87.98	85.59
Cyantaraniliprole	73.78	68.79	65.32	58.72	77.68	73.43	61.34	53.98	87.98	85.69
Abamaectine	73.78	69.25	47.31	38.21	73.68	69.14	51.55	43.18	71.99	67.15

Biological trial.

Impact of biological control treatments on thrips species is presented in Table 6. On the first day prior to application, no significant differences were observed among treatments, including the control ($F_{(6,18)} = 0.98$; $p = 0.44$), indicating comparable initial thrips densities. On the first day after treatment, differences between treatments remained non-significant ($F_{(6,18)} = 1.67$; $p = 0.36$), with the control recording 7.05±0.86 thrips per tap and the lowest count observed for the Pyrethrum + azadirachtin mixture (4.18±0.50). On the third day post-treatment, thrips populations remained relatively stable across treatments ($F_{(6,18)} = 0.38$; $p = 0.89$), with the control at 5.31±0.48 and the lowest density observed in the Azadirachtin + paraffinic mineral oil mixture (4.31±0.66). By the seventh day, significant differences emerged between treatments and the control ($F_{(6,18)} = 3.36$; $p = 0.05$), with the control maintaining a high thrips population (7.43±0.61), whereas the Azadirachtin + oil mixture showed the greatest reduction (3.62±0.43). On the fourteenth day, most treatments significantly reduced thrips numbers compared to the control ($F_{(6,18)} = 3.74$; $p = 0.013$), with thrips densities of 0.56±0.18 for *B. bassiana*, 0.50±0.42 for Azadirachtin + paraffinic mineral oil, and 0.75±0.21 for Neem oil, compared to 1.93±0.32 in the control. On the twenty-first day, no significant differences were noted between treatments ($F_{(6,18)} = 0.29$; $p = 0.94$), as all biological treatments maintained low thrips densities (0.50-0.93 individuals per tap), indicating sustained efficacy over time (Table 6).

Overall, these results demonstrate that the biological treatments particularly Azadirachtin + paraffinic mineral oil, *B. bassiana*, and Neem oil effectively suppressed thrips populations, with maximal reductions observed between 7 and 14 days post-application, and maintained low densities through day 21, supporting their potential integration into sustainable thrips management strategies in citrus orchards.

Table 6. Average number of Thrips captured by tapping (% ± SE), ANOVA with one Factor used to compare the effect of biological treatments on captured populations before and after treatment in Marrakech region during 2021

Treatment	BFA	d+1	d+3	d+7	d+14	d+21
Control	7.25±0.9a	7.05±0.86	5.31±0.48	7.43±0.61b	1.93±0.32b	0.93±0.39b
Pyrethrum	6.93±0.97a	5.37±0.86	5.87±0.88	6.56±0.75ab	1.37±0.31ab	0.81±0.33
Neem oil	7.12±1.59a	5.81±0.78	5.12±0.87	7.12±0.27b	0.75±0.21a	0.81±0.20
B. bassiana	8.75±1.34a	6.00±0.85	5.81±0.73	4.37±0.85ab	0.56±0.18a	0.81±0.20
Azadirachtin + paraffinic mineral oil	8.87±1.00a	5.75±0.88	4.31±0.66	3.62±0.43a	0.50±0.42a	0.81±0.24
Pyrethrum + neem oil	7.93±1.37a	4.06±0.69	4.81±0.47	7.50±1.03b	1.50±0.42ab	0.93±0.29
Pyrethrum + Azadirachtin	11.87±2.26a	4.18±0.50	5.75±0.64	6.18±0.83ab	1.93±0.29ab	0.50±0.24
F	F(6,18)=0.98	F(6,18)=1.67	F(6,18)=0.38	F(6,18)=3.36	F(6,18)=3.74	F(6,18)=0.29
P	0.44>0.05	1.36>0.05	10.888>0.05	0.05>0.05	0.013<0.05	0.939>0.05

* BFA: before foliar product application, d: day

The efficacy and reduction rates of the tested biological treatments are summarized in Table 7. Among the different options, the Azadirachtin + paraffinic mineral oil mixture demonstrated the highest and most consistent efficacy, reaching 78.82% at day 14 with a reduction rate of 74.09%. *B. bassiana* also performed strongly, with an efficacy of 75.96% and a reduction rate of 70.98% at the same period. In contrast, treatments such as Neem oil alone and the Pyrethrum + neem oil mixture provided only moderate suppression, with efficacy values peaking at 60.43% and 47.35%, respectively, and showing a decline by day 21 (Table 7).

Overall biological treatments contributed to reducing adult thrips densities. However, because larval stages were not assessed in this study, the full effectiveness of the tested products cannot be fully determined. Within these limitations, the results nonetheless indicate that Azadirachtin + paraffinic mineral oil and *B. bassiana* showed the most consistent reductions in adult populations. These two treatments may therefore represent promising options for integration into Integrated Pest Management (IPM) programs aiming to limit reliance on synthetic insecticides, although further evaluations including effects on larvae are required before drawing definitive conclusions about their overall efficacy.

Table 7. Reduction rate (R) and efficacy (E) of treatments against thrips populations assessed using the beating method ([j + n] = days after treatment) in Marrakech region during 2021

Treatment	d+1		d+3		d+7		d+14		d+21	
	R(%)	E(%)	R(%)	E(%)	R(%)	E(%)	R(%)	E(%)	R(%)	E(%)
Pyrethrum	23.83	20.31	0.00	0.00	11.71	7.63	29.02	25.74	12.90	8.88
Neem oil	17.59	16.08	3.58	1.82	4.17	2.42	61.14	60.43	12.90	11.31
B. bassiana	14.89	29.48	0.00	9.50	41.18	51.27	70.98	75.96	12.90	27.83
Azadirachtin + paraffinic mineral oil	18.44	33.34	18.83	33.66	51.28	60.18	74.09	78.82	12.90	28.81
Pyrethrum + neem oil	42.41	47.35	9.42	17.18	0.00	7.71	22.28	28.94	0.00	8.58
Pyrethrum + azadirachtin	40.71	63.79	0.00	33.86	16.82	49.20	0.00	38.92	46.24	67.16

DISCUSSION

This study provides new insights into the composition, abundance, and population dynamics of thrips and their natural enemies in Moroccan citrus orchards, while also evaluating the efficacy of chemical and biological control methods. The identification of *F. occidentalis* and *T. tabaci* as dominant species, along with the detection of *P. kellyanus* at low abundances, is consistent with findings from other Mediterranean citrus-growing regions, such as Tunisia, Spain, and Cyprus (Belaam, and Boulahia-Kheder 2012, Navarro-Campos et al. 2012, Vassiliou 2010). Although *P. kellyanus* is considered a key pest in Spain and Italy, causing up to 70–80% fruit damage (Conti et al. 2001, Navarro-Campos et al. 2011), its low density in Marrakech region suggests that the species is not yet a major economic threat in Morocco. This situation is similar to observations in Tunisia, where *P. kellyanus* was reported but remained below damaging thresholds (Belaam, and Boulahia-Kheder 2012). However, regular monitoring remains essential given the capacity of this species to rapidly increase under favorable ecological conditions.

The relatively low diversity of thrips in Moroccan citrus orchards, compared to regions such as Florida with 36 species, including several predators (Childers, and Nakahara 2006), may be linked to the sampling strategy, the dominance of monoculture citrus systems, and the absence of surrounding host plants that act as thrips reservoirs. Indeed, studies from Tunisia and Spain have shown that orchards bordered by mixed fruit trees or vineyards host higher thrips populations, due to continuous availability of flowering hosts (Navarro-campos et al. 2013). The predominance of *F. occidentalis* and *T. tabaci* also reflects their well-documented polyphagy, as they are known to infest a wide range of crops, including vegetables and ornamentals (Deligeorgidis et al. 2005).

The occurrence of *C. carnea*, *C. septempunctata*, predatory thrips, and spiders confirms their role in thrips suppression, as previously documented in Mediterranean orchards (Elimem, and Chermiti 2012; Smaili et al. 2020).

However, in this study their abundance was lower than other citrus growing area like Australia where diverse and abundant predator guilds contribute significantly to natural regulation (Baker et al. 2011). This discrepancy may be explained by the intensive use of insecticides, which reduce beneficial insect populations, as well as by climatic conditions such as high summer temperatures (> 40 °C) that may limit predator survival. The seasonal activity of *C. carnea* and *C. septempunctata*, peaking during flowering and petal fall, matches observations from Tunisia and Italy (Belaam Kort et al. 2020, Perrotta, and Conti, 2008), suggesting a strong phenological synchronization with thrips populations. This highlights their potential for augmentative release programs, which have been successful in California against *Scirtothrips citri* (Khan, and Morse 1999).

Chemical control trials confirmed the efficacy of several insecticides (Flonicamid, Formetanate, Cyantraniliprole, Abamectin) in reducing thrips populations, consistent with previous studies in Cyprus and Greece (Vassiliou 2007, 2008, Deligeorgidis et al. 2005). However, their declining performance after 21 days, coupled with concerns about resistance development in *F. occidentalis* (Bielza et al. 2007, Reitz et al. 2020), raises questions about the sustainability of relying solely on chemical strategies. Reports from Spain and Australia have already documented resistance to spinosad and acrinathrin (Herron, and James 2005), reinforcing the need for rotational use and integration with non-chemical tools.

Biological control trials revealed that while most botanical insecticides (Pyrethrum, Neem oil, Azadirachtin) showed limited field persistence, the combination of Azadirachtin + paraffinic mineral oil and the entomopathogenic fungus *B. bassiana* achieved significant reductions at day 14, with efficacy exceeding 70%. These results are comparable to studies conducted in semi-arid environments, where environmental stressors (high temperatures, low humidity) reduced the persistence of microbial and botanical formulations (Lewis et al. 1997, Mouden et al. 2017). To enhance their performance, future research should focus on improved formulations with higher stability under heat stress, as well as optimized application timing (i.e., evening sprays to reduce UV degradation).

Overall, the results demonstrate that biological treatments, particularly *B. bassiana* and Azadirachtin + paraffinic mineral oil, represent promising alternatives to chemical insecticides**,** and should be considered as core elements of Integrated Pest Management (IPM) programs in Moroccan citrus orchards. However, their efficacy remains dependent on environmental conditions and should be complemented with conservation strategies, such as the introduction of flowering cover crops to sustain predator populations (Kirk, and Terry 2003).

This study is a first step toward a more comprehensive understanding of thrips dynamics in Moroccan citrus orchards. Its limitations include more precise identification of species based on microscopic preparations, the short trial duration, the focus on a single region, not having considered the larvae (the most important instar for damage), and the restricted sampling of associated vegetation. Future work should expand monitoring across different agro-ecological zones and for several years, assess inter-annual variability, and evaluate the compatibility of biocontrol agents with pollinators and other beneficial organisms. Such efforts will be crucial for developing sustainable and

locally adapted IPM strategies, particularly in the context of growing demand for residue-free and organic citrus production.

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RESUME

Khallou A., Smaili M.C., Mennani M., Haddad N., Kokiçi H., And Boutaleb-Joutei A. 2025. Management des espèces de thrips dans les vergers d'agrumes de la région de Marrakech: Exploration des méthodes de lutte biologique comme alternatives aux méthodes chimiques. Tunisian Journal of Plant Protection 20 (2): 97-116.

Les thrips, historiquement responsables de dégâts limités dans les vergers d'agrumes au Maroc, sont devenus des ravageurs préoccupant depuis 2018. Une étude a été menée de mars à juillet 2021 dans un verger d'agrumes de la région de Marrakech afin d'identifier les espèces de thrips et les ennemis naturels, de suivre la dynamique des populations et d'évaluer des méthodes alternatives de lutte. Deux essais ont été réalisés pour évaluer l'efficacité de différents produits contre les thrips dans les vergers d'agrumes. Le premier essai de lutte chimique a porté sur les insecticides Flonicamid, Spirotétramat, Formétanate, Acétamipride, Cyantraniliprole et Abamectine. Le deuxième essai de lutte biologique a évalué les traitements aux Pyrèthre, Huile de neem, *Beauveria bassiana*, mélange Azadirachtine et huile minérale paraffinique, mélange Pyrèthre et huile de neem ainsi que le mélange Pyrèthre et azadirachtine. Six espèces de thrips ont été recensées: *Frankliniella occidentalis*, *Thrips tabaci*, *Pezothrips kellyanus*, *Scirtothrips* sp., *Aeolothrips* sp. et *Haplothrips* sp. Les pics de population ont varié selon la variété d'agrumes. Les adultes ont atteint leurs niveaux les plus élevés pendant la période de la chute des pétales chez la clémentine Nules (4 ± 1 individus/frappage) et pendant la pousse estivale chez la mandarine Afourer (8,8 ± 1 individus/frappage), tandis que les populations larvaires ont été observées dix jours plus tard chez Nules et sont restées faibles chez Afourer. Quatre prédateurs ont été observés sur les agrumes : *Coccinella septempunctata*, *Orius* sp., la chrysope verte *Chrysoperla carnea* et *Aeolothrips* sp., avec des pics d'abondance durant la chute des pétales. Le Formétanate a montré l'efficacité la plus élevée (88,7 % après 3 jours), tandis que le Spirotétramat s'est révélé moins efficace contre les thrips. En lutte biologique, *Beauveria bassiana* ainsi que le mélange azadirachtine et huile minérale paraffinique ont montré l'efficacité la plus élevée significativement (75,9% et 78.82 %, respecivement, après deux semaines). Ces résultats mettent en évidence la menace croissante que représentent les thrips dans les vergers d'agrumes commerciaux au Maroc et soulignent l'intérêt de stratégies de lutte intégrée combinant méthodes chimiques et biologiques pour une gestion durable de ces ravageurs.

Mots clés: Agrumes, Biopesticides, Insecticides, Maroc, *Pezothrips kellyanus*, *Scirtothrips* sp.

ملخص

خلّو، عبد الحق ومولاي شريف سماعيلي ومحمد مناني ونجاة حداد وحسين كوكيتشي وعبد الملك بوطالب-جوطي. 2025. إدارة حشرة التربس في بساتين الحمضيات بجهة مراكش: استكشاف أساليب المكافحة البيولوجية كبديل للمكافحة الكيميائية. Tunisian Journal of Plant Protection 20 (2): 97-116.

تُعدّ حشرات التربس من الآفات التي كانت تُسبب تاريخياً أضراراً محدودة في بساتين الحمضيات بالمغرب، غير أنها أصبحت منذ سنة 2018 تشكل مشكلة متزايدة الأهمية. أُجريت دراسة خلال الفترة الممتدة من مارس إلى يونيو 2021 في بستان حمضيات بمنطقة مراكش، وهدفت إلى تحديد أنواع التربس وأعدائها الطبيعيين، وتتبع ديناميكية تجمعاتها، وتقييم بعض وسائل المكافحة البديلة. تم تنفيذ تجربتين لتقييم فعالية عدة معاملات ضد التربس في بساتين الحمضيات. شملت التجربة الأولى للمكافحة الكيميائية كلًّا من المبيدات فلونيكاميد وسبيروتيترامات وفورمِتانيت وأسيتامبيريد وسيانترانيليبرويل وأبامكتين. أما التجربة الثانية للمكافحة البيولوجية فقد شملت المعاملات بواسطة البيريثروم وزيت النيم والفطر *Beauveria bassiana* ومزيج الأزاديراختين + زيت معدني ومزيج البيريثروم + زيت النيم ومزيج البيريثروم + الأزاديراختين. تم تسجيل ستة أنواع من التربس وهي *Frankliniella occidentalis* و *Thrips tabaci* و *Pezothrips kellyanus* و *Scirtothrips* sp. و *Aeolothrips* sp. و *Haplothrips* sp.. وقد اختلفت ذروات الكثافة العددية باختلاف صنف الحمضيات، حيث بلغت أعداد البالغات ذروتها خلال مرحلة تساقط البتلات في صنف الكليمنتين نوليس (4 ± 1 أفراد/ضربة) وخلال النمو الصيفي لصنف المندرين أفورير (8.8 ± 1 أفراد/ضربة)، في حين بلغت كثافة اليرقات ذروتها بعد عشرة أيام في صنف نوليس، وبقيت منخفضة في صنف أفورير. كما تم رصد أربعة مفترسات طبيعية على أشجار الحمضيات وهي *Coccinella septempunctata* و *Orius* sp. والدعسوقة الخضراء *Chrysoperla carnea* و *Aeolothrips* sp.، حيث تزامنت ذروة كثافتها مع مرحلة تساقط البتلات. أظهر مبيد فورمِتانيت أعلى فعالية في المكافحة (88.7% بعد ثلاثة أيام)، في حين كان المبيد سبيروتيترامات أقل فعالية ضد التربس. وفي المكافحة البيولوجية، كان الفطر *Beauveria bassiana* والمزيج الأزاديراختين مع الزيت المعدني هما الأكثر فعالية بشكل معنوي (75.9% و 78.82% على التوالي، بعد أسبوعين) .تؤكد هذه النتائج تزايد خطورة التربس في بساتين الحمضيات التجارية بالمغرب، وتبرز أهمية اعتماد استراتيجيات المكافحة المتكاملة التي تجمع بين الوسائل الكيميائية والبيولوجية لتحقيق إدارة مستدامة لهذه الآفة.

كلمات مفتاحية: المغرب، حمضيات، مبيدات كيميائية، مبيدات بيولوجية، *Pezothrips kellyanus*، *Scirtothrips* sp.

LITERATURE CITED

Abbassi, M. 2008. Note au sujet de la virulence de ravageurs considérés comme secondaires au Maroc et aspects pratiques de lutte. Pages 253-258. In: Proceedings du Symposium Méditerranéen sur la Protection Phytosanitaire des Agrumes, 9-11 juin 2008, Rabat, Maroc.

Abbassi, M. 2014. Les thrips des agrumes : Données préliminaires. Agriculture du Maghreb, 76: 32.

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267.

Baker, G.J. 2016. Kelly citrus thrips management. Factsheet. Government of South Australia, Primary Industries and Regions SA. Available at: http://www.pir.sa.gov.au/__data/assets/pdf_file/0010/285517/Kellys_Citrus_Thrips_Management_Fact_Sheet.pdf

Baker, G.J., Keller, M., Crisp, P., Purvis, S., Jackman, D.J., and Barbour, D. 2004. The biological control of Kelly's citrus thrips in Australian citrus orchards. Pages 267-274. In: Proceedings of the XXII International Congress of Entomology, 15-21 August 2004, Brisbane, Australia.

Baker, G.J., Keller, M.A., Crisp, P., Jackman, D., Barbour, D., and Purvis, S. 2011. The biological control of Kelly's citrus thrips in Australian citrus orchards. IOBC/WPRS Bulletin 62: 267-274.

Belaam Kort, I., Moraza, M. L., Attia, S., Mansour, R., and Boulahia Kheder, S. 2020b. Beneficial arthropods as potential biocontrol candidates of thrips (Thysanoptera: Thripidae) occurring in Tunisian citrus orchards. Biologia 75: 2261-2270. https://doi.org/10.2478/s11756-020-00487-x

Belaam, I., and Boulahia-Kheder, S. 2012. Inventory of thrips species in citrus orchards and assessment of scarring fruits in two citrus-producing regions of Tunisia. Tunisian Journal of Plant Protection 7: 1-9.

Belaam-Kort, I., and Boulahia-Kheder, S. 2017. Thrips in citrus orchards, emerging pests in Tunisia. Faunistic Entomology 70: 77-87. https://doi.org.10.25518/2030-6318.3677

Belaam-Kort, I., Marullo, R., Attia, S., and Boulahia-Kheder, S. 2020a. Thrips fauna in citrus orchards in Tunisia: An update. Bulletin of Insectology 73: 110.

Bielza, P., Quinto, V., Contreras, J., Torne, M., Martin, A., and Espinosa, P. J. 2007. Resistance to spinosad in the western flower thrips, *Frankliniella occidentalis* (Pergande), in greenhouses of South-Eastern Spain. Pest Management Science 63: 682-687. https://doi.org/10.1002/ps.1388

Blank, R.H., and Gill, G.S.C. (1997). Thrips (Thysanoptera: Terebrantia) on flowers and fruit of citrus in New Zealand. New Zealand

Journal of Crop and Horticultural Science 25: 319-332.

Childers, C.C., and Nakahara, S., 2006. Thysanoptera (thrips) within citrus orchards in Florida: Species distribution, relative and seasonal abundance within trees, and species on vines and ground cover plants. Journal of Insect Science 6: 3-7.

Childers, C.C. 1992. Suppression of *Frankliniella bispinosa* (Thysanoptera: Thripidae) and the fungal pathogen *Colletotrichum gloeosporioides*, with pesticides during the bloom cycle and improved fruit set on 'Navel' Orange in Florida. Journal of Economic Entomology 85: 1330-1339.

Cloyd, R.A. 2016. Western flower thrips (Thysanoptera: Thripidae) and insecticide resistance: An overview and strategies to mitigate insecticide resistance development. Journal of Entomological Science 51: 257-273. https://doi.org.10.18474/JES16-15.1

Colloff, M.J., Fokstuen, G., and Boland, T. 2003. Toward the triple bottom line in sustainable horticulture: Biodiversity, ecosystem services and an environmental management system for citrus orchards in the Riverland of South Australia. CSIRO entomology Canberra, Australia, 112 pp.

Conti, F., Tumminelli, R., Amico, C., Fisicaro, R., Frittitta, C., Perrotta, G., and Marullo R. 2001. Monitoring *Pezothripskellyanus* on citrus in eastern Sicily, pp. 207-210. Thrips and tospoviruses. Pages 207-210. In R. Marullo & L. Mound (Eds.), Proceedings of the 7th international symposium on Thysanoptera, 1-8 July 2001, Reggio Calabria, Italy.

Costa, L., Mateus, C., Zur, S.R., and Franco, J.C. 2006. Thrips (Thysanoptera) associated to lemon orchards in the ouster region of Portugal. IOBC/WPRS Bulletin 29: 285-291.

Crisp, P., Baker, G., and Wheeler, S. 2011. Agronomic outcomes of IPM control of citrus thrips in Riverland soils. IOBC/WPRS Bulletin 62: 275-283.

De Grazia, A., and Marullo, R. 2013. The spreading of *Frankliniella occidentalis* (Pergande) over citrus orchards: The impact on pest thrips fauna and the evaluation of economic thresholds. Page 267. Abstract in: Book of abstracts, future IPM in Europe, 2013, Riva del Garda, Italy.

Deligeorgidis. PN., Ipsilandis. CG., Vaiopoulou. M., Kaltsoudas. G., Sidiropoulos G 2005. Predatory effect of *Coccinella septempunctata* on *Thrips tabaci* and *Trialeurodes vaporariorum*. J. Appl. Entomol. 129: 246-249. https://doi.org/10.1111/j.1439-0418.2005.00959.x

Derolez, B., Orczyk N., Declercq, S. 2014. Clé d'identification des coccinnelles du nord-Pas de Calais [Identification Key of Ladybirds from Nord-Pas-de-Calais]. Available on http://gon.fr/gon/wp-content/uploads/2015/03/cle_cox_NPdC_version4_1.pdf

Donghwang, K., Hyeogmo, K., & Kwangsik, K. 2000. Current status of the occurrence of the insect pests in the citrus orchard in Cheju Island. Korean Journal of Applied Entomology 39: 267-274.

Elimem, M., and Chermiti, b. 2013. Identification et caractérisation morphologique des différentes espèces de thrips (Insecta; Thysanoptera) sur culture de rosier dans différentes localités du Sahel Tunisien. Biodiversité et Sécurité Alimentaire 5 (1): 2.

Elimem M, and Chermiti B. 2012. Use of the predators *Orius laevigatus* and Aeolothrips spp. to control *Frankliniella occidentalis* populations in greenhouse peppers in the region of Monastir, Tunisia. IOBC/WPRS Bulletin 80:141-146.

Flint, M.L., Kobbe, B., Clark, J.K., Dreistadt, S.H., and Pegrson, J.E. 1991. Integrated Pest management for citrus 2nd edition. University of California, Oakland, CA, USA, 144 pp.

Gao, Y., Lei, Z., and Reitz, S.R. 2012. Western flower thrips resistance to insecticides: Detection, mechanisms and management strategies. Pest Management Science 68: 1111-1121. https://doi.org/10.1002/ps.3305

Henderson, C.F., and Tilton, E.W. 1955. Tests with acaricides against the brown wheat mite. Journal of Economic Entomology 48: 157-161.

Gao, Y., Lei, Z., and Reitz, S.R. 2012. Western flower thrips resistance to insecticides: Detection, mechanisms and management strategies. Pest Management Science 68: 1111-1121.

Immaraju, J., Morse, J.G., and Jefri Kersten, D. 1989. Citrus thrips (Thysanoptera: Thripidae) pesticide resistance in the Coachella and San Joaquin valleys of California. Journal of Economic Entomology 82: 374-380.

Immaraju, J., Morsei, J.G., and Hobzn, R.F. 1990. Field evaluation of insecticide rotation and mixtures as strategies for Citrus thrips (Thysanoptera: Thripidae) resistance Management in California. Journal of Economic Entomology 83: 306-314.

Jacas, J.A., Karamaouna, F., Vercher, R., and Zappalà, L. 2010. Citrus pest management in the northern Mediterranean Basin: Spain, Italy and Greece. Pages 3-26. In a. Ciancio & K.G Mukerji (Eds.), integrated Management of Arthropod Pests and Insect Borne Diseases Volume 5. Springer, Netherlands. https://doi.org/10.1007/978-90-481-8606-8_1

Kirk, W.D.J., and Terry, L.I. 2003. The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). Agricultural and Forest Entomology 5: 301-310.

Lewis, T. 1997. Thrips as Crop Pests, CAB International, Wallingford, UK, 740 pp.

Makabe, T., Futamura, T., Noudomi, T., Wakakuwa, M., and Arikawa, K. 2014. Phototaxis of western flower thrips, *Frankliniella occidentalis* and onion thrips, *Thrips tabaci* and the possibility of controlling Thrips using ultraviolet temitting trap in the greenhouse of Satsuma mandarin (Citrus unshiu). Japanese Journal of Applied Entomology and Zoology 58: 187-195.

Mansour, R., Belzunces, L.P., Suma, P., Zappalà, L., Mazzeo, G., Grissa-Lebdi, K., Russo, A., and Biondi, A. 2018. Vine and citrus mealybug pest control based on synthetic chemicals. A review. Agronomy for Sustainable Development 38: 37 https://doi.org/10.1007/s13593-018-0513-7

Marullo, Rita. 2003. Conoscere i tisanotteri: guida al riconoscimento delle specie dannose alle colture agrarie. [Knowing Thrips. A Guide to Identifying Species Harmful to Agricultural Crops], Bologna Edagricole, Italy, 75 pp.

Marullo, R. 1998. *Pezothrips kellyanus*, un nuovotripideparassita delle colturemeridionali. Informatore Fitopatologico 48: 72-75.

Morse, J. G., and Hoddle, M. S. 2006. Invasion biology of thrips. Annual Review of Entomology 51: 67-89.

Mouden, S., Sarmiento, K.F., Klinkhamer, P.G., and Leiss, K. A. 2017. Integrated pest management in western flower thrips: Past, present and future. Pest Management Science 73: 813-822. https://doi.org/10.1146/annurev.ento.51.110104.151044

Mound, L.A, and Jackman, D.J. 1998. Thrips in the economy and ecology of Australia, pp. 472-478. In: Pest management - Future challenges, proceedings of the sixth Australian applied entomological research conference. M.P. Zalucki, R.A.I. Drew & G.G. White (Eds.), University of Queensland, St Lucia, Australia.

Mound, L.A., and Palmer, J.M. 1981. Identification, distribution and host-plants of the pest species of *Scirtothrips* (Thysanoptera: Thripidae). Bulletin of Entomological Research 71 (3): 467-479.

Mound, L.A., Wang, Z., Lima, É.F., and Marullo, R. 2022. Problems with the concept of "pest" among the diversity of pestiferous thrips. Insects 13 (1): 61. https://doi.org/10.3390/insects13010061

Mueller, T.G., Kahl, H.M., Cass, B.N., Grafton-Cardwell, E.E., and Rosenheim, J.A. 2019. Differential impacts of citrus thrips across sweet orange and mandarin species. Journal of Economic Entomology 112: 2767-2773. https://doi.org/10.1093/jee/toz178

Nault, B.A., and Shelton, A.M. 2010. Impact of insecticide efficacy on developing action thresholds for pest management: A case study of onion thrips (Thysanoptera: Thripidae) on onion. Journal of Economic Entomology 103: 1315-1326. https://doi.org/10.1603/EC10096

Navarro-Campos, C., Aguilar, A., and Garcia-Marí, F. 2011. Population trend and fruit damage of *Pezothrips kellyanus* in Citrus orchards in Valencia (Spain). Integrated control in Citrus fruit crops. IOBC/WPRS Bulletin 62: 285-292.

Navarro-Campos, C., Aguilar, A., and Garcia-Mari, F. 2012. Aggregation pattern, sampling plan, and intervention threshold for *Pezothrips kellyanus* in citrus groves. Entomologia Experimentalis et Applicata 142 : 130-139. https://doi.org/10.1111/j.1570-7458.2011.01204.x

Navarro-Campos, C., Beltrà, A., Calabuig, A., Garcia-Marí, F., Wäckers, F.L., and Pekas, A. 2020. Augmentative releases of the soil predatory mite *Gaeolaelaps aculeifer* reduce fruit damage caused by an invasive thrips in Mediterranean citrus. Pest Management Science 76: 2500-2504. https://doi.org/10.1002/ps.5791

Navarro-Campos, C., Pekas, A., Aguilar, A., and Garcia-Marí, F. 2013. Factors explaining variation in citrus fruit scarring by *Pezothrips kellyanus* (Thysanoptera: Thripidae). Integrated control in Citrus fruit crops. IOBC/WPRS Bulletin 95: 71-76.

Perrotta, G., and Conti, F. 2008. A threshold hypothesis for an integrated control of thrips infestation on citrus in southeastern Sicily. IOBC/WPRS Bulletin 38: 204-209

Purvis, S. 2002. Talking Thrips in citrus. Issue 1. Available on

http://www.sardi.sa.gov.au/__data/assets/pdf_file/0003/44922/talking_ thrips_1.pdf

Rao, C.N., George, A., and Rahangadale, S. 2019. Monitoring of resistance in field populations of *Scirtothrips dorsalis* (Thysanoptera: Thripidae) and *Diaphorina citri* (Hemiptera: Liviidae) to commonly used insecticides in Citrus in Central India. Journal of Economic Entomology 112: 324-328. https://doi.org/10.1093/jee/toy311

Reitz, S.R. 2009. Biology and ecology of the western flower thrips (Thysanoptera: Thripidae): The making of a pest. Florida Entomologist 1: 7-13.

Reitz, S.R., Gao, Y.L., Kirk, W.D.J., Hoddle, M.S., Leiss, K.A., and Funderburk, J.E. 2020. Invasion biology, ecology, and management of western flower thrips. Annual Review of Entomology 65: 17-37. https://doi.org/10.1146/annurev-ento-011019-024947

Reitz, S.R., Gao, Y.-L., and Lei, Z.-R. 2011. Thrips: Pests of concern to China and the United States. Agricultural Sciences in China 10: 867=892.

Reynaud, P. 2010. Thrips (Thysanoptera). BioRisk, 4: 767-791.

Smaili M.C., Boutaleb J.A. and Blenzar A. 2020. Beneficial insect community of Moroccan citrus groves: assessment of their potential to enhance biocontrol services. Egyptian Journal of Biological Pest Control 30: 47. https://doi.org/10.1186/s41938-020-00241-0.

Smaili, M.C., Benyahia, H., and Kabbage, T. 2018. Alerte! Invasion des populations du thrips *Pezothrips kellyanus* (Thysanoptera, Thripidae) sur différentes variétés d'agrumes dans la région du Souss-Massa. Agriculture du Maghreb 114 : 82-85.

Trabelsi, I., and Boulahia-Kheder, S. 2009. Sur la présence en Tunisie du thrips des agrumes *Pezothrips kellyanus* (Thysanoptera: Thripidae). Annales INRAT 82: 181-186.

Tsuchiya, M., and Furuhashi, K. 1993. Outbreak of western flower thrips (*Frankliniella occidentalis* (Pergande)), its damage to Satsuma mandarin growing in vinyl greenhouses, and its distribution in Shizuoka prefecture, Japan. Proceedings of Kanto-Tosan Plant Protection Society 40: 265-268.

Vassiliou, V.A. 2007. Chemical control of *Pezothrips kellyanus* (Thysanoptera: Thripidae) in citrus plantations in Cyprus. Crop Protection 26: 1579-1584. https://doi.org/10.1016/j.cropro.2007.02.004

Vassiliou, V.A. 2008. Bio-ecology and control of *Pezothrips kellyanus* (Thysanoptera: Thripidae) on Citrus in Cyprus. The European Journal of Plant Science and Biotechnology 2: 69-73.

Vassiliou, V. A. 2010. Ecology and behavior of *Pezothrips kellyanus* (Thysanoptera: Thripidae) on citrus. Journal of Economic Entomology 103: 47-53. https://doi.org/10.1603/EC09114

Wu, S.Y., Gao, Y.L., Smagghe, G., Xu, X.N., and Lei, Z.R. 2016. Interactions between the entomopathogenic fungus Beauveria bassiana and the predatory mite *Neoseiulus barkeri* and biological control of their shared prey/host *Frankliniella occidentalis*. Biological Control 98: 43-51. https://doi.org/10.1016/j.biocontrol.2016.04.001

Plant Protection Events

Report

on

National Workshop on Strengthening the Capacities of Biological Control Stakeholders: Towards Sustainable and Innovative Agriculture

Hammamet, Tunisia, September 17-19, 2025

A National Workshop on Strengthening the Capacities of Biological Control Stakeholders: Towards Sustainable and Innovative Agriculture was held from 17 to 19 September 2025 in Hammamet, Tunisia. The event was organized within the framework of the BIOREST project "Support to Sustainable and Climate-Resilient Organic Agriculture" and the TCP/TUN/4001 project on "Emergency assistance for the management of the cactus cochineal in Tunisia". The workshops brought together more than 40 participants representing startups specialized in bio-agents, agricultural technical centers, research

institutes, universities, and national authorities, including the General Directorate of Plant Health and Organic Agriculture.

The opening session highlighted the strategic importance of biological control as a key component of Integrated Pest Management (IPM) and as a sustainable alternative to chemical pesticides. National authorities and FAO representatives emphasized the need to strengthen coordination among stakeholders, capitalize on research achievements, and address technical and regulatory constraints to scale up biological control solutions in Tunisia.

The main objectives of the workshop were to build a national network for the production and dissemination of bio-agents and biopesticides, strengthen the technical capacities of startups and institutions, and carry out a participatory diagnosis of existing infrastructures, skills, regulatory challenges, and market opportunities. Special focus was placed on the local production of beneficial insects, particularly the predator *Hyperaspis trifurcata* for the control of the cactus cochineal, in order to reduce dependence on costly imports and promote green job creation.

Over two days, a series of technical presentations were delivered by national experts on integrated pest management strategies, biological control research, insect mass rearing, sterile insect technique, microbial and plant-based biopesticides, and successful national experiences in different crop systems. These were followed by group work sessions focused on five strategic value chains: date palm, olive tree, vegetable crops, cactus, and fruit trees and citrus.

The group discussions identified major phytosanitary constraints affecting each sector, including *Tuta absoluta* and mildew in vegetables, olive fly and *Verticillium* in olives, cochineal in cactus, pests of date palm, and *Ceratitis capitata* and mites in fruit and citrus orchards. While several biological and cultural control solutions already exist, key limitations were highlighted, notably the lack of locally produced auxiliaries, insufficient availability of certified organic inputs, regulatory constraints, high costs, and limited farmer awareness.

Cross-cutting needs were identified in the areas of research and innovation, local production of biological control agents, capacity building, public–private partnerships, infrastructure development, and improvement of the regulatory framework. Priority actions include supporting applied research on local strains of beneficial microorganisms, establishing decentralized production units for auxiliaries, strengthening farmer training through Farmer Field Schools, and promoting green entrepreneurship through startups.

The third day was dedicated to a field visit to the Technical Center of Citrus, where participants observed the rearing facilities and protocols for the mass production of *Hyperaspis trifurcata* under the TCP/TUN/4001 project. The workshop concluded with strong commitments from national institutions to continue joint efforts for the development and scaling up of biological control in Tunisia.

Key recommendations include the creation of a national multi-actor biological control network, regulatory reforms to facilitate the registration of biopesticides and auxiliaries, establishment of local production units, reinforcement of laboratories, development of farmer training programs, and the creation of national platforms for knowledge exchange and technology transfer.

Overall, the workshop underscored the need for an integrated, collaborative, and innovative approach to make biological control a central pillar of a sustainable, resilient, and competitive agricultural system in Tunisia.

Prof. Naima Mahfoudhi,
DG of DG.SVCIA,
Ministry of Agriculture, Tunisia

Report

on

The 14th Arab Congress of Plant Protection (ACPP)

Algeris, Algeria, November 03-07, 2025

The 14th Arab Congress of Plant Protection (ACPP 2025) has been held in Algeria, during the period 03-07 November 2025 under the theme "Plant Health for Sustainable Food security".

The Organizing Committee was happy with the success of this scientific event co-organized by the Arab Society of Plant Protection (ASPP), the National Higher School of Agronomy (Algerian Ministry of Higher Education and Scientific Research) and the Directorate of Plant Protection and Technical Control (Algerian Ministry of Agriculture, Rural Development and Fisheries).

The congress was attended by more than 250 participants from Arab and non-Arab countries. It started on Monday 3rd November, 2025, with an opening ceremony under the patronage of his excellency the Minister of Higher Education and Scientific Research, and his excellency the Minister of Agriculture, Rural Development and Fisheries. It was, followed by a keynote address by Dr. Thaer Yaseen (Near East and North Africa Regional Office of FAO, Cairo, Egypt), entitled "The role of plant protection in achieving food security in the Arab region".

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

1. Use of artificial intelligence and other new innovations in optimizing pest management.
2. Innovations to improve pest management and enhance plant health under climate change conditions.
3. Invasive and newly emerging pests in Arab region and means to reduce their negative effect on food security.
4. Plant health and agricultural quarantine in the Arab region and means of improving monitoring quarantine pests.

The symposia plenary sessions included presentations by eminent invited speakers from well-known Research Centers or Universities or from International Organizations (CIMMYT, CIHEAM, FAO, ICARDA, NEPPO, AOADA, IITA…). These sessions included presentations on important plant protection topics such as:

- Use of decision-making tools to enhance implementation of integrated pest management.
- Advances in use of High Throughput Sequencing (HTS) technology to detect plant pathogens and its adoption in implementing agricultural quarantine regulations.
- Use of satellite remote sensing for crop disease surveillance and forecasting.
- Plant protection as the intersection of biotechnologies and innovative technologies.
- Effects of climate change on plant health: are beneficial microbes and their metabolites a possible solution?
- The role of CropLife Africa Middle East in advancing sustainable agriculture and food security.
- Addressing transboundary diseases is crucial for safeguarding fruit crops in NENA Countries.
- Management of North Africa-Middle East (NAFME) cryptic whitefly haplotypes to mitigate Begomovirus spread for Arab food security.
- Date palm invasive and newly emerging pests and innovative measures to reduce their negative impact on date production.
- The role of CGIAR Germplasm Health Units in enhancing germplasm phytosanitary safety and mitigation of transboundary pest spread.
- The importance of Phytosanitary Measures in mitigating the spread of transboundary plant pests in the NENA region.
- Working together for clean plants: the National Clean Plant Network example for an Arab regional network to support agricultural quarantine and develop plant disease control programs.
- Pest general and specific surveillance based on IPPC recommendations to improve plant health of important crops.

In addition to the symposia, thirty-six oral paper presentation sessions and three poster sessions were organized where more than 300 scientific papers, focusing on specialized topics in all plant protection disciplines were presented and discussed such as:

- Soil-borne pathogens,
- Fungal diseases,
- Bacterial diseases,
- Virus and phytoplasma diseases,
- Weeds,
- Economic entomology,
- Beneficial insects,
- Biological control,
- Plant extracts,
- Food security and plant protection,
- Climate change and plant protection.

During the congress, a new ASPP Executive Committee for the 2026-2028 period was elected and is composed as follows:

- Dr. Ahmad M. Katbeh-Bader (Jordan): President,

- Dr. Safaa Kumari (Syria): Vice President,
- Dr. Zinette Melhem Moussa (Lebanon): Secretary-Treasurer,
- Dr. Emad M. Ghalib Al-Maaroof (Iraq): Member & Chairman of Translation Committee,
- Dr. Houda Boureghda (Algeria): Member & Chairman of Publiction Committee,
- Dr. Hassan Dahi (Egypt): Member & Chairman of Membership Committee,
- Dr Abdulrahman Saad Aldawood (Kingdom of Saudi Arabia): Member & Chairman of Honour and Awards Committee,
- Dr. Ibrahim Al-Jboory (Iraq): Member & Editor-in-Chief of ANEPPB,
- Dr. Khaled Makkouk (Lebanon): Member & Editor-in-Chief of AJPP.

During the closing ceremony, the organizing committee thanked all sponsors for their financial support which contributed to the success of ACPP 2025 and as is customary at ASPP Congresses, the Honour and Awards Committee announces the names of colleagues and PhD students who received the "ASPP Honorary awards", the "ASPP Fellow Award" and the "Students awards".

Prof. Asma Najjar,
INRAT,
University of Carthage, Tunis, Tunisia

TUNISIAN JOURNAL OF PLANT PROTECTION

Vol. 20 No. 2 DECEMBER 2025

CONTENTS

Photo of the cover page: *Aeolothrips* sp. (Courtesy *Abdelhak Khalibu*)

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

Plantae Senae in Terra Sena