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Contents

Photo of the cover page: *Helicoverpa armigera* larva molting (Courtesy *Thameur Bouslama*)

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Guest Editorial

The International Year of Plant Health (IYPH) 2020 "Protecting Plants, Protecting Life"

In December 2018, the United Nations General Assembly declared 2020 as the International Year of Plant Health (IYPH). The year is a once in a lifetime opportunity to raise global awareness on how protecting plant health can help end hunger, reduce poverty, protect the environment, and boost economic development. The Food and Agriculture Organizations of the United Nations (FAO), in collaboration with the International Plant Protection Convention (IPPC), is the lead UN agency for the event.

Healthy plants constitute the foundation for all life on Earth, ecosystem functions, as well as food security. Plants make up 80 percent of the food we eat and produce 98 percent of the oxygen we breathe. Plant pests and diseases leave millions of persons without food to eat and negatively affect agriculture, which is the primary source of income of rural poor communities.

Plant health is fundamental for reaching the 2030 Agenda for UN Sustainable Development Goals. Hence, sustaining plant health is an integral part of the sustainable development of agriculture to feed the growing global population by 2050. Protecting plants from pests and keeping plants healthy support efforts to end hunger, malnutrition and poverty; protect the environment, forests and biodiversity; address the effects of climate change; and boost economic development.

The IYPH is a unique opportunity to raise awareness on the important role of plant health for life of earth and to promote activities in favor of preserving and sustaining global plant resources. It will also raise public awareness globally about the importance of plant health and improve plant health policies, capacity development and resource mobilization opportunities for plant health institutions.

IYPH aims to mobilize governments, industries, civic organizations, scientists, and the public to work together in protecting the world's plants against the spread of devastating pests; encourage scientific innovation to address pest threats; promote responsible practices that reduce the spread of pests; and increase public and private sector support and investment for more sustainable plant health strategies and services.

Plant Health starts with prevention, so everyone is invited to contribute to protecting plant health inspired by the following key messages:

- *Keep plants healthy to achieve Zero Hunger and the Sustainable Development Goals. Policies and actions to promote plant health are fundamental for reaching the Sustainable Development Goals, in particular those aimed at eliminating hunger and malnutrition and reducing poverty and threats to the environment.*
- *Be careful when bringing plants and plant products across borders. Help reduce the spread of plant pests and diseases, which can seriously damage*

national food security, the environment and economies. Make sure that the phytosanitary requirements are applied when bringing plants or plant products when travelling, and be careful when ordering plants and plant products online, or through postal services, since packages can easily bypass regular phytosanitary controls.

- *Make trading in plants and plant products safe by complying with the international plant health standards. It is important to implement international plant health standards and norms, such as those developed by the International Plant Protection Convention (IPPC) and FAO to make trade safe. This reduces the negative impact of pests and pesticides on human health, economies and the environment. It also makes it easier to prevent and control the spread of pests and diseases without setting up unnecessary barriers to trade.*
- *Keep plants healthy while protecting the environment. When combatting pests and diseases, farmers should adopt, and policymakers should encourage the use of, environmentally friendly methods such as integrated pest management.*
- *Invest in plant health capacity development, research and outreach. Governments should invest more in plant health related research and outreach, as well as innovative practices and technologies, and empower plant protection organizations and other relevant institutions, and provide them with adequate human and financial resources.*
- *Strengthen monitoring and early warning systems to protect plants and plant health. Regularly monitoring plants, and receiving early warning information about emerging threats, helps governments, agricultural officers and farmers take preventive and adaptive measures to keep plants healthy.*

The FAO will officially launch the IYPH, presided by the UN Secretary General and FAO Director General, through a FAO Council Special Session on 2 December 2019 at FAO Headquarters in Rome.

Everyone should promote the IYPH! To help you do this, FAO's IYPH team has set up a website with the IYPH logo in your language, downloadable communications resources including posters, videos and toolkits, and a list of global, regional and national IYPH events.

Many global, regional and national IYPH events are being scheduled, including the IYPH global key event, and the International Plant Health Conference "Protecting plant health in a changing world" to be held from 5 to 8 October 2020 in Helsinki, Finland.

Many of the events will be organized by national and regional plant protection organizations and institutions, and we encourage you to get in contact with relevant organizers to join these activities; as well as organize your own events.

For more information on the IYPH and program of events, please visit IYPH webpage: www.fao.org/plant-health-2020 or write: iyph@fao.org

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Salt Stress Responses of Six Grapevine Genotypes from the Oases of Tozeur

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ABSTRACT

Habib, A., Ben Maachia, S., Sahli, A., Harbi-Ben Slimane, M. 2019. Salt stress responses of six grapevine genotypes from the oases of Tozeur. Tunisian Journal of Plant Protection 14 (2): 1-16.

In order to study the responses to salinity of the principal grapevine genotypes cropped in the oases of Tozeur, Tunisia, six grapevine genotypes were subjected to gradual salt stress. After 8 weeks of exposure to different salinity doses, 50, 75, 100 and 150 mM NaCl, length and dry weight of shoots and roots, and leaf number were measured. The high salt dose 150 mM NaCl decreased the shoot length for the grapevine genotypes Chetoui, Guelb Sardouk and Muscat d'Italie. Furthermore, the damages of salinity were displayed by the decrease of leaf number for all the tested grapevine, particularly under the high salt dose. The long-term exposure to salt stress increased the aerial part (shoots and leaves) dry weight for all the tested grapevines. For Arbi, an increase of shoot dry weight was obtained under 150 mM NaCl. The root dry weight of the local grapevine Chetoui was increased under salt stress. The local grapevine genotypes revealed accumulation of Na^+ in roots greater than in leaves while Cl^- levels were greater in leaves than in roots. Local grapevines performed Na^+ exclusion from leaves to reduce sodium damages on vegetative growth and/or high Na^+ accumulation in roots which would confer for them a mechanism to manage salinity damages. However, the introduced grapevine Muscat d'Italie accumulated high level Cl^- in leaves. Indeed, the high salinity dose 150 mM NaCl induced important accumulation of Na^+ in the introduced grapevine leaves that exceeded roots level. Yet, roots content on saline ions (Na^+ and Cl^-) decreased under salt stress.

Keywords: Dry matter, grapevine genotypes, salinity, toxic ions (Na^+ and Cl^-), vegetative growth

The salinization is becoming a worldwide menace for the soil and the plants. Zhang et al. (2012**)** estimated saline-affected farmland at 80 million hectares; they were generally located in arid and semi-arid climatic zones might aggravate the influence of (Nachshon 2018). In fact, the arid climate salinization on irrigated soils, especially in the absence of periodic leaching. Moreover, the soil salinization had relevant influences on agricultural sustainability and environmental health leading to severe

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losses of soil productivity and desertification processes (Rengasamy 2006; Tóth et al. 2008).

The salinity exerted also drastic effects on plants in term of low productivity and adverse effects on germination, plant vigor and crop yield (Munns and Tester 2008). Under salt stress, plants could be submitted to ion toxicity, osmotic stress, nutrient balance disturbance and lower rate of photosynthetic assimilation (Flowers 2004; Munns and Tester 2008). Shortly exposed to high salinity, plants suffered mainly from water stress. Once the salt stress still prolonged, ionic toxicity may be more aggravated. It has been proposed that the damages in plants were due to chloride ions or to the cationic increase caused by sodium ions (Garcia and Charbar 1993). In fact, an excessive accumulation of Na^+ was considered responsible for growth inhibition and yield reduction under salinity (Hong et al. 2009). Though, Cl^- was also accumulated by plants growing in saline condition (Gorham 1990).

Salinity actions on grapevines were manifested by the reduction of shoot, bunch and root number, decreased vigor, growth rate and photosynthetic rate decline, necrosis of leaf margin, adult leaf premature senescence due to ionic stress thus leaf number decline, with lower yield and death of the grapevine (Downton and Crompton 1979; Downton et al. 1990; Fisarakis et al. 2001; Ha et al. 2008; López-Aguilar et al. 2003; Prior et al. 1992; Shani and Ben-Gal 2005; Walker et al. 1997, 2002). It was found that salinity decreased the crop yields and its component by reducing root water uptake for potato in the southern of Tunisia (Nagaz et al. 2007) and for grapevines (Aragüés et al. 2015). In previous study, almost 25% of yield decrease occurred at 2.7 $dS.m^{-1}$ and 50% at 4.5 $dS.m^{-1}$ (Grattan 2002; Mass and Hoffmann 1976; Rhoades 1992).

In Tunisia, 1.5 million ha of soils, almost 10% of the total area of the country, are affected by salinity. The soil salinization was more intensified at the main vineyard areas in Tunisia (Hachicha 2007). The increased soil salinization was also considered as a serious threat to grapevine growing with the risk of salt dissolvability in water for most irrigated vineyards, especially those deficit-irrigated (Keller 2010).

The viticulture is spread almost over all the country of Tunisia. Currently, grapevines are grown throughout Tunisia mainly for fruit production (Harbi Ben Slimane 2001). The principal production areas are localized in Ben Arous, Nabeul in the north-east and Sidi Bouzid in the center of Tunisia with productions in 2015 of 56094, 14845 and 18003 tons. respectively (GIFruit 2016). Despite the arid climate, the production of table grapevines is relatively important in the south Tunisia, especially in Gabes and Medenine (800 and 1970 tons, respectively) (GIFruit 2015).

In 2015, the oases of Tozeur, southwest of Tunisia, registered 88 tons of grapevines yield (GIFruit 2015). According to an investigation carried out in the oases of Tozeur on 2012, five local populations with an introduced table grapevine variety were found showing important adaptation to the area conditions, particularly the high salt load of irrigation water (Ben Maachia 2014).

Furthermore, the oases of Tozeur were endowed with resources of irrigation water with high salt loads. Irrigation water was provided by the Agricultural Development Groups (GDA) with a salinity about 1.8 to 3 g/l or by farmers' wells (surface or deep wells) with a salinity about 1.9 to 10.62 g/l (Ben Maachia 2014). The present study investigated responses to salt stress of principal grapevine genotypes in the oases of Tozeur. In fact, the objective of the study was to classify local grapevines genotypes according to their tolerance to salinity and thus better value the grapevine heritage of the oases of Tozeur as well as its salinized soils.

MATERIALS AND METHODS

Plant material and salt treatment conduct.

The study was carried out in the open-air conditions in the Regional Center for Research in Oasis Agriculture of Degueche, Tozeur, Tunisia. This region was characterized by a dry and arid climate. Indeed, the geographical location of Tozeur and the averages of recorded rainfall explained its classification in the upper Saharan bioclimatic level (Table 1).

Table 1. Monthly distribution of rainfall (mm) in the Governorate of Tozeur

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2013	0	0	0	7.7	0	3	0	0	20.8	0	0	0
2014	9.8	1.3	16.2	21.5	1.8	1.9	0	4.4	0	0	23	5.9

Source: INM, Directorate Tozeur, Tunisia (2013/14)

For this assay, the principal grapevines in the oases of Dgueche, Chamsa, Mrah Lahouar and Nefta (Tozeur, Tunisia) were selected namely Arbi, Chetoui, Guelb Sardouk, Kahla and Sfaxi which are local grapevine populations and Muscat d'Italie which is an introduced variety. The cuttings were collected on December 2012 from these oases.

Three buds' cuttings were planted in plastic pots (3 kg) containing peat and sand (2:1; w:w). Ten replicates were considered for each genotype. The culture substrate (sand + peat) was non-saline, well provided with organic matter (Tables 3, 4) and warmed up quickly at low temperatures leading to an early start of root activity (Dhaouadi, 2017). The sand was characterized by a fast mineralization (Dhaouadi, 2017) due to its C/N ratio (Table 3).

The plantlets were irrigated twice a week with tap water with 1.77 g/l salt load, or 28 mM NaCl, (Table 2) before the beginning of the stress, from January 2013 to April 2014. The rainfall during this period was law and recorded on few months (Table 1). The maximum rainfall was recorded on September 2013 and April 2014, respectively 20.8 mm and 21.5 mm (Table 1).

Table 2. Chemical analysis of the tap water used for irrigation

pH	ECe (dS/m)	Soluble cations				Soluble anions				SAR
		Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}	
7.91	2.52	178.7	74.0	302.3	17.0	0.0	124.7	491.7	569.3	10.59

Source: SONEDE, Tozeur, Tunisia (2014)

Once the one-year old cuttings reached up to a homogenous stage (6-7 leaves), the salt treatment was started. The salt stress was applied by adding gradually 25 mM NaCl to the tap water (Table 2) each week until reaching the final doses (50, 75, 100 and 150 mM NaCl). The stress lasted 8 weeks (May and June 2014) during which the rainfall was 1.8 and 1.9 mm, respectively (Table 1).

Table 3. Chemical analysis of the used sand

pH	MO (%)	ECe (dS/m)	N^- org$^+$ NO_3^-	P_2O_5 assim.	K_2O exch.	Ca exch.	Mg exch.	Na exch.	C/N
8.86	0.08	0.17	0.20	36.57	81.17	4715.02	155.68	85.44	2.33

Source: Food Quality Laboratory, Tunis, Tunisia (2014)

After 5 weeks, the final concentrations 50, 75, 100 and 150 mM NaCl were reached. Irrigation with the final concentrations was continued for 3 weeks. A control group irrigated by tap water (28 mM NaCl) was also used.

The plantlets were watered twice a week with an alternating cycle of irrigation with saline solutions followed by an irrigation with nutrient solution (Stimulant containing 13% nitrogen, 40% P_2O_5, 13% K_2O and 3% MgO); between these irrigations, a leaching was performed with tap water (ECe = 2.52 dS/m) weekly to avoid salt build-up in the substrate.

The objective of this pot experiment was to evaluate the responses of the six grapevine genotypes to the salt stress.

Table 4. Chemical analysis of the used peat

pH	MO (%)	MS (%)	ECe (dS/m)	N	P_2O_5	K_2O	S	Mg
6	90	25	0.4	210	150	270	150	100

Source: Klasmann-Deilmann Compagny, GmbH, Germany (2013)

Morphological parameters.

At the end of the salt treatment, the cuttings were collected from pots with the sand mixture (sand and peat). To guarantee the collect of all the roots, “cuttings + sand mixture” were gently washed under the tap until there was no sand in the plantlet roots; this procedure facilitated the collect of all the roots even the fine ones. The cuttings were then washed with distilled water and dried from water at room temperature.

To investigate the impact of salt on photosynthetic organs and to note the changes that would be involved in the salt tolerance of the six studied grapevine genotypes, the following measures were performed:

- Length of the root part: length of part below the ground with considering the principal root,
- Length of the vegetative part: length of part above the ground to the apex of the principal branch,
- Number of leaves: the total leaf number per cuttings.

Ion analyses.

Following this, local cuttings were divided into root and aerial parts (above and below the ground), weighted and dried in the oven at 110 °C until their weight became stable. Then, their dry matter content was determined.

The dried leaves and roots were grounded into powder and then the mineral content (Na^+ and Cl^-) were determined in the Food Quality Laboratory, Megrine-Tunis, Tunisia.

The sodium content was measured by a mineralization carried out with ashes of the dried samples (leaves and roots) with adding 10 ml of nitric acid (HNO_3). Sodium contents were determined by flame photometry on extracts obtained by mineralization. Yet, chloride content was measured on dried samples and the dosage was carried out by titration. These analyses were triplicate.

Statistics.

A stat view ANOVA program was used for statistical analysis of the data. Values for different treatments within each parameter were compared using the variance analysis (ANOVA) with repeated measures. Means were tested by Newman-Keul's with a significance level of $P \leq 0.05$.

RESULTS

Salt stress effects on growth parameters.

According to the daily observations, the first symptoms of stress (chlorosis) were observed for Kahla cuttings irrigated by the 75 mM NaCl five weeks after the salt treatment application (Fig. 1A). The stress action on leaves was aggravated at the end of the treatment especially under 75, 100 and 150 mM NaCl (Fig. 1BCD).

On the other hand, no mortality was recorded for all the genotypes under the different tested salinities.

In the present study, the shoot length of Guelb Sardouk cuttings increased slightly from 58 cm to 65 cm at 75 NaCl and to 71 cm at 100 mM NaCl while it decreased significantly under the high salt dose 150 mM NaCl, 45% less than control level (Fig. 2). The salt doses 50 and 100 mM NaCl induced also the decline of shoot length for Kahla (Fig. 2).

The local grapevine Sfaxi revealed significant decline of root length under all the salt doses of the stress; Sfaxi roots were more than 28% shorter compared to control roots (Fig. 2).

Else, the damages of long-term salt stress were more pertinent on leaves in term of number (Fig. 3). The leaf number was reduced progressively with the increment of salinity from control dose to 150 mM NaCl for the grapevine genotypes Chetoui, Kahla, Sfaxi, Arbi and Muscat d'Italie (Fig. 3).

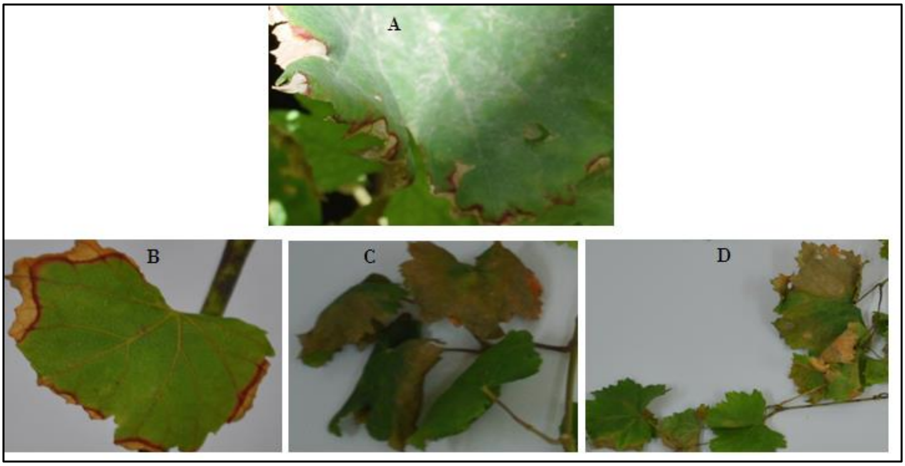

Fig. 1. Gradual salt stress symptoms on leaves. A: symptom on Kahla leaves after five weeks under 75 mM NaCl. B: symptom on Kahla leaves at the end of 75 mM NaCl stress. C-D: symptom on Muscat d'Italie leaves at the end of 100 and 150 mM NaCl stress, respectively.

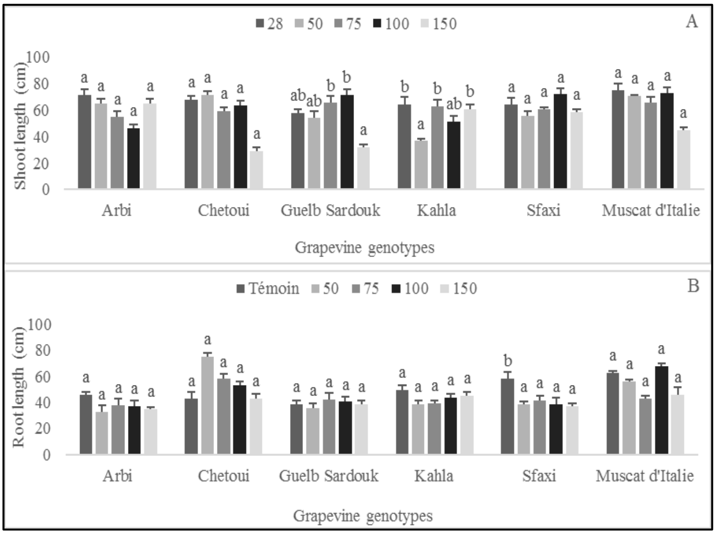

Fig. 2. Variations of shoot mean length (A) and root mean length (B) in cm for six grapevine genotypes submitted to salt stress. The dose of 28 mM NaCl was the salt load of the control treatment; 50, 75, 100 and 150 mM NaCl were the salt doses added progressively. Data represent the mean ± SE of three replicates. Different letters indicate significant difference for each grapevine genotype, according to Newman-Keuls test ($P \leq 0.05$).

At 50 mM NaCl, the reductions of leaf number were from 5% (Kahla and Muscat d'Italie) to 29% (Sfaxi) while at 75 mM NaCl, they were from 26% (Kahla) to 51% (Arbi). With increasing the stress to 100 mM NaCl, the declines of leaf number were noted from 23% (Sfaxi) to 51% (Muscat d'Italie).

The dose 150 mM NaCl exerted drastic effects on the leaf number average for the studied grapevine genotypes (Fig. 3); the decreases varied from 46% (Chetoui) to 81% (Muscat d'Italie). Otherwise, Guelb Sardouk registered an increase on leaf number under 75 mM NaCl (+10%) and 100 mM NaCl (+31%), while the dose 150 mM NaCl reduced the leaf number from 50 leaves (control) to 27 leaves (Fig. 3).

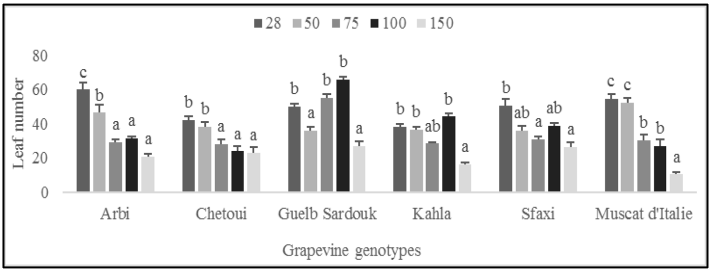

Fig. 3. Variations of leaf number average for six grapevine genotypes submitted to salt stress. The dose of 28 mM NaCl was the salt load of the control treatment; 50, 75, 100 and 150 mM NaCl were the salt doses added progressively. Data represent the mean ± SE of three repetitions. Different letters indicate significant difference for each grapevine genotype, according to Newman-Keuls test ($P \leq 0.05$).

Salinity effects on dry matter.

According to our data, long term exposure to gradual salt stress, at all salt doses, increased the dry weight of the aerial part (shoots + leaves) for the grapevine genotypes. For Arbi cuttings, the shoots dry weight increased at $P \leq 0.05$ only under the high salt dose 150 mM NaCl (Fig. 4A), while for Guelb Sardouk cuttings, the shoot dry weight decreased under the gradual stress of 150 mM NaCl (Fig. 4A).

The roots dry weight increased significantly at 75 mM NaCl for Sfaxi, at 100 mM NaCl for Guelb Sardouk and at 150 mM NaCl for Kahla, whereas the other salt doses had no significant effect on their root dry weight (Fig. 4B). Chetoui cuttings also showed an increase of their root dry weight under salt stress (Fig. 4B). The gradual salt stress did not induce any variation on root dry weight for Muscat d'Italie cuttings (Fig. 4B).

Otherwise, the higher ratio root/shoot dry weight (2.0) was registered by Sfaxi cuttings under control condition (Table 5). Under salt stress, Sfaxi and Kahla cuttings revealed the highest root/shoot dry weight ratio (1.5) at 75 mM NaCl comparable to that of control (Table 5). The root/shoot dry weight ratio decreased by increasing the salinity when the salt dose was 100 mM NaCl for Arbi (Table 5).

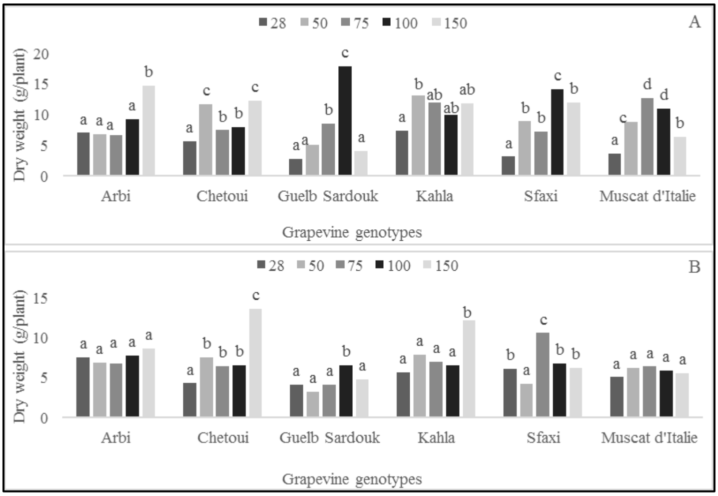

Fig. 4. Salt stress effects on dry matter distribution for six grapevine genotypes submitted to salt stress. A: shoot dry matter variations. B: root dry matter variations. Salt doses: 28 mM NaCl (control) and 50, 75, 100 and 150 mM NaCl were the salt doses added progressively. Data represent the mean ± SE of three repetitions. Different letters indicate significant difference for each grapevine variety, according to Newman-Keuls test ($P \leq 0.05$).

After long term exposure to salt stress, Chetoui cuttings showed root/shoot dry weight ratio comparable to that of the control ratio while at 150 mM NaCl, this ratio was slightly higher (1.2). However, Guelb Sardouk revealed high root/shoot dry weight ratio at control condition with a ratio slightly higher than 1 (1.2) at 150 mM NaCl (Table 5). Muscat d'Italie revealed also a high root/shoot dry weight ratio (1.4) at control condition which decreased with increasing the salinity; at 150 mM

NaCl, the ratio root/shoot dry weight increased to 0.9 but still lower than control level (Table 5).

Low root/shoot dry weight ratios were registered with Guelb Sardouk and Muscat d'Italie at 50, 75 and 100 mM NaCl, with Kahla and Sfaxi at 50 and 100 mM NaCl and with Arbi at 150 mM NaCl (Table 5). These low ratios suggested the favorized aerial part development than root growth by grapevine genotypes at low salinities for Guelb Sardouk, Muscat d'Italie, Kahla and Sfaxi and at the high salinity 150 mM NaCl for Arbi.

Table 5. Salt stress effects on root/shoot dry weight ratio of the grapevine genotypes

Grapevine genotypes	Salt doses (mM NaCl)				
	28	50	75	100	150
Arbi	1.1	1.0	1.1	0.8	0.6
Chetoui	0.9	0.7	0.9	0.9	1.2
Guelb Sardouk	1.5	0.6	0.5	0.4	1.2
Kahla	0.8	0.5	1.5	0.7	1.0
Sfaxi	2.0	0.5	1.5	0.5	1.0
Muscat d'Italie	1.4	0.7	0.5	0.5	0.9

Ion analysis.

There were significant differences ($P \leq 0.05$) between grapevine leaf and root Na^+ and Cl^- contents under salt stress (Tables 6, 7).

Leaves.

For the local genotypes Arbi, Chetoui, Kahla and Sfaxi, Na^+ leaf content decreased with increasing salinity level, while it increased for Guelb Sardouk and Muscat d'Italie (Table 6).

In fact, Chetoui, with the highest Na^+ leaf content (0.49% dry weight) at control condition, revealed decline of Na^+ leaf level with the increment in salinity from 50 to 150 mM NaCl (0.33% dry weight). Yet, Muscat d'Italie, with the lowest content (0.27% dry weight) at control condition, recorded important accumulation (0.65% dry weight) of Na^+ in its leaves at 150 mM NaCl (Table 6).

Chloride ions were more accumulated in leaves than sodium ions for the studied grapevines (Table 6). Under salt stress, Cl^- leaf content increased for the local genotype Arbi from 1.1% dry weight at 28 mM NaCl to 1.52% dry weight at 150 mM NaCl (Table 6). However, Sfaxi recoded high accumulation at control condition (1.88% dry weight) and under low salt dose 50 mM NaCl (1.95% dry weight). With the increment of salinity from 50 to 150 mM NaCl, leaf content on Cl^- decreased to 1.03% dry weight at 150 mM NaCl for Sfaxi cuttings (Table 6). The salt dose 75 mM NaCl induced important amount of Cl^- in Kahla leaves (2.02% dry weight) while under 100 mM NaCl, the highest leaf content on Cl^- was recorded by the introduced variety (Table 6). Under the high salt dose 150 mM NaCl, Arbi, Chetoui and Kahla revealed similar Cl^- leaf content (Table 6).

Roots.

According to our results, sodium ions were more accumulated in grapevine roots than leaves (Tables 6, 7). In fact, Na^+ Arbi root content increased with increasing the salt dose, from 0.46% dry weight at control condition to 0.57% dry weight under 150 mM NaCl (Table 7). Despite the recorded arise at 75 mM NaCl, Chetoui revealed low root content under 50 and 100 mM NaCl compared to control level, respectively 0.33% and 0.34%. At 150 mM NaCl, Chetoui Na^+ root content was similar to control level (Table 7). The gradual salt stress reduced Na^+ root content for Kahla and Muscat d'Italie; 100 mM NaCl increased their Na^+ roots content to a similar content to control level (Table 7). However, Guelb Sardouk and Sfaxi, cuttings, revealed important accumulations of Na^+ within their roots under 150 mM NaCl, respectively 0.68% and 0.85% dry weight (Table 7).

Table 6. Variations of Na^+ leaf content under gradual salt stress for the tested grapevine genotypes

Grapevine genotype	Salt dose (mM NaCl)									
	28		50		75		100		150	
	Na^+	Cl^-	Na^+	Cl^-	Na^+	Cl^-	Na^+	Cl^-	Na^+	Cl^-
Arbi	0.32 b	1.1a	0.33 b	1.52 b	0.2 a	1.49 b	0.27 ab	1.42 b	0.25 ab	1.52 b
Chetoui	0.49 b	-	0.25 a	1.6 b	0.31 a	1.24 a	-	-	0.33 a	1.52 b
Guelb Sardouk	0.34 ab	-	0.27 a	-	0.38 b	1.74 c	0.39 b	1.31 bc	-	-
Kahla	0.36 c	-	0.16 a	1.35 a	0.24 b	2.02 c	0.23 b	1.17 a	0.28 b	1.6 b
Sfaxi	0.39 c	1.84 c	0.13 a	1.95 c	0.24 b	1.6 b	0.15 a	1.03 a	-	-
Muscat d'Italie	0.27 b	-	0.17 a	-	0.28 b	1.21 a	0.22 ab	1.88 d	0.65 c	-

Data represent the mean of three repetitions. Different letters indicate significant difference for each grapevine genotype, according to Newman-Keuls test ($P \leq 0.05$).

Table 7. Variations of Na^+ root content under gradual salt stress for the tested grapevine genotypes

Grapevine cultivar	Salt dose (mM NaCl)									
	28		50		75		100		150	
	Na^+	Cl^-	Na^+	Cl^-	Na^+	Cl^-	Na^+	Cl^-	Na^+	Cl^-
Arbi	0.46 a	0.96 a	0.48 b	1.24 b	0.51 c	1.06 a	0.55 d	0.96 a	0.57 d	1.21 b
Chetoui	0.58 b	0.99 ab	0.33 a	0.85 a	0.87 c	0.89 a	0.34 a	0.82 a	0.57 b	1.06 b
Guelb Sardouk	0.42 a	1.03 b	0.48 a	1.17 b	0.94 b	1.31 b	0.48 a	0.71 a	0.68 b	1.1 b
Kahla	0.58 b	0.78 a	0.42 a	0.92 ab	0.41 a	1.21 c	0.59 b	0.82 a	0.53 ab	0.99 b
Sfaxi	0.64 b	0.92 a	0.38 a	0.96 a	0.4 a	1.21 b	0.37 a	0.85 a	0.85 c	1.49 c
Muscat d'Italie	0.69 b	1.38 c	0.4 a	-	0.38 a	0.71 a	0.7 b	1.06 b	0.34 a	-

Data represent the mean of three repetitions. Different letters indicate significant difference for each grapevine genotype, according to Newman-Keuls test ($P \leq 0.05$).

Otherwise, long term exposure to salinity increased Cl^- root contents for Kahla, Arbi and Chetoui from 0.78%, 0.96% and 0.99 % dry weight under control condition to 1.21% dry weight at 75 mM NaCl, 1.21% and 1.06% dry weight at 150 mM NaCl respectively (Table 7).

The salt dose 100 mM NaCl decreased Cl^- root content for Guelb Sardouk, from 1.03% dry weight under control condition to 0.71% dry weight at 100 mM NaCl; the other salt doses had no significant effects on Cl^- root content (Table 7). The gradual salt stress decreased Cl^- root content for Muscat d'Italie from 1.38% dry weight under control condition to 0.71% and 1.06% dry weight under 75 and 100 mM NaCl respectively (Table 7).

DISCUSSION

Salinity induced severe damages on plants growth; it resulted in a reduction of the growth rate and a decrease of shoot, bunch and root numbers (Troncoso at al. 1999). Under salt stress, plant roots are in direct contact with saline solution in the soil as well as shoots since it supplies water for the other plant organs. So, roots and shoots are more susceptible to translated salinity effects. Thus, the depressive action of salt was more obvious on shoots and roots growth and then on leaves (Chookhampaeng et al. 2011; Munns 2005; Shani and Ben-Gal 2005; Troncoso at al. 1999). These previous findings were confirmed by local grapevine study with shoot and root lengths decreasing with the increment of salinity from 50 to 150 mM NaCl. Kahla and Guelb Sardouk genotypes revealed significant decreases in their shoot lengths at 50 to 150 mM NaCl. These decreases of shoot length confirmed the results of one-year-old Sultana grapevine genotype, own-rooted or grafted on 41B, 110R, 140Ru, 1103P and SO4; its shoot length is reduced at all salinity levels (5, 25, 50 and

100 mM of NaCl) (Fisarakis et al. 2001). The local cultivar Sfaxi revealed significant decline of root length under all salt doses. In fact, the sand used in this experiment is characterized by a quick heat up at low temperature that favorized an early start of root activity (Dhaouadi, 2017). Thus, plants could consume their reserves quickly to meet their needs early and to ensure a good root development that allowed them to better withstand summer difficulties such as heat, wind, lack of water (Dhaouadi, 2017). This could explain the good root development and the non-affected root length by salinity of local grapevine cuttings.

Moreover, the tested grapevine genotype leaves were more damaged by long-term salt stress. The foliar damages were observed after 8 weeks of exposure to 75 mM NaCl from -26% for Kahla to -51% for Arbi while for clementine Hernandina the leaf number reductions were recorded after 16 weeks of 35 and 70 mM NaCl stress (Askri et al. 2017). With the increment of salinity to 150 mM NaCl, these damages became more prominent for local grapevines; the reductions in leaf number reached 81.2% at 150 mM NaCl for Muscat d'Italie. These results confirmed previous studies on three grapevine genotypes. Seedless Red and Ghezel Uzum (Karimi et al. 2013) and on one-year-old Sultana, own-rooted or grafted on 41B, 110R, 140Ru, 1103P and SO4 (Fisarakis et al. 2001). Moreover, four rootstocks 1103 Paulsen, 110 Richter, 140 Ruggeri and SO4 showed leaf number reduction after long term exposure to 100 mM NaCl added progressively (Hanana et al. 2015). However, for the Tunisian wild grapevine 'Séjnène', the total leaf number/ plant did not vary significantly after 60 days exposure to gradual salt stress of 100 mM NaCl (Hamrouni et al. 2011).

Our results agreed with Munns (2005) who suggested the decrease in vegetative growth, expressed by the reduction in the number of leaves as first reactions of glycophytes to salt stress. Under saline condition, the adult leaves accumulated sodium chloride in the cell walls and the cytoplasm. Then, the sap of the vacuoles could not amass more salt, causing the decline of salt concentration in the cells, thus, the rapid death and reduction of leaves (Munns 2002).

Furthermore, the reduction of vegetative growth was proposed as a salt adaptation mechanism performed by plants to avoid salinity-related damage (Ben Mahioul et al. 2009; Zhu 2002). Plants involved the reduction of vegetative growth to accumulate energy and resources for the stress combat before increasing the imbalance between the inside and the outside of the organ to an irreversible point of damage. Accordingly, plant growth was inversely correlated with salt stress resistance of a species or variety (Zhu 2002).

Otherwise, plant dry matter content was considered as a functional parameter used to assess the plant strategy for resource acquisition and use (Duru et al. 2009). Our data showed that salt stress increased the dry matter of the aerial part (shoots and leaves) for the tested grapevines.

Moreover, root dry weight measure was considered important in the evaluation of plant responses to salt stress. For grapevines, increases of root dry matter were induced by different salt doses: for Sfaxi at 75 mM NaCl, Guelb Sardouk at 100 mM NaCl and Kahla at 150 mM NaCl. The gradual salt stress increased root dry mater of Chetoui cuttings at all salt doses while the root dry matter of Muscat d'Italie cuttings was not affected.

These increases of dry matter were also reported by *Pennisetum alopecuroides* at 100 mM salinity (Mane et al. 2011) and by Purslane (Portulaca oleracea L.) at 16, 24 and 32 $dS.m^{-1}$ (Alam et al. 2016). Xu et al. (2008) suggested the accumulation of inorganic ions and organic solutes for osmotic adaptation to explain this stimulation in dry matter production in response to salt stress. The important accumulation of Cl^- in leaves could explain the increase of shoot dry weight for local grapevine genotypes. Furthermore, Na^+ root content for local grapevine genotypes was higher than lea content. Associated with the decline of Na^+ leaf content, we could suggest that local grapevine genotypes favorited the accumulation of Na^+ in roots. Thus, the sodium ion damages on aerial part growth could be reduced which was supported by the shoot dry weight increase.

For the glycophytes, the salinity tolerance was associated with the ability to limit uptake and/or transport of saline ions (principally Na^+ and Cl^-) from root zone to aerial parts (Greenway and Munns, 1980). The reduction of Na^+ leaf content for Arbi, Chetoui, Kahla and Sfaxi suggested the inhibition of Na^+ accumulation in their leaves under salt stress. In addition, Muscat d'Italie also revealed the ability to exclude sodium from the leaves and store it at the root level, while at high salinity 150 mM NaCl, a significant increase in Na^+ at the leaf level associated with a reduction in Na^+ root content was observed in Muscat d'Italie. For the local cultivar Guelb Sardouk, the inclusion capacity of Na^+ at the leaf level was observed with the salinity 75 mM NaCl. The Tunisian wild grapevine 'Séjnène' also adopted an "includer" behavior for sodium in the aerial parts (shoots and leaves) under gradual stress of 100 mM NaCl (Hamrouni et al. 2011). The local genotypes Arbi, Chetoui, Kahla and Sfaxi seem to be "excluder" for sodium from their leaves.

The ionic levels of shoot tissue consisted on the summation of integrated processes: transport of ions from roots to shoots, relative growth rates, tissue selectively of the shoot sand rates of ion re-translocation from shoots to roots (Pitman, 1975; Greenway and Munns, 1980). In fact, Na^+ re-translocation from shoots to roots has been reported (Pitman, 1975). These finding could explain the decline of Na^+ leaf content for the local genotypes Arbi, Chetoui, Kahla and Sfaxi Indeed, Sfaxi followed by Kahla, Arbi and Chetoui, in descending order, were the least accumulating sodium in the leaves. Muscat d'Italie revealed low Na^+ leaf content under 50, 75 and 100 mM NaCl, while at the high salinity (150 mM NaCl), the Na^+ leaf content increased remarkably with a decline of Na^+ root level; thus, we could suggest that, at 150 mM NaCl, Muscat d'Italie could not limit the uptake and/or transport of saline ions (Na^+) from root zone to aerial parts.

Local grapevine genotypes accumulated high Cl^- amounts in both parts; Cl^- levels were greater in leaves than in roots which could be due to the illimitation of the uptake and/or transport of Cl^- from roots to leaves. Therefore, we could suggest the adoption of an "includer" behavior for Cl^- by the tested grapevines while the Tunisian wild grapevine Séjnène excluded Cl^- ions from its aerial parts (Hamrouni et al. 2011). In fact, Fisarakis et al. (2001) reported that higher leaf Cl^- accumulation associated with low Na^+ content did not mean that Cl^- is responsible for toxic symptoms. Na^+ was considered as main responsible for leaf burn even if it occurred at lower level than Cl^- (Fisarakis et al., 2001).

Besides, the irrigation water chemical proprieties, the SAR, representing sodium ions activity, was higher than 10 which indicated the possibility of sodium accumulation in soil (N'Diaye et al., 2010). Consequently, with the fact of the favorized accumulation of

Na^+ in roots than in leaves, we could suggest the limitation of the uptake and/or transport of Na^+ to leaves by local grapevine genotypes. Indeed, the salt stress increased the Na^+ root content for Arbi, Chetoui and Guelb Sardouk which could provide a mechanism to manage salinity in the rooting medium (Fisarakis et al., 2001). This finding was associated with unaffected root length and dry weight for Arbi, Chetoui and Guelb Sardouk. However, the increase of Na^+ and Cl^- leaf content associated to the reduction of Na^+ and Cl^- root contents could reveal the sensitivity of Muscat d'Italie to salinity.

To conclude, the pronounced effect of the gradual salt stress was the decrease of leaf number for all the tested grapevines. We could propose that the local cultivar Arbi involved the reduction of salt uptake rate with the control of translocation to leaves to control the salt concentration in the aerial parts. Arbi has the least Cl^- accumulation in leaves than in roots under salinity followed by Chetoui and Sfaxi. Muscat d'Italie accumulated the highest amounts of Cl^- in leaves not in roots.

Else, Arbi root/shoot dry weight ratio was 1 or almost 1 at control condition and under 50 and 75 mM NaCl. Thus, we could suggest that for Arbi, shoot and root growth were similarly affected upon low salinities. By increasing the salt dose to 100 mM NaCl, the root growth was reduced slightly; the decrease was bigger under 150 mM NaCl. The decrease of root volume could reduce the drastic effect of salinity on shoot growth by limiting the accumulation of toxic ions in shoots.

We could classify local local grapevine genotypes according to their response to salinity: Arbi was the most tolerant one followed by Sfaxi, Kahla and Chetoui. The grapevine genotypes Guelb Sardouk and Muscat d'Italie could be considered as sensitive to long-term gradual salt stress.

RESUME

Habib A., Ben Maachia S., Sahli A. et Harbi Ben Slimane M. 2019. Réponses au stress salin de six génotypes vigne des oasis de Tozeur. Tunisian Journal of Plant Protection 14 (2): 1-16.

Afin d'étudier les réponses à la salinité des principaux génotypes de vigne, cultivés dans les oasis de Tozeur, Tunisie, six génotypes de vigne ont été soumis à un stress salin progressif. Après 8 semaines d'exposition à différentes doses de sel 50, 75, 100 et 150 mM NaCl, la longueur et le poids sec des sarments et des racines et le nombre de feuilles ont été mesurés. La salinité de 150 mM NaCl a réduit la longueur du sarment chez Chetoui, Guelb Sardouk et Muscat d'Italie. Les dégâts de la salinité se sont aussi manifestés par la diminution du nombre de feuilles pour les vignes locales, notamment sous 150 mM NaCl. Le stress salin à long terme a augmenté le poids sec de la partie aérienne (tige et feuilles) pour les vignes testées. Pour Arbi, une augmentation du poids sec de la partie végétative a été enregistrée sous 150 mM NaCl. Le poids sec des racines de la vigne locale Chetoui a augmenté sous stress salin. Les génotypes locaux de vigne ont révélé des accumulations de Na^+ plus importantes au niveau des racines qu'au niveau des feuilles alors que les teneurs des Cl^- étaient plus élevées dans les feuilles que dans les racines. Les vignes locales ont révélé l'exclusion de Na^+ des feuilles pour réduire les dégâts du sodium sur la croissance végétative et/ou la forte accumulation de Na^+ dans les racines qui semble leur conférer un mécanisme pour gérer les dégâts de salinité. Cependant, la variété introduite Muscat d'Italie a accumulé des quantités élevées de Cl^- au niveau des feuilles. En outre, la forte salinité 150 mM NaCl a induit une importante accumulation de Na^+ au niveau des feuilles de la vigne introduite qui dépassait le contenu des racines. Toutefois, la teneur des racines en ions salins (Na^+ et Cl^-) a diminué sous stress salin.

Mots clés : Croissance végétative, génotypes de vigne, ions toxiques (Na^+ et Cl^-), matière sèche, salinité

ملخص

الحبيب، عبير وسهام بن معاشية وعلي الساحلي ومنيرة حربي-بن سليمان. 2019. استجابات ستة أنماط جينية للكروم من واحة توزر للإجهاد الملحي. Tunisian Journal of Plant Protection 14 (2): 1-16.

بهدف دراسة الاستجابات للملوحة لأهم الأنماط الجينية للكروم المزروعة في واحة توزر، تونس، عُرّضت ستة انماط جينية لإجهاد ملحي تدريجي. بعد 8 أسابيع من التعرض لمختلف جرعات الملوحة 50 و75 و100 و150 ميليمول من كلوريد الصوديوم، تم قياس الطول والوزن الجاف للبراعم والجذور وعدد الأوراق. أدت الجرعة 150 ميليمول من كلوريد الصوديوم الى انخفاض طول البراعم لدى الأنماط الجينية "شتوي" و"قلب سردوك" و"مسكي إيطاليا". كما تجسدت الأضرار الناجمة عن الملوحة من خلال انخفاض عدد الأوراق لدى الكروم المحلية، وخاصة تحت تأثير أعلى جرعة من الملح (150 ميليمول من كلوريد الصوديوم). أدى التعرض الطويل الأمد لإجهاد الملح إلى زيادة الوزن الجاف للجزء الهوائي (الجذع والأوراق) للكروم المدروسة. بالنسبة لـ"عربي"، ارتفع الوزن الجاف للبراعم فقط تحت تأثير 150 ميليمول من كلوريد الصوديوم. بينما أظهر الكرم المحلي "شتوي" زيادة في الوزن الجاف للجذور تحت ضغط إجهاد الملح. كشفت الكروم المحلية عن تخزين Na^+ في الجذور أكثر من الأوراق؛ اما كميات Cl^- فكانت أعلى في الأوراق من الجذور. تقوم الكروم المحلية بإقصاء Na^+ من الأوراق لتقليل الضرر الناجم عن الصوديوم على النمو الخضري و/أو بمراكمة عالية لـ Na^+ في الجذور كآلية ممكنة تمنحهم التحكم في الأضرار الناتجة عن الملوحة. أما الكرم الدخيل "مسكي إيطاليا" فيخزن كميات عالية من Cl^- في الأوراق. كما تسببت الملوحة العالية 150 ميليمول من كلوريد الصوديوم في تراكم كمية هامة من Na^+ في أوراق الكرم الدخيل لتتجاوز محتوى الجذور. ولكن انخفضت كميات أيونات الملح (Na^+ و Cl^-) في الجذور تحت تأثير الإجهاد ملحي.

كلمات مفتاحية: أنماط جينية للكروم، أيونات سامة (Na^+ وCl^-)، ملوحة، مادة جافة، نمو خضري

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Observations of some Biological Characteristics of *Helicoverpa armigera* Reared under Controlled Conditions

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ABSTRACT

Bouslama, T., Chaieb, I., Jerbi-Elayed, M., and Laarif, A. 2019. Observations of some biological characteristics of *Helicoverpa armigera* reared under controlled conditions. Tunisian Journal of Plant Protection 14 (2): 17-27.

Helicoverpa armigera is a polyphagous pest causing significant economic losses on different host crops. Investigating the biological characteristics of this pest is essential to develop efficient integrated pest management strategies. Several collections of larvae were taken from pepper field crops in the governorate of Kairouan, Tunisia. Larvae were reared on semi artificial diet under laboratory conditions till adults' emergence. Thirty newly emerged couples were chosen for the oviposition and the rearing starting-up. A mean number of 226 eggs were laid per female and 37% of the eggs have hatched. After hatching, 100 larvae were chosen to follow and describe the different stages of the insect development. Duration of 32 days has elapsed between the egg stage and the adult emergence. Neonate mortality was higher than the other stages of the insect development. A cannibalism test was performed on first-instar and third-instar larvae of *H. armigera*. The test showed that third-instar larvae have the most cannibalistic behavior. This test has also shown that cannibalism increases with crowding.

Key words: Biological characteristics, cannibalism, *Helicoverpa armigera*, rearing

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Helicoverpa armigera (Lepidoptera: Noctuidae) known as old world bollworm, African bollworm or cotton bollworm is a polyphagous pest of tropical origin (Denlinger 1986).

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H. armigera is widely distributed in Europe, Africa, Asia and Australia; it recently invaded South America and is likely to spread to North America (Downes et al. 2017; Kriticos et al. 2015).

It can attack more than 172 plant species from 68 different families (Cunningham and Zalucki 2014; Fitt 1989; Singh et al. 2002; Zalucki et al. 1986; 1994), including cotton, sorghum, tomato, sunflower, beans, maize, numerous leguminous, ornamental plants and fruit trees (Razmjou et al. 2014; Sun et al. 2004).

This insect pest is characterized by its high reproductive potential and high capacity for dispersion and survival (Amer and El-Sayed 2014). *H. armigera* caterpillar can damage both the vegetative and the reproductive phases of the plant, feeding on leaves, stems, buds, inflorescences, fruits and pods (Reed 1965; Wang and Li 1984).

In Tunisia, *H. armigera* damage was reported for several years only on tomato fruits in open field and greenhouse cultures (Boukhris-Bouhachem et al. 2007; Chermiti et al. 1996).

Direct damage to the structures of flowering and fruiting of host plants by *H. armigera* caterpillars and the extensive use of insecticides result in low productivity and high costs of control (Fathipour and Naseri 2011; Lammers and MacLeod 2007). That needs to seek other alternative methods for *H. armigera* control.

The study of insect in the laboratory is important to develop later modern pest control strategies (Greene et al. 1976; Hamed and Nadeem 2008). To reach this aim, it is necessary to succeed in the rearing of the insect in the laboratory.

This work is a study of the biological characteristics of *H. armigera* under controlled conditions in order to settle a permanent rearing of the insect in the laboratory.

MATERIAL AND METHODS

H. armigera collection and rearing.

Several collections of larvae were taken from pepper field crops in the governorate of Kairouan, Tunisia. The insect rearing was held on a chickpea-based artificial diet in a rearing room under controlled conditions at a temperature of 25 ± 2°C, relative humidity of 70 ± 5% and 16 hours day light.

This artificial diet is inspired by the diet developed by Poitout and Bues (1974). It is composed of 800 ml of distilled water, 1.5 g of ascorbic acid, 1 g of benzoic acid, 1.5 g of nepagin, 1.5 ml of formaldehyd, 105 g of chickpea powder, 10 g of yeast and 12 g of agar.

Differentiation between the two sexes was performed in the chrysalid stage by examining the ventral side of the last abdominal segments (Armes et al. 1992).

When adults emerged, they were fed with a sweet solution (10% sucrose).

Study of the biological characteristics of *H. armigera*.

Thirty newly emerged couples were placed separately in 30 plastic jars (5 cm × 6 cm) covered by muslin. These jars were lined with sterile and humidified sponges. The sponges containing the eggs were plucked in hermetic boxes until hatching. Neonates were placed in Petri dishes containing the artificial diet.

Hundred larvae were taken at random from the obtained batch to follow their development. Larval length, mortality rate and period of each larval stage were studied based on moults. During pupation, the number of males and females, as well as the weight of each pupa, was determined. The number of emerged males and females was also registered.

Study of the cannibalism of *H. armigera*.

Cannibalism may become common in *H. armigera* larval populations when physiological needs increase whereas larvae molt from a stage to another (Tang et al. 2016).

To estimate cannibalism of first instar and third instar *H. armigera* larvae, 4 densities of each stage were tested (2, 4, 8 and 16 larvae). The larvae of each larval stage corresponding to one of each density were put in Petri dishes containing cubes of about 1 g of artificial diet. Each density of each larval stage is repeated 5 times. The Petri dishes were checked after 48 h to record the number of cannibalized larvae.

Cannibalism in first and third instar larvae for the four densities was analyzed using the Student's *t*-test, ($P < 0.05$). Difference between the two instars was considered significant when the *t*-stat value was greater than the *t*-critical value and the $P < 0.05$. Cannibalism in each larval stage was analyzed using a variance analysis test (ANOVA), $P < 0.05$, averages were analyzed by a test of Student Newmann and Keuls (SNK). All tests were performed using the software SPSS 21.

RESULTS

Study of the biological characteristics.

Eggs. Eggs were laid individually on sponges (Fig. 1). Eggs were spherical and about 0.5 mm in diameter with longitudinal ridges. Newly laid eggs were creamy white. Fertile eggs turned brown and became black before hatching. Infertile eggs were yellow.

The results of the experiment showed that a female can lay up to 226 eggs, after a period of 3 days in laboratory conditions. A number of 37% of eggs hatched after 4 days from the oviposition date (Table 1).

Table 1. Eggs and adult's lifetime of *Helicoverpa armigera*

Laid eggs per female	Hatched eggs per female	Number of days till		Adult's lifetime (Days)	
		Oviposition	Hatching	Males	Females
226.13 ± 7.16	82.96 ± 2.29	2.61 ± 0.58	4.33 ± 0.73	4.2 ± 1.6	4.1 ± 1.68

Larvae. Neonates were pale cream with faint dark longitudinal lines, brown-black dorsally distributed spots, brown head capsules and brown thoracic legs (Fig. 2).

A rate of 17% of first-instar larvae died after hatching. *H. armigera* larvae passed by five instars in laboratory conditions. The second instar larvae were light brown. The third instar larvae were brown and presented larval morphological characteristics of *H. armigera*. Fourth and fifth instar larvae were characterized by the combination of different colors (green yellow and brown).

The 5 larval stages lasted about 15 days. Larvae length ranged from 2 mm for L1 to 29 mm for L5. Mortality was higher after hatching than during the other stages of the insect development (Table 2).

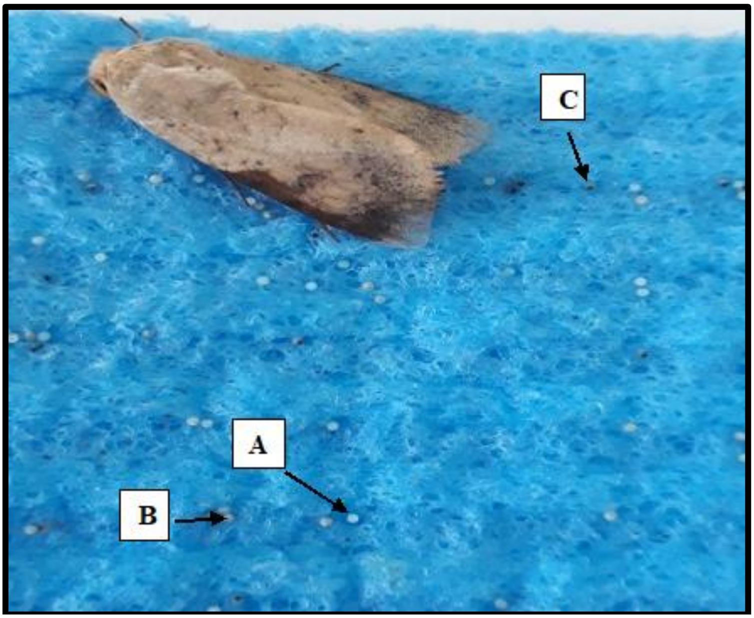

Fig. 1. *Helicoverpa armigera* female's oviposition on sponges (A: freshly laid egg; B: infertile egg; C: fertile egg)

Table 2. *Helicoverpa armigera* larval instars charateristics and dead larvae at each instar

Instar	Lenght (mm)	Instar duration (days)	Dead larvae
L1	1.61±0.28	2.16±0.37	14.10±0.39
L2	4.18±0.85	1.9±0.3	9±0.288
L3	5.99±0.87	1.43±0.5	0.00±0.00
L4	13.96±1.14	3.8±0.4	0.00±0.00
L5	28.95±1.8	5.07±0.25	0.00±0.00

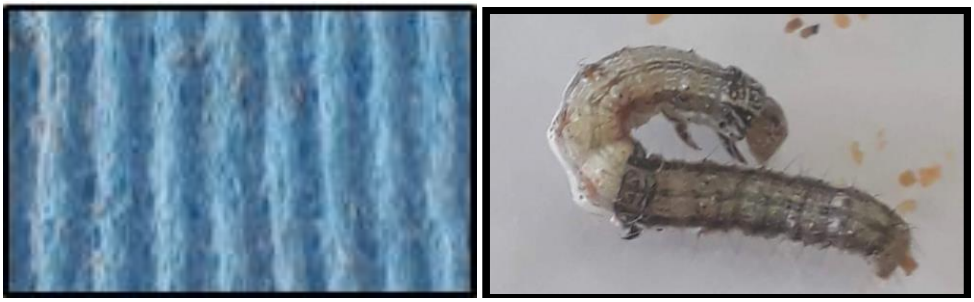

Fig. 2. *Helicoverpa armigera* neonates on the left; larva molting on the right

Pupae. When the larvae were fully grown and the pupation began, pupae were initially creamy white. The color changed gradually to brown and became darker with time. Pupae were soft and smooth surfaced (Fig. 3).

Female's genital groove was located in the middle of the 8th abdominal segment whereas the male's genital groove, observed under a binocular loupe, was located in the 9th abdominal segment (Figs. 4, 5). Pupae had an average weight of 201 mg. The duration of pupation was about 10 days (Table 3).

Table 3. Pupation charateristics and dead pupae of *Helicoverpa armigera*

Weight (mg)	Pupation duration (days)	Number of males	Number of females	Number of dead pupae
200.91±40.75	10.34±1.51	39.00±0.00	38.00±0.00	14.00±0.35

Fig. 3. Variation in color between young (on the left) and old (on the right) pupae of *Helicoverpa armigera*.

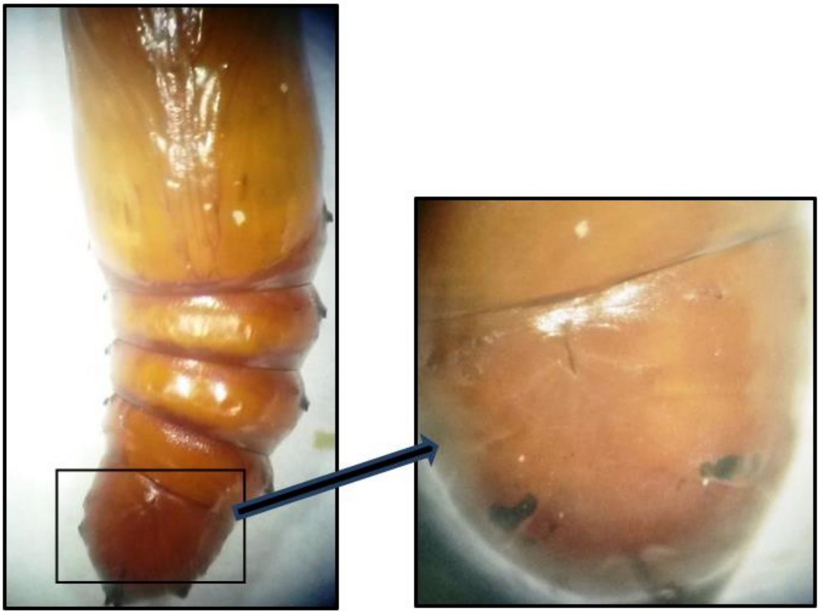

Fig. 4. Ventral view of female's *Helicoverpa armigera* pupae

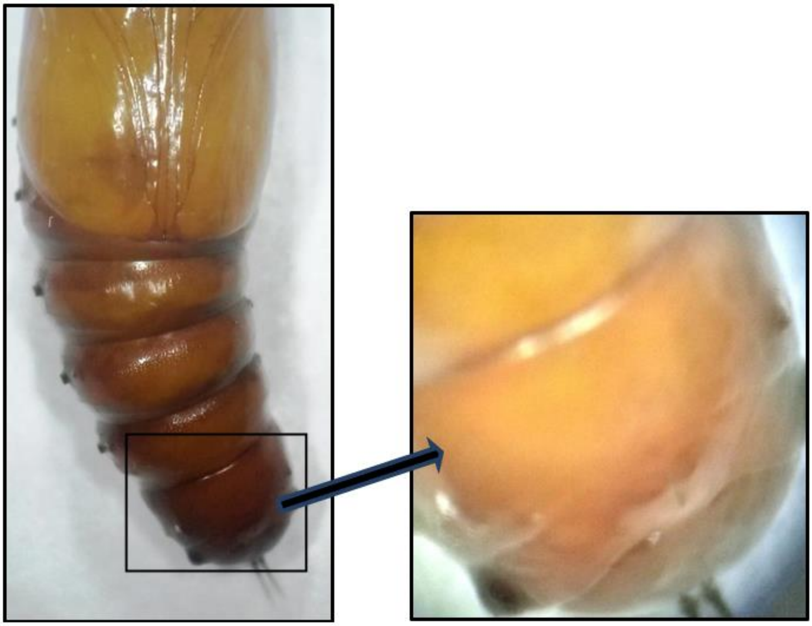

Fig. 5. Ventral view of male's *Helicoverpa armigera* pupae

Adults. Adult appearance was variable depending on the sex of moth. Males had greenish forewings and females had orange-brown forewings. Adults also showed similar characteristics, the middle of their forewings had a dark kidney-colored marking and their hindwings were lighter colored with a creamy white patch in the dark of the outer margin (Fig. 6).

In laboratory conditions, the longevity of adults was about 4 days and a female laid up to 226 eggs (Table 2).

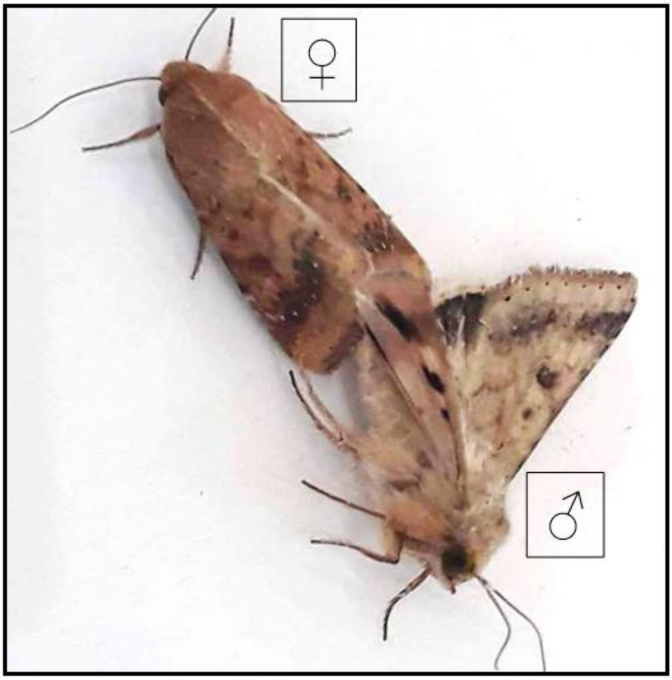

Fig. 6. Male and female adults of *Helicoverpa armigera* mating

Cannibalism test.

The results of the cannibalism test showed that cannibalism was more important in third instar larvae.

For first instar larvae, there was no cannibalism for the first two densitie (2 and 4 larvae) however larvae began to show cannibalistic behaviour in densities 3 (8 larvae) and 4 (16 larvae). It reached 2.5% for density 3 and 8.75% for density 4.

For third instar larvae, cannibalism was observed in all densities. Density 1 showed the lowest cannibalism with 30% cannibalized larvae, and density 4 showed the highest cannibalism, with 88.75% cannibalized larvae (Fig. 7).

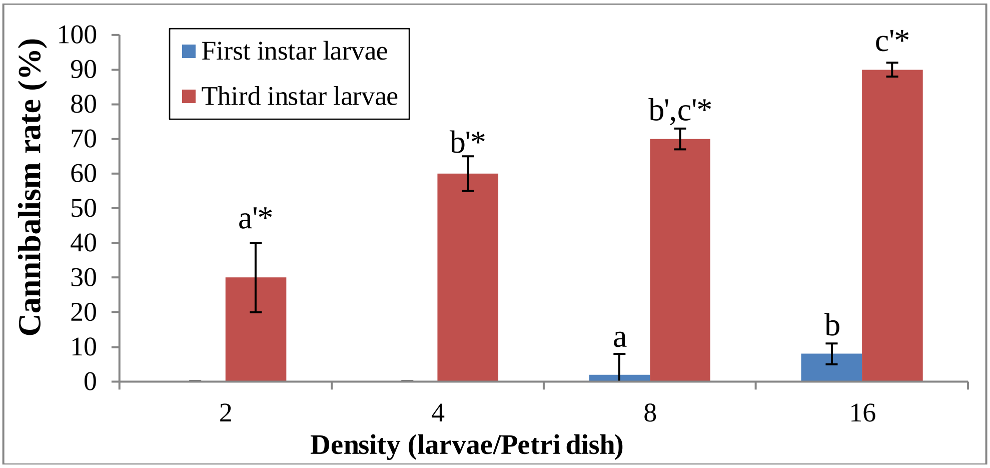

Fig. 7. Cannibalism observed for different densities of first and third instar larvae of *Helicoverpa armigera*. Error bars represent standard deviations. Different lowercase letters represent statistically significant differences among mortalities for each day of treatment according to SNK multiple comparison test ($P < 0.05$).
* : Statistically significant difference among mortalities according to Student test ($P < 0.05$).

DISCUSSION

Under laboratory conditions, *H. armigera* larvae had 5 stages based on moults. Kumar et al. (2009) observed 5 instars while Jha et al. (2014) and Liu et al. (2004) reported 6 instars.

The larval development period was about 15 days under controlled conditions. Mironidis and Savopoulou-Soltani (2009) stated that at 25°C, larval development period could last about 16 days. Barbosa et al. (2016) reported 13 days and Genç et al. (2017) reported a period between 13 and 21 days depending on temperature and humidity.

The pupal development period was about 10 days. These results are similar to the results reported by Mironidis and Savopoulou-Soltani (2009) which claimed that if the temperature is between 20 and 27.5°C, the pupal development period could last between 9 and 12 days according to a decreasing temperature gradient.

The number of laid eggs was about 226 eggs per female. These results are similar to those reported by Nasreen and Mustafa (2000) which stated 249 eggs per female reared with a 10% sucrose solution.

The results of the cannibalism test showed that *H. armigera*is cannibalism is more important in the third instar larvae than in the first instar larvae; larger is the larva, more it shows cannibal behavior. It has also been shown that cannibalism increases with crowding; greater is the number of larvae in a restricted space, more they show cannibal behavior. These results could be explained by the fact that there is a competition between the larvae for food and space. Fox (1975) and Kirkpatrick (1957) have noted that cannibalism may be widespread among herbivorous insects. Other authors stated that cannibalism can be observed in *H. armigera* even at first instars if larvae are kept at high density (Armes et al. 1992; Kakimoto et al. 2003). Cannibalism can be reduced by increasing the surface area available for feeding by using strips of diet (Giret and Couilloud 1987; Griffith and Haskell 1988) or vertical sticks as refuges to larvae (Giret and Couilloud 1987).

The results of this study could help in the settlement of a permanent rearing of *H. armigera* under laboratory conditions.

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RESUME

Bouslama T., Chaieb I., Jerbi-Elayed M. et Laarif A. 2019. Observations de quelques caractéristiques biologiques de *Helicoverpa armigera* élevée sous des conditions contrôlées. Tunisian Journal of Plant Protection 14 (2): 17-27.

Helicoverpa armigera est un ravageur polyphage qui provoque des pertes économiques importantes sur différentes plantes hôtes. Etudier les caractéristiques biologiques de cet insecte nuisible est essentiel au développement de stratégies de lutte intégrée efficaces. Plusieurs collectes de larves ont été réalisées à partir de cultures de piment au champ dans le gouvernorat de Kairouan, Tunisie. Les larves ont été élevées sur milieu artificiel au laboratoire jusqu'à l'émergence des adultes. Trente couples d'adultes qui venaient d'émerger ont été choisis pour la ponte et le démarrage des élevages. Un nombre moyen de 226 œufs ont été pondus par femelle et 37% des œufs ont éclos. Après l'éclosion, 100 larves ont été choisies pour suivre et décrire les différents stades de développement de l'insecte. Une durée de 32 jours s'est écoulé entre le stade œuf et l'émergence des adultes. La mortalité des jeunes larves était supérieure à celle des autres stades de développement de l'insecte. Un test de cannibalisme a été réalisé sur les larves de premier et troisième stades de *H. armigera*. Le test a montré qu'un cannibalisme est observé surtout chez les larves du troisième stade. Ce test a également montré que le cannibalisme augmente avec la surpopulation.

Mots clés: Cannibalisme, caractéristiques biologiques, élevage, *Helicoverpa armigera*

ملخص

بوسلامة، ثامر وإقبال الشايب ومي الجربي-العايد وأسماء العريف. 2019. ملاحظات لبعض الخصوصيات البيولوجية للعثة *Helicoverpa armigera* المرباة تحت ظروف محكّمة.
Tunisian Journal of Plant Protection 14 (2): 17-27.

الحشرة *Helicoverpa armigera* هي عثة متعددة العوائل تتسبب في خسائر اقتصادية كبيرة على محاصيل زراعية مختلفة. تعد دراسة الخصوصيات البيولوجية لهذه الآفة أساسية لتطوير استراتيجيات فعالة لحماية النباتات. جُمعت اليرقات من حقول زراعات فلفل في ولاية القيروان، تونس. ربيت اليرقات على نظام غذائي اصطناعي في المختبر حتى بزوغ البالغين. تم اختيار 30 زوجا من البالغين لوضع البيض وانطلاق التربية. وضعت كل أنثى معدل 226 بيضة، فقّس منها 37%. بعد الفقس، تم اختيار 100 يرقة لمتابعة ووصف المراحل المختلفة لتطور الحشرة .مرت فترة بـ 32 يومًا من مرحلة البيض إلى بزوغ البالغين. كان معدل وفيات اليرقات صغيرة السن أعلى من وفيات المراحل الأخرى من تطور الحشرة. تم إجراء اختبار الاعتراك على يرقات الطوريْن الأول والثالث. بين الاختبار وجود اعتراك أكثر لدى يرقات الطور الثالث. أظهر هذا الاختبار أيضًا أن الاعتراك يرتفع مع ارتفاع الاكتظاظ.

كلمات مفتاحية: اعتراك، تربية، خصوصيات بيولوجية، *Helicoverpa armigera*

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Mites Associated with the Red Palm Weevil (*Rhynchophorus ferrugineus*) in Tunisia

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ABSTRACT

Slimane-Kharrat, S., and Ouali, O. 2019. Mites associated with the red palm weevil (*Rhynchophorus ferrugineus*) in Tunisia. Tunisian Journal of Plant Protection14 (2): 29-38.

The red palm weevil (*Rhynchophorus ferrugineus*) is one of the main pests on Canary palm trees in Tunisia. Adults of this pest were captured in pheromone-baited traps in Canary infested palm orchards then dissected. Three mite species were found associated with this beetle: *Centrouropoda almerodai* attached to the underside of elytra, *Uroobovella marginata* adhered to the pygidium, thorax and head, and Ur*oobovella javae* fixed to the antenna, thorax and legs. The highest deutonymph load was found in the elytral space of red palm weevil adults. This study was carried out, for the first time in Tunisia, to identify mite species associated with the red palm weevil and to study the distribution pattern of these mites on the pest adult's body.

Keywords: *Centrouropoda almerodai, Rhynchophorus ferrugineus,* Tunisia, *Uroobovella javae, Uroobovella marginata*

Red palm weevil (RPW, *Rhynchophorus ferrugineus*; Coleoptera: Curculionidae) is the most important pest of date and Canary palm in the Middle East, Europe and North Africa. RPW adult is a strong beetle flyer, male and female weevils are able to fly to the longest average distances (25–35 km) in 24h under an average laboratory temperature of 27°C (Hoddle et al. 2015). Many organisms, including viruses, bacteria, fungi, nematodes, mites, insects and vertebrates, have been found in association with the red palm weevil (Mazza et al. 2014; Murphy and Briscoe 1999).

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Mites are considered as one of the most inadequately studied groups (Dilipkumar et al. 2015), with 26 identified species from different taxa (Dilipkumar et al. 2015; Kontschán et al. 2014). So far, 11 known and four unidentified species of the cohort Uropodina have been found to be related to this beetle from different Asian and European countries (Dilipkumar et al. 2015; Kontschán et al. 2014).

Among the mites reported in association with the red weevil we cite: *Hypoaspis* sp. (Gomaa 2006; Peter 1989); *Rhynchopolipus rhynchophori* (Ewing 1924; Husband and Flechtmann 1972; Ochoa et al. 1995) *Rhynchopolipus swiftae* (Husband and O'connor 1999), *Eutogenes punctata* (Gomaa 2006; Zaher and Soliman 1966) *Uroobovella javae*

(Wisniewski 1981), *Uroobovella marginata* (Koch 1839; Ragusa et al. 2009), *Centrouropoda almerodai* (Hiramatsu and Hirschmann1992; Longo and Ragusa 2006; Ragusa et al. 2009) and *Uropoda orbicularis* (Atakan al. 2009; Müller 1776). These mites are not considered to have a role in red palm weevil control (Longo and Ragusa 2006) as the relation between them is considered as a phoretic relationship (without any host impact). Phoresy is a phenomenon by which an organism (phoront) is actively on or in another organism (host) for a limited time period to complete their dispersal strategy to favorable habitats (Kaliszewski et al. 1995; O'Connor 1982).

However, a recent laboratory study by Mazza et al. 2011 showed that the life span of carried red palm weevil by mites was reduced by one third and not infested individuals living longer than affected one.

The purpose of this article is to introduce uropodine mites associated with the red palm weevil in Tunisia.

MATERIALS AND METHODS

Samples collection.

The red palm weevil adults were collected from pheromone traps containing ethyl acetate and food bait (Fig. 1). The traps were installed in sites containing infested Canary palm trees (*Phoenix canariensis*): Bizerte from April 2017 to August 2018 and in Tunis from June 2017 to August 2018 with one-week interval for sampling. Adults were collected and conserved in 75% ethanol under a temperature of 4°C.

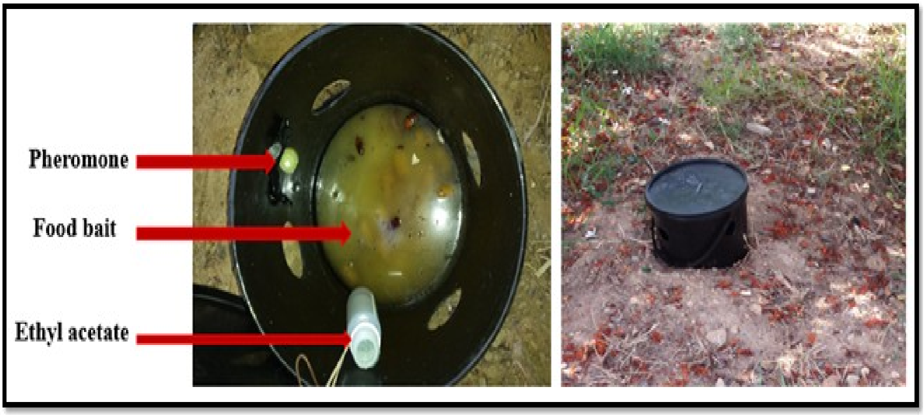

Fig. 1. Pheromone trap baited with pheromone, ethyl acetate as kairomone and food bait.

Mite identification.

Collected adults were individually dissected under a binocular loupe, and mites were separated from weevil's body using a camel hairbrush and then preserved in 75% ethanol. These mites were then cleared and mounted on microscopic slides for identification using an optical microscope at powers up to ×1000 magnification. The identification was based on some morphological criteria like the size and the color of the anal pedicels and also on the mite fixation locality on the red palm weevil body since each species has a preferred location on the weevil body as reported in many studies (Al-Deeb et al. 2011; Al-Dhafar and Al-Qahtani 2012; El-Bishlawy and Allam 2007; Dilipkumar et al. 2015; El-Sharabasy 2010; Farmahiny et al. 2016; Mesbah et al. 2008; Porcelli et al. 2009).

RESULTS

Among a total of 825 collected adult weevils using pheromone baited traps, 513 individuals were found to have deutonymphs of phoretic mites under the elytra, snout, pygidium, femur, thorax and head.

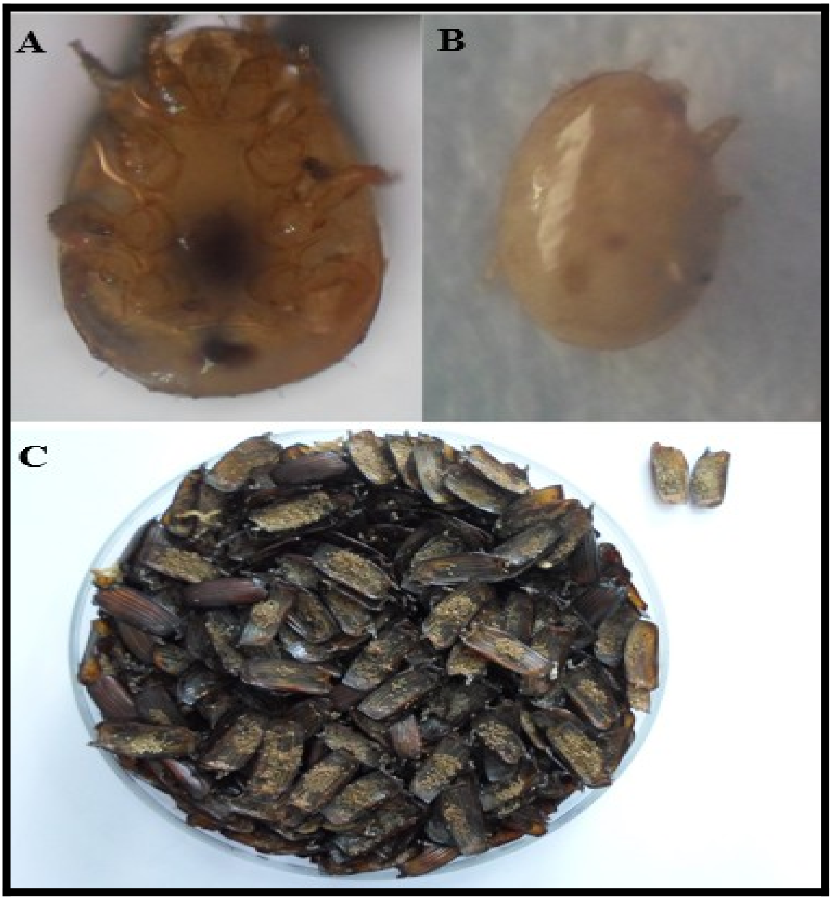

Fig. 2. *Centrouropoda almerodai*, A: Ventral view (×100), B: Dorsal view (×100), C: Elytra lifted from red palm weevil adult that show aggregation of mites.

The most abundant species was *Centrouropoda almerodai* (Uropodidae) (Fig. 2), it was isolated from the majority of collected weevils during all samplings. The number of mites per insect can be from ten to hundreds. *C. almerodai* mites were attached to the elytra under surfaces by short, broad and dark brown anal pedicels. From the 825 captured insects there were 497 individuals containing this species. Within these 497, there were 450 containing only *Centrouropoda almerodai* under elytra, 9 insects containing simultaneously *Centrouropoda almerodai* and *Uroobovella marginata*, 33 insects containing simultaneously *Centrouropoda almerodai* with Wisniewski 1981) and 5 insects containing the three mite species together (Table 1).

Table 1. Number of red palms weevil adults containing mites per site

Mite species	Gender	Tunis	Bizerte
Centrouropoda almerodai	Male	126	16
	Female	278	30
Uroobovella marginata	Male	0	2
	Female	2	2
Uroobovella javae	Male	2	1
	Female	4	1
Centrouropoda almerodai + Uroobovella marginata	Male	6	0
	Female	3	0
Centrouropoda almerodai + Uroobovella javae	Male	14	0
	Female	19	0
Uroobovella javae + Uroobovella marginata	Male	1	0
	Female	1	0
Centrouropoda almerodai + Uroobovella javae + Uroovella marginata	Male	2	0
	Female	3	0
No associated mites	Male	58	42
	Female	137	75
Total		656	169

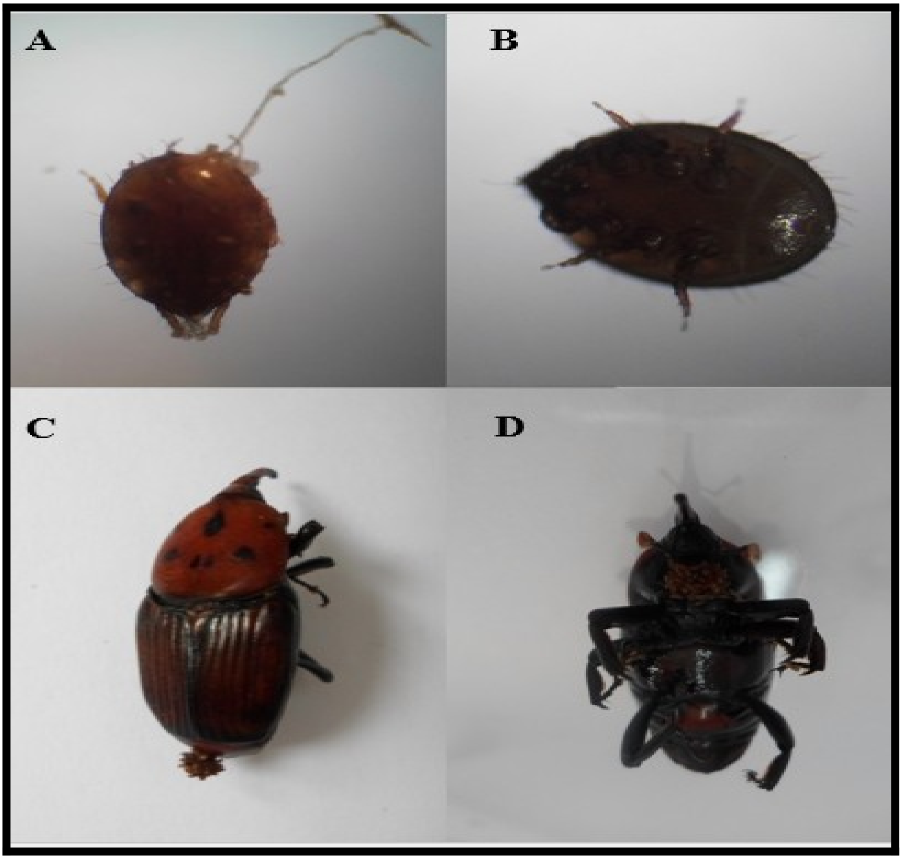

Fig. 3. *Uroobovella marginata*, A: Dorsal view (×100), B: Ventral view (×100), C: Aggregation of mites on pygidium, D: Mites aggregated on thorax.

The identification of the second mite species is *Uroobovella marginata* (Dinychidae) (Fig. 3). It was based on morphological descriptions and according to the location on the insect body. This mite was found adhered by long, flexible, slim pale and brown anal pedicels to the pygidium, thorax and head of the weevil body. The number varied between twenty and eighty. A number of 22 insects from the whole collected population contained *Uroobovella marginata*; 6 individuals contained only *Uroobovella marginata*, 9 insects contained *Uroobovella marginata* associated with *Centrouropoda almerodai*, 2 insects contained *Uroobovella marginata* with *Uroobovella javae* and 5 insects contained the 3 species: *Uroobovella marginata, Centrouropoda almerodai* and *Uroobovella javae* (Table 1).

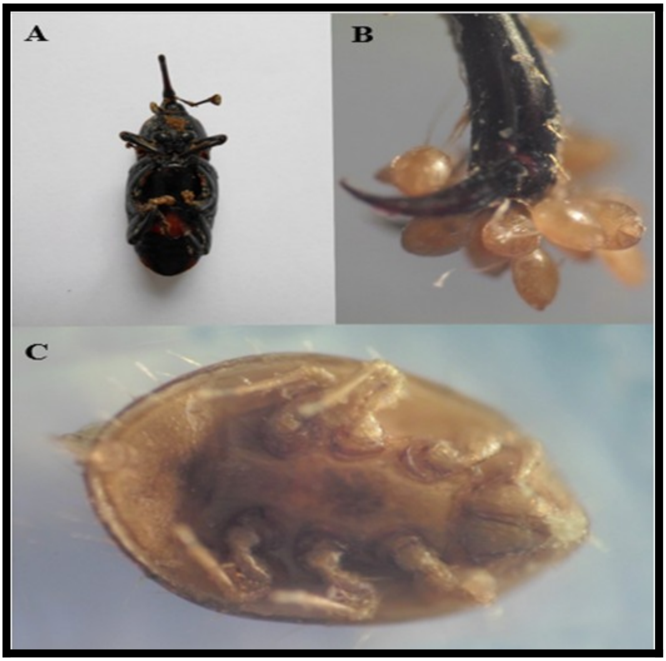

Fig. 4. *Uroobovella javae*, A: Mites aggregated on thorax, legs and antenna, B : Mites aggregated on legs (×40), C: Ventral view (×400).

The recognition of *Uroobovella javae* (Dinychidae) (Fig. 4), the third mite species was based on morphology, especially the very small size of this species compared to other ones, and according to the fixation locality. It was attached to the insect body by a medium to long flexible and light brown anal pedicels to ventral parts of antenna, thorax and legs. Twenty to hundred mites per insect were counted. Among 48 insects containing this mite, there were 8 insects containing only *Uroobovella javae* , 33 containing *Uroobovella javae* associated with *Centrouropoda almerodai*, 2 containing *Uroobovella javae* with *Uroobovella marginata* and 5 insects containing the three identified mite species: *Uroobovella javae* , *Uroobovella marginata* and *Centrouropoda almerodai* (Table 1).

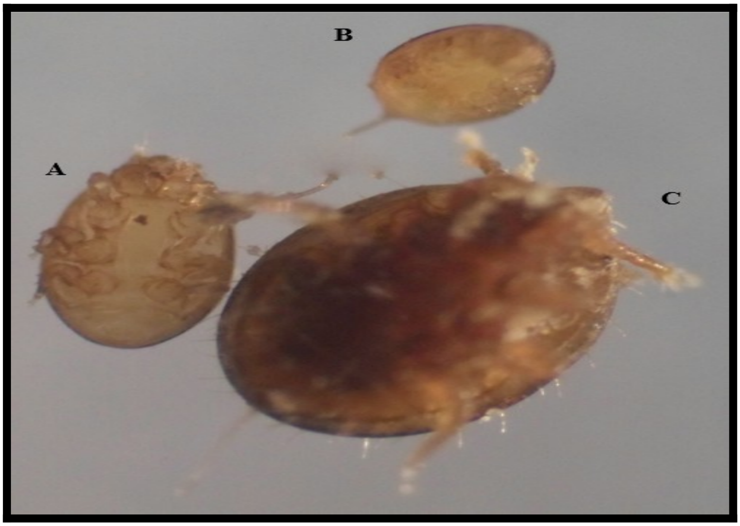

Fig. 5. Three mite species, A: *Centrouropoda almerodai* (×100), B: *Uroobovella javae* (×100) and C: *Uroobovella marginata* (×100).

Fig. 6. Two conidia (red arrows) on deutonymph *Centrouropoda almerodai* (×100).

Among the 3 species (Fig. 5), on some specimens of *Centrouropoda almerodai*, we observed conidia of unknown hyperphoretic fungus attached to the ventral part of mites (Fig.6).

From the 826 examined mites, 32 contained fungi conidia with a number varying from 2 to 28 conidia/mite.

DISCUSSION

In the present study, we found three mite species associated with red palm weevil in Tunisia. The deutonymph *C. almerodai* was found in high abundance under elytra of the weevil bodies and rarely on wings and on first abdominal tergum. This result is in line with previous studies of Al-Deeb et al. (2011), Farmahiny et al. (2016), Mazza et al. (2011), Porcelli et al. (2009) who confirmed that the greatest number of C. *almerodai* was found located on the elytra of the red palm weevil body. According to Al-Deeb et al. (2011), mites attached under elytra have more protection against high temperature and desiccation and when the insect walks through palm trees and dense fibers. Besides, elytra are not actively used for flight like membrane wings, which can give more protection to mites located under elytra.

Ragusa et al. (2009) collected the mites on membranous wings and under elytra of *R. ferrugineus* and reported that mites discriminately choose any body regions that could hide and provide protection against unfavorable conditions, once the elytra space was fully occupied.

U. marginata was found adhered to pygidium, thorax and head of *R. ferrugineus*. This mite was previously found associated with red palm weevil in Egypt, Italy, Malta, Turkey, UAE and Iran (Dilipkumar et al. 2015). Other authors detected this species also on some other parts of the weevil's body: on snout, prothorax, inter-segmental cuticle and ventral abdomen (Mesbah et al. 2008), beneath the elytra (Atakan et al. 2009), mainly on the sternum, pygidium, thorax and head, and rarely on legs and tarsi (Porcelli et al. 2009), on head, thorax, terminal part of abdomen and legs (Ragusa et al. 2009).

We detected *U. javae* in association with red palm weevil in Tunisia. This deutonymph was adhered to the antenna, thorax and legs and have been already known to be associated with *R. ferrugineus* according to same pattern distribution on the weevil body (Longo and Ragusa, 2006; Mazza et al. 2011; Porcelli et al. 2009; Ragusa et al. 2009; Wiśniewski1981).

The interactions of these mites with red palm weevil have been reported in previous studies. Al-Deeb et al. (2011) reported that excessively large number of phoretic mites on *R. ferrugineus* may limit the insect's flight ability. According to Mazza et al. (2011), the presence of *C. almerodai* affects the life span of the infested weevil becoming 1.4-times shorter than the non-infested weevils. The same author considers that association between these mites and red palm weevil is not merely phoretic as the mites may possibly prey on weevil's larvae and exploit their protein source. In the same context, Al-Deeb et al. 2011, Cardoza et al. 2008, Ouck and Cohen 1995 and Mazza et al. 2011, reported that phoretic mites act as parasites to their respective hosts. Along these lines, further studies need to be done to better investigate the interaction between mites and red palm weevil host in Tunisia and to determine if these mites could be an alternative biocontrol agent against this pest.

RESUME

Slimane-Kharrat S. et Ouali O. 2019. Acariens associés au charançon rouge du palmier (*Rhynchophorus ferrugineus*) en Tunisie. Tunisian Journal of Plant Protection 14 (2): 29-38.

Le charançon rouge du palmier (CRP, *Rhynchophorus ferrugineus*) est l'un des principaux ravageurs du palmiers canarien en Tunisie. Des adultes du CRP ont été capturés dans des pièges à appâts de phéromones dans des palmeraies infestées, puis disséqués. Trois espèces d'acariens ont été trouvées associées au CRP: *Centrouropoda almerodai* attaché à la face inferieur de l'élytre, *Uroobovella marginata* adhéré au pygidium, au thorax et à la tête, et *Uroobovella javae* fixé à l'antenne, le thorax et les jambes. La charge la plus élevée en deutonymphe a été trouvée dans l'espace des élytres des adultes du CRP. Cette étude a été réalisée pour la première fois en Tunisie, pour identifier les espèces d'acariens associées au CRP et étudier leurs répartitions sur le corps de l'adulte ravageur.

Mots clés: *Centrouropoda almerodai, Rhynchophorus ferrugineus,* Tunisie, *Uroobovella javae, Uroobovella marginata*

ملخص

سليمان-خراط، سعيدة وعثمان والي. 2019. الأكاروسات المرتبطة بسوسة النخيل الحمراء (*Rhynchophorus ferrugineus*) في تونس. Tunisian Journal of Plant Protection 14 (2): 29-38.

سوسة النخيل الحمراء هي واحدة من الآفات الرئيسية لأشجار نخيل الزينة الكناري في تونس. تم اصطياد سوسة النخيل الحمراء في طورها البالغ بواسطة مصائد فيرومونية وُضعت قرب النخيل، ثم تجفيفها. وجدنا ثلاثة أنواع من الأكاروسات مرتبطة بحشرة السوسة: الأكاروس *Centrouropoda almerodai* على الجانب السفلي من الجناح الغمدي والأكاروس *Uroobovella marginata* على العجز والصدر والرأس والأكاروس *Uroobovella javae* على قرون الاستشعار والصدر والسيقان. تمت هذه الدراسة للمرة الأولى في تونس لتشخيص أنواع الأكاروسات المرتبطة بسوسة النخيل الحمراء وتوزعها على جسم الطور البلغ لهذه الآفة.

كلمات مفتاحية: تونس، *Centrouropoda almerodai*، *Rhynchophorus ferrugineus*، *Uroobovella javae*، *Uroobovella marginata*

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Chemical Composition and Allelopathic Activity of Lemongrass (*Cymbopogon citratus*) Essential Oils Growing in Tunisia

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ABSTRACT

Trabelsi, I., Souihi, M., Amri, I., and Ben Brahim, N. 2019. Chemical composition and allelopathic activity of lemongrass (*Cymbopogon citratus*) essential oils growing in Tunisia. Tunisian Journal of Plant Protection 14 (2): 39-48.

Essential oils isolated by hydrodistillation from lemongrass (*Cymbopogon citratus*) cultivated in Tunisia, were analyzed by gas chromatography-mass spectrometry (GC-MS). Their allelopathic effects were studied on four weed species. The results showed that the average yield of essential oils was 0.85 (v/w). Four main components representing 91.08% of the total oils were identified. Monoterpenes aldehydes were dominants (79.80%) and the major component was citral present with both isomers the *(E)*-citral (geranial), 46.01% and *(Z)*-citral (neral), 34.39%. The results also showed that the germination reduction depends on the concentration level of the essential oils and the weed species. They inhibited significantly the germination of *Phalaris paradoxa*, *Lolium rigidum*, *Cichorium intybus* and *Convolvulus arvensis* seeds. However, seed germination of grassy weeds *P. paradoxa*, *L. rigidum* was completely inhibited at very low concentration 0.1 µl/ml when compared to bindweeds *Ci. intybus* and *Co. arvensis*. More investigations in the effects of the lemon grass essential oils at the seedling stage of weeds are needed in order to confirm their potential use as a bioherbicide.

Keywords: Allelopathic activity, chemical composition, *Cymbopogon citratus*, essential oils, weeds

Cymbopogon citratus popularly known as "lemongrass" is an aromatic and medicinal perennial grass belonging to Poaceae family. It is originated from the Asian Southwest, mainly India, and considered as the most widely distributed all over the word. *C. citratus* is commercially cultivated in the Democratic Republic of Congo (DRC), Madagascar, and the Comoros Island. However, the leader exporter of these plants is Guatemala, trading about 250,000 kg/year (Avoseh et al. 2015). Their ability to grow in moderate and extremely difficult climatic conditions contributed in

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enhancing their commercial value (Padalia et al. 2011).

C. citratus is consumed as an aromatic drink and used in traditional cuisine for its lemon flavor; it is also employed in popular and traditional medicine, for cosmetics, pharmaceuticals, and perfumery applications (Avoseh et al. 2015). It is well known for its high content of essential oils which are complex volatile compounds produced in different plant parts. Their chemical composition varies according to the geographical origin. However, citral remains the major content of *C. citratus* essential oils composition (Negrelle and Gomez 2007).

Essential oils are known to have various functions in plants including conferring pest and disease resistance. Several studies reported antimicrobial (Daferera et al. 2003; Ohno et al. 2003; Plotto et al. 2003), antifungal (Tzortzakis and Economakis 2007) and insecticidal (Nonviho et al. 2010) activities of *C. citratus* essential oils. The use of natural plant protectants with pesticidal activity is considered as a safe alternative to replace synthetic chemical pesticides which became very expensive and detrimental on human health and environment (Aktar et al. 2009).

Towards abandoning use of herbicides and the speed evolution of weed herbicides resistance, the researchers were oriented to search for new and innovation tools for weed management. Natural products such as essential oils could be part of the solution. The efficient control of weeds needs the integration of farming techniques, where the allelopathy and the bioherbicide are considered as an alternative of the chemical control.

The aim of this study was to determine the chemical composition of lemongrass essential oils cultivated in Tunisia and to explore their efficacy on weed germination control of four species unstudied before: *Phalaris paradoxa*, *Lolium rigidum*, *Cichorium intybus* and *Convolvulus arvensis* which cause serious yield losses for agricultural crops especially wheat and forage crops.

MATERIALS AND METHODS

Plant material.

Fresh leaves of *C. citratus* were collected in February 2017 from the Botanical garden of the National Institute of Agricultural Research of Tunisia (INRAT). A voucher specimen for each plant has been deposited in the herbarium of the Institute. Leaves were dried and powdered until eventual use.

Seed weeds were collected in 2016 from mature plants of paradoxa grass (*P. paradoxa*), annual ryegrass (*L. rigidum*), chicory (*Ci. intybus*) and bindweed (*Co. arvensis*).

Extraction and chemical analysis of essential oils.

The essential oils of *C. citratus* were extracted by hydrodistillation using a modified Clevenger-type apparatus. The supernatant was separated by decantation, dried over anhydrous Na_2SO_4 overnight and kept in sterile flasks. The oils were preserved in a sealed vial at 4°C. Yield was calculated based on dried weight of the sample.

GC-MS analyses of the essential oils were performed by a gas chromatograph HP 5890 (II) interfaced with a HP 5972 mass spectrometer with electron impact ionization (70 eV). A HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm film thickness) was used. The column temperature was programmed to rise from 50°C to 240°C at a rate of 5°C/min. The carrier gas was helium with a flow rate of 1.2 ml/min; split ratio was 60:1. Scan time and mass range were 1 s and 40-300 m/z respectively. The relative percentage-concentration of compounds was obtained by integrating the peak area of the chromatograms.

All constituents were identified by gas chromatography by comparison of their Kovats retention indices (RI) with either those cited in the literature or the authentic compounds available in the laboratorie. The Kovats retention indices were determined in relation to a homologous series of *n*-alkanes (C10–C35) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 02 and Wiley 275 libraries or with mass spectra from the literature (Davies 1990; Adams 2007).

Seed germination and allelopathic activity of essential oils.

Seeds were sterilized with 5% sodium hypochlorite for 10 min, and then rinsed with abundant distilled water. Empty and undeveloped seeds were removed. Twenty seeds of each plant species were placed between two filter papers in Petri dish (90 mm diameter). The essential oils were dissolved in tween-water solution (1 %; v/v). Concentrations (C) used in the different treatments varied from 0.1 µl/ml (C1) to 1 µl/ml (C10). Distilled water without essential oils was used as control. Petri dishes were sealed with parafilm and incubated in the dark at 23°C. The number of germinated seeds was recorded daily over one week.

The seed vigor was calculated using the formula (Agrawal, 1980; AOSA, 1996; ISTA, 1996):

$$\text{Seed vigor} = \sum \frac{\textit{Daily counts of number of germinated seeds}}{\textit{number of days}}.$$

Statistical analyses.

Statistical analyses were performed using the SPSS software (Version 20.0 for Windows). Mean comparison was based on Tukey's multiple ranges classification test at P ≤ 0.05. Each experiment was replicated three times (n = 3).

RESULTS

Yield and chemical composition of lemongrass essential oils.

The average yield of the essential oils extracted from dry leaves of lemongrass (*C. citratus*) was 0.85 (v/w). Analyze of essential oils composition by GC-MS showed the presence of four main components representing 91.08% of the total oils (Table 1). The major components were monoterpenes aldehydes (79.80%) mainly the citral present with both isomers the dominant *(E)*-citral (geranial), 46.01% and *(Z)*-citral (neral), 34.39%. Nerol (8.94%) and the miner component L-limonene (1.74%) were also present in the essential oils of lemongrass (Table 1).

Allelopathic activity of lemongrass essential oils on weeds.

Lemongrass essential oils effect on germination of four weeds species: paradoxa grass (*P. paradoxa*), annual ryegrass (*L. rigidum*), chicory (*Ci. intybus*) and bindweed (*Co. arvensis*) was studied. Different concentrations of essential oils (C1-C10) were used and germination rate was recorded daily over 6 days.

Table 1. Rate (%), retention time and index of different components obtained from lemongrass essential oils analyzed by GC-MS

N°	Components	Retention time (RT min)	Retention index (RI)	Area (%)
1	**L-limonene**	7.933	1029	1.74
2	***(Z*** **)-citral (neral)**	13.794	1238	34.39
3	***(E)*****-citral (geranial)**	14.625	1267	46.01
4	**Nerol**	17.500	1229	8.94
	Total			91.08

The kinetic of germination of annual ryegrass (*L. rigidum*) showed significant differences between treatments with essential oils and the control with distilled water over 6 days. Towards the end of the experiment, the control presented 80% of seed germination (Fig. 1). However, among the used concentrations only C1 (0.1 µl/ml) allowed germination of few numbers of ryegrass seeds and the germination rate did not exceeded 3.33%. Concentrations from C2 to C10 inhibited completely seed germination (Fig. 1).

The effect of lemongrass essential oils on paradoxa grass (*P. paradoxa*) seed germination showed similar results to those observed for ryegrass. The control presented significantly the highest germination rate (73.33%) after 6 days of incubation compared to C1 (23.33%). The remaining concentrations (C2-C10) inhibited seed germination (Fig. 2).

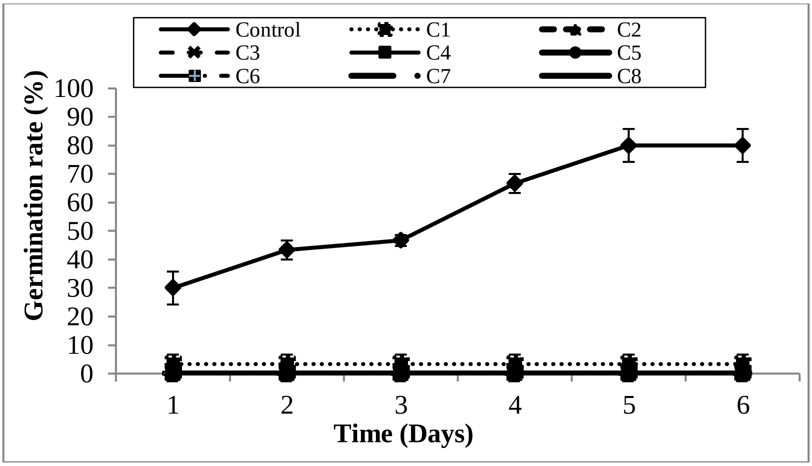

Fig. 1. Effect of lemongrass essential oils on annual ryegrass (*Lolium rigidum*) seed germination. Data are means ± SE, (n = 3).

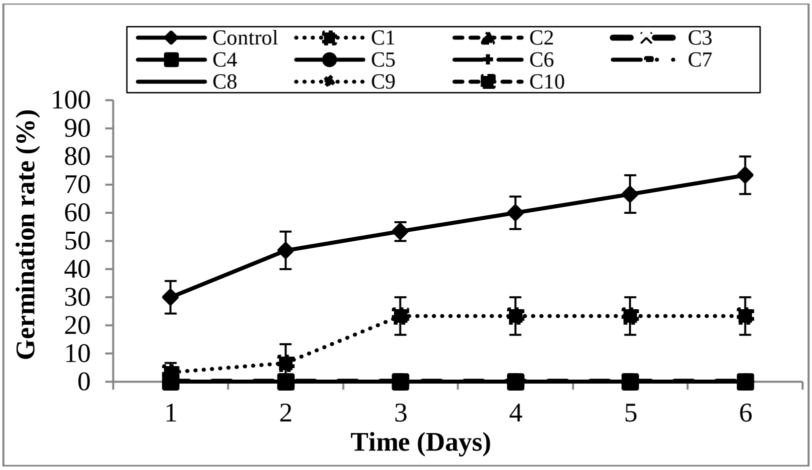

Fig. 2. Effect of lemongrass essential oils on paradoxa grass (*Phalaris paradoxa*) seed germination. Data are means ± SE, (n = 3).

Regarding bindweed, the control presented 83.33% of germinated seeds. The concentrations from C1 to C4 showed significantly lower seed germination; it did not exceeded 33.33% for C1, C2 and C3 and 40% for C4 after 6 days of incubation (Fig. 3). The germination was completely inhibited by the remaining concentrations (C5-C10).

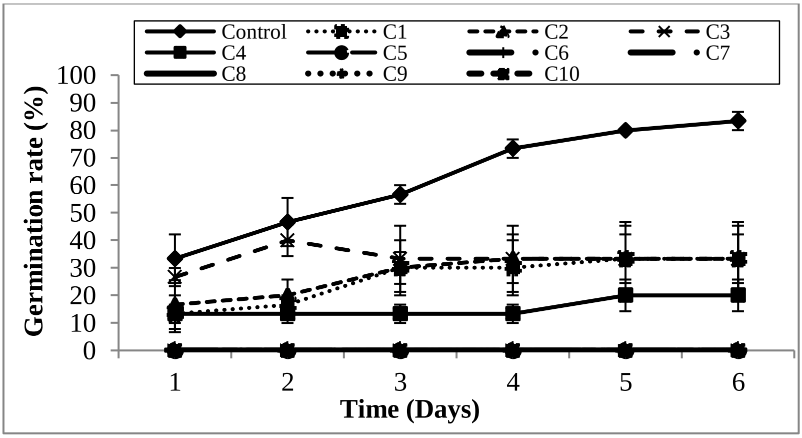

Fig. 3. Effect of lemongrass essential oils on bindweed (*Convolvulus arvensis*) seed germination. Data are means ± SE, (n = 3).

Chicory seeds were less sensitive to lemongrass essential oils compared to other studied weeds. Seed germination was significantly inhibited in higher concentration C8 (0.8 µl/ml) to attend 6.66% against 80% for the control. Seed germination was completely inhibited with concentration C9 (0.9 µl/ml) and C10 (1 µl/ml) (Fig. 4).

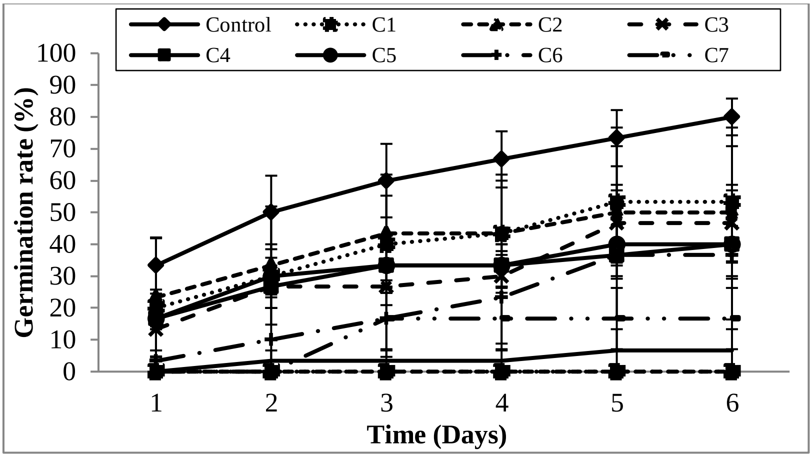

Fig. 4. Effect of lemongrass essential oils on chicory (*Cichorium intybus*) seed germination. Data are means ± SE, (n = 3).

Seed vigor was seriously affected by 0.2 µl /ml of essential oils for *P. paradoxa* and *L. rigidum* as compared to the control (Fig. 5). However, this parameter was less affected for *Ci. intybus* and *Co. arvensis*. Seed germination was completely inhibited at 0.8 µl/ml and 0.5 µl/ml, respectively.

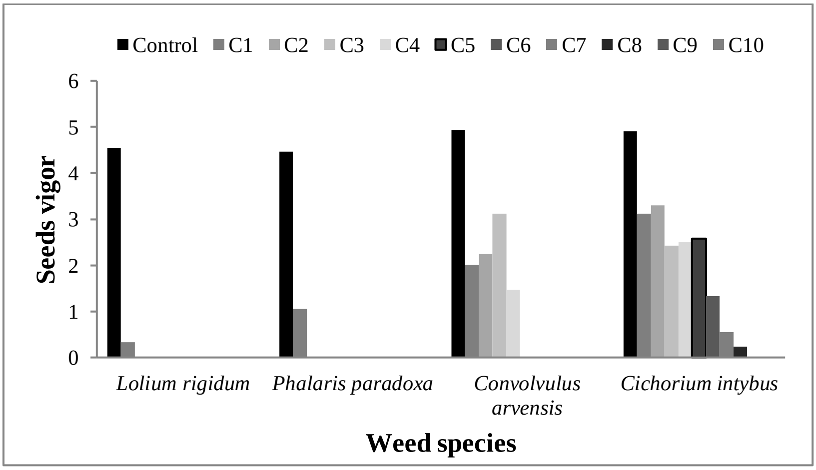

Fig. 5. Seed vigor of weed species exposed to different concentrations of lemongrass essential oils after 6 days of incubation. Data are means ± SE, (n = 3).

DISCUSSION

In the present study, lemongrass (*C. citratus*) essential oils were extracted by hydrodistillation. The chemical composition was analyzed and the allelopathic effect on weed seed germination was studied. The results showed that the average yield of essential oils obtained from dry leaves of lemongrass was 0.85 (v/w). In a previous works, it was reported that the essential oils yield varied from 0.28% to 1.4% according different region except in Zambia, it reached 3% (Negrelle and Gomes 2007). Compared to the reported results, the lemongrass cultivated in Tunisia is considered moderately productive in essential oils.

Chemical composition of lemongrass essential oils was well studied around the world. Results showed that the amount of essential oils and the chemical composition depend on the geographic origin (Kumar et al. 2011). However, regardless its origin, lemongrass is characterized by a high production of citral (70-80%) with variable amounts as compared to other species from the genus *Cymbopogon* (Ganjewala 2009; Gbenou et al. 2013; Kpoviessi et al. 2014; Solórzano-Santos and Miranda-Novales 2012). Our results also showed that citral with both isomers neral and geranial presented 80.4% of the chemical composition.

The ability of some essential oils to inhibit seed germination or plant growth has been well studied for *C. citratus*. It was reported that essential oils of lemongrass has an herbicidal effect on seed germination and seedling growth of *Sinapsis avensis* (Rida et al. 2015), *Sinapis nigra*, *Amaranthus blitoides*, *Amaranthus palmeri*, *Euphorbia hirta* (Dudai et al.1999), *Digitaria horizontalis*, *Sorghum halepense*, *Bidens pilosa*, *Euphorbia heterophylla* and *Raphanus raphanistrum* (Valarini et al. 1996). Weeds are considered as major constraints to crop productivity. In our study, the allelopathic effects of *C. citratus* essential oils on seed germination of four weed species including two grass weeds (paradoxa grass and annual ryegrass), and two broad leaved species (bindweed and chicory) were studied. Paradoxa grass (*P. paradoxa*) and annual ryegrass (*L. rigidum*) are major constraints limiting cereal crop productivity. Generally, herbicides are effective in their control, but herbicides resistance in weed populations makes weed control more difficult (Cruz-Hipolito et al. 2012; Owen et al. 2012). Bindweed (*Co. arvensis*) is considered also among top ten weeds of the world and is reported from about 54 countries as a weed in 32 different crops. It spreads by rhizome and seed making its control exceptionally difficult (Kaur and Kalia 2012). Moreover, Fateh et al. (2012) reported its allelopathic activity on seed germination and yield of wheat. They significantly decreased by 14% and 80%, respectively when influenced by bindweed (*Co. arvensis*) residues extract. Chicory (*Ci. intybus*) is also as harmful as other weeds in cultivated areas eventhough if it could be used as a forage plant or in salad. In this study, the results showed an interesting allelopathic activity against the previously mentioned species. Seed germination of *L. rigidum* and *P. paradoxa* was totally inhibited by the concentration C2 (0.2 µl/ml) of *C. citratus* essential oils, whereas *Co. arvensis* and *Ci. intybus* were less sensitive to those essential oils. Seed germination was inhibited by C5 (0.5 µl/ml) and C9 (0.9 µl/ml) respectively for both latter species. Allelopathic activity of lemongrass essential oils was more noticeable for the two grass weeds (paradoxa grass and annual ryegrass), than the broad leaved species (bindweed and chicory) suggesting that they can be very helpful for their management.

The allelopathic properties of lemongrass essential oils is due mainly to the presence of the monoterpene, citral (Rida et al. 2015). Those properties were confirmed on the four common weeds species: *P. paradoxa*, *L. rigidum*, *Ci. intybus* and *Co. arvensis*, in the present work. Thus, they are suggested to be used as a biological herbicide against the four studied species.

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RESUME

Trabelsi I., Souihi M., Amri I. et Ben Brahim, N. 2019. Composition chimique et activité allélopathique des huiles essentielles de la citronnelle (*Cymbopogon citratus*) cultivée en Tunisie. Tunisian Journal of Plant Protection 14 (2): 39-48.

Les huiles essentielles isolées par hydrodistillation à partir de la citronnelle (*Cymbopogon citratus*) cultivée en Tunisie ont été analysées par spectrométrie de masse couplée à la chromatographie en phase gazeuse (GC-MS). Leur effets allélopathiques sur quatre espèces de mauvaises herbes ont été étudiés. Les résultats ont montré que le rendement moyen en huiles essentielles de la citronnelle est de 0,85 (v/w). Quatre composants principaux représentant 91,08% des huiles totaux ont été identifiés. Les monoterpènes aldéhydes sont dominants (79,80%) et le principal composant détecté est le citral présent avec ces deux isomères le (*E*)-citral (géranial) à 46,01% et le (*Z*)-citral (néral) à 34,39%. Les résultats ont également montré que la réduction du taux de germination dépend de la concentration des huiles essentielles et des espèces de mauvaises herbes. Ces huiles ont inhibé significativement la germination des semences de *Phalaris paradoxa*, *Lolium rigidum*, *Cichorium intybus* et *Convolvulus arvensis*. Cependant, la germination des semences des mauvaises herbes monocotylédones *P. paradoxa* et *L. rigidum* a été inhibée à une très faible concentration (0.1 µl/ml) en comparaison avec les espèces dicotylédones *Ci. intybus* et *Co. arvensis*. Plus d'investigations sur les effets des huiles essentielles de la citronnelle sur le stade plantule des mauvaises herbes sont nécessaires afin de confirmer leur utilisation potentielle comme bioherbicide.

Mots-clés: Activité allélopathique, composition chimique, *Cymbopogon citratus*, huiles essentielles, mauvaises herbes

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ملخص

الطرابلسي، إيمان ومنى سويهي وإسماعيل عمري ونادية بن إبراهيم. 2019. التركيبة الكيميائية ونشاط المجاهضة للزيوت الأساسية لعشب الليمون (*Cymbopogon citratus*) المزروع في تونس.
Tunisian Journal of Plant Protection 14 (2): 39-48.

تم عزل الزيوت الأساسية لعشب الليمون (*Cymbopogon citratus*) المزروع في تونس عن طريق التقطير المائي وتحليلها بواسطة الكروماتوغرافيا الغازية مع مطياف الكتلة (GC-MS) وتمت دراسة نشاط مجاهضتها على أربعة أنواع من الأعشاب الضارة. أظهرت النتائج أن الإنتاج المتوسط من الزيوت الأساسية 0.85 (حجم/وزن). تم تحديد أربعة مكونات رئيسية تمثل 91.08% من إجمالي الزيوت. كانت الألديهيدات أحادية التربين هي المهيمنة (79.80%) وكان مكونها الرئيسي هو السيترال بأيزومراته *E*-سيترال (جرانيال) بـ 46.01% و*Z*-سيترال (نيرال) بـ 34.39%. كما أظهرت النتائج أيضا أن تخفيض نسبة الإنبات يرتبط بمستوى تركيز الزيوت الأساسية وأنواع الأعشاب. منعت هذه الزيوت الأساسية بشكل كبير إنبات بذور الأعشاب الضارة *Phalaris paradoxa* و *Lolium rigidum* و *Cichorium intybus* و *Convolvulus arvensis*. لكن كان إنبات بذور الأعشاب الضارة ذات الفلقة (*P. paradoxa* و *L. rigidum*) مثبط مع تركيز ضعيف للزيوت الأساسية (0.1 مكل/مل) مقارنة بالأعشاب ذات الفلقتين (*Ci. intybus* و *Co. arvensis*). هناك حاجة إلى المزيد من الدراسات حول تأثير الزيوت الأساسية لعشب الليمون على مرحلة بادرات الأعشاب الضارة لتأكيد إمكانية استخدامها كمبيدات عشبية بيولوجية.

كلمات مفتاحية: أعشاب ضارة، تركيبة كيميائية، زيوت أساسية، نشاط مجاهضة، *Cymbopogon citratus*

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Consumption Risk Assessment of Pesticides Residues in Yam

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ABSTRACT

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Chemical pesticides have contributed significantly to agriculture production throughout the world. However, human exposure to pesticides remains a critical concern. One important source of human exposure to pesticides is through food consumption. The potential negative effects of pesticides have resulted in stringent regulation in the production and use of the products, especially in the developed countries. To limit the potential negatives effects of pesticides, risk assessments are usually conducted by scientific experts to establish the risk levels and to offer risk management strategies. Yam is a food commodity widely consumed by Africans both home and by the diaspora. Yam farmers have been using pesticides in yam production over years. The public is concerned about the health impacts that may result from exposure to residues. This study was designed to assess the risk of dietary intake of 12 pesticides, including five insecticides (cadusafos, fenitrothion, imidacloprid, profenofos and propoxur), four fungicides (carbendazim, fenpropimorph, metalaxyl, propiconazole) and three herbicides (bentazone, glyphosate and pendimethalin) in yam cropped by farmers in the Nanumba traditional area of Ghana. Residue and consumption data were collected and combined to derive Estimated Daily Intake (EDI). Three approaches were adopted in the calculation of EDI (deterministic, simple distribution and probabilistic) and the EDI values were compared with Acceptable Daily Intake (ADI) values. The study revealed that farmers' EDI to the twelve pesticides, according to the deterministic and the simple distribution approaches were lower than their respective ADI set by the EU Commission. However, the EDI of about 10% of the farmers to fenpropimorph and fenitrothion were higher than their ADI.

Keywords: Consumption, deterministic, pesticide risk assessment, probabilistic, yam

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Agriculture represents an important economic sector in Ghana, accounting for about 40% of the country's gross domestic product and offering employing to over 50% of the economically active population of the country (FAO 2018; MoFA 2010). With an annual average growth rate of 2.5%, the population is estimated at 25 million. Like in many other countries throughout the world, the need to increase

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food production to meet the demands of the burgeoning population has led to the adoption of chemical pesticides. The use of pesticides does not only boost crop production, but also serves as an insurance policy against devastating crop losses due to diseases and pests attacks (Bempah 2012). As a result, there has been increasing use of pesticides in the country over the last decade (FAOSTAT 2018). When pesticides are used correctly, they clearly produce tangible benefits and increase crop yields. However, their misuse can possibly lead to the presence of residues in the environment and in food products which could trigger negative effects on human health (de Gavelle et al. 2016; Illyassou et al. 2018; Nougadère et al. 2012). Chronic exposure to certain categories of pesticides have been linked to diseases such as Parkinson's, cancers and Alzheimer's (Chourasiya et al. 2015; Darko and Akoto 2008; Gorell et al. 1998; Ouédraogo et al. 2014). Many reports showed pesticide residues in vegetables, fruits, cereals and yam in Ghana (Bempah et al. 2011; Fosu et al. 2017; Samuel et al. 2012; Wumbei et al. 2018).

To ensure food safety for the public, consumption risk assessment is often carried out to determine the risk levels and to take appropriate risk management decisions. As part of consumption risk assessment, exposure assessment to pesticides residues is done by combining the amount of food consumed and the amount of pesticides residues present in the food. The obtained exposure values are compared with Acceptable Daily Intake (ADI) for chronic risk assessment and with the Acute Reference Dose (ARfD) for acute risk assessment in order to make a decision regarding food safety (Mekonen et al. 2015).

When a certain fraction of the population (e.g. median or 95th percentile) is exposed to higher levels of the concerned chemical than the ADI or ARfD, they have a potential risk of illness (Hamilton et al. 2004). The risk index (RI) for human exposure can be calculated using the following formula:

$$\mathrm{RI} = \frac{\mathrm{Exposure}}{\mathrm{ADI\ or\ ARfD}},$$

where: RI = Risk Index, ARfD = Acute Reference Dose and ADI = Acceptable Daily Intake.

Exposure assessment is defined as "the qualitative and/or quantitative evaluation of the likely intake of biological, chemical or physical agents via food as well as exposure from other sources". It involves the estimation of how likely an individual or a population will be exposed to a chemical of concern and how much of that chemical is taken up in the body through consumption of food, drinking water and others (Lammerding and Fazil 2000).

It requires many data sources, such as: supervised field residue trials, national pesticides monitoring programs and food consumption surveys. Exposure assessment can be calculated for both chronic (long term) and acute (short term) scenarios. When samples residues are below Limit of Detection (LOD) or Limit of Quantification (LOQ), different scenarios can be adopted: lower bound (replacing non-detects by 0), medium bound (replacing non-detects by ½ of LOD/LOQ) and upper bound (replacing non-detects by the LOD/LOQ). The adoption of the lower bound is sometimes called the 'optimistic scenario' and the adoption of the upper bound is called the 'pessimistic scenario' (Kettler et al. 2015).

The two well-known dietary exposure assessment methods are the deterministic and probabilistic methods. The deterministic exposure model can be used as a simple exposure modeling tool on fixed values (point estimates) derived from residue concentrations and consumption data. The deterministic calculation is done by multiplying a fixed value of the food consumed and residue concentration, usually the mean or 97.5 percentile values (worst case scenario) (FAO/WHO 1997; Kroes et al. 2002). Deterministic exposure models are used as a low tier approach to determine whether there is an indication of concern for the given exposure. They form part of the regulatory decision making guidelines because of their simplicity, rapidity and inexpensive character (EFSA 2012; Hamilton et al. 2004). The deterministic model does not include information about variability in potential exposure of the population.

Probabilistic dietary exposure models are the most preferred exposure models, because they take into account the distribution of one or more parameters to represent variation and uncertainty and generate more realistic exposure estimates. Most of these distributional models are based on Monte Carlo simulations and are referred to as Monte Carlo models (EFSA 2012; Hamilton et al. 2004). The simulation is repeated for a certain number of iteration (e.g. 10,000) using statistical software such as @Risk or Monte Carlo Risk Assessment and results in intake curve of the concerned population (Kettler et al. 2015).

Yam (*Dioscorea* spp.), as one of the important staples and cash crops in Ghana, is consumed by many people, especially, people in the Nanumba traditional area. There is public concern regarding pesticides residues and their possible health risks. This study was initiated based on residues analysis in Wumbei et al. (2018) and Wumbei et al. (2019), to estimate farmers risk of dietary intake of 12 pesticides (bentazone, carbendazim, cadusafos, fenitrothion fenpropimorph, glyphosate, imidacloprid, metalaxyl, pendimethalin, profenofos, propiconazole and propoxur) in yam, using deterministic, simple distribution and probabilistic methods and to determine the risk associated with such exposures.

MATERIALS AND METHODS

Consumption survey.

To be able to estimate intake of pesticides, consumption data were collected from 100 farmers from randomly selected households in eight communities in the Nanumba traditional area (Nanumba South and North districts) during the yam planting season of March, 2016. During the survey, socio-demographic data including weight of the farmers were collected. The consumption data were collected in a repeated 24 hours recall interview in accordance with standard practice (Illyassou et al., 2018). The interviews were done in two non-consecutive days separated by 15 days. The interviews were done face-to-face by trained interviewers using a structured questionnaire. The type of yam dish eaten and the amount of yam consumed was estimated using pictures of various portion sizes. The average of the two recall interviews was taken and used to calculate yam consumption on a daily basis. The average daily consumption (kg/kg bodyweight (BW)/day) of yam for each person was calculated.

Yam sampling and sample preparation for residues analysis.

A total of 328 yam samples were collected. Out of this number, 150 samples were collected from Ghanaian markets, 100 samples were collected from households in Ghana, 48 samples were collected from a field trial in Ghana and 30 samples were collected from shops in Ghent, Belgium. The yam samples collected from Ghent were imported from Ghana. Sample preparation, extraction and analysis for pesticides residues were done in Wumbei et al. (2018) and Wumbei et al. 2019.

Exposure analysis.

Pesticides residue data of Wumbei et al. (2018) and Wumbei et al. (2019) were pooled for the consumer exposure assessment. This was done by calculating the EDI *i.e.* chronic exposure. The residues of each pesticide were mostly below the LOD. Therefore, in the dietary exposure assessment, the upper bound scenario, also known as the pessimistic was adopted. The exposure was compared with the toxicological limits *i.e.* ADI of the pesticides. Three approaches were adopted for the exposure assessment, *i.e.* the deterministic, the simple distribution and the probabilistic, as prescribed by the European Food Safety Authority (EFSA 2012). Fenitrothion and fenpropimorph were assessed deterministically and probabilistically and the rest were assessed by simple distribution. An independent t-test was conducted to compare the estimated dietary exposure of the farmers to the two pesticides derived from the probabilistic and deterministic approaches.

Dietary exposure to fenitrothion and fenpropimorph was calculated based on the daily yam consumption data and the pesticide residues in yam, using the deterministic approach. This is a method that makes use of point estimations. In this study, the dietary exposure was estimated by multiplying a single value of consumption and a single value of concentration and dividing by the body weight as in the equation below:

$$\mathrm{EDI}\left({}^{\mathrm{mg}}\!/_{\mathrm{kg\ bodyweight}}\!/_{\mathrm{day}}\right) = \frac{\mathrm{Residue}\ \left(({}^{\mathrm{mg}}\!/_{\mathrm{kg}})\ \mathrm{x\ Consumption}\ \left({}^{\mathrm{kg}}\!/_{\mathrm{day}}\right)\ \mathrm{x\ E}\right)}{\mathrm{Bodyweight\ (kg)}}$$

where: E = correction factor for the edible portion.

However, since the yam samples were peeled before being analyzed Wumbei et al. (2018) and Wumbei et al. (2019), there was no need to correct for the edible part, hence E was taken as 1. Statistical means and percentiles (P 50, P 75, P 90, P 95, P 97.5 and P 99) were calculated for the consumption and concentration data, and combined to generate the corresponding exposures.

Probabilistic dietary exposure assessment was done using @Risk® 5.7 software, version 6.0, a Microsoft Excel add in program from Palisade Corporation, USA. In contrast to the deterministic approach, here both the consumption and concentration data were fitted to distributions after which the consumption and concentration distributions were combined to obtain an exposure distribution. Subsequent to the generation of the exposure distribution, first-order Monte-Carlo simulation was performed with 10,000 iterations. From the simulated results the means and relevant percentiles (P 50, P 75, P 90, P 95 P 97.5 and P 99) of the estimated exposure to the two pesticides were determined. In the process of the probabilistic risk assessment, the residue data of fenitrothion and fenpropimorph could not be fitted directly to a distribution. Hence, the data were grouped into high values (above the LOQ), medium values (LOQ) and low values (LOD). The high values were fitted to a distribution, after which the IF function in @Risk® was used to generate a random distribution for the residue data (lower bound values and upper bound values).

The IF function for the lower bound distribution was: IF(RAND() < fraction of LOD;0;IF(RAND() < fraction of LOQ; LOD;distribution of fraction above LOQ)).

The IF function for the upper bound distribution was: IF(RAND() < fraction of LOQ; LOD;IF(RAND() <

fraction above LOQ ; LOQ; distribution of fraction above LOQ)).

The consumption data on the other hand, had only one zero and therefore, it was possible to fit it directly to a distribution. Subsequent to the generation of the random distributions (lower bound and upper bound) for both pesticides, exposure was calculated by adding output in @Risk® and multiplying the random distributions by the consumption distribution.

With the simple distribution approach, a single residue value is combined with a distribution of consumption to obtain an exposure estimate. The 10 pesticides detected with concentrations below their LOD/LOQ, were converted to the LOD/LOQ. These single values were combined with the distribution of the yam consumption data to obtain exposure estimates for the pesticides. Like in the case of the probabilistic approach, the exposure estimates were subjected to Monte-Carlo simulation with 10,000 iterations and from the simulated results the means and the relevant percentiles (P 50, P 75, P 90, P 95, P 97.5 and P 99) of the estimated exposure were determined.

RESULTS

Socio-demographic data.

The majority (99%) of the farmers interviewed were adult men with their ages ranging between 21 and 65 years. The weight of the famers ranged between 55 and 99 kg with an average of 69 kg. The farmers consume yam a maximum of three times and a minimum of one time per day with the individual consumption ranging between 0.12 kg and 0.85 kg/day, with an average of 0.4 kg/day.

Pesticides residue concentration and consumption data.

Residues data of twelve pesticides and consumption data of 100 people were used for the dietary intake assessment. Out of the twelve pesticides, there were 314 residues detects for fenpropimorph, 288 for cadusafos and 257 for fenitrothion, out of 328 samples. The rest of the pesticides had more non-detects (zeros) than detects. Among them, there were 58 residue detects for metalxyl, 41 for propiconazole, 19 for propoxur, 14 for glyphosate, 6 for bentazone and 2 for carbendazim. The rest (imidacloprid, pendimethalin and profenofos) had only one residue detect each.

Exposure assessment.

The deterministic and probabilistic methods of dietary exposure assessment were used to evaluate farmers EDI towards fenitrothion and fenpropimorph while simple distribution was used to evaluate farmers EDI to the rest of detected pesticides. The levels of exposure to the pesticides between the two methods (deterministic and probabilistic) were compared.

Dietary exposure assessment by the deterministic method was carried out based on single point estimation. The concentration data of the pesticides, the yam consumption data and the resultant EDI are presented in Table 1. The mean and 99th percentile concentrations of fenitrothion were 0.0029 and 0.014 mg/kg respectively, while those of fenpropimorph were 0.0003 and 0.003 respectively. The mean and 99th percentile yam consumption were 0.006 and 0.013 kg/kgBW/day respectively.

Table 1. Estimated daily intake of fenpropimorph and fenitrothion through deterministic exposure assessment and corresponding ADIs

Exposure	Residues (mg/kg)	
	Fenitrothion	**Fenpropimorph**
Mean	0.0043	0.0003
Median	0.0023	0.0002
P75	0.0069	0.0002
P90	0.0069	0.0006
P95	0.0079	0.0007
P97.5	0.0097	0.0013
P99	0.0144	0.0031

Yam consumption (kg/kgBW/day)

Mean	Median	P75	P90	P95	P97.5	P99
0.006	0.006	0.008	0.009	0.01	0.011	0.013

Exposure	EDI (mg/kgBW/day)	
	Fenitrothion	**Fenpropimorph**
Mean	0.000026	0.000002
Median	0.000014	0.000001
P75	0.000055	0.000002
P90	0.000062	0.000006
P95	0.000082	0.000007
P97.5	0.000110	0.000014
P99	0.000187	0.000040
ADI (mg/kgBW/day)	**0.005**	**0.003**

Simple distribution method of dietary exposure assessment.

The intake results for the 10 pesticides, whose EDI were assessed through the simple distribution method, and their respective ADIs are presented in Table 2.

Table 2. Estimated daily intake of used pesticides through simple distribution and corresponding ADIs

	Residues (mg/kg)				
	Cadusafos	**Carbendazim**	**Glyphosate**	**Imidacloprid**	**Metalaxyl**
	0.0005	0.0007	0.12	0.0007	0.0009
Statistical dist. of yam consumption (kg/kgBW/day) = Log logistic(-0,017192;0,02288;14,635)					
Exposure	**EDI (mg/kgBW/day)**				
	Cadusafos	**Carbendazim**	**Glyphosate**	**Imidacloprid**	**Metalaxyl**
Mean	0.0000029	0.0000041	0.00070	0.0000041	0.0000053
Median	0.0000028	0.0000039	0.00068	0.0000039	0.0000051
P75	0.0000037	0.0000052	0.00089	0.0000052	0.0000067
P90	0.0000047	0.0000066	0.00113	0.0000066	0.0000084
P95	0.0000054	0.0000075	0.00129	0.0000075	0.0000097
P97.5	0.0000061	0.0000085	0.00146	0.0000085	0.000011
P99	0.0000071	0.00001	0.00169	0.00001	0.000013
ADI (mg/kgBW/day)	**0.0004**	**0.02**	**0.5**	**0.06**	**0.08**
	Residues (mg/kg)				
	Pendimethalin	**Profenofos**	**Propiconazole**	**Propoxur**	**Bentazone**
	0.0003	0.0004	0.0002	0.0004	0.0007
Exposure	**EDI (mg/kgBW/day)**				
	Pendimethalin	**Profenofos**	**Propiconazole**	**Propoxur**	**Bentazone**
Mean	0.0000018	0.0000023	0.0000012	0.0000023	0.0000041
Median	0.0000017	0.0000022	0.0000011	0.0000022	0.0000039
P75	0.0000022	0.0000029	0.0000015	0.0000029	0.0000052
P90	0.0000028	0.0000037	0.0000019	0.0000037	0.0000066
P95	0.0000032	0.0000043	0.0000021	0.0000043	0.0000075
P97.5	0.0000036	0.0000049	0.0000024	0.0000049	0.0000085
P99	0.0000042	0.0000056	0.0000028	0.0000056	0.00001
ADI (mg/kgBW/day)	**0.125**	**0.03**	**0.04**	**0.02**	

With the probabilistic method of dietary exposure assessment, the yam consumption data and the concentration data of fenpropimorph and fenitrothion were fitted to distributions in @Risk®, the palisade Microsoft excel add in program. In the case of the concentration data, where the data were grouped, the best fitting distribution was chosen for the high values (>LOQ) prior to the use of the help function to find a random distribution. The best fitting distribution was also chosen for the yam consumption data, considering the χ^2 value, the shape of the graphs (PP and QQ plots) and the closeness of the distribution data to the input data. The fit comparison curves and the PP and QQ plots are presented in Figs. 1, 2, respectively.

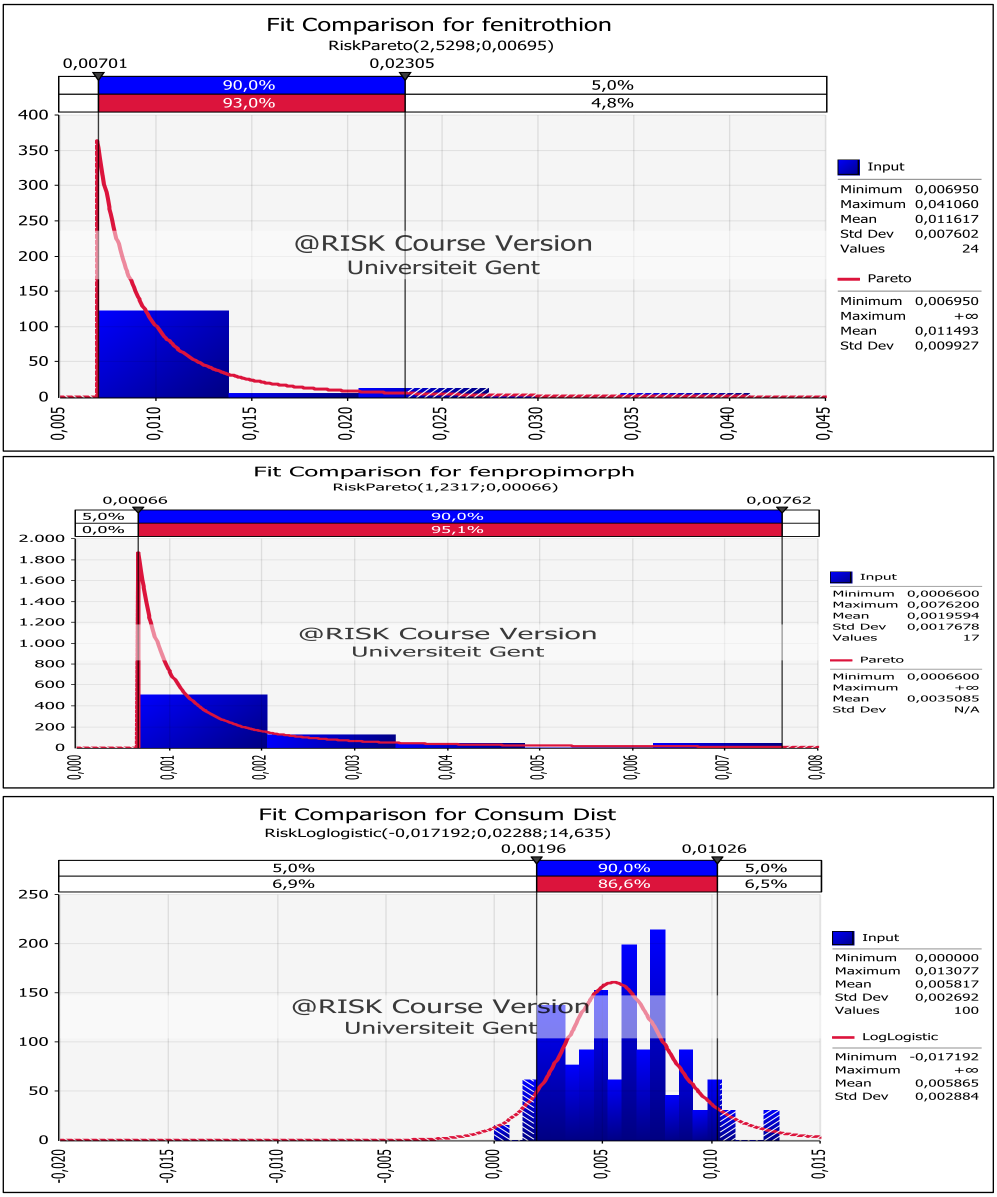

Fig. 1. Fit comparison curves for probabilistic risk assessment.

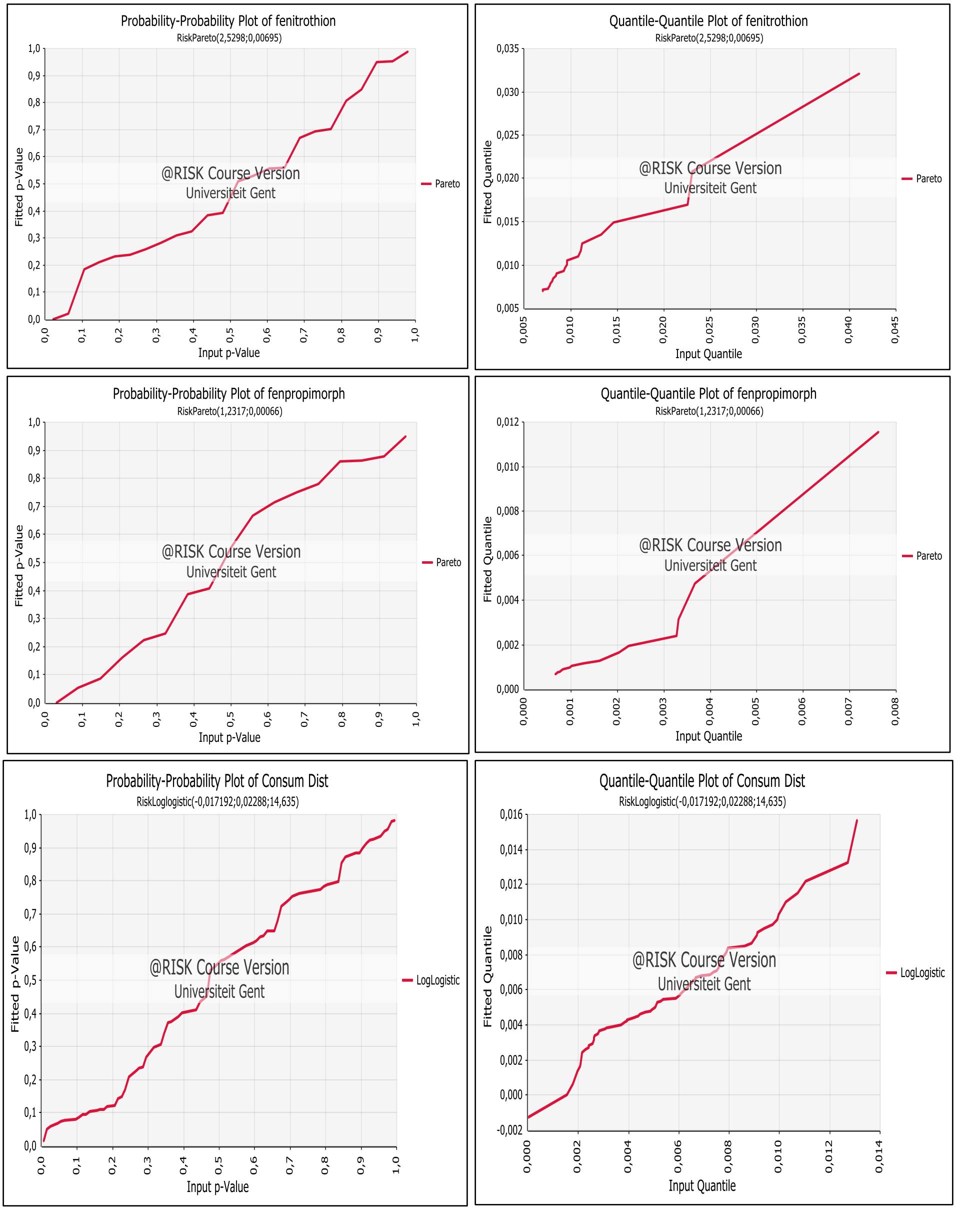

Fig. 2. PP and QQ Plots for probabilistic risk assessment.

The distribution for the concentration data and that of the consumption were multiplied by each other to obtain the intake or exposure distribution. The distribution for the consumption data, the distribution for the concentration data of the pesticides and the estimated daily intake from the probabilistic method are presented in Table 3.

Table 3. Estimated daily intake of fenpropimorph and fenitrothion through probabilistic exposure assessment

Statistical Distributions of Residue and Consumption Data				
Fenpropimorph (mg/kg)	**Fenitrothion (mg/kg)**		**Yam consumption (kg/kgBW/day)**	
LB (0), UB (0.0035)	LB (0) UB (0.0115)		Loglogistic(-0,017192;0,02288;14,635)	
	EDI (mg/kgBW/Day)			
Percentile	**Fenpropimorph**		**Fenitrothion**	
	Lower Bound	**Upper Bound**	**Lower Bound**	**Upper Bound**
Mean	0.000008	0.00058	0.000024	0.0012
Median	0.000000	0.00001	0.000000	0.0001
P75	0.000000	0.00002	0.000043	0.0022
P90	0.000006	0.0023	0.000076	0.0042
P95	0.000011	0.0051	0.0001	0.0052
P97.5	0.000019	0.0066	0.00013	0.0060
P99	0.000044	0.0082	0.0002	0.0071
ADI(mg/kgBW/Day)	**0.003**	**0.003**	**0.005**	**0.005**

LB = Lower bound, UB = Upper bound.

DISCUSSION

From the yam consumption survey, it was found that majority (99%) of the farmers interviewed were adult men having ages ranging between 21 and 65 years. The weight of the famers ranged between 55 and 99 kg with an average of 69 kg. Similar to the per capita yam consumption in Ghana as reported by the FAO (FAOSTAT, 2019), the farmers consume yam on the average 0.4 kg/day.

From the deterministic exposure assessment, it was found that, fenitrothion and fenpropimorph did not exceed their ADI. For those pesticides whose EDI were determined through the simple distribution approach, it was found that none of them had its EDI exceeding its respective ADI.

From the probabilistic dietary exposure assessment, it was found that the estimated daily intake for fenitrothion and fenpropimorph, for the lower bound scenario, was lower than their respective ADI, implying that there was no dietary intake risk. However, with the upper bound scenario, about 10% of the farmers had their EDI to the two pesticides above their respective ADI. The EDI of the 10% farmers exceeded the ADI of fenitrothion by about 4% and exceeded the ADI of fenpropimorph by about 70%. This means that those farmers had dietary intake risk to fenpropimorph and fenitrothion. The 99th percentile EDI for fenitrothion was 0.0002 mg/kgBW/day for the lower bound scenario and 0.0071 mg/kgBW/day for the upper bound scenario. This implies that 1% of the farmers had their EDI exceeding the ADI of fenitrothion by about 42% under the upper bound scenario. The 99th percentile EDI for fenpropimorph was 0.000044 mg/kgBW/day for the lower bound scenario and 0.0082 mg/kgBW/day for the upper bound scenario. This implies that 1% of the farmers had their EDI exceeding the ADI of fenpropimorph by about 173%. The results imply that, the 10% farmers with dietary intake risk to fenitrothion and fenpropimorph, are more at risk to fenpropimorph than to fenitrothion. This could be attributed to the low ADI of fenpropimorph (0.003 mg/kgBW/day) compared to the high ADI of fenitrothion (0.005 mg/kgBW/day).

Generally, the exposure assessment showed that the farmers have minimal exposure to the twelve pesticides with only about 10% having intake risk under the upper bound scenario. These minimal exposures to the pesticides could be attributed to the very low concentrations of the pesticides detected in the yam samples (Wumbei et al. 2018 and Wumbei et al. 2019), which in turn could be attributed to the fact that the yam samples were peeled before being analyzed, as peeling is found to reduce pesticide residues in yam and other root vegetables by about 40% (Clostre et al. 2014).

The farmers' exposure to the pesticides could even go further lower considering the fact that yam is not eaten raw, but rather boiled, fried or roasted, each of which can contribute to reducing the residues of pesticides in food. In a study of Kumari (2008) to monitor the levels of organochlorines, organophosphates, synthetic pyrethroids and carbamates in processed and unprocessed vegetables, it was found that boiling reduced residues by 32-100%. Household processing such as boiling is found to reduce pesticides residues in food by 20 to 100% (Kumari, 2008). In a study of Bonnechère et al. (2012), to measure the effect of household and industrial processing on pesticides, it was found that washing vegetables with tap water could reduce pesticides in the vegetables by 10-50%. In another study by Bonnechère et al. (2012) to assess the effect of processing on pesticide residues in carrots, it was found that washing and peeling each decreased the concentration of the pesticides in the carrots by 90%. In other studies (Keikotlhaile 2010; Soliman 2001) it was found that frying combined with washing could reduce pesticides residues in food crops up to 50%.

A comparison between the results of the deterministic and probabilistic methods (Fig. 3) showed that there is a significant difference ($P < 0.05$) between the two methods. The exposure values were consistently higher under the probabilistic approach than under the deterministic approach. This confirms the assertion that the deterministic dietary exposure assessment method has the tendency to either underestimate or overestimate exposure while the probabilistic method gives more accurate estimates of exposure (Finley and Paustenbach 1994; Kirchsteiger 1999; Rivera-Velasquez et al. 2013).

Human exposure to pesticides through the consumption of food is as important as any of the other routes of exposure. As a result there has been research in Ghana to determine human exposure to pesticides via the consumption of fruits and vegetables, maize, cowpea and Bambara beans (Akomea-Frempong et al. 2017; Akoto et al. 2013; Bempah et al. 2016; Donkor et al. 2015). The results as observed in this study, conform with some of the studies done in Ghana and in the UK (Akomea-Frempong et al. 2017; Bempah et al. 2016; Donkor et al. 2015) on fruits and vegetables, including yam, in which the produce were contaminated with pesticides, but no risk of dietary intake was observed. The results were different from other studies in Ghana (Akoto et al. 2015;) in which risk of intake to organochlorine and organophosphate pesticides was observed. These studies considered children and also cumulative risk of the various pesticides when one eats all the vegetables concerned. The results, as far as glyphosate is concerned also conform with the studies of EFSA (2016).

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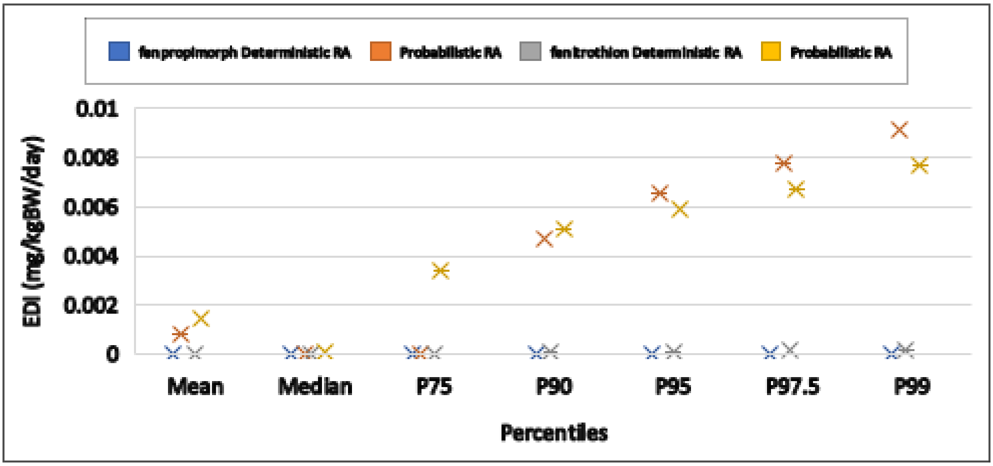

Fig. 3. Comparison of EDI of farmers to fenpropimorph and fenitrothion between the deterministic and the probabilistic methods.

RESUME

Wumbei A., Issahaku A., Abubakari A., Lopez E. et Spanoghe, P. 2019. Evaluation des risques de consommation des résidus de pesticides dans l'igname. Tunisian Journal of Plant Protection 14 (2): 49-64.

Les pesticides chimiques ont largement contribué à la production agricole à travers le monde. Cependant, l'exposition humaine aux pesticides reste une préoccupation majeure. La consommation alimentaire est une source importante d'exposition humaine aux pesticides. Les effets négatifs potentiels des pesticides ont entraîné une réglementation stricte de la production et de l'utilisation des produits, en particulier dans les pays développés. Pour faire face aux effets négatifs potentiels des pesticides, les évaluations des risques sont généralement effectuées par des experts scientifiques afin d'établir les niveaux des risques et de proposer des stratégies de gestion des risques. L'igname est un produit alimentaire largement consommé par les africains à la maison et par la diaspora. Les producteurs d'igname utilisent des pesticides pour la production de cette culture au fil des années. Le public est préoccupé par les effets sur la santé de l'exposition aux résidus. Cette étude visait à évaluer le risque d'ingestion de 12 pesticides par le régime alimentaire, dont cinq insecticides (cadusafos, fénitrothion, imidaclopride, profénofos et propoxur), quatre fongicides (carbendazime, fénpropimorphe, métalaxyl, propiconazole) et trois herbicides (bentazone, glyphosate et pendhalin) dans l'igname cultivé par des agriculteurs de la zone traditionnelle du Nanumba au Ghana. Les données de résidus et les données de consommation ont été collectées et combinées pour obtenir l'absorption journalière estimée (EDI). Trois approches (déterministe, distribution simple et probabiliste) ont été adoptées dans le calcul de l'EDI et les valeurs de l'EDI ont été comparées à la dose journalière admissible (ADI) des différents pesticides afin de déterminer s'il existait un risque d'ingestion. L'étude a révélé que l'EDI des agriculteurs utilisant les douze pesticides visés par l'approche déterministe et la distribution simple, était inférieur à leur ADI fixé par la Commission de l'UE. Cependant, l'EDI d'environ 10% des producteurs de fenpropimorphe et de fénitrothion était supérieur à leur ADI.

Mots clés: Consommation, déterminisme, évaluation des risques liés aux pesticide, probabiliste, l'igname

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ملخص

ويمباي، أبوكاري وعبد الرحمان إسحاق وعبد الله أبوبكر وإيديليبيس لوبيز وبيتر سبانوغ. 2019. تقييم مخاطر استهلاك بقايا المبيدات في زراعة اليام. Tunisian Journal of Plant Protection14 (2): 49-64.

ساهمت المبيدات الكيميائية بشكل كبير في الإنتاج الزراعي في جميع أنحاء العالم. ومع ذلك، لا يزال التعرض البشري للمبيدات مصدر قلق بالغ. أحد المصادر المهمة للتعرض البشري للمبيدات هو استهلاك الغذاء. أدت الآثار السلبية المحتملة للمبيدات إلى تنظيم صارم في إنتاج واستخدام المنتجات، وخاصة في العالم المتقدم. لمعالجة الآثار السلبية المحتملة للمبيدات، عادة ما يتم إجراء تقييمات للمخاطر من قبل خبراء علميين لتحديد مستويات المخاطر وتقديم استراتيجيات لإدارة هذه المخاطر. يام هي سلعة غذائية يستهلكها الأفارقة على نطاق واسع في الوطن وفي الشتات على حد سواء. يستخدم مزارعو اليام (نوع من البطاطا الحلوة) المبيدات في إنتاج هذه الزراعة على مر السنين. يشعر عموم الناس بالقلق إزاء الآثار الصحية التي قد تنجم عند التعرض إلى بقايا المبيدات. صُممت هذه الدراسة لتقييم مخاطر ابتلاع 12 من مبيدات الآفات، بما في ذلك خمسة مبيدات حشرية (كادوسافوس وفينيتروثيون وإيميداكلوبريد وروفينوفوس وبروبوكسور) وأربعة مبيدات فطرية (كاربيندازيم وفينوبربيمورف وميتالاكسيل وبروبايونازيل) وثلاثة مبيدات عشبية (بانتازون وغلايفوزات وبنذالين) في زراعة اليام في منطقة نانومبا التقليدية في غانا. تم جمع بيانات البقايا وبيانات الاستهلاك ودمجها لاستخراج الامتصاص اليومي المقدر (EDI). تم اعتماد ثلاثة مناحي (حتمي، توزيع بسيط، احتمالي) في احتساب الامتصاص اليومي المقدر وتمت مقارنة قيم هذا الامتصاص اليومي المقدر مع الجرعة اليومية المقبولة (ADI) لمختلف المبيدات وذلك لتحديد ما إذا كان هناك مخاطر مع الابتلاع. وكشفت الدراسة أن الامتصاص اليومي المقدر عند المزارعين للمبيدات الاثنتي عشر المستهدفة بالمنحى الحتمي وبمنحى التوزيع البسيط، كان أقل من الامتصاص اليومي المقبول الذي ضبطته مفوضية الاتحاد الأوروبي. ومع ذلك، كان الامتصاص اليومي المقدر لدى حوالي 10% من المزارعين للمبيدَيْن فينوبربيمورف و فينيتروثيون أعلى من الجرعة اليومية المقبولة عندهم.

كلمات مفاتيح: الاستهلاك، الحتمية، الاحتمالية، تقييم مخاطر المبيدات، اليام

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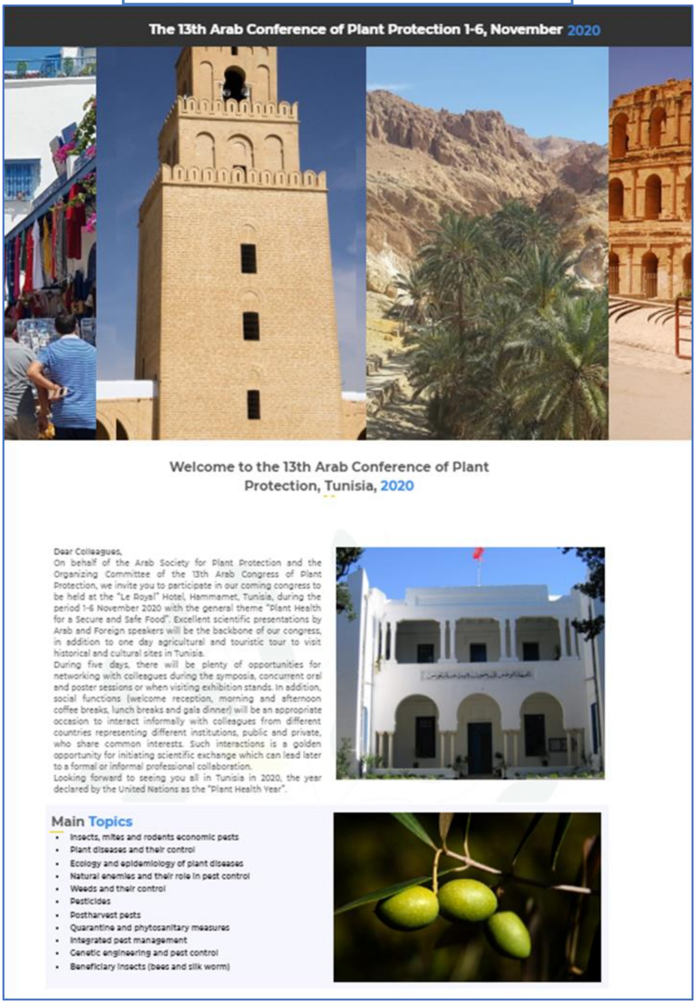
Plant Protection Events

Announcing

of

The 13th Arab Conference of Plant Protection

Hammamet, Tunisia, 1-6 November, 2020



Announcing

of

The 4th Africa-International Allelopathy Congress (AIAC-2020)

Sousse, Tunisia, 9-11 April, 2020

Tunisian Association for Sustainable Agriculture

Higher Agronomic Institute of Chott-Mariem

« ALLELOPATHY FOR ORGANIC AGRICULTURE»

Contact: tunisiallelopathy@yahoo.com

Invitation

After the success of the three editions of Africa-International Allelopathy Congresses (AIAC-2014, AIAC-2016 and AIAC-2018), the Higher Agronomic Institute of Chott-Mariem, the Tunisian Association for Sustainable Agriculture (**ATAD**) and the Local Organizing Committee, cordially invite scientists to attend the **Fourth Africa-International Allelopathy Congress (AIAC-2020)**, which will be held in Sousse, Tunisia, from 9 to 11 April, 2020.

This scientific event is organized biannually and constitutes an important meeting ground for researchers within all aspects of allelopathy in African countries and worldwide. The congress organizing committee has chosen "***ALLELOPATHY FOR ORGANIC AGRICULTURE***" as the topic for this meeting. The overall goal of the congress is to promote the active exchange of innovations in Allelopathy and their potential use for organic farming.

About ATAD

The Tunisian Association for Sustainable Agriculture "*Association Tunisienne pour une Agriculture Durable*, ATAD" was created in March 2013 for scientific purposes, specifically to promote the cooperation between Tunisian scientists and to develop such scientific collaborations throughout the world. Further informations on ATAD activities are now available at the following link: **https://atad-isa.com/**.

Theme of the Congress: *Allelopathy for Organic Agriculture*

The International Federation of Organic Agriculture Movements (IFOAM) defined the organic farming as "*a production system that maintains the health of soils, ecosystems and people. It is based on ecological processes,* *biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science for the benefit of the common environment*".

Wendell Berry, wrote that *"A biological farm, strictly speaking, is not the one that uses certain methods and substances and avoids others, it is a farm whose structure mimics the structure of a natural system that has the integrity, the independence and benign dependence of an organism"*.

Thus, the bases of organic agriculture are ecological processes, biodiversity and biogeochemical cycles. Allelopathy perfectly meets the requirements of the organic farming. Hence, how allelopathy can serve organic agriculture? How to use its principles in this field? How to exploit this natural process to overcome the great problems caused by the excessive use of industrial inputs? Participants to AIAC-2020 will try to answer to all of these questions.

The 4th AIAC 2020 is open to all researchers working on the implementation of organic agriculture, its principles and difficulties as well as those working on the use of natural resources in agriculture and seeking to exploit biological processes as functional inputs.

Language

The official language of the Fourth Africa-International Allelopathy Congress will be English. All abstracts, posters and oral presentations will be in English without translation.

Registration

Registration is available at the following link: **https://atad-isa.com/aiac-2020/**

Submission

In addition to invited lecturers, the symposium will be based on oral and poster presentations. To participate, researcher should submit the title and a one-page abstract of his contribution via e-mail to: tunisiallelopathy@yahoo.com

Important dates:

- Deadline for abstract submission: **February 15th, 2020**
- Notification of abstract acceptance: **March 15th, 2020**
- Submission of full papers: **April 30th, 2020** (**http://www.tjpp.tn/PaperSubmission.php**).

Accepted papers will be published in a special issue of Tunisian Journal of Plant Protection (**http://www.tjpp.tn**) devoted to the publication of the most relevant presentations in AIAC-2020.

Registration Fees

FULL Local Participants: 400 TND
FULL Foreign Participants: 250 Euros
ONE DAY Participants: 150 TND
Full registration fees include: Accommodation (2 nights), breakfast, lunches, dinners, refreshment breaks, registration kit.

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FINANCIAL SUPPORT/SPONSORS

Report

on

Information Day on Sustainable Organic Vegetable Production

INAT, Tunis, Tunisia, Friday 20th September 2019

Introduction

Dr. Ferdinando Branca is associated professor of Catania University where he has studied aspects related to diversification and innovation of vegetable production by studying the variation of plant bio-morphology, primary and secondary metabolites and genetic profiles of landraces and of crop wild relatives, of species widespread in other geographic regions, and of species neglected or underutilized, such as wild species utilized as vegetables. He is actually coordinator of the H2020 BRESOV (Breeding for Resilient, Efficient and Sustainable Organic Vegetable Production).

During the conference, which took place on 20th September 2019 at the Auditorium of the National Agronomic Institute of Tunisia (INAT), he presented two talks.

1) Breeding for resilient efficient and sustainable organic vegetable production.

For several wild species utilized as vegetables in Italy, he has individuated diffusion sites, collected and conserved propagation materials, studied biological characteristics of the collected materials and evaluated their adaptability in different environmental conditions in the frame of several research projects. Since 1994, he has collected, characterized and evaluated wild and cultivated vegetable species, and in particular Brassicaceae ones. Among them, has studied Sicilian germplasm of cauliflower, broccoli, kale, and kohlrabi of which he has collected, conserved and evaluated several landraces for their main morpho-biological, agronomical, nutraceutical and organoleptic traits, evaluated glucosinolate profiles, individuated resistance sources for *Xanthomonas campestris* pv. *campestris*, and obtained breeding and androgenic lines.

2) Exploitation of Brassicaceae for sustainable agriculture and innovation of production chains.

On another hand, we evaluate and validate soil biofumigation by the use of glucosinolate (GLS) compounds which are extracted from Brassicaceae. Among them, sinigrin showed biocontrol activities against several pests and diseases via nematotoxic action. Among the Brassicaceae species rich in sinigrin, he choses *Brassica macrocarpa* (BM) because its leaves show 90% of all GLS, and he could better estimate the action of this single GLS.

Indeed, tomato crops are affected in Mediterranean cold-greenhouse agrosystems by soilborne diseases, such as root-knot nematodes (*Meloidogyne* spp.), which represent a serious problem leading to losses in production. Agroecological soil management based on biocontrol agents and natural compounds has had increased grower interest in order to reduce chemical residues in the produce and to adopt environmentally friendly farming methods. Therefore, different dosages of BM leaf flour, containing 200 to 300, 350, 400, 450, and 650 µmol.m^{-2} of sinigrin, were inserted into soil already infected by *Meloidogyne* spp. for evaluating their effects on tomatoes grown in cold greenhouses in comparison to absolute control (CTRL) and to the chemical one, Vydate 5G® (CCTRL). The root disease index, caused by nematode attack, was the highest in CTRL, and a reduction of about 50% was observed with the 300 to 650 µmol.m^{-2} sinigrin dosage. The CCTRL showed twofold marketable yield increase, and a fourfold increase was found in 650 µmol.m^{-2} of sinigrin dosage, in comparison to the CTRL. Biofumigant applications improved tomato plant growth and development, and fruit quality, significantly for dry matter and soluble sugars (°Brix). BM leaf flour inserted into the soil, at a dose of 300 µmol.m^{-2} of sinigrin, showed similar effects to the CCTRL on root disease index, root weight, and marketable yield. Data showed the nematotoxic effect of sinigrin for the control of *Meloydogine* spp. by the use of *B. macrocarpa* leaves, very rich in this GLS compound, which represent a new tool for agroecological soil management and for organic farming.

Prof. Walid Hamada
INAT, University of Carthage, Tunis
Tunisia

Report

on

The Organization of a Business Forum: “Climate change and Plant Health” under the CLICHA Project

INAT, Tunis, Tunisia, 17 December, 2019

Presentation of the Project "CLICHA"

In addition to causing extreme weather, drought, flooding and other disasters, climate change's effect in Mediterranean regions is expected to exacerbate existing problems such as water scarcity, groundwater salinization, soil desertification, biodiversity loss and extension of distribution. In this context, the CLICHA project-*Climatic Change in Agriculture* was initiated in October 2017 for 3 years. It's an innovative Erasmus+ project involving 10 institutions from 4 countries: Italy, Latvia, Greece and Tunisia, for capacity building in Higher Education that aims to share good practices in the field of climate change and resilience of agriculture. In fact, the role of agriculture is not only crucial in mitigating, but also in adapting to climate change. Hence the goal of CLICHA project is to educate future generations to enable them to develop and change agricultural systems to both limit their impact on and to be resilient to climate change, to ensure food security and sustainable development in a changing climate. In Tunisia, there is a need for such program because the curricula of Tunisian High Education Institutes (HEIs) are mostly based on "conventional" agriculture in the sense that courses do not include sufficiently the component "climate change". To improve this situation in order to make future agricultural engineers and scientists equipped with the necessary skills and knowledge to cope with climatic change in their specific local context, the five Tunisian HEIs involved in the project, will benefit from the experience of the EU HEIs. The latter will contribute to the modernization of university courses in Tunisia at several levels: engineer, master and doctorate. ICT will be more integrated in courses and educational material (e-learning platforms, websites etc.) and research and innovation capacities will be strengthened by networking and collaborations as well as the cooperation between enterprises-HEIS is expected to be fostered. Finally, general public and stakeholders will also benefit from the outputs of CLICHA project through dissemination in TV, social media etc. The CLICHA project Tunisian coordinators are Dr Fatma Trabelsi (National level) and Dr Layla Ben Ayed (University of Carthage).

The Business forum on "Climate change and Plant Health"

This event was organized by the University of Carthage and INAT (Dr L. Ben Ayed, Dr K. Lebdi and Dr S. Attia) where it took place December 17, 2020. Given the risks of emergence of new pests and diseases and also extending of geographical distribution of already known pests consequently to new climatic conditions (higher temperatures, precipitation patterns), research in plant health field as well as techniques of pest and pathogen surveillance and detection must be improved and international collaborations must be enhanced. The organization of trainings, seminars and information days involving all plant protection stakeholders are excellent opportunities for exchanges and partnerships. The CLICHA business forum held at INAT was such an opportunity to discuss adaptation strategies of crop protection in the context of climate change. For this forum, INAT has invited experts, researchers and practitioners in Agriculture and Plant Protection from Tunisia, Italy and Greece to share their experiences, good practices and actions. A session was dedicated to start-ups active in climate change adaptation, to present their activities and possible collaborations with research and farmers to help agriculture adaptation to climate change.

For more information on CLIHA business forum on Climate change and Plant Health or on CLICHA project, please visit the website <www.clicha.eu>.

Dr. Synda Boulahia-Khedher
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Tunisia

Report

on

A National Training on Desert Locust Control

Sousse, Tunisia, 28 October - 1 November, 2019

The Desert Locust is a terrifying pest because of the extent of the damage that can cause in agro-sylvo-pastoral production on large area during the invasion and due to the significant socio-economic and environmental disturbances caused. The control strategy of Desert Locust is a prevention strategy which is recognized by the international community as the most effective and the only sustainable economic strategy taking into account the protection of human health and the environment. It is within this framework that CLCPRO intervenes, whose headquarters are in Algiers, Algeria, and which aims to promote at the national, regional and international level all actions, including research and training with a view to ensuring rational and sustainable management of the preventive fight and coping with Desert Locust invasions in the western region of its habitat area, grouping the West and the North-West of Africa.

The "Training" aspect received special attention from both General Directorate of Plant Health and Control of Agricultural Inputs (DG/SVCIA), to strengthen central and regional capacities, and CLCPRO, either in programming of regional and national activities or in putting interesting amounts of the funding. Three regional training plans (PFR) were thus developed and implemented, in 2007-2009 (PFR I), in 2011-2014 (PFR II) and finally in 2015-2018 (PFR III). The fourth one (PFR IV) is in progress 2019-2022.

In order to further consolidate the important achievements of the implementation of these regional training plans, CLCRO supports member countries in organizing and achieving national trainings. Tunisia benefits since 2015 of technical and financial support of the Commission to hold such events.

In October 2019, the National Unit of Desert Locust Control in Tunisia, using the technical and financial contribution of CLCPRO, organized a training about the technics of spraying in Desert Locust Control. The session held in Sousse lasted five days from October 28 to November 1, 2019, in which the technical staff of the regional representatives of Plant Health Directorates and DG/SVCIA attended the course. Theoretical and technical aspects of the course have been raised.

Sixteen regional participants involved have participated coming from the governorates of the front line: Tataouine, Medenine, Gafsa, Tozeur and Kébili and those of the second line: Kasserine, Gabes, Sfax, Kairouan and Sidi Bouzid.

Theoretical themes treated in the training were: Bio-ecology of Desert Locust, methods of prospection of the pest, **Spraying technics in control**: its principals parameters to efficient chemical intervention by using Ultra Low Volume pesticides (ULV) and sprayer calibration.

Practical aspects involved a test with a sprayer carried by man (AU8000) and another test with a sprayer carried by vehicle (Ulvamast V3).

Recommendations:

- Continuing to organize national trainings.
- The sessions organized are considered as trainings of trainers.
- Each participant attended at least two or three sessions.
- Each participant should organize training sessions concerning different aspects of Desert Locust Control for the benefit of local technicians in his region. A support of DG/SVCIA can be added.

Eng. Mouna Mhafdhi
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Photo of the cover page: *Helicoverpa armigera* larva molting (Courtesy *Thameur Bouslama*)

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