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Photo of the cover page: ***Oryzeaphilus surinamensis*** (Courtesy *Abir Soltani*)

Sincere homage to the soul of Prof. Dr. Bassam Bayaa

With big sorrow, and full acceptance of God's willing and destiny, the Arab plant protection family mourns the departure of the late Prof. Dr. Bassam Bayaa, who died January, the 3rd, 2022 in the city of Aleppo, Syria. This world's plant protection scientist has endowed almost his 76-year life to the field of science and sober research, and he entrusted it with honesty and creativity in the minds of many who drew from his knowledge and enrich their senses with the supply of his long academic and practical experience that he never kept for himself.

The Arab Society for Plant Protection has lost one of its pillars and an element that laid its foundation in the city of Aleppo in 1979. The late Prof. Dr. Bassam Bayaa linguisted all the Society's publications, including books, magazines, brochures, as well as the Arabization of plenty of plant protection vocabulary that we are presently using. His loss will surely leave a great void in the Arabic plant health field.

Guest Editorial

Old data with new uses: the host reproduction number for fruit fly management and trade

Fruit flies are the most significant insect pest in the fruit industry and are present on all continents except Antarctica. Most countries have endemic fruit flies to manage and to minimize fruit damage due to fruit flies. Importers need good quality and uninfested fruit, and exporters of traded fruit must create confidence that these commodities do not contain immature stages of fruit flies. Additionally, countries must be alert to incursions of exotic fruit flies and early warning surveillance is used by many countries.

These activities (surveillance, management and trade) must continue in a world where the number of acceptable pesticides is declining, where the carbon footprint of commodities is becoming more important, and where our climate is changing. So, the operating environment for fruit production and trade is changing, and historical management strategies are being challenged. There is merit in revisiting what we do and assessing if this is still the best way.

There is much historical data available and can be used to modify what we do. One area of merit is to re-assess risk provided by different hosts that support the production of large pest populations. It has long been known that different host fruit have different capacities to support different levels of fruit fly populations. Historically, these hosts were called "good" hosts or "poor" hosts although the quantification of these terms was not done until recently.

In a literature review of tephritids in the Pacific region, hosts were ranked into five categories using published data on infestations. These categories were termed the host suitability index and provided guidance on host suitability ranging from 0.1 to >100 adults per kg. Field harvested fruit is more likely to provide more realistic data, compared to artificial laboratory experimental conditions. These five categories were based on the number of tephritid adults that emerged from a kilogram of fruit. This metric was recently termed the host reproduction number or HRN.

An understanding of HRN can provide insights into tephritid management strategies. There are three important work areas for HRN. The first is surveillance. Surveillance traps should be placed in hosts with a high HRN because these hosts are likely to support

higher adult populations and hence adults are more likely to enter traps sooner. In monoculture orchards, this is not an option. However, many farms have home orchards and these hosts need to be monitored and treated similarly to the commercially produced fruit.

In management, there are several factors that might be combined to provide a better management outcome. Tephritids are arboreal pests and generally fly from tree to tree; they do not move or disperse in the same way as aphids. Many tephritids are attracted to shapes and colors and make short hopping flights between trees. Flies spend much of their time in non-flight activities. They are not known to fly over pastures as they know there is no fruit in gramineous plants. In temperate climates, tephritids usually fly or disperse less than 500 m in their lifetime. This dispersal distance is less clear in tropical environments. All flies seek water and sustenance in trees, and use leaves to hide from predators. Mature males look for mating sites while mature mated females look for egg laying opportunities. So, tephritids use these tree corridors of non-commercial hosts for dispersal and potential population expansion.

Often, tree corridors contain many and different host species with their different capabilities to support tephritid populations. Most tree corridors are not managed or treated with pesticides. Therefore, fruit producers need to be aware of the higher HRN hosts in tree corridors and act to minimize the capacity of tephritids to enter their properties. Tree corridor hosts that produce 200 adults per kg should be treated differently to hosts that support 2 adults per kg. Ideally, higher HRN hosts should be removed (poisoned or cut down) to prevent an annually reoccurring problem. Alternatively, these trees should be fruit stripped before fruit is susceptible to egg laying. If these trees provide other valuable merits to a community, then these trees should be treated the same way as commercial produce.

The third potential work area is in new trade protocols. Currently, most produce must undergo end point disinfestation for fear that some eggs or larvae are present in fruit. Usually, these trade protocols are required to be equivalent to probit 9 at least. However, this requirement assumes that all fruit and fruit infestation is the same. The published HRN data for Africa indicates that this is not true. Would all commodities (with a low HRN or a high HRN) require the same end-point treatment to achieve the same level of confidence?

The end point treatments are not a fix-all for every commodity in all growing conditions. What if growers could achieve the same level of confidence without the endpoint treatment? One of the newer International Standards for Phytosanitary Measures is the "Systems Approach" where several independently acting risk mitigation techniques can be combined to minimize the risk of infestation. A systems approach may include intense pest exclusion on the property boundary (such as male annihilation technique), surveillance in production blocks, preharvest fruit inspection, examination

of reject fruit for infestation, and HRN to demonstrate that the commodity has a low likelihood of infestation. A systems approach may decrease the need for methyl bromide, a recognized greenhouse gas. Another end point treatment uses temperature control for about 3 weeks but this treatment has a large carbon footprint. Some countries are increasing concerned that these large carbon footprint commodities are contributors to climate change. Given global concerns about climate change, these end-point treatments may be less preferred, compared with previous decades.

HRN has several areas where surveillance scientists, fruit fly managers and market access regulators could benefit from increased HRN knowledge. Hence, there is merit in scientists collecting more information in the HRN format for more commodities. Additionally, the role of environmental conditions on HRN metrics needs to be evaluated. For instance, altitude reportedly influences HRN in several species. In the future, HRN knowledge could assist tephritid scientists to change how they do their business.

Dr. Bernie Dominiak,
Scientific Publications Coach, Chief Scientist Branch
Department of Primary Industries, Adjunct Fellow Macquarie University
Sydney, Australia

Behavior of New Entries and Developed Tomato Hybrids Carrying *Ty-2* Gene

Mounira Elbaz, Monaam Timoumi, LR21AGR03/Production et Protection pour une Horticulture Durable, CRRHAB, Université de Sousse, BP. 57, Chott Meriem 4042, Sousse, Tunisia, **and Peter Hanson,** AVRDC/The World Vegetable Center, West and Central Africa (Coastal and Humid Regions), IITA-Benin Campus, 08 BP 0932 Tri Postal, Cotonou, Benin **(*Tunisia/Benin*)**
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ABSTRACT

Elbaz, M., Timoumi, M., and Hanson, P. 2022. Behavior of new entries and developed tomato hybrids carrying *Ty-2* genes. Tunisian Journal of Plant Protection 17 (1): 1-14.

Tomato yellow leaf curl disease (TYLCD) is a serious problem hampering tomato production worldwide. In the Mediterranean Basin, disease incidence and severity are higher in the dry season increasing whitefly (*Bemisia tabaci*) populations. Effectiveness of resistance to *Tomato yellow leaf curl virus* (TYLCV) depends on both tomato host resistance and TYLCV complex species. So far, six different *Ty* tomato resistance genes have been identified. Two main TYLCV complex species, *Tomato yellow leaf curl virus-Israel* (TYLCV-Is) and *Tomato yellow leaf curl Sardinia virus* (TYLCSV), have been identified in Tunisia. The present work aimed to evaluate entries heterozygous for *Ty-2* gene to help predict hybrid performance. Two tomato entries homozygous for the *Ty-2* TYLCV resistance gene, one tomato hybrid homozygous for *Ty-2* and two heterozygous hybrids were included, besides two susceptible tomato entries. Resistance response to TYLCD was recorded based on disease incidence and severity levels. Data analysis was performed according to presence/absence of *Ty-2* gene and taking into account homozygosity and heterozygosity of *Ty-2*. Generalized linear model analysis was applied to check significance of individual factors' effects (*i.e.* effect of tomato entries or tomato groups of entries based on presence or absence of homozygous/heterozygous *Ty-2* gene, block unit within the field trial and the year of the trial) on the dependent variables (disease incidence and severity). Further multi-comparison tests gave evidence on significant effect of *Ty-2* homozygous gene tomato entries on TYLCD incidence and severity levels. The results were discussed with special focus on the relevance use of heterozygous hybrid tomato varieties.

Keywords: Heterozygous, resistance genes, tomato, *Tomato yellow leaf curl virus* (TYLCV), Tunisia

Tomato yellow leaf curl disease (TYLCD) is caused by a complex of monopartite and bipartite begomoviruses belonging to the family Geminiviridae and vectored by the adult sweet potato whitefly (*Bemisia tabaci*). Begomoviruses infect a wide range of botanical families, including Solanaceae (tomato, tobacco, pepper, petunia), Cucurbitaceae (melon, watermelon, squash, gourd), Fabaceae (common bean, soybean, lima bean, mung bean, cowpea), Euphorbiaceae (cassava) and Malvaceae (cotton, okra) (Seal et al. 2006). Begomoviruses cause serious

Corresponding author: Mounira Elbaz
Email address: mounira_elbaz@yahoo.com

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tomato (*Solanum lycopersicum*) yield losses particularly in tropical and subtropical regions (Cohen and Lapidot 2007; Ji et al. 2007b; Moriones & Navas-Castillo 2000; Varma et al. 2003; Rybicki 2015).

Strategies to control the disease focus on reducing sources of inoculum, controlling *B. tabaci* population, use of physical (*e.g.* whitefly-proof screens, UV-absorbing plastic sheets, and reflective plastic mulches), chemical (insecticide applications) or cultural (virus-free seeds, virus-free transplants, crop-free periods, rouging symptomatic plants and weed management) methods (Riley and Srinivasan 2019). Besides, another alternative control method using salycilic acid as a resistance-inducing factor was shown to effectively enhance TYLCD tomato resistance in both resistant and susceptible tomato cultivars (Li et al. 2019). However, in general TYLCD control is not possible after infection because of very low efficient spray level of *B. tabaci* and whitefly resistance to some commonly used pesticides (Antignus et al. 2001; Horowitz et al. 2007). Thus, the use of resistant varieties combined with other strategies of control can contribute to significantly reduce the incidence of the disease. So far, six TYLCD resistance *Ty* genes have been identified (Dhaliwal et al. 2020) and their chromosomal locations mapped, *i.e.*, *Ty-1* (Zamir et al.1994) and its allele *Ty-3* on chromosome 6 (Ji et al. 2007a; Verlaan et al. 2013), *Ty-2* on chromosome 11 (Kalloo & Banerjee 1990; Hanson et al. 2000; Yang et al. 2014), *Ty-4* on chromosome 3 (Ji et al. 2009), recessive *ty-5* on chromosome 4 (Anbinder et al. 2009; Hutton et al. 2012; Wang et al. 2018), and *Ty-6* on chromosome 10 (Hutton and Scott 2014; Gill et al. 2019). These resistance genes were originally discovered and introgressed from several tomato wild species including *S. peruvianum* (*ty-5*), *S. chilense* (*Ty-1* and *Ty-3*, *Ty-4* and *Ty-6*), and *S. habrochaites* (*Ty-2*) (Kasrawi et al. 1988; Pico et al. 1996. Hanson et al. 2000; Hanson et al. 2006; Ji et al. 2007a, c). Gene function studies were conducted for *Ty-1/Ty-3*, *ty-5* and *Ty-2*. *Ty-3* encoding for a DFDGD-class RNA-dependent RNA polymerase which function is still unclear (Verlaan et al. 2013). Lapidot et al. (2015) discovered that *ty-5* gene is a loss-of-function mutant allele of the Pelota gene due to a T-to-G transversion in the coding region in cultivated tomato. *Ty-2* was found to be synonymous with *TYNBS1*, a functional resistance gene which is an *NB-LRR* (nucleotide-binding domain and leucine-rich repeat-containing) gene (Yamaguchi et al. 2018). In general, TYLCD resistance is positively correlates with lower levels of virus accumulation: *i.e.* the virus level was found to be 10% lower in tomato lines carrying *Ty-1/Ty-3* compared to virus level in susceptible tomato cultivars (Verlaan et al. 2013). Similar results were reported with *Ty-2* (Barbieri et al. 2010).

The present work aimed to evaluate heterozygous entries for one *Ty* gene intending to help predict hybrid performance. The impact of *Ty-2* gene at heterozygous status has not been tested in tomato yet. Hence, two tomato entries homozygous for the *Ty-2* TYLCV resistance gene, one tomato hybrid homozygous for this gene and two heterozygous hybrids were involved in this work. Additionally, two susceptible tomato entries, with no *Ty* gene, were added as susceptible controls. Resistance response to TYLCD was recorded based on incidence and severity levels of the disease. Data analysis was performed according to presence/absence of *Ty-2* resistance gene only. Results were discussed with special attention to relevance of heterozygous hybrid tomato varieties utilization.

MATERIALS AND METHODS

Experimental site.

Field trials were conducted in the Marj Ellil - Route Regueb in Kairouan in central region of Tunisia, during two consecutive seasons in 2015 and 2016 from February to August. Kairouan is under a Mediterranean climate.

Plant material and experimental design.

Seven tomato entries (E1 to E7) were included in the study (Table 1): two tomato lines and one hybrid homozygous for *Ty-2* and two hybrids heterozygous for *Ty-2*. Additionally, two susceptible tomato entries, lacking Ty genes, were added as susceptible controls. Resistance response to TYLCD was recorded based on incidence and severity levels of the disease. Tomato hybrids E3, E4 and E5 were obtained from crosses using either tomato accessions, obtained from The World Vegetable Center gene bank in Taiwan (WorldVeg), or with the inbred line variety Rio Grande (Table 1). The cross schemes are written as female parent crossed with the male parent so in all crosses resistant parents were used as the pollen donor. Tomato entries were categorized into 3 groups (Grp1 to Grp3) based on presence/absence of the homozygous/heterozygous *Ty-2* gene (Table 1). The experimental design was a Randomized Blocks Design (RBD), with an arrangement of grouping the heterogeneous units into homogenous blocks, using 3 blocks and 16-20 plants per plot. All entries were represented in each block. Plant spacing was 40 cm within- and 100 cm between- rows. In addition, in order to ensure homogeneous infection of the experimental site by *B. tabaci*, tomato Rio Grande variety, known as a susceptible genotype to TYLCD, was planted in the border of the entire field trial.

Table 1. Tomato entries (E) and corresponding groups (Grp) representing different combinations of *Ty* resistance genes screened for TYLCD resistance in Tunisia

Current code	Original code/Name	Status of *Ty-2* gene	Group Nb.	Source
E1	CLN2498E	*Ty* 2 Hm	Grp1	WorldVeg
E2	CLN2498D	*Ty* 2 Hm	Grp1	WorldVeg
E3	E2* E1	*Ty* 2 Hm	Grp1	This work
E4	E6*E1	*Ty* 2 Ht	Grp2	This work
E5	Rio Grande*E1	*Ty* 2 Ht	Grp2	This work
E6	CLN1466P	None	Grp3	WorldVeg
E7	CLN2026D	None	Grp3	WorldVeg

E2*E1, E6*E1 and Rio Grande*E1 represent the crosses' codes for hybrid production; Hm and Ht refer to homozygous and heterozygous, respectively.

Disease incidence and severity scoring.

TYLCD incidence and severity were recorded weekly for two months, starting the fifth week after planting when TYLCD symptoms occurred first. Symptom severity assessment was performed based on a scoring scale from 1 to 5 adapted from Lapidot et al. (2006) and Lapidot et al. (1997) where:

1: no symptoms.
2: slight leaf curl.
3: substantial curl with or without lightyellowing.
4: substantial curl with substantial yellowing.
5: substantial curl + yellowing + stunting or death of the plant.

A single score was assigned to each tomato plant according to detectable symptoms.

Incidence and severity scores (I% and S%, respectively) were calculated for each plot using the following formula:

$$I\,(\%) = \frac{PA \times 100}{PT}$$

where I: disease incidence; PA: number of symptomatic plants as soon as a visible symptom was observed; PT: total number of plants in the plot.

$$S(\%) = \sum_{i=1}^{5} \frac{iYi \times 100}{5N}$$

where S: disease severity; i: class; Yi: number of plants in class i; N: total number of plants.

Data analysis.

Only data from the last evaluation were subjected to statistical analysis to detect possible effect of tomato entries on the disease incidence and severity levels. Similarly, based on the presence or absence of homozygous/heterozygous *Ty-2* gene, effect of tomato entries group (Grp) on the disease incidence and severity was investigated.

Then, two generalized linear models (GLM) were written as:

$$Y_{ijk} = \mu + E_i + B_j + Yr_k + e_{ijk}$$

where μ: mean; E_i: tomato entry i effect (from 1 to 7); B_j: block j effect (from 1 to 3); Yr_k: year k effect (1 or 2, respectively for year 2015 and 2016); e_{ijk}: residual for E_i at B_j and Yr_k; Y_{ijk}: severity or incidence of E_i at B_j and Yr_k, and

$$Y_{ijk} = \mu + Grp_i + B_j + Yr_k + e_{ijk}$$

where μ: mean; Grp_i: group of tomato entries i effect (from 1 to 3); B_j: block j effect (from 1 to 3); Yr_k: year k effect (1 or 2, respectively for year 2015 and 2016); e_{ijk}: residual for Grp_i at B_j and Yr_k; Y_{ijk}: severity or incidence of Grp_i at B_j and Yr_k.

Data analyses were applied using Generalized Linear Model (GLM) to check significance of the effects of individual factors on the dependent variables (*i.e.* incidence and severity rates).

Following GLM analyses, data distribution was tested for normality using the Shapiro-Wilk test and Q-Q plot of residuals. ANOVA (analysis of variance) was applied followed by an adequate post hoc test for mean comparison if data were normally distributed. The Tukey Kramer multi-comparison test was used if results of the Levene's test showed equal variances, whereas Dunnett's test was applied in the case of heterogeneous variances. The Kruskal Wallis test, a non-parametric test, was applied when normality was rejected. If a significant effect was shown using Kruskal Wallis test, a post hoc test, Bonferroni multi-comparison test was used. The 95% confidence level was applied with all the tests. All of the statistical analyses were carried out using the SPSS 25 program (IBM 2017).

RESULTS

Experimental field infestation.

High levels of both incidence and severity scores were recorded from the Rio Grande plots (Table 2). The field trial was obviously highly infected by *B. tabaci* during the experimental trials over the two trial periods year 1 and year 2 (2015 and 2016, respectively). Consequently, homogeneous infection over the trial plots was observed.

Table 2. Disease severity and incidence rates of susceptible Rio Grande variety around the experimentation plot

Plot	Year	N	Severity (%)			Incidence (%)		
			Mean ± SE	Min (%)	Max (%)	Mean ± SE	Min (%)	Max (%)
1	**1**	**3**	65.1±1.9	62.3	67.0	96.7±2.4	93.0	100
1	**2**	**3**	63.2±2.0	60.2	65.3	96.3±2.7	92.3	100
2	**1**	**3**	66.6±1.8	65.3	69.3	97.8±3.0	93.3	100
2	**2**	**3**	68.9±1.6	66.7	71.3	97.8±1.5	95.7	100
3	**1**	**3**	69.9±1.0	68.3	71.3	98.3±1.1	97.0	100
3	**2**	**3**	68.2±2.7	64.3	72.3	98.3±1.6	96.0	100
4	**1**	**3**	68.9±2.3	66.3	72.3	99.0±1.3	97.0	100
4	**2**	**3**	64.4±1.6	62.7	66.7	97.5±1.8	94.7	100

Generalized linear model analysis.

GLM analysis was applied to quantify the degree of association between three independent variables (E/Grp, B and Yr) to a dependent variable, TYLCD incidence or severity. Significant results of composite tests were found with both incidence and severity, either in the case of models including E or Grp. Models of incidence based on E, B and Yr independent variables or Grp, B and Yr independent variables came out with p = 0.036 and 0.021, respectively. The p values were lower than 0.001 in both GLMs with severity as the dependent variable (severity by E, B and Yr and severity by Grp, B and Yr). Accordingly, significant global effect of all tested models is concluded.

Furthermore, effects of each GLM were tested using Wald χ^2 test. Independent variable Yr showed no significant effect with all GLMs, whereas independent variable B had a significant effect on severity only when associated to E and Yr (p = 0.041). The independent variable E had a significant effect on both dependent variables, incidence and severity, with a p = 0.008 and $p < 0.001$, respectively. Similarly, independent variable Grp was significantly associated with incidence and severity with a p = 0.004 and $p < 0.001$, respectively.

TYLCD resistance response based on incidence.

Incidence data set and corresponding residuals were not normally distributed either if incidence is expressed by E or by Grp. Kruskal Wallis test, a non-parametric test, was conducted to examine differences on TYLCD incidence according to E and Grp factors. Significant differences were found for E (χ^2 = 13.183,

$p = 0.040$ and df = 6) and Grp ($\chi^2 = 10.162$, $p = 0.006$ and df = 2). A pairwise comparison test showed no significant differences of the effect of E on TYLCD incidence based on adjusted significance using the Bonferroni correction ($p = 0.05$) (Figure 1), while the same comparison test showed significant differences between Grp on TYLCD incidence. Indeed, Grp1 (entries homozygous for *Ty-2*) was significantly different from Grp2 (heterozygous for *Ty-2*) ($p = 0.017$) and Grp3 (homozygous for susceptible allele) ($p = 0.031$) (Figure 2). Grp2 and Grp3 were not significantly different. TYLCD incidences of Grp1, Grp2 and Grp3 were 96.72%, 99.54% and 99.07%, respectively. *Ty-2* gene seems to have no effect on TYLCD incidence when heterozygous.

Tomato entries heterozygous for *Ty-2* behaved similarly as susceptible tomato entries lacking *Ty-2*.

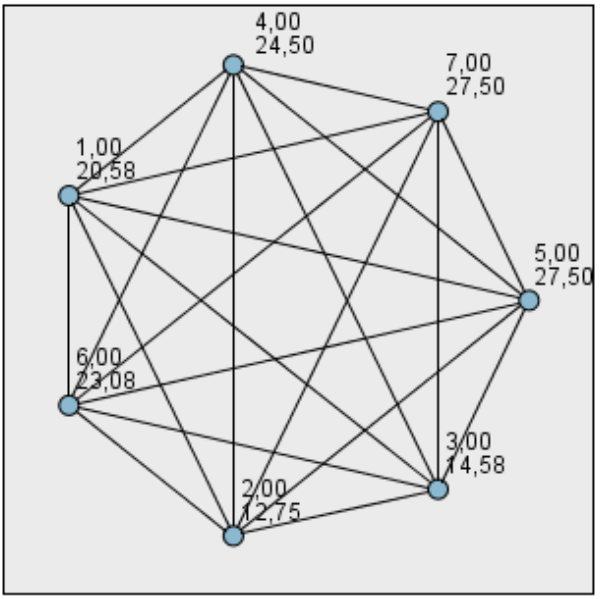

Fig. 1. Pairwise comparisons of E mean effect on TYLCD incidence by Bonferroni test using Bonferroni significance correction. Each node shows the sample average rank of E.

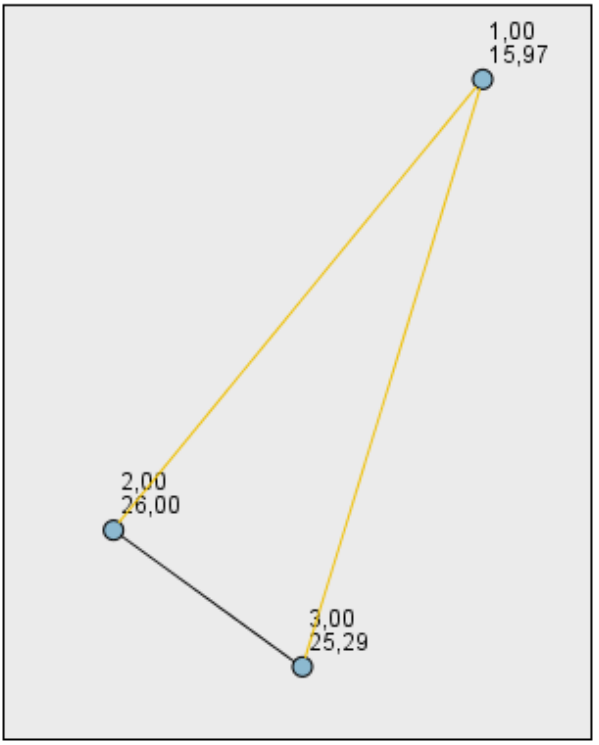

Fig. 2. Pairwise comparisons of Grp mean effect on TYLCD incidence by Bonferroni test using Bonferroni significance correction. Each node shows the sample average rank of Grp.

TYLCD resistance response based on severity.

GLM analysis showed that both E and B independent variables were significantly associated to TYLCD severity. Considering variable B, severity data were normally distributed, and heterogeneous variance was shown based on Levene's test. Subsequently, a post hoc multi-comparison test was required. Data analysis using Dunnett's test showed no significant differences between B1, B2 and B3 (blocks 1, 2, 3, respectively). Hence, no significant effect of individual levels of B on severity was found.

Severity data set and corresponding residuals were shown to be normally distributed either when severity was expressed by E or by Grp. Variance homogeneity was proven using Levene's test within severity by Grp data (p = 0.070), so multi-comparison was carried out using Tukey Kramer's test, whereas heterogeneous variance was shown within severity by E data (p = 0.211). Then, multi-comparison Dunnett's test was performed.

Mean's multi-comparison tests showed significant differences between E and Grp on severity levels (Table 3). In fact, TYLCD severity levels of *Ty-2* homozygous lines E1, E2 and E3 with severity rate of 50.88%, 52.49 % and 50.76 %, respectively, were significantly different from TYLCD severity levels of susceptible tomato lines (E6 and E7), with severity rate of 73.89% (E6) and 68.64%

(E7). On the other hand, heterozygous tomato hybrid E5 behaved similarly as a susceptible line based on TYLCD severity. Indeed, E5 was significantly different from homozygous tomato lines with higher TYLCD severity level. In addition, it showed no significant differences compared to susceptible tomato lines (Table 3). However, heterozygous *Ty-2* tomato line E4 showed no significant difference of TYLCD severity compared to all other tomato entries: E4 seemed to have an intermediate behavior, since it showed a severity level that was not significantly different neither from homozygous *Ty-2* entries nor from susceptible tomato controls.

TYLCD severity data analyses based on Grp of tomato lines showed significant differences of Grp1 to Grp2 and Grp3 (Table 3). Grp1 included tomato lines homozygous for *Ty-2* resistance gene, whereas Grp2 comprised tomato lines heterozygous for *Ty-2* resistance gene, and Grp3 had no resistance gene. Definitely, effectiveness of *Ty-2*, as a resistance gene to TYLCD, was only demonstrated with tomato lines harboring homozygous *Ty-2* gene.

Table 3. TYLCD severity as expressed among tomato entries (E1-E7) and *Ty-2* gene groups (Grp1-Grp3)

Entry/Group of Entries	N	Mean ± Std Error	Minimum (%)	Maximum (%)
Severity by entry (Dunnett's test)				
E1	6	50.88 ± 1.91a	44	55
E2	6	52.49 ± 2.56a	43	60
E3	6	50.76 ± 1.69a	48	59
E4	6	66.01 ± 3.27ab	56	79
E5	6	71.13 ± 1.68b	66	76
E6	6	73.89 ± 3.83b	60	84
E7	6	68.64 ± 2.76b	60	77
Severity by Group of entries (Tukey Kramer's test)				
Grp1	18	51.38 ± 1.15a	43	60
Grp2	12	68.57 ± 1.91b	56	79
Grp3	12	71.27 ± 2.39b	60	84

In summary, tomato entries homozygous for *Ty-2* (Grp1) were significantly different from susceptible tomato entries (Grp3) for TYLCD severity. The group of heterozygous tomato (Grp2) behaved similarly as the group of susceptible tomato entries (Grp3). These results suggest that *Ty-2* should be homozygous to achieve better TYLCD resistance, especially under high whitefly and inoculum pressure.

DISCUSSION

In Tunisia, TYLCD is one of the major threats particularly occurring during dry season in open field tomato growing areas due to high whitefly population density. Both TYLCV-Is and TYLCSV do exist in Tunisia all over tomato production areas. Mnari-Hattab et al. (2014) reported the occurrence of both TYLCV species. Many TYLCD resistant tomato hybrid varieties have been developed harboring

one or more *Ty* resistance genes. In Tunisia, all commercial tomato varieties, even those commonly known as highly tolerant to TYLCV (*e.g.* Saada, Savera, Tisey, Berna and Kismat varieties), still show TYLCD symptoms under high inoculum pressure and/or early infection.

In a previous work, we carried out a tomato field trial aiming to screen TYLCD resistance resources (Elbaz et al. 2016). The screening was applied to a set of tomato entries homozygous for one or several *Ty* genes, including*Ty-1/Ty-3*, *Ty-2* and *ty-5*. The work demonstrated that tomato lines with *Ty-1*/*Ty-3* and *Ty-2* genes offered the highest levels of resistance to begomoviruses, whereas a line homozygous for *Ty-2* alone displayed relatively low levels of TYLCD resistance. Similarly, the combination of *Ty-2* and *ty-5* in homozygous condition showed better resistance than *ty-5* alone. In similar work performed in Oman, Al-Shihi et al. (2018) found that tomato lines homozygous for *ty-5* performed the best, possibly due to the presence of a different begomovirus species than those occurring in Tunisia.

Ty-2 resistance gene was shown to have an additive effect of tomato resistance against begomoviruses in Tunisia (Elbaz et al. 2016). This additive effect of *Ty-2* gene was confirmed by Tabein et al.(2017), when pyramiding *Ty-1*/*Ty-3* and *Ty-2* in tomato hybrids. Accordingly, the *Ty-2* gene, a dominant resistance gene, was shown to act with a minor additive effect against this disease. Moreover, the impact of *Ty-2* gene at heterozygous status has not been tested in tomato yet.

As a case study, the present work aimed to evaluate TYLCD resistance of tomato entries heterozygous for *Ty-2* gene compared to tomato entries homozygous for *Ty-2*. The study also included susceptible tomato entries lacking *Ty* genes.

All tomato entries showed symptoms caused by TYLCV infection. TYLCD incidence of tomato entries in this study varied from 95.95% to 100%. These tomato entries showed severity levels higher than 68% over the two year trials. These registered rates of TYLCD incidence and severity indicated high begomovirus pressure. Moreover, tomato entries homozygous for *Ty-2* gene (E1, E2 and E3) were significantly different from all other entries except E4 tomato line. E4, heterozygous for *Ty-2*, had an intermediate behavior between resistant (E1, E2 and E3) and susceptible (E6 and E7) entries. The occurrence of the intermediate genotype such as E4 line indicated that resistance level response might be explained not only based on genetic effect but also by other factors like plant vigor (Kasrawi et al. 1988), weather conditions and whiteflies abundance (Moriones and Navas-Castillo 2000). In addition, the mean of tomato entries of Grp1 with homozygous *Ty-2* gene, either line entries or hybrid entry, showed the lowest levels of TYLCD severity compared to Grp2 (hybrids heterozygous for *Ty-2* gene) and Grp3 (susceptible lines with no *Ty* gene).

Our work showed that *Ty-2* was more efficient when homozygous. Consequently, resistance levels of tomato hybrids heterozygous for *Ty-2* are questionable especially in case of severe epidemic. To address this issue, we suggest that TYLCD resistant tomato varieties should be homozygous for *Ty-2*. This implies that the two parental lines involved in a genetic cross aiming to produce a tomato TYLCD resistant F_1 hybrid, are both homozygous for *Ty-2*. Similar results were reported by Vijeth et al. (2018). They showed that when challenged to a high disease pressure by using controlled inoculation by whiteflies, all tomato TYLCD resistant lines crossed with a susceptible tomato parent produced

susceptible hybrids except for two crosses (among a total of seven hybrids) where the hybrids were classified as tolerant. On the other hand, most crosses involving two resistant lines produced resistant hybrids. Nevertheless, conclusion from the present work needs to be further verified with other *Ty* genes since this study involved only *Ty-2*. In particular, *Ty-1/Ty-3* gene needs to be considered during future investigation in the context of Tunisian epidemiological TYLCD. Based on our results and those of Vijeth et al. (2018), we expect that the highest resistance against TYLCD in Tunisia would be obtained from tomato hybrids homozygous for *Ty1*/*Ty-3* and *Ty-*

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RESUME

Elbaz M., Timoumi M. et Hanson P. 2022. Comportement de nouvelles entrées et hybrides de tomate développés portant le gène *Ty-2*. Tunisian Journal of Plant Protection 17 (1): 1-14.

La maladie de la feuille jaune en cuillère de la tomate (Tomato yellow leaf curl disease, TYLCD) est une menace sérieuse pour la production de tomate dans le monde entier. Dans le bassin méditerranéen, l'incidence et la sévérité de la maladie sont plus élevées pendant la saison sèche qui favorise les populations élevées de la mouche blanche (*Bemisia tabaci*). L'efficacité de la résistance au virus de la maladie de la feuille jaune en cuillère de la tomate (*Tomato yellow leaf curl virus*, TYLCV) dépend à la fois de la résistance de la plante hôte, ici la tomate, et des espèces présentes du complexe TYLCV. Jusqu'à présent, six gènes différents Ty de résistance chez la tomate ont été identifiés. En Tunisie, deux principales espèces du complexe TYLCV, *Tomato yellow leaf curl virus-Israel* (TYLCV-Is) et *Tomato yellow leaf curl Sardinia virus* (TYLCSV), ont été identifiées. Le présent travail vise à évaluer les entrées de tomate hétérozygotes pour le gène *Ty-2* afin d'aider à prédire les performances des hybrides. Deux entrées de tomates homozygotes pour le gène de résistance *Ty-2* au TYLCD, un hybride de tomate homozygote pour *Ty-2* et deux hybrides hétérozygotes ont été inclus, en plus de deux entrées de tomates sensibles. La réponse de résistance au TYLCD a été enregistrée en fonction de l'incidence et des niveaux de sévérité de la maladie. L'analyse des données a été réalisée selon la présence/absence du gène *Ty-2* et en tenant compte de l'homozygotie et de l'hétérozygotie de *Ty-2*. L'analyse en modèle linéaire généralisé a été appliquée pour vérifier la signification des effets des facteurs individuels (c'est-à-dire l'effet des entrées de tomate ou des groupes d'entrées de tomate en fonction de la présence ou de l'absence du gène *Ty-2* homozygote/hétérozygote, de l'unité expérimentale dans l'essai sur le terrain et de l'année de l'essai) sur les variables dépendantes (incidence et sévérité de la maladie). D'autres tests de comparaisons multiples ont révélé un effet significatif des génotypes de tomate ayant le *Ty-2* à l'état homozygote sur l'incidence et les niveaux de sévérité de la maladie TYLCD. Les résultats ont été discutés avec une attention particulière à la pertinence des variétés de tomates hybrides hétérozygotes.

Mots clés: Hétérozygote, gènes de résistance, tomate, *Tomato yellow leaf curl virus* (TYLCV), Tunisie

الملخص

الباز، منيرة ومنعم تيمومي وبيتر هانسون. 2022. سلوك إدخالات جديدة وهائجن من الطماطم مطوَّرة حاملة للجين 2-yT.
Tunisian Journal of Plant Protection 17 (1): 1-14.

يعد مرض تجعد أوراق الطماطم الصفراء (TYLCD) تهديدا خطيرا لإنتاج الطماطم في جميع أنحاء العالم. في حوض البحر الأبيض المتوسط تكون معدلات الإصابة بالأمراض وشدتها أعلى في موسم الجفاف الذي يسمح بوجود أعداد كبيرة من الذبابة البيضاء (*Bemisia tabaci*). تعتمد فعالية مقاومة فيروس تجعد أوراق الطماطم الصفراء (TYLCV) على كل من المقاومة الجينية لصنف الطماطم وأنواع الفيروس الموجودة. حتى الآن تم تحديد ستة جينات Ty مختلفة لمقاومة هذا المرض. في تونس تم التعرف على نوعيْن رئيسييْن من نوع هذا الفيروس وهما فيروس تجعد الأوراق الصفراء للطماطم-إسرائيل (TYLCV-Is) وفيروس سردينيا لتجعد أوراق الطماطم الصفراء (VSTYLC). يهدف هذا العمل إلى تقييم إدخالات مختلفة من الطماطم حاملة للجين في صيغته المتماثلة أو الهجينة غير المتماثلة للجين للمساعدة على التنبأ بالأداء الجيني في مقاومة المرض. تم تضمين اثنين من إدخالات الطماطم ذات الصيغة الجينية المتماثلة للجين Ty-2 مع هجين طماطم واحد متماثل الصيغة للجين Ty-2 وهجينيْن اثنين غير متماثلي الصيغة الجينية لجين المقاومة Ty-2، بالإضافة إلى صنفيْن من الطماطم الخالية من أي مقاومة للمرض. تم تسجيل مدى مقاومة المرض بناء على نسبة حدوث المرض ومدى شدته. وقد تم إجراء تحليل البيانات باعتبار تواجد أو عدم تواجد الجين Ty-2 مع مراعاة صيغة التماثل من عدمها التي يتواجد عليها هذا جين. التحليل الإحصائي للمعطيات مكن من التحقق من مدى أهمية تأثيرات العوامل الفردية (أي تأثير إدخالات الطماطم أو مجموعات إدخالات الطماطم المكونة بناء على وجود أو عدم وجود الجين Ty-2 باعتبار صيغة التماثل الموجود عليها هذا الجين، ووحدة التجربة الميدانية وسنة التجربة) على المتغيرات صلب الدراسة (نسبة المرض وشدته). أعطت اختبارات المقارنة المتعددة دليلا على التأثير المعنوي لجين المقاومة المتماثل yT-2 على مستوى نسبة مرض TYLCD وشدته صلب مختلف إدخالات الطماطم. تمت مناقشة النتائج مع إيلاء اهتمام خاص لأهمية أصناف الطماطم الهجينة غير المتماثلة.

كلمات مفتاحية: اللاتماثل الجيني، تونس، جينات المقاومة، طماطم، فيروس تجعد أوراق الطماطم الصفراء (TYLCV)

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Assessing the Insecticidal Impact of Rosemary Essential Oils on the Saw-toothed Grain Beetle *Oryzeaphilus surinamensis*

Abir Soltani, Soumaya Haouel-Hamdi, **Insaf Ajmi**, **Maha Ben Abada, Tasnim Djebbi, Hadhami Chargui, Imen Mathlouthi, Amina Laabidi,** Laboratoire de Biotechnologie Appliquée à l'Agriculture (LR11INRAT06), Institut National de la Recherche Agronomique de Tunisie, Université de Carthage, Rue Hedi Karray, 1004 El Menzah, Tunisia, **Hela Mahmoudi,** Département de Biologie, Institut Supérieur de Biotechnologie de Béja, Université de Jendouba, Beja, Tunisia, **Jouda Mediouni-Ben Jemâa** Laboratoire de Biotechnologie Appliquée à l'Agriculture (LR11INRAT06), Institut National de la Recherche Agronomique de Tunisie, Université de Carthage, Rue Hedi Karray, 1004 El Menzah, Tunisia

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ABSTRACT

Soltani, A., Haouel-Hamdi, S., Ajmi, I., Ben Abada, M., Djebbi, T., Chargui, H., Mathlouthi, I., Laabidi, A., Mahmoudi, H., and Mediouni-Ben Jemâa, J. 2022. Assessing the insecticidal impact of rosemary essential oils on the saw-toothed grain beetle *Oryzeaphilus surinamensis*. Tunisian Journal of Plant Protection 17 (1): 15-28.

This work studied the fumigant toxicity of free and encapsulated rosemary (*Rosmarinus officinalis*) essential oils against adults of the saw-toothed grain beetle (*Oryzeaphilus surinamensis*) for three storage periods: 30, 45 and 60 days. Chitosan was used as encapsulation matrix. GC/MS analysis results showed that camphor and 1,8-cineole were the major components with respectively 18.04% and 39.67%. Mortality rates caused by the essential oils at 300 µL/L air after 10 days of storage were about 85.48%. The median lethal concentration (LC_{50}) was 124.80 µL/ L air. Encapsulation efficacy was 25.8% and loading capacity was 1.9%. Encapsulated essential oils achieved an efficacy of 82%, 100% and 100% respectively after 30, 45 and 60 days of storage. Reference treatment with Phosphine revealed a toxicity of 100%, 96% and 71% after 30, 45 and 60 days of storage respectively. Results showed that encapsulated essential oils caused a very slight modification on semolina properties. Protein contents decreased at the end of the storage period less than 1% (from 13.61% after 30 days to 12.91% after 60 days of storage). Encapsulated essential oils might be considered as an alternative fumigant control way for semolina without deterioration of its quality during storage.

Keywords: Chitosan, encapsulation, fumigant toxicity, *Oryzeaphilus surinamensis*, *Rosmarinus officinalis*, semolina quality

Durum wheat (*Triticum turgidum*) is the basic component of the daily human food worldwide (Durante et al. 2012; Shewry and Hey 2015). It provides carbohydrates, food calories and nutriments such as vitamin E as tocopherols and tocotrienols (Breiman and Graur 1995; De Santis et al. 2021). Even though, Bushuk (1997) and Pompa et al. (2021) reported that durum wheat constitutes only 5% to 8% of world production, it is considered as one of the

Corresponding author: Abir Soltani
Email: soltany.aby@gmail.com

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most economically important crop. The economic and dietary importance of this crop, forced the researchers as well as producers to focus on improving its production in the field and its conservation during storage (Saari and Prescott 1985). Durum wheat productivity is profoundly affected by biotic and abiotic stresses (Xynias et al. 2020). In this context, insects are considered as the most damaging pests of stored cereals (Alonso-Amelot and Avila-Núñez 2011). Ahmed (1983) and Hinton (1945) revealed that stored products may be attacked by more than 600 of beetles' species. Various treatments and technologies have been adopted in order to overcome the problems especially stored insect pests (Owusu 2000).

Rosemary (*Rosmarinus officinalis*) is a botanical species known by its a insecticidal potential against insects during storage, due to its major component such as 1,8-cineole, α-pinene, camphor, camphene (Katerinopoulos et al. 2005; Krzyżowski et al. 2020; Silvestre et al. 2019). Due to its properties such as volatility, absorption, bioavailability and tolerance to storage conditions such as temperature and oxygen, essential oils of rosemary are used as an alternative way of pest control (Turasan et al. 2015; Yadav et al. 2017)). Alternative methods are well needed such as encapsulation by using different matrix, which is considered as one of the most effective technique that preserve essential oils (Raza et al. 2020). Previous works demonstrated that chitosan matrix is a good approach to preserve essential oils properties and improve its insecticidal toxicity during storage (Salar et al. 2019).

The objective of this work was (i) to evaluate the fumigant toxicity of rosemary essential oils against one important stored durum wheat insect pest, the saw-toothed grain beetle, *Oryzeaphilus surrinamensis* (Silvanidae, Coleoptera), during different storage periods 30, 45 and 60 days, and (ii) to determine the insecticidal potential of encapsulated rosemary essential oils into chitosan matrix. Consequently, these objectives show the way of another objective, which aspire to understand the effect of those free and encapsulated essential oils of rosemary on some physical and chemical properties of the treated semolina (humidity, gluten, protein, and lipid) during the storage periods.

MATERIALS AND METHODS

Plant material and essential oil extraction.

Aerial parts of rosemary leaves were collected from the region of Korbos (846 m 36° 48'54" N 10° 34' 14" E) situated in North Tunisia during February and March 2021. Leaves were dried at room temperature for 10 days at darkness before distillation (Soltani et al. 2020). The extraction was made by hydrodistillation using Clevenger-type apparatus during 4 hours.

Gas Chromatography-Mass Spectrometry (GC-MS).

Quantitative and qualitative analysis of the essential oils was performed using an Agilent-technologies CPG-SM as described in previous research reported by Soltani et al. (2020).

Insect rearing.

Adults were collected from the rearing colony carried out in laboratory of the National Institute of Agronomic Research of Tunisia (INRAT). Rearing of *O. surinamensis* was carried out on semolina in a temperature of 25± 1°C, a relative humidity of 65 ± 5% and a photoperiod of 12 h Light/12 h Dark (Dal Bello et al. 2000; Soltani et al. 2020). The boxes were covered with muslin cloth. After 14 days, adults were removed by sieving.

Preliminary bioassay.

The preliminary fumigant test was conducted by using three doses 26.3, 52.5 and 105 µL; in order to evaluate the toxicity and to determine the median lethal concentration (LC_{50}) after 10 days of storage with occupation of 100%. A density of 1 adult newly emerged were placed per 10 g into 1000 mL glass bottles contained 420 g of semolina during 10 days of storage. Essential oils were deposited on filter paper disks of 7.5 cm² (Whatman N° 1) using a micropipette. These doses were converted into concentrations, namely 75, 150 and 300 µL/L air. Corrected mortality percentages were calculated by using Abbott's (1925) formula. Additionally, the lethal concentration CL_{50} and CL_{95} were evaluated using the Probit Analysis (Finney 1971).

Encapsulation.

The formulation was performed according to the protocol of Keita (2010) with some modifications. Rosemary essential oils were diluted in acetone at concentration (10%). The obtained solution was mixed with gum Arabic and chitosan. Two ratios of chitosan: gum Arabic: essential oils (w/w/w) were used namely 0.5:0.5:0.5 and 0.5:0.5:0.25. After 5 min of manual stirring, the mixture was placed in water bath at 30 °C until complete evaporation of acetone. Similarly, a suspension of the powder (gum Arabic and chitosan) was mixed only with acetone in order to serve as a control. The flavored powders were stored in brown bottles and tightly closed using parafilm and placed at 4 °C.

Encapsulation efficiency (EE %) and loading capacity (LC %).

Encapsulation Efficiency (EE %) and Loading Capacity (LC %) were calculated according to the methods and formula described by Keawchaoon and Yoksan (2011) with some modifications. The amount of essential oils charged was determined from a calibration curve prepared with rosemary essential oils in 95% ethyl alcohol (Abs = 006 [conc] + 0.220; R^2 = 0.577). Each sample was measured three times. These parameters were calculated according to the following formula:

$$EE\ (\%) = \frac{mass\ of\ loaded\ oils}{initial\ mass\ of\ oils} \times 100$$

and

$$LC\ (\%) = \frac{mass\ of\ charged\ oils}{mass\ of\ sample} \times 100$$

FTIR characterization.

Fourier-Transform InfraRed (FTIR) spectroscopy was used to determine the information about functional groups or chemical band in the samples. The FTIR spectra of the microcapsules were achieved by using a Fourier-Transform infrared spectro-photometer JASCO (FT/IR4700 type A). Dried samples of chitosan: gum Arabic and chitosan: gum Arabic: essential oils at two ratio (1:1:0.2; 1:1:0.5) were analyzed. The analysis was accomplished as cited by Chaib et al. (2021). Each spectrum was recorded in a frequency range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}.

Fumigation tests for free and encapsulated essential oils.

A number of 42 newly emerged adults of *O. surinamensis* were placed in 1000 mL glass bottles containing 420 g of semolina. Regarding the free essential oils, the bottle was secured as described in previous section, the used concentration was the same used in the preliminary bioassay. The insecticidal activity of the formulation chitosan: gum Arabic: essential oils was determined according to the protocol described by Soltani et al. (2022). A mass of 1.75 g of capsule was

placed in a thin tissue that was glued to the suburb of the bottle and closed hermetically. Tests were replicated three times for each treatment. Untreated boxes were used as a control. Treated and untreated boxes were placed under the same conditions. Mortality assessment was carried out after 30, 45 and 60 days of storage. Phosphine (PHOSTOXIN®) was a chemical treatment that used as a reference at doses of 3 mg/L air.

The inhibition emergence percentage was determined using the method from Tapondjou et al. (2003) described by the following formula:

Inhibition (%) = $(Cn - Tn)/Cn*100$

where Cn = number of adults emerged in untreated boxes (control) and Tn = number of adults emerged in the boxes treated with the essential oils.

Proximate composition.

The impact of free and encapsulated essential oils as well as chemical treatment (Phosphine) on semolina properties such as humidity, gluten, protein, and lipid were evaluated according to the standard methods of analysis (AOAC 1984).

Protein content.

Protein content was quantified through the determination of the total nitrogen determined according to Kjeldahl method (1883). This method consists of three phases: mineralization, distillation and titration. Around 1 g of powder dates was hydrolyzed with 15 mL concentrated sulfuric acid (H_2SO_4) containing two copper catalyst tablets in a heat block at 420 °C for 2 h. Behind cooling, we added H_2O to the hydrolysate thereafter, neutralization and titration were done. The total nitrogen was calculated according to the formula:

$$Total\ nitrogen\ (\%) = \frac{VHCl * 0.0875}{WRow\ material}$$

where V_{HCL} = Volume of HCl and $W_{Row\ material}$: Weight of row material.

The protein content was calculated by multiplying the total nitrogen rate N (%) by the coefficient 6.25 (Thabet et al. 2007):

Protein content = Total nitrogen content × 6.25.

Lipid content.

Extraction of free lipids from durum wheat semolina was carried out according to the method described by Ounane et al. (2006) with some modifications. The extraction was performed into Soxhlet extraction apparatus by using ether. In fact, 20 g of substrate were introduced into cellulose cartridge. A volume of 60 ml of ether was placed into bucket that was stirred at 110°C until exhaustion of the lipids entrained by the organic solvent. The semolina was dried in a vacuum oven at 60 °C for 24 h to remove traces of solvent. Then, the buckets were weighed again. The lipid content was determined by the difference in mass. All measurements were repeated thrice.

Statistical analyses.

Data were analyzed using SPSS statistical software version "20". For each parameter, mortality percentage and semolina property data were analyzed using two-way ANOVA.

RESULTS

The chromatographic analyses (GC-MS) of rosemary essential oils indicated the presence of 23 components with a percentage of about 99.3%. The identified components were divided into three chemical classes (Table 1). The major component (> 5 %) were identified by matching their spectra with accessible ones according to the database of bibliography. The major class was represented by oxygenated monoterpenes

(58.87%), followed by sesquiterpenes hydrocarbons (21.36 %) and oxygenated monoterpenes (12.27 %). Rosemary leaves were characterized by the highest fraction of 1,8-cineole with (39.67 %), followed by camphor (18.04 %) and bornneol (10.5 %). The obtained retention indexes (RI^a /RI^b) are summarized in Table 1.

Table 1. Chemical compounds and identified constituents (%) of essential oils extracted from rosemary

N°	Component	Fraction (%)	RI^a /RI^b
	Monoterpene hydrocarbons	**12.27**	
1	α-Pinene	6.33	937/913
2	α-Terpinene	0.39	1021/-
3	β-pinene	2.84	982/965
4	γ-Terpinene	0.7	1107/1041
5	ʋ-Terpinolene	0.62	1092/-
6	β-Myrcene	0.93	992/991
7	Bornylacetane	2.52	
	Oxygenated Monoterpenes	**58.87**	
8	O-cymene	1.33	1028/-
9	1,8-Cineole	39.67	**1036**/1041
10	Borneol	10.51	1185/1164
11	Carvacrol	0.86	1433/-
12	Linalool	1.48	1151/1175
13	Terpinene-4-ol	1.31	1200/1164
14	ʋ-terpineol	4.17	1208/-
	Sesquiterpenes hydrocarbons	**21.36**	
15	α-Caryophyllene	0.22	1459/-
16	β-Caryophyllene	3.1	1429/1421
17	Camphor	18.04	1175/1119
	Other compounds	**6.88**	
18	Heptane	2.58	
19	Cycloheptasiloxane	0.33	
20	1,2-Phenylene	1.02	
21	Tétracyclohexane	0.25	
22	α-Phellandrène	0.18	
	Minor compounds	**1.83**	
	Major compounds	**97.5**	
	Total identified compounds (%)	**99.33**	

RI^a: Retention Index determined according to the homologous series of n-alkanes (C9-C24); RI^b; Literature Retention Index.

Preliminary Bioassay.

Exposed *O. surrinamensis* adults to free essential oils during 10 days of storage showed different mortality rates at different concentrations of free essential oils (Fig 1). Results showed that mortality rate increased with increasing concentration with significant differences between concentrations ($df = 2$, $f = 896.12$, $p \leq 0.01$).

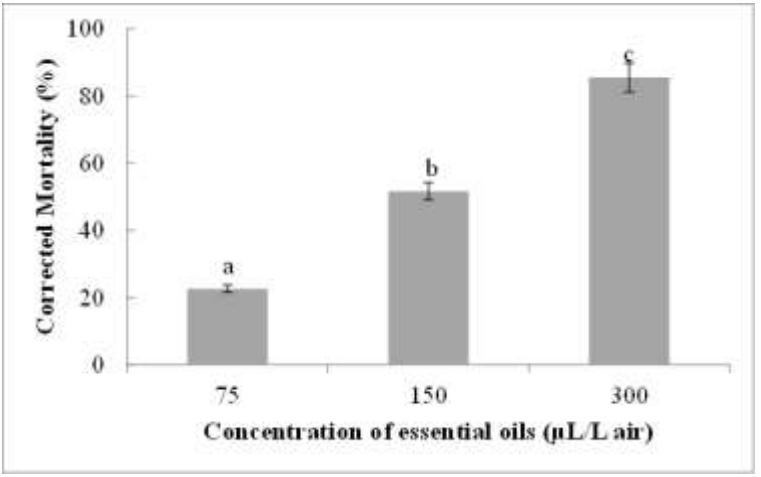

Fig. 1. Corrected mortality rates of *Oryzeaphilus surinamensis* during 10 days of storage treated by rosemary essential oils. Comparison was made between concentrations. The values followed by different letters are significantly different ($p \leq 0.05$).

Regarding lethal concentrations (LC_{50} and LC_{95}) of rosemary essential oils after 10 days of storage, obtained results are shown in Table 2. The LC_{50} and LC_{95} values of the essential oils were respectively 124.8 and 231.3 µL/L air. The median lethal concentration was used for the fumigation and encapsulation.

Table 2. LC_{50} and LC_{95} values (µL/L air) of rosemary essential oils from the North West of Tunisia after 10 days of storage used against *Oryzeaphilus surrinamensis*.

Insect	Slope ± ES*	χ^2	LC_{50}**(µL/L air)	LC_{95}***(µL/L air)
Oryzaephilus surinamensis	0.015±0.002	5.85	124.80	231.30

Data tested by χ^2-test for homogeneity of 1:1 ratio; * ES = Standard error, **LC_{50} = Median lethal concentration; *** LC_{95} =Lethal concentration at 95% of the insect population.

Encapsulation efficiency (EE %) and loading capacity (LC %) for two ratio chitosan: gum Arabic: Essential oils with (w:w:w; 0.5:0.5:0.5 and 0.5:0.5:0.25) are shown in Table 3. Highest values of EE% and LC% were about 25.8 and 1.49% recorded for Ratio 1 (0.5:0.5:0.5).

Table 3. Encapsulation efficiency (EE %) and Loaded capacity (LC %) of rosemary essential oils (EO) loaded in chitosan matrix determined by UV-Vis spectrophotometry

Chitosan : EO (W/W)	EE (%)	LC (%)
R1=0.5:0.5:0.5	25.8	1.49
R2=0.5:0.5:0.25	12.9	0.6

Fourier Tranfsorm Infrared Spectroscopy (FTIR) characterization.

Fig 2 reported the FTIR spectra of chitosan: gum Arabic (50%) and rosemary essential oil loaded into chitosan and gum Arabic matrix with ratio 0.5:0.5:0.5 and 0.5:0.5:0.25.

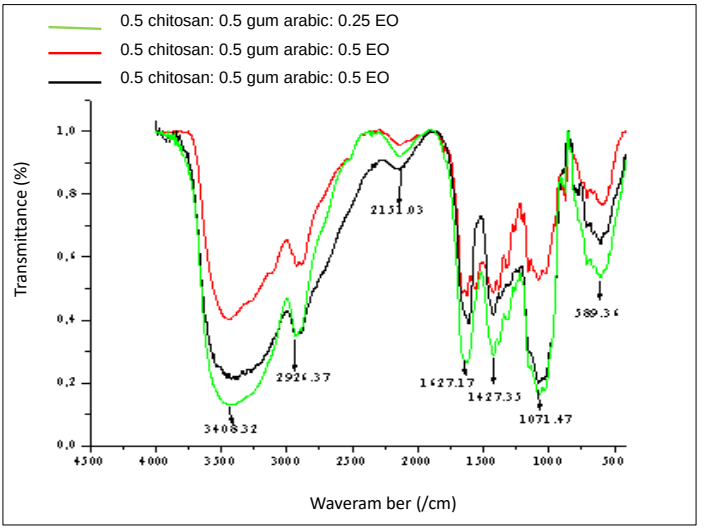

Fig. 2. Spectro FTIR of chitosan: gum Arabic for one ratio 0.5: 0.5 and chitosan: gum Arabic: Essential oils for two ratios 1 :0.5 et 1 :0.2.

In general, chitosan powder revealed peaks characteristics. Results showed peaks at 35779 (-OH and NH_2), at 2947(-CH), 1631 (Amide I), 1438 (C-O-C) and finally a characteristic peak by the establishment of a complex through an interaction between NH_3^+ groups and chitosan: gum Arabic powder at 389.76 and 3474 cm^{-1} respectively for the two ratios 0.5:0.5:0.5 and 0.5:0.5:0.25. Besides, new peaks appeared at 1056.59, 1353.98, 1025.3 and 935.1 cm^{-1}. In fact, obtained results confirm those reported by Hosseini et al. (2013) and Yoksan et al. (2010). These authors revealed that appearance of new peaks of C-O-C and the appearance of Amide II are due to the interaction between the NH_3^+ groups of chitosan and the chemical group within the formulation.

Fumigant toxicity and comparison between free and encapsulated essential oil effect against *O. surinamensis.*

Corrected mortality rates obtained for *O. surinamensis* showed different results according to adults during three storage periods (30, 45 and 60 days under chemical), free essential oils, chitosan: essential oils treatments.

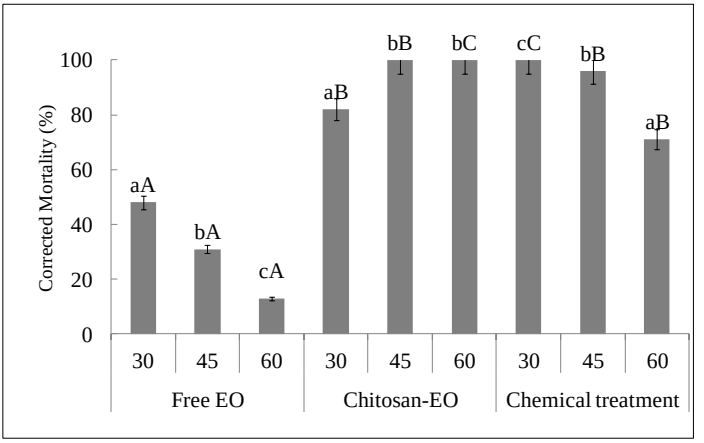

Fig. 3. Mortality percentages of *Oryzeaphilus surrinamensis* caused by free and encapsulated essential oils during storage periods of 30, 45 and 60 days. Bars represent means ± standard error of three replicates. Different letters a, b and c indicated significant differences at ($p < 0.001$) for storage periods; A, B and C indicated significant differences at ($p < 0.01$) for treatment according Duncan test).

O. surinamensis varied in their susceptibility to treatments and storage periods. Mortality percentage for the control was 0%, 2.31% and 4.17% after 30, 45 and 60 days of storage. Corrected mortality was high for chitosan: essential oils and chemical treatments under the same conditions. However, for chemical treatment, there was a decrease of mortality percentages from 100% after 30 days to 61.2% after 60 days of storage. Likewise, for free essential oils the mortality percentages were 48% and 13% after 30 and 60 days of storage. Statistical analyses revealed high significant differences between treatments against *O. surinamensis* during different storage periods (df = 2, f = 28357.28, $p \leq 0.001$). Equally, high significant differences on mortality percentages were observed between storage periods for free essential oil treatment (df = 3, f = 1654.06, $p \leq 0.001$). For encapsulated essential oils and chemical treatment, differences were observed (at $p \leq 0.05$). At the same time, the results obtained for inhibition of emergence was zero after 30, 45 and 60 days of exposure for encapsulated essential oils. On the other hand, emergence inhibition rate was 0% after 30 days and reached 5.36% and 8.12% after 45 and 60 days of storage for chemical treatment, respectively. However, free oils present an inhibition rate of 21.54%, 36.1% and 43.12% after 30, 45 and 60 days of storage, respectively.

Impact of infestation by *O. surinamensis* and different treatments on semolina quality.

Impact of free and encapsulated essential oils as well as phosphine treatment on humidity, Gluten, Protein and Lipid are reported in Table 4.

Table 4. Properties of semolina treated with free and encapsulated essential oils (EO) compared to phosphine.

Treatment	Storage periods	Humidity (%)	Gluten (%)	Protein (%)	Lipid (%)
Free EO	30	11.7±0.57 a	25.2±1.0 c	13.49±0.01 c	1.75±0.5 b
	45	12.1±0.57 b	24.2±1.51 b	13.0±0.34 b	0.92±0.35 a
	60	12.8±0.57 b	22.7±1.06 a	12.7±0.71a	0.82±0.52 a
Encapsulated EO	30	10.02±1.15 a	26.2±1.53 c	13.61±0.5 b	1.81±0.57 b
	45	11.98±1.15 b	24.1±1.75 b	13.26±0.5 b	0.98±0.57 a
	60	12.03±1.15 c	23.0±1.52 a	12.91±0.57 a	0.91±0.6 a
Chemical treatment (Phosphine)	30	10.91±0.57 a	25.1±1.0 c	13.14±0.01 b	1.71±0.5 c
	45	11.2±0.57 b	24.1±1.0 b	12.89±0.1 a	1.01±0.5b
	60	12.5±1.15 c	22.9±1.2 a	12.64±0.01 a	0.89±0.5 a
Infested and not treated	30	13.5±0 a	23.8±1.53 c	11.1±0.01 b	1.15±0.01 b
	45	13.7±0.57 b	22.4±1.2 b	11.03±0.01 b	0.72±0.01 a
	60	14.02±0 c	21.1±1.50 a	10.8±0.01 a	0.7±0.01 a
Control	30	12.01±0.6 b	26.8±2.1 b	13.4±0.01a	1.9±0.57 a
	45	11.08±0.57 a	23.1±2.18 a	13.3±0.01 a	1.7±0.6 a
	60	11.03±0.57 a	23.7±1.9 a	13.1±0.57 a	1.05±0.5a7

For each treatment, values followed by different letters are significantly different at $p < 0.5$.

Chemical compositions of untreated and treated semolina with free and encapsulated essential oils, and chemical treatments during different storage periods are reported in Table 4. Results revealed that storage periods and treatments have an effect on the semolina quality.

The humidity percentage of semolina increased during storage periods. In fact, it was about 11.7 % for semolina treated with essential oils, 10.02 % for semolina treated with chitosan: essential oils, 10.91 % for semolina treated by phosphine, 13.7 % for infested semolina and 12.01% for the control, after 30 days. And the increase reaches 12.8, 12.03, 12.5, 14.02, 11.03 % after 60 storage days for semolina treated with essential oils, chitosan: essential oils, phosphine, infested semolina and the control, respectively. Besides, significant differences at $p \leq 0.05$ in humidity were recorded between treatments.

The gluten content of semolina decreased during storage from 25.2, 26.2, 23.8 and 26.8 after 30 days to 22.7, 23.0, 22.9, 21.1 and 23.7 after 60 days of storage for free essential oils, chitosan: essential oils, phosphine, infested semolina and control, respectively. Significant differences have also been observed between treatments (df = 3, f = 2.92, $p \leq 0.03$). In addition, the highest gluten amount was observed for semolina treated with encapsulated essential oils after 30 days of storage (Table 4), while lowest value was recorded for infested semolina after 60 days of storage. Thus, gluten content showed no significant differences ($p \geq 0.05$) during the storage.

The protein contents of semolina stored during different periods under various treatments were reported in Table 4. The protein content in infested semolina was 11.1, 11.03 and 10.8% after 30, 45 and 60 days of storage, respectively. However, for treated semolina with chitosan: gum Arabic: essential oil protein content reached its maximum 13.61% after 30 days of storage. A decrease on protein values have been observed after 45 and 60 days of storage (Table 4). The storage period tended to cause a decrease in protein content of semolina. Significant

differences were registered between treatments (df = 4, f = 66.87, $p \leq 0.01$).

The lipid percentages of semolina during storage periods ranged between 0.7 and 1.15 for infested semolina while it was comprised between 1.05 and 1.9 for control. Furthermore, for treated semolina with free essential oils, chitosan: gum Arabic: essential oils and chemical treatment lipid content varied from 0.82 to 1.75 and from 0.91 to 1.81 and from 0.89 to 1.71, respectively. No significant differences were observed on lipid content between treatments (df = 4, f = 2.04, $p \leq 0.1$). Moreover, significant differences were registered between storage periods (df = 2, f = 0.3, $p \leq 0.01$).

DISCUSSION

During this study, the GC-MS analysis was conducted to determine chemical composition of rosemary essential oils. Results showed that 1,8-Cineole, Borneol, and α–Pineneas are the major components with more than 5% of rosemary essential oils, and which can be confirmed by previous work conducted on rosemary by Kadri et al. (2011). According to Prakash et al. (2021), different factors may affect the percentages of the chemical components such as plant species, parts of plant, seasons, extraction methods and geographical conditions. Subsequently, the median lethal concentration will be used for encapsulation with chitosan and gum Arabic matrix. In fact, the encapsulation inhibits the degradation of food ingredients under different conditions such as oxygen, heat and moisture (Maswal and Dar 2014). According to Taylor et al. (2007), the increase of matrix concentration makes encapsulation efficiency values significantly increase. One of the most known matrix chitosan-encapsulated essential oils could be utilized as an encapsulating matrix due to various characteristics such as ecological safety, low toxicity and excellent biodegradability (Prashanth and Tharanathan 2007; Zhang et al. 2022). This study has been made in order to evaluate the efficacy of free and encapsulated rosemary essential oils as an ecofriendly alternative to chemical treatment like phosphine. Due to their major component like 1,8-cineole, these oils had a high fumigant toxicity for controlling *O. surinamensis*. In the same context, Lee et al. (2002) demonstrated that rosemary essential oils showed high fumigant toxicity against insect pests of stored products, whereas results revealed that toxicity potential of free essential oils depend on storage durations. An interesting decrease has been shown on corrected mortality caused by free essential oils from 4 % after 30 days to 3 % after 45 days to reach finally 1 % after 60 days of storage. This is due probably to the volatile compounds. During three storage periods, it has been shown that encapsulated essential oils were relatively more toxic against *O. surinamensis*. In addition, considerable differences were observed in mortality of insects between different treatments. Regarding fumigant test, toxicity of encapsulated essential oils is due to major components. According to previous researches, major compounds such as phenol, camphor and 1,8-cineole had a high insecticidal toxicity against various insect pests (López and Pascual-Villalobos 2010).

The humidity percentage of semolina in the present study varied upon storage durations. These results showed that chemical proprieties of semolina changed during storage periods with deterioration on semolina quality in infested semolina by *O. surinamensis* compared to control and treated semolina. Results showed that lowest values were registered in infested semolina with

chemical factors such as gluten, protein, and lipid. Similarly, Haouel-Hamdi et al. (2020), Saeed Mohamed et al. (2012), Panth and Susheela (1977) revealed that infestation by insects during storage periods cause high deterioration and damages in cereal. In addition, Sanchez-Marinez et al. (1997) reported that insect infestation leads to protein degradation and modification in gluten structure. Results showed high variation in gluten quantity in infested semolina compared to control and treated semolina. Furthermore, these results are confirmed by those reported by Mohammad et al. (2012), which demonstrated that insect infestation caused the decline in the gluten content which gives the flour certain liquidity and lack of rubber and cohesion. The results regarding protein contents during different storage periods were less than those obtained in earlier study conducted by Erbas et al. (2005), which revealed that protein percentage (15.35%) decreased through the storage periods, but our results confirm those reported by Haouel-Hamdi et al. (2020).

RESUME

Soltani A., Haouel-Hamdi S., Ajmi I., Ben Abada M., Djebbi T., Chargui H., Mathlouthi I., Laabidi A., Mahmoudi H. et Mediouni-Ben Jemâa J. 2022. Évaluation de l'impact insecticide des huiles essentielles du romarin sur le cucujide dents de scie des grains *Oryzeaphilus surinamensis*. Tunisian Journal of Plant Protection 17 (1): 15-28.

L'étude explore la toxicité fumigène des huiles essentielles du romarin (*Rosmarinus officinalis*) libres et encapsulées contre les adultes du cucujide dents de scie des grains (*Oryzeaphilus surinamensis*) pendant trois périodes de stockage, 30, 45 et 60 jours. Le chitosane a été utilisé comme matrice d'encapsulation. Les résultats GC/MS ont montré que le 1,8-cinéole et le camphre étaient les principaux composants avec 39.67% et 18.04%, respectivement. Les huiles essentielles ont causé une mortalité de 85.48% à 300 µL/L air après 10 jours de stockage. La concentration létale médiane (CL_{50}) était de 124,80 µL/L d'air. L'efficacité d'encapsulation était de 25.8% et la capacité de charge était de 1.9%. Les huiles essentielles encapsulées ont atteint une efficacité de 82%, 100% et 100% après 30, 45 et 60 jours de stockage, respectivement. Le traitement de référence à la phosphine a révélé une toxicité de 100%, 96% et 71% après 30, 45 et 60 jours de stockage. En revanche, les résultats ont montré que les huiles essentielles encapsulées entraînaient une très faible modification sur les proprietes de la semoule. Les teneurs en protéine ont diminué à la fin de la durée de stockage de moins de 1% seulement (de 13.61% après 30 jours à 12.91% après 60 jours de stockage). Ainsi, les huiles essentielles encapsulées pourraient être considérées comme un moyen de fumigation alternatif pour la semoule sans détérioration de sa qualité pendant le stockage.

Mots clés: Chitosane, encapsulationqualité de la semoule, *Oryzeaphilus surinamensis*, *Rosmarinus officinalis*, toxicité de fumigants

ملخص

سلطاني، عبير وسمية حوال-حمدي وإنصاف عجمي ومهى بن عبادة وتسنيم دجبي وحذامي شرقي وإيمان مثلوثي وأمينة عبيدي وهالة محمودي وجودة مديوني بن جماعة. 2022. تقييم تأثير الزيوت العطرية لإكليل الجبل كمبيد حشري على خنفساء الحبوب المنشارية. Tunisian Journal of Plant Protection 17 (1): 15-28.

تستكشف الدراسة سمية التدخين للزيوت العطرية لإكليل الجبل (*Rosmarinus officinalis*) الحرة والمغلفة ضد الطور البالغ لخنفساء الحبوب المنشارية (*Oryzeaphilus surinamensis*) لمدة ثلاث فترات تخزين هي 30 و 45 و 60 يومًا. تم استخدام الشيتوزان كمصفوفة تغليف. أظهرت نتائج التحاليل الكيميائية GC/MS أن 1.8-سينيول والكافور كانا المكونان الرئيسيين بنسبة 39.67% و 18.04%، على التوالي. أظهرت الزيوت العطرية نسبة وفيات 85.48% عند تركيز 300

ميكرولتر/لتر هواء بعد 10 أيام من التخزين. كان متوسط التركيز المميت 124.80 ميكرولتر/لتر هواء. كانت فعالية التغليف 25.8% وسعة التحميل 1.9%. حققت الزيوت العطرية المغلفة 82% و 100% و 100% بعد 30 و 45 و 60 يومًا من التخزين، على التوالي. أظهرت المعاملة المرجعية بالفوسفين سمية 100% و 96% و 71% بعد 30 و 45 و 60 يوماً من التخزين. من ناحية أخرى، أوضحت النتائج أن الزيوت العطرية المغلفة تسبب تغيير طفيف في خصائص السميد، حيث انخفض محتوى البروتين في نهاية فترة التخزين بأقل من 1% فقط (من 13.61% بعد 30 يومًا إلى 12.61% بعد 60 يومًا من التخزين). وبالتالي، يمكن اعتبار الزيوت العطرية المغلفة وسيلة تبخير بديلة للسميد دون تدهور جودته أثناء التخزين.

كلمات مفتاحية: تغليف، جودة السميد، سمية التبخير، كيتوزان، *Oryzeaphilus surinamensis*، *Rosmarinus officinalis*

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Repellency and Insecticidal Activities of *Thapsia garganica* Crude Extract against Some Important Pests

Ghofrane Jmii, Rabiaa Haouala, Samir Gharsallaoui, LR/Agrobiodiversité et Ecotoxicologie (LR21AGRO2), Institut Supérieur Agronomique de Chott-Mariem, Université de Sousse, 4042, Chott-Mariem, Tunisia, **Ikbal Chaieb, and Asma Laarif,** LR21AGR03-LR/Production et Protection pour une Agriculture Durable (2PHD), Centre Régional des Recherches en Horticulture et Agriculture Biologique de Chott-Mariem, Université de Sousse, 4042, Chott-Mariem, Tunisia **(*Tunisia*)**
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ABSTRACT

Jmii, G., Haouala, R., Gharsallaoui, S., Chaieb, I., and Laarif, A. 2022. Repellency and insecticidal activities of *Thapsia garganica* crude extract against some important pests. Tunisian Journal of Plant Protection 17 (1): 29-42.

Repellency and insecticidal activities of *Thapsia garganica* leaf methanolic extract were investigated against *Tribolium castaneum*, *Myzus persicae*, *Phthorimaea operculella*, and *Spodoptera littoralis*. Repellency and toxic activities (by ingestion and topical application) were evaluated on *T. castaneum* nymphs and adults. Topical application treatment caused total larval growth inhibition at 10%, until mortality after 7 days. The highest mortality was recorded with 94% at the same concentration. Methanolic extracts incorporation into *T. castaneum* larvae artificial diet at 10% caused 100% mortality after 3 days. The extract at 1% cause high repellent effect on *T. castaneum* after 60 min of exposure, while *M. persicae* was less sensitive. *P. operculella* female's showed sensitivity by a repellent effect at oviposition. Egg's number laid on treated tubers at 1% and 2% decreased significantly to 32% and 72%, respectively. In addition, methanolic extracts had a preventive effect on *P. operculella* larval penetration. In fact, the number of larvae was reduced by 30.46% and 76.12% in the treated tubers at 1% and 2%, respectively. For *S. littoralis*, a low antifeeding effect was recorded. However, the relative growth rate (RGR), conversion of ingested and digested food to biomass, were decreased. The approximate digestibility increased. Moreover, a delay in larval development was observed. This study suggests that the leaf extract of *T. garganica* could be applied as bio-insecticide.

Keywords: Antifeeding proprieties, crude extract, insecticidal activity, *Thapsia garganica*

Food provision has always been a challenge facing mankind; but the competition from insect pests is the main factor in this challenge. Hence, insect pests are responsible for large losses to stored products and crops through feeding damage; but also as vectors of plant pathogens such as viruses. The application and the development of synthetic insecticides have made it possible to suppress populations of pests in order to achieve an adequate supply of food. However, the use of chemicals has been associated with environmental pollution and adverse effects on human health and non-target organisms and frequent applications of insecticides have led to the

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

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occurrence of resistance in many insect populations (Sparks et al. 2021; Wang et al. 2022). Researchers are therefore turning towards natural active substances capable to interfere with the development and physiology of insect pests (Tlak Gajger and Dar 2021; Yactayo-Chang et al. 2020).

Plants are a rich source of bioactive compounds against pests. For example, the alkaloid nicotine, extracted from *Nicotiana tabacum*, was one of the first natural products used as an insecticide (Kortbeek et al. 2019). Another example is azadirachtin, which was isolated from neem seeds. This compound was long used in agriculture (Kortbeek et al. 2019).

The present article is focused on four relevant insect species: *Tribolium castaneum* (Coleoptera, Tenebrionidae), *Myzus persicae* (Hemiptera, Aphididae), *Phthorimaea operculella* (Lepidoptera, Gelechiidae) and *Spodoptera littoralis* (Lepidoptera, Noctuidae). *T. castaneum* is one of the most important insects damaging stored grains and related products (particularly flour and the other processed grain products) (Hamouda et al. 2015a). *M. persicae* is highly polyphagous species. It is able to feed on over 400 host plant species, including several major crop types (Hemming et al. 2022). Regarding *P. operculella*, it is a cosmopolitan insect of solanaceous crops. It causes relevant damage to potato (storage and field) (Hamouda et al. 2015a). Concerning *S. littoralis*, it attacks around 44 plant families and deciduous fruit trees (Lopez et al. 2006). The voracious caterpillars attack plants (leaves, flowers, and stems) (Alford 1995).

The aim of this work was to evaluate the insecticidal activity of *T. garganica* leaf methanolic extract on the insect species previously cited. *T. castaneum* was subjected to assays evaluating the effect of the extract through topical application, repellency, and ingestion. The insecticidal potential of the methanolic extract was also evaluated on *M. persicae*, and *P. operculella* through larval penetration and oviposition-preference activity tests, and on *S. littoralis* through feeding assays and morphological malformations evaluation.

MATERIALS AND METHODS

Plant material.

Leaves of *T. garganica* were collected in February 2021 from the region of Sousse (35° 49' 31" N and 10° 38' 13" E) in the Sahel of Tunisia. *T. garganica* was in the vegetative growth stage when sampling. The collected plant was identified using the flora of Tunisia (Pottier-Alapetite, 1981). Leaves were dried at room temperature in complete darkness and grind. Powdered plant tissues (100 g) were macerated in methanol. To remove peel particles, the extracts were filtered through 0.22-µm-pore paper in a Büchner funnel coupled to a vacuum pump. They were evaporated to dryness under reduced pressure at 45-50°C, using Rotavapor R-114 (Buchi, France) and stored at 4°C until use (Jmii et al. 2020).

Insects.

M. persicae was collected from pepper crop grown in greenhouses located in the Regional Center of Research on Horticulture and Organic Agriculture (CRRHAB), Chott Mariem, Sousse, Tunisia.

P. operculella was reared on potato tubers in the laboratory of entomology in the CRRHAB. Insects were kept at (27 °C), constant day/night cycle (photoperiod of 12 h), and ambient relative humidity of 65%.

T. castaneum was reared on wheat semolina (*Triticum durum*) mixed with beer yeast and corn flour (100/5/50, w/w/w) at 30°C in complete darkness.

Toxicity assays were carried out both on nymph and adult insects. Tested larvae were in the third instar of development (3 mm of length), and the age of adult insects analyzed was 14 days old.

S. littoralis was reared starting from caterpillars collected from pepper crops grown in greenhouses, and maintained in the laboratory of entomology in the RCRHOA. Caterpillars were reared in Petri dishes at 25°C, 70% humidity, and a 16h:8h photoperiod (L:D). They were fed with artificial diet composed of 800 mL water, 150 g chickpea powder, 20 g agar, 40 g beer yeast, 1 g benzoic acid, 5 g ascorbic acid, and 1 g nipagin. When caterpillars reached third instar, they were reared individually to avoid cannibalism. At the last (the sixth) instar, they were transferred to round plastic bowls (15 cm in depth and 70 cm in diameter) furnished with moistened saw dust to serve as pupating medium. Resulted pupae were daily collected and transferred to adult rearing cages (nesting cages). Cages were cubic (measuring 50 × 50 × 50 cm). The top, the floor, and the three sides of each cage were wooden, and covered with kraft paper serving as oviposition support. The fourth side of the cage was composed of wire screen (acts as a door and allows the ventilation of the cage). In the cages, small pots containing pieces of cotton wool soaked with 10% sugar feeding solution were placed. The kraft paper (on which the eggs were deposited) was cut into pieces. These pieces were placed in Petri dishes containing the artificial diet of chickpea. Newly emerged third instar caterpillars were used for toxicity assays.

Insecticidal activity against *T. castaneum*.

Topical application test. The dry extract was dissolved in distilled water to obtain final concentrations of 0.1, 1 and 10%. 1 µL of each solution was applied on the topical part of the thorax of nymphs and adults. The control insects received 1 µL of distilled water. For each assay, 10 larvae and adults were used. The mortality was assessed daily via direct observation for a period of 7 days. Insect mortality was calculated using the Abbott correction formula for natural mortality in untreated controls (Abbott 1925). The increase in length was also determined for both treated and control groups.

Repellency assay. An area preference method was adopted to assess the repellent activity of *T. garganica* essential oils against *T. castaneum* adults. Repellency assay was carried out into Petri dishes measuring 9 × 1.3 cm (diameter/height). Each Petri dish contained a disc of Whatman paper N°1 of the same diameter, cut into two equal halves. The first semi-circle was soaked in methanol (200 µL) to be the control part, while the second semi-circle was treated with the same quantity of the extract at the concentrations of 0.1 and 1%. After the evaporation of the solvent, both treated and untreated halves were then attached with cellophane tape and placed in Petri dish. Twenty *T. castaneum* adults were deposited into the center of the dish (Hamouda et al. 2015b).

The measured parameter was the number of adults in each semi-circle, after 15, 30, 60, and 120 min. Repulsion percentage (PR) was calculated according to the formula (McDonald et al. 1970): PR = [(N control - N treated) / (N control + N treated)] × 100, where N control: number of insects on the control area and N treated: number of insects on the treated area.

The repulsion average percentage was calculated and attributed according to McDonald's classification (McDonald et al. 1970) to one of the different repellent classes varying from 0 to V:

Repulsion Percentage (PR)	Class
PR ≤ 0.1 %	0
0.1 % < PR ≤ 20 %	I
20.1 % < PR ≤ 40 %	II
40.1 % < PR ≤ 60 %	III
60.1 % < PR ≤ 80 %	IV
80.1 % < PR ≤ 100 %	V

Ingestion assay. This assay aims evaluating the potential of the extract to be used as poisoned bait for the control of *T. castaneum*, through its incorporation into larvae's food (wheat semolina). The extract was first dissolved in methanol at 0.1, 1, and 10% concentrations, and 10 mL of each solution were added to 20 mg of wheat semolina. Once the solvent has evaporated, the treated diet was introduced in Petri dishes, containing 20 *T. castaneum* larvae. For control samples, semolina (20 mg) was treated with 10 mL of methanol following the same procedure, and larvae mortality and adults' emergence percentages were assessed daily via direct observation for a period of 21 days (Hamouda et al. 2015b).

Insecticidal activity against *M. persicae*.

The dry methanolic extract was dissolved in distilled water at the concentrations of 0.1, 1, and 2%. These solutions were tested by spray treatment, for which 5 µL were homogeneously sprayed on pepper leaves infested by *M. persicae*. The number of insects used was 120, 132, 127, and 141 for the control, and the different treatments were 0.1, 1, and 2%, respectively. In the control experiment, the insects received the same quantity of distilled water. The infested leaves were then introduced in Petri dishes (9 cm × 1.3 cm), coated with filter paper, and introduced into a climatic chamber. They were kept for 24 h with a photoperiod of 12h:12h (L:D), while maintained at a temperature of 25±2°C, and relative humidity of 70±10%.

The mortality rate assessment was then recorded using a binocular stereomicroscope. Insects were considered dead when no leg or antennal movements were observed after stimulation with a soft paint brush. The mortality rate assessment was corrected according to Abbott's (1925) correction formula.

Insecticidal activity against *P. operculella.*

Each non infested potato tuber was dipped in 10 mL of 1% and 2% methanolic extract of *T. garganica*. When tubers dried and solvent evaporated, five potato tubers per treatment were transferred into plastic boxes with ventilated lids kept at 25°C, photoperiod of 16h:8h (L:D), and relative humidity of 70±10%. In each box, five infested tubers were introduced, and larval penetration was recorded with the number of larvae moving into potatoes. For the oviposition-preference activity, the egg number was determined under a binocular stereomicroscope (Hamouda et al. 2015a).

Insecticidal activity against *S. littoralis.*

Third instar caterpillars were weighed and placed individually in Petri dishes, to be fed with artificial diet, previously treated with the methanolic extract. This food consisted of a 1 g disc prepared by mixing the diet based on chickpea with the methanolic extract. The weight of this extract was 0.1 g and 1 g per 100 g diet. For the negative control, caterpillars were fed with chickpea mixed with distilled water.

Every two days, each disc was weighed before being presented to the caterpillars, and reweighed and replaced by a new weighed disc, for a total time of 10 days. The five gravimetric indices defined by Waldbauer (1968) were calculated, which allow to understand the use of food by the caterpillars. The

measured parameters to perform these calculations were the weights of consumed food, the rejected excrement, and the biomass variations in the insect after the treatment period. The five indices were:

The relative ingestion rate (RIR) = Fi / (P × Td),

The approximate digestibility (AD) = 100 × (Fi - Fp) / Fi,

The efficiency of converting digested food into biomass (ECD) = 100 × WG / (Fi - Fp),

The efficiency of converting ingested food into biomass (ECI) = 100 × WG / Fi

The relative growth rate (RGR) = WG / (P × Td),

where Fi: Food ingested (mg), Fp: Faeces produced (mg), Wg: Weight gain (mg) = (Wf - Wi), Td: Development time (day), Wf: Final weight of the caterpillar (mg), Wi: Initial weight of the caterpillar (mg), P: Average weight of the caterpillar = ((Wf - Wi)/log ((Wf / Wi)).

The antifeeding activity of the methanolic extract was determined according to Simmonds et al (1989): AFI = (C - T / C + T) × 100, where C: Consumption of control caterpillar, T: Consumption of treated caterpillar.

The antifeeding activity classification was carried out according to Liu et al (2007):

* AFI < 20%: no antifeeding activity (–),
* 50% > AFI ≥ 20%: low antifeeding activity (+),
* 70% > AFI ≥ 50%: medium antifeeding activity (++),
* AFI ≥ 70%: strong antifeeding activity (+++).

Malformations (like head capsule persistence, pupae and larvae having reduced size) which could make *S. littoralis* vulnerable to several sorts of mortality (such as molting and exuviations difficulties) were also evaluated during this experiment.

Statistical analyses.

The data were reported as mean ± standard deviation (SD) in five replicates. ANOVAs followed by Duncan's test were performed by IBM SPSS Statistics version 20.0 to analyze the differences between treatments. Differences were considered statistically significant at the 5 % level ($p < 0.05$).

RESULTS

Contact effect of the methanolic extract on *T. castaneum*.

Larval development. Table 1 shows that *T. garganica* leaf methanolic extract (at 0.1%, 1%, and 10%) significantly ($p < 0.05$) inhibited larval length growth. The larva increase in length was only 0.32 mm at 0.1%, after 7 days of the treatment, versus 0.54 mm for the control. At the highest concentration, the larval growth was totally inhibited.

Table 1. Increase in length (mm) of *T. castaneum* larvae after 7 days of the topical application treatment with *T. garganica* leaf methanolic extract at 0.1%, 1%, and 10%, as compared to the control

	Control	0.1%	1%	10%
Larva increase in length (mm)	0.54 ± 0.05^{d}	0.32 ± 0.04^{c}	0.12 ± 0^{b}	0 ± 0^{a}

Values are means ± standard deviations (SD) (n = 5). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test).

Toxic effect. The mortality rate of *T. castaneum* 3rd instars caused by *T. garganica* leaf methanolic extract after 7 days are presented in Table 2. This experiment showed a mortality rate of 48% in the case of larvae treated by topical application with the extract at 0.1%. In the case of larvae treated with methanolic extract at 1% and 10%, the mortality rate reached 96% and 100%, respectively

For *T. castaneum* adults treated with methanolic extract at 0.1% and 1%, the mortality rate reached 28% and 86%, respectively, after 7 days of the treatment. At the highest concentration, adults exhibited the highest mortality with 94% (Table 2).

Table 2. Mortality rate (%) of *T. castaneum* larvae and adults after 7 days of the topical application treatment with *T. garganica* leaf methanolic extract at 0.1%, 1%, and 10%, as compared to the control. Mortality rate was corrected using Abbott's formula (1925)

Insect mortality rate	0.1%	1%	10%
Larvae	48 ± 8.36^{a}	96 ± 5.47^{b}	100 ± 0^{c}
Adults	28 ± 13.03^{a}	86 ± 11.4^{b}	94 ± 8.94^{c}

Values are means ± standard deviations (SD) (n = 5). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test).

Repellency assay.

The repellency percentage calculated for adults exposed to methanolic extract at 0.1% and 1% showed that the extract at 0.1% has a moderately repellent effect in the order of 53% after 60 min of exposure. Nevertheless, from the first 15 min, the extract at 1% noted a repellent effect with 68%. This effect became very repulsive (84%) after 30 min of exposure to reach its maximum after 60 min (100%) (Table 3).

Ingestion assay.

The methanolic extract incorporation into *T. castaneum* larva artificial diet at 10% caused 100% larvae mortality after 3 days. As a result, the percentage of emergence of *T. castaneum* adults from larvae subjected to treated food was 0% at the highest concentration (Table 4).

Table 3. Repulsion average percentages of *T. casataneum* adults exposed to *T. garganica* leaf methanolic extract at 0.1% and 1%

Extract concentration	Exposure (min)	Average repulsion (%)	Repulsive class	Repellency effect
0.1%	15	$14\pm0.49^{a**}$	I	Very weak repellent
	30	$14\pm0.49^{a**}$	I	Very weak repellent
	60	$53\pm0.22^{b**}$	III	Moderately repellent
	120	$53\pm0.22^{b**}$	III	Moderately repellent
1%	15	68 ± 0.36^{a}	IV	Repellent
	30	84 ± 0.47^{b}	V	Very repellent
	60	100 ± 0^{c}	V	Very repellent
	120	100 ± 0^{c}	V	Very repellent

Values are means ± standard deviations (SD) (n = 5). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test). ** means are significantly different using means variation test ANOVA at ($p < 0.01$).

Table 4. Percentage of emergence (%) of *T. castaneum* adults from larvae fed on artificial diet mixed with *T. garganica* leaf methanolic extracts at 0.1%, 1%, and 10%

Extract concentration	Control	0.1%	1%	10%
Larvae mortality (%)	0 ± 0^{a}	21 ± 1^{b}	72 ± 1^{c}	100 ± 0^{d}
Percentage of emergence of *T. castaneum* adults from larvae subjected to ingestion assay (%)	100 ± 0^{d}	79 ± 1.73^{c}	28 ± 2.64^{b}	0 ± 0^{a}

Values are means ± standard deviations (SD) (n = 5). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test).

Insecticidal activity against *M. persicae*.

Percentage of mortality in adults of *M. persicae* attacking pepper plants treated with *T. garganica* leaf methanolic extract at 0.1%, 1%, and 2% is represented in Table 5. Aphids in this treatment showed the highest mortality with 7.09% at the highest concentration. However, the mortality rate did not exceed 1.51% and 4.72% at 0.1% and 1%, respectively.

Table 5. Mortality rate (%) in adults of *M. persicae* adults treated by foliar application with *T. garganica* leaf methanolic extract at 0.1%, 1%, and 2% as compared to control. Mortality rate was corrected using Abbott's formula (1925)

Extract concentration	Numbers tested (n)	Mortality (%)
0.1%	132	1.51 ± 0.14^{a}
1%	127	4.72 ± 0.23^{b}
2%	141	7.09 ± 0.18^{c}

Values are means ± standard deviations (SD). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test).

Insecticidal activity against *P. operculella.*

Table 6 shows that the number of larvae decreased with 30.46% and 76.12% in tubers treated with leaf methanolic extract at 1% and 2%, respectively. Similarly, the number of eggs laid by *P. operculella* on treated tubers was reduced by 32% and 72% in the presence of the extracts at 1% and 2%, respectively.

Table 6. Mean numbers of larvae and eggs of *P. operculella* in potato tubers treated with *T. garganica* leaf methanolic extract at 1% and 2%, as compared to the control

Extract concentration	Mean numbers of larvae/Potato tuber	Mean numbers of eggs/Potato tuber
Control	15.33±0.57^{c}	16.66±0.57^{c}
1%	10.66±1.15^{b}	11.33±0.57^{b}
2%	3.66±0.57^{a}	4.66±0.57^{a}

Values are means ± standard deviations (SD) (n = 5). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test).

Insecticidal activity against *S. littoralis.*

Effect on nutrient utilization. The methanolic extract incorporation into the artificial diet of *S. littoralis* caterpillars significantly affected all gravimetric indices. The caterpillars fed on artificial diet supplemented with distilled water (control) showed the highest relative growth rate (RGR = 0.48 mg/mg/day). In the presence of the methanolic extract, the relative growth rate decreases significantly ($p < 0.05$) especially at the concentration of 1% (RGR = 0.35 mg/mg/day) (Table 7).

The caterpillars subjected to a diet supplemented with methanolic extracts from *T. garganica* leaves at 0.1% and 1% showed a RIR which exceeded than the control group (RIR = 1.78 mg/mg/day). The incorporation of the extract into *S. littoralis* diet at the highest concentration had the highest RIR effect on caterpillars (RIR = 4.02 mg/mg/day) (Table 7).

The data of the AD showed a significant difference between treatments ($p < 0.05$). Caterpillars fed on artificial diet containing the methanolic extract at 1% had the highest approximate digestibility with 96.24%. However, the control showed the lowest approximate digestibility, which was around 82.95%. The extract at 0.1% induced an average rate of 89.95% (Table 7).

For ECI and ECD, statistical analysis made possible to classify these two parameters into three groups: The control presented the highest ECI and ECD, with 28.20% and 34% respectively, while the caterpillars fed on artificial diet supplemented with the methanolic extract at 1% had two lowest values with 9.49% and 9.89%. The treatment at 0.1%, demonstrated an ECI and ECD of 20.31% and 22.70%, respectively.

Table 7. Relative growth rate (RGR) (mg/mg/day), relative ingestion rate (RIR) (mg/mg/day), approximate digestibility (AD) (%), efficiency of converting ingested food into biomass (ECI) (%), and efficiency of converting digested food into biomass (ECD) (%) of *S. littoralis* caterpillars subjected to a diet supplemented with *T. garganica* leaf methanolic extracts at 1% and 2%, as compared to the control

Extract concentration	RGR	RIR	AD	ECI	ECD
Control	0.48 ± 0.01^{c}	1.78 ± 0.36^{a}	82.95 ± 4.71^{a}	28.20 ± 5.67^{c}	34 ± 6.77^{c}
0.1%	0.42 ± 0.02^{b}	2.23 ± 0.52^{b}	89.95 ± 2.02^{b}	20.31 ± 5.94^{b}	22.7 ± 6.9^{b}
1%	0.35 ± 0.02^{a}	4.02 ± 1.01^{c}	96.24 ± 1.3^{c}	9.49 ± 2.97^{a}	9.89 ± 3.23^{a}

Values are means ± standard deviations (SD) (n = 5). Means with the same letterare not significantly different at $p < 0.05$ (Duncan's test).

Antifeeding activity. The methanolic extract at 0.1% and 1% had low antifeeding effects (50% > AFI ≥ 20%) on *S. littoralis* caterpillars (Table 8).

Table 8. Antifeeding activity (%) of *T. garganica* leaf methanolic extract at 1% and 2% on *S. littoralis* caterpillars. Low antifeeding activity (+)

Extract concentration	Antifeeding activity (AFI)
0.1%	22.40 ± 9.29 (+) **
1%	31.16 ± 8.38 (+)

Values are means ± standard deviations (SD) (n = 5). Data were analyzed by ANOVA test. **: Statistically significant differences ($p < 0.01$).

Toxicity and malformations. The incorporation of methanol extract in *S. littoralis* artificial diet caused a mortality of larvae and pupae. The development of larvae fed on diet treated by the extract at 0.1% and 1% was delayed. After 15 days of delay, the mortality rate of larvae (having a reduced size) fed on diet mixed with the extract at 0.1% reached 36%. The larvae died because of a molting difficulty. Only 52% of dwarf pupae were formed from dwarf larvae (Table 9). Twenty percent (20%) of dwarf adults were emerged from the dwarf pupae. In the case of the treatment at 1%, after more than one month of development delay, 74% of dwarf larvae died. All (100%) of malformed pupae (head capsule persistence) after exuviation difficulties died (Table 9).

Table 9. Larval and pupal mortality (%) of *S. littoralis* caterpillars subjected to a diet supplemented with *T. garganica* leaf methanolic extracts at 1% and 2%, as compared to the control

Extract concentration	Larval mortality (%)	Pupal mortality (%)
0.1%	36[b]	48[b]
1%	74[c]	100[c]
Control	0[a]	0[a]

Values are means ± standard deviations (SD) (n = 5). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test).

DISCUSSION

T. garganica leaf methanolic extract showed an insecticidal activity against stored product pests *T. castaneum*, and against crop pests *P. operculella* and *S. littoralis*. Apiaceae is one of the most known and used plants for their richness of secondary metabolites. Our previous work (Jmii et al. 2020) showed that *T. garganica* leaf methanolic extract is a source of four molecules: Two flavonoid glycosides, luteolin 7-*O*-glucoside and apigenin7-*O*-glucoside and two sesquiterpene lactones, thapsigargin, and 10β-acetoxy-8α-butyryloxy-11α-hydroxy-2β-((2-methyl-butanoyl) oxy)-1β*H*,6α*H*,7α*H*,11β*H*-guaian-3-en-12,6-olide. These molecules could be responsible for the toxicity, causing *T. castaneum* larvae total mortality (following ingestion and topical application treatment), repellent activity (on *T. castaneum* and *P. operculella*), larval growth delay until mortality and pupal malformations (in the case of *S. littoralis*). In fact, plants have developed an array of defensive strategies by producing biochemical defenses that restrict insect pests, to avoid herbivore damage. The biochemical traits include various toxic secondary metabolites (Ben-Khalifa et al. 2018). These authors indicated that many several compounds synthesized as secondary metabolites have repellent and antifeeding activity against insect pests. Such metabolites can also disturb the insect growth and development and inhibit their oviposition (Ben-Khalifa et al. 2018). It was reported for example that flavonoids influence the insect behavior, growth, and development. A number of flavones such as isoflavonoids, proanthocyanidins, flavonols, and flavonones have been investigated as feeding deterrents. A flavonoid isolated from *Tephrosia vogelii* (5-methoxyisoronchocarpin) has been found as feeding deterrent against *S. littoralis*. Judaicin was also found to be deterrent to the same insect (War et al. 2012). Additionally, it has been found that tannins and procyanidin polymers act as feeding deterrents, affecting the growth of some insects (such as *Euproctis chrysorrhoea* for tannins and *Aphis Craccivora* for procyanidin polymers). Condensed tannins from *Betula Neoalaskana* (Alaska paper birch), coated on birch leaves at 3% dry weight, reduced *Rheumaptera hastata* larva survival and pupal mass (War et al. 2012). Glycoalkaloids (solamargine, solasonine, and solasodine) were responsible for *T. castaneum* mortality (Hamouda et al. 2015a). Saunders et al (1992) determined that steroidal alkaloids possess insect repellent activity.

Methanolic extracts addition to *S. littoralis* diet caused developmental disturbances, demonstrated by weight gain inhibition and malformations at pupation. These results highlight two hypotheses: (1) the antifeeding proprieties of *Thapsia* leads to a reduction in the weight of caterpillars and therefore of the dwarf nymphs, and (2) reduced size can also be explained by the disruption of the insect's hormonal balance, thus causing early pupation (without going through all larval stages) (Chaieb 2005). Certainly, the leaves showed low antifeeding activity; so, the second hypothesis is more likely. RIR increase can prove also the low antifeeding effect of *T. garganica* leaf methanolic extracts. Caterpillars feeding on treated artificial diet showed an AD greater than control. Digestion increase could be a detoxifying way to get rid of toxins contained in *T. garganica*. Thus, Haubruge and Amichot (1998) showed that the insect contact with an insecticide leads to increased activity of xenobiotic degradation systems. ECD decrease could be explained by a decrease in the ability to detoxify toxic compounds present in the methanolic extracts that affect the conversion of the absorbed food into biomass. The reduction in ECD associated with secondary metabolite ingestion is a frequent phenomenon (Reese 1978; Lindroth et al. 1988; Koul et al. 1990; Appel and Martin 1992). This may be due to the interaction of the secondary metabolites with certain metabolic processes (Slansky 1992) or an indirect slowdown in growth, thereby by passing a greater proportion of absorbed food in the breath (Appel and Martin 1992). Amr (2001) found that the reduction in the efficiency of *S. littoralis* caterpillars to convert digested and ingested food causes a weight reduction. This explanation was confirmed by Reese and Beck (1976 a, b) and Dahlman (1977) results who suggested that ECI reduction is the result of TRC decrease.

A low mortality rate in *M. persicae* treated adults could be explained by their resistance to secondary metabolites contained in *T. garganica* leaf methanolic extract. In fact, it was reported that *Shistocerca gregaria* has the ability to tolerate tannins by hydrolyzing them rapidly, to avoid their damaging effects. In addition, they have the ability to adsorb them (for example on the thick peritrophic membrane) to restrict their passage (War et al. 2012). Furthermore, various detoxifying enzymes (increased activities of cytochrome P450 and esterase, involvement of glutathione S-transferases in the metabolism of secondary metabolites) could be induced by insects to avoid the damaging effects of reactive toxicants. It is the case for example for aphids (War et al. 2018).

To conclude, the present study showed that leaves from *T. garganica* have repellent and toxic activity against larvae and adults of *T. castaneum. P. operculella* showing sensitivity by a repellent effect at oviposition and a decrease of potato tuber moth larval penetration. Leaves were also able to delay larval growth until mortality and induce pupal deformities in *S. littoralis*. *M. persicae* was less sensitive to the treatment. Secondary metabolites contained in *T. garganica* leaf methanolic extract may prove to be important for the formulation of effective bio-insecticides. *T. garganica* methanolic leaf extract should be further investigated in order to elucidate more their insecticidal potentialities and to identify the bioactive molecules.

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RESUME

Jmii G., Haouala R., Gharsallaoui S., Chaieb I. et Laarif, A. 2022. Activités répulsives et insecticides de l'extrait brut de *Thapsia garganica* contre quelques ravageurs importants. Tunisian Journal of Plant Protection 17 (1): 29-42.

L'activité répulsive et insecticide des extraits méthanoliques de feuilles de *Thapsia garganica* (Apiacées) a été étudiée contre *Tribolium castaneum*, *Myzus persicae*, *Phthorimaea operculella* et *Spodoptera littoralis*. Une activité répulsive et toxique (par ingestion forcée et application topique) contre les larves et les adultes de *T. castaneum* a été démontrée. Le traitement par application topique a provoqué une inhibition totale de la croissance larvaire à 10%, jusqu'à la mortalité après 7 jours. La mortalité la plus élevée a atteint 94% à la même concentration. L'incorporation d'extraits méthanoliques dans l'alimentation artificielle des larves de *T. castaneum* à 10% a causé 100% de mortalité après 3 jours. L'extrait à 1% a eu un effet répulsif élevé sur *T. castaneum* après 60 min d'exposition tandis que *M. persicae* a été moins sensible. Le taux de mortalité a atteint 7,09 % à la concentration de 2%. Les femelles de *P. operculella* ont montré une sensibilité par un effet répulsif lors de la ponte. Le nombre d'œufs pondus sur les tubercules traités à 1% et 2% a diminué de manière significative avec 32% et 72%, respectivement. De plus, les extraits méthanoliques ont eu un effet préventif sur la pénétration larvaire de *P. operculella*. En fait, le nombre de larves a diminué de 30,46 % et 76,12 % dans les tubercules traités à 1% et 2%, respectivement. Pour *S. littoralis*, un faible effet anti-appétant a été enregistré sur ces chenilles. Cependant, la conversion des aliments ingérés et digérés en biomasse a diminué. De même, le taux de croissance a diminué. Quant à la digestibilité approximative, elle a augmenté. De plus, un retard de développement larvaire a été observé. Cette étude suggère que l'extrait des feuilles de *T. garganica* pourraient être employées comme bio-insecticides.

Mots clés: Activité insecticide, extrait brut, propriétés antiappétantes, *Thapsia garganica*

ملخص

جميعي، غفران وربيعة حوالة وسمير غرسلاوي وإقبال الشايب وأسماء لعريف. 2022. الأنشطة الطاردة والمبيدة للحشرات للمستخلص الخام من *Thapsia garganica* ضد بعض الآفات الهامة.

Tunisian Journal of Plant Protection 17 (1): 29-42.

تم تقييم الأنشطة الطاردة والمبيدة للحشرات للمستخلصات الميثانولية لأوراق *Thapsia garganica* ضد الحشرات *Tribolium castaneum* و *Myzus persicae* و *Phtorimaea operculella* و *Spodoptera littoralis*. تم إثبات الأنشطة الطاردة والمبيدة للحشرات (عن طريق الابتلاع القسري والتطبيق الموضعي) ضد *T. castaneum*. تسببت المعاملة الموضعية في عرقلة نمو اليرقات بنسبة 10% حتى النفوق بعد 7 أيام. تم تسجيل أعلى معدل وفيات بنسبة 94% وبنفس التركيز. أدمجت المستخلصات الميثانولية في النظام الغذائي الصناعي ليرقات *T. castaneum* بنسبة 10% مما تسبب في وفيات بنسبة 100% بعد 3 أيام. كان للمستخلص بنسبة 1% تأثير طارد عالي على *T. castaneum* بعد 60 دقيقة من التعرض، بينما كان *M. persicae* أقل حساسية. بلغ معدل الوفيات 7.09%بتركيز 2%. أظهرت *P. operculella* الأنثى حساسية من خلال تأثير طارد في وضع البيض. انخفض عدد البيض الذي تم وضعه على درنات بطاطا معاملة بنسبة 1% و 2% بشكل ملحوظ بنسبة 32% و 72%، على التوالي. بالإضافة إلى ذلك، كان للمستخلصات الميثانولية تأثير وقائي على اختراق يرقات *P. operculella*. لكن، انخفض عدد اليرقات بنسبة 30.46% و 76.12% في الدرنات المعاملة بنسبة 1% و 2%، على التوالي. بالنسبة للحشرة *S. littoralis*، تم تسجيل تأثير منخفض ضد التغذية على اليرقات، حيث انخفض تحويل الطعام المهضوم والمبتلع إلى كتلة حيوية. نفس الشيء بالنسبة إلى معدل النمو الذي أظهر انخفاضًا، لكن قابلية الهضم التقريبية ازدادت، إضافة إلى ذلك حدوث تأخير في نمو اليرقات. تشير هذه الدراسة إلى أنه يمكن استخدام مستخلص أوراق *T. garganica* كمبيد حشري حيوي.

كلمات مفتاحية: خصائص مضادة للتغذية، مستخلص خام، نشاط مبيد للحشرات، *Thapsia garganica*

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Plant Protection Events

Announcing/Postponement

of

The 13th Arab Conference of Plant Protection

Hammamet, Tunisia, 16-21 October, 2022

Welcome to the 13th Arab Conference of Plant Protection, Tunisia, 2022

Dear Colleagues,

On behalf of the Arab Society for Plant Protection and the Organizing Committee of the 13th Arab Congress of Plant Protection, we invite you to participate in our coming congress to be held at the "Le Royal" Hotel, Hammamet, Tunisia, during the period October 16-21, 2022 with the general theme "Plant Health for a Secure and Safe Food". Excellent scientific presentations by Arab and Foreign speakers will be the backbone of our congress, in addition to one day agricultural and touristic tour to visit historical and cultural sites in Tunisia.

During five days, there will be plenty of opportunities for networking with colleagues during the symposia, concurrent oral and poster sessions or when visiting exhibition stands. In addition, social functions (welcome reception, morning and afternoon coffee breaks, lunch breaks and gala dinner) will be an appropriate occasion to interact informally with colleagues from different countries representing different institutions, public and private, who share common interests. Such interactions is a golden opportunity for initiating scientific exchange which can lead later to a formal or informal professional collaboration.

Looking forward to seeing you all in Tunisia in 2022, the year declared by the United Nations as the "Plant Health Year".

Dr. Ibrahim Al-Jboory
President
Arab Society for Plant Protection

Dr. Asmaa Najar
Chairperson, ACPP 2021
Organizing Committee, INRAT

Main Topics

- Insects, mites and rodents economic pests
- Plant diseases and their control
- Ecology and epidemiology of plant diseases
- Natural enemies and their role in pest control
- Weeds and their control
- Pesticides
- Postharvest pests
- Quarantine and phytosanitary measures
- Integrated pest management
- Genetic engineering and pest control
- Beneficiary insects (bees and silk worm)

Postponement of the 13th Arab Congress of Plant Protection

Due to the continuation of the Covid-19 pandemic and unstable health situation in Tunisia, the Congress Organizing Committee decided to postpone the congress to October 2022. The Society Executive Committee endorsed the new date and ensured getting the same hotel prices offered earlier.

1-The new congress date is October 16-21, 2022.

2-Registration deadline: September 1, 2022.

3-Abstract's submission deadline (confirmation of earlier submitted abstracts or presenting new ones): April 30, 2022.

4-Acceptance of abstracts: 30 June, 2022.

5-Deadline for submitting proposals for invited concurrent oral research papers sessions: March 31, 2022

6-Second congress announcement: May 31, 2022.

7-Third and final congress announcement: July 1, 2022.

8-Deadline for hotel booking: July 31, 2022.

9-The email address and the website of the congress will continue to be: **info@acpp-aspp.com** and **www.acpp-aspp.com**

We apologize for any inconvenience caused by this postponement imposed on us by conditions beyond our control, and we appreciate your kind understanding. We are looking forward to meeting you all in the fall of 2022 in Tunisia.

The Arab Society for Plant Protection

The Organizing Committee of the 13th Arab Congress of Plant Protection in Tunisia

Recent Doctorate Theses in Plant Protection (2021/22)

Ben Abada, Maha. 2021. New post-harvest control approaches against the date moth *Ectomyelois ceratoniae* (Zeller, 1839) by micro-encapsulations of essential oils. Doctorate Thesis in Agronomic Sciences (Phytiatry), INAT, University of Carthage, Tunis, Tunisia, 211 pp. (*Public Defense*: *22 November 2021*).

Essential oils (EOs) are natural complex mixtures of volatile secondary metabolites. Nowadays, extensive research has been dedicated EOs aiming their use as natural alternatives to synthetic antimicrobial, antioxidants, anti-inflammatories and insecticides. However, EOs exhibited a low water solubility, high volatility, high light and thermal sensitivity that limit their further use. This current study aimed to encapsulate *Rosmarinus officinalis* EOs in cage molecules namely Cyclodextrins (CDs) and clays, in order to develop natural systems with potential applications in the postharvest control of the date moth *Ectomyelois ceratoniae*. It focused on four main research axes. The first part deals with the study of the chemical composition of rosemary essential oils collected from eight sites in Tunisia, the determination of their chemotypes as well as the evaluation of their fumigant potential. The second part was devoted to the preparation and characterization of CD (β-CD and its derivative HP-β-CD)/ guest (essential oils, and their major compounds: 1,8-cineole, α- pinene and camphor) inclusion complexes both in solution and in solid state. The insecticidal activity of essential oils and their components encapsulated in CDs was evaluated against the fifth instar larvae of *E. ceratoniae*. The third part aimed the development of clay / EOs formulations. Four clays were tested, namely smectite, bentonite, chlorite1 and chlorite 2. These formulations were characterized and their larvicidal potential by fumigation was evaluated. The last part was devoted to the evaluation of the effect of the fumigation with rosemary EOs and formulations on the physicochemical and organoleptic properties of treated dates. Results showed that CDs as well as clays could successfully encapsulate the essential oils, reduce their volatility, enhance their solubility and photo-stability and generate controlled release systems. In addition, the encapsulation maintained the insecticidal properties of EOs. On the other hand, this study showed that the fumigation of dates using encapsulated EOs in the CD or in clay preserves their organoleptic quality and improves their nutritional value and their physicochemical properties. This study suggests that the encapsulated EOs can be used in an IPM approach against pests of the stored dates.

Ayed, Fakher, 2022. Biological characterization of *Sclerotium rolfsii* and search for active biomolecules from various composts and their associated microorganisms. Doctorate Thesis in Agronomic Sciences (Phytiatry), INAT, University of Carthage, Tunis, Tunisia, 315 pp. (*Public Defense*: *14 July 2022*).

Following the emergence of stem and root rot disease induced by *Sclerotium rolfsii* on various crops, a biological characterization was carried out for some isolates collected. The study showed that 30°C is the most optimal temperature for pathogen mycelial growth whereas those comprised between 25 and 35°C corresponded more to the optima of production and germination of sclerotia. The pH values ranging between 6 and 7 were favorable for mycelial growth while mycelial biomass and production of sclerotia were highest between pH 4 and 7. Aeration is favorable to the production of sclerotia without any effect on their germination. Pathogen mycelial growth is optimal on Oat Meal Agar medium and moderate on Czapek-Dox, Malt Yeast Extract Agar, Yeast Dextrose Agar and Potato Dextrose Agar media that are more favorable to the production of sclerotia as Water Agar. As for the effects of the osmotic potential, the mycelial growth of the fungus and the production of sclerotia are optimum between -0,1 and -1,9 MPa and at -0,1 and/or at -1,1 MPa, respectively. Ammonium chloride is the nitrogen source the most favorable for mycelial growth whereas potassium nitrate and sodium nitrate have more favorized the production of sclerotia. Concerning their effects on the germination of sclerotia, L-arginine, ammonium chloride and L-asparagine had favored more this process. The study also showed that D-mannitol, maltose and D-glucose are the carbon sources the most appropriate for *S. rolfsii* growth and survival. Aiming to search for sustainable and efficient alternatives to control this fungus, five proportions of organic wastes were placed into piles in open conditions and followed all over the composting process that lasted eight months. Some physico-chemical, microbiological and phytotoxicity analyses were used for the determination of the maturation and the stability of the composts studied before their valorization for tomato production and the management of the target disease. Firstly, these composts and their extracts were evaluated for their antifungal activity against *S. rolfsii* using two confrontation techniques. At the end of this screening, the extract of the compost C4 was found to be the more active against pathogen mycelial growth and the germination of sclerotia. Tested as tomato cv. Rio Grande plants treatment, the majority of composts tested and their extracts, excepting C1, had limited stem rot severity (mainly C3 and C4 and their extracts) and had improved the growth of plants (C2, C3 and C4 and their extracts). The physico-chemical and microbiological characteristics of the selected composts and their richness in secondary metabolites seem to be involved directly and/or indirectly in disease suppression and plant growth biostimulation. Seventeen culturable actinobacterial isolates, issued from the most active composts (C3 and C4), were selected and screened for their suppressive and biostimulating potentials. The first screening, carried out in nursery, allowed the selection of 11 non phytopathogenic isolates active against the fungus. Five isolates had limited pathogen mycelial growth by more than 78% and the presence of bioactive secondary metabolites was confirmed through the test of the antifungal activity of their culture filtrates (20% v/v). Tomato plants treated with actinobacterial suspensions and culture filtrates of the selected isolates showed a decrease in disease severity compared to inoculated and untreated control where the isolates A5-3, A2-4, A3-4, A4-4 and A5-4 were found to be the most active in suppressing disease and in promoting plant growth. The 16S rRNA gene sequencing from these isolates revealed their affiliation to three genera i.e. *Streptomyces*, *Micromonospora* and *Saccharomonospora*. Results concerning the secondary metabolites involved in the plant growth stimulation showed that all isolates are able to produce the indole-3-acetic acid (AIA). The phosphate solubilization potential was confirmed for *Streptomyces* sp. A5-3, *S. palmae* A5-4, and Streptomyces sp. A4-4, respectively, that also

showed an important capacity to solubilize zinc. *Streptomyces* sp. A3-4, *S. palmae* A5-4, and *Micromonospora* sp. A3-3 are shown able to fix nitrogen. As for mechanisms involved in *S. rolfsii* suppression, siderophores production was demonstrated for *Saccharomonospora cyanea* A2-4 and *Streptomyces* sp. A4-4, the production of hydrocyanic acid was confirmed for A4-3, A5-3, A1-4, A4-4 and A6-4 isolates. Concerning the enzymatic activities, all isolates, excepting *S. cyanea* A2-4 and A8-3, showed positive chitinase activity, *Streptomyces* sp. A5-3 and *S. palmae* A5-4 exhibited important proteolytic and lipolytic activities, and *Streptomyces* sp. A5-3 showed the highest amylase and pectinase production potential as compared to the other target isolates.

Elbekkay, Mokhtar, 2022. Diversity, selection and agronomic value of local Cucurbitaceae cultivars collected from the oases in Southern Tunisia. Thesis in Biological Sciences. Center of Biotechnology of Borj Cedria & University of Tunis ElManar, Tunisia, 141 pp. (*Public Defense*: *15 July 2022*).

The oases in Tunisia are characterized by varied plant diversity subject to several agronomic, socio-economic and environmental constraints. As a result, they suffer from a loss of agrobiodiversity, which continues to accelerate, particularly for species of the Cucurbitaceae family which it represent one of the pillars of the oases cropping systems. Therefore, the exploration, preservation and enhancement of the oases phytogenetic resources is essential to guarantee the sustainability of these agrosystems. Thus, the objectives of the thesis work are (i) the evaluation of the diversity of vegetables species in the oases agrosystems and the collection for the ex-situ preservation of local cucurbits cultivars, (ii) the study of the genetic diversity and evaluation of agronomic potential and resistance to certain fungal and viral diseases in local cultivars of watermelon and melon.

The study of the diversity of vegetables species in the oases of Nefzaoua (Kébili) showed that 18 species of them have been identified, of which the Cucurbitaceae family represents 22% with the highest average specific cover of 202.89 m²/ha. Species richness, average recovery and Shannon Weaver index of vegetable crops are higher in modern private oases, compared to modern public oases and old oases, which is attributable to the availability of water. The old oases shelter the highest number of local cultivars of cucurbits, which shows their interest in the in-situ conservation of the local genetic heritage of these vegetable species. Thus, 261 cucurbit cultivars belonging to the melon, watermelon and squash species were collected, morphologically characterized and multiplied ex-situ in the IRA experimental plots. The study of the diversity of height local cultivars and two varieties Sugar Baby and Giza of watermelon was based on 15 morphological traits, which describe 67.85% of the total variability. The local cultivars are characterized by their precocity and their high average fruit weight compared to the controls. The genetic diversity of local cultivars and commercial varieties of watermelon was assessed using nine RAPD markers that showed a polymorphism rate of 98.4%. Principal coordinate analysis (PCoA) along the first three axes, which sum up 94.9% of the total variability, indicates a clear separation between commercial varieties and local cultivars, reflecting the genetic specificity of local biological resources. The study of correlations showed that seed weight and pericarp thickness are positively correlated with the geographical origin of local watermelon cultivars,

and seed weight and fruit acidity are positively correlated with some RAPD molecular markers.

The study of the diversity of 22 local cultivars of melon based on 32 morphological traits showed that the local cultivars of the South differ from the commercial variety Ananas. Indeed, the local cultivars of the South are characterized by their earliness with fruits of larger size and low sugar content. The genetic diversity of melon cultivars, assessed using four RAPD markers, showed a polymorphism rate about 95%. The PCA analysis according to the first three axes (54.7% of the total variability) indicates a clear separation between the Ananas variety and the local cultivars. Correlations between distance matrices of morphological data and RAPD molecular data showed that the fruit length, fruit diameter, size of pistil scar and maximum thickness of flesh were significantly correlated with several RAPD markers.

The evaluation of resistance to seven viruses (CMV, ZYMV, WMV, PRSV, MWMV, MNSV and TolCNDV) and two pathogenic fungi *Fusarium oxysporum* f. sp. *melonis* (FOM) and Podosphaera xanthii (powdery mildew) showed that the local cultivars of melon, watermelon and squash present sources of resistance for different viral and fungal strains to which the studied commercial varieties are sensitive. In melon, 19 cultivars presented a source of resistance to the MNSV-Ved virus and two cultivars to WMV-FR, in watermelon the cultivars P35 and P55 have a source of resistance to the MWMV virus and in squash a resistance to 5 viruses WMV-FR, PRSV-E2, MNSV-Ved, CMV-14, MWMV was observed in cultivar C65. The local melon cultivars from the South are susceptible to all five strains of powdery mildew. However, the northern local cultivars M120 and M122 showed resistance to strains Sm3 and 00Sm39 and the commercial variety Jaune Canari showed resistance to strains Sm3, S87-7 and 00Sm39. For resistance to FOM, five local melon cultivars presented resistance to race 1 while all the cultivars tested were susceptible to race 2.

The results of this thesis present a real practical impact through the creation and characterization of a local collection of three species of cucurbits which will be the starting point for the development of a selection and improvement program of cucurbits species in Tunisia.

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Photo of the cover page: ***Oryzeaphilus surinamensis*** (Courtesy *Abir Soltani*)

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