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Effects of exogenous abscisic acid on antioxidant system of salt tolerant and salt sensitive cotton cultivars

K.M. Kuldoshova*, A.A. Akhunov, N.R. Khashimova and J.F. Ziyavitdinov

Summary Salinity is one of the most imperative global problems that affect crop productivity on a large scale. Salinity impairs plant growth and development by imposing various stresses. Therefore it is vital to decode those stress factors and identify possible solutions to improve agriculture productivity. However, the adaptive mechanisms under saline conditions of glycophytes have not been studied. The present study was undertaken to determine the effects of exogenous abscisic acid (ABA) on salinity tolerance in cotton plants. Some patterns of resistance development were revealed on the seedlings of two cotton cultivars, a salt-tolerant (Gulistan) and a salt-sensitive one (C-4727). Moreover, the antioxidant potentials of these cultivars were compared. The activities of ascorbate peroxidase (APX), superoxide dismutase (SOD), as well as the quantity of endogenous ABA, malondialdehyde (MDA), and free proline (Pro) were determined in control and post treatment. Our results demonstrate significant differences between the salt- tolerant and sensitive cotton seedlings in response to saline stress, i.e., high levels of Pro and endogenous ABA, but lower MDA concentrations, and higher activity of APX and SOD for the salt-tolerant cultivar, Gulistan, as compared to the salt stress-sensitive cultivar C-4727.

Additional keywords: ascorbate peroxidase, free proline, *Gossypium hirsutum* L., lipid peroxidation, salinity, superoxide dismutase

Introduction

Soil salinity is a major environmental limitation to world agriculture affecting 800 million hectares of land throughout the world. This is over 6 % of the world's total land area, affected either by salinity (397 million hectares) or associated condition of sodicity (434 million hectares) (Munns, 2005). Salt stress alters various biochemical and physiological responses in plants and thus affects almost all plant processes including photosynthesis, growth, and development (Iqbal *et al*., 2006). In several crops negative effects of soil salinity have been well described. In tomato, salinity was determined to affect the crop yield, i.e., salt stress reduced dry and fresh weight of tomatoes and the biomass of tomato plants (Ghorbani *et al*., 2018, 2019). Studies on salinity stress will be a stride towards the urgent need of developing crop varieties possessing a higher growth rate and yield in salt-affected environments.

Phytohormones, as the main components of the plant regulatory system, play a key role not only in plant growth and morphogenetic processes but also in adaptive reactions associated with exposure to unfavorable factors (Hojin and Yong, 2015). To improve the adverse outcome of salinity stress on plant growth, diverse phytohormones are extensively used (Hojin and Yong, 2015). In this context, the plant hormone abscisic acid (ABA) is considered an important agent in the mechanisms of resistance and adaptation in plants against salt stress conditions (Bakhsh *et al*., 2011). Thus, ABA acts as a mediator in controlling adaptive plant responses to environmental stresses. It has been well documented that endogenous ABA accumulates in plants under abiotic stresses (Xiong *et al*., 2002). Nevertheless, ABA is involved in several other physiological processes, such as stomatal closure, embryo morphogenesis, development of seeds, synthesis of storage proteins and lipids (Rock and Quatrano, 1995), as well as germination, leaf senescence and defense against pathogens (Richardson *et al*., 1987). Additional-

Institute of Bioorganic Chemistry, Academy of Sciences of Uzbekistan, M. Ulughbek St. 83, Tashkent, Uzbekistan.
* Corresponding author: zarabarlos90@bk.ru

ly, exogenous application of ABA enhances the tolerance of plants to various stresses including cold, heat, drought, heavy metals, anoxia, and other environmental factors (Ahmad *et al*., 2010). These and other findings suggest that ABA has a great agronomic potential for improving the stress tolerance of important crops.

Enzymes of the antioxidative system play a considerable role in the resistance of the cotton plant to stress factors (Meloni *et al*., 2002). The antioxidant state of the plant is identified by the balance between prooxidant and antioxidant reactions occurring in cells (Mandhania *et al*., 2006). Characterization of antioxidant system functions is important to evaluate how the plant adapts to environmental changes (Mittal *et al*., 2012).

The present work examines the effect of exogenous ABA on enhancing salt resistance of a salt-tolerant (Gulistan) and a salt-sensitive (C-4727) cotton cultivar. The enzymic activities of superoxide dismutase **(**SOD) and ascorbate peroxidase (APX), and quantity changes of malondialdehyde (MDA), free proline and endogenous ABA in these cultivars were also studied to establish the differences in antioxidative potential.

Materials and methods

Plant materials and stress treatment

Seven-day-old seedlings of two cotton (*Gossypium hirsutum* L.) cultivars with varying degrees of salt tolerance, i.e., a salt-tolerant (Gulistan) (Akhmedov *et al*., 2022) and a salt-sensitive one (C-4727) (Babaeva *et al*., 2020), were used in this study. The cotton seeds were treated with concentrated sulfuric acid and washed under tap water for 10-15 minutes. The seeds were further soaked in distilled water for 12 hours. The swollen seeds were wrapped in paper rolls and incubated for germination for 7 days in the moist chamber at 27°C. After the 7th day of germination, half of seedlings remained in the distilled water as control. The second half of the seedlings were divided into 4 parts and kept in saline solutions: 1% NaCl; 1% NaCl + 10^{-7} M ABA; 4% NaCl; 4% NaCl + 10^{-7} M ABA. The cotton seedlings were homogenized in liquid nitrogen and used for biochemical analysis.

Extraction of enzyme extract

About 200 mg of seedlings of both varieties were homogenized in 5 ml of 50 mM phosphate buffer (pH 7.8) containing 1% polyvinylpyrrolidone (PVP), 1 mM ascorbic acid and 1 mM phenylmethylsulphonyl fluoride (PMSF) as described by Moran *et al.* (1994). After centrifugation at 7, 000 x g for 15 min at 4-8°C the supernatant was dialyzed against the same extraction buffer to be used as an enzyme extract.

Ascorbate peroxidase

The APX was assayed according to Nakano and Asada (1981). To a reaction mixture containing 1 ml 50 mM phosphate buffer (pH 7.0), 0.2 mM ascorbic acid, 0.2 mM ethylenediaminetetraacetic acid (EDTA) and the enzyme, 20 mM H_2O_2 was added. The absorbance was recorded at 290 nm on the UV-Vis spectrophotometer (ELICO, India) (extinction coefficient of 2.8 mM^{-1} $1cm^{-1}$ at 30s intervals up to 7 min). A correction was made for the low, non-enzymatic oxidation of ascorbic acid by H_2O_2. The specific activity of the enzyme was expressed as units/mg protein.

Superoxide dismutase

The SOD activity was determined as described by Giannopolitis and Ries (1977). Assays were carried out on a rotating plate under illumination. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of p-nitro blue tetrazolium chloride reduction at 560 nm.

Total protein determination

To measure the activity of antioxidative enzymes, such as APX and SOD, to make a standard curve and to read the absorbance, quantitation of total protein content of samples was performed according to the Lowry method (1951) using bovine serum albumin

(BSA; Sigma-Aldrich, USA).

Lipid peroxidation

The Rogozhin *et al.* (1998) methodology of thiobarbituric acid (TBA) reaction was followed to determine the level of lipid peroxidation in terms of MDA concentration in the samples. For these analyses, about 500 mg of seedlings were homogenized with 5 ml of 0.1 % trichloroacetic acid (TCA) and then centrifuged at 7, 000 x g for 15 min. For every 1 ml of aliquot, 4 ml of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95°C for 30 min and then cooled immediately on an ice bath. The resultant mixture was centrifuged at 7, 000 x g for 15 min, and the absorbance of the supernatant was recorded at 532 nm. The concentration of MDA was calculated by using an extinction coefficient of 156 $mM^{-1}cm^{-1}$.

Determination of proline

The method of Bates *et al.* (1973) was used for the determination of proline. About 500 mg of leaf tissue was homogenized in 5 ml of 3% aqueous sulphosalicylic acid. The homogenate was centrifuged at 7, 000 x g for 15 min. In a test tube, 1 ml of the extract was mixed with 2 ml of acid-ninhydrin containing 1.25 g of ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid. Subsequently, 2 ml of glacial acetic acid was added and the mixture was heated for an hour at 100°C. Later, 4 ml of toluene was used to extract the reaction mixture. Further, the reaction mixture was vortexed for 20-25 sec. The chromophore containing toluene was aspirated from the aqueous phase, and the absorbance of the toluene layer was measured at 520 nm with toluene as blank. Values were expressed as millimole per gram of fresh weight.

Abscisic acid quantitation by HPLC

The quantitation of ABA was performed using Agilent Technologies 1200 series for HPLC (USA). The column 250 x 4.6-mm of strong anion exchanger (5- µm spherical particles of Adsorbosphere SAX, Alltech Associates, USA) was used for the next step of purification. The samples were dissolved in the mixture of acetonitrile (30%) and 0.1% phosphoric acid (70%). Acetonitrile was a mobile phase solvent A; trifluoroacetic acid was used as a solvent B at 1 ml min-1. The solvent B concentration gradient was 30–50% at 10 min, 50–50% at 5 min, and 50–30% at 2 min. A Waters model 440 fixed-wavelength UV absorbance detector (Waters Corporation, USA) (254 nm) was used to detect ABA.

Statistical analyses

The results were statistically analyzed using Graph Pad Prism 8. Values are expressed as mean ± SD for three biological replicates per treatment. The analysis was conducted using T-test at P= 0.05.

Results and discussion

To assess the activity of antioxidant systems of a salt-tolerant (Gulistan) and a salt-sensitive (C-4727) cotton cultivar, we determined the effects of the main enzymes of their antioxidant protective system, i.e., the APX and SOD. The response of this system to salinization is known to depend on the variety and type of plant, its physiological state, as well as on the level and duration of stress (Miguel *et al*., 2006). In our study, we managed to identify the direct correlation between the levels of antioxidant system induction and the degree of plant resistance to salinization.

APX is a key enzyme in the ascorbate-glutathione cycle playing a critical role in H_2O_2 scavenging (Chawla *et al.,* 2013). APX uses ascorbate as an electron donor to reduce H_2O_2 to water. Our findings showed that the resistant cultivar had a much higher APX level in 1 hour (32.23 units) as compared to the sensitive cultivar C-4727 (20.6 units) (Table 1). There was no appreciable change in APX activity in the tolerant variety, but the sensitive one demonstrated APX increase at both levels of salinity stress. The 4% NaCl solution enhanced APX activity in C-4727, but it was much lower than in the tolerant cultivar. These results are supported by Benavides *et*

Table 1. The exogenous effect of ABA on the activities of APX and SOD in seedlings of two cotton varieties**,** Gulistan (salt-tolerant) and C-4727 (salt-sensitive), under conditions of NaCl salinity for 1 h and 24h.

Samples	Gulistan (salt-tolerant)		C-4727 (salt-sensitive)	
	1 Hour	24 Hours	1 Hour	24 Hours
APX, units/mg protein				
Control	27.6 ± 1.2c	25.4 ± 1.2c	22.7 ± 1.0d	21.0 ± 0.5d
1% NaCl	32.2 ± 1.4b	34.0 ± 1.5b	20.6 ± 0.9d	16.0 ± 0.7d
1% NaCl + ABA	39.4 ± 1.5a	30.7 ± 1.1c	24.6 ± 1.2d	20.5 ± 0.9d
4% NaCl	32.3 ± 1.0b	28.8 ± 1.2c	21.0 ± 1.0d	26.1 ± 1.2c
4% NaCl + ABA	38.7 ± 1.3a	33.3 ± 1.5b	22.1 ± 1.0d	23.0 ± 1.1d
SOD, units/mg protein				
Control	4.0 ± 0.1c	3.9 ± 0.1c	2.7 ± 0.1d	2.7 ± 0.1d
1% NaCl	5.5 ± 0.2b	5.1 ± 0.2b	3.0 ± 0.1d	3.5 ± 0.1d
1% NaCl + ABA	6.3 ± 0.2b	6.0 ± 0.3b	3.4 ± 0.1d	3.2 ± 0.1d
4% NaCl	3.9 ± 0.1d	6.2 ± 0.2b	4.6 ± 0.2c	2.6 ± 0.1d
4% NaCl + ABA	4.4 ± 0.2c	9.1 ± 0.4a	5.1 ± 0.2b	4.1 ± 0.2c

Means in each column followed by the same letter(s) do not differ significantly at $P \leq 0.05$.

al. (2000) who reported a more significant elevation of the APX levels in salt-tolerant potato clones than the one in a salt-sensitive clone. Similarly, Meneguzzo *et al.* (1999) observed a greater increase in APX activity in a salt-sensitive cultivar than in a salt-tolerant one of wheat when exposed to NaCl stress. With the increase in salt stress, APX activity also increased in wheat (Heidari and Mesri 2008), cotton (Desingh and Kanagaraj 2007), stevia (Omran *et al*., 2021) and sea rocket (Amor *et al*., 2007) suggesting that high level of APX and salt-induced increase in APX activity could impart tolerance by detoxifying H_2O_2 generated upon exposure of plants to saline conditions.

Regarding the effect of exogenous ABA, the activity of APX in the seedlings of the salt-tolerant cotton cultivar was significantly higher in samples treated with 1% NaCl + ABA, as compared with the ones treated with NaCl (39.43 units versus 32.23 units) while the control sample exhibited a significantly lower APX activity (27.6 units) (Table 1). Our results suggest that oxidative stress can play a key role in the salt tolerance of the seedlings of a cotton cultivar under the salt stress; our salt-tolerant cultivar, Gulistan, turned out to have more efficient antioxidant protection promoting resistance to the oxidative stress.

The SOD activity increased progressively with the increase of salinity in the salt-resistant cultivar but receded in the sensitive one (Table 1). After 1 and 24 hours in 1% NaCl solution, the level of SOD activity was significantly higher in the salt-tolerant cultivar than the one in the salt-sensitive cultivar (5.53, and 5.1 units in Gulistan and 3.0 and 3.55 units in C-4727, respectively). After 24 hours in 4% NaCl + ABA solution, the increase of the enzyme activity was observed in both cultivars. Possibly, the SOD activity increase allows reducing lipid peroxidation under higher salt stress. These observations are in agreement with those reported earlier in *Solanum tuberosum* (Benavides *et al*., 2000), *Brassica juncea* (Chawla *et al.,* 2012) and *Najas graminia* (Rout and Shaw, 2001) where the salt-tolerant cultivars had a higher level of the enzyme activity compared to that in the salt-sensitive ones. Similar to the present findings, the SOD activity increased in the salt-tolerant cultivars but reduced in the salt-sensitive cultivar of wheat (Mandhania *et al*., 2006), cotton (Meloni *et al*., 2002)

and *Catharanthus roseus* (Jaleel, 2009). Contrarily, salinity has been reported to stimulate the SOD activity in both salt-tolerant and salt-sensitive cultivars of *Brassica juncea* with a higher level in the salt-tolerant ones (Chawla *et al*., 2012). An increase in the SOD activity upon salinization in leaves of all the salt-tolerant cotton cultivars could accelerate the dismutation of superoxide ions generated upon salt-treatment, which may allow these varieties to survive under oxidative stress (Desingh and Kanagaraj, 2007). In the salt-sensitive cultivar, reduction in the SOD activity would limit its metabolic capacity to withstand oxidative stress.

Under high salinity stress, the increase of reactive oxygen in plant species leads to lipid peroxidation (LPO) in the cell membrane. MDA is the main product of membrane lipid peroxidation when plants are under salt stress, and its levels reflect the degree of cell membrane damage (Mittal *et al*., 2012). Therefore, MDA concentrations can be a marker of a plant salt tolerance. In our study, MDA concentrations were found to lower by 8% at salinization after 1 hour in a 1% NaCl solution in Gulistan variety (Fig. 1). After 24-hour exposure to 1% NaCl, we observed a more significant decrease of MDA in Gulistan cultivar (52%). When ABA was added, a considerable reduction in MDA concentrations occurred (16.7%). After 1-hour and 24-hour exposures to 1% and 4% NaCl, MDA concentrations in Gulistan cultivar declined by 16% and 9.2%, respectively. ABA declined the MDA concentrations by 30.5% and 21%, respectively. Hence, salt resistance seems to stimulate the capability of protection from oxidative damage by both exposures. In contrast, in the salt-sensitive C-4727 cultivar, MDA concentrations increased considerably at any degree of salinization (Fig. 3). When ABA was added, further reduction in MDA was observed which was more significant than the one observed in the salt-tolerant cultivar. Increased MDA levels in tomato leaves under different salinity stress (Ghorbani *et al*., 2018) support our results. Our findings demonstrate that Gulistan variety is more resistant to salt stress than C-4727. The accumulation of lipid peroxidation products may not occur in the seedlings of Gulistan due to higher activity of antioxidant enzymes.

A considerable increase in the concentrations of proline took place under the effect of 1% NaCl within 1 hour in the salt-resistant Gulistan cultivar (Fig. 2). The accumulation

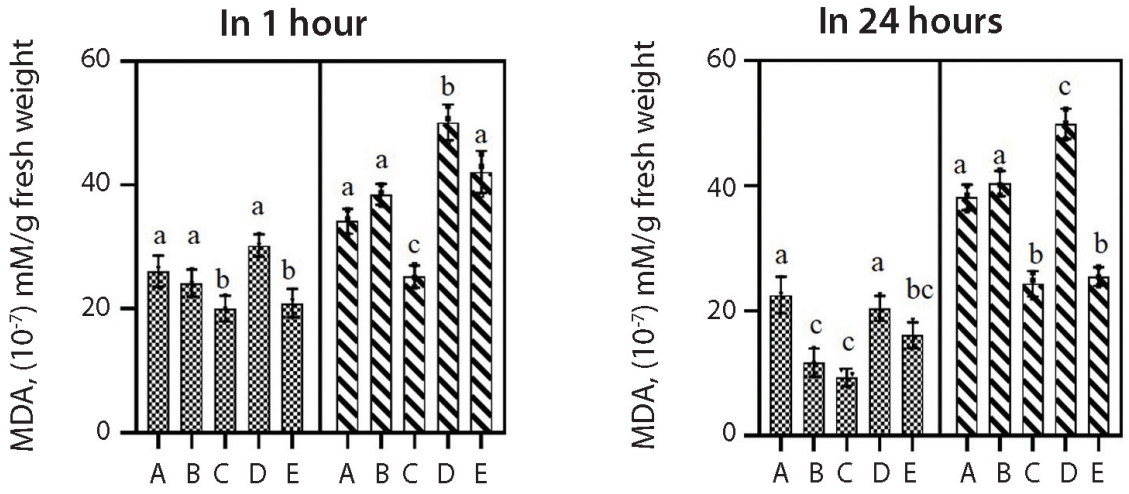

Figure 1. Effect of exogenous ABA on MDA concentrations in cotton seedlings under conditions of NaCl salinity for 1h and 24h.

Note: ▩ - salt-tolerant Gulistan cotton cultivar; ◪ - salt- sensitive C-4727 cotton cultivar; A: Control; B: 1% NaCl; C: 1% NaCl + ABA; D: 4% NaCl; E: 4% NaCl + ABA. Means with the same letter(s) on top of the columns do not differ significantly.

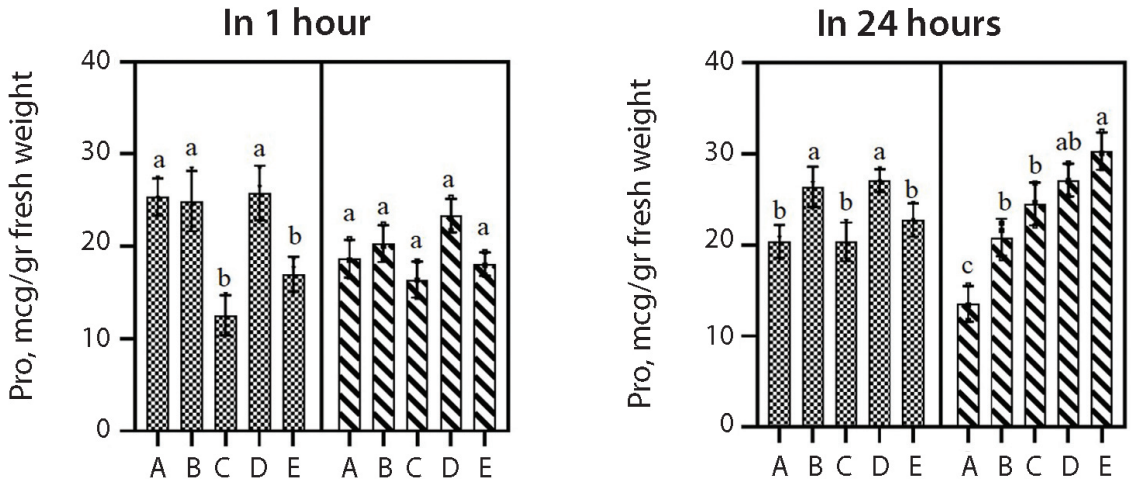

Figure 2. Effect of exogenous ABA on concentration of free proline in cotton seedlings under conditions of NaCl salinity for 1h and 24h.

Note: ▩ - salt-tolerant Gulistan cotton cultivar; ◪ - salt- sensitive C-4727 cotton cultivar; A: Control; B: 1% NaCl; C: 1% NaCl + ABA; D: 4% NaCl; E: 4% NaCl + ABA. Means with the same letter(s) on top of the columns do not differ significantly.

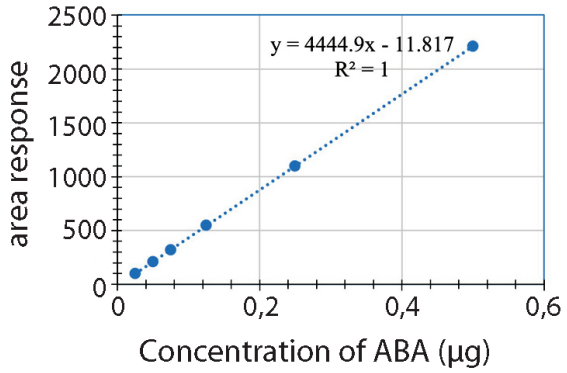

Figure 3. Calibration curve for determination of endogenous ABA concentration.

of proline under salinity stress has been reported in *Pisum sativum* (Najafi *et al*., 2006), *Brassica juncea* (Rais *et al*., 2013) and *Triticum aestivum* (Ashfaque *et al*., 2014). Proline performs a protective function against salinity stress in plants (Kishor *et al*., 2005; Verbruggen and Hermans, 2008). It acts as a compatible osmolyte, enzyme protectant, free radical scavenger, cell redox balancer, cytosolic pH buffer and stabilizer for subcellular structures (Kishor *et al*., 2005; Verbruggen and Hermans, 2008) to bring about salinity tolerance. Proline could also act as a major source of energy and nitrogen during immediate post-stress metabolism. Accumulating in plants, proline supplies energy for their growth and survival, thereby inducing salt tolerance (Silveira *et al*., 2003). Similar findings were reported by Hassine and Lutts (2010). The exogenous ABA was found to decrease the amount of proline after 1-hour exposure in 1% and 4% NaCl solutions, in both cultivars. After 24 hours, 1 and 4% salinization caused a rapid increase in proline levels (Fig. 2). According to Nikolaeva *et al*. (2015), exogenous ABA inhibits the rate of synthesis of proline or stimulates its degradation intensity in saline conditions.

In our study, a calibration curve was used to determine the concentration of endogenous ABA (Fig. 3). The treatment with 1% and 4% NaCl solutions led to a significantly higher accumulation of endogenous ABA in cotton seedlings in 1-hour and 24-hour exposures (Fig. 4). Upon addition of exogenous ABA, the concentration of endogenous ABA increased in the seedlings of Gulistan in 1 hour by 15% (1% NaCl+ABA) and by 20% (4% NaCl+ABA). After 24 hours, endogenous ABA concentration doubled in the seedlings exposed to 1% NaCl, while no significant changes were observed in the seedlings treated with 4%NaCl. In the control seedlings of Gulistan cultivar, high concentrations of endogenous ABA were observed, i.e., after 1-hour

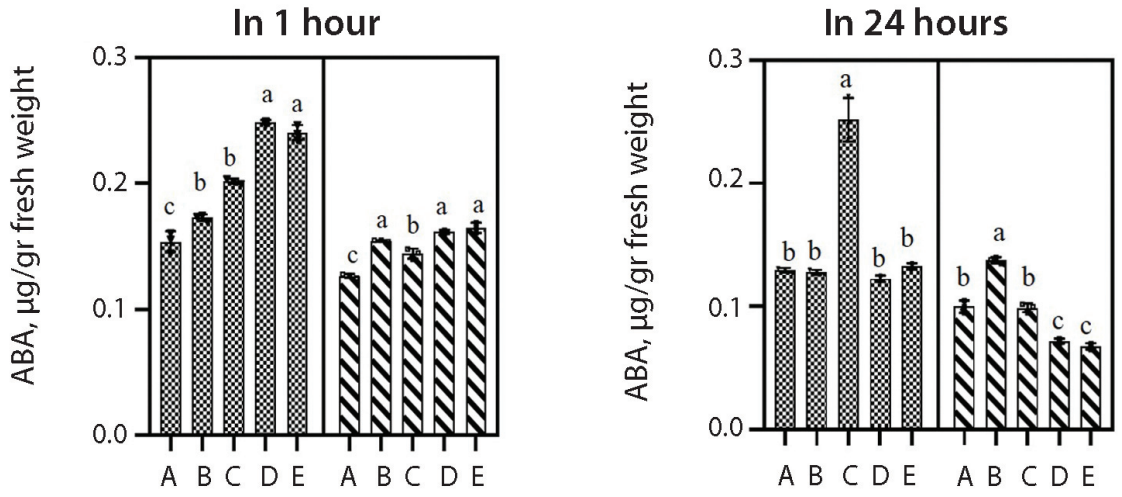

Figure 4. Effect of exogenous ABA on concentration of endogenous abscisic acid in cotton seedlings under conditions of NaCl salinity for 1h and 24 h.

Note: ▩ - salt-tolerant Gulistan cotton cultivar; ◥ - salt- sensitive C-4727 cotton cultivar; A: Control; B: 1% NaCl; C: 1% NaCl + ABA; D: 4% NaCl; E: 4% NaCl + ABA. Means with the same letter(s) on top of the columns do not differ significantly.

exposure to 4% NaCl, the ABA concentration increased by 61.7% on average. Prolonged exposure of plants to salinization reduced their survival and the level of endogenous ABA, especially at higher concentrations of salts. The resistance of cotton to the short-term effect of chloride salinization is associated with an increased level of ABA. It should be emphasized that the accumulation of endogenous ABA in cotton seedlings under salinization with low concentrations of salt was temporary, starting to decline with the increase in salt concentration. The exposure of plants to high salt stresses is known to rapidly induce a proportional increase in the endogenous level of ABA (Kang *et al*., 2005) and the expression of its biosynthetic genes in the plant. The elevated endogenous ABA helps plants to acclimate under lower water availability by closing stomata and accumulating numerous proteins and osmoprotectants for an osmotic adjustment (Hojin Ryu and Yong-Gu Cho, 2015). Talanova *et al*., 1994 demonstrated that in plants grown in saline soil endogenous ABA manifests itself either as a factor that triggers the formation of increased resistance or as one of the direct participants providing an increase in resistance in the first hours of exposure to this factor.

Conclusions

Our work demonstrated significant differences in the response of sensitive and resistant cotton cultivars to salt stress, which are closely associated with differences in the activity of antioxidant enzymes and the concentrations of MDA. The resistance of cotton cultivars to salinity is associated with the high efficiency of the enzymatic system for neutralizing the reactive oxygen intermediates, thus increasing the oxidation-reduction homeostasis and the preservation of the cell components. The introduction of exogenous ABA can change the intensity of LPO as well as the activity of antioxidant enzymes in cotton, making a possible decrease of lipid peroxidation, depending on the sensitivity of the cultivars, the salinity levels and the exposure. Overall, our results indicate an important role of ABA in tolerance to salt stresses and provide new insights into the possible biochemical mechanism of enhanced salt tolerance.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Literature Cited

Ahmad, P., Jaleel, C.A., Salem, M.A., Nabi, G. and Sharma, S. 2010. Roles of enzymatic and non-enzymatic antioxidants in plants during abiotic stress. *Critical Reviews in Biotechnology*, 30: 161-175.

Akhmedov, D.Kh., Jabborov, J.S. and Mannopov R.S. 2022. IOP Conf. Ser.: *Earth Environmental Science* 1068 012027.

Babaeva, D., Esanov, R., Akhunov, A., Gafurov, M., Khashimova, N. and Matchanov, A. 2020. Biological activity of the supramolecular complex of glycyrrhizic and salicylic acids. *Chemistry of Natural Compounds*, 56: 278-281.

Bakhsh, I., Awan, I., Sadiq, M., Niamatullah, M., Zaman, Ku. and Aftab, M. 2011. Effect of plant growth regulator application at different growth stages on the economical yield potential of coarse rice *Oryza Sativa* L. *Journal of Animal and Plant Science,* 21: 612-616.

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

Benavides, M.P., Marconi, P.L., Gallego, S.M., Comba, M.E. and Tomaro, M.L. 2000. Relationship between antioxidant defence systems and salt-tolerance in *Solanum tuberosum*. *Journal Australian Journal of Plant Physiology*, 27: 273-278.

Desingh, R. and Kanagaraj, G. 2007. Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. Journal General and Applied Plant Physiology, 33: 221-234.

Chawla, S., Jain, S. and Jain, V. 2012. Salinity induced oxidative stress and antioxidant system in salt-tolerant and salt-sensitive cultivars of rice (*Oryza sativa* L.). *Journal Plant Biochemistry and Biotechnology,* 22: 27-34.

Ghorbani, A., Razavi, S.M., Omran, V.O.G. and Pirdashti, H. 2018. *Piriformospora indica* inoculation alleviates the adverse effect of NaCl stress on growth, gas exchange and chlorophyll fluorescence in tomato (*Solanum lycopersicum* L.). *Journal of Plant Biology*, 20: 729-736.

Ghorbani, A., Razavi, S.M., Omran, V.O.G. and Pirdashti, H. 2018. *Piriformospora indica* alleviates salinity by boosting redox poise and antioxidative potential of tomato. *Russian Journal Plant Physiology,* 65: 898-907.

Ghorbani, A., Omran, V.O.G., Razavi, S.M., Pirdashti, H. and Ranjbar, M. 2019. *Piriformospora indica* confers salinity tolerance on tomato (*Lycopersicon esculentum* Mill.) through amelioration of nutrient accumulation, K^+/Na^+ homeostasis and water status. *Plant Cell Reports,* 38: 1151-1163.

Giannopolitis, N. and Ries, S.K. 1977. Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiology,* 59: 309-314.

Greenway, H. and Munns, R. 1980. Mechanisms of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology*, 31: 149-190.

Hassine, A.B. and Lutts, S. 2010. Differential responses of saltbush *Atriplex halimus* L. exposed to salinity and water stress in relation to senescing hormones abscisic acid and ethylene. *Journal of Plant Physiology*, 167: 1448-1456.

Heidari, M. and Mesri, F. 2008. Salinity effects on compatible solutes, antioxidants enzymes and ion content in three wheat cultivars. *Pakistan Journal of Biological Sciences,* 11: 1385-1389.

Hojin, R., and Yong, G.Ch. 2015. Plant hormones in salt stress tolerance. *Journal Plant Biology*, 58: 147-155.

Iqbal, M., Ashraf, M, Jamil, A. and Rehman, S. 2006. Does seed priming induce changes in the levels of some endogenous plant hormones in hexaploid wheat plants under salt stress. *Journal of Integrative Plant Biology,* 48: 181-189.

Jaleel, C.A. 2009. Soil salinity regimes alters antioxidant enzyme activities in two varieties of *Catharanthus roseus*. *Journal of the Botanical Research Institute of Texas*, 2: 64-68.

Kang, D., Seo, Y., Lee, J.D., Ishii, R., Kim, K.U., Shin, D.H., Park, S.K. and Lee, I. 2005. Jasmonic Acid differentially affects growth, ion uptake and abscisic acid concentration in salt-tolerant and salt-sensitive rice cultivars. *Journal of Agronomy and Crop Science*, vol. 191: 273–282.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with folin phenol reagent. *Journal Biological Chemistry*, 193: 265-275.

Mandhania, S., Madan, S. and Sawhney, V. 2006. Antioxidant defense mechanism under salt stress in wheat seedlings. *Journal Biologia Plantarum*, 50: 227-231.

Meloni, D.A., Oliva, M.A., Martinez, C.A. and Cambraia, J. 2002. Photosynthesis and activity of superoxide dismutase and glutathione reductase in cotton under salt stress. *Journal of Environmental and Experimental Botany*, 49: 69-76.

Meneguzzo, S., Navari-Izzo, F. and Izzo, R. 1999. Antioxidative responses of shoots and roots of wheat to increasing NaCl concentration. *Journal of Plant Physiology*, 155: 274-280.

Miguel, A.T., Jonathan, D.G. and Jeffery, L. D. 2006. Reactive oxygen species signaling in response

to pathogens. *Journal Plant Physiology*, 141: 373–378.

Mittal, S., Kumari, N. and Sharma, V. 2012. Differential response of salt stress on *Brassica juncea* photosynthetic performance, pigment, proline D1 and antioxidant enzymes. *Journal Plant Physiology Biochemistry*, 54: 17-26.

Mitteler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Journal Trends in Plant Science*, 7: 405-410.

Moran, J.F., Becana, M., Iturbe-Ormaetxe, I., Frechilla, S., Klucas, P. and Aparicio-Tejo, R.V. 1994. Drought induced oxidative stress in pea plants, *Journal Planta*, 194: 346-352.

Munns, R. 2005. Genes and salt tolerance: bringing them together. *Journal New Phytologist*. 167: 645-663.

Nakano, Y. and Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiology*, 22: 867-880.

Nikolaeva, M.K., Maevskaya, S.N. and Voronin, P.Y. 2015. The activity of antioxidant and osmoprotective systems and photosynthetic gas exchange of seedlings of corn under drought conditions. *Plant Physiology*, 62: 340-348.

Omran, V.O.G., Ghorbani, A. and Otaghsara, S.A. 2021. Melatonin alleviates NaCl-induced damage by regulating ionic homeostasis, antioxidant system, redox homeostasis, and expression of steviol glycosides-related biosynthetic genes in *in vitro* cultured *Stevia rebaudiana* Bertoni. *In Vitro Cellular and Developmental Biology – Plant*, 57: 319-331.

Ozgur, R., Uzilday, B., Sekmen, A.H. and Turkan, I. 2013. Reactive oxygen species regulation and antioxidant defence in halophytes. *Journal Functional Plant Biology,* 40: 832-847.

Richardson, M., Valdez-Rodriguez, S. and Blanco-Lahra, A. 1987. A possible function for thaumatin and a TMV-induced protein suggested by homology to a maize inhibitor. *Journal Nature*, 327: 432-438.

Rock, C. and Quatrano, R.S. 1995. The role of hormones during seed development. P. Davies (Ed.), plant hormones, physiology, biochemistry and molecular biology *Dordrecht: Kluwer Academic Publishers,* pp. 671-697.

Rogozhin, V.V., Kurilyuk, T.T. and Kershengolts, B.M. 1998. A method for determining the concentration of malondialdehyde using thiobarbituric acid. *Patent of the Russian Federation* No. 2112241.

Rout, M.P. and Shaw, B.P. 2001. Salt tolerance in aquatic macrophytes: Possible involvement of the antioxidative enzymes. *Journal Plant Science*, 160: 415-423.

Seo, H.S., Kim, S.K., Jang, S.W., Choo, Y.S., Sohn, E.Y. and Lee, I.J. 2005. Effect of jasmonic acid on endogenous gibberellins and abscisic acid in rice under NaCl stress. *Journal Biologia Plantarum*, 49: 447-450.

Silveira, J., Almeida, V. R., De Da Rocha, I., Moreira, A., De Azevedo, M. R. and Oliveira, J.T. 2003. Proline accumulation and glutamine synthetase activity are increased by salt-induced proteolysis in cashew leaves. *Journal Plant Physiology*, 160: 115-123.

Xiong, L., Schumaker, K. and Zhu, J.K. 2002. Cell signaling during cold, drought and salt stress. *Journal Plant Cell*, 14: 165-183.

Talanova, V.V. and Titov, A.F. 1994. Endogenous abscisic acid content in cucumber leaves under the influence of unfavourable temperatures and salinity. *Journal of Experimental Botany*, 45: 1031-1033.

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Επιδράσεις του εξωγενούς αμπσισικού οξέος στο αντιοξειδωτικό σύστημα ανθεκτικών και ευαίσθητων στην αλατότητα ποικιλιών βαμβακιού

K.M. Kuldoshova, A.A. Akhunov, N.R. Khashimova και J.F. Ziyavitdinov

Περίληψη Η αλατότητα είναι ένα από τα πιο επιτακτικά προβλήματα παγκοσμίως που επηρεάζουν σε μεγάλο βαθμό την παραγωγικότητα των καλλιεργειών. Η αλατότητα μειώνει την αύξηση και ανάπτυξη των φυτών προκαλώντας ποικίλες καταπονήσεις. Επομένως είναι ζωτικής σημασίας να αποκωδικοποιηθούν αυτοί οι παράγοντες καταπόνησης και να βρεθούν πιθανές λύσεις για τη βελτίωση της γεωργι-

κής παραγωγής. Εντούτοις, δεν έχουν μελετηθεί οι μηχανισμοί προσαρμογής των γλυκοφύτων σε συνθήκες αλατότητας. Η παρούσα μελέτη διενεργήθηκε με σκοπό τον προσδιορισμό των επιδράσεων του εξωγενούς αμπσισικού οξέος (ABA) στην αντοχή φυτών βαμβακιού στην αλατότητα. Διαπιστώθηκαν μερικοί μηχανισμοί ανάπτυξης αντοχής στην αλατότητα σε σπορόφυτα δύο ποικιλιών βαμβακιού, μιας ανθεκτικής (Gulistan) και μιας ευαίσθητης (C-4727) στην αλατότητα. Επιπλέον, συγκρίθηκαν οι αντιοξειδωτικές ιδιότητες αυτών των ποικιλιών. Έγινε προσδιορισμός της δράσης της ασκορβικής υπεροξειδάσης (APX), της δισμουτάσης του υεροξειδίου του υδρογόνου (SOD), καθώς και της ποσότητας του ενδογενούς ABA, της μαλονδιαλδεΰδης (MDA) και της ελεύθερης προλίνης (Pro) στο μάρτυρα και μετά την επέμβαση. Τα αποτελέσματα δείχνουν σημαντικές διαφορές μεταξύ ανθεκτικών και ευαίσθητων στην αλατότητα σποροφύτων βαμβακιού ως απόκριση των φυτών στην καταπόνηση από υψηλή συγκέντρωση αλάτων. Στην ανθεκτική ποικιλία Gulistan διαπιστώθηκαν υψηλότερα επίπεδα Pro και ενδογενούς ABA, αλλά χαμηλότερες συγκεντρώσεις MDA και μεγαλύτερη δραστηριότητα APX και SOD, σε σύγκριση με την ευαίσθητη ποικιλία C-4727.

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Repellent effect of *Gardenia jasminoides* ethanol extracted oil on *Blattella germanica* and *Monomorium pharaonis*

T.A. Wagan[1, 2], C. Hamada[1], W. Cai[1], H. Hongxia[1], G.S. Solangi[3*] and R.H. Lyu[4*]

Summary The study examined the repellency of *Gardenia jasminoides* ethanol-extracted oil against the German cockroach, *Blattella germanica*, and the pharaoh ant, *Monomorium pharaonis*, which are serious pests in areas of public health hygiene. For the repellency tests, 31.4 µg of the oil was applied per cm^2 on one half of filter paper discs (9 or 15 cm diameter for the ant and cockroach, respectively), whereas the other half was treated as control (DMSO + Tween). Repellency effects were observed 1, 2, 3 and 4 h after the insect release. The oil showed high repellency against all life stages of cockroaches and worker ants. The maximum repellency was observed for the cockroach adults (81.7 ± 3.1%) followed by the fourth, third and second nymphal stages (76.7 ± 4.2, 75.0 ± 3.4, and 56.7 ± 8.4%, respectively), after 1h exposure. The repellence effect was strong against worker ants (78.3 ± 4.8%) after 1 h exposure. The repellence effect can last at least four hours for both species. Analysis of *Gardenia* oil with Gas chromatography-mass spectrometry identified 14 major chemical components.

Additional keywords: ants, cockroaches, ethanol-extracted oil, *Gardenia jasminoides*, repellent

Introduction

German cockroaches (*Blattella germanica*) and pharaoh ants (*Monomorium pharaonis*) are the most common indoor and outdoor pests in urban areas; both insect species thriving in most of the world's big cities, wherever they have access to water and food (Bosik, 1997; Hansen, 2011; Mahmoud *et al*., 2013). Numerous findings have demonstrated that both species cause direct damage by infesting food and structural damage in homes, and indirectly as vectors for many disease-producing pathogens such as fungi, bacteria, viruses, protozoa, and helminths, exposing humans to a contamination risk in residential and public health hygiene areas (Dobesh *et al.,* 1993, Fu *et al*., 2009, Pavlik and Falkinham, 2009; Menasria, 2009). Some cockroach species emit bad odor from their body, which makes the surrounding environment to smell bad for a long time (Cochran, 1982).

For the control of these species infestation, synthetic chemicals have been introduced in households, hospitals, stores, and gardens, because of their rapid action for repelling, killing, or paralyzing the insects (Kostyukovsky, 2002). Although synthetic pesticides timely kill or repel the targeted insect species, the extensive and regular application of these substances increases the risk of resistance development, environmental pollution and damaging effects on biodiversity, bees and pollination, water resources, and food security (Parveen and Dhandapani, 2001; Malaj *et al*., 2014; Queyrel *et al.,* 2016).

Pest control by natural products such as plant essential oils is a promising alternative to chemicals, as their application is considered to be safe and environmentally friendly (Nerioa *et al.,* 2009). Most of the plant essential oils are not pest killers, but they can

[1] Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, 430070 China.
[2] Department of Plant Protection, Shaheed Zulfiqar Ali Bhutto Agricultural College, Dokri 77060, A constitute College of Sindh Agriculture University, Tandojam, Pakistan.
[3] Department of Entomology, Shaheed Zulfiqar Ali Bhutto Agricultural College, Dokri 77060, A constitute College of Sindh Agriculture University, Tandojam, Pakistan.
[4] Division of International Cooperation, Guangxi Academy of Agricultural Sciences, Nanning 530007, China.
* Corresponding authors: solangi_sarwar@yahoo.com; lvronghua99@126.com

repel insect pests and affect their growth. Moreover, they are cheap and non-toxic for other organisms (Geetha and Anitha, 2014). Due to these reasons, research on the insecticidal and repellent effects of essential oils on insects is increasing every day (Ayvaz *et al.,* 2010; Suthisut *et al.,* 2011; Faraone *et al*., 2015). The present study aims to investigate the repellency of *Gardenia jasminoides* ethanol-extracted oil against German cockroaches and pharaoh ants.

Material and Methods

Insect collection and rearing

German cockroaches and pharaoh ants were reared at the laboratory of Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory at the Huazhong Agricultural University, China (30.5931°N, 114.3054°E) at 25°C, 50% relative humidity (RH), and 14:10 h (L:D). The German cockroaches were originally collected from public kitchens and were reared on waste (fruit, biscuit, newspapers). The workers of pharaoh ants were reared on aphid honeydew provided by aphid colonies on maize crops.

Biopesticide formulations

Dry fruits of *G. jasminoides* were obtained from a franchise of the Beijing Tongrentang Group located in Wuhan, China. Methodology by Su *et al.* (2009) with some modifications was used for the extraction of the oil. Briefly, dried seeds were cleaned and dried for 3 days at 455°C to get constant weight. Once the plant material was dried, it was ground into powder and passed through a 40 mm mesh sieve. The powder was mixed in a brown bottle with a ratio of 1 g of plant powder and 5 mL of 95% ethanol for 7 days. The bottle was shaken twice a day for maximum dissolution and mixing the powder in the ethanol and placed in a dark room at 20–25°C. The ethanol was filtered, and the residual plant material was again diluted with ethanol at 2.5 mL of ethanol for 1 g of residuals and placed under the same conditions. After all the mixing and dissolving processes, both filtered solutions were mixed and dried in a rotary evaporator until all the liquid evaporated and glue-like consistency with an oil-type liquid on the surface appeared. The final product was collected in a brown bottle, weighed (159.28 g of crude oil was obtained from 589.54 g of seeds) and stored at 4°C.

For the preparation of the working solution, 0.05 g of *G. jasminoides* crude extract was dissolved in 0.3 mL of dimethyl sulfoxide (DMSO); 1% Tween 20 was added to the solution, and the final volume was adjusted to 5 mL at a final concentration of 10,000 ppm (10 mg/ml) by adding double-distilled water. For the control solution, 0.3 mL of DMSO and 1% Tween 20 were added to the water to make 5 mL.

Gas chromatography and mass spectrometry analysis

Gas chromatography-mass spectrometry (GC-MS) Varian 450-GC/320-MS (Varian, Inc., Walnut Creek, California) was used to separate and identify the chemical components of ethanol-extracted oil. The methodology followed was previously described by Caballero-Gallardo *et al.* (2014). The same column and analysis conditions were used for both the GC and MS. The spectrophotometer was equipped with a flame ionization detector and an HP-5ms capillary column (30 m × 0.25 mm × 0.25 µm). For the GC, the initial oven temperature was held at 60°C for 3 min, increased at 10°C/min to 180°C for 1 min, and then increased at 20°C/min to 280°C for 15 min. The injector temperature was maintained at 270°C. The samples (1 µL diluted to 1% with hexane) were injected with a split ratio of 1:10, and the column pressure was 100 kPa. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The electron ionization source of the detector had an electron energy of 70 eV. Spectra were scanned from 35 to 1,200 m/z at 2 scans per second. MS quad temperature was 150°C, the ion source temperature was 230°C, the transmission line temperature was 250°C, and the column pressure

was 100 kPa. Most constituents were identified from the gas chromatography using MANLIB, REPLIB, PMWTox3N, and Wiley (NIST 2011). The retention indices were determined in relation to a homologous series of n-alkanes (C8-C24) under the same operating conditions.

Ant and cockroach repellency test

An area preference test was performed on 9 cm diameter filter paper for ant bioassay and 15 cm filter paper for cockroaches in Petri dishes. Filter paper discs of either diameter were cut into two halves. With the help of a micropipette, 0.1 mL of the working solution was applied uniformly on one half of the filter paper disc, and the same volume of control solution was applied uniformly on the other half of the filter paper disc, to get a final concentration of 31.4 µg of the ethanol-extracted oil per cm^2. Both halves were placed at room temperature to dry for one hour. When dry, the two halves were placed into the Petri dishes. Six holes of 1 mm were made on the Petri dish cover for ventilation. Twenty pharaoh ant workers were released in the center of the Petri dishes using an aspirator. Twenty adult cockroaches were released in the center of the Petri dishes using a fine brush. All dishes were covered with black plastic tape (leaving space of 5 cm for ventilation) to provide darkness and kept in the same room as described for the rearing conditions. Individuals were counted on both treated and control halves of the filter paper 1, 2, 3, and 4 h after the insect release. The same experiment was repeated with the fourth, third, second and first nymphal stages of the cockroach. The repellency test was repeated using fresh insects. A total of 8 replications were conducted for each release time of the ant and 6 replications of each treatment combination (life stage x release time) of the cockroach.

For the calculation of the percentage of repellency (PR) the formula "PR = [(Nc-Nt)/20]*100%" was used, where Nc was the number of ants/cockroaches present in the control area, and Nt was the number of ants/cockroaches present on the treatment area (Liu *et al*., 2013).

Statistical analysis

A Chi-square test was conducted to compare the repellent effect in the treatment and control parts within the same developmental stage and exposure time. One-way ANOVA and Turkey test was used to achieve the comparisons of the means between the developmental stages within the same exposure time and between exposure times within the same developmental stage. Data were analyzed using SPSS (Ver. 20) at $\alpha = 0.05$ significance level. All the graphs were plotted in Sigma Plot 10.

Results

GC-MS analysis of the *G. jasminoides* oil

Ethanol-extracted oil of *G. jasminoides* was analyzed by GC-MS and 14 major chemical components were identified. The chemicals, their percentage, retention time, and formula are presented in Table 1 (also presented in Wagan *et al*., 2018).

Repellency of *G. jasminoides* oil to the German cockroach

The ethanol-extracted oil from *G. jasminoides* showed high repellency against all growth stages of German cockroach and pharaoh ant workers in the area preference test in Petri dishes.

Chi-square test showed a significant difference in repellency between the treated and the corresponding control area within each developmental stage and time exposure: in the adult stage at 1, 2, 3, and 4 h exposure: $\chi^2 = 48.02$, 31.72, 30.00, and 23.74, respectively, df = 1, $P < 0.01$; in the fourth nymphal stage at 1, 2, 3, and 4 h exposure: $\chi^2 = 41.34$, 29.56, 25.21 and 20.95, respectively df = 1, $P < 0.01$; in the third nymphal stage at 1, 2, 3, and 4 h exposure: $\chi^2 = 39.27$, 28.34, 20.95, and 18.37, respectively, df = 1, $P < 0.01$; in the second nymphal stage at 1, 2, 3, and 4 h exposure ($\chi^2 = 20.95$, 17.16, 13.82, and 10.89, respectively, df = 1, $P < 0.01$; in the first nymphal stage at 1, 2, 3, and 4 h: $\chi^2 =$

Table 1. GC-MS results of the ethanol-extracted oil from *Gardenia jasminoides*.

Components	Retention Time	Percentage of Total	Formula
Isolongifolene	10.10	0.48	$C_{15}H_{24}$
Thujopsene	10.37	0.24	$C_{15}H_{24}$
Diethyl phthalate	11.21	0.14	$C_{12}H_{14}O_4$
8.beta.h-cedran-8-ol	11.51	0.16	$C_{15}H_{26}O$
Alpha-bisabolol	11.81	0.10	$C_{15}H_{26}O$
n-Hexadecanoic acid	13.17	0.60	$C_{16}H_{32}O_2$
Hexadecanoic acid, ethyl ester	13.30	0.76	$C_{18}H_{36}O_2$
Ethylene brassy-late	13.62	0.41	$C_{15}H_{26}O_4$
9-12-Octadecadienoic acid	14.03	1.81	$C_{18}H_{32}O_2$
Ethyl linoleate	14.12	1.30	$C_{20}H_{36}O_2$
9-Octadecenoic acid ethyl ester	14.14	0.99	$C_{20}H_{38}O_2$
Octadecanoic acid, ethyl ester	14.24	0.14	$C_{20}H_{40}O_2$
9-12-Octadecadienoyl chloride	15.28	0.16	$C_{18}H_{31}ClO$
Squalene	17.54	0.37	$C_{30}H_{50}$

Source: Wagan *et al.* (2018)

8.35, 10.00, 5.52, and 6.86, respectively, df = 1, $P < 0.01$ (Fig. 1).

The repellency of the oil differed significantly between the cockroach stages (ANOVA) within each exposure time: 1hr (F = 11.21, df = 4, 25, $P = 0.00$), 2 h (F = 9.06, df= 4, 25, $P = 0.00$), 3 h (F = 1.76, df = 4, 25, $P = 0.00$) and 4 h (F = 7.52, df = 4, 25, $P = 0.00$) of exposure (Fig. 2). Moreover, in each developmental stage the repellency differed significantly between exposure times: in the adult stage: F = 7.61, df = 3, 20, $P = 0.01$; in the fourth instar: F = 6.84, df = 3, 20, $P = 0.01$; in the third instar: F = 9.62, df = 3, 20, $P = 0.00$; in the second instar: F = 1.31, df = 3, 20, $P = 0.30$; in the first instar: F = 0.61, df = 3, 20, $P = 0.62$ (Fig. 3).

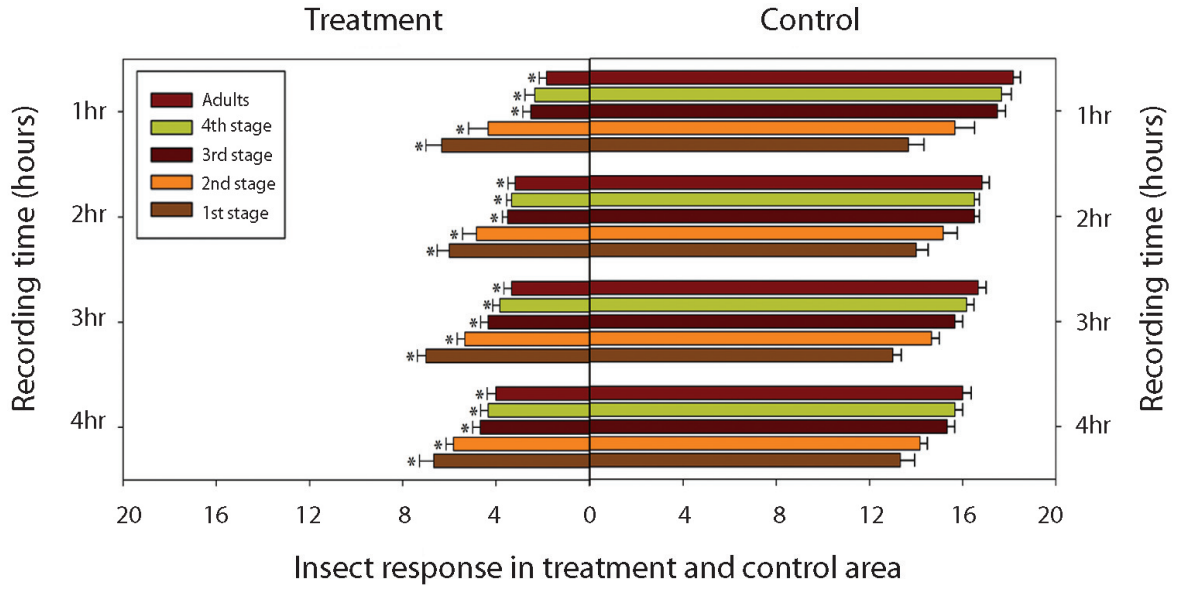

Figure 1. Chi-square test on repellency between the treated area with ethanol-extracted oil from *Gardenia jasminoides* (31.4 µg oil/cm^2) and the corresponding control area, to adults and nymphs of the German cockroach, *Blatella germanica*. Bars indicate the standard error.

The maximum repellency was recorded during the first hour of exposure in all stages except for first instar nymphs, and the repellency decreased with the increase of exposure time (Fig. 3). The oil was more effective on the adult cockroaches with a repellency percentage of 81.7 ± 3.1% at 1 h exposure while this percentage was 76.7 ± 4.2, 75.00 ± 3.4, and 56.7 ± 8.4%, on the fourth, third and second instar nymphs, respectively (Fig. 2). In first instar nymphs, the maximum repellency percentage (40.0 ± 5.2%) was recorded after 2 h exposure to the oil (Fig. 3).

Repellency of *G. jasminoides* oil to worker ants

The chi-square analysis of worker ant repellency showed significant differences between the treated and controlled area during 1, 2, 3, and 4 h of exposure (χ^2 = 43.49, 37.28, 35.36, and 30.00, respectively, df = 1, $P < 0.01$) (Fig. 4). The maximum repellency percentage was recorded after 1h exposure (78.3 ± 4.8%), whereas the repellency decreased with increasing exposure time (Fig. 5). ANOVA analysis showed a significant difference in repellency between exposure times (F = 15.35, df = 3, 20, $P = 0.00$).

Repellency comparison of *G. jasminoides* oil between worker ants and adult cockroaches

The repellency of *G. jasminoides* oil to adult stages of the insects was higher in the cockroach compared to the ant at 1, 3 and 4h exposure time (ANOVA: 1h: F = 5.29, df = 1, 10, $P = 0.04$; 3h: F = 8.00, df = 1, 10, $P = 0.02$; 4h: F = 14.76, df = 1, 10, $P = 0.01$). No significant difference was observed at 2h exposure time (F = 0.12, df= 1, 10, $P = 0.76$) (Fig. 6).

Discussion

The ethanol extracted oil from *G. jasminoides* showed a repellent effect to both the German cockroach, *B. germanica*, and the pharaoh ant, *M. pharaonis*, at the concentration of 31.4 µg of oil per cm^2 of filter paper in Petri dishes of 15 and 9 cm diameter, respectively.

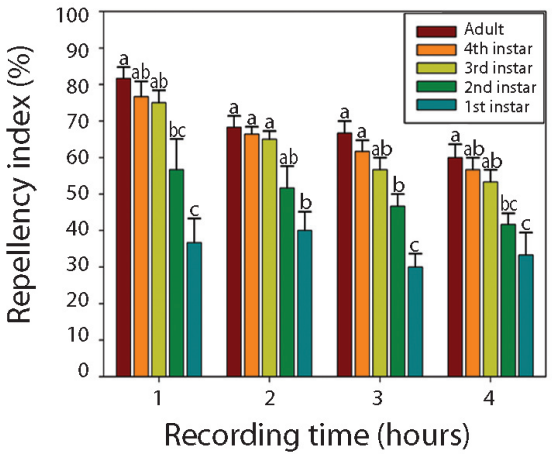

Figure 2. Repellency percentage (Mean + SE) of ethanol-extracted oil from *Gardenia jasminoides* to adults and nymphs of the German cockroach, *Blatella germanica,* at 31.4 µg oil/cm^2. Letters on the top of the columns show significant differences between developmental stages within the same exposure (recording) time.

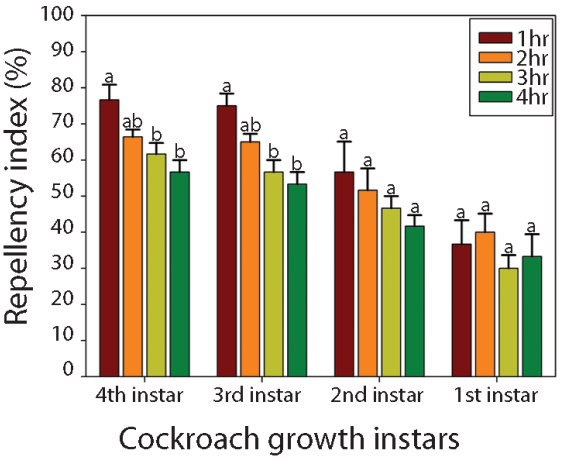

Figure 3. Repellency percentage (Mean + SE) of ethanol-extracted oil from *Gardenia jasminoides* to all nymphal instars of the German cockroach, *Blatella germanica,* at 31.4 µg oil/cm^2. Letters on top of the columns show significant differences between exposure times within the same instar.

Studies on bioactivity of *G. jasminoides* ethanol-extracted oil on insects are scarce. A repellent effect of *G. jasminoides* oil has been reported for *Tetranychus urticae* (Tetranychidae) 24h after treatment and the repellency was decreased with increasing of exposure time at 72 h (Kim *et al.*, 2005). Potential toxicity, repellency, and anti-oviposition activities of the oil have been reported against *Bemisia tabaci* (Hemiptera: Aleyrodidae) and *T. urticae* in laboratory and greenhouse ex-

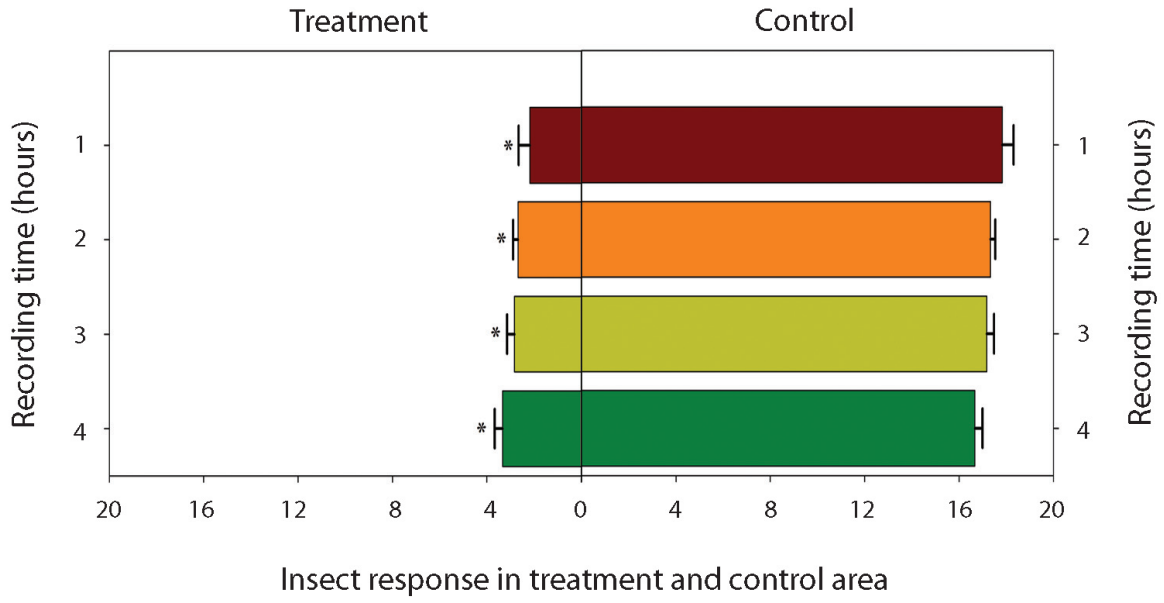

Figure 4. Chi-square test on repellency between the treated area with ethanol-extracted oil from *Gardenia jasminoides* (31.4 µg oil/cm^2) and the corresponding control area, to worker ants of *Monomorium pharaonic*. Bars indicate the standard error.

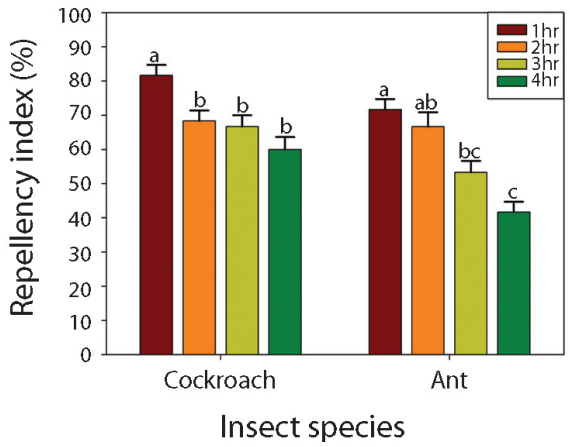

Figure 5. Repellency percentage (Mean + SE) of ethanol-extracted oil from *Gardenia jasminoides* to adults of the German cockroach, *Blatella germanica,* and the worker ants of *Monomorium pharaonic* at 31.4 µg oil/cm^2. Letters on the top of the columns show significant differences at different exposure times (1, 2, 3, 4 h) for each insect species.

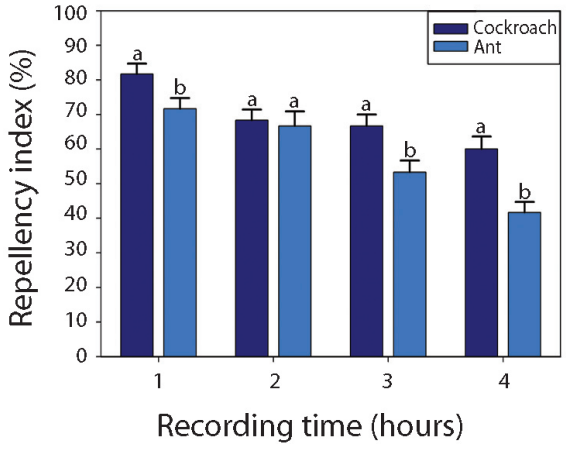

Figure 6. Repellency percentage (Mean + SE) of ethanol-extracted oil from *Gardenia jasminoides* to adults of the cockroach *Blatella germanica* and the worker ants of *Monomorium pharaonic* at 31.4 µg oil/cm^2. Letters on the top of the columns show significant differences between the insect species at different exposure times (1, 2, 3, 4 h).

periments (Wagan *et al*., 2018).

In German cockroaches, the *G. jasminoides* oil was more repellent to adults compared to nymphal stages. The weak effect of the oil on first instar nymphs could be related to differences in their chemoreceptor organs which probably are not fully developed in this stage. Several studies have proved that essential oils of several plant species have repellent activities against German cockroaches. For example, Zibaee *et al.* (2016) evaluated the repellency from the mixture of *Eucalyptus globulus*, *Rosmarinus officinalis* essential oils against *B. ger-*

manica, the American cockroach *Periplaneta americana* (Blattodea: Blattidae), and the brown banded cockroach *Supella longipalpa* (Blattodea: Ectobiidae, formerly Blattellidae), which showed 95%, 100%, and 100% repellency, respectively in laboratory experiments. The same study demonstrated 97% reduction of cockroaches in the field when these oils were applied as a mixed formulation with 10% active ingredient in water. The essential oil of *Citrus hystrix* showed 100% repellency against *B. germanica* and *P. americana* and 87.5% repellency against *Neostylopyga rhombifolia* in a laboratory experiment, whereas it reached 86% repellency when *Citrus hystrix* essential oil was formulated as a 20% active ingredient in ethanol (Thavara *et al*., 2007).

Similarly, *G. jasminoides* oil showed repellent activity against worker pharaoh ants in area preference tests up to 4h exposure. Some studies have already proved the repellency of botanical substances introducing new plant-based repellents against different ant species. For example, the aqueous extracts of a plant mixture of cucumber-mint, lemon-garlic, and garlic-mint exhibited 100% repellency against ants (Chaudhari *et al*., 2013). Chemical component cineole and D-camphor from *Artemisia annua* L oil can repel red imported fire ant workers. These substances showed significant repellent effect at 100, 10, and 1 mg/kg (Zhang *et al*., 2014).

Fourteen major chemical components were found in *G. jasminoides* ethanol-extracted oil. The bioactivities of these chemicals on insects, mites, rodents and some pathogens are already identified by previous researchers (Wagan *et al*., 2018; Parthipan *et al*., 2015; Sato *et al*., 2007; Friedman *et al*., 2002). *Gardenia jasminoides* essential oil and its four major compounds, i.e., squalene, ethyl linoleate, n-hexadecanoic acid and 9-12-octadecadienoic, effectively control the adult and nymphal stages of whiteflies and mites and they affect whitefly oviposition (Wagan *et al*., 2018).

In conclusion, the ethanol-extracted oil of *G. jasminoides* possesses repellent activities against German cockroaches and pharaoh ants, which could last for four hours. Further research is necessary to elaborate in these results with different concentrations and field tests. As ethanol-extracted oils are non-toxic for mammalians, the *G. jasminoides* could be used as an alternative natural product in urban pest management and in the areas of public health hygiene (Kumar *et al*., 2008; Ling *et al*., 2009).

Literature Cited

Ayvaz, A., Sagdic, O., Karaborklu, S. and Ozturk, I. 2010. Insecticidal activity of the essential oils from different plants against three stored-product insects. *Journal of Insect Science*, 10: 21.

Bosik, J.J. 1997. Common names of insects and related organisms. Entomological Society of America, Lanham, Maryland.

Caballero-Gallardo K., Pino-Benitez N., Pajaro-Castro, N., Stashenko, E. and Olivero-Verbel, J. 2014. Plants cultivated in Choco, Colombia, as source of repellents against *Tribolium castaneum* (Herbst). *Journal of Asia-Pacific Entomology*, 17: 753-759.

Chaudhari, P.S, Jangam, S.S., Chaudhari, S.V., Baheti, K.G., Patil, S.U., and Jadhav, G.B. 2013. Herbal plants as an ant repellent. *International Journal of Bioassays*, 2(6): 883-886.

Cochran, D.G. 1982. *Cockroach: Biology and control*. WHO/VBC/82.856: 52 pp.

Dhifi, W., Bellili, S., Jazi, S., Bahloul, N. and Mnif, W. 2016. Essential oils' chemical characterization and investigation of some biological activities: A Critical review. Medicines: 3 (25) doi:10.3390/medicines3040025.

Dobesh, S.M, Cress, D.C., Bowden, R., Henderson, E.R. and Mock, D.E. 1993. Public Health Pest Control, Pesticide Application Training. Kansas State University, Kansas. Available online: https://www.bookstore.ksre.ksu.edu/pubs/S14.pdf (last accessed 14 July 2014).

Faraone, N., Hillier, N.K. and Cutler, G.C. 2015. Plant essential oils synergize and antagonize toxicity of different conventional insecticides against *Myzus persicae* (Hemiptera: Aphididae). PLoS ONE 10(5): e0127774. https://doi.org/10.1371/journal.pone.0127774.

Friedman, M., Henika, P. R., and Mandrell, R. E. 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *Journal of Food Protection,* 65: 1545-1560.

Fu, X., Ye, L. and Ge, F. 2009. Habitat influences on di-

versity of bacteria found on German cockroach in Beijing. *Journal of Environmental Science*, 21: 249-254.

Geetha, R.V. and Anitha, R. 2014. Essential Oil Repellents- A short Review. *International Journal of Drug Development and Research* 6(2): 20-27.

Hansen, L. 2011. Identification and habits of key ant pests in the Pacific Northwest. A Pacific Northwest Extension Publication (PNW624), Washington State University, Oregon State University, University of Idaho.

Kim, D.I., Park, J.D., Kim, S.G., Kuk, H., Jang, M.S. and Kim, S.S. 2015. Screening of some crude plant extracts for their acaricidal and insecticidal efficacies. Journal of Asia-Pacific Entomology, 8: 93–100 doi.org/10.1016/S1226-8615(08)60076-X.

Kostyukovsky, M., Rafaeli, A., Gileadi, C., Demchenko, N. and Shaaya, E. 2002. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect Pests. *Pest Management Science*, 58: 1101-1106.

Kumar, A., Shukla, R., Singh, P., Prasad, C.S. and Dubey, N.K. 2008. Assessment of *Thymus vulgaris* L. essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities. *Innovative Food Science and Emerging Technologies,* 9: 575-580.

Ling. A., Sulaiman, S. and Othman. H. 2009. Evaluation of *Piper aduncum* Linn. essential oil (Fam: Piperaceae) against *Periplaneta americana* (L.), *Iranian Journal of Arthropod Borne Disease*, 3(2): 1-6.

Liu, X.C., Li, Y.P., Li, H.Q., Deng, Z.W., Zhou, L., Liu, Z.L. and Du, S.S. 2013. Identification of repellent and insecticidal constituents of the essential oil of *Artemisia rupestris* L. aerial parts against *Liposcelis bostrychophila* Badonnel. *Molecules*, 18: 10733–10746.

Mahmoud, M.F., El-Bahrawy, A.F., El-Sharabasy, H.M., El-Badry, Y.S., El-Kady, G.A. 2013. Ecological investigation, density, infestation rate and control strategy of German cockroach, *Blattella germanica* (L.) in two hospitals in Ismailia, Egypt. *Arthropod,* 24: 216-224.

Malaj, E., von der Ohe, P.C., Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Brack, W., Schäfer, R.B. 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proceedings of the National Academy of Science*, 111(26): 9549–9554.

Menasria, T., Samir, T., EL-Hamza, S., Mahcene, D., Moussa, F., Leyla, B., Mekahlia, M.N. 2014. A survey of the possible role of German cockroaches as a source for bacterial pathogens. *Journal of Advanced Science of Applied Engineering*, 1: 67-70.

Nerioa, L.S., Olivero-Verbela, J. and Stashenko. E. 2009. Repellent activity of essential oils: A review. *Bioresource Technology,* 101(1): 372-378.

NIST (National Institute of Standards and Technology). 2011. NIST/EPA/NIH Mass Spectral Database (NIST 11) and NIST Mass Spectral Search Program (Version 2.0g) 2011. U.S. Department of Commerce, National Institute of Standards and Technology, Standard Reference Data Program, Gaithersburg, Maryland.

Parthipan, B., Suky, M. G.T., and Mohan, V. R. 2015. GC-MS Analysis of Phytocomponents in *Pleiospermium alatum* (Wall. Ex Wight & Arn.) Swingle, (Rutaceae). *Journal of Pharmacognosy and Phytochemistry,* 4: 216-222.

Pavlik, I, Falkinham, J.O. 2009. The Occurrence of Pathogenic and Potentially Pathogenic Mycobacteria in Animals and the Role of the Environment in the Spread of Infection, In: J. Kazda, I. Pavlik, J. O. Falkinham, K. Hruska, (eds.) The Ecology of Mycobacteria: Impact on Animal's and Human's Health. Springer, 199-281.

Queyrel, W., Habets, F., Blanchoud, H., Ripoche, D., Launay, M. 2016. Pesticide fate modeling in soils with the crop model STICS: Feasibility for assessment of agricultural practices. *Science of the Total Environment*, 542: 787–802.

Sato, S., Kitamura, H., Chino, M., Takei, Y., Hiruma, M. and Nomura, M. A. 2007. 13-week oral dose subchronic toxicity study of gardenia yellow containing geniposide in rats. *Food and Chemical Toxicology*, 45: 1537-1544.

Su, Y.P., Yang, C.J., Hua, H.X., Cai, W.L., Lin, Y.J. 2009. Bioactivities of ethanol extracts from thirteen plants against *Nilaparvata lugens* (Stål). Chinese *Agricultural Science Bulletin*, 25: 198–202.

Suthisut, D.1., Fields, P.G. and Chandrapatya, A. 2011. Contact toxicity, feeding reduction, and repellency of essential oils from three plants from the ginger family (Zingiberaceae) and their major components against *Sitophilus zeamais* and *Tribolium castaneum*. *Journal of Economic Entomology*, 104(4): 1445-54.

Thavara, U., Apiwat, T., Payu, B., Prapai, W., Boonruad, T., Jaree, B., Pranee, C., Narumon, K., Pader, S. and Mulla, M.S. 2007. Repellent activity of essential oils against cockroaches (Diptera: Blattiddae, Blattelidae and Blaberidae) in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health,* 38: 663-673.

Wagan, T.A, Wanlun, Cai and Hongxia, Hua. 2018. Repellency, toxicity, and anti-oviposition of essential oil of *Gardenia jasminoides* and its four major chemical components against whiteflies and mites. *Scientific Reports*, 8: 9375 | DOI:10.1038/s41598-018-27366-5.

Zhang, N, Tang, L., Hu, W., Wang, K., Zhou, Y., Li, H., Huang, C., Chun, J. and Zhang, Z. 2014. Insecticidal, fumigant, and repellent activities of sweet wormwood oil and its individual components against red imported fire ant workers (Hy-

menoptera: Formicidae). *Journal of Insect Science*, 14: 241, doi: 10.1093/jisesa/ieu103. PMID: 25525106; PMCID: PMC5634023.

Zibaee, I, Khorram, P.B. and Hamoni, M. 2016. Evaluation of repellent activity of two essential oils and their mixed formulation against cockroaches (Dictyoptera: Blattidae, Blattellidae) in Iran. *Journal of Entomology and Zoology Studies,* 4(4): 106-113.

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Απωθητική δράση αιθανολικού εκχυλίσματος ελαίου του φυτού *Gardenia jasminoides* στην κατσαρίδα *Blattella germanica* και το μυρμήγκι *Monomorium pharaonis*

T.A. Wagan, C. Hamada, W. Cai, H. Hongxia, G.S. Solangi και R.H. Lyu

Περίληψη Η μελέτη εξέτασε την απωθητική δράση αιθανολικού εκυλίσματος ελαίου του φυτού *Gardenia jasminoides* στη Γερμανική κατσαρίδα *Blattella germanica* και στο μυρμήγκι Φαραώ *Monomorium pharaonis*, τα οποία είναι σοβαροί εχθροί υγειονομικής σημασίας. Στις δοκιμές απωθητικής δράσης εφαρμόστηκαν 31,4 μg ελαίου/cm^2 στο μισό δίσκου διηθητικού χαρτιού (διαμέτρου 9 ή 15 cm για τα μυρμήγκια και τις κατσαρίδες, αντίστοιχα), ενώ το άλλο μισό αποτέλεσε τον μάρτυρα (DMSO + Tween). Παρατηρήθηκε απωθητική δράση 1, 2, 3 και 4 ώρες μετά την απελευθέρωση των εντόμων. Το έλαιο έδειξε υψηλή απωθητική δράση σε όλα τα στάδια ανάπτυξης των κατσαρίδων και στα μυρμήγκια-εργάτες. Στις κατσαρίδες, η μέγιστη απωθητική δράση παρατηρήθηκε στα ενήλικα άτομα (81,7 ± 3,1%) και ακολούθως στις νύμφες τετάρτου, τρίτου και δεύτερου σταδίου (76,7 ± 4,2, 75,0 ± 3,4 και 56,7 ± 8,4%, αντίστοιχα), μετά από μία ώρα έκθεσης. Στα μυρμήγκια-εργάτες, η απωθητική δράση ήταν ισχυρή (78,3 ± 4,8%) μετά από μία ώρα έκθεσης. Η απωθητική δράση μπορεί να διαρκέσει τουλάχιστον τέσσερις ώρες και για τα δύο είδη εντόμων. Η χημική ανάλυση του ελαίου *Gardenia* με αέρια χρωματογραφία-φασματομετρία μάζας εντόπισε 14 κύρια χημικά συστατικά.

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Effect of nano-silica extracted from two different plant sources on survival and development of *Phthorimaea operculella* (Zeller) larvae

I. Idris[1*], M. Naddaf[2], H. Harmalani[2], R. Alshater[2] and R. Alsafadi[1]

Summary The study examined the effect of nano-silica extracted from two different plant sources on the survival and development of the potato tuber moth, *Phthorimaea operculella*. The silica powder was derived from two different agricultural byproducts, olive stones and corncobs. Characterization by X-ray diffraction revealed that the extracted powder has an amorphous silica phase. The nitrogen adsorption-desorption measurements revealed that both extracted and treated silica have mesoporous structure, with a specific surface area of around 300 m^2/g and 270 m^2/g for the silica derived from olive stones and corncobs, respectively. The silica nanoparticles (SiO_2 NPs) prepared from the silica derived from olive stones showed higher larvae mortality, pupae weight, and larval and pupal developmental time, compared to the silica derived from corncombs. The results show that the nano-silica derived from agriculture byproducts can be as effective as the synthetic insecticide (deltamethrin) utilized in control of the potato tuber moth, with lower environmental impact in terms of preventing pesticide residue accumulation. In addition, the efficiency of SiO_2 NPs applications depends on the source of the silica nanoparticles and the applied concentration to achieve the optimum results for the pest control.

Additional keywords: BET, corncobs, olive, potato tuber moth, SiO_2 NPs, XRD

Introduction

Potato (*Solanum tuberosum* L.) is the second most widely cultivated edible crop of Syria, after wheat (*Triticum aestivum* L.); the annual cultivation area is 22 thousand hectares and the production is 562 thousand tons of potato. Furthermore, potato is one of the most important economic crops in the southern and central regions of Syria (e.g., mainly in Damascus suburbs and Homs provinces) (Statistical data, 2019). Indeed, potato crop has been identified as a key element for ensuring national food security and reducing poverty levels (Zhang *et al*., 2021).

The potato tuber moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelatiidae), can damage potato crop by 50% to 100% in tropical and subtropical regions of America, Africa, Europe, Oceania, Australia and Asia (Clough *et al*., 2010). The pest causes about 20-30% infestation in the field while the infestation can reach 100% during the storage period (Ahmed *et al*., 2016; Idris and Shoaib, 2019), thus it can seriously disturb the success of potato as a commercial crop.

Phthorimaea operculella larvae primarily infest potato by feeding on leaves, stems and petioles, and by making tunnels in potato tubers, causing damage to the crop (Chandel *et al*., 2010; Zhang *et al*., 2021). Pesticide applications are the dominant method for the control of the pest. However, due to their non-selectivity properties, resistance development and environmental issues (Idris and Hussian, 2021), there in an increased interest by the global potato industry to find effective alternative methods to control *P. operculella* (Harba and Idris, 2018; Zhang *et al*., 2021).

The technique of nanoparticles supports more environmentally friendly pesticides (Papanikolaou *et al*., 2018; Wang *et*

[1] Department of Molecular Biology and Biotechnology, AECS, P. O. Box 6091 Damascus, Syria.
[2] Department of Chemistrry, AECS, P. O. Box 6091 Damascus, Syria.
[3] Qualitative Analysis Office, AECS, P. O. Box 6091 Damascus, Syria.
* Corresponding author: scientific@aec.org.sy

al., 2022). Compared with nanoparticles used in other fields, agricultural nanoparticles, such as TiO_2, Al_2O_3 and SiO_2 nanoparticles (NPs), are generally much more affordable and easier to produce than traditional nanoparticles (Biswal *et al.,* 2011; El-Bendary and El-Helaly, 2013; El-Helaly *et al*., 2016). Silica, silicon dioxide, nanoparticles (SiO_2, NPs) are increasingly used in plant treatments in the field of plant protection. They have shown great potential when they are applied appropriately in agriculture. Researchers report that the development of nanotechnology has increased the efficiency of chemical and organic pesticides, e. g. nanosilica has been found to be effective against the polyphagous plant pest *Spodoptera littoralis* (Lepidoptera: Noctuidae) (El-Bendary and El-Helaly, 2013). Moreover, nano-silica can reduce biotic and chemical stress on plants, such as salt, metal toxicity, and nutrition deficiency (Hersanti *et al*., 2018). Due to its simplicity, efficiency and low cost, the extraction of silica from plants and agriculture byproducts is increasingly used as a competent method for preparation of SiO_2 NPs (Goswami and Mathur, 2022, Naddaf *et al*., 2020, Mor *et al*., 2017). However, obtaining methods of the silica has been found to affect the SiO_2, NPs efficiency for agricultural applications (Karunakaran *et al*., 2013).

The purpose of this study is to: a) characterize the microstructure properties of silica powder extracted from olive stones and corncobs, by X-ray diffraction (XRD); b) determine the specific surface area and total pore volume of silica by measuring the nitrogen absorption-desorption; c) investigate whether the produced silica nanoparticles affect the life course of *P. opercullela* larvae including larvae mortality, pupae weight, and development time of larvae and pupae.

Materials and Methods

Insects rearing

Phtorimea operculella larvae were obtained from our laboratory stock cultures. They were reared on wax coated potato slices, maintained at a constant temperature of 25 ± 1°C, with 70 ± 5% relative humidity, and 12-hour light/darkness cycle as described by Makee and Saour (2003).

Silica preparation and characterization

Silica powder was extracted from corncobs and olive stones by the alkali leaching extraction method which is described in details in the study by Naddaf *et al*. (2020). The microstructure properties of the extracted silica powder were characterized by x-ray diffraction (XRD), using a STOE Powder diffractometer. Nitrogen adsorption-desorption by the Brunauer-Emmett-Teller (BET) method was used to determine the specific surface area and the pore size distribution of the studied samples. The measurements were performed at 77 K using Quantachrome NOVA 2200 BET surface area analyzer. The sample was vacuum-outgassed before the measurements for 12 hours at 150°C. The nitrogen adsorption-desorption isotherms were carried out in the range of relative pressure [P/P_0] from 0.05 to 1 atm.

Application of SiO_2 NPs on *P. operculella*

SiO_2 NPs were prepared by milling the extracted silica powder in a porcelain mortar, dispersing it in double distilled water (1.5 mg/ml), and ultrasonication for 30 min at a frequency of 30 kHz using an ultrasonic homogenizer with a tip in order to break big cluster agglomerates. By diluting the stock solution (1500 ppm), two more concentrations of SiO_2 NPs were prepared: 1000 and 500 ppm.

Potato tubers of the cv. "Draja" were planted in 10 L plastic (N=8 pots) containers containing moistened soil (three tubers/pot). Plants were grown in greenhouses at 25°C during the day and 23°C at night with daylight of 16 hours and a relative humidity of 85-95%. All plants were watered and fertilized the same way. Groups of eight fresh leaves were excised randomly from potato plants (six to seven weeks old) and sprayed with three concentrations (1500 ppm, 1000 ppm, 500 ppm) of amorphous olive nanoparticles and amorphous corncobs nano-

particles. A pyrethroid insecticide (deltamethrin) was used as a positive control and sterile water as a negative control.

A total of 200 potato tuber moth larvae (24h old) were fed on leaves in 10 plastic boxes (18 x 12 x 8 cm) for each treatment (20 larvae per box). The boxes were sealed with parafilm to prevent larvae from escaping until they reached the pupa stage. Incubation took place at 25 ± 1˚C with daylight 12h and relative humidity of 70%. Development time was recorded for 30 pupae and larvae in each treatment. The number of emerging pupae was recorded and the percentage of larval mortality was calculated: Percentage of mortality = (number of larvae tested - number of pupae emerging) / number of larvae tested x 100. The experiment was repeated three times. Twenty five pupae per treatment (four days old) were randomly selected and weighed.

Statistical analysis

The statistical analysis was done using the STATISTIC program version 6 (Statsoft, Inc. 2003) at 5% significance level (p=0.05). Analysis of variance was performed on the data of mortality and pupae weight and Tukey HSD test was used for the separation of means.

Results and discussion

Characterization of SiO_2 NPs by XRD technique

The obtained silica from corncobs shows XRD pattern similar to the reported XRD pattern for the olive stones silica (Naddaf *et al.*, 2020) (Fig. 1). Figure 1 shows very broad peak centered at 2θ=20.26°, which corresponds to typical features of amorphous silica (Mor *et al.,* 2017). The XRD results were assisted by nitrogen adsorption-desorption measurements using the BET method. Figure 2 shows the nitrogen adsorption-desorption isotherm of the studied silica samples. Based on the six types of isotherm proposed by the International Union of Pure and Applied Chemistry (IUPAC) for classification of porous materials (ALOthman, 2012), the observed isotherms in our case exhibit characteristics of IV type which corresponds to a mesoporous material. Moreover, the specific surface areas of the studied samples were determined as follows: The silica derived from olive stones has a specific surface area of 300 m^2/g and a total pore volume of 0.327 cm^3/g. The corresponding values for the silica from corncobs were 270 m^2/g and 0.678 cm^3/g, respectively. The inset in Figure 2 shows the pore size distribution of the studied silica samples obtained from the nitrogen adsorption-desorption measurements by the Barrett-Joyner-Halenda (BJH) method. Both samples show pore diameter distribution that consists of mesoporous materials. However, the sample extracted from olive stones shows a maximum pore value of ~ 3.7 nm, which is more than three times lower than the maximum pore value (~ 12.4 nm) of the sample extracted from corncobs. This shows that the extraction source has an important impact on both porosity and particle size of the produced silica. This is in agreement with reported results by Karunakaran *et al*. (2013).

Effect of SiO_2 NPs on mortality and development of *P. operculella*

Mortality of the silica-treated *P. operculella* larvae at 1500 ppm was similar (corncob)

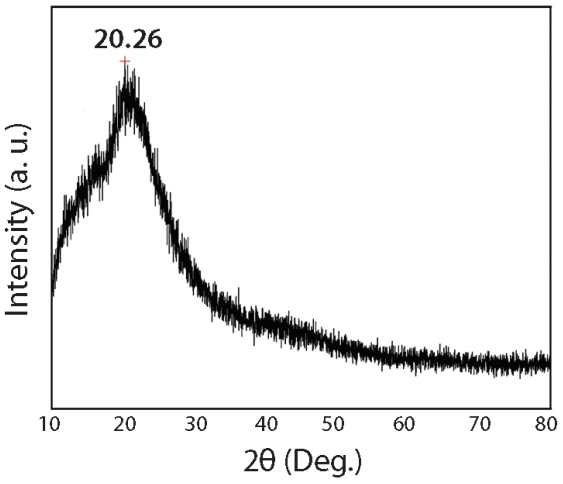

Figure 1. XRD of the extracted silica powder from corncobs, the very broad peak centered at 2θ=20.26° indicates the amorphous nature of the extracted silica.

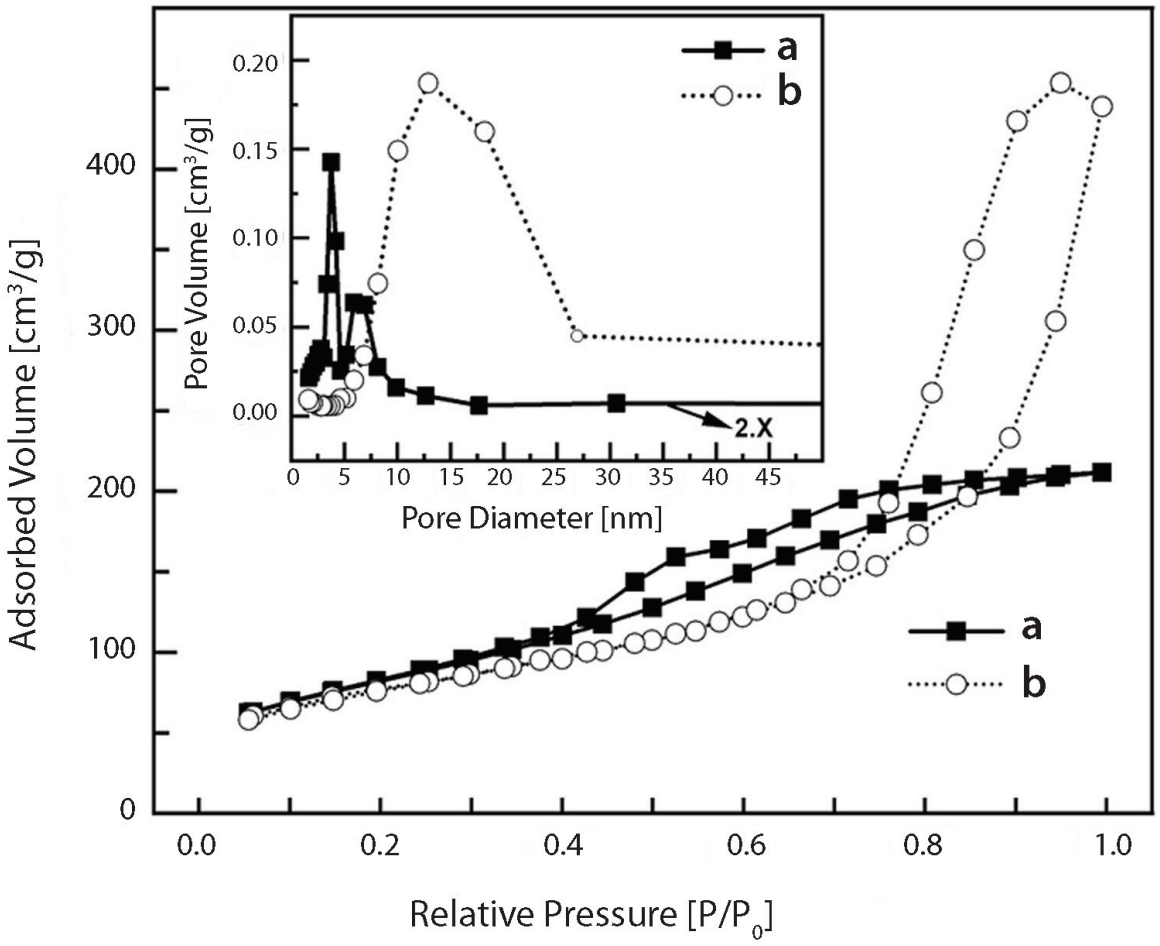

Figure 2. Nitrogen adsorption-desorption isotherm of silica samples extracted from: (a) olive stones and (b) corncobs; the corresponding pore distribution of each sample is depicted in the inset of the figure.

or statistically higher (olive stone) compared to that in deltamethrin treatment whereas larval mortality in the untreated control was significantly low (Fig. 3). Larval mortality in corncob SiO_2 NPs-treated larvae at the concentration of 1500 ppm did not statistically differ with that of the pesticide -treated larvae (DF=7, F-value=63.82, p<0.0001). The mortality of olive SiO_2 NPs-treated larvae was higher than the mortality of the corncob SiO_2 NPs -treated larvae. The effectiveness of silica nanoparticles in controlling insect pests has been demonstrated by numerous studies, and these results corroborate those findings. Previous studies on three mosquito species show a significantly higher mortality rate for larvae and pupae following exposure to SINPs (Cáceres *et al*., 2019). Usha *et al*. (2014) showed that terpene compounds significantly increased the toxicity of SiO_2 NPs in two lepidopterous pest species when combined with chemical compounds. The silica gel formulation with essential oils was also used to control two grain storage beetles, *Tribolium confusum* and *Sarocladium oryzae,* and resulted in high mortality rates for both species, which lasted for 7, 14 and 21 days after exposure (Athanassiou *et al*., 2013).

The amorphous phase of silica powder can cause more damage to larvae's midgut epithelium due to the mechanism of action leading to the insect's death (Mommaerts *et al*., 2012; Santo-Orihuela *et al*., 2016). Nevertheless, it is possible that the amorphous nanoparticles from olive-stones caused a higher mortality rate for larvae because the particles were smaller than corncob nanoparticles (Fig.1). In addition, silica obtained from olive stones has a higher specific sur-

face area, and lower total pore volume (300 m^2/g, 0.327 cm^3/g) than silica obtained from corncobs (270 m^2/g, 0.678 cm^3/g). In an assessment of nanoparticle nanoformulations, researchers found that nanoparticle toxicity was improved in smaller size (Debnath *et al*., 2011). On the other hand, Santo-Orihuela *et al*. (2016) detected significant differences in the concentration of nanoparticles in *Spodoptera frugiperda* Sf9 cells as a size-dependent effect of nanoparticles.

Pupae in SiO_2 NPs treatments had statistically lower weight when compared with the control pupae. The lowest weight was observed in olive SiO_2 NPs treatment at 1500 ppm (DF=7, F-value=15.23, p<0.0001) (Fig. 4). Studies on the effect of silica nanoparticles on insects report cuticular damage, desiccation (Benelli *et al*., 2018; Cáceres *et al*., 2019), cuticle abrasion and spiracle obstruction (Shoaib *et al*., 2018). These factors could possibly contribute to the mortality and the reduction of pupae weight as a result of physiological disruption caused by SiO_2 NPs.

Larval development time differed between the treatments: it was statistically longer in the olive nanoparticles (1500 ppm, 1000 ppm) and corncobs (1500 ppm) treatments compared to other treatments and the control (DF=7, F-value=12.976, p<0.0001) (Table 1). The reason for the prolonged time of larval development could be attributed to the difficulties that larvae experience in feeding due to the nanoparticle treatment. As a result, larvae at high nanoparticle concentrations require longer time to reach the final instar and to pupate. This is in agreement with previous studies showing that larvae treated with toxic agents have longer development time (Adam *et al*., 2016; Idris *et al*., 2019; Idris and Hussian, 2021). Additionally, pupation time differed between treatments (DF=7, F-value=9.23, p<0.0001). The longest pupation time was recorded under the treatment with SiO_2 NPs of olive stone, at a concentration of 1500 ppm, compared to the other treatments and the control. According to El-Bendary *et al*. (2013), when larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae) ingested nanoparticles of silica, the pupal period was extended in comparison with the control.

Conclusions

Nanoparticles of amorphous silica powders extracted from olive stones and corncobs are effective against *P. operculella,* with

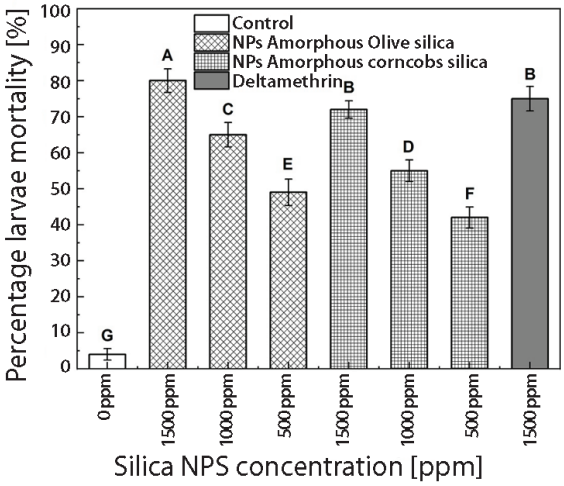

Figure 3. Effect of SiO_2 nanoparticles, derived from corncobs and olive stones, on larvae mortality of *Phthorimea operculella.* Different capital letters on top of columns indicate significantly different means at p<0.05 (Tukey HSD test).

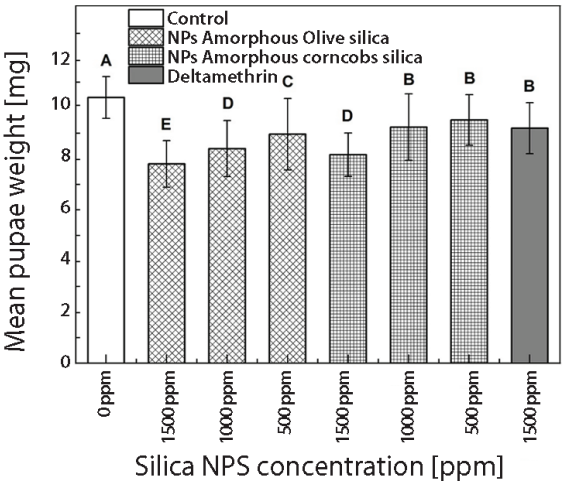

Figure 4. Effect of SiO_2 nanoparticles, derived from corncobs and olive stone, on pupae weight of *Phthorimea operculella.* Different capital letters on top of columns indicate significantly different means at p<0.05 (Tukey HSD test).

Table 1. Effect of SiO_2 nanoparticles, derived from corncobs and olive stones, on the development time (days) of larvae and pupae of *Phthorimea operculella*.

Treatments	Concentrations	Larval development time (days) (mean ± s.e.)	Pupae development time (days) (mean ± s.e.)
Control	0 ppm	18.2 ± 0.8cd	8.3 ± 0.7c
Olive stone nanoparticles	1500 ppm	21.1 ± 1.0a	9.9 ± 0.9a
	1000 ppm	20.1 ± 1.0b	9.2 ± 1.1b
	500 ppm	19.1 ± 1.1c	8.7 ± 0.7bc
Corncob nanoparticles	1500 ppm	20.8 ± 1.0b	9.2 ± 1.2b
	1000 ppm	18.9 ± 1. 3c	8.9 ± 0.6bc
	500 ppm	18.8 ± 1.0cd	8.8 ± 1.0bc
Deltamethrin	1500 ppm	19.1 ± 3.5c	8.4 ± 0.7c

Means ($\bar{x}$±SE) followed by different capital letters in columns are significantly different at P<0.05 (Tukey HSD test).

most effective the ones from olive stones at 1500 ppm. Further research is needed to determine the influence of morphological and microstructural aspects, such as porosity and crystallinity, on the efficiency of the produced silica nanoparticles against the pest.

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Literature cited

Adam, A., Idris, I., Khalil, N. and Houssian, K. 2016. Induced resistance in potato plants by a non-pathogenic Pseudomonas putida BTP1 against potato tuber moth (*Phthorimaea operculella* Zeller). *Advances in Horticultural Science*, 30(1): 47-52.

ALOthman, Z.A., 2012. A review: fundamental aspects of silicate mesoporous materials. *Materials*, 5(12): 2874-2902.

Athanassiou, C.G., Kavallieratos, N.G., Evergetis, E., Katsoula, A.M. and Haroutounian, S.A. 2013. Insecticidal efficacy of silica gel with *Juniperus oxycedrus* ssp. *oxycedrus* (Pinales: Cupressaceae) essential oil against *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae). *Journal of Economic Entomology,* 106(4): 1902-1910.

Benelli, G. 2018. Mode of action of nanoparticles against insects. *Environmental Science and Pollution Research*, 25(13): 12329-12341.

Biswal, S.K., Nayak, A.K., Parida, U.K. and Nayak, P.L. 2012. Applications of nanotechnology in agriculture and food sciences. *International Journal of Science Innovations* and *Discoveries*, 2(1): 21-36.

Cáceres, M., Vassena, C.V., Garcerá, M.D. and Santo-Orihuela, P.L. 2019. Silica nanoparticles for insect pest control. *Current pharmaceutical design*, 25(37): 4030-4038.

Clough, G.H., Rondon, S.I., DeBano, S.J., David, N. and Hamm, P.B. 2010. Reducing tuber damage by potato tuberworm (Lepidoptera: Gelechiidae) with cultural practices and insecticides. *Journal of Economic Entomology*, 103(4): 1306-1311.

Data of the statistical group of the Syrian Ministry of Agriculture and Agrarian Reform 2019.

Debnath, N., Das, S., Seth, D., Chandra, R., Bhattacharya, S.C. and Goswami, A. 2011. Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae* (L.). *Journal of Pest Science*, 84(1): 99-105.

El-Bendary, H.M. and El-Helaly, A.A. 2013. First record nanotechnology in agricultural: Silica nano-particles a potential new insecticide for pest control. *Applied of Scientific Reports*, 4(3): 241-246.

El-Helaly, A.A., El-Bendary, H.M., Abdel-Wahab, A.S., El-Sheikh, M.A.K. and Elnagar, S. 2016. The silica-nano particles treatment of squash foliage and survival and development of *Spodoptera littoralis* (Bosid.) larvae. *Journal of Entomology and Zoology Studies*, 4(1): 175–180.

Goswami, P. and Mathur, J. 2022. Application of agro-waste-mediated silica nanoparticles to sustainable agriculture. *Bioresources and Bio-*

processing, 9(1): 1-12.

Harba, M. and Idris, I. 2018. The effect of host density and viability on superparasitism behavior of *Trichogramma cacoeciae* and *T. principium* females. *Agriculture, Forestry and Fisheries* 7(1): 11-18.

Hersanti, Hidayat, S., Susanto, A., Virgiawan, R. and Joni, I.M. 2018. The effectiveness of *Penicillium sp*. mixed with silica nanoparticles in controlling *Myzus persicae*. In *AIP Conference Proceedings* (Vol. 1927, No. 1, p. 030029). AIP Publishing LLC.

Idris, I. and Hussian, K. 2021. Effects of gamma radiation and *Bacillus thuringiensis* on F1 progeny of *Cydia pomonella*. *Hellenic Plant Protection Journal*, 14(2): 99-107.

Idris, I. and Shoaib, A. 2019. Efficiency of AFLP markers to detect genetic variation in *Phthorimaea operculella* (Lepidoptera: Gelechiidae) offspring irradiated males. *Advances in Horticultural Science*, 33(3): 321-326.

Idris, I., Hussian, K., Alali, N. and Ikhtiar, A. 2019. irreversible fertility of irradiated *Phthorimaea operculella* (Lepidoptera: gelechiidae) females. *Journal of Bio Innovation*, 8(4): 517-531.

Karunakaran, G., Suriyaprabha, R., Manivasakan, P., Yuvakkumar, R., Rajendran, V., Prabu, P. and Kannan, N. 2013. Effect of nanosilica and silicon sources on plant growth promoting rhizobacteria, soil nutrients and maize seed germination. *IET Nanobiotechnology*, 7(3): 70-77.

Makee, H. and Saour, G. 2003. Noninherited sterility in irradiated *Phthorimaea operculella* females. *Journal of Applied Entomology*, 127(9-10): 489-493.

Mommaerts, V., Jodko, K., Thomassen, L.C., Martens, J.A., Kirsch-Volders, M. and Smagghe, G. 2012. Assessment of side-effects by Ludox TMA silica nanoparticles following a dietary exposure on the bumblebee *Bombus terrestris*. *Nanotoxicology*, 6(5): 554-561.

Mor, S., Manchanda, C.K., Kansal, S.K. and Ravindra, K. 2017. Nanosilica extraction from processed agricultural residue using green technology. *Journal of Cleaner Production*, 143: 1284-1290.

Naddaf, M., Kafa, H. and Ghanem, I. 2020. Extraction and characterization of nano-silica from olive stones. *Silicon*, 12(1): 185-192.

Papanikolaou, N.E., Kalaitzaki, A., Karamaouna, F., Michaelakis, A., Papadimitriou, V., Dourtoglou, V. and Papachristos, D.P., 2018. Nano-formulation enhances insecticidal activity of natural pyrethrins against *Aphis gossypii* (Hemiptera: Aphididae) and retains their harmless effect to non-target predators. *Environmental Science and Pollution Research*, 25: 10243-10249.

Santo-Orihuela, P.L, Foglia, M.L., Targovnik, A.M., Miranda, M.V., and Desimone, M.F. 2016. Nanotoxicological effects of SiO2 nanoparticles on *Spodoptera frugiperda* Sf9 cells. *Current Pharmaceutical Biotechnology*, 17(5): 465-470.

Shoaib, A., Elabasy, A., Waqas, M., Lin, L., Cheng, X., Zhang, Q. and Shi, Z.H. 2018. Entomotoxic effect of silicon dioxide nanoparticles on *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) under laboratory conditions. *Toxicological and Environmental Chemistry*, 100(1): 80-91.

Usha Rani, P., Madhusudhanamurthy, J. and Sreedhar, B. 2014. Dynamic adsorption of α-pinene and linalool on silica nanoparticles for enhanced antifeedant activity against agricultural pests. *Journal of Pest Science*, 87(1): 191-200.

Wang, D., Saleh, N.B., Byro, A., Zepp, R., Sahle-Demessie, E., Luxton, T.P., Ho, K.T., Burgess, R.M., Flury, M., White, J.C. and Su, C., 2022. Nano-enabled pesticides for sustainable agriculture and global food security. *Nature Nanotechnology*, 17(4): 347-360.

Zhang, M., Yan, J., Ali, A. and Gao, Y. 2021. Different Performance of *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) among four potato tuber varieties under laboratory condition. *Insects*, 12(7): 580.

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Επίδραση του νανο-πυριτίου που εξάγεται από δύο διαφορετικές φυτικές πηγές στην επιβίωση και την ανάπτυξη των προνυμφών της φθορίμαίας της πατάτας, *Phthorimaea operculella*

I. Idris, M. Naddaf, H. Harmalani, R. Alshater και R. Alsafadi

Περίληψη Εξετάστηκε η επίδραση νανο-πυριτίου φυτικής προέλευσης στην επιβίωση και την ανάπτυξη των προνυμφών της φθορίμαίας της πατάτας, *Phthorimaea operculella* (Zeller). Παραλήθφηκε

σκόνη οξειδίων του πυριτίου από δύο διαφορετικά γεωργικά υποπροϊόντα, τον πυρήνα ελαιοκάρπου και τον σπάδικα αραβοσίτου. Ο χαρακτηρισμός με περίθλαση ακτίνων Χ έδειξε ότι η εκχυλισμένη σκόνη περιέχει μια άμορφη φάση πυριτίου. Οι μετρήσεις προσρόφησης-εκρόφησης αζώτου έδειξαν ότι τόσο το εκχυλισμένο πυρίτιο όσο και το επεξεργασμένο πυρίτιο έχουν μεσοπορώδη δομή, με ειδική επιφάνεια περίπου 300 m^2/g και 270 m^2/g για το πυρίτιο που προέρχεται από τον πυρήνα του ελαιοκάρπου και τον σπάδικα του αραβοσίτου, αντίστοιχα. Τα νανοσωματίδια πυριτίου (SiO_2 NPs) που παρασκευάστηκαν από πυρίτιο που προέρχεται από πυρήνες ελαιοκάρπου είχαν μεγαλύτερη επίδραση στη θνησιμότητα των προνυμφών, στο βάρος των νυμφών και στο χρόνο ανάπτυξης των προνυμφών και των νυμφών της φθοριμαίας, σε σύγκριση με τα νανοσωματίδια πυριτίου από σπάδικες καλαμποκιού. Τα αποτελέσματα δείχνουν ότι το νανο-πυρίτιο που προέρχεται από γεωργικά υποπροϊόντα μπορεί να είναι εξίσου αποτελεσματικό με το συνθετικό εντομοκτόνο (δελταμεθρίνη) που χρησιμοποιείται για την αντιμετώπιση της φθοριμαίας, με μικρότερη περιβαλλοντική επιβάρυνση όσον αφορά στην πρόληψη συσσώρευσης υπολειμμάτων γεωργικών φαρμάκων. Επιπλέον, η αποτελεσματικότητα των επεμβάσεων εξαρτάται από την πηγή προέλευσης των νανοσωματιδίων πυριτίου και την εφαρμοζόμενη συγκέντρωση για να επιτευχθούν τα βέλτιστα αποτελέσματα αντιμετώπισης του εντόμου.

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Behavioral and histopathological changes of *Clarias gariepinus* as a predatory fish against *Culex pipiens* larvae following exposure to sublethal concentration of quinclorac and bensulfuron-methyl based herbicide

I.A. Mohamed[1,*], M. Fathy[1], A.I.A. Farghal[1], S.A.H. Temerak[1], S.Kh. Abd El-Ghaffar[2] and S.K.A. Idriss[3]

Summary *Clarias gariepinus* is one of the widespread culturable freshwater fish species in Africa, which is prevalent in various natural and human-made aquatic habitats including rice-fish system. This fish species displays predation potential on the aquatic stages of mosquitoes. Bensulfuron-methyl and quinclorac are herbicide active substances that have been extensively applied in rice culture in Egypt and other countries worldwide. This study assessed the adverse effects of sublethal concentration of a commercial herbicide formulation containing quinclorac and bensulfuron-methyl on the predation potential of *C. gariepinus* female and male predatory fish on *Culex pipiens* mosquito larvae. Also, stomach and intestine histopathology of the treated fish was investigated. The exposure of *C. gariepinus* to sublethal concentration of quinclorac and bensulfuron-methyl based herbicide produced detrimental effects on prey consumption and histopathological changes in the stomach and intestine of the fish. The mosquito consumption by the treated female and male fish decreased significantly compared to the untreated fish of both sexes. The histological changes in the intestines were hyperplasia of the intestinal epithelium and goblet cells; edema of lamina propria and broad intestinal villi, and distortion in intestinal villi in comparison to control. The stomach histopathology changes were necrosis and sloughing of mucosal epithelium with severe damage of sub-mucosa. Thus, the tested herbicide at sublethal concentration on *C. gariepinus* decreased the prey consumption on mosquito larvae and caused histopathological alterations in the fish that may impair its digestive physiology. These findings suggest a threat of the tested herbicide to *C. gariepinus* survival and potential as a native successful biocontrol agent against *Cx. pipiens* larvae.

Additional keywords: *Clarias gariepinus*, *Culex pipiens*, herbicide, histopathology, predatory fish, prey

Introduction

Several fish species have been used successfully as effective, low-cost, and eco-friendly biocontrol approaches against aquatic stages of mosquitoes (Chandra *et al.,* 2008; Bhattacharjee *et al.,* 2009). Since 1900, *Gambusia* spp. and *Poecilia* spp. are native larvivorous fish in American regions and have been widely used as exotic predators against mosquitoes in different aquatic habitats all over the world (Pyke *et al.,* 2008). However, negative impacts of *Gambusia* spp. on several aquatic beneficial invertebrates, amphibians, and other native fish species have been recorded in many countries (Pyke *et al.,* 2008). This has led researchers to look for different indigenous predatory fish species in local aquatic environments in Africa, Asia, and South America (Ghosh and Dash, 2007; Chandra *et al.,* 2008). Therefore, about 315 different fish species, from many different countries, have been recorded as being effective and potentially suitable indigenous predatory fish for mosquito control (Ghosh and Dash, 2007). For example, many edible fish such as *Clarias gariepinus* (Burchell), *Ore-*

[1] Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt.
[2] Pathology Department, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt.
[3] Fish Diseases and Management Department, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt.
* Corresponding author: ibrahim.mahmoud@agr.aun.edu.eg

ochromis mossambica (Peters), *Oreochromis spilurus* (Gunther), *Oreochromis niloticus* L., *Tilapia zillii* (Gervais), and *Ctenopharyngdon idella* (Valenciennes) are farmed in natural habitats or human-made aquatic culture for human consumption and they also provide effective control of mosquito larvae and pupae of *Anopheles*, *Aedes*, and *Culex* species (Chandra *et al.,* 2008; Chala *et al.,* 2016; Abebe *et al.,* 2018; Das *et al.,* 2018; Mohamed *et al.,* 2021).

Clarias gariepinus is a native African freshwater fish, which is prevalent in various natural and human-made aquatic habitats or even in sewage systems in urban regions worldwide, especially in African and Asian countries (Ponzoni and Nguyen, 2008). It is an omnivore fish that regularly feeds on a large variety of aquatic invertebrates, aquatic insect larvae and pupae, small fishes, algae, and aquatic plants (Ghosh *et al.,* 2008; Ponzoni and Nguyen, 2008). It is a hardy fish and tolerant to difficult environmental conditions (e.g., polluted water) (Ponzoni and Nguyen, 2008). The immature stages of mosquitoes may occur in aquatic habitats of *C. gariepinus* (Gashaw *et al.,* 2008). All these reasons make *C. gariepinus* a suitable candidate biocontrol agent against aquatic animal pests, mainly mosquitoes. In Ethiopia, *C. gariepinus* has the potential to control the larvae of *Culex* spp. and *Anopheles arabiensis* Patton (Chala *et al.,* 2016) and the aquatic snail *Biomphalaria pfeifferi* Krauss (Gashaw *et al.,* 2008).

Egypt is the largest producer of various edible fish mainly *O. niloticus*, *C. gariepinus*, and *Mugil cephalus* L. in Africa (Soliman and Yacout, 2016). In Egypt, rice-fish farming produced about 51.31% of *O. niloticus* and 29.93% of *C. gariepinus* from the total production of rice-fish system in 2012 (Soliman and Yacout, 2016). However, according to the best of our knowledge, limited studies have been published assessing the predatory efficiency of *C. gariepinus* against aquatic stages of mosquitoes, and no studies on the mosquito *Culex pipiens* L. *Culex pipiens* is a main efficient vector of lymphatic filariasis worms and several serious arboviruses to humans and is the most widely distributed mosquito in temperate regions worldwide, including Egypt (Mohamed *et al.,* 2021).

Predation of larvivorous fishes may be affected by different biotic and abiotic factors, which are related to the fish (predator), mosquito (prey), and its aquatic habitats (Yildirim and Karacuha, 2007; Chala *et al.,* 2016; Mohamed *et al.,* 2021). Chala *et al.* (2016) indicated that the predation efficacy of *C. gariepinus* against mosquito larvae was significantly affected by the mosquito genera, larvae number exposed, hours of feeding, and the size of predatory fish. Interestingly, predation of larvivorous fishes is also negatively impacted by the presence of toxicant chemical residues, mainly pesticides (insecticides and herbicides) in their aquatic habitats (Kerby *et al.,* 2012). Sublethal concentrations of different chemical pesticides induced larger adverse effects on the behavior of predatory fish than on their prey (Kerby *et al.,* 2012). For example, the insecticide diazinon at sub-lethal concentrations significantly reduced the activity and attack rates of *Gambusia affinis* (Baird and Girard) on *Pseudacris regilla* (Baird and Girard) tadpole prey which likely reflects the negative effects of the pesticide on the predatory behavior of *G. affinis* (Kerby *et al.,* 2012). Also, several herbicides produced adverse effects on different freshwater fish such as changes in mobility, feeding, orientation behavior, enzymes, biochemical and hematological modifications, histopathological disturbances, oxidative stress, genotoxicity, endocrine toxicity, and neurotoxicity (Ullah *et al.,* 2014; Stanley and Preetha, 2016; Fathy *et al.,* 2019; Saleh *et al.,* 2022).

Bensulfuron-methyl (sulfonylurea) and quinclorac (quinoline) are among the most commonly and widely applied selective herbicides in rice, various agricultural crops, and turfgrass lands worldwide. The residues of both herbicides are persistent in different aquatic systems that may result in adverse effects on aquatic biota, mainly fish (Okamoto *et al.,* 1998). Bensulfuron-methyl has been detected in water of rice fields at a range of <0.01–139.97 µg/L (Parveen *et al.*, 2005),

while quinclorac was present at 1.34–6.97 µg/L (Resgalla *et al.,* 2007). Recently, both herbicides are mixed in a post-emergence herbicide (trade name Repare®), which has also been extensively applied in rice culture in Egypt and other countries worldwide (APC 2021). Exposure of *O. niloticus* to a sublethal dose of bensulfuron-methyl and quinclorac individually altered the behavior of fish, induced oxidative stress, and damaged the liver and spinal cord (Fathy *et al.,* 2019; Saleh *et al.,* 2022). The 96-h exposure of silver catfish, *Rhamdia quelen* to LC_{50} of quinclorac (395 mg/L) elicited adverse behavioral changes, oxidative stress, biochemical alternations, and induced histological lesions in the liver, kidney, and gill on the fish (Miron *et al.,* 2005; Persch *et al.,* 2017; Persch *et al.,* 2018). Histopathological examination variables are frequently used as potential biomarkers in the pesticide toxicological studies to provide information about organ damage and the physiological, functional, and health status of fish after exposure to these toxic agents (Vander Oost *et al.,* 2003; Fathy *et al.,* 2019; Saleh *et al.,* 2022). *C. gariepinus* is continuously exposed to a great range of pesticide residues (e.g. herbicides), in its aquatic habitats, especially present in or surrounding agricultural regions like rice-fish culture (Chala *et al.,* 2016).

This study was designed in order to evaluate the effects of sublethal concentration of a quinclorac and bensulfuron-methyl based commercial herbicide on the predation potential of *C. gariepinus* against *Cx. pipiens* larvae under laboratory conditions and to assess the histopathological changes in the stomach and intestine of the fish.

Materials and Methods

Collection and maintenance of the fish *Clarias gariepinus*

Females and males of *C. gariepinus* fish were collected from a private local fish farm in Assiut Governorate, Egypt, and transferred to the Environmental Toxicology laboratory in the Plant Protection Department, Faculty of Agriculture, Assiut University. The female and male fish were stocked separately in 70 L boxes and acclimated for 4 weeks under laboratory conditions at 25 ± 1°C and 12:12 h (L:D) photoperiod.

Culex pipiens larvae

Field populations of *Cx. pipiens* larvae were collected from the sewage treatment plant in the Arab El-Madabegh region, Assiut city, Egypt. The samples were transfered to the laboratory and kept at 25 ± 1°C. The collected *Cx. pipiens* larvae were fed on a diet containing fine ground dry bread and yeast and then late third to early fourth instar larvae were used in this study.

Determination of sublethal concentration of herbicide on *C. gariepinus*

The tested commercial mixture herbicide formulation was Repare® 18% TB (16.5% quinclorac and 1.5% bensulfuron-methyl; Starchem Chemical Manufacturing, Egypt). A preliminary experiment was conducted to define the sub-lethal concentration of the herbicide on *C. gariepinus* fish. In this experiment *C. gariepinus* fish (72 fish, 28.5–30.0 cm and 167–180 g) were divided to six experimental groups (12 fish/group), the non-exposed fish (control) group and five experimental groups exposed to different concentrations of the tested quinclorac and bensulfuron-methyl herbicide. Each group of *C. gariepinus* fish was kept in three separate 70 L boxes (4 fish/box). In the non-exposed control group, the fish remained in water, while in the herbicide treatment groups, the fish were exposed to 390, 780, 1560, 3120 and 6240 mg of Repare® 18% TB/L water for 96 hours. Fish were maintained in the laboratory at the same conditions as described above. Mortality was recorded every 24 hours over 96 h and dead fish were removed from boxes immediately. Finney's probit analysis (Finney, 1971) was used to estimate lethal concentration (LC_{50} and LC_{90}) values of *C. gariepinus* after 96 h exposure using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA, 2).

Predatory potential of *C. gariepinus* fish on *Culex pipiens* larvae after exposure to sublethal concentration of herbicide

After the acclimatization, eighteen females (30.0–30.76 cm and 180.00 ± 2.77 g) and eighteen males (29.50–31.00 cm and 162.5 ± 2.51 g) of *C. gariepinus* were selected for the experiments. The aforementioned female and male fish were divided into two groups (9 fish/sex/group) as follows: the first group was the non-exposed fish (control) and the second group was exposed to the determined sub-lethal concentration of quinclorac and bensulfuron-methyl based mixture herbicide in water (390 mg of Repare® 18% TB/L) for 15 days and placed in 70 L boxes. The water in boxes was changed every 48 h and the herbicide was again added to maintain the concentration constant as described by Doherty *et al.* (2016) and Hamed and Osman (2017). Fish were fed once a day with commercial dry food pellets (25% crude protein, Al-Salam Company, Egypt). On the day 14 of exposure, all *C. gariepinus* fish in all groups were left over without food for 24 h to starve. After the exposure period and before the predation experiment, the treated fish of each sex were transferred carefully to separate boxes containing clean water to wash the fish off herbicide before starting the predation experiments. Each fish was placed in 8 L water with 600 mosquito larvae for 12h, from 7:30 a.m. to 7:30 p.m. (day time predation experiment) and then the same fish was transferred to new box with fresh 8 L water and new batch of 600 larvae from 7:30 p.m. to 7:30 a.m. (night time predation experiment). This procedure was repeated every 12 hours for a total period of 5 days (120 hours).

The total number of *Cx. pipiens* larvae consumed by both pre-treated and untreated female and male fish groups was recorded every 12 h over 120 hours. The means of *Cx. pipiens* larvae consumption by pre-treated and untreated female and male *C. gariepinus* fish groups were estimated and expressed as mean ± SE. The t-test for independent samples was performed to compare the mean number of consumed larvae between treated and untreated group at a= 0.05 signifficance level.

Histopathological studies

Three *C. gariepinus* fish from each of the pre-treated and untreated (control) groups were randomly selected after 15 days of exposure. Tissue samples of the stomach and intestine were anatomically dissected from each fish in both groups, rapidly removed, washed with neutral saline, and fixed in neutral buffered formalin 10%. Post-fixed tissue specimens of each group were dehydrated in a graded alcohol series, cleared with methyl benzoate, and then embedded in a paraffin wax (Bancroft and Stevens, 1982). Thin sections were cut at 5 µm thickness and stained with the hematoxylin–eosin stain (HE) technique (Bancroft *et al.,* 1996). All sections in each group were histopathologically examined and photographed using an Olympus CH30 microscope.

Results

Sublethal concentration of tested herbicide on *C. gariepinus*

All fish exposed to 3120 and 6240 mg Repare® 18%/L water died within 24 h, but for those exposed to 390 and 780 mg of the herbicide there was no mortality after 24 h and even after 96 h of treatment, respectively. The concentration of 1560 mg/L produced 41.67% mortality after 24 h and 96 h of treatment. The LC_{50} of the herbicide against *C. gariepinus* at 96 h was 1607 mg/L (Table 1). The selected sublethal concentration of Repare® herbicide in our study was 390 mg/L, which is almost the 1/4 LC_{50} value at 96 h and approximately 1/27 from the recommended application rate (10416.67 mg Repare® 18%/L) of the herbicide for rice weeds in Egypt (APC, 2021).

Predatory potential of *C. gariepinus* fish on *Culex pipiens* larvae after exposure to sublethal concentration of herbicide

The data regarding the predatory efficacy of the treated female and male of *C.*

gariepinus fish after exposure to sublethal concentration of quinclorac and bensulfuron-methyl based herbicide (390 mg Repare® 18%/L) and the control against third to fourth -instar larvae of *Cx. pipiens* under laboratory conditions are showed in Table 2. The sublethal concentration of the tested herbicide affected negatively the feeding potential of the female and male of *C. gariepinus*. The daily consumption (sum of day and night time records) of pre-treated and untreated females of *C. gariepinus* on *Cx. pipiens* larvae was 669.17 ± 3.72 and 1018.17 ± 10.27 larvae, respectively, i.e., significantly reduced by 34.28%. Also, the daily consumption of pre-treated and untreated males of *C. gariepinus* on *Cx. pipiens* larvae was 471.25 ± 10.78 and 595.92 ± 24.92 larvae, respectively, i.e., significantly reduced by 20.92%. The daily larval consumption by the pre-treated and untreated *C. gariepinus* was 570.21 ± 2.68 and 794.65 ± 5.93, respectively, regardless the gender (Table 2), hence, the sublethal concentration of the herbicide reduced the consumption potential of *C. gariepinus* on *Cx. pipiens* larvae by 28.23%.

Larval consumption by the pre-treated and untreated female of *C. gariepinus* was higher than in the pre-treated male by 1.71 and the untreated male fish by 1.42 times. The larval consumption by the pre-treated and untreated male fish was also higher during the nighttime by 1.91 and 1.71 times than during the daytime, respectively.

Histopathological studies

In the present study, no visible changes or no pathologic changes were observed in

Table 1. Lethal concentrations (LC_{50} and LC_{90}) along with 95% confidence limits of quinclorac and bensulfuron-methyl based herbicide (Repare® 18%) on *Clarias gariepinus* after 96 h exposure.

Value	Concentration (mg/L)	95% confidence limits		Slope±S.E.
		Lower	Upper	
LC50	1607.0	1542.72	1794.84	0.004 ± 0.001
LC90	1941.0	1770.05	2957.69	0.004 ± 0.001

Table 2. Predation of untreated and treated females and males of *Clarias gariepinus* after exposure with sublethal concentration of quinclorac and bensulfuron-methyl based herbicide (390 mg Repare® 18%/L water) for 15 days against third-fourth instar larvae of *Culex pipiens*.

Fish sex	Treatment	Mean Fish size (±SE) (cm)	Mean Fish weight (±SE) (g)	Mean (±SE) number of consumed *Cx. pipiens* larvae per fish		
				Day time†	Night time†	Daily‡
Female	Untreated	30.00 ± 1.38	180.00 ± 2.77	461.50 ± 3.04	556.67 ± 10.97	1018.17 ± 10.27
	Treated	30.76 ± 0.33	180.00 ±2.77	309.67 ± 7.20	359.50 ± 5.79	669.17 ± 3.72
P-value				0.001*	0.0001*	0.0001*
Male	Untreated	29.50 ± 1.50	162.50 ± 2.51	196.38 ± 5.89	374.75 ± 1.25	571.13 ± 4.64
	Treated	31.00 ± 1.00	162.50 ± 2.51	174.13 ± 6.64	297.13 ± 4.14	471.25 ± 10.78
P-value				0.013*	0.0001*	0.001*
Adult	Untreated	29.75 ± 1.44	171.25 ± 2.64	328.94 ± 0.39	465.71 ± 5.70	794.65 ± 5.93
	Treated	30.88 ± 0.67	171.25 ± 2.64	241.90 ± 2.51	328.31 ± 4.63	570.21 ± 2.68
P-value				0.001*	0.0001*	0.0001*

Signifficant differences between means using t-test ($p < 0.05$) are indicated with an asterisk (*). † Day time (from 7.30 am to 7.30 pm) and night time (from 7.30 pm to 7.30 am). ‡ Daily is the sum of day and night time records.

the stomach (normal histological structure in Figure 1A, 1B) and the intestine (Figure 2A) of C. gariepinus in the control group. Exposure for 15 days to 390 mg Repare® 18%/L altered the stomach histology resulting in a focal area of damage in stomach mucosa (Figure 1C, 1D), accompanied by necrosis and sloughing of mucosal epithelium that causes severe damage to sub-mucosa (Figure 1C, 1D). After 15 days, the fish intestinal in the treated group showed: hyperplasia of intestinal epithelium and goblet cells (Figure 2B), edema of lamina propria and broad intestinal villi in some cases (Figure 2C), and distortion in intestinal villi (Figure 2D) in comparison to control.

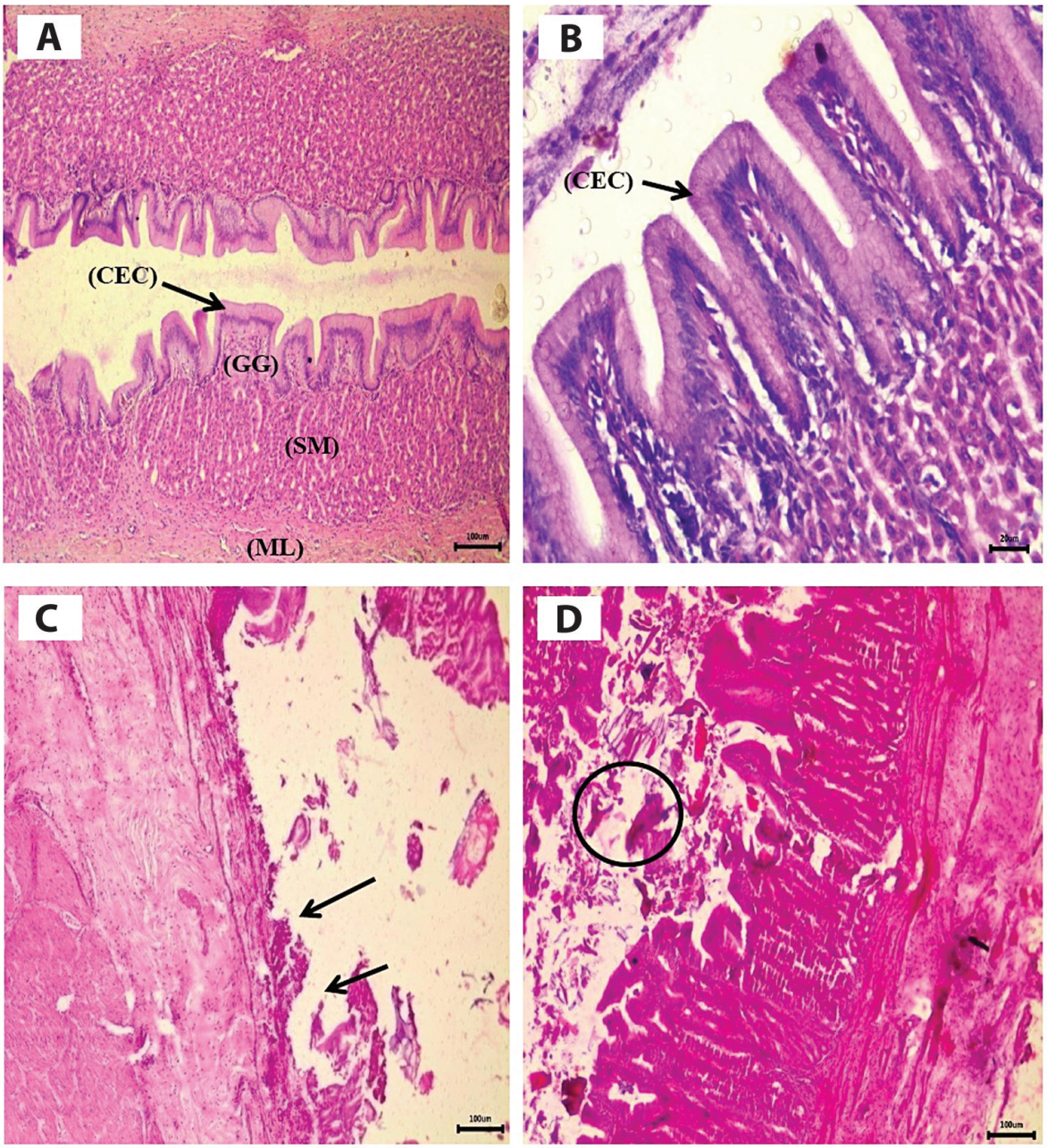

Figure 1. Stomach sections of *Clarias gariepinus* (H & E stain, 400×): A and B) Control stomach showing normal mucosa with a single layer of columnar epithelial cells (CEC) and gastric glands (GG), sub mucosa (SM), and muscle layer (ML). C) and D) Exposed to 390 mg Repare® 18%/L for 15 days showing necrosis (circle), sloughing of mucosal epithelium (arrow). Scale bars: A, C, D (100 µm) and B (20 µm).

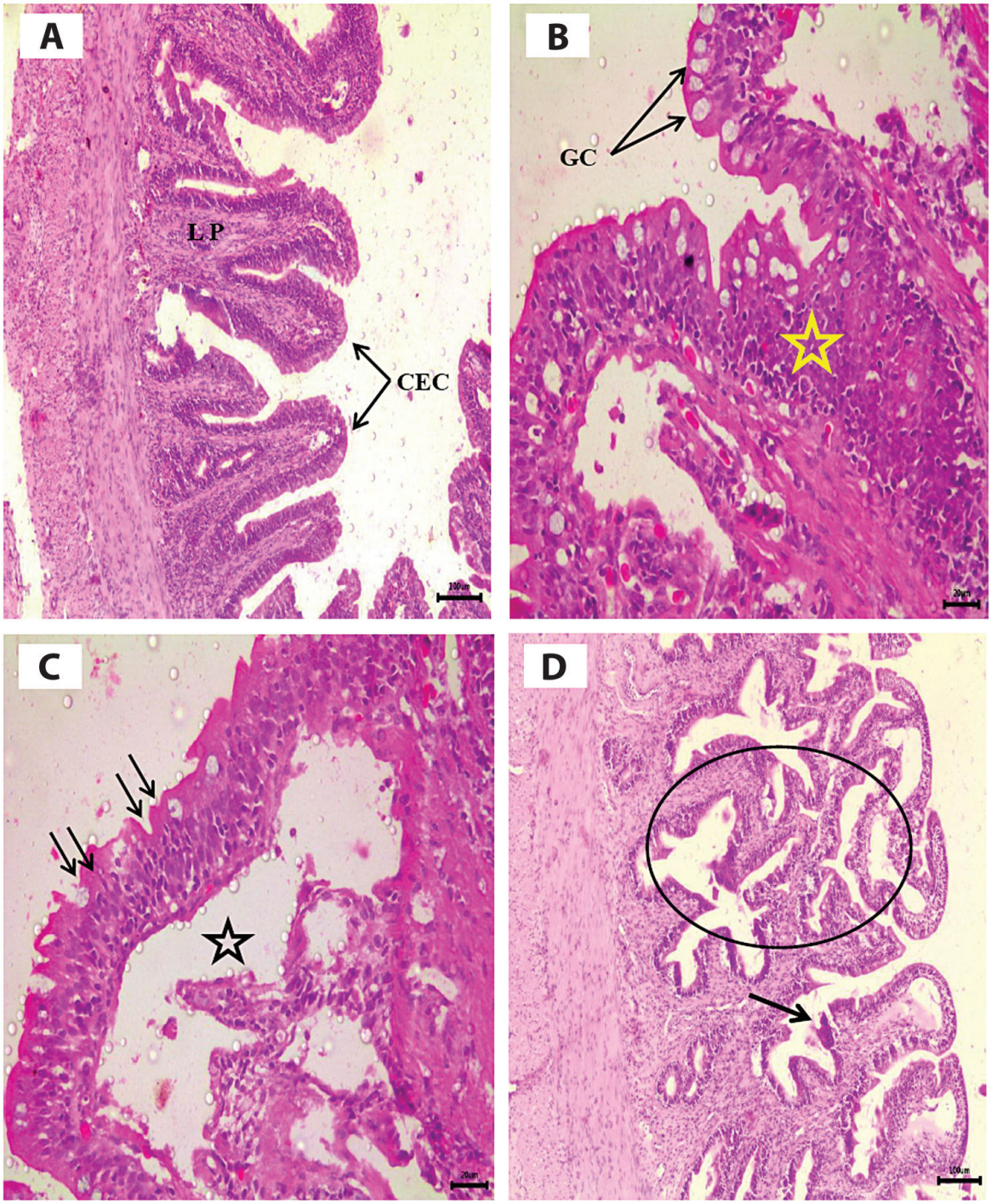

Figure 2. Intestine sections of *Clarias gariepinus* (H & E stain): A) Control intestine tissue showing normal lamina propria (LP), and normal columnar epithelial cells (CEC) with distinct nucleus. B, C, D) Exposed to 390 mg Repare® 18%/L for 15 days; B) Showing hyperplasia of intestinal epithelium (yellow star) with hyperplasia of goblet cells (GC) (arrow); C) Showing Edema of lamina propria (star) and broad intestinal villi (double arrow); D) Showing damaged CEC (arrow) and distortion of villi and lamina propria (oval). Magnification: A (100×) and B, C, D (400×). Scale bars: A, D (100 µm) and B, C (20 µm).

Discussion

This is the first report indicating that the exposure of *C. gariepinus* fish species to sublethal concentration (almost 1/4 of the LC_{50} concentration for 96 h exposure) of a com-

mercial herbicide containing quinclorac and bensulfuron-methyl has negative effects on the predation potential of the fish, female and male. Kerby *et al.* (2012) indicated that exposure of *G. affinis* to sublethal concentrations (0.5 and 1.0 mg/L) of the pesticide diazinon for 48 h decreased its consumption by 90-100% on *P. regilla* tadpole prey, whereas the presence of the pesticide resulted in a significant reduction in activity and attack rates of *G. affinis* against the target prey.

In the case of bensulfuron-methyl and acetochlor mixture rice herbicide, the LC_{50} values against *Procambarus clarkii* fish were 191.25 and 145.24 mg/L at 24 and 96 h, respectively. Tthe herbicide at 1/2 of the 96-h LC_{50} (72.6 mg/L) caused behavioral and morphological changes and induced severe pathological alterations in the gill, heart, muscle, perigastric organ, midgut, and stomach of the fish (Yu *et al.,* 2017). The 96-h LC_{50} of bensulfuron-methyl on *Cyprinus carpio* was 1620 mg/L (Rahmani et al. 2020) and the 96-h LC_{50} of quinclorac on *Rhamdia quelen* fingerlings was 395 mg/L (Miron *et al.* 2005).

This is the first report for the predatory activity of *C. gariepinus* fish collected in Egypt against *Cx. pipiens* larvae under laboratory conditions, indicating its potential use as an eco-friendly and effectively indigenous biocontrol agent against the aquatic developmental stages of *Cx. pipiens*. The larvivorous efficiency of *C. gariepinus* has been reported in Ethiopia against larvae of both *An. arabiensis* and *Culex* sp. in laboratory and semi-field experiments; the larvae consumption by the fish was significantly increased during the nighttime than during the daytime (Chala *et al.,* 2016). In India, *C. gariepinus* exhibited a highest capacity to feed on *An. stephensi* (Liston) larvae compared with *C. idella*, *Cyprinus carpio* L., and *O. niloticus* (Ghosh *et al.,* 2005). Earlier studies demonstrated that the predation rates of *C. gariepinus* and other indigenous and exotic larvivorous fish species were greatly associated with different biological traits such as the biotype, size, developmental stages and sex of the larvivorous fish, and the genera, developmental stages and population density of mosquito prey (Seng *et al.,* 2008; Chala *et al.,* 2016; Mohamed *et al.,* 2021), or the presence of different chemical and physical stressors such as pesticides, light, and salinity in aquatic habitats (Kerby *et al.,* 2012; Yofukuji *et al.,* 2021).

In our study, the untreated female of *C. gariepinus* consumed more *Cx. pipiens* larvae than the untreated male fish. Moreover, the daily predation rates of the pre-treated and untreated groups of female *C. gariepinus* against *Cx. pipiens* larvae were higher than those of the male fish in both groups, indicating that the predation rates of *C. gariepinus* were strongly associated with the sex of fish. Similarly, mosquito larval consumption is strongly correlated with the sex of predatory fish *Poecilia reticulata* (Seng *et al.,* 2008; Saleeza *et al.,* 2014).

Female and male of *C. gariepinus* exposed to sublethal dose of the tested quinclorac and bensulfuron-methyl based herbicide showed decreased predation rates on *Cx. pipiens* larvae compared to non-exposed fish. This might be associated with the direct deleterious effect of the herbicide on the behavioral, physiological, pathological, and metabolic functions in the treated fish, e.g. to reduce the activity and change swimming speed behavior (rest and slow swim) that may decrease the attack capability rate on mosquito larvae. The presence of chemical contaminants such as heavy metals and pesticides in aquatic habitats may affect the survival and predatory ability of predatory fish by decreasing the predation rates, altering their swimming ability or reducing their vigor (Kerby *et al.,* 2012; Monde *et al.,* 2016; Yofukuji *et al.,* 2021). Changes in ecological functions of mobility and predation may be due to the inhibitory effect of the toxic compounds on fish's acetylcholinesterase (Banaee, 2012). The hybrid catfish, which was exposed to sub-lethal doses of endosulfan insecticide (0.03-1.0 µg/L) exhibited behavioral changes and decreased its predation capacity on *Bulinus globosus* (Morelet) prey (Monde *et al.,* 2016). Kerby *et al.* (2012) found that diazinon insecticide at low concentrations significantly decreased the predation rate of mosquitofish *G. affinis* against tad-

poles by reducing the fish activity and vigor.

Pesticides and other chemical toxicants in water can enter the digestive tracts of fish during feeding and can also cause histopathological injuries in the digestive organs of fish that may negatively influence the fish feeding activity (Banaee 2012; Yu *et al.,* 2017; Saleh *et al.,* 2022). Histological analyses in the present study showed histological alternations in the stomach and the intestine tissue of *C. gariepinus* fish after exposure to the quinclorac and bensulfuron-methyl based herbicide at a sublethal concentration of 390 mg Repare® 18%/L water for 15 days. Similarly, Samanta *et al.* (2016a) reported that exposure of *O. niloticus* to Almix® herbicide (metsulfuron-methyl+chlorimuron-ethyl) for 30 days led to degenerative changes like distorted mucosal folds, damage in columnar epithelial cells and submucosa, and merged mucosal folds in stomach tissues. Pathological changes in the stomach, midgut, and intestine were also found in *Procambarus clarkia* (Girard) after exposure to the mixture bensulfuron-methyl+acetochlor (Yu *et al.,* 2017). Exposure of *Cirrhinus mrigala* (Hamilton) for 96 h to 1.5-3.0 µg/L fenvalerate insecticide altered intestine histology resulting in necrosis of the epithelial cells linning intestinal villi, sloughing of the mucosal epithelium and lymphocytic cell reaction in the lamina propria (Velmurugan *et al.,* 2007).

Fish stomach and intestine are primarily responsible for the digestion of ingested food materials and are vital organs, which are affected by different xenobiotic compounds, mainly pesticides (Braunbeck and Appelbaum, 1999). The histological changes in stomach and intestine of *C. gariepinus* exposed to quinclorac and bensulfuron-methyl based herbicide could be related to the deformity structures and functions of these organs due to the herbicide toxicity and cause a negative effect on the prey consumption by the fish. The prey consumption of a bug predator, *Podisus nigrispinus* (Dallas) was decreased significantly by prey exposure to permethrin, thiamethoxam, and *Bacillus thuringiensis* insecticides causing morphological and histological alternation in the midgut of the predator that impaired its digestibility (Silva *et al.*, 2021). Samanta *et al.* (2016b) described similar lesions formation in villi of *Anabas testudineus* (Bloch) after exposure to glyphosate herbicide.

In conclusion, this study showed that the fish *C. gariepinus* collected from Egypt can consume *Cx. pipiens* larvae, and fish females exhibit higher consumption capacity than the males. Our results suggest that the native to Egypt fish, *C. gariepinus* could be considered as a pioneer and a promising biocontrol agent against *Cx. pipiens* larvae. However, exposure of *C. gariepinus* to sublethal concentration of a herbicide containing quinclorac and bensulfuron-methyl (390 mg Repare® 18%/L water) for 15 days reduced the predatory potential of the fish female and male against mosquito larvae of *Cx. pipiens*. The herbicide also caused histopathological changes in the stomach and intestine of this predatory fish that may compromise predation and affect digestion and absorption of nutrients. These findings suggest the necessity of appropriate application of the tested herbicide according to the authorized uses indicated in the label of the product in agricultural lands and fish farms in order to prevent or reduce harmful effects on the fish. The effects of pesticides on predatory fish, their prey, and their interactions require further laboratory and field studies.

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Compliance with ethical standards

All laboratory experiments were approved by the Committee of the Faculty of Veterinary of Assiut University, Egypt.

Literature Cited

Abebe, A., Natarajan, P. and Getahun, A. 2018. Efficacy of tilapia, *Oreochromis niloticus* and *Tilapia zilli* for the control of mosquito larvae around

Fincha Valley, Oromia region, Ethiopia. *International Journal of Mosquito Research*, 5: 35–41.

APC 2021. Agricultural Pesticide Committee. [online] URL: http://www.apc.gov.eg/en/default.aspx. (accessed March 1, 2021).

Banaee, M. 2012. Adverse effect of insecticides on various aspects of fish's biology and physiology, in: Soloneski, S., Larramendy, M.L. (Eds.), Insecticides Basic and Other Applications. InTech, Rijeka, Croatia., pp. 101-126.

Bancroft, J.D., Stevens, A. 1982. Theory and Practice of Histological Techniques, second ed. Edinburgh, Churchill Livingstone, London, UK.

Bancroft, J.D., Stevens, A. and Turner, D.R. 1996. Theory and Practice of Histological Techniques, fourth ed.Churchill Livingstone, New York, USA.

Bhattacharjee, I., Aditya, G. and Chandra, G. 2009. Laboratory and field assessment of the potential of larvivorous, air-breathing fishes as predators of culicine mosquitoes. *Biological Control*, 49: 126–133.

Braunbeck, T. and Appelbaum, S. 1999. Ultrastructural alterations in the liver and intestine of carp *Cyprinus carpio* induced orally by ultra-low doses of endosulfan. *Diseases of Aquatic Organisms*, 36: 183–200.

Chala, B., Erko, B., Animut, A., Degarege, A. and Petros, B., 2016. Assessment of *Clarias gariepinus* as a biological control agent against mosquito larvae. *BMC Ecology,* 16: 27.

Chandra, G., Bhattacharjee, I., Chatterjee, S. and Ghosh, A. 2008. Mosquito control by larvivorous fish. *The Indian Journal of Medical Research,* 127: 13–27.

Das, M.K., Rao, M. and Kulsreshtha, A. 2018. Native larvivorous fish diversity as a biological control agent against mosquito larvae in an endemic malarious region of Ranchi district in Jharkhand, India. *Journal of Vector Borne Diseases,* 55: 34–41.

Doherty, V.F., Ladipo M.K., Aneyo, I.A., Adeola, A. and Odulele, W.Y. 2016. Histopathological alterations, biochemical responses and acetylcholinesterase levels in *Clarias gariepinus* as biomarkers of exposure to organophosphates pesticides. *Environmental Monitoring Assessment*, 188: 312.

Fathy, M., Mohamed, I.A., Farghal, A.I.A., Temerak, S.A.H. and Sayed, A.E.H. 2019. Hemotoxic effects of some herbicides on juvenile of Nile tilapia *Oreochromis niloticus*. *Environmental Science and Pollution Research*, 26: 30857–30865.

Gashaw, F., Erko, B., Teklehaymanot, T. and Habtesellasie, R. 2008. Assessment of the potential of competitor snails and African catfish (*Clarias gariepinus*) as biocontrol agents against snail hosts transmitting schistosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 102: 774–779.

Ghosh, A., Mandal, S., Bhattacharjee, I. and Chandra, G. 2005. Biological control of vector mosquitoes by some common exotic fish predators. *Turkish Journal of Biology,* 29: 167–171.

Ghosh, S.K. and Dash, A.P. 2007. Larvivorous fish against malaria vectors: a new outlook. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 101: 1063–1064.

Hamed, H.S. and Osman, A.G.M. 2017. Modulatory effect of lycopene against carbofuran toxicity in African catfish, *Clarias gariepinus*. *Fish Physiology and Biochemistry*, 43: 1721–1731.

Kerby, J.L., Wehrmann, A. and Sih, A. 2012. Impacts of the insecticide diazinon on the behavior of predatory fish and amphibian prey. *Journal of Herpetology,* 46: 171–176.

Miron, D.S., Shettinger, M.R., Morsch, V.M., Baldisseroto, B., Tierno, M.A., Moraes, G. and Vieira, V.L.P. 2005. Effects of the herbicides clomazone, quinclorac, and metsulfuron methyl on acetylcholinesterase activity in the silver catfish (*Rhamdia quelen*) (Heptapteridae). *Ecotoxicology and Environmental Safety*, 61: 398–403.

Mohamed, I.A., Fathy, M., Farghal A.I.A., Temerak, S.A.H. and Sayed, A.E.H. 2021. Efficacy of nile tilapia (*Oreochromis niloticus*) juveniles and spinosyns bioinsecticides against aquatic stages of *Culex pipiens*: An experimental study. *Journal of Asia-Pacific Entomology,* 24: 190–194.

Monde, C., Syampungani, S. and Van den Brink, P.J. 2016. Effects of endosulfan on predator-prey interactions between catfish and schistosoma host snails. *Archives of Environmental Contamination and Toxicology,* 71: 257–266.

Okamoto, Y., Fisher, R.L., Armbrust, K.L. and Peter, C.J. 1998. Surface water monitoring survey for bensulfuron methyl applied in paddy fields. *Journal of Pesticide Science,* 23: 235–240.

Parveen, S., Kohguchi, T., Biswas, M. and Nakagoshi, N. 2005. Predicting herbicides concentrations in paddy water and runoff to the river basin. *Journal of Environmental Science*, 17: 631–636.

Persch, T., da Silva, P.R., Dos Santos, S., de Freitas, B.S. and Oliveira, G.T. 2018. Changes in intermediate metabolism and oxidative balance parameters in sexually matured three-barbeled catfishes exposed to herbicides from rice crops (Roundup®, Primoleo® and Facet®). *Environmental Toxicology and Pharmacology,* 58: 170–179.

Persch, T., Weimer, R.N., Freitas, B.S. and Oliveira, G.T. 2017. Metabolic parameters and oxidative balance in juvenile *Rhamdia quelen* exposed to rice paddy herbicides: Roundup®, Primoleo®, and Facet®. *Chemosphere,* 174: 98–109.

Ponzoni, R.W. and Nguyen N.H. 2008. Proceedings of a Workshop on the Development of a Genetic Improvement Program for African catfish *Clarias gariepinus*. World Fish Center Conference Proceedings Number 1889. The WorldFish Center, Penang, Malaysia.

Pyke, G.H. 2008. Plague minnow or mosquito fish?

A review of the biology and impacts of introduced *Gambusia* species. *Annual Review of Ecology, Evolution and Systematics,* 39: 171–191.

Rahmani, K.F., Omidzahir, S., Movahedinia, A. and Akhoundian, M. 2020. Determination of median lethal concentration of herbicide bensulfuron methyl in common carp (*Cyprinus carpio*). *Iranian Scientific Fisheries Journal*, 29: 65–72.

Resgalla, C.Jr., Noldin, J.A., Tamanaha, M.S., Deschamps, F.C., Eberhardt, D.S. and Rorig, L.R. 2007. Risk analysis of herbicide quinclorac residues in irrigated rice areas, Santa Catarina, Brazil. *Ecotoxicology*, 16:565–571.

Saleeza, S.N., Norma-Rashid, Y. and Sofian-Azirun, M. 2014. Guppies as predators of common mosquito larvae in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health,* 45: 299–308.

Saleh, S., Mohamed, I.A., Fathy, M. and Sayed, A.E.H. 2022. Neuro-hepatopathological changes in juvenile *Oreochromis niloticus* exposed to sublethal concentrations of commercial herbicides. *Environmental Toxicology and Pharmacology,* 93: 103871.

Samanta, P., Pal, S., Mukherjee, A.K., Senapati, T., Kole, D. and Ghosh, A.R. 2016a. Effects of Almix® herbicide on oxidative stress parameters in three freshwater teleostean fishes in natural condition. *Biochemical and Pharmacology (Los Angels),* 5: 209.

Samanta, P., Pal, S., Mukherjee, A.K., Senapati, T., Kole, D. and Ghosh, A.R. 2016b. Histopathological and ultrasturctural alternations in *Anabas testudineus* exposed to glyphosate-baded herbicide, excel mera 71 under field and laboratory conditions. *Journal of Aquacultural Resserch Development,* 7: 436–441.

Seng, M.C., Setha, T., So Nealon, J., Socheat, D., Chantha, N. and Nathan, B.M. 2008. Community-based use of the larvivorus fish *Poecilia reticulata* to control the dengue vector *Aedes aegypti* in domestic water storage container in rural Cambodia. *Journal of Vector Ecology,* 33: 139–144.

Silva, W.M., Martínez, L.C., Plata-Rueda, A., Serrão, J.E. and Zanuncio, J.C. 2021. Exposure to insecticides causes effects on survival, prey consumption, and histological changes in the midgut of the predatory bug, *Podisus nigrispinus* (Hemiptera: Pentatomidae). *Environmental Science and Pollution Research International,* 28: 57449–57458.

Soliman, N.F. and Yacout, D.M. 2016. Aquaculture in Egypt: status, constraints and potentials. *Aquaculture International, 24*: 1201–1227.

Stanley J. and Preetha G. 2016. Pesticide Toxicity to Non-target Organisms: Exposure, Toxicity and Risk Assessment Methodologies. Springer, Dordrecht.

Ullah, S. and Zorriehzahra, M.J. 2015. Ecotoxicology: a review of pesticides induced toxicity in fish. *Advances in Animal and Veterinary Sciences,* 3: 40–57.

Velmurugan, B., Selvanayagam, M., Cengiz, E.I. and Unlu, E. 2007. The effects of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala*. *Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants and Agricultural Wastes,* 42: 157–163.

Yildirim, Ö. and Karacuha, A. 2007. A preliminary study on determination of *Aphanius chantrei's* feeding behaviour on mosquito larvae. *Acta Tropica,* 102: 172–175.

Yofukuji, K.Y., Gonino, G.M., Alves, G.H., Lopes, T.M. and Figueiredo, B.R. 2021. Acute ecotoxicity of exposure to sugarcane ashes on the behaviour of predator and prey fish species. *Water, Air and Soil Pollution,* 232: 312.

Yu, J., Xu, E.G., Ren, Y., Jin, S., Zhang, T., Liu, J. and Li, Z. 2017. Mixture toxicity of bensulfuron-methyl and acetochlor to red swamp crayfish (*Procambarus clarkii*): behavioral, morphological and histological effects. *International Journal of Environmental Research and Public Health,* 14: 1466.

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Συμπεριφορικές αλλαγές και ιστοπαθολογικές αλλοιώσεις στο ψάρι *Clarias gariepinus* ως αρπακτικό προνυμφών του κουνουπιού *Culex pipiens* μετά από έκθεση σε υποθανατηφόρο συγκέντρωση ζιζανιοκτόνου με δραστικές ουσίες quinclorac και bensulfuron-methyl

I.A. Mohamed, M. Fathy, A.I.A. Farghal, S.A.H. Temerak, S.Kh. Abd El-Ghaffar και S.K.A. Idriss

Περίληψη Το *Clarias gariepinus* είναι ένα καλλιεργούμενο είδος ψαριού του γλυκού νερού, ευρέως διαδεδομένο στην Αφρική, το οποίο κυριαρχεί σε διάφορα φυσικά και ανθρωπογενή υδάτινα οικο-

συστήματα, συμπεριλαμβανομένου του μικτού συστήματος ιχυοκαλλιέργειας - καλλιέργειας ρυζιού. Αυτό το είδος ψαριού εμφανίζει αρπακτική ικανότητα στα υδρόβια στάδια ανάπτυξης των κουνουπιών. Οι bensulfuron-methyl και quinclorac είναι ζιζανιοκτόνες δραστικές ουσίες που έχουν εφαρμοστεί εκτενώς σε καλλιέργειες ρυζιού στην Αφρική και άλλες χώρες παγκοσμίως. Η μελέτη αυτή αξιολόγησε τις αρνητικές επιδράσεις υποθανατηφόρου συγκέντρωσης ενός εμπορικά διαθέσιμου ζιζανιοκτόνου που περιέχει τις δραστικές ουσίες quinclorac and bensulfuron-methyl, στην αρπακτική ικανότητα των θηλυκών και αρσενικών ατόμων του ψαριού *Clarias gariepinus* σε προνύμφες του κουνουπιού *Culex pipiens*. Επίσης, μελετήθηκε η ιστοπαθολογία του στομάχου και του εντέρου των ψαριών στα οποία έγινε η εφαρμογή του ζιζανιοκτόνου. Η έκθεση του *C. gariepinus* στην υποθανατηφόρο συγκέντρωση του ζιζανιοκτόνου με δραστικές ουσίες quinclorac και bensulfuron-methyl προκάλεσε αρνητικές επιδράσεις στην κατανάλωση λείας και ιστοπαθολογικές αλλοιώσεις στον στόμαχο και στο έντερο του ψαριού. Η κατανάλωση προνυμφών κουνουπιών από τα θηλυκά και αρσενικά ψάρια στα οποία είχε γίνει εφαρμογή του ζιζανιοκτόνου μειώθηκε σημαντικά σε σχέση με εκείνη των ψαριών και των δύο φύλων που δεν εκτέθηκαν στο φάρμακο. Οι ιστοπαθολογικές αλλοιώσεις στο έντερο ήταν υπερπλασία του επιθηλίου του εντέρου και των καλυκοειδών κυττάρων, οίδημα της υποβλεννογόνιου στοιβάδας και διεύρυνση των εντερικών λαχνών, και παραμόρφωση των εντερικών λαχνών, σε σύγκριση με το μάρτυρα. Οι ιστοπαθολογικές αλλοιώσεις του στομάχου ήταν νέκρωση και αποκόλληση του επιθηλίου του βλεννογόνου με σοβαρή βλάβη του υποβλεννογόνου. Ως εκ τούτου, το δοκιμαζόμενο ζιζανιοκτόνο σε υποθανατηφόρο συγκέντρωση στο ψάρι *C. gariepinus* μείωσε την κατανάλωση προνυμφών κουνουπιών και προκάλεσε ιστοπαθολογικές αλλοιώσεις στο ψάρι, οι οποίες ενδεχομένως επηρέασαν τη φυσιολογίας της πέψης. Τα ευρήματα της μελέτης δείχνουν ότι το δοκιμαζόμενο ζιζανιοκτόνο μπορεί να αποτελέσει απειλή για την επιβίωση του ψαριού *C. gariepinus* και τη δυνατότητα χρήσης του ως επιτυχούς ιθαγενή παράγοντα βιολογικής καταπολέμησης προνυμφών του κουνουπιού *Cx. pipiens*.

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SHORT COMMUNICATION

New data on the parasitization of *Aleurothrixus floccosus* (Maskell) (Hemiptera: Aleyrodidae) in Greece

G.J. Stathas[1*], N.G. Kavallieratos[2], L.N. Cheliotis[1], P.J. Skouras[1], M.V. Giakoumaki[3] and P.G. Milonas[3]

Summary *Signiphora flavella* (Girault) (Hymenoptera: Signiphoridae) was recorded in 2022 as a new parasitoid species of the serious pest of citrus *Aleurothrixus floccosus* (Maskell) in Greece, in two different areas, i.e., the provinces of Laconia and Messinia. Previously, *Signiphora flavella* was recorded in Greece parasitizing *Hemiberlesia rapax* (Comstock) and *H. lataniae* (Signoret) in early 60s. It is mainly a parasitoid species of scale insects belonging to Diaspididae (Hemiptera: Coccomorpha), whiteflies, or a hyperparasitoid of aphelinids.

Additional keywords: biological control, parasitoid, *Signiphora flavella,* whitefly

The woolly whitefly, *Aleurothrixus floccosus* (Maskell, 1896) (Hemiptera: Aleyrodidae) was firstly recorded in Greece during 1991 at several urban areas of Athens (Greece) heavily infesting citrus trees (Katsoyannos, 1991). Later, it was confirmed invading all citrus cultivated areas of Greece. Successful biological control of this noxious species was conducted by introducing the exotic parasitoid *Cales noacki* Howard, 1907 (Hymenoptera: Aphelinidae) from Spain and France (Katsoyannos, 1991). The parasitoid was reared and massively released to the infested trees in numerous citrus growing areas of Greece (Katsoyannos, 1991; 1994; Katsoyannos *et al.,* 1997; 1998). The spectrum of natural enemies of *A. floccosus* consisted of the introduced parasitoid (*C. noacki*) and the native predator *Clitostethus arcuatus* (Rossi) (Coleoptera: Coccinellidae) feeding on eggs and early-instar nymphs of *A. floccosus*. During the period 1991-2005 studies on the ecology of *A. floccosus* in citrus cultivated areas (36 areas of Attica, Greece and 34 in the rest country), recorded that *C. noacki* was the main natural enemy of *A. floccosus*. In high infestation levels, the predators *C. arcuatus* and *Oenopia conglobata* (L.) have been drastically feeding upon *A. floccosus* (Kontodimas *et al.,* 2005).

Natural enemies of *A. floccosus* are referred in other areas of the world; Chile: *C. noacki, Eretmocerus paulistus* Hempel (Hymenoptera: Aphelinidae)*, Amitus spiniferus* (Brethes) (Hymenoptera: Platygastridae)*, Signiphora* sp. Ashmead (Hymenoptera: Signiphoridae) (Tello-Mercado and Zarzar-Maza, 2021), *Oligota pygmaea* and *Parastethorus histrio* (Coleoptera: Coccinellidae) (Rioja, *et al.,* 2015); Mexico: *Eretmocerus naranjae* Myartseva (Hymenoptera: Aphelinidae) (Myartseva and Coronado-Blanco, 2007); Brazil *Signiphora* sp. and *C. noacki* (Marsaro Júnior, *et al.,* 2015); California (USA): *Cales rosei* Mottern (Hymenoptera: Aphelinidae) (Mottern and Heraty, 2014); Dominican Republic and Florida (USA): *Encarsia dominicana* Evans (Hymenoptera: Aphelinidae) (Evans and Serra, 2002); France: *C. noaki* and

[1] Laboratory of Agricultural Entomology and Zoology, Department of Agriculture, University of the Peloponnese, GR-241 00 Kalamata, Greece.
[2] Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens, 75 Iera Odos Str., GR-118 55, Athens, Greece.
[3] Laboratory of Biological Control, Scientific Directorate of Entomology & Agricultural Zoology, Benaki Phytopathological Institute, 8 Stefanou Delta Str., GR-145 61 Kifissia, Athens, Greece.
* Corresponding author: g.stathas@uop.gr

Amitus spiniferus (Brèthes) (Hymenoptera: Platygastridae) (Onillon, 1988); Turkey: *C. noacki*, *C. arcuatus* Risso, *Cryptoleamus montrouzieri* Mulsant, *Chilocorus bipustulatus* L., *Serangium parcesetosum* Sicard (Coleoptera: Coccinellidae), *Conwentzia* sp. (Neuroptera: Coniopterygidae), and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) (Telli and Yiğit, 2012); India: *Encarsia guadeloupae* Viggiani (Hymenoptera: Aphelinidae), *Pseudomallada astur* (Banks) (Neuroptera: Chrysopidae), and the entomopathogenic fungus, *Isaria fumosorosea* Wize (Hypocreales: Clavicipitaceae) (Sundararaj *et al.,* 2021).

Within the genus *Signiphora*, 50 species and numerous undescribed species have been particularly recorded in the Neotropical region. They have been reported as primary or secondary parasitoids of Diaspididae, Coccidae, Pseudococcidae, Aleyrodidae, while rarely of other Homoptera, and parasitoids of pupae of Tachinidae and Drosophilidae (Diptera) (Gibson *et al.,* 1997; Woolley, 1988; Noyes, 2023).

Signiphora flavella is a cosmopolitan and polyphagous species. It is distributed to Algeria, Argentina (Buenos Aires), Australia, Brazil, Chile (Pocochay, Santiago), Honduras, Greece (Crete), India (Shillong), Indonesia, Israel (Rehovot), Mexico (Amecameca), Morocco, New Zealand, Peru (Lima), Puerto Rico (Central Aguirre), South Africa, Spain, USA (California: Santa Barbara, Miami, New Orleans, San Antonio, San Diego), Venezuela (Trinidad, Tobago) (Woolley and Dal Molin, 2017; Schmidt *et al.,* 2019).

The hosts of *S. flavella* are mainly scale insects of the family Diaspididae: *Aonidiella aurantii* (Maskell), *A. ensifera* McKenzie, *Aspidiotus nerii* Bouché, *Chrysomphalus dictyospermi* (Morgan), *Comstockaspis perniciosa* (Comstock), *Hemiberlesia cyanophylli* (Signoret), *Hemiberlesia lataniae* (Signoret), *Hemiberlesia palmae* (Cockerell), *Lepidosaphes ulmi* (L.), *Oceanaspidiotus spinosus* (Comstock), *Pseudaonidia trilobitiformis* (Green), and *Pseudaulacaspis pentagona* (Targioni Tozzetti) (García Morales *et al.,* 2016). In a recent review, Woolley and Dal Molin (2017) have reported more hosts such as *Hemiberlesia rapax* (Comstock), *Aspidiotus cyanophylli* Signoret, *Serenaspis minima* (Maskell) under the synonym *Hemichionaspis minor* (Maskell), *Diaspis pentagona* Targioni Tozzetti, *Lepidosaphes beckii* (Newman), *Parlatoria pittospori* Maskell, *Pseudaulacaspis cockerelli* (Cooley), *Parlatoria pergandii* Comstock (Hemiptera: Diaspididae), and *A. floccosus*. Furthermore, *S. flavella* is referred as hyperparasitoid of Aphelinidae parasitoids (Hymenoptera), i.e., *Coccophagus* sp. Westwood (attacking *Saissetia persimilis* (Newstead)) and *Aspidiotiphagus citrinus* (Craw) (Woolley and Dal Molin, 2017).

In Greece, *S. flavella* has been found by DeBach on *H. rapax* and *H. lataniae* in early 60s (Woolley Dal Molin, 2017). In 2022, *S. flavella* was found to highly parasitise *A. floccosus* that heavily infested orange trees, *Citrus sinensis* (L.) Osbeck var. Valencia, in the area Vlachioti (Laconia province, Peloponnese, Greece) (36˚51′ N, 22˚41′ E; altitude: 14 m above sea level (a.s.l.)) (Fig. 1). The same year it was recorded parasitizing *A. floccosus* on *C. sinensis* var. Navelina in the area Asprochoma (Messenian province, Peloponnese, Greece) (37˚03′ N, 22˚03′ E; altitude: 5 m a.s.l.). In preliminary studies in Vlachioti, we found that *S. flavella* effectively parasitized *A. floccosus* infesting *C. sinensis* and developed higher population than *C. noacki.* In this area, the population composition of *S. flavella*/*C. noacki* was 69.4/30.4% during November 2022 on the examined samples of infested leaves. Considering the fact that *S. flavella* is recorded for the first time on *A. flocossus* in Greece after its first report on other hosts 70 years ago, further studies are needed on the biology and ecology of this species, that will clarify its role and importance on the biological control of the wooly whitefly.

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Figure 1. *Signiphora flavella* adults parasitizing *Aleurothrixus floccosus* colony infesting citrus.

Literature Cited

Evans, G.A. and Serra, C.A. 2002. Parasitoids associated with whiteflies (Homoptera: Aleyrodidae) in Hispaniola and descriptions of two new species of *Encarsia* Förster (Hymenoptera: Aphelinidae). *Journal of Hymenoptera Research*, 11(2): 197-212.

García Morales, M., Denno, B.D., Miller, D.R., Miller, G.L., Ben-Dov, Y. and Hardy, N.B. 2016. ScaleNet: A literature-based model of scale insect biology and systematics. Database. doi: 10.1093/database/bav118. http://scalenet.info [accessed on: 04-05-2023].

Gibson, G.A.P., Hubner, J.T. and Woolley, J.B. 1997. Annotated keys to the Genera of Nearctic Chalcidoidea (Hymenoptera). Ottawa, Canada: NCR Research Press. 794 p.

Katsoyannos, P. 1991. First record of *Aleurothrixus floccosus* (Mask.) (Homoptera: Aleyrodidae) in Greece and some observations on its phenology. *Entomologia Hellenica*, 9: 69-72.

Katsoyannos, P. 1994. Rearing method of *Cales noacki*, natural enemy of the wooly whitefly. *Agriculture Crop and Animal Husbandry*, 4: 32-34 (in Greek).

Katsoyannos, P., Ifantis, K. and Kontodimas, D.C. 1997. Phenology, population trend and natural enemies of *Aleurothrixus floccosus* (Hom.: Aleyrodidae) at a newly invaded area in Athens, Greece. *Entomophaga,* 42(4): 619-628. doi:10.1007/BF02769820.

Katsoyannos, P., Kontodimas, D.C. and Stathas, G.J. 1998. The innudative release of *Cales noacki* Howard (Hymenoptera: Aphelinidae), for curative treatment of *Aleurothrixus floccosus* (Maskell) (Homoptera: Aleyrodidae) on heavily infested citrus in Greece. *Annals del' Institut Phytopathologique Benaki,* (N.S.), 18: 121-134.

Kontodimas, D.C., Papanicolaou, N., Milonas, P., Stathas, G.J., Beris, E., Bertsouklis, K. and Lagoudakis, E. 2005. Wooly whitefly on citrus in Greece, during 1991-2005. *Proceedings of the 11th Panhellenic Entomological Congress*, 11-14 October, Karditsa – Greece, pp 12-20 (in Greek).

Marsaro Júnior, A.L., Filho, F.R., Raga, A. and Costa, V.A. 2015. New records of whiteflies (Hemiptera: Aleyrodidae) in Rio Grande do Sul state, Brazil. [Nuevos registros de moscas blancas (Hemiptera: Aleyrodidae) en Rio Grande do Sul, Brasil] *Idesia,* 33(1) : 143-145. doi:10.4067/S0718-34292015000100015.

Mottern, J.L. and Heraty, J.M. 2014. The dead can talk: Museum specimens show the origins of a cryptic species used in biological control. *Biological Control, 71*, 30-39. doi: 10.1016/j.biocontrol.2014.01.002.

Myartseva, S.N. and Coronado-Blanco, J.M. 2007. Species of *Eretmocerus* Haldeman (Hymenoptera: Aphelinidae) - parasitoids of *Aleurothrixus floccosus* (Maskell) (Homoptera: Aleyrodidae) of Mexico, with a description of a new species. Acta *Zoologica Mexicana*, 23 (1): 37-46.

Noyes, J.S., 2023. Universal Chalcidoidea Database. World Wide Web electronic publication. http://www.nhm.ac.uk/chalcidoids [accessed on: 11-05-2023].

Onillon, J.C. 1988. Lutte biologique et intégrée dans les vergers de citrus en zone méditerranéenne. *Entomophaga*, 33(4): 481- 494. doi:10.1007/BF02373184.

Rioja, T., Ceballos, R., Rebolledo, R. and Vargas, R. 2015. Rearing and development of *Oligota pygmaea* and *Parastethorus histrio* (Coleoptera: Staphylinidae, Coccinellidae) feeding on *Oligonychus yothersi* (Acari: Tetranychidae) and survival on non-mite foods under laboratory conditions, *International Journal of Acarology,* 41(8): 681-687.

Schmidt, S., Hamid, H., Ubaidillah, R., Ward, S. and Polaszek, A. 2019. A review of the Indonesian species of the family Signiphoridae (Hymenoptera, Chalcidoidea), with description of three new species. *ZooKeys*, (897): 29-47. doi:10.3897/zookeys.897.38148.

Sundararaj, R., Krishnan, S. and Sumalatha, B.V. 2021, Invasion and expansion of exotic whiteflies (Hemiptera: Aleyrodidae) in India and their economic importance. *Phytoparasitica,* 49(5): 851-863.

Telli, Ö. and Yiğit, A. 2012. Natural enemies of citrus woolly whitefly, *Aleurothrixus floccosus* (Maskell) and nesting whitefly, *Paraleyrodes m*inei laccarino (Hemiptera: Aleyrodidae) in Hatay province, Turkey. [Hatay ili turunçgillerinde zararli Turunçgil pamuklu beyazsinegi, *Aleurothrixus floccosus* (Maskell) ve Turunçgil Ipek beyazsinegi, *Paraleyrodes minei* laccarino (Hemiptera: Aleyrodidae)' nin dogal düsmanlari] *Turkiye Entomoloji Dergisi*, 36(1): 147-154.

Tello-Mercado, V. and Zarzar-Maza, M. 2021. Population fluctuation and parasitism of *Aleurothrixus floccosus* (Hemiptera: Aleyrodidae) in citrus trees from Atacama Desert, Chile. [Fluctuación poblacional y parasitismo de *Aleurothrixus floccosus* (Hemiptera: aleyrodidae) en cítricos del desierto de Atacama, Chile]. *Revista Colombiana De Entomologia,* 47(1) doi:10.25100/SOCOLEN.V47I1.7806.

Woolley, J.B. and Dal Molin, A. 2017. Taxonomic revision of the *flavopalliata* species group of *Signiphora* (Hymenoptera: Signiphoridae). *Zootaxa*, 4315(1): 1-150. doi:10.11646/zootaxa.4315.1.1.

Woolley, J.B. 1988. Phylogeny and classification of the Signiphoridae (Hymenoptera: Chalcidoidea). *Systematic Entomology,* 13(4): 465-501. doi:10.1111/j.1365-3113.1988.tb00256.x.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Νέα στοιχεία επί του παρασιτισμού του *Aleurothrixus floccosus* (Maskell) (Hemiptera: Aleyrodidae) στην Ελλάδα

Γ.Ι. Σταθάς, Ν.Γ. Καβαλλιεράτος, Λ.Ν. Χελιώτης, Π.Ι. Σκούρας, Μ.Β. Γιακουμάκη και Π.Γ. Μυλωνάς

Περίληψη Το είδος *Signiphora flavella* (Girault) (Hymenoptera: Signiphoridae) καταγράφηκε στην Ελλάδα κατά το έτος 2022 ως παρασιτοειδές του *Aleurothrixus floccosus* (Maskell) σε σοβαρές προσβολές του σε εσπεριδοειδή, στις περιοχές Λακωνίας και Μεσσηνίας. Προγενέστερα, κατά τις αρχές της δεκαετίας του 1960, το *Signiphora flavella* είχε καταγραφεί στην Ελλάδα ως παρασιτοειδές των *Hemiberlesia rapax* (Comstock) και *H. lataniae* (Signoret). Είναι παρασιτοειδές κυρίως κοκκοειδών εντόμων της οικογένειας Diaspididae (Hemiptera: Coccomorpha), αλλά και αλευρωδών, καθώς και υπερπαρασιτοειδές παρασιτοειδών που ανήκουν στην οικογένεια Aphelinidae.

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