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RESEARCH ARTICLE

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Residual toxicity of insecticides used in Tunisian citrus orchards on the imported parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae): Implications for IPM program of *Ceratitis capitata* (Diptera: Tephritidae)

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Abstract

Citrus agro-industry is globally harshened mainly by *Ceratitis capitata* (Wiedemann), the most worldwide destructive tephritid fruit fly species. Citrus agro-industry is one of the pillars of Tunisia economy, and by hence, harshened by this species. Tunisia has established an Integrated Pest Management (IPM) programme against citrus pests, including *C. capitata*, that rely on the structured use of pesticides, on the application several trapping protocols, along with pilot-scale sterile insect technique program and, since 2013, with pilot-scale releases of the braconid parasitoid *Diachasmimorpha longicaudata* Ashmed (Hymenoptera: Braconidae). Insecticide side-effects on parasitoids and other natural enemies are being requested for a successful implementation of biological control within any IPM programme. However, these data are almost scarce for the braconid species *D. longicaudata*. To this end, we have determined the side-effects of malathion, methidathion, acetamiprid, azadiractin, abamectin, deltametrin+thiacloprid and spinosad, as the most popular insecticides used in Tunisia either as fresh residues or at several aged time points, on the parasitoid *D. longicaudata* according the IOBC pesticide harm-classification. IOBC classification evolution of residues over time had allowed determining the best combination of pesticide applications in a structured fashion with the viable releases of *D. longicaudata* for the control of *C. capitata* in Tunisian citrus agro-ecosystems.

Additional keywords: integrated pest management; natural enemy; pesticide persistence; IOBC

Abbreviations used: APPPC (Asia & Pacific Plant Protection Commission); CBC (Classical Biological Control); EPPO (European Plant Protection Organization); IOBC (International Organisation for Biological and Integrated Control); IPM (Integrated Pest Management); NAPPO (North American Plant Protection Organization).

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Introduction

In Tunisia, citrus cultivation is an important agricultural sector that covers about 21,000 hectares with 6.4 million trees which represent 0.3% of the total useful agricultural area and 3.4% of total fruit crop area. Annual production is estimated to approximately 393,000 tons, 9.45% of fruit production value (FAO, 2016). Citrus agro-ecosystems are threatened by a plethora of pest arthropods, among these, the true fruit flies (Diptera: Tephritidae) are considered as key pests worldwide (Liquido *et al.,* 1990; Jerraya, 2003;

Primo *et al.,* 2003; Urbaneja *et al.,* 2009). One of the key tephritid fruit fly species that is threatening the Tunisian citrus agro-industry is *Ceratitis capitata* Wiedemann, commonly known as the Mediterranean fruit fly or medfly. This consideration is not only for direct damage it produces to citrus crops, but also for the associated phytosanitary restrictions to the export market and indirect fruit loses by secondary pathogens proliferation (Jerraya, 2003). This species is an European Plant Protection Organization (EPPO) A2 quarantine pest and considered of quarantine significance throughout the world (APPPC, NAPPO), especially for Japan, USA and New Zealand (EPPO, 2014), with more than 300 plant species recorded as putative viable hosts (White & Elson-Harris, 1992). For this reason, many countries forbid the import of susceptible fruits without strict pre- and post-harvest control treatments having been applied by the exporter.

During many decades, the suppression of *C. capitata* in Tunisian citrus orchards relied on calendar broad-spectrum applications of synthetic insecticides, mainly carbamates and organophosphates (Jerraya, 2003; Braham *et al.,* 2007; Boulahia-Kheder *et al.,* 2012). More recently, the new European legislations regarding the use of insecticides and their tolerated residues in imported commodities (OJEU, 2009), as well as the emergence of resistance and cross resistance to many commonly used insecticides in Mediterranean populations of *C. capitata* forced stakeholders to review control strategies adopted against this economic pest (Magaña *et al.,* 2007; Couso-Ferrer *et al.,* 2011; Vontas *et al.,* 2011; Arouri *et al.,* 2015). As result, alternative control tactics were introduced and promoted in Tunisia including mass trapping and attract-and-kill techniques leading, when properly applied, to a relative decrease in the frequency of insecticide applications (Boulahia-Kheder *et al.,* 2012; Braham, 2013; Navarro Llopis *et al.,* 2013; Hafsi *et al.,* 2015). Furthermore, a Classical Biological Control (CBC) program was initiated through the screening for local natural enemies and by the introduction of available efficient exotic parasitoids. As result of this first screening, the pupal parasitoid *Pachycrepoideus vindemmiae* Rondani (Hymenoptera: Pteromalidae) was recorded performing very low parasitism rates (Harbi *et al.,* 2015). This wasp is an idiobiont parasitoid of pupae of a wide range of Diptera species in the families Anthomyiidae, Calliphoridae, Drosophilidae, Muscidae, Sarcophagidae, Tachinidae and Tephritidae (Wharton, 1989; Marchiori & Barbaresco, 2007; Tormos *et al.,* 2009). Besides, the larval-pupal endoparasitoid *Dichasmimorpha longicaudata* Ashmed (Hymenoptera: Braconidae) was introduced into Tunisia from Spain in 2012 (the authors).

To enhance the establishment and adaptation of an exotic parasitoid into a new environment, and to guarantee efficient Integrated Pest Management (IPM) strategies, many factors should be taken into consideration. Among these factors, one arises, the response of the exotic parasitoid to the portfolio of available pesticides for each crop. The assessment of acute toxicity of pesticides constitute a corner stone for CBC programmes within and IPM programme, as the degree of toxicity of a pesticide could reduce the establishment and survival of non-target arthropods (Jerraya, 2003; Suma *et al.,* 2009; Urbaneja *et al.,* 2009; Biondi *et al.,* 2012a, 2013, 2015; Juan-Blasco *et al.,* 2013; Vanaclocha *et al.,* 2013).

Diachasmimorpha longicaudata is considered an exotic parasitoid in many parts of the world, originally coming from Southeast Asia where it attacks other tephritid fruit fly species (mainly from *Bactrocera* genus). This parasitoid species has been successfully used in many countries for the control of *Anastrepha*, *Bactrocera* and *Ceratitis* species, by being released mainly in combination with sterile insects (Baranowski *et al.,* 1993; Vargas *et al.,* 2001; Orozco *et al.,* 2002; Benelli *et al.,* 2014). Despite its wide use as biological control agent in other countries, a limited number of studies reflect its susceptibility to pesticides, being in most of the cases, tested in a one by one basis (Stark *et al.,* 1992, 2004; Purcell *et al.,* 1994; Vargas *et al.,* 2001). This scarcity highlights the need of pesticide toxicity studies required for successful implementation of biological control programmes.

The objective of this study is to fill-in this knowledge gap by determining the toxicity level of seven pesticides commonly used to control citrus pest species in Tunisia, based on the International Organization for Biological and Integrated Control (IOBC)- Working group “Pesticides and Beneficial Organisms” standards toxicity classes.

Material and methods

Pesticides

The pesticides used in this work are listed in Table 1, reporting their trade names, formulations, suppliers, active ingredients, chemical families, field rates and modes of action. All tested

Table 1. Insecticides evaluated for their acute toxicity to *Diachasmimorpha longicaudata*, characteristics and application dose.

Trade name, formulations and suppliers	Active ingredient (%, w/v)	Field rate (mL/hL)	Chemical family	Target pests in citrus orchards	Mode of action[1]
Mospilan®- SL, SEP-CM, Tunisia	Acetamiprid 20	30	Neonicotinoid	Leafminers, mites	AChE[1] inhibitor
Oïkos® -EC, Agrosys-teme, Tunisia	Azadirachtin 3.2	60	Botanical	Leafminers	Agonism/perturbation of ecdysone.
Vertimec®-EC, Biopro-tection, Tunisia	Abamectin 0.8	60	Avermetin	Mites	Activation of chloride channel. Interference with the GABA[2] recep-tor in insects
Proteus®-OD, Promo-chimie, Tunisia	Deltametrin + Thiacloprid 15 + 2	60	Pyrethroid+ Chloronicotinyl	Aphids, leafmin-ers, fruit flies, scales	Modulator of the sodi-um channel. + AChE inhibitor
Fyfanon®-EC, SEPCM, Tunisia	Malathion 50	200	Organophos-phate	Aphids, fruit flies, mites and thrips	AChE inhibitor
Ultracide®-EC, Biopro-tection, Tunisia	Methidathion 50	150		Scales, whiteflies	Modulator of the sodi-um channel.
Success-Appât®-CB, Chimic Agri, Tunisia	Spinosad 0.024	1250	Spynosin	Leafminers, thrips, fruit flies	Disruption of nicotinic/ gamma amino butyric acid-gated chloride channels

[1]AChE, acetylcholinesterase; [2]GABA, gamma-aminobutyric acid.

insecticides were stocked, prepared and applied according to manufacturer guidelines. The concentration tested was the maximal recommended by the manufacturer for citrus. (Table 1).

Insect

Medflies and parasitoids were obtained from laboratory colonies maintained in the facilities of the High Agronomic Institute of Chott-Mariem (ISA-CM), Sousse (Tunisia). Both insects, *D. longicaudata* and *C. capitata*, were reared under controlled conditions (25±2ºC, 65±10% RH and 16:8 h L:D) in climatic chambers (constant climate chamber in-house built, Memmert® GmbH, D-91126 Schwabach, Germany).

Ceratitis capitata colony was established in 2012 from infested citrus fruits (collected at Chott-Mariem, Tunisia). Adults are maintained in plastic cages (40 × 30 × 30 cm) with fine mesh framed lateral sides to allow oviposition and egg collection according to the rearing procedure described by Sabater-Muñoz *et al.* (2009) and Martins *et al.* (2010). Adults were provided with a*d libitum* water, sugar and a mixture 1:4 of yeast hydrolysate: household sugar. Larvae of the medfly were fed on artificial diet based on wheat bran yeast and sugar (Sabater-Muñoz *et al.,* 2009; Martins *et al.,* 2010).

Diachasmimorpha longicaudata colony was also established in 2012 with imported parasitized pupae from the Instituto Valenciano de Investigaciones Agrarias (IVIA) research station (Valencia, Spain). Adults were maintained in plastic cages (40 × 30 × 30 cm), similar to those of medfly, with approximately 4,000 to 5,000 females per cage, and provisioned with *ad libitum* water, honey and household sugar. Medfly third instar larvae (within its artificial diet) were exposed daily to the parasitism of *D. longicaudata* females through a fine mesh framed window located on the upper side of the rearing cages. Exposed larvae were allowed to develop in separate cages until the emergence of the new parasitoid generation approximately two weeks later. A cohort of 6-8-d-old parasitoids (≈1:1 sex ratio female:male) were established from this rearing colony for each treatment.

IOBC bioassays for toxicity assessment

To evaluate the residual toxicity of pesticides in *D. longicaudata*, a laboratory method was used (Contreras *et al.,* 2005). Briefly described, sour orange (*Citrus* × *aurantium*) fully expanded young leaves (approx. 10 days after flush fully expanded) were collected from 10-yr-old ornamental untreated trees located at ISA-CM (Chott-Mariem, Tunisia) and delicately brushed then rinsed in distilled water to remove dust, bird feces or other accidently present arthropods, prior treatment.

Clean leaves were treated with selected pesticides (Table 1) by the leaf-dip method (Immaraju *et al.,* 1990). Distilled water was used as control throughout the bioassay. Treated leaves were placed individually in experimental units (isolators) in the laboratory to allow aging of pesticides for 1 hour (fresh residue), 3, 7, 14, 21 or 28-d under ambient conditions, including light exposure (natural photoperiod) to simulate open field conditions. Ambient conditions including sunlight exposure (what we call field conditions) have been stated to decrease the activity and cause degradation (by sun fotobleaching) of several insecticide residues, which are not taken up by tree leaves, as abamectin and spinosad (Demchak & Dybas, 1997; Urbaneja *et al.,* 2009). Isolator consisted on two superposed plastic glasses (600 mL and 100 mL). The top glass had a central hole on its bottom to allow citrus leaf petiole to reach the water present in the bottom glass allowing to keep leaf turgidity and metabolic actions during the pesticide aging process. A fine mesh cloth was fixed on the upper opening of the top glass to allow ventilation. This cup system was previously validated in similar side-effects studies by Zappalà *et al.* (2012) and Biondi *et al.* (2013).

The bioassays were conducted under controlled environmental conditions (25±2°C, 60±10% RH, 16:8 h L:D) in a climatic chamber. Bioassay arena consisted in a polypropylene transparent box (14 × 10 × 9 cm) with a mesh covered aeration window (10 cm^2) in the lid. Treated leaves were transferred individually to bioassay arena, replacing the 100 mL water container by a 1.5 mL micro-centrifuge vial attached with modeling clay to the bottom of bioassay arena. A cohort of 40 6-8 days-old *D. longicaudata* adults (≈ 1:1 females: males ratio) was used in each replica, being provided with a honey solution (in a cylindrical 0.5 × 1.5 cm (diameter × length) dental cotton roll) as food source. Residues of the selected pesticides were assayed at 1h (fresh), 3, 7, 14, 21 or 28 days-old post application in a sequential way (*i.e.* aged residues, which do not show significant differences with control, were not further continued). Each treatment was replicated three times, assessing mortality after three days of exposure to treated leaves. Parasitoid specimens were considered as dead if no response was observed under stereomicroscope after being stimulated with the tip of a soft paintbrush.

Statistical analyses

Data were first tested for normality and homogeneity of variance using Shapiro-Wilk test (SPSS, 2011). The mortality was then compared using one-way analysis of variance (ANOVA) at $p<0.05$. Means were then separated using the LSD *post hoc* test. When significant differences were detected between the control and the pesticides, the mortality values were corrected using Abbot's formula (Abbott, 1925). Then, corrected mortality percentages were used to rank insecticides and their residues according to the IOBC Working group "Pesticides and Beneficial Organisms" standards toxicity classes (Sterk *et al.,* 1999) as follows: (1) harmless, mortality <30%; (2) slightly harmful, 30-79%; (3) moderately harmful, 80-99%; and (4) harmful, mortality >99%.

Results

Three days after exposure to fresh (1 h) and 3-d-old residues were enough to indicate acute toxicity of almost all tested pesticides to *D. longicaudata* (Table 2). The highest mortality rates (100%) were registered for acetamiprid, deltametrin+thiacloprid, malathion, and methidathion without statistical differences among fresh residues. These insecticides were classified as harmful (IOBC class 4) according to the IOBC standards. The rest were classified as slightly harmful (IOBC class 2) causing mortality rates ranging between 65.25 and 78.81% (Table 3). When considering the effect of 3-d-old residues, only acetamiprid, deltametrin+thiacloprid and methidathion reduced their toxicity categories. Toxicity evolution was slow for acetamiprid, malathion and methidathion from 3-d-old residues onwards.

After 14-d-post-treatment, malathion and methidathion were still classified as moderately harmful (IOBC class 3), abamectin and spinosad classification decreased to become harmless (IOBC class 1) whereas acetamiprid toxicity decreased from moderately harmful (IOBC class 3) to slightly harmful (IOBC class 2). After 21 days post-treatment, malathion remained as moderately harmful, methidathion, acetamiprid remained slightly harmful (IOBC class 2) and deltametrin+thiacloprid and abamectin become harmless (IOBC class 1) even if they induce mortality at 15-24%, with statistical difference among them (Table 2 and 3). At this residue age, malathion and methidathion residues remained the most toxic to *D. longicaudata* adults, even mortality percentage allowed to assign both pesticides to different IOBC classes, they do not show statistical differences in induced mortality (Table 2). At the most distantly treatment time point, 28-d, malathion still remained as moderately harmful (IOBC class 3), methidathion and acetamiprid, even if show a reduction in induced mortality, remained as slightly harmful (IOBC class 2).

Taking all these data we have determined a structured high to low acute toxicity aggrupation to *D. longicaudata* for the seven tested pesticides as: malathion, methidathion >> acetamiprid > deltametrin+thiacloprid > abamectin, spinosad > azadirachtin.

Table 2. Mean percent mortality (±SE) of *Diachasmimorpha longicaudata* adults after 3 days of exposure to selected chemicals, as fresh or aged residues, compared to control (water treated) leaves.

Active ingredient	Residue age					
	Fresh (1 h)	3 d	7 d	14 d	21 d	28 d
Abamectin	78.81 ± 6.39c (78.81) [1]	79.16 ± 6.29c (78.44)	61.66 ± 11.27c (59.64)	30.0 ± 7.5b (23.63)	20.83 ± 5.77ab(-)	
Acetamiprid	100.0 ± 0.00d (100)	96.66 ± 2.88de (96.55)	87.5 ± 4.33d (86.84)	80.0 ± 7.5d (78.18)	77.5 ± 6.61c (76.10)	54.16 ± 10.10cd (52.58)
Azadirachtin	71.18 ± 5.29bc (71.18)	35.83 ± 2.88b (33.62)	15.0 ± 2.5ab (-) [2]			
Deltametrin+ Thiacloprid	100.0 ± 0.0d (100)	90.83 ± 5.20d (90.51)	83.33 ± 6.29d (82.45)	65.0 ± 9.01 c (61.81)	28.33 ± 9.46b (23.89)	20.83 ± 5.77b (18.10)
Malathion	100.0 ± 0.0d (100)	100.0 ± 0.0e (100)	100.0 ± 0.00e (100)	95.0 ± 4.33e (94.54)	90.83 ± 3.81d (90.26)	81.66 ± 5.20d (81.03)
Methidathion	100.0 ± 0.0d (100)	94.16 ± 3.81de (93.96)	85.83 ± 2.88d (85.08)	86.66 ± 2.88de (85.45)	80.0 ± 9.01cd (78.76)	46.66 ± 5.20c (44.82)
Spinosad	65.25 ± 10.58b (100)	39.16 ± 5.20b (37.06)	20.0 ± 2.5b (15.78)	20.83 ± 7.63ab(-) [2]		
Control	0.0 ± 0.0a	3.33 ± 3.81a	5.00 ± 4.33a	8.33 ± 2.88a	5.83 ± 1.44a	3.33 ± 1.44a
Statistics						
F	149.21	218.08	148.79	81.12	83.43	67.50
d.f.	7	7	7	6	5	4
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[1] Corrected mortality (Abbott) showed between brackets. [2] (-); as indicated in the text, data were not determined as in the precedent residue age, no statistical differences were observed with control. Different letters within each set of columns indicate significantly different values of mortality ($p<0.05$; LSD test).

Discussion

In the present study we have determined the toxicity level for *D. longicaudata* of the seven most used pesticides in Tunisian citrus orchards. Following the achieved classification (Table 3), we will develop a structured scheme of pesticides applications that guaranty the establishment of *D. longicaudata* in Tunisian citrus orchards. To facilitate the discussion, we followed the aggrupation based on toxicity of tested insecticides to *D. longicaudata*: malathion, methidathion >> acetamiprid > deltametrin+thiacloprid > abamectin, spinosad > azadarachtin.

Malathion, methidathion

Malathion and methidathion belong to the same organophosphate chemical family but with differentiated mode of action (Table 1). These insecticides have been recently banned from the European Union (OJEU, 2015) by their human health concerns (Flessel *et al.,* 1993; Marty *et al.,* 1994) and their harmful effects on non-target arthropods (Ehler & Endicott, 1984; Urbaneja *et al.,* 2009) which lead in some cases to outbreaks of secondary agricultural pests that were under control of natural enemies. Despite this banning, Malathion is still used in the Mediterranean area [including some EU members by some considerations in the OJEU (2015)], including Tunisia, as efficient pesticide against *C. capitata*. However, in some of these countries, it has been reported the presence of malathion resistant medfly populations after an increase of the number of treatments by the citrus growers (Magaña *et al.,* 2007; Vontas *et al.,* 2011). These results for *C. capitata* along with the ones presented here, which classifies Malathion as IOBC class 4 even at 7-d-post-treatment to *D. longicaudata*, allowed us to ask for a complete removal from the Tunisian IPM programme against the medfly. Concerning methidathion, in Tunisia it is used against scales and whiteflies, which to date are not showing any resistance. But, due to the human health and environmental concerns (Flessel *et al.,* 1993; Marty *et al.,* 1994), along with the residual toxicity for *D. longicaudata* determined in this work, our proposal is similar to those of malathion, just to be replaced by other less harmful substances.

Acetamiprid

Acetamiprid belongs to the neonicotinoids pesticide family and have been considered as rational

Table 3. IOBC toxicity classification of selected pesticides[1] at different residue ages on *Diachasmimorpha longicaudata* 6-8 d-old adults.

Active ingredient	Residue age					
	Fresh (1 h)	**3d**	**7d**	**14d**	**21d**	**28d**
Abamectin	2	2	2	1	1	-
Acetamiprid	4	3	3	2	2	2
Azadirachtin	2	2	1	-	-	-
Deltametrin+ Thiacloprid	4	3	3	2	1	1
Malathion	4	4	4	3	3	3
Methidathion	4	3	3	3	2	2
Spinosad	2	2	1	1	-	-

[1] IOBC toxicity categories: (1) harmless, mortality lower than 30%; (2) slightly harmful, between 30 and 79%; (3) moderately harmful, between 80 and 99%; and (4) harmful, mortality higher than 99% (Sterk *et al*., 1999).

alternative to the organophosphates due to their high specificity, elevated efficacy and relatively low toxicity to the environment (Tomizawa & Casida, 2005). Neonicotinoids are widely used in Tunisian citrus orchards to control hemipteran pests considering their ovicidal and larvicidal activities and systemic action. However, in the past 3-5 years, eco-toxicological studies revealed a wide range of adverse side effects on non-target arthropods, including the worldwide-threatened honey bee *Apis mellifera* L. (Hymenoptera: Apidae), which remains the model organism against the use of neonicotinoids (Laurino *et al.,* 2011). Regarding tephritid fruit fly parasitoids, neonicotinoids have been previously tested, being the active ingredient imidacloprid, not the acetamiprid used in this work. Liburd *et al.* (2004) determined lethal effects of imidacloprid on *D. longicaudata* when in use in treated spheres for the management of key fruit fly pests. Whereas Adán *et al.* (2011) assessed lethal and sublethal toxicity of imidacloprid on *Psyttalia concolor* Szépligeti (Hymenoptera: Braconidae), depending on the application mode (cover sprays become lethal whereas bait sprays remained sublethal). So taking into consideration our results, we can confirm that neonicotinoids should be used with caution when beneficial hymenopterans are present in the agro-ecosystem.

Deltametrin+Thiacloprid

Whilst the first belongs to the pyrethroids class, the second belongs to the neonicotinoids class, and many of the effects detected can be attributed to the lethal effects of the neonicotinoid as the observed in past works. But neonicotinoids can have either a nitro group or a cyano. Those that have the cyano group, as thiacloprid, show relative lower toxicity attributed to different receptors, metabolism and secondary metabolites production (Suchail *et al.,* 2004; Jones *et al.,* 2006). Despite this lower toxicity, some beneficial arthropods exhibit sublethal side effects, like flight-navigation problems, reduction in attack rate, increase of handling time or even reduced emergence success or sex ratio distortion, factors that decrease the chance for parasitoid establishment in new territories (Krepsi *et al.,* 1991; Garcia *et al.,* 2009; Carmo *et al.,* 2010; Wang *et al.,* 2012a,b; Fischer *et al.,* 2014). In our study, although determined an initial acute toxicity, its effect on *D. longicaudata* significantly decreased with time (Tables 2 and 3), rendering it as compatible within the IPM for citrus pests. Despite this, more research is needed especially regarding long-term toxicity and sublethal side effects for *D. longicaudata* not done within this work.

Abamectin, Spinosad

Abamectin, a mixture of avermectins, is a natural insecticidal, acaricidal and nematicidal compound derived from the bacteria *Streptomyces avermitilis*. This agricultural compound was approved as a plant protection agent and as a veterinary drug for control of endo- and ecto-parasites (FAO, 1996). Spinosad, a bacterial insecticide derived from the actinomycete *Saccharopolyspora spinosa*, shares some relations with abamectin.

Even if we have classified both as slightly harmful for *D. longicaudata* (IOBC class 2) as in other risk assessment studies (Stark *et al.,* 2004), opinions are still divergent about their compatibility with many biocontrol agents of other plant pests, especially parasitoids with emphasis on its possible trans-generational sublethal side effects (Bueno & Freitas, 2004; Biondi *et al.,* 2012b, 2013; Costa *et al.,* 2014; Abbes *et al.,* 2015). As for the precedent substance, more research is required to determine the sublethal side effects on *D. longicaudata*.

Azadirachtin

Azadirachtin, a limonoid tetranor-triterpenoid chemical derived from neem tree (*Azadiracta indica*), has been extensively used in other countries for the control of phytophagous (Stark *et al.,* 1992; Stara *et al.,* 2011; Alvarenga *et al.,* 2012) and livestock arthropod (Ruiu *et al.,* 2008) pests by its environmental compatibility and extremely low acute mammalian toxicity (Schmutterer, 1990 and references herein). Our results indicate that this was the less harmful pesticide for *D. longicaudata*, even as fresh residue, as similarly determined by Stark *et al.* (1992). However, its application form and dose could be the responsible of the differential results observed by other authors. When *D. longicaudata* hosts were fed with neem seed cake, with a high content in azadirachtin, the parasitoid emergence rate was significantly lower than the control not feed with this neem cake (Alvarenga *et al.,* 2012). Whereas *D. longicaudata* survival and emergence rate did not differ from control when hosts (either *Dacus dorsalis* Hendel (Diptera: Tephritidae) or *C. capitata*) were subjected to azadirachtin sprays at doses that inhibits host emergence (Stark *et al.,* 1992).

Conclusions

Considering data from the literature and our results, it can be concluded that the most harmful tested pesticides named in decreasing order of toxicity, malathion and methidathion, acetamiprid, deltametrin+thiacloprid should be avoided in broad sprays, switching their use to mainly bait stations or localized bait sprays, in accordance with the new regulations of the European Union while protecting other natural enemies. Although abamectin, azadirachtin and spinosad were classified as slightly harmful (IOBC class 2), they should be used in a differential way, being favored azadirachtin when possible. Abamectin and spinosad should be used in bait stations with *C. capitata* specific attractants to reduce its impact on predators, when present in the citrus orchards, and to avoid any sublethal effect on other parasitoids and beneficial pollinators. If aerial sprays are required, augmentative releases of *D. longicaudata* should be performed 14 days after treatment with abamectin or spinosad, or could be reduced to 7 days after azadirachtin aerial spray treatment. In addition, further studies are needed to address their possible sublethal effects on *D. longicaudata*, as has been addressed in other natural enemies' species. As final remark, our study could be the first step aiming to integrate *D. longicaudata* with chemical control against *C. capitata*, within an IPM program in Tunisian citrus agro-ecosystems, while preserving its establishment possibilities and enhancing its biological impact on target pest reduction.

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