

Review

Side Effects of Pesticides on the Olive Fruit Fly Parasitoid *Psytalia concolor* (Szépligeti): A Review

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Abstract: Pesticide applications in olive orchards could alter the biological control of parasitoid *Psytalia concolor* Szépligeti (Hymenoptera: Braconidae) on the key pest *Bactrocera oleae* Rossi (Diptera: Tephritidae). *Psytalia concolor* adults can be contaminated by exposure to spray droplets, contact with treated surfaces or oral uptake from contaminated food sources. Pesticides impact both pest and parasitoid populations when they coexist in time and space, as they reduce pest numbers available for parasitoids and might cause toxic effects to parasitoids from which they need to recover. Therefore, the appropriate timing and application of selective chemical treatments provides the opportunity to incorporate this parasitoid in the IPM of *B. oleae*. This manuscript reviews the current literature on lethal and sublethal effects of insecticides, fungicides, herbicides, and biopesticides on *P. concolor*. Insecticides were generally more toxic, particularly organophosphates and pyrethroids, while herbicides and biopesticides had less effects on mortality and reproductive parameters. Some fungicides were quite harmful. Most of the studies were conducted in laboratory conditions, focused on reproduction as the only sublethal effect, exclusively considered the effect of a single pesticide and persistence was hardly explored. Field studies, currently quite scarce, are absolutely needed to satisfactorily assess the impact of pesticides on *P. concolor*.

Keywords: biological control; *Bactrocera oleae*; biopesticides; insecticides; fungicides; herbicides; integrated pest management

1. Introduction

Substantial quantitative and qualitative crop losses are caused by the incidence of pests and pathogens [1]. These organisms can be reduced and eliminated with the application of biological, chemical, physical, or cultural control measures. Traditionally, agriculture has extensively relied on pesticides for pest control [2]. Application of pesticides offers advantages such as efficacy and low cost, minimizing work force and reducing costly inputs as labor or fuel [3]. However, undesirable effects include side effects on non-target organisms, pest resistance to active substances, secondary pest outbreaks, or residue persistence in water, soil, and food chain [4–7].

The increasing number of farmers consciously alerting for a sustainable use of pesticides contributed to the implementation of integrated pest management (IPM) [8,9], mandatory in the

European Union (EU) under the directive 2009/128/EC [10]. In this context, an essential component of IPM is the use of macrobial (predators or parasitoids) or microbial (bacteria, viruses, fungi, or nematodes) agents against insect and mite pests [11]. However, in some cases biological control is not as robust to reduce pest levels below the economic threshold, where the pest population or the extent of crop damage exceeds the cost of controlling the pest, despite obvious advantages such as the minimal disruption to agroecosystems. Up to date, the best strategy is the optimal management of natural enemies, with actions targeted to enhance their establishment, conservation or increase, supplemented with selective chemical treatments applied at an appropriate timing to maintain key and secondary pest populations below economic thresholds [12]. In such scenario, the application of pesticides impacts not only the pest but also the natural enemies' population. The susceptibility of natural enemies to pesticides will depend on the guild, developmental stage, sex, age, type of pesticide, application method and timing, dose rate, and mode of action [7]. According to directive 2009/128/EC, it is essential to understand the risks of pesticides and adopt more effective and selective substances [10]. Because of that, the first step for developing a successful IPM is the accurate evaluation of the potential lethal and sublethal side effects of pesticides on the biological control agents [13–15]. Although sublethal concentrations do not directly kill the exposed natural enemies, they may interfere with their physiological and behavioral traits, which can significantly affect their fitness and compromise the success of biological control [5,16].

1.1. *Bactrocera oleae*—*Psyttalia Concolor*: Compatibility between Chemical and Biological Control

The olive fruit fly *Bactrocera oleae* Rossi (Diptera: Tephritidae) is a key pest of olive orchards (*Olea europaea* L.) worldwide, the larvae of which are strictly monophagous feeding on the olive mesocarp. Females lay their eggs in olive fruits, larvae feed off the pulp and finally pupate inside the olive or exit to pupate on the ground [17]. This causes a premature fruit drop and fruit weight loss. Moreover, microorganism growth inside the fruit increases the acidity of olive oils [18]. Not all cultivars are equally susceptible to the olive fruit fly, being the severity of the damage influenced by olive characteristics such as fruit size, color, exocarp hardness and chemical composition, geographical area, and weather conditions [18,19].

Control methods are based on the use of (a) insecticide treatments (bait applications (insecticides mixed with an attractant) and cover sprays); and (b) traps [20]. Organochlorides, organophosphates and carbamates were the first insecticides used against the olive fruit fly, followed by the introduction of pyrethroids, neonicotinoids, and spinosad as bait treatment, which is recognized to be an integral component of IPM as it reduces pesticide levels and environmental impact on natural enemies and pollinators [21–25]. Kaolin works as a physical barrier that prevents egg laying in the olive fruit [26]. Although insecticides have been efficient and cost-effective, this pest has developed resistance to the most commonly used active substances [20,22,27–29]. Entomopathogenic nematodes are also effective and safe control agents against larvae and pupae in laboratory experiments [30,31], although nematode efficacy in field conditions is still a challenge. In the Mediterranean area, biological control is mainly achieved by chalcidoidea ectoparasitoids *Eupelmus urozonus* Dalman (Eupelmidae), *Phygadeuonidae* *Pnigalio mediterraneus* Ferriere and Delucchi (Eulophidae), *Eurytoma martellii* Domenichini (Eurytomidae) and *Cyrtoptyx latipes* Rondani (Pteromalidae), and the ichneumonoida endoparasitoid *Psyttalia concolor* Szépligeti (Braconidae) [32,33]. All of them are native, except *P. concolor*, whose presence is linked to historical and recent releases. Since these releases were not as effective as desirable to control *B. oleae* in some of the regions, at present, most of the studies are focused on conservation biological control, which seeks to contribute to pest management by supporting populations of the natural enemies present in the agroecosystems [34]. Unfortunately, up to date, the control of *B. oleae* still relies on the use of insecticides. *Psyttalia concolor* is a synovigenic—females mature eggs throughout their life—, koinobiont—the host continues its development after parasitization—, larval–pupal endoparasitoid of many Tephritidae (Diptera), released or already established in Mediterranean and Californian olive groves with varying levels of success [17,33,35,36]. *Psyttalia concolor* was originally

described by Szépligueti from *B. oleae* infested olives in Tunisia in 1910. It is a member of a complex of closely related species from Africa, which also includes *P. humilis* and *P. perproximus*, which have been treated as synonyms of one another, amongst others. In fact, shortly after being described in Tunisia, it was introduced to olive-growing regions of Italy, Greece and France [17,37]. It is able to attack at least sixteen tephritids on different wild and/or cultivated plants, but only two are known as typical hosts in its native range: The olive fruit fly and the medfly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) [38]. This parasitoid has been used in different Mediterranean areas for the biological control of *B. oleae* by inundative and propagative releases and recently released in Californian olive-groves as a part of classical biological control programmes [17]. Parasitism rates of *P. concolor* are low, ranging between 22.4 and 23.4% in Spanish organic orchards in the Balearic Islands [35]. The presence of ecological infrastructures in olive groves influences the compatibility of *P. concolor* with pesticides. Flowering strips, banker plants and hedgerows provide food (pollen, nectar and hemipteran honeydew), alternative hosts and refuges, which are important resources for parasitoid establishment in agroecosystems [39,40]. However, these structures should also be free of pesticides because of the possible risk of contamination. *Psytalia concolor* can be contaminated by contact with pesticide droplets or residues, or oral uptake from contaminated food sources. On one hand, pesticide residues persist in plant tissues long enough to contaminate pollen and nectar [41,42], essential source of energy and protein for parasitoid survival, host foraging and reproduction [43]. *Psytalia concolor* may also feed on the liquid exuded from the host, particularly important for egg maturation of synovigenic females [44,45], thus increasing the risk of exposure. On the other hand, parasitoids are also exposed to pesticides by direct contact with leaves during host searching, feeding, mating, and resting activities [46–48]. Pesticides induce changes in the chemical constituents of flowering or host plants, which may decrease their nutritional value or became less attractive to parasitoids [49–51], interfere with olfactory orientation during oviposition (reduced capacity to find the host or respond to host kairomones), change foraging patterns or sex pheromonal communication [46,47,52,53].

1.2. Standard Methods for Testing Side Effects of Pesticides on Natural Enemies

Side effects of pesticides on biological control agents have been extensively studied in the last forty years. In 1974, the working group “Pesticides and Beneficial Organisms” of the International Organization for the Biological Control (IOBC) was established with the major aim at encouraging “the development of standard methods for testing the side effects of pesticides on natural enemies” [54]. IOBC test methods are based on a sequential scheme of three levels (laboratory, semi-field, and field) [55,56]. This sequence assumes that pesticides that are harmless at laboratory level will also be safe in semi-field and field conditions, and do not need to be further evaluated. However, when a chemical is categorized as harmful in laboratory conditions, its effect cannot be inferred in the next level, and the sequential scheme must be followed until (1) it displays no negative effects at semi-field level or (2) the evaluation finishes at field conditions because the chemical was also harmful at semi-field level. Laboratory methods evaluate the lethal effect (mortality) and sublethal effects (typically reproductive parameters) of pesticide residues on an inert substrate, although topical and ingestion uptake routes have been also included in this review. Semi-field tests are performed as direct application of pesticides on plants, and field studies are carried out in semi-natural field conditions [55–57]. Pesticides are ranked for (a) laboratory studies in (1) (harmless, <30% corrected mortality with the control treatment), (2) (slightly harmful, 30–79%), (3) (moderately harmful, 80–98%) and (4) (harmful, >99%); and (b) semi-field and field studies in (1) (harmless, <25%), (2) (slightly harmful, 25–50%), (3) (moderately harmful, 51–75%), and (4) (harmful, >75%) [58].

This work aims at reviewing the available literature on the side effects of active substances, currently registered in the EU, tested on *P. concolor*, categorized as insecticides, fungicides and herbicides according to their mode of action, discussing their implications on parasitoids in IPM. Moreover, we have included the available literature concerning ecotoxicology of microbial and botanical

compounds on this parasitoid, even if their current use in olive groves can be questioned except for *Bacillus thuringiensis*.

2. Side Effects of Pesticides on *Psytalia concolor*

In addition to the insecticides registered in olive groves against *B. oleae*, other pesticides can be applied against other olive pests, such as the moth *Prays oleae* Bernard (Lepidoptera: Praydidae) or the scale *Saissetia oleae* Olivier (Hemiptera: Coccidae), fungal diseases and weeds. For instance, anthracnose (*Colletotrichum* spp. complex) and olive leaf spot (*Venturia oleaginea* (Castagne) Rossman and Crous) are two important diseases causing significant yield losses and reduction of the quality of the olive oil and table olives [59,60]. Traditionally, they were controlled with copper-based fungicides [59,61], however site specific fungicides such as difenoconazole and tebuconazole, trifloxystrobin or dodine are applied nowadays [60,62]. Sulfur and mancozeb are contact fungicides with protective activity used to reduce the incidence of anthracnose [61]. Thus, although insecticides would presumably cause the most damage to *P. concolor*, the complex of plant protection products applied in olive trees has been included in this review, as these compounds could also have toxic effects on this parasitoid.

2.1. Insecticides

Organophosphates (group 1B) [63], acetylcholinesterase inhibitors, are broad spectrum insecticides with low selectivity, high acute toxicity and environmental impact [2]. Phosmet exhibited high toxicity in glass residual contact laboratory tests, but it was harmless when the substrate was treated parasitized *C. capitata* pupae (Table 1) [64]. Toxicity of organophosphates has been reported for other braconids, such as *Aphidius gifuensis* Ashmead [65] or *Diachasmimorpha longicaudata* Ashmead [66].

Pyrethroids (group 3A) [63] are sodium channel modulators rapidly absorbed by the insect tegument. They have a quick action, causing hyperactivity, convulsions and an immediate “knockdown” paralysis [67]. Beta-cyfluthrin, lambda-cyhalothrin, alpha-cypermethrin, zeta-cypermethrin, and deltamethrin greatly compromised *P. concolor* survival when females were exposed to residual contact and ingestion tests, but emergence was not affected when parasitized pupae were treated (Table 1) [64,68]. Short-term mortality of adults due to pyrethroids [65,66,69–72] and negative sublethal effects on larval and pupal development, fecundity, sex-ratio or oviposition [70,73–75] have been well documented in families Aphelinidae, Encyrtidae, Braconidae, Mymaridae, Trichogrammatidae, and Scelionidae. Pyrethroids can interfere with the mobility and orientation of parasitoids searching for food sources or host plants [76]. Other studies report that adults surviving to residual exposure retain their ability to orient to host odors [77], or that impaired foraging and orientation can be recovered after exposure [46,52]. As far as side effects are concerned, pyrethroids should not be recommended in IPM programs including *P. concolor* due to the high risk of mortality and interference with multiple behavioral functions [5].

Neonicotinoid imidacloprid (group 4A) [63] is a nicotinic acetylcholine receptor (nAChR) competitive modulator, which blocks the transmission of stimuli in the insect nervous system. Imidacloprid was harmless for *P. concolor* females in bait spraying but caused high mortality and sublethal effects on parasitization rate and progeny when applied as cover spray in glass surface or semi-field (Table 1) [78,79]. This revealed that ecological selectivity may result through the use of bait treatment. High toxicity of imidacloprid and moderate toxicity of acetamiprid have been reported with residues of up to 28 days in other braconids [65,66,72,75].

Spinosad (group 5) [63] is a combination of two fermentation factors, spinosyns A and D, produced by the actinomycete *Saccharopolyspora spinosa* [80]. Spinosyns disrupt nicotinic acetylcholine receptors and present high selectivity and reduced risks to the environment [80,81]. However, evidence about the compatibility with natural enemies is still inconclusive, particularly parasitoids, as acute lethal and multiple sublethal effects have been identified [82]. Spinosad is usually harmless for predators but moderately harmful for parasitoids [81]. Spinosad was harmful to *P. concolor* via ingestion, residual contact and topical application in laboratory conditions, but not in bait spraying of olive

leaves (Table 1) [78,83]. Similarly, acute toxicity was found for braconids *Bracon nigricans* Szépligeti, *A. gifuensis* and *Aphidius colemani* Viereck [65,75,84], but not for *D. longicaudata* [66]. Bait spraying in olive orchards had no harmful effects on braconids *Fopius arisanus* Sonan and *Psytalia fletcheri* Silvestri [84]. Spinosad residues degrade quickly, with little residual toxicity after 3–7 days post-application [66,81], although high toxicity has also been reported after 10 days [85].

Insect growth regulators (IGRs) interfere with development and reproduction [24]. Fenoxycarb (group 7B) [63] presents low soil mobility, non-accumulation and quick degradation. Potential toxicity on non-target insects has already been reviewed [86]. Fenoxycarb did not affect the longevity and emergence of *P. concolor* (Table 1) [64,87]. More recently, it was found that fenoxycarb was compatible with *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) [88], but slightly harmful to *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) [89]. Although it is important to increase the knowledge on *P. concolor*, it could be a rational candidate for IPM. Pyriproxyfen (group 7C) [63], was slightly harmful to *P. concolor* survival but harmless for its reproduction (Table 1) [90]. In contrast, pyriproxyfen caused high mortality on *E. eremicus* and *E. formosa* larvae and pupae [88,89]. A recent study showed a strong evidence of vertical transmission of pyriproxyfen from the treated female to the egg of parasitoid *Trissolcus japonicus* (Hymenoptera: Scelionidae) [91].

Diflubenzuron, an inhibitor of chitin biosynthesis type 0 (group 15) [63] drastically decreased *P. concolor* emergence when *C. capitata* larvae were fed at 0.2 g a.i./kg diet compared to 0.02 g a.i./kg diet (Table 1) [87]. A similar effect was reported in braconid *Apanteles melanoscelus* Ratzeburg [92].

Tebufenozide, an ecdysone receptor agonist (group 18) [63], decreased *P. concolor* emergence by 35% when the host larvae were treated at 6 g a.i./kg diet compared to 0.6 g a.i./kg diet (Table 1) [87]. On the contrary, it was completely harmless at a lower concentration by ingestion, residual contact or topical application (Table 1) [83]. Methoxyfenozide, another ecdysone agonist, was harmless to braconid *A. gifuensis* [65]. Therefore, it is important to clarify the compatibility of IGRs under field conditions.

The target protein responsible for the biological activity of azadirachtin, a limonoid tetranor-triterpenoid chemical derived from the neem tree, is unknown [63]. Azadirachtin impaired the beneficial capacity of *P. concolor* females when it was applied via treated host, residual contact or ingestion (Table 1) [83,87]. Particularly, it provoked a large reduction in the longevity, which might be related to the reported antifeeding effect of this product. Azadirachtin fresh residues also produced lethal and sublethal effects on braconids *B. nigricans* and *D. longicaudata*; however, toxicity decreased rapidly after 7–10 days [66,85].

Kaolin is a white aluminosilicate clay, non-porous, low-abrasive, and chemically inert over a wide pH range [63,93]. Kaolin was harmless to *P. concolor* at laboratory and semi-field conditions (Table 1) [26,78,94]. A reduction of abundance and diversity in the arthropod community of kaolin-treated olive groves has been highlighted [95–97], probably due to its deterrent effect [93]. As a general conclusion, kaolin is a recommendable pest control product in olive groves [98–100], with a low impact on non-target organisms [95,97].

Table 1. Toxicity of insecticides, currently registered in the European Union, tested on *Psytalia concolor* adults.

Active Ingredient (a.i.)	MoA ¹	Trademark	Concentration	Exposure ²	Substrate	Mortality ³	Emergence ⁵	Attacked Hosts (%) ⁶	Progeny (%)	Longevity (Days)	Reference
Phosmet	1B	Imidan [®] , ICI	0.125% a.i.	R	glass	4 (IOBC)					[64]
				T	parasitized pupa		1 (IOBC)				
beta-Cyfluthrin	3A	Bulldock [®] , Nufarm	0.03 mL/100 mL	I	adult	3 (IOBC)					Unpublished data
				R	olive leaves	4 (IOBC)					
lambda-Cyhalothrin	3A	Karate [®] , ICI	0.0038% a.i.	R	glass	4 (IOBC)					[64]
				T	parasitized pupa		1 (IOBC)				
alpha-Cypermethrin	3A	Fastac [®] , Shell	0.004% a.i.	R	glass	0.013 (0.012-0.014) (LT ₅₀)					[68]
				R	glass	4 (IOBC)					
				T	parasitized pupa		1 (IOBC)				
zeta-Cypermethrin	3A	Fury [®] , FMC	10 µL/100 mL	I	adult	2 (IOBC)					Unpublished data
				R	olive leaves	4 (IOBC)					
Deltamethrin	3A	Decis Evo [®] , Bayer	50 µL/100 mL	I	adult	4 (IOBC)					Unpublished data
				R	olive leaves	4 (IOBC)					
Imidacloprid	4A	Confidor [®] , Bayer + Biocebo [®] , Bioibérica	150 mg a.i./L + 1500 mg a.i./L (bait spray)	R	olive leaves	8.0 ± 2.0% (0 DAT ⁴); 1 (IOBC)			35.9 ± 4.8		[78]
				R	olive leaves	0.0 ± 0.0% (0 DAT); 1 (IOBC)			55.4 ± 4.8		
		Confidor [®] , Bayer + Biocebo [®] , Bioibérica	150 mg a.i./L + 2250 mg a.i./L (bait spray)	R	olive leaves	8.0 ± 3.7% (0 DAT); 1 (IOBC)		74.0 ± 5.0	77.1 ± 4.5		
				R	olive leaves	0.0 ± 0.0% (0 DAT); 1 (IOBC)		72.4 ± 5.4	74.6 ± 5.5		[79]
Confidor [®] , Bayer	150 mg a.i./L (cover spray)	R	glass	75.0 ± 9.2%; 3 (IOBC)							
		Sf	small olive tree	15.6 ± 8.9%; 3 (IOBC)		38.1 ± 2.7	32.5 ± 13.1				

Table 1. Cont.

Active Ingredient (a.i.)	MoA ¹	Trademark	Concentration	Exposure ²	Substrate	Mortality ³	Emergence ⁵	Attacked Hosts (%) ⁶	Progeny (%)	Longevity (Days)	Reference							
Spinosad	5	Tracer [®] , Dow Agrosiences	120 mg a.i./L	I	water	4 (IOBC)				0.5 ± 0.1	[83]							
				R	glass	4 (IOBC)			0.2 ± 0.4									
				T	adult	4 (IOBC)			0.0 ± 0.0									
Fenoxycarb	7B	Insegar [®] , Maag	20 mg a.i./L (bait spray)	R	olive leaves	4.0 ± 2.5% (0 DAT); 1 (IOBC)			30.7 ± 5.1		[78]							
				0.15% a.i.	R	glass	1 (IOBC)					[64]						
				5 g a.i./kg diet	Th	parasitized pupa	1 (IOBC)											
Pyriproxyfen	7C	Juvinal [®] , Kenogard	50 g i.a./ha	I	adult	46.6% (0 DAT); 2 (IOBC)	43.9 ± 4.7%			29.2 ± 2.1	[87]							
												50 g a.i./kg diet	Th	larvae of <i>C. capitata</i>	40.1 ± 4.6%	29.8 ± 2.6		
												75 g i.a./ha	I	adult	53.8% (0 DAT); 2 (IOBC)			
Diflubenzuron	15		0.02 g a.i./kg diet	Th	larvae of <i>C. capitata</i>		44.2 ± 1.6%			28.5 ± 1.6	[87]							
												0.2 g a.i./kg diet	Th	larvae of <i>C. capitata</i>	3.3 ± 1.4%			
												0.6 g a.i./kg diet	Th	larvae of <i>C. capitata</i>	40.8 ± 6.3%	30.7 ± 1.7		
Tebufenozide	18	Mimic [®] , Certis	6 g a.i./kg diet	Th	larvae of <i>C. capitata</i>		26.5 ± 8.4%			31.3 ± 2.0	[87]							
												180 mg a.i./L	I	water	1 (IOBC)	91.3 ± 3.1	70.0 ± 5.4	26.3 ± 0.4
													R	glass	1 (IOBC)	88.8 ± 2.0	71.2 ± 2.9	22.2 ± 1.1
													T	adult	1 (IOBC)	88.3 ± 2.2	73.9 ± 3.4	27.8 ± 1.0

Table 1. Cont.

Active Ingredient (a.i.)	MoA ¹	Trademark	Concentration	Exposure ²	Substrate	Mortality ³	Emergence ⁵	Attacked Hosts (%) ⁶	Progeny (%)	Longevity (Days)	Reference	
Azadirachtin	unknown	Align [®] , Sipcam	0.015 g a.i./kg diet	Th	larvae of <i>C. capitata</i>		27.3 ± 4.0%			32.0 ± 2.0	[87]	
			0.15 g a.i./kg diet	Th	larvae of <i>C. capitata</i>		0.0 ± 0.0%					
			48 mg a.i./L	I	water	3 (IOBC)		64.1 ± 8.2	43.9 ± 4.8	4.9 ± 0.1		[83]
	R	glass	2 (IOBC)		86.4 ± 2.8	43.7 ± 5.0	21.2 ± 1.8					
	T	adult	1 (IOBC)		96.5 ± 1.7	71.5 ± 4.7	28.8 ± 0.4					
Kaolin	unknown	Surround [®] , Basf	4.75 kg/hL	R	olive leaves	2.0 ± 2.0% (0 DAT); 1 (IOBC)			54.2 ± 9.0		[77]	
			5 kg/hL	R	glass	1 (IOBC)		1 (IOBC)	2 (IOBC)		[94]	
				R	glass		0.0 ± 0.0% (0 DAT); 1 (IOBC)		97.7 ± 1.3	65.0 ± 3.9		
			5 kg/hL	T	parasitized pupa		66.5 ± 6.6%	96.4 ± 1.5	38.6 ± 8.3		[26]	
				R	olive leaves			89.4 ± 2.2	70.8 ± 1.4			
				Sf	olive tree		1.1 ± 1.1% (0 DAT); 1 (IOBC)		94.3 ± 2.7	45.2 ± 4.3		

¹ IRAC Mode of Action (MoA) classification: 1B: Organophosphates (nerve action); 3A: Pyrethrins (nerve action); 4A: Neonicotinoids (nerve action); 5: Spinosins (Nerve action); 7: Juvenile hormone mimics (growth regulation); 15: Inhibitors of chitin biosynthesis type 0 (Benzoylureas); 18: Ecdysone receptor agonists (Diacylhydrazines); Unknown: Compounds of unknown or uncertain MoA [63]. ² Intake route: R: Residual contact; T: Topical; I: Ingestion; Sf: Semifield; Th: Treated host. ³ Mortality is given as category of International Organization for the Biological Control (IOBC), LT₅₀ (lethal time that kills 50% of the treated insects), or percentage. ⁴ DAT: days after treatment (residue age). ⁵ Emergence is given as category of IOBC or percentage. ⁶ Pupae without fly emergence.

2.2. Fungicides

Information on the side effects of fungicides on *P. concolor* is scarce. The oximino-acetate trifloxystrobin (group C3) [101] did not cause any deleterious effect on *P. concolor* mortality, female activity and progeny (Table 2). Triazoles difenoconazole, penconazole and tebuconazole (group G1) [101] were slightly or moderately harmful to females exposed to a fresh residue on glass surface, but harmless when the parasitized *C. capitata* pupae were treated (Table 2) [64]. Cyproconazole was harmless even at glass residual contact tests (Table 2) [64]. Difenoconazole and tebuconazole were harmless or slightly harmful in ingestion tests, respectively, harmless in olive leave residual contact tests, and had no sublethal effects on female activity or progeny (Table 2).

Copper-based fungicides (group M01) [101], copper oxychloride, cuprous oxide and Bordeaux mixture, a mixture of copper(II) sulphate (CuSO_4) and slaked lime (Ca(OH)_2), were slightly harmful or harmful to females exposed to glass residual contact, but harmless when the parasitized pupae were treated (Table 2) [64]. Conversely, other formulations of copper oxychloride and Bordeaux mixture did not cause any deleterious effect on mortality neither sublethal effects on progeny in residual contact and semi-field experiments (Table 2) [26]. Similarly, copper oxychloride was completely safe to braconids *A. gifuensis* and *A. colemani* [65,72]. In this context, copper-based compounds can be considered compatible with *P. concolor*.

Sulfur (group M02) [101] had negative effects to *P. concolor* survival in glass residual contact tests, but not on parasitized pupae (Table 2) [64]. There is evidence that sulfur negatively impacts natural enemies, namely parasitoids [54,102]. Mancozeb (group M03) [101], its mixture with copper sulphate, and dithianon (group M09) [101] were completely harmless to *P. concolor* (Table 2) [64]. Literature indicates that mancozeb is not toxic to *Anagyrus* sp. near pseudococci Girault and *Coccidoxenoides perminutus* Timberlake (Hymenoptera: Encyrtidae) [103]. Dodine (group U12) [101] was harmful in ingestion tests but harmless in olive leave contact tests (Table 2).

Another important aspect to consider is that fungicides can reduce the diversity and richness of nectar microorganisms (fungus and bacteria) in flowers, which decreases nectar quality, sugar concentration or pH, having consequences for the feeding of natural enemies [104]. Therefore, consequences of the application of fungicides on parasitoids should be further studied.

Table 2. Toxicity of fungicides, currently registered in the European Union, tested on *Psytalia concolor* adults.

Active Ingredient (a.i.)	MoA ¹	Trademark	Concentration	Exposure ²	Substrate	Mortality ³	Emergence (%)	Attacked hosts (%) ⁵	Progeny (%)	Reference	
Trifloxystrobin	C3	Flint [®] , Bayer	10 mg/100 mL	I	adult	1 (IOBC)		99.8 ± 0.3	56.7 ± 3.5	Unpublished data	
				R	olive leaves	1 (IOBC)		99.8 ± 0.3	52.2 ± 3.2		
Cyproconazole	G1	Alto [®] , Sandoz	0.0186% a.i.	R	glass	1 (IOBC)				[64]	
Difenoconazole	G1	Score [®] , Ciba Geigy	0.125% a.i.	R	glass	2 (IOBC)				[64]	
				T	parasitized pupa	1 (IOBC)					
				I	adult	1 (IOBC)		99.8 ± 0.3	52.1 ± 4.6	Unpublished data	
R	olive leaves	1 (IOBC)		100 ± 0.0	52.8 ± 1.6						
Penconazole	G1	Omnex [®] , Ciba Geigy	0.0025% a.i.	R	glass	2 (IOBC)				[64]	
				T	parasitized pupa	1 (IOBC)					
Tebuconazole	G1	Folicur [®] , Bayer	0.0938% a.i.	R	glass	3 (IOBC)				[64]	
				T	parasitized pupa	1 (IOBC)					
				I	adult	2 (IOBC)				Unpublished data	
				R	olive leaves	1 (IOBC)		99.3 ± 0.8	51.1 ± 2.3		
Copper oxychloride	M01	Cupravit [®] , Bayer	0.196% a.i.	R	glass	4 (IOBC)				[64]	
				T	parasitized pupa	1 (IOBC)					
				R	glass	0.0 ± 0.0% (0 DAT ⁴); 1 (IOBC)		92.2 ± 5.8	52.1 ± 4.4		
Copper oxychloride	M01	ZZ-cuprocol [®] , Syngenta Agro	250 mL/hL	T	parasitized pupa		63.6 ± 3.6 ⁵	98.8 ± 0.3	30.0 ± 13.9	[26]	
				R	olive leaves			98.3 ± 0.8	78.6 ± 3.6		
				Sf	olive tree	1.1 ± 1.1% (0 DAT); 1 (IOBC)		93.3 ± 3.9	45.3 ± 4.1		
Cuprous oxide	M01	Oxiram [®] , Ciba-Geigy	0.3% a.i.	R	glass	3 (IOBC)				[64]	
				T	parasitized pupa	1 (IOBC)					

Table 2. Cont.

Active Ingredient (a.i.)	MoA ¹	Trademark	Concentration	Exposure ²	Substrate	Mortality ³	Emergence (%)	Attacked hosts (%) ⁵	Progeny (%)	Reference	
Bordeaux mixture	M01	C. Bord Vallès®, I. Q. Vallès	0.2% a.i.	R	glass	2 (IOBC)				[64]	
				T	parasitized pupa	1 (IOBC)					
		Poltiglia®, Manica SPA	1 kg/hL	R	glass	0.0 ± 0.0% (0 DAT); 1 (IOBC)		91.8 ± 3.7	60.2 ± 4.8		[26]
				T	parasitized pupa		64.8 ± 4.3	98.7 ± 0.9	37.9 ± 10.4		
				R	olive leaves			94.5 ± 1.1	72.3 ± 4.9		
				Sf	olive tree	3.3 ± 1.9% (0 DAT); 1 (IOBC)		90.2 ± 6.5	54.0 ± 3.5		
Sulfur	M02	Microtox®, Agrocros	0.432% a.i.	R	glass	3 (IOBC)				[64]	
				T	parasitized pupa	1 (IOBC)					
Mancozeb	M03	Dithane M-45®, Rohm and Haas	0.208% a.i.	R	glass	1 (IOBC)				[64]	
Mancozeb + copper sulphate	M03, M01	Cuprodithane® + Fuerte®, Rohm and Haas	0.048% a.i. + 0.144% a.i.	R	glass	1 (IOBC)				[64]	
Dithianon	M09	Delan 75®, Shell	0.075% a.i.	R	glass	1 (IOBC)				[64]	
Dodine	U12	Syllit Flow®, Kenogard	0.225 mL/100 mL	I	adult	3 (IOBC)				Unpublished data	
				R	olive leaves	1 (IOBC)		99.8 ± 0.3	51.4 ± 2.0		

¹ FRAC Mode of Action (MoA) classification: G1: Sterol biosynthesis in membranes (Triazoles); M01: Chemicals with multi-site activity (Inorganic copper); M02: Chemicals with multi-site activity (Inorganic sulfur); M03: Chemicals with multi-site activity (Dithio-carbamates and relatives); M09: Chemicals with multi-site activity (Quinones); U: Unknown mode of action [101].

² Intake route: I: Ingestion; R: Residual contact; T: Topical; Sf: Semifield. ³ Mortality is given as category of IOBC or percentage. ⁴ DAT: days after treatment (residue age). ⁵ Pupae without fly emergence.

2.3. Herbicides

From an integrated weed control strategy perspective, it is important to select herbicides compatible with natural enemies. The only study available on the effects of herbicides on *P. concolor* adult females found that the pyridyloxy-carboxylate fluroxypyr (group 4) [105] and the triazone metamitron (group 5) [105] were slightly harmful in residual contact assays, but harmless when the parasitized pupa was treated (Table 3) [64]. The phosphonate glyphosate (group 9); [105] was slightly harmful in ingestion tests but safe in olive leave residual contact tests, without any deleterious effect on female activity or progeny (Table 3). The benzofurane ethofumesate (group 15) [105] was completely safe, even when tested at glass surface (Table 3) [64]. In conclusion, although these herbicides did not prove to be toxic to *P. concolor*, literature is scarce and further studies are needed to understand the persistence of herbicides on olive groves and, consequently, the real implications on parasitoids. As mentioned for fungicides, some herbicides can also decrease pollen production and viability [50]. Besides that, the indiscriminate application of herbicides can reduce floral community diversity, removes refuges for natural enemies or plants with alternative hosts [106]. Herbicide studies have been traditionally neglected, especially in the case of pre-emergence herbicides, due to its uncommon presence in the exposure methodology proposed by IOBC. Nevertheless, from a broader perspective, all herbicides can indirectly affect *P. concolor* population as they limit the growth of plants providing food or shelter. In addition, post-emergence herbicides might potentially reduce the survival and reproduction of *P. concolor* adults.

Table 3. Toxicity of herbicides, currently registered in the European Union, tested on *Psytalia concolor* adults.

Active Ingredient (a.i.)	MoA ¹	Trademark	Concentration	Exposure ²	Substrate	Mortality ³	Attacked Hosts (%) ⁴	Progeny (%)	Reference
Fluroxypyr	4	Starane 180 [®] , Dow Elanco	0.09% a.i.	R	glass	2 (IOBC)			[64]
				T	parasitized pupa	1 (IOBC)			
Metamitron	5	Goltix [®] , Bayer	1.75% a.i.	R	glass	2 (IOBC)			[64]
				T	parasitized pupa	1 (IOBC)			
Glyphosate	9	Touchdown [®] , Special	0.7 mL/100 mL	I	adult	2 (IOBC)			Unpublished data
				R	olive leaves	1 (IOBC)	99.8 ± 0.3	53.4 ± 4.2	
Ethofumesate	15	Tramat 500 [®] , Schering	0.5% a.i.	R	glass	1 (IOBC)			[64]

¹ HRAC Mode of Action (MoA) Classification: 4: Auxin mimics (Pyridyloxy-carboxylates); 5: D1 Serine 264 binders (Triazinones); 9: Inhibition of enolpyruvyl shikimate phosphate synthase; 15: Inhibition of very long-chain fatty acid synthesis (Benzofuranes) [105]. ² Intake route: R: Residual contact; T: Topical, I: Ingestion. ³ Mortality is given as category of IOBC.

⁴ Pupae without fly emergence.

2.4. Microbial Insecticides

Microorganisms (bacteria, fungi, viruses, and others) have good potential as biopesticides because of their high selectivity and safety to non-target organisms [107,108]. *Bacillus thuringiensis*, a microbial disruptor of midgut membranes (group 11A) [63], has a relatively broad spectrum of activity against coleopterans (subspecies *tenebrionis* and *lentimorbus*), dipterans (subspecies *israelensis*) and lepidopterans (subspecies *kurstaki* and *aizawai*) [109]. Subspecies *kurstaki* was compatible with *P. concolor* (Table 4) [64]. Eleven field isolates of subspecies *israelensis* collected in several countries, which produced significant mortality to *B. oleae*, were safe for *P. concolor* adults in ingestion tests (Table 4) [110]. Likewise, subspecies *kurstaki* was harmless for braconids *B. nigricans* and *A. colemani* [72,85]. On the other hand, subspecies *kurstaki* did not affect the reproductive success of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) while a significant effect was observed on longevity and the time spent on host eggs patches [111].

Fungal agent *Beauveria bassiana* is another biopesticide used to control the olive fruit fly [112]. *Beauveria bassiana* did not cause any mortality on *P. concolor* (Table 4) [14]. However, their beneficial capacity was negatively affected via residual contact or treated host (lower progeny size), or by ingestion (fewer attacked hosts). Also, *P. concolor* emergence decreased by 50% compared to control when the fungal treatment was applied to parasitized pupae (Table 4) [113]. Although some studies evidenced that some predators and parasitoids are susceptible in laboratory conditions, impact is minimum in the field [114]. On the other hand, *Metarhizium anisopliae* did not pose any adverse effect on the emergence of *P. concolor* (Table 4) [115]. Entomopathogenic nematode species *Heterorhabditis megidis* Poinar, *Steinernema feltiae* Filipjev and *H. bacteriophora* are not entirely safe for *P. concolor*. Although they did not cause direct mortality to *P. concolor*, they decreased the progeny size when females parasitized on treated larvae, compared to control, but significant reductions were only observed for *S. feltiae* (Table 4) [14].

For a safer use of microorganism-based biopesticides that can be used concomitantly with *P. concolor*, more studies about side effects are needed. Also, microorganisms present in the field should be further studied as landscape structure, crucial for the establishment of arthropod community, constitutes an additional reservoir of genetic diversity with potential for pest management [39,40].

2.5. Botanical Compounds

Plant extracts and oils might be also used as alternative to conventional insecticides. Extracts of *Trichilia havanensis* Jacq. (Meliaceae) seeds, F12 limonoids (azadirone) and F18 (1.7 + 3.7-di-O-acetyl-havanensin), and *Teucrium viscidum* (Lamiaceae) seeds, M1 (Teucjaponic B) and M9 (Teucvin), did not cause mortality on *P. concolor*, neither reduced the beneficial capacity in topical or ingestion laboratory conditions (Table 4) [13,116]. Similar results were obtained when the olive fruit was treated with *Peganum harmala* L. (Zygophyllaceae) seed extract. There was no difference in the emergence of *P. concolor* between treated and untreated fruit, wasps actively searched for hosts and parasitoid mortality was not registered (Table 4) [117]. Conversely, *Melaleuca alternifolia* (Myrtaceae) essential oil was slightly to moderately harmful to *P. concolor*, although it was considered more toxic for the Mediterranean fruit fly, *C. capitata*, than for the parasitoid (Table 4) [118]. Finally, the mixture of essential oils of *Cymbopogon citratus* (DC.) Stapf (Poaceae), *Cedrus atlantica* (Endl.) Manetti ex Carrière (Pinaceae) and *Corymbia citriodora* (Hook.) K.D. Hill and L.A.S. Johnson (Myrtaceae) was harmless to *P. concolor* (Table 4) [119].

Despite the limitations of botanical compounds on pest control, some authors have reported a certain degree of efficacy of these products on pests *B. oleae* and *C. capitata* [117–119]. Thus, there is a need to extend the research and use of these promising compounds within IPM programs of target pests. Overall, microbial and botanical pesticides are harmless to *P. concolor* and should be used to increase the sustainability of agricultural systems as these products have environmental safety, target-specificity, efficacy and biodegradability [120].

Table 4. Toxicity of microbial insecticides and botanical compounds tested on *Psytalia concolor* adults.

Active Ingredient (a.i.)	MoA ¹	Trademark	Concentration	Exposure ²	Substrate	Mortality ³	Emergence ⁶	Attacked Hosts (%) ⁷	Progeny (%)	Reference
Entomopathogenic bacteria										
<i>Bacillus thuringiensis</i> var. <i>kurstaki</i>	11A	Bactospeine [®] , S.C. Agrocrós	1.7 × 10 ⁷ IU/L	R	glass	1 (IOBC)				[64]
				T	parasitized pupa	1 (IOBC)				
		Delfin DG [®] , Sandoz	3.2 × 10 ⁷ IU/L	R	glass	1 (IOBC)				[64]
<i>Bacillus thuringiensis</i> var. <i>israelensis</i>	11A	Field isolates	10 ⁶ IU/L	I	water	1 (IOBC)				[110]
Entomopathogenic fungus										
<i>Beauveria bassiana</i>	-	Naturalis L [®] , Agrichem	2.3 × 10 ¹² conidia/hL	R	glass	10.8 ± 2.0% (0 DAT ⁵)		42.8 ± 2.0	81.2 ± 5.5	[14]
				T	drop in the prothorax ⁴	2.9 ± 0.9% (0 DAT)		60.0 ± 2.8	71.7 ± 2.8	
				I	water	3.6 ± 1.2% (0 DAT)		31.9 ± 3.1	81.7 ± 3.0	
				Th	larvae of <i>Ceratitis capitata</i>			69.4 ± 3.5	48.6 ± 3.4	
			2.3 × 10 ¹⁰ conidia/hL	T	parasitized pupa		2 (IOBC)			[113]
<i>Metarhizium anisopliae</i>	-	Field isolate	4 × 10 ¹² conidia/ha	T	parasitized pupa		54.2 ± 12.6%			[115]
Entomopathogenic nematodes										
<i>Heterorhabditis bacteriophora</i>	-	Larvanem [®] , Koppert	100 infective juveniles/cm ²	Th	larvae of <i>C. capitata</i>				15%	[14]
<i>Heterorhabditis megidis</i>	-	Heterorhabditis system [®] , Biobest	100 infective juveniles/cm ²	Th	larvae of <i>C. capitata</i>				20%	[14]
<i>Steinernema feltiae</i>	-	Steinernema system [®] , Biobest	100 infective juveniles/cm ²	Th	larvae of <i>C. capitata</i>				9.5%	[14]
Botanical compounds										
F12 Limonoids (azadirone)	-	-	1000 mg a.i./L	I	water	3.3 ± 3.3% (0 DAT); 1 (IOBC)		89.1 ± 1.9	50.2 ± 4.5	[116]
				T	drop in the prothorax ⁴	3.3 ± 3.3% (0 DAT); 1 (IOBC)		87.0 ± 3.6	51.7 ± 3.9	
				I	water	10.0 ± 3.3% (0 DAT)		74.2 ± 3.5	63.6 ± 3.4	

Table 4. Cont.

Active Ingredient (a.i.)	MoA ¹	Trademark	Concentration	Exposure ²	Substrate	Mortality ³	Emergence ⁶	Attacked Hosts (%) ⁷	Progeny (%)	Reference
F18 (1.7 + 3.7-di-O-acetilhavanensin)	-	-	1000 mg a.i./L	I	water	3.3 ± 1.9% (0 DAT); 1 (IOBC)		85.0 ± 2.6	48.2 ± 5.9	[116]
				T	drop in the prothorax ⁴	6.7 ± 2.7% (0 DAT); 1 (IOBC)		85.5 ± 3.4	51.2 ± 4.4	
				I	water	11.7 ± 3.2% (0 DAT)		70.6 ± 5.5	57.5 ± 6.9	[13]
M1 (Teucjaponic B)	-	-	1000 mg a.i./L	I	water	11.7 ± 1.7% (0 DAT)		64.9 ± 5.2	49.4 ± 4.4	[13]
M9 (Teucvin)	-	-	1000 mg a.i./L	I	water	6.7 ± 3.8% (0 DAT)		66.9 ± 6.0	51.2 ± 3.8	[13]
<i>Peganum harmala</i> seed extract	-	-	2% (w/w)	Th	larvae of <i>Bactrocera oleae</i>	1 (IOBC)	1 (IOBC)			[117]
<i>Melaleuca alternifolia</i> oil	-	-	0.1 to 2% (w/w)	I	water	0.639% (LD ₅₀); <35%				[118]
			0.1 to 3 µL/cm ²	R	filter paper	0.147% (LC ₅₀); >60%				
			0.15 to 18 µL/L air	F	filter paper	9.348% (LC ₅₀); >50%				
<i>Cymbopogon citratus</i> , <i>Cedrus atlantica</i> , <i>Corymbia citriodora</i> oils	-	-	4.8% (w/w)	Sf	orange tree	0.0 ± 0.0% (0 DAT); 1 (IOBC)				[119]

¹ IRAC Mode of Action (MoA) classification: 11A: Microbial disruptors of insect midgut membranes [63]. ² Intake route: R: Residual contact; T: Topical; I: Ingestion; Th: Treated host; F: Fumigation; Sf: Semifield. ³ Mortality is given as category of IOBC, percentage, or LD₅₀/LC₅₀ (lethal dose/lethal concentration that kills 50% of the treated insects). ⁴ 0.5 microliters/insect. ⁵ DAT: days after treatment (residue age). ⁶ Emergence is given as category of IOBC or percentage. ⁷ Pupae without fly emergence.

3. Conclusions

- Insecticides cause more negative effects on *P. concolor* survival and reproduction than fungicides, herbicides or biopesticides. Neurotoxic organophosphates and pyrethroids are the most toxic insecticides to *P. concolor* adults, however they are usually harmless when applied in host pupae stage, the most protected life stage. IGRs and kaolin are alternative pesticides compatible with *P. concolor*, however the variety of responses urges for more research on side effects of this parasitoid.
- Despite that the impact of fungicides on *P. concolor* is milder, some are harmful at residual contact tests, thus negative effects due to foliar application on the olive canopy should not be ignored.
- Most studies focus on mortality and reproduction, but other sublethal effects such as longevity, learning performance, behavior, neurophysiology, physiological or immunology should also be considered.
- Most studies only consider a single compound but not the synergies of several substances applied at the same time. For example, there are no data on the joint use of fungicides and insecticides.
- Literature about laboratory studies supported with field data is very scarce, especially for herbicides, biopesticides and botanical extracts. Field ecotoxicological studies would allow the optimization of the management of parasitoids after pesticide applications, establishing the use of harmless pesticides for *P. concolor* as a pre-requisite for the control of *B. oleae*.
- The number of independent publications related to scientific ecotoxicological studies, beyond those required for the registration of plant protection products, have sharply decreased in the EU. Consequently, there is a need for updated data on the toxicity of novel substances on important natural enemies of relevant crops, such as *P. concolor*.

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