Research Article

Lethal and Sublethal Effects of Pesticides and Their Consequences on *Trichogramma* (Hymenoptera: Trichogrammatidae) Biocontrol Agents

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Abstract | Pesticides constitute a predominant and inevitable part of plant protection worldwide. However, their extensive use results in many undesirable ecological consequences, including the disruption of natural enemies and biological control agents of insect pests such as insect parasitoids. *Trichogramma* wasps are one of the most studied and widely used insect parasitoid groups. These parasitoids have been demonstrated effective against a wide array of insect pest species under laboratory, greenhouse, semi-field and field conditions. This review encompasses a wide range of studies to date on the direct effect of different pesticides and their sublethal effects on the biological and physiological traits of *Trichogramma* wasps. Lethal effects of pyrethroid pesticides have been widely studied on 10 of 12 *Trichogramma* species, and are considered safe for 8 of 10 species. Of all biological parameters, the adult emergence is the foremost trait being affected by most of the synthetic insecticides among 20 *Trichogramma* wasp species investigated, but T. pretiosum appears to be an exception.

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Introduction

Pesticides mitigate populatio' ns of insect pests in both agronomic and horticultural crop production, providing prompt control of pests due to their rapid knockdown action. However, the indiscriminate and extensive use of these hazardous products has resulted in many non-target effects e.g. disruption of non-target organisms, contamination of soil, air and water resources, insecticide resistance in populations of pests, outbreaks and emergence of secondary insect pest species, and even human health hazards (Kumari and John, 2018; Pathak *et al*., 2022; Tang *et al*., 2025).

Therefore, it is of utmost importance to divert attention towards the development of more sustainable, reliable and environmentally friendly pest control methods. In biological control, beneficial organisms such as insect entomopathogens, parasitoids and predators are utilized to suppress insect pest populations. A wide range of biological control programs have been successfully deployed against many insect pest species worldwide (Narendran, 2001; Cock *et al*., 2016). Successful integration of pesticides with biological control agents (entomopathogens, parasitoids and predators) has also been achieved in some cases (Croft, 1990). These integrated pesticides must be compatible with the biological control agents, i.e., not affecting the population of the biocontrol agents, but with efficacy toward the agricultural insect pests (Ruberson *et al*., 1998; Charleston *et al*., 2005; Cloyd, 2005). Pesticides have a positive correlation between their activity and their chemical classification, which kills the insect pests directly or lethally (Cloyd and Bethke, 2011).

Impacts of pesticides on biocontrol agents are usually a consequence of direct effects, such as mortality (24 to 96 hours) (Stapel *et al*., 2000; Nozad-Bonab *et al*., 2021). But indirect effects of pesticides can also be important (Desneux *et al*., 2007). These indirect effects include sublethal influences on longevity, oviposition, developmental period, emergence, parasitism rate, survival, fecundity, sex ratio, mating and fertility (Croft, 1990; Ruberson *et al*., 1998; Desneux *et al*., 2007; Ochiai *et al*., 2007).

Many natural enemies from the Hymenoptera used as biological control agents are good alternatives to chemical pest control (Wu and Guo, 2005). Greathead (1986) elucidated that out of 393 species of parasitoids which have been established in different classical biological control programs, 344 (87%) belong to the Hymenoptera. Similarly, Shaw and Hochberg (2001) reported that about 25% of the total insect fauna in Great Britain is comprised of parasitic Hymenoptera. Moreover, the Trichogrammatidae is the most important and extensively studied family of minute parasitic wasps and are being used in several biological control programs. About 200 insect species belonging to 70 families of 8 insect orders are parasitized by these wasps in diverse habitats (aquatic and arboreal) (Knutson, 1998; Sumer *et al*., 2009; Consoli *et al*., 2010; Jalali, 2013; Wang *et al*., 2025). *Trichogramma* wasps are minute and very uniform morphologically, creating difficulty in species identification and differentiation (Thomson *et al*., 2003). *Trichogramma* species (0.9 mm in size) parasitize a large number of lepidopteran insect pests belonging to 18 genera in nine families. These important lepidopteran insect pest species include cotton bollworms, sugarcane and rice borers, corn earworms, armyworms and loppers, in different horticultural and agronomic crops including corn, cotton, rice, sugarcane and tomatoes etc. (Nordlund *et al*., 1997; Cherif *et al*., 2021).

For many years direct effects of pesticides were estimated using median lethal concentrations (LC_{50}), but more recently, estimates of indirect or delayed effects of pesticides sublethally have been investigated, viz., different physiological and behavioral parameters of test insects hindered by pesticides (Wang *et al*., 2012). The present review focuses on lethal and sublethal effects of pesticides on *Trichogramma* based on previously published studies found on a master list of Clarivate Analytics.

Impact of pesticides on Trichogramma species

Pesticide application is aimed at direct control of pests, but indirectly, it can influence natural biological control agents like *Trichogramma*. This influence may be in the form of acute toxicity in *Trichogramma* or without apparent mortality, but adverse effects on the biology of this parasitoid (Desneux *et al*., 2007; Zhao *et al*., 2012). In this section, pesticides with lethal effects are classified according to the principles of the Environmental Protection Agency (EPA) (EPA, 1997) while sublethal effects on *Trichogramma* biological parameters are classified according to the principles of the International Organization of Biological Control (IOBC) (Hassan *et al*., 1991).

Lethal effects

The direct or acute lethal effects of pesticides on *Trichogramma* have been estimated quantitatively as risk quotients under field conditions (**Table 1**). Risk assessment depends on the toxicity exposure of *Trichogramma* to a pesticide and their quantitative interaction known as a Risk Quotient (RQ) (Damalas and Eleftherohorinos, 2011; Zhao *et al*., 2012). These toxicity classes are classified based on risk quotient values (safe = less than 50 gha^{-1}/ppm, slightly to moderately toxic = 50-2500 gha^{-1}/ppm, and dangerously toxic = greater than 2500 gha^{-1}/ppm)

(EPA, 1997). The following 22 pesticidal groups are organized to assess risk to *Trichogramma* species (Table 1).

Table 1: *Risk assessment of trichogramma species by different pesticidal groups.*

Tricho-gramma specie[s]*a*	Pesticide's common or trade name	Pesticide group	Risk quotient[b] (gha^{-1}/ppm)	Class[c]	References
Tricho-gramma brassicae	Abamectin, Emamectin benzoate	Avermectin	9,1	1	(Parsaeyan, Safavi, Saber, and Poorjavad, 2018)
	Chlorpyrifos, Profenophos		1, 1	1	(El-Sebai and El-Tawil, 2011)
	Diazinon	Organophos-phate	10000	3	(Ghorbani, Saber, Bagheri, and Vaez, 2016)
	Fipronil	Phenylpyrazole	48	1	
	Cypermethrin	Pyrethroid	25	1	(Parsaeyan *et al.*, 2018)
T. cacoe-ciae	Fenpyroximate	Acaricide	1	1	(Saber, 2011)
	Neemazal	Azadirachtin	1	1	(Saber, Hejazi, and Has-san, 2004)
	Imidacloprid	Neonicotinoid	56	2	(Saber, 2011)
T. chilo-nis	Abamectin, Emamectin benzoate	Avermectin	254,8	2,1	(Cheng *et al.*, 2018)
	BPMC- fenobucarb	Carbamate	20478	3	(Preetha, Stanley, Suresh, Kuttalam, and Samiyap-pan, 2009)
	Cartap		8	1	(D.-S. Wang *et al.*, 2012)
	Chlorantraniliprole	Diamide	13	1	(Preetha *et al.*, 2009)
	Butralin, Napropamid, Pendi-methalin	Herbicide	524,235,158	2	(Cheng *et al.*, 2018)
	Bacillus thuringiensis	Microbial pes-ticides	0	1	(Amichot, Curty, Benguettat-Magliano, Gallet, and Wajnberg, 2016)
	Acetamiprid, Dinotefuran, Imidacloprid, Thiamethoxam	Neonicotinoid	114, 434,79,915	2	(Cheng *et al.*, 2018)
	Clothianidin, Imidacloprid, Thiamethoxam		2212,9259, 17857	2,3,3	(Preetha *et al.*, 2009)
	Thiamethoxam+chlorantra-niliprole	Neonicotinoid+ Diamide	16949	3	
	Endosulfan	Organochlorine	324	2	
	Acephate	Organophos-phate	134	2	
	Fipronil	Phenylpyrazole	10	1	(D.-S. Wang *et al.*, 2012)
	Pymetrozine	Pyridine azomethine	156	2	(Preetha *et al.*, 2009)
	Beta-cypermethrin	Pyrethroid	0	1	(D.-S. Wang *et al.*, 2012)
			2	1	(D. Wang, Lu and He, 2018)
	Alpha-cypermethrin, Bifen-thrin, Cyhalothrin, Deltame-thrin		51,915,3950,192	2,2,3,2	(Cheng *et al.*, 2018)
	Ethofenprox		11111	3	(Preetha *et al.*, 2009)

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	Chlorfenapyr	Pyrrole	30	1	(D.-S. Wang *et al.*, 2012)
	Spinosad	Spinosyn	1	1	
			143	2	(D. Wang *et al.*, 2018)
T. confusum	Abamectin, Emamectin benzoate, Ivermectin	Avermectin	31,0,4	1	(Y. Wang *et al.*, 2013)
	Carbaryl, Carbosulfan, Isoprocarb, Metolcarb, Promecarb	Carbamate	1433,112,3529,2348,1587	2,2,3,2,2	
	Chlorfluazuron, Fufenozide, Hexaflumuron, Tebufenozide	Insect growth regulator	0	1	
	Acetamiprid, Imidacloprid, Imidaclothiz, Nitenpyram Thiacloprid, Thiamethoxam	Neonicotinoid	0,0,0,36,1,94	1,1,1,1,1,2	
	Chlorpyrifos, Fenitrothion, Phoxim, Profenofos, Triazophos	Organophosphate	16216,2264,13500,2400, 2069	3,2, 3,2,2	
	Butene-fipronil, Ethiprole, Fipronil	Phenylpyrazole	48,1,26	1	
	Cyhalothrin, Cypermethrin, Fenpropathrin, Lambda-cyhalothrin	Pyrethroid	8,9,6,8	1	
	Sulfoxaflor	Sulfoximine	305	2	(Jiang, Liu, Zhang, Liu, and Mu, 2019)
T. cordubensis	Deltamethrin	Pyrethroid	8	1	(Garcia, Cabral, Oliveira, and Rodrigues, 2006)
T. dendrolimi	Abamectin, Emamectin benzoate	Avermectin	636,54	2	(Cheng *et al.*, 2018)
	Butralin, Napropamid, Pendimethalin	Herbicide	757,395,1032	2	
	Acetamiprid, Dinotefuran, Imidacloprid, Thiamethoxam	Neonicotinoid	189,4577,458,3296	2,2,2,3	
	Alpha-cypermethrin, Bifenthrin, Cyhalothrin, Deltamethrin	Pyrethroid	2027,1177,2568,968	2,2,3,2	
	Sulfoxaflor	Sulfoximine	813	2	(Jiang *et al.*, 2019)
T. evanescens	Abamectin, Emamectin benzoate, Ivermectin	Avermectin	17,1,3	1	(Y. Wang *et al.*, 2014)
	Carbaryl, Carbosulfan, Isoprocarb, Metolcarb, Promecarb	Carbamate	2277,98,1935,13500,2381	2,2,2,3,2	
	Chlorfluazuron, Fufenozide, Hexaflumuron, Tebufenozide	Insect growth regulator	0	1	
	Acetamiprid, Imidacloprid, Imidaclothiz, Nitenpyram Thiacloprid, Thiamethoxam	Neonicotinoid	1,1,0,10,6,20	1	
	Chlorpyrifos, Fenitrothion, Phoxim, Profenophos, Triazophos	Organophosphate	18750,4800, 3600, 273, 1176	3,3,3,2,2	
	Butene-fipronil, Ethiprole, Fipronil	Phenylpyrazole	811,5,321	2,1,2	
	Cyhalothrin, Cypermethrin, Fenpropathrin, Lambda-cyhalothrin	Pyrethroid	7,6,14,13	1	
	Lambda-cyhalothrin		1	1	(El-Sebai and El-Tawil, 2011)

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T. exiguum	Thiodicarb	Carbamate	1	1	(Suh, Orr, and Van Duyn, 2000)
	Profenophos	Organophosphate	17	1	
	Cypermethrin, Lambda-cyhalothrin	Pyrethroid	0	1	
	Spinosad	Spinosyn	21	1	
T. japonicum	Abamectin, Emamectin benzoate, Ivermectin	Avermectin	65,2,5	2,1,1	(Zhao *et al*., 2012)
	Carbaryl, Carbosulfan, Isoprocarb, Metolcarb, Promecarb	Carbamate	2656,59,1224,15429,1515	3,2,2,3,2	
	Chlorfluazuron, Fufenozide, Hexaflumuron, Tebufenozide	Insect growth regulator	0	1	
	Acetamiprid, Imidacloprid, Imidaclothiz, Nitenpyram Thiacloprid, Thiamethoxam	Neonicotinoid	1,0,0,42,1,55	1,1,1,1,1,2	
	Chlorpyrifos, Fenitrothion, Phoxim, Profenofos, Triazophos	Organophosphate	15000,3000,4909,1463,1429	3,3, 3,2,2	
	Butene-fipronil, Ethiprole, Fipronil	Phenylpyrazole	16,4,25	1	
	Cyhalothrin, Cypermethrin, Fenpropathrin, Lambda-cyhalothrin	Pyrethroid	5,4,4,12	1	
T. nubilale	Abamectin, Emamectin benzoate, Ivermectin	Avermectin	9,0,2	1	(Y. Wang, R. Yu, *et al*., 2012)
	Neemazal	Azadirachtin	1	1	(Chen, Song, Qi, and Wang, 2013)
	Carbaryl, Carbosulfan, Isoprocarb, Metolcarb, Promecarb	Carbamate	1433,190,5000,1019, 1042	2,2,3,2,2	(Y. Wang, R. Yu, *et al*., 2012)
	Methoxyfenozide	Diacylhydrazine	0	1	(Chen *et al*., 2013)
	Chlorfluazuron	Insect growth regulator	0	1	
	Chlorfluazuron, Fufenozide, Hexaflumuron, Tebufenozide		0	1	(Y. Wang, R. Yu, *et al*., 2012)
	Acetamiprid, Imidacloprid, Imidaclothiz, Nitenpyram, Thiacloprid, Thiamethoxam	Neonicotinoid	1,0,0,7,2,12	1	
	Acetamiprid, Imidacloprid		38,13	1	(Chen *et al*., 2013)
	Chlorpyrifos, Fenitrothion, Phoxim, Profenofos, Triazophos,	Organophosphate	7407,57, 3857,3333,332	3,2,3,3,2	(Y. Wang, R. Yu, *et al*., 2012)
	Chlorpyrifos, Triazophos		15000,561	3,2	(Chen *et al*., 2013)
	Indoxacarb	Oxadiazine	0	1	
	Butene-fipronil, Ethiprole, Fipronil	Phenylpyrazole	4412,13,78	3,1,2	(Y. Wang, R. Yu, *et al*., 2012)
	Cypermethrin, Cyhalothrin, Fenpropathrin, Lambda-cyhalothrin	Pyrethroid	5,5,1,2	1	
	Chlorfenapyr	Pyrrole	9	1	(Chen *et al*., 2013)
	Rotenone	Rotenone	0	1	
	Spinosad	Spinosyn	345	2	

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T. ostriniae	Abamectin, Emamectin benzoate	Avermectin	148,6	2,1	(Cheng *et al.*, 2018)
	Abamectin, Emamectin benzoate, Ivermectin		7,0,2	1	(Y. Wang, L. Chen, *et al.*, 2012)
	Carbaryl, Carbosulfan, Isoprocarb, Metolcarb, Promecarb	Carbamate	1433,79,1071,10800,758	2,2,2,3,2	
	Butralin, Napropamid, Pendimethalin	Herbicide	453,126,978	2	
	Chlorfluazuron, Fufenozide, Hexaflumuron, Tebufenozide	Insect growth regulator	0	1	
	Acetamiprid, Imidacloprid, Imidaclothiz, Nitenpyram Thiacloprid, Thiamethoxam	Neonicotinoid	1,0,0,6,0,9	1	
	Dinotefuran, Imidacloprid, Nitenpyram, Thiamethoxam		750,31,38,322	2,1,1,2	(Li *et al.*, 2015)
	Acetamiprid, Dinotefuran, Imidacloprid, Thiamethoxam		44,1471,135,5493	1,2,2,3	(Cheng *et al.*, 2018)
	Chlorpyrifos, Fenitrothion, Phoxim, Profenofos, Triazophos	Organophosphate	12000,4000, 3857,265,252	3,3, 3,2,2	(Y. Wang, L. Chen, *et al.*, 2012)
	Butene-fipronil, Ethiprole, Fipronil	Phenylpyrazole	100,1,161	2,1,2	
	Alpha-cypermethrin, Bifenthrin, Cyhalothrin, Deltamethrin	Pyrethroid	147,411,1712,138	2	(Cheng *et al.*, 2018)
	Cyhalothrin, Cypermethrin, Fenpropathrin, Lambda-cyhalothrin		4,1,9,6	1	(Y. Wang, L. Chen, *et al.*, 2012)
	Pymetrozine	Pyridine azomethine	0	1	(Li *et al.*, 2015)
	Sulfoxaflor	Sulfoximine	406	2	(Jiang *et al.*, 2019)
T. pretiosum	Neemazal	Azadirachtin	0	1	(Parreira *et al.*, 2018)
	Oxamyl	Carbamate	5	1	(Williams and Price, 2004)
	Spinosad	Spinosyn	435	2	

Acaricides

Acaricides are pesticides used to kill the mites, and fenpyroximate is a widely used acaricidal chemical worldwide (Abd Al-Rahman *et al.*, 2012). Although it interferes with the mitochondrial electron transport chain in *Trichogramma* species and inhibits mitochondrial NADH-CoQ reductase (Nicotinamide Adenine Dinucleotide plus Hydrogen-Coenzyme Q) in epidermal cells and ovaries (Degli Esposti, 1998). It has only been tested against *T. cacoeciae* and classified as "safe".

Avermectins

Avermectins, a group of macrocyclic lactones, are a mixture of natural products produced by a soil actinomycete, *Streptomyces avermitilis*. They act as neurotoxins by blocking the transmission of electrical activity in insect nerves and muscle cells by enhancing the effect of glutamate. This causes an influx of chloride ions into the cells, leading to hyperpolarisation and subsequent paralysis of insect neuromuscular systems (Lumaret *et al.*, 2012). This group has shown effects on 8 *Trichogramma* species. Abamectin is slightly to moderately toxic to *T. chilonis*, *T. dendrolimi*, *T. japonicum* and *T. ostriniae*, whereas emamectin benzoate is only toxic to *T. dendrolimi*. All remaining pesticides in this group are considered safe for all *Trichogramma* species (Parsaeyan *et al.*, 2018).

Azadirachtin

Azadirachtin is a limonoid triterpene naturally occurring in *Azadirachta indica* seeds. This substance inhibits feeding but also has multiple physiological effects on growth and reproduction, disrupting moulting hormones and blocking vitellogenesis and testis development (Morgan, 2009). This pesticide is considered "safe" for all 3 *Trichogramma* species tested, including *T. cacoeciae*, *T. nubilale* and *T. pretiosum*.

Carbamates

Carbamates are synthetic insecticides that act as synaptic poisons through inhibition of acetylcholinesterase (AChE). Eight *Trichogramma* species, including *T. chilonis*, *T. japonicum*, *T. confusum*, *T. nubilale T. evanescens*, *T. japonicum* and *T. ostriniae*. Some carbamates are slightly to moderately toxic, such as carbaryl (for *T. confusum*, *T. evanescens*, *T. nubilale* and *T. ostriniae)*; carbosulfan (for *T. confusum*, *T. evanescens*, *T. japonicum*, *T. nubilale*, *T. ostriniae)*; isoprocarb (for *T. evanescens*, *T. japonicum*, *T. ostriniae)*; metolcarb (for *T. confusum*, *T. nubilale)* and promecarb (for *T. confusum*, *T. evanescens*, *T. japonicum*, *T. nubilale* and *T. ostriniae)*.

Diacylhydrazines

Diacylhydrazined are a group of ecdysone receptor agonist insecticides, which induce precocious molting in insects by mimicking the action of the molting hormone. They bind at the ecdysone binding site of the ecdysone receptor-ultraspiracle protein-dimer, causing it to activate ecdysone-responsive genes that are normally activated during molting and metamorphosis (Minakuchi, 2005). Methoxyfenozide is the only pesticide of this group tested against *T. nubilale* and without any toxic effect.

Diamides

These are ryanodine receptor modulators, which are calcium-activated channels in the sarcoplasmic reticulum of muscle cells. Its function is to amplify a small trigger calcium signal to produce the massive calcium release from intracellular stores that is needed for muscle contraction. Ryanodine receptors are also found in neurons of the central nervous system, where they may be involved in Ca^{2+}-signaling. Direct activation of ryanodine receptors by diamides causes sustained muscle contractions, leading to rapid feeding (Cordova *et al.*, 2006). Chlorantraniliprole is the only diamide tested thus far and showed no toxic effect to *T. chilonis*.

Herbicides

Herbicides are used specifically for vegetation (weed) management, but their impacts on beneficial insects have been documented (Region, 1977). Butralin, napropamide and pendimethalin are herbicides tested against 3 *Trichogramma* species (*T. chilonis, T. dendrolimi* and *T. ostriniae*) and classified as slightly to moderately toxic.

Insect growth regulators (IGRs)

Insect growth regulators inhibit molting processes and prevent insects from reaching maturity. All such pesticidal chemicals tested (chlorfluazuron, fufenozide, hexaflumuron, tebufenozide) have been found safe to 5 *Trichogramma* species (*T. confusum*, *T. evanescens*, *T. japonicum*, *T. nubilale* and *T. ostriniae*).

Microbial pesticides

Microbial pesticides are the major group of biopesticides used in pest management. Bacillus thuringiensis (Bt), a spore-forming bacterium, is widely used for insect control. Bt spores are packaged within crystals that release toxins when dissolved after ingestion by a susceptible insect. These protein toxins destroy the gut lining, causing leakage of gut contents into the hemocoel (Bravo *et al.*, 2007). Bacillus thuringiensis applied to T. chilonis had no toxic effect.

Neonicotinoids

Neonicotinoids are a group of compounds that bind to nicotinic acetylcholine receptors (nAChRs) of cells present in the central nervous system of the insect. Acetylcholinesterase breaks down acetylcholine to terminate signals from these receptors, but acetylcholinesterase cannot break down neonicotinoids, and their binding is irreversible. Low to moderate activation of nAChRs causes nervous stimulation, paralysis and ultimately insect death. (Taillebois *et al.*, 2018). This group has shown effects on 8 *Trichogramma* species. Imidacloprid is very toxic to *T. chilonis* and *T. dendrolimi,* as is thiamethoxam to *T. chilonis. In contrast,* acetamiprid is *slightly to moderately toxic to T. chilonis* and *T. dendrolimi*; clothianidin to *T. chilonis*; dinotefuran to *T. chilonis*, *T. dendrolimi* and *T. ostriniae*; imidacloprid to *T. cacoeciae*, *T. chilonis* and *T. dendrolimi* and thiamethoxam to *T. chilonis*, *T. confusum*, *T. japonicum* and *T. ostriniae* (Tai *et al.*, 2022; Manzoor *et al.*, 2024).

Neonicotinoid+Diamide

Neonicotinoid is a nerve poison and diamide is a muscle poison. The combined effect of these two poisons are synergistic (Dhang, 2018) But the mechanisms of action of these pesticides have yet to be studied in Trichogramma. T. chilonis is strongly affected by application of neonicotinoid+diamide (thiamethoxam+chlorantraniliprole); therefore, this group is classified as dangerously toxic.

Organochlorines

Organochlorines are chlorinated hydrocarbons, classical axonic poisons, some of which inhibit the normal activity of gamma-aminobutyric acid (GABA). Abnormal activity of GABA leads to blockage of GABA-gated chloride channels that cause spasm and overstimulation in insects (Vale *et al*., 2003). One such organochlorine, endosulfan, is slightly to moderately toxic against *T. chilonis.*

Organophosphates

Organophosphates are a group of chemical insecticides whose primary action is the inhibition of acetylcholinesterase (AChE) that degrades the neurotransmitter acetylcholine (ACh) into choline and acetic acid in the nervous system. Organophosphates inactivate AChE by phosphorylating the serine hydroxyl group at the active site of AChE (Colovic *et al*., 2013). Eight *organophosphate insecticides* have been investigated in *Trichogramma* species. Chlorpyrifos is dangerously toxic to *T. confusum*, *T. evanescens*, *T. japonicum*, *T. nubilale,* and *T. ostriniae*; diazinon to *T. brassicae*; fenitrothion to *T. evanescens*, *T. japonicum,* and *T. ostriniae*; phoxim to *T. confusum, T. evanescens*, *T. japonicum, T. nubilale,* and *T. ostriniae* and profenofos to *T. nubilale*. On the other hand, acephate is slightly to moderately toxic to *T. chilonis*; fenitrothion to *T. confusum* and *T. nubilale;* profenofos to *T. confusum*, *T. evanescens*, *T. japonicum,* and *T. ostriniae*; triazophos to *T. confusum*, *T. evanescens*, *T. japonicum*, *T. nubilale,* and *T. ostriniae* (Delpuech and Meyet, 2003; Nidagundi *et al*., 2022).

Oxadiazines

Oxadiazines are a group of insecticides that block sodium channels in insect nerve cells. An esterase leaves the carbomethoxy group from the urea linkage, liberating free urea, which then acts as the voltage-dependent sodium channel blocker (Wing *et al*., 2000). *Only 1 Trichogramma species* has been studied to date, and with no harmful effects from indoxacarb.

Phenylpyrazoles

Phenylpyrazoles are a chemical group that acts by blocking glutamate-activated chloride channels in insects (Vidau *et al*., 2009). Seven *Trichogramma* species have been evaluated for risk from phenylpyrazones. Butene-fipronil is the only pesticide that is dangerously toxic to *T. nubilale,* but it is slightly to moderately toxic to *T. evanescens* and *T. ostriniae*. Fipronil is also classified as "slightly to moderately toxic" to *T. evanescens*, *T. nubilale* and *T. ostriniae*. Four remaining *Trichogramma* species (*T. brassicae*, *T. chilonis*, *T. confusum* and *T. japonicum*) are considered safe from pesticides of this group.

Pyrethroids

Pyrethroids are axonic toxins, which prevent the closure of the voltage-gated sodium channels in the axonic membrane of insect neurons (Soderlund *et al*., 2002). This group influences 10 *Trichogramma* species. Cyhalothrin is dangerously toxic to *T. chilonis* and *T. dendrolimi*. Alpha-cypermethrin, bifenthrin, and deltamethrin are slightly to moderately toxic to *T. chilonis* and *T. dendrolimi*. Eight other *Trichogramma* species (*T. brassicae*, *T. confusum*, *T. cordubensis*, *T. evanescens*, *T. exiguum*, *T. japonicum* and *T. nubilale*) have been tested with no harmful effects from these pesticides.

Pyridine azomethine

Pyridine azomethine is a new pesticide with a unique mode of action. It completely blocks the penetration of insect stylets into plants, preventing feeding (Fuog *et al*., 1998). Pymetrozine is slightly to moderately toxic to *T. chilonis* but safe to *T. ostriniae*.

Pyrroles

Pyrroles act by disrupting metabolic respiratory pathways (oxidative phosphorylation) in the mitochondria of cells. Two *Trichogramma* species (*T. chilonis* and *T. nubilale*) appear to be unaffected by chlorfenapyr.

Rotenone

Rotenone is the pesticide that blocks the electron transport chain in mitochondria by inhibiting electron transfer from iron-sulfur centers in complex I, which can ultimately damage the DNA and other components of mitochondria in insects (Mehta and Li, 2009). Only *T. nubilale* has been studied, with no harmful effects of rotenone.

Spinosyns

Spinosyns target the binding sites on nicotinic acetylcholine receptors (nAChRs) of the insect nervous system by disrupting acetylcholine neurotransmission that ultimately kills the insect by hyperexcitation (Salgado, 1998). Four *Trichogramma* species have been tested with this group, and among these *T. nubilale* and *T. pretiosum* are slightly to moderately affected by spinosad. According to one study, spinosad is slightly to moderately toxic to *T. chilonis* but another study indicates it is safe and *T. exiguum* is also not harmed by spinosad. However, a recent study warns about the side-effects of spinosad on *Trichogramma* wasps (Papari *et al*., 2024).

Sulfoximine

Sulfoximine modulates the nicotinic acetylcholine receptor (nAChR) in the insect nervous system and causes uncontrolled nerve impulses , resulting in paralysis and death (Casida and Durkin, 2013). Sulfoxaflor is dangerously toxic to *T. confusum*, *T. dendrolimi* and *T. ostriniae* (Schofer *et al*., 2024).

Sublethal effects

Indirect effects correlate to sublethal effects of pesticides, tabulated as Table 2, because no apparent mortality ensues from pesticide exposure, but other adverse effects on different traits of parasitoid biology can occur (Desneux *et al*., 2007). It is important to understand the sublethal effects of pesticides as part of an integrated pest management (IPM) system by investigating a pesticide's side effects on *Trichogramma* wasps. The reduced-biological trait is calculated by the following equation:

$$E\ (\%) = \left(\frac{1-\text{biological parameter}}{\text{Control}}\right) \times 100$$

In this regard, different toxicity classes have been established, as follows; class 1, harmless (E = <30%); class 2, slightly harmful (E = > 30% but < 79%); class 3, moderately harmful (E = > 80% but < 99%); and class 4, harmful (E = > 99%) (Hassan *et al*., 1991; Sterk *et al*., 1999). These organized classes provide an easy way to understand quantitatively the reduction of any biological trait of natural enemies by different pesticides, such as development period, emergence, fecundity, fertility, longevity, mating time, oviposition, parasitism, sex ratio and survival of *Trichogramma* species.

Table 2: *Development reduction (%) of Trichogramma egg parasitoid species by different pesticide groups.*

Trichogramma Species[a]	Pesticide common or trade name	Pesticide group	Development reduction (%)	Class[b]	References
Trichogramma achaeae	Abamectin, Emamectin benzoate	Avermectin*	9,7	1	(Fontes *et al*., 2018)
	Neem seed oil	Azadirachtin*	2	1	
	Merthiocarb, Methomyl, Pirimicarb	Carbamate*	0,8,6	1	
	Chlorantraniliprole	Diamide*	5	1	
	Vegetable oil	Essential oil**	0	1	
	Azoxystrobin, Sulfur	Fungicide**	0	1	
	Cyromazine, Hexythiazox, Lufenuron	Insect growth regulator*	0,6,5	1	
	Bacillus thuringiensis	Microbial pesticides*	5	1	
	Acetamiprid, Imidacloprid, Thiamethoxam	Neonicotinoid*	5,9,10	1	
	Acrinatrin, Deltamethrin, Lambda-cyhalothrin	Pyrethroid*	0	1	
	Spinosad	Spinosyn*	0	1	
T. atopovirilia	*Beauveria bassiana, Metarhizium anisopliae*	Microbial pesticides*	0	1	(Polanczyk, Pratissoli, Dalvi, Grecco, and Franco, 2010)
T. brassicae	Indoxacarb	Oxadiazine*	4	1	(Liu and Zhang, 2012)
	Spinosad	Spinosyn*	11	1	

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T. cordubensis	Acetamide+ dithiocarbamate	Acetamide+ Carbamate**	0	1	(Vieira, Oliveira, and Garcia, 2001)
	Basic copper sulphate	Fungicide**	0	1	
	B. thuringiensis	Microbial pesticides*	0	1	
	Endosulfan	Organochlorine*	8	1	
	Trichlorfon	Organophosphate*	0	1	
	Deltamethrin, Lambda-cyhalothrin	Pyrethroid*	0	1	
T. embryophagum	*Ferula assafoetida*	Essential oil**	0	1	(Poorjavad *et al.*, 2014)
T. evanescens			0	1	
T. pretiosum	*B. bassiana, M. anisopliae*	Microbial pesticides*	0	1	(M Potrich, Alves, Lozano, Bonini, and Neves, 2017)
	M. anisopliae		7	1	(Michele Potrich *et al.*, 2009)
	Indoxacarb	Oxadiazine*	0	1	(Liu and Zhang, 2012)
	Spinosad	Spinosyn*	6	1	

Some pesticidal groups whose mode of action is not explained in the section "lethal effects" of pesticides are described here in Table 2 because only the sublethal effects of these pesticides have been tested with different *Trichogramma* species.

Acetamide+Carbamate

Acetamide is a monocarboxylic acid amide with major antimicrobial activity. When applied with dithiocarbamate, a carbamate, on *T. cordubensis* no side effects on the development period, emergence, longevity and parasitism were observed (Mansour *et al*. 2023).

Benzoylureas

These are derivatives of N-benzoyl-N`-phenylurea that inhibit the synthesis of chitin in the insect integument (Junquera *et al*., 2019). Only one *Trichogramma* species has been studied with exposure to teflubenzuron. Teflubenzuron did not decrease adult emergence of *T. pretiosum* and was considered "harmless," whereas another study determined it to be slightly "harmful". This difference may be due to different doses of pesticide used. Longevity of *T. pretiosum* was not affected by this pesticide. Teflubenzuron reduces the number of parasitized host eggs by *T. pretiosum* females and was considered "moderately harmful," whereas another study concluded that this pesticide was "harmless". The sex ratio of *T. pretiosum* was unaffected by this pesticide (Cost *et al*., 2022).

Benzimidazole+Bisdithiocarbamate

Benzimidazole inhibits cuticular growth (Kamil *et al*., 2015) whreas bisdithiocarbamate inhibits the molting process of insects (McMullen, 1959). When carbendazin and thiram were assessed in *T. pretiosum* for adult emergence and parasitism, the study determined it to be slightly harmful and harmless, respectively.

Cyflumetofen

This pesticide interferes with the mitochondrial electron transport chain in insects and inhibits mitochondrial NADH-CoQ reductase in mitochondria of epidermal cells (Degli Esposti, 1998). It did not diminish the adult emergence and parasitism by *T. pretiosum*.

Diamide+Pyrethroid

Diamide is a muscle poison, and pyrethroid is a nerve poison. The combined effect of chlorantraniliprole and lambda-cyhalothrin slightly decreased adult emergence of *T. pretiosum* whereas parasitism is moderately reduced (Ajudia *et al*., 2025).

Dipyridyl+Substitued urea

Dipyridyl and substituted ureas are both used widely as herbicides, but to a lesser degree as insecticides

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(Retnakaran and Wright, 1987). The combined effect of paraquat and diuron slightly reduced adult emergence and parasitism of *T. pretiosum* (Aliyah *et al.*, 2022).

Plant oils

Plant oils are hydrophobic volatile oils that often provide the distinguishing aroma to a plant like citrus, lavender or rosemary. These are used as repellents, feeding deterrents or insecticides (Isman, 2019; Isman, Miresmailli, and Machial, 2011). Different plant oils have an influence on 9 biological traits of *Trichogrmma* species. The vegetable oil did not interfere with the normal development time of *T. achaeae*. Moreover, adult emergence, fertility and sex ratio of *T. achaeae* were not affected. In contrast, female adult longevity and parasitism was slightly reduced. Karanjin oil is slightly harmful to *T. chilonis* and *T. japonicum* because it reduces adult female emergence and parasitism. *Ferula assafoetida* oil is harmless to *T. embryophagum* and *T. evanescens* because it did not decrease adult emergence and female adult longevity, but this oil is slightly harmful for *T. evanescens* for mating time, parasitism and sex ratio. Matrine oil is harmless for *T. cacoeciae* with reference to parasitism and adult female survival but slightly reduces adult longevity. Essential oil of *Reynoutria sachalinensis* is also harmless for parasitism by *T. cacoeciae*. Essential oils of *Citrus sinensis* and *Zingiber officinale* are slightly harmful for female adult longevity and parasitism by *T. galloi,* but harmless to the sex ratio. *Crotalaria* oil is slightly harmful for parasitism by *T. ostriniae*. The oils of *Ageratum fastigiatum*, *Chromolaena chaseae*, *Eremanthus Elaeagnus*, *Lepidaploa lilacina*, *L. rufogrisea*, *Lychnophora ramosissima*, *Ly. ericoides*, *Mikania nummularia*, *Trichogonia villosa*, *Trixis glutinous* and *Vernonia holosenicea* are harmless for the adult emergence of *T. pretiosum,* but *A. sativum, Me. piperita* are slightly harmful to adult longevity. The oviposition and parasitism by female *T. pretiosum* were not affected by *Leptospermum petersonii* and *Trichilia pallida* oils. Generally, the essential oils are harmless to slightly harmful for *Trichogrmma* species (Sombra *et al.*, 2022).

Fungicides

Fungicides are used to control different fungal diseases but their impact on *Trichogramma* biology has not been well studied (Wajnberg and Vinson, 1991). Six biological traits of *Trichogramma* have been observed following exposure to different fungicides. Azoxystrobin and sulfur are harmless to *T. achaeae* concerning the development period, emergence and sex ratio, whereas fertility, longevity, and parasitism were not reduced by azoxystrobin but were slightly decreased by sulfur. Azoxystrobin, mancozeb, tebuconazole and thiophanate-methyl did not reduce adult emergence, parasitism or alter sex ratio for *T. atopovirilia* but chlorothalonil is slightly harmful because it decreases parasitism. Among different fungicides used, dichlofluanid, thiram® and sulphur are harmful to parasitism by *T. cacoeciae,* but sulphur is harmless to adult emergence. For *T. chilonis* alone, parasitism is slightly reduced by a combination of fungicides (pyraclostrobin + Metiram), but myclobutanil and trifloxystrobin+tebuconazole did not affect parasitism. Basic copper sulphate is harmless to *T. cordubensis* concerning its development period, emergence, and longevity, whereas parasitism is slightly reduced, although another study indicates parasitism is not affected by basic copper sulphate. Among 15 fungicides tested on *T. pretiosum*, 11 are harmless to emergence and parasitism as detailed in Table 2

Insect growth regulator+Organophosphate

Combined application of a molting inhibitor (lufenuron) and a nerve poison (profenofos) slightly reduced the emergence of *T. pretiosum,* but longevity was not decreased.

Minerals

Minerals are natural inorganic compounds mined from the earth in crystal form and used as a physical pesticide (Murray *et al.*, 2013). The effect of different minerals on *Trichogramma* biology showed that the emergence and parasitism of *T. cacoeciae* was not affected by kaolin, kemesol and super misrona.

Organic tin

This product is mostly used as a wood preservative (Wanda *et al.*, 1998). Fentin hydroxide, an organic tin, did not reduce the parasitism capacity of the *T. pretiosum* as shown in Table 2. A study by Bastos *et al.* (2006) showed moderate effects of fentin hydroxide on *T. pretiosum* developing in *E. kuehniella* and *S. cerealella* eggs under laboratory conditions.

Pyrethroid+Neonicotinoid

The combined effect of two nerve poisons (lambda cyhalothrin and thiamethoxam) was studied on four biological traits of two *Trichogramma* species. Adult emergence, parasitism and sex ratio of *T. galloi* were slightly reduced by this combination of pesticides, whereas these biological parameters in *T. pretiosum* were not affected by these pesticides, although longevity was slightly reduced (Tuncbilek and Ayvaz, 2003).

Pyrazole

Pyrazole is a respiratory poison that inhibits the mitochondrial electron transport chain, ultimately disrupting adenosine triphosphate (ATP) formation (Wu *et al*., 2012). Tolfenpyrad and fipronil are phenyl pyrazoles that are slightly harmful to the emergence and harmful for the parasitism capacity of *T. pretiosum* and *T. brassicae*, respectively (Saber *et al*., 2020).

Pyridinecarboxamide

The foremost insecticidal mechanism of this pesticide is starvation, based on the inhibition of stylet penetration into plants (Morita *et al*., 2007). Flonicamid is moderately harmful to emergence but harmless to the parasitism capability of *T. pretiosum*.

Plant growth regulators

Plant growth regulators (PGRs) may act as growth inhibitors or promoters, but can be used as substitutes to traditional insecticides for controlling the economically important insect pests. Synthetic PGRs mimic natural endogenous PGRs to stimulate or retard plant growth and reduce the insect pest infestation on plants by direct death or impairement of their reproductive potential and other physiological processes (Ahmad *et al*., 2003; De Mendonca *et al*., 2006; Gupta *et al*., 2009; Kaur *et al*., 2016). Therefore, they are marketed frequently as insecticides and their impact on *Trichogramma* biology showed that sulfometuron-methyl is harmless to adult emergence of *T. galloi,* but trinexapae-ethyl is slightly harmful. Mepiquat chloride is also slightly harmful for *T. pretiosum* concerning emergence. Regarding parasitism capacity, sulfometuron-methyl and trinexapae-ethyl are harmless to *T. galloi* as is mepiquat chloride for *T. pretiosum*.

Spirocyclic tetronic acids

This is a new class of chemical insecticides, derived from tetronic acid, that act as an inhibitor of acetyl-coenzyme A carboxylase, a lipid metabolism enzyme, resulting in the decrease of total lipids in insects (Parmar *et al*., 2019). Spiromesifen and spirotetramat are tetronic acid derivatives that have been tested on the biological parameters of four *Trichogramma* species. Adult emergence of *T. achaeae*, *T. cacoeciae* and *T. pretiosum* was slightly reduced by spiromesifen but for *T. chilonis*, it was harmless. Fecundity, longevity, parasitism and survivorship of *T. achaeae* were not harmed by spiromesifen. The parasitism capacity of *T. chilonis* and *T. pretiosum* was not affected by spiromesifen or spirotetramat but spiromesifen slightly reduced survivorship of *T. cacoeciae* (Tabebordbar *et al*., 2020; Khan, 2022).

Surfactants

Surfactants are used to remove the surface tension of two states of matter and improve the efficacy of pesticides (Castro *et al*., 2013). Tween was not observed to reduce adult emergence or alter sex ratio of *T. galloi*.

Effect of pesticides on trichogramma biology

The sublethal effects of pesticides on non-target species, because the consequences are not observed during short-term acute (lethal) exposure to pesticides (Desneux *et al*., 2007). The biological parameters that can be influenced sublethally are further subgrouped into physiological and behavioral classes. In this review, we have focused only on the physiological bio-traits of *Trichogramma* influenced by pesticides (De Paiva *et al*., 2018; Costa *et al*., 2023; Theenoor *et al*., 2024).

Development period

The development period is the days required for the insect from egg to imago, and it may be reduced when exposed to pesticides (Khan and Ahmad, 2019) The development time for seven species of *Trichogramma,* such as *T. achaeae*, *T. atopovirilia*, *T. brassicae*, *T. cordubensis*, *T. embryophagum*, *T. evanescens,* and *T. pretiosum* was not reduced by pesticides tested (Tonga, and Erkek, 2024).

Emergence

The emergence of an endoparasite, such as *Trichogramma,* from host eggs as an adult can be highly related to pesticide exposure (Asma *et al*., 2018). Twenty species of *Trichogramma* have been assessed for adult emergence following pesticide exposure. The adult female emergence of *T. acacioi*, *T. bennetti*, *T. brasiliensis*, *T. bruni*, *T. demoraesi*, *T. embryophagum*, *T. evanescens*, and *T.* soaresi has only been assessed under one pesticidal group, which appears to be harmless for all these species. *T. pretiosum* is an important species, as thirty pesticidal groups have been assessed for their effects on adult emergence.

The acaricides, benzimidazole+bisdithiocarbamate, diamide+pyrethroid, dipyridyl+substituted, insect growth regulator+organophosphate, phenylpyrazol, plant growth regulator, pyrazole, and spirocyclic tetronic acid were found to be slightly harmful to adult emergence of *T. pretiosum,* whereas organochlorine is moderately harmful, and carbamate is moderately harmful to harmful.

Fecundity

Interference of pesticides with reproductive processes in *Trichogramma* usually causes reductions in fecundity (Consoli *et al.*, 1998). Only two *Trichogramma* species, *T. achaeae* and *T. chilonis,* have been evaluated in this regard by seven and eleven pesticidal groups, respectively. Azadirachtin is slightly harmful to *T. achaeae* but all remaining pesticidal groups are harmless. Avermectin, azadirachtin and carbamate are slightly harmful but phenylpyrazole moderately reduced the fecundity of *T. chilonis* (Costa *et al.*, 2023).

Fertility

The fertility of *Trichogramma* females is estimated by the number of black spots observed on host eggs, but it can be reduced when the fertility of female *Trichogramma* is affected by pesticides (Fontes *et al.*, 2018). Fertility of only one *Trichogrmma* species, *T. achaeae,* has been observed under the influence of eleven pesticidal groups. Insect growth regulators and microbial pesticides are slightly harmful, whereas avermectins and spinosyns are harmful, because they made female *T. achaeae* infertile. The pyrethroid group is slightly harmful to harmful (Papari *et al.*, 2024).

Longevity

Longevity is often used as an indicator of wasp quality. Longevity of parasitoids may rely on such factors as the type of insecticides and the parasitoid species (Saber, 2011). Thirteen species of *Trichogramma* have been observed for longevity reduction. The longevity of *T. achaeae* and *T. pretiosum* has been evaluated under the maximum number of pesticidal groups and among those, essential oil is slightly harmful, whereas organophosphate and spinosyn are moderately harmful in the longevity reduction of *T. achaeae.* The pyrethroid + neonicotinoid is slightly harmful, whereas organophosphate and pyrrole are moderately harmful to *T. pretiosum* (Ray *et al.*, 2022; Cost *et al.*, 2023).

Mating time

Mate finding and mating time are considered crucial steps in the mating system of an endoparasitoid (Poorjavad *et al.*, 2014). Pesticide exposure may cause a reduction in mating time. The essential oil of *F. assafoetida* caused a slight reduction in mating time for female *T. embryophagum,* but *T. evanescens* females mated normally.

Oviposition

There is a close association between the oviposition and parasitism rate for all host-parasitic wasp interactions (Knutson, 1998). Oviposition reduction has been observed for three *Trichogramma* species exposed to ten pesticide groups. Among those pesticide groups, seven were tested for oviposition reduction in *T. chilonis*. Azadirachtin, insect growth regulator and oxadiazine are considered slightly harmful for oviposition of *T. chilonis* whereas, avermectin is moderately harmful (Wang *et al.*, 2016; Salim *et al.*, 2025).

Parasitism

Parasitism of hosts is done specifically by female *Trichogramma,* and contact action is involved directly by inserting the ovipositor into a host egg (Amaro *et al.*, 2015). Thirty-one pesticide groups have been tested for parasitism reduction in *T. pretiosum*. Among these groups, the acaricides, avermectin, carbamate, diamide+pyrethroid, dipyridyl+substitutedurea, insect growth regulator+organophosphate, organochlorine, phenylpyrazole, pyrazole, pyrethroid, and pyrrole reduced the parasitism capacity of *T. pretiosum* (De Paiva *et al.*, 2018; Salim *et al.*, 2025).

Sex ratio

The sex ratio of the progeny was determined by dividing the number of females by the number of females + males in the offspring (Delpuech and Meyet, 2003). Changes in sex ratio have been estimated for sixteen *Trichogrmma* exposed to twenty-two pesticide groups. The sex ratio of ten *Trichogramma* species - *T. acacioi*, *T. achaeae*, *T. atopovirilia*, *T. bennetti*, *T. brasiliensis*, *T. bruni*, *T. cacoeciae*, *T. demoraesi*, *T. exiguum,* and *T. soaresi* - was not reduced by any of the different pesticide groups (Tai *et al.*, 2022).

Survival

The impact of pesticides must be assessed not only on adult survival but also on pupae of *Trichogramma*

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(Gallego *et al*., 2019). The pupal survival of *T. brassicae* was not reduced by pesticides, nor was adult survival of *T. confusum*, *T. dendrolimi*, *T. ostriniae* or *T. pretiosum* reduced by the pesticides tested (Bastos *et al*., 2006; Cost *et al*., 2022).

Conclusions and Recommendations

World cropping systems do not rely exclusively on biological control of insect pests, and the use of pesticides remains mandatory for combating agricultural pests. However, broad-spectrum and non-selective pesticides often have deleterious, lethal, or sub-lethal effects on natural enemies of insect pests, including parasitic Hymenoptera. Therefore, lethal and sublethal effects of each pesticide on non-target and beneficial organisms, such as *Trichogramma* parasitoids, should be critically determined as part of the regulatory approval process. More research is needed to determine the side effects of synthetic pesticides on pollinators, predators, and parasitoids. Furthermore, insecticides intended for incorporation into any IPM program should be efficacious against the target pests, but not against their natural enemies. In this respect, all pesticide groups discussed above have been categorized into different side effect classes to allow for recommendations to farmers on chemical use.

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Novelty Statement

Trichogramma is one of the most widely studied and practically-demonstrated biological control agents being used against different insect pests, particularly against lepidopterous ones. This review paper presents a consolidated information on various lethal and sublethal effects of different groups of pesticides on these tiny wasps.

Author's Contribution

Kanwer Shahzad Ahmed: Conceived the review idea and designed the protocol for data collection.
Muhammad Zeeshan Majeed: Provided technical assistance. KSA prepared the preliminary manuscript.
Abu Bakar Muhammad Raza: Performed the technical proofreading and revision of the draft.

Generative AI and AI-assisted technology statement
The authors stated that they didn't use generative AI and AI-assisted technology in preparing this manuscript.

Conflicts of interest
Authors have declared no conflict of interest.

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