Review Article

Invasive Alien Plant species (IAPs) for Sustainable Pest Management: A Review

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Abstract

Invasive alien plants (IAPs) threaten biodiversity and agricultural productivity via disruption of ecological processes. Managing IAPs is challenging as they spread across Nepal. Prevention is now challenging, and eradication is only feasible in small, managed areas. IAPs have great potential as sustainable source of bio-pesticides. The use of synthetic pesticides for controlling agricultural pests, household insects, and mosquitoes has significant drawbacks. They are expensive, persist long-term in soil and food, negatively impact human health and environment, and lead to pest resistance. Hence, IAPs can be the alternative for synthetic pesticides. This review was conducted to examine the insecticidal activities of Nepal's IAPs to generate alternative solutions for pest and IAPs management by utilizing them. The study involved a comprehensive literature review of the insecticidal properties of 30 IAPs of Nepal. Data were collected from various published studies and the focus was on identifying the plant parts used for formulations, the insect and their stages targeted, and the overall effectiveness of different extract formulations. Among the 30 IAPs of Nepal, 18 species have been studied at many countries for their insecticidal properties against various insects, including mosquitoes and agricultural pests. Most studies were conducted in India and Nigeria. Leaves were the most frequently used plant part for formulations. The larval stage of insects was the preferred stage for studying insecticidal properties. Essential oils were found to be more effective than other extract formulations. All studies demonstrated toxicity to different insects at different level. IAPs of Nepal possess insecticidal activity against harmful insects and pests, offering potential for sustainable pest management.

Keywords: Bio-pesticides, Insecticides, Invasive plants, Mosquito

Introduction

Synthetic pesticides pose significant issues related to human health, soil quality and impact on nontarget organisms thereby causing devastating impact on floral, faunal and microbial diversity. Their persistent nature, high cost, environmental impact, and development of pest resistance have raised concerns, highlighting the need for eco-friendly, cost-effective, and sustainable bio-pesticides with specific modes of action (Hicks et al., 2018). Unlike synthetic insecticides, which have eco-toxicological impacts, negatively affect non-target organisms, and cause genetic drift in targeted species; bio-pesticides offer a safer alternative (Ayilara et al., 2023) They can repel insect pests, disrupt their development, affect reproduction, or kill live organisms on contact. Bio-pesticides are eco-friendly and accessible to resource-poor smallholder farmers and locals in regions with food insecurity (Kumar et al., 2021). Bio-pesticides exhibit various modes of action, such as denaturing protein structures, inhibiting growth rates, causing metabolic disorders, and releasing bioactive compounds (Sparks & Nauen, 2015).

Several plants and micro-organisms have been utilized for bio-insecticide formulations. For example, *Azadirachta indica* A. Juss. (neem) is used by farmers in developing countries as an insecticide. It contains the bioactive compound azadirchtin, which exhibits pronounced insecticidal activity, affecting egg hatchability, fecundity inhibition, oviposition repellence, larval, pupal, and adult lifespan, insect growth regulation, ovicidal effects, and anti-feedant properties (Chaudhary et al., 2017). Extracts from various plants (Rengül Demirak &

Canpolat, 2022), such as *Adhatoda vasica* L. leaves, Annona squamosa L. seeds, Nerium oleander L. stem, Volkameria inermis L., Pongamia pinnata (L.) Pierre seeds, *Prosopis chilensis* (Molina) Stuntz stems, Vitex negundo L. leaves, Madhuka indica J.F. Gmel, Azadirchta indica and Aegle marmelos (L.) Corrêa had shown significant insecticidal activity against Nilaparvata lugens (Stal) (Hiremath et al., 1997). Additionally, ethanol extracts from tropical plants, including Nikotina tobaccum L., Dioscorea pallens Schltdl., Cuscuta americana L., Bontia daphnoides L., Capsicum annuu L., Artocarpus altilis (Parkinson) Fosberg, Cyclopeltis semicordata (Sw.) J.Sm., Hibiscus rosa -sinensis L., Annona reticulate L., Mimosa pudica L., Gliricidia sepium (Jacq.) Kunth and Chromolaena odorata (L.) R.M.King & H.Rob. have demonstrated insecticidal activity with 50-100% mortality of Tribolium confusum Jacquelin du Val, 1863 adults (Williams & Mansingh, 1993).

Building upon the use of varied plants species for the bio-pesticides formulations, IAPs offer twin benefit by addressing the need of sustainable pest management and the ecological burden of IAPs themselves. IAPs is emerging as a unavoidable environmental problem, threatening biodiversity and agricultural productivity (Roy et al., 2024). In Nepal, 182 alien plant species have naturalized, with 30 classified as invasive (Shrestha et al., 2024). Their distribution and impact is increasing, complicating the management of these species (Shrestha & Shrestha, 2021). Among these, four species - Lantana camara L., Mikania micrantha Kunth, Pontederia crassipes Mart. and Chromolena odorata (Spreng.) R.M.King & H.Rob- are listed among the world's 100 invasive species (Shrestha, 2016). Also, recently reported Leucaena leucocephala (Lam) de Wit is one of the 100 worst invasive species (Shrestha et al., 2024). The invasive species problem is global but is particularly pronounced in developing countries like Nepal, where management efforts are hampered by a lack of expertise and limited resources.

Invasive plant species produce allelopathic compounds (Kalisz et al., 2021) which have potential to suppress native plants. Also, invasive plants has high chemical diversity and novel secondary

metabolites than native plants (Macel et al., 2014). Wide variety of phenolic compounds, alkaloids, volatile organic compounds from the invasive plants have shown the bio-medical importance presenting anti-cancer, anti-bacterial, anti-fungal, anti-oxidant activities. These secondary metabolites can also have the potential source of bio-pesticides. The review aims to explore the management of problematic IAPs in Nepal through their economic utilization for insecticide formulation. Authors synthesized existing but scattered literature on the insecticidal activity of various parts of IAPs, specifically those invasive in Nepal. The paper discusses the prospects and opportunities for using IAPs as bio-insecticides for controlling agricultural pests, protecting the environment, and addressing human health concerns. Finally, the paper highlights trends in bio-pesticide use, identify research gaps, and recommend bioassay-guided studies of IAPs as insecticide for future research.

Materials and Methods

Search strategy

The information presented in this review was sourced from journal articles relevant to the insecticidal properties of IAPs in Nepal. Scientific papers were obtained from diverse sources, including Google Scholar, PubMed, DOAJ and EBSCO. Systematic keywords used in the search included "invasive alien plants," "insecticidal," mosquitocidal potential," along with the scientific names and synonyms of each plant reported to possess insecticidal properties. Boolean operators (OR) were used to refine the search for more focused and productive results. The literature search was conducted during May 2024.

Inclusion and exclusion criteria

Only literature focusing on insecticidal properties was included. Out of 143 published papers retrieved, 60 were reviewed. Excluded papers were those published before 2014, those involving plants not declared invasive in Nepal, ethno-botanical studies, clinical trials, studies on non-IAPs of Nepal, insect repellency, ovicidal effect; and those focused on impact and distribution.

Data extraction and analysis

Data recorded included the percentage of insect mortality, LC₅₀ and LC₉₀ values (converted to ppm where possible), country of study, IAP names, plant parts used, formulations, target insects, developmental stages at which the formulation was applied, highest percentage mortality, observation periods, and reported LC₅₀ and LC₉₀ values. Microsoft tools and reference manager (Zotero) were used for data analysis and reference management, respectively. The ranking of studied articles was assessed using the SCImago ranking website. But both ranked and non-ranked journal were studied.

Results and Discussion

Case studies of insecticidal potential of IAPs in different countries

From the literature survey, 60 studies published in the last ten years globally, reporting on the insecticidal activities of 18 plant species that are invasive to Nepal. The highest numbers of published case studies were from India and Nigeria, with 20 and 15 studies, respectively. Other countries, such as Egypt, Brazil, China, Ethiopia, Ghana, Kenya, Colombia, Thailand, Vietnam, Ivory Coast, Burkina Faso, Slovenia, the Philippines, and the United States, each contributed fewer than five studies. No records were obtained from Nepal regarding the insecticidal potential of IAPs (Figure 1), despite extensive studies on their distribution and impact (Pandey et al., 2020). It can be hypothesized that the large number of research papers from India,

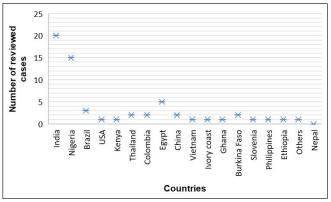


Figure 1: Case studies of insecticidal potential of IAPs in different countries

Nigeria, and other developing countries is due to the availability of those IAPs in these countries than other; and the need of local people to manage insect pests of agricultural, environmental and medical importance.

Insecticidal potential of different IAPs of Nepal

The published articles (Table 1) on the insecticidal potential of Nepal's IAPs, represents species names, plant parts used, test sample formulations, targeted insects and stages, percentage mortality, and/or LC₅₀, LC₉₀ and duration of observation. Information regarding the phytochemicals data and methods of nanotechnology-based formulations are not mentioned here, but presented the dependency of insecticidal effect on concentration and formulation of test sample, biological assay and post-exposure time for varied insects. Additionally, only a few cases were recorded to have performed toxicity tests on albino mice or brine shrimp, which are also excluded from the data. Gas Chromatography Mass Spectrometry (GC-MS) data was not the focus point of this review.

These studies utilized different plant parts to formulate essential oils, crude drugs, or extracts with various solvents. Notably, no studies were found that employed bioassay-guided fractionation or examined effects on non-target organisms. This limits the identification of potential compound responsible for the insecticidal activities and maintenance of regulatory standards and environmental integrity. Most studies used sample sizes of 20-25 for mosquito species and 10 for others. The World Health Organization (WHO) methods were commonly used for mosquito applications, with slight modifications based on available instruments. Other insects, both adults and larvae, were studied for contact toxicity, fumigant toxicity, anti-feedant activity, and habitat alteration. The lethal concentration in all cases was reported with a 95% confidence level for significant differences.

Table 1: Insecticidal potential of different invasive alien plant species of Nepal

N.	Name of species/family	Part used	Solvent/ Formulation	Insect tested	Biological assay/stage	% mortality	LC ₅₀	LC ₉₀	Time	References
	Lantana camara L.	Leaves	Methanol (sachet)	Mosquito species	Larvicidal (2,3 Instar)	40 ± 1.91 at 1	NM	NM	48 h	(Kandalkar et al., 2020)
	(Verbenaceae)	Leaves	Hydro-distillation	Anopheles subpictus Grassi Aedes aegypti L. Culex quinquefasciatus Say	Larvicidal (4 Instar)	mg/ml 94-96% at 100 ppm	45.32 ± 1.66 ppm	96.62 ± 1.99 ppm	24 h	(Sharma et al., 2021
		Leaves Stem Flower Synergy	Methanol (cold maceration)	Aedes aegypti L.	Larvicidal (4instar)	100% at 250, 500 and 1000 ppm	22.55 ppm 1695.51 ppm No activity >2000	96.44 ppm 9643.95 ppm No activity >2000	24 h	(Eze et al., 2022)
		Leaves	Methanol Aqueous (maceration)	Anopheles sp.	Larvicidal	100% at 100 mg/ml (Aq)	22.91 ppm 17.38 ppm	NM	6 h	(Ekemezie,2021)
		Leaves	Hexane Ethanol	Aedes aegypti L.	Larvicidal (4 instar)	100% at 1000 ppm		159.96 ppm	24 h	(Sharma et al.,2016)
		Stem	Hexane Ethanol				89.62 ppm -	125.89 ppm		
		Leaves	Chloroform	Aedes aegypti L. Culex quinquefasciatusSay.	Larvicidal (4 th or late 3 rd instar)		>300 ppm 55.92 ppm	>300 ppm 146.61 ppm	48 h	(Hari &Mathew,2018)
			Petroleum ether	Aedes aegypti L. Cu. Quinquefasciatus	,		74.93 ppm 10.63 ppm	149.4 ppm 92.98 ppm		
			Methanol (soxhlet)	Say			>300 ppm	>300 ppm		
				Aedes aegypti L. Culex Quinquefasciatus Say			-	-		
		Leaves	Essential oil loaded nanoemulsion	Aedes aegypti L.	Larvicidal I-instar II-instar III-instar iv- instar Pupicidal Adulticidal	-	18.18 ppm 23.34 ppm 29.73 ppm 38.94 ppm 45.30 ppm LD50: 11.95 mg/cm ²	46.96 ppm 56.48 ppm 71.67 ppm 82.02 ppm 85.99 ppm LD90: 47.72 mg/cm ²	-	(Udappusamy et al.,2022)
		NM	Acetone	Anopheles gambie Giles Sensitive strain local strain	Larvicidal Sensitive Local	100% in 160-320 ppm of hexane	32.62 ppm 106.09 ppm	180.29 ppm 91.19 ppm	24 h	(Wangrawa et al.,2016)
			Ethanol	loval stain	Sensitive Local	extract.	29.23 ppm 122.82 ppm	63.13 ppm 202.34 ppm		
			Hexane		Sensitive		15.94 ppm	42.65 ppm		
		Leaves	Methanol	Anopheles arabiensis Patton	Local Larvicidal (III &IV) Adulticidal (3-5day, Nonblood		20.19 ppm 89.48 ppm 0.42 % mg/l	49.29 ppm 178.02 ppm 0.81 % mg/l	24 h	(Wangrawa et al.,2023)

S.N.	Name of species/family	Part used	Solvent/ Formulation	Insect tested	Biological assay/stage	% mortality	LC ₅₀	LC ₉₀	Time	References
		Different parts	Acetone Hydrodistillation	Aedes aegypti L.	Larvicidal Adulticidal Larvicidal RS DS	0. 49	3.72 ppm 43 % mg/l 9 ppm 1.7 ppm	143.97 ppm 0.80 % mg/l	24 h	(Luz et al.,2022)
		NM	Aqueous extract	Pest of cabbage: Plutella xylostella L. Brevicoryne brassicae L.	Adulticidal	when compare		in untreated plot ted plot. All the n.	4 weeks	(Amoabeng et al.,2020)
				Hellula undalis Fab Araneae						
				Coccinellidae				2		
		Fresh leaves	Hydro-distillation	Rice weevil: Sitophilus oryzae L.	Adulticidal	-	9.81 mg/cm ²	41.20 mg/cm ²	72 h	(Tawfeek et al.,2021)
		Leaves	Hexane, Ethyl acetate,	Stored grain pest:	Adulticidal Fumigant	100% mortality was	440.05		24 h	(Rajashekar et al.,2014)
			Acetone Methanol (as all	Sitophilus oryzae (L.),	assay/ contact	observed in C. chinensis	128.07 ppm 0.16 mg/cm ²			
			are dissolved before application.)	Callosobruchus chinensis (Fab.),	toxicity assay	after 7 days.	130.32 ppm 0.14 mg/cm ²	-		
			apprication.)	Tribolium castaneum (Herbst.)			0.14 mg/cm			
				(178.7 ppm 0.21	-		
		Leaves	Essential oil	Pomacea canaliculata Lamark	Adulticidal (Mollusca)		mg/cm2 23.6–40.2 ppm	-		(Huy Hung et al.,2021)
				Gyraulus convexiusculus T. Hutton	"		7.9–29.6 ppm	-		
				Tarebia granifera Lamark	"		15.0–29.6 ppm		24 h	
				Aedes aegypti L.	Larvicidal		15.1–29.0	29.27-62.93		
				AedesalbopictusSkuse	"		ppm,	ppm		
				Culex quinquefasciatus	,		26.4–53.8 ppm	46.35-81.83 ppm		
		Leaves	Hydrodistillation	Tribolium castaneum Herbst	Adulticidal Fumigant/		20.8–59.3 ppm 16.70 ppm 8.93 mg/cm2	64.43-88.70 ppm 23.21 ppm 13.54 mg/cm2	24 h	(Aisha et al.,2024)
				Lasioderma serricorne Fabricius	toxicity		4.141 ppm 4.82	10.91 ppm 17.47 mg/cm2		
				Callosobruchus chinensis L.			mg/cm2	27.36 ppm		

S.N.	Name of species/family	Part used	Solvent/ Formulation	Insect tested	Biological assay/stage	% mortality	LC ₅₀	LC ₉₀	Time	References
	, .,	Leaves	Juice	Aedes aegypti L.	Larvicidal	-	6.25 ppm 6.24 mg/cm2 52.06 ppm	6.27 mg/cm2 104.33 ppm	24 h	(Sharma et al.,2023)
				Anopheles subpictus Grassi	(III &IV)		70.91 ppm	218.10 ppm		
				Culex quinquefasciatusSay			47.47 ppm	106.70 ppm		
		Leaves	Copper nanoparticles	Anopheles multicolor Combouli	Larvicidal (III instar)	100% at 20 ppm	12.5 ppm	18.4 ppm	24 h	(Abd El Hafiz Hassanain et al2019)
			Petroleum ether extract			140 ppm	63.5 ppm	119.9 ppm		
2.	Parthenium hysterophorus L. (Asteraceae)	Leaves	Methanol sachet	Mosquitospecies	Larvicidal (III&IV instar)	78 ± 1.70% at 1mg/ml	NM	NM	48 h	(Kandalkar et al.,2020)
		Leaves	Hexane, Acetone, Petrolium ether Ethanol	Anopheles arabiensis Patton	Larvicidal (IV instar)	Stem Petroleum ether: 90% at 360 ppm;	368 ppm 478 pmm 393.1 ppm	809.2 ppm 1358.2 ppm 1352.6 ppm	24 h	(Tarekegn et al.,2021)
		Stem				Hexane: 85- 97% at 480 ppm. (Higher)	112.4 ppm 259.4 ppm 701.1 ppm	331.9 ppm 1814.6 ppm 701.1 ppm		
		Root				<50% in ethanol	87.9 ppm 342.1 ppm 10.7 ppm	338.3 ppm 769.7 ppm 105.5 ppm		
		Leaves	Methanol	Aedes aegypti L.	Larvicidal (3 rd and 4 th instar)	$66 \pm 0.44\%$ at 300 ppm	-	-	72 h	(Jayaraman et al.,2023)
		Whole plant	Parthenin: Ethylene glycol Azide	Anopheles gambiae	Larvicidal (III instar)		154 ppm 37 ppm 66 ppm	313 ppm 698 ppm 282 ppm	72 h	(Milugo et al.,2021)
3.	Chromolena odorata (Spreng.) R.M.King & H.Rob	Leaves	Petrolium ether, Chloroform Ethanol (maceration)	Culex quinquefasciatus	Larvicidal (IV instar)	90% in 2.5 and 5 ppm petroleum ether.	0.16 ppm 0.24 ppm 0.64 ppm	9.19 ppm 52.30 ppm 38.75 ppm.	72 h	(Kumar et al.,2023)
	(Asteraceae)	Leaves	Methanol (maceration)	Anopheles stephensi Culex	Larvicidal (III and IV instar)	100% at 1000 ppm	1613 ppm	8306 ppm	24 h	(Sukhthankar et al.,2014)
				quinquefasciatus Aedes aegypti L.		220 ppm	43 ppm	110 ppm		
		Leaves	Methanol (maceration)	AnophelesGambiae	Larvicidal (III &IV) Pupicidal	900 ppm 100 % at 160 mg/l to larva and pupa but	138 ppm 31.33 ppm 53.61 ppm	463 ppm 95.73 ppm 117.66 ppm	24 h	(Ileke &Olabimi,2019)
		Leaves Stem Root	Aqueous	Culex pipiens	adulticidal Adulticidal	not adult Whole part most potent	296.20 ppm -	2221.05 ppm -	Spray tech.	(Sasan et al.,2021)

S.N.	Name of species/family	Part used	Solvent/ Formulation	Insect tested	Biological assay/stage	% mortality	LC ₅₀	LC ₉₀	Time	References
		Leaves	Fresh juice	Aedes Culex	Larvicidal (III instar)	100% at 4 mg/ml	-	-	72 h	(Rathy et al.,2015)
		Leaves	Ethanol (soxhlet)	Anopheles gambiae	Adulticidal	60% at 5 mg/ml	4.52 ppm	8.32 ppm	24 h	(Afolabi et al.,2018)
		Leaves	Methanol	Spodoptera litura Fabricius	Ovicidal	73.33 ± 0.57%	-	-	96 h	(Gorawade et al.,2022)
				(tobacco eating)	Antifeedant	82.45 ± 0.16%				, ,
					larvicidal	68.33 ± 0.05%				
			Acetone Water							
						Less mortality				
		Leaves	Aqueous n-butanol	Anopheles gambiae	Larvicidal (III and early IV)	-	3497.27 ppm 3117.97	9707 ppm 9283.08 ppm	24 h	(Imam et al.,2018)
		Leaves	Fresh pulverized	Periplanata	Adulticidal	36.63 % at 1	ppm -	-	48 h	(Udebuani et
		Leaves	juice Aqueous	Americana L. Culex	Larvicidal	ml 30.67 ± 0.33	396.8 ppm	716.8 ppm	24 h	al.,2015) (Elemike et al.,2017)
				quinquefasciatus	III/IV	26.67 ± 0.33 (At 250 ppm)	(III) 448.3 ppm	803.9 ppm		
			AgNO3 nanoparticles			$81.33 \pm 0.88 70.67 \pm 0.88$	(IV)	337.5 ppm (III) 438.7 ppm (IV)		
							95.9 ppm (III)			
							166.4 ppm (IV)			
4.	Spergula arvensis L.	Whole plant	Methanol	Annopheles. Culicifacies Giles	Larvicidal (IV instar)	100% at 40 μg/ml at	6.84 ppm	24.816 ppm	24 h	(Sogan et al.,2021)
	(Caryophyllaceae)		Hexane			Methanol	2265.007 ppm	5738.69 ppm		
			Dichloromethane (Soxhlet)				1951.79 ppm	7238.69 ppm		
5.	Argemone mexicana L. (Papaveraceae)	Leaves	Methanol (maceration)	Anopheles subpictus Grassi	Larvicidal (IV instar)	83.33 ± 0.37% at 400 ppm	224.45 ppm	420.34 ppm	24 h	(Maheshwari et al.,2023)
6.	Ageratum conyzoidesL.	Leaves	Ethanol (maceration)	Culex quinquefasciantus	Larvicidal		-	-	48 h	(Ojianwuna et al.,2021)
	(Asteraceae)	Leaves	Ethanol (maceration)	Aedes aegypti L.	Larvicidal (IV instar)	No effect	-	-	24 h	(Pintong et al.,2020)
		different colored flower	EO (hydrodistillation)		Adulticidal (LP)		LD ₅₀ =0.84%	LD ₉₀ =1.76%		
		Leaves	Petrolium ether (Soxhlet)	Aedes aegypti L.	Larvicidal (IV instar)	78 % 77%	1.92 ppm	2.99 ppm	24 h	(Ramasamy et al.,2021)
				Anopheles stephensi Culex		73% (150 ppm)	1.95 ppm	2.33 ppm		
				quinquefasciatus		(FF/				

S.N.	Name of species/family	Part used	Solvent/ Formulation	Insect tested	Biological assay/stage	% mortality	LC ₅₀		LC ₉₀	Time	References
	·	Aerial parts	Hydrodistillation EO	Sitophilus oryzae	Adulticidal Contact	73.6± 1.2%/	>10000 ppm	>100	00 ppm	12days	(Nenaah,2014)
				Rhyzopertha dominica Fabricius	toxicity/ fumigant	100%	>10000 ppm	1000	0 ppm		
				Tribolium castaneum Herbst	toxicity	60 20g/kg	>10000 ppm	>100 (LC ₉ :			
		Leaves	Steam distillation	Anopheles gambiae	Larvicidal (III)	100% at 0.30 mg/ml	190 ppm	420 p	,	48 h	(Adelaja et al.,2023)
					A 1 10' '1 1	3.3% at 0.05 mg/ml					
		NM	Aqueous extract	Pest of cabbage: Plutella xylostella L.	Adulticidal Adulticidal	when compare plants helped i	ss densities were observed in untreated plot en compared with the treated plot. All the nts helped in pest suppression.				(Amoabeng et al.,2020)
				Brevicoryne brassicae L.		4 weeks					
				Hellula undalis Fab							
				Araneae)							
				Coccinellidae							
7.	Bidens pilosa L. (Asteraceae)	Leaves	Ethanol (maceration)	Culex quinquefasciantus	Larvicidal	100% at 0.6 m	1 -		-	48 h	(Ojianwuna et al.,2021)
8.	Ageratina adenophora (Spreng.) R.M.King & H.Rob (Asteraceae)	Leaves	Methanol Chloroform Petroleum ether	Culex quinquefasciatus	Larvicidal (III instar)	55% 100% 100% At 500 ppm	335.	93 ppm 10 ppm 98 ppm	611.29 ppm 768.36 ppm 514.24 ppm	24 h	(Samuel et al.,2014)
	(1300	Leaves & Stems	Petroleum ether (Partitioning) Com:1 Com:2 Com:3	Psoroptes cuniculi Delafond	Adult (Acaricidal)	100% at 4-6 h % extract	at 50 0.35' 0.07 0.58	%	NM	4-6 h	(Nong et al.,2014)
9.	Mesosphaerum suaveolens (L.)Kuntze (Lamiaceae)	Leaves	Ethanol (soxhlet)	Anopheles gambiae	Larvicidal Pupicidal Adulticidal	100% at 0.8% 100% at 1% 100% at 0.8%	NM	70	NM	48Hrs "4hrs	(Obembe et al.,2024)
	(Eumaceae)	Leaves	Hydrodistillation	Anopheles sp.	Larvicidal (III&IV)	100% at 50 pp 44% dead at 51		ppm	13.83 ppm	6 h	(Duniya et al., 2022)
					Adulticidal	90 min.	6.20	ppm	28.28 ppm	1.5 h	
		Leaves	Ethanol Aqueous	Anopheles sp.	Larvicidal (I-IV)	75% at 0.1 mg 0.50% at 1 mg			- -	6 h	(Sani et al.,2021)
		Leaves	(cold maceration) Methanol	Culex quinquefasciatus/ Aedes aegypti L.	Larvicidal (III or IV)	-	>300 >300	ppm ppm	>300 ppm >300	12 h 48 h	(Hari &Mathew,2018)
			Petroleum ether	0/1				ppm ppm	ppm 78.11		

S.N.	Name of species/family	Part used	Solvent/ Formulation	Insect tested	Biological assay/stage	% mortality	LC ₅₀)	LC ₉₀	Time	References
	,		Chloroform (soxhlet)		v o	·		41.39 ppm >300 ppm	ppm 139.28 ppm		
		NM.			Landaldal	1000/ 220			81.68 ppm >300 ppm	241	(Westernand
		NM	Acetone	Anopheles Gambiae Sensitive/ local strain	Larvicidal ((III&IV)	100% at 320 ppm hexane and acetor extract		95.66 ppm 201.66 ppm	196.76 ppm 341 ppm	24 h	(Wangrawa et al.,2016)
			Ethanol					78.88 ppm 206.49 ppm	193.49		
			Hexane (maceration)					77.33 ppm 109.72 ppm	ppm 339.97 ppm		
									120.7 ppm 179.32 ppm		
		Leaves	EO (Clevenger)	Aedes aegypti L.	Larvicidal (III)	100% at 100 ppm	1	32.85 ppm	69.27 ppm	24 h.	(Moola et al.,2023)
		Leaves	Aqueous n-butanol	Anopheles gambiae	Larvicidal (III&IV)	-		2613.01 ppm 2167.92	8553.77 ppm 7307.60	24 h	(Imam et al.,2018)
		Leaves	EO Steam distillation	Anopheles gambiae	Larvicidal (III)	100% at 0.30 mg/	/ml	ppm 240 ppm	ppm 320 ppm	24 h	(Adelaja et al.,2023)
					Adulticidal	36.67% at 0.50 mg/ml		-	-		
		Leaves	Aqueous	Helicoverpa armigera Hubner	Larvicidal			30.25%	748.61%	72 h	(Bini et al.,2023)
		Leaves	EO nanoemulsion	Culex quinquefasciatus	Larvicidal (III)	100% at 250 ppm	1	102.41 ppm	168.03 ppm	24 h	(Peniche et al.,2022)
		Leaves	Acetone Methanol Hexane Aqueous	Culex quinquefasciatus	Larvicidal (IV)	59.58% 52.92% 90.42% 59.58%		>10000 ppm >10000 ppm	>10000 ppm >10000 ppm	72 h	(Aremu et al.,2022)
						At 250 mg/ml		>10000 ppm >10000 ppm	>10000 ppm >10000 ppm		
		Leaves	EO Steam distillation	Spodoptera frugiperda J.E. Smith	Larvicidal	$6.67 \pm 0.5\%$		-	-	72 h	(de Menezes et al.,2020)
		Leaves	Hexane, Methanol, Acetone, Isopropanol Di-methyl- sulphoxide (soxhlet)	Aedes albopictus	Larvicidal (III)	-		689.69 ppm 310.47 ppm 258.39 ppm 349.77 ppm 569.73 ppm	-	24 h	(Yadav et al.,2014)

S.N.	Name of species/family	Part used	Solvent/ Formulation	Insect tested	Biological assay/stage	% mortality	C ₅₀	LC ₉₀	Time	References
10.	Tithonia diversfolia (Hemsl) A.Gray (Asteraceae)	Leaves	Methanol Aqueous (maceration)	Annopheles sp.	Larvicidal	100% at 100 mg/m at 4 hrs.	>10000 ppm >10000 ppm	-	6 h	(Ekemezie,2021)
		Leaves	Butanol eluent Crude extract	Spodoptera frugiperda	Larvicidal (II and neonate) (Diet incorporation toxicity bioassay)	22.22% 13.33%	-	-	72 h	(Oluwamodupe et al.,2024)
		Leaves	Hexane 70% ethanol Dichloromethane	Leaf-cutter ant Atta cephalotes L.	Adulticidal	- - 70-90% at 1000 pp.	- n	-	14 days	(Pantoja-Pulido et al.,2020)
			Ethyl acetate Butanol Aqueous			-				
		Stem and leaves	Green manure mulch	Atta cephalotes	Adult	50% decline in ant activity.	-	-	12 weeks.	(Rodríguez et al.,2015)
11.	Pontederia Crassipes Mart. (Pontederiaceae)	Leaves	Hexane Benzene Ethyl acetate Methanol Aqueous (soxhlet)	Culex quinquefasciatus	Larvicidal (IV)		80.54 ppm 1371.78 ppm 645.33 ppm 135.70 ppm 983.18 ppm	1744.50	48 h	(Annie et al.,2015)
		Leaves	Ethanol (75%) Petroleum ether	Culex pipiens L.	Larvicidal (III)	100% at 3000 ppm 1000 ppm	-	-		(Hasaballah,2015)
		Root				2000 ppm 500 ppm				
		Whole plant	Acetone Ethanol	Aphis craccivora Koch	Adulticidal	11	64 ppm 140 ppm	-	24 h	(Abdelkhalek et al.,2022)
		Whole plant	Ethanol (95%)	Spodoptera frugiperda	Neonate larva	98 ± 1.38% surviva		-	4 days	(Fu et al.,2020)
12.	Senna occidentalis (L) Link. (Fabaceae)	Leaves Stem	Ethanol Hexane (maceration)	Aedes aegypti	Larvicidal (IV)	90-100%	117.451 ppm 149.698 ppm	-	24 h	(Sharma et al.,2016)
13.	Xanthium strumarium L. (Asteraceae)	Leaves and stem	Ethanol Hexane (maceration)	Aedes aegypti L.	Larvicidal (IV)		586.185 ppm 460.923 ppm	-	24 h	(Sharma et al.,2016)
14.	Mimosa pudica L. (Fabaceae)	Whole body	Fresh juice	Aedes	Larvicidal (III)	100% at 8 m <i>l</i>	-	-	48hrs	(Rathy et al.,2015)

S.N.	Name of species/family	Part used	Solvent/ Formulation	Insect tested	Biological assay/stage	% mortality	LC ₅₀	LC ₉₀	Time	References
15.	Ageratum houstonianum Mill.	Flower Leaves stem	Ethanol	Culex pipines	Larvicidal (III)	·	259.79 ppm 266.85 ppm 306.86 ppm	ppm 991.71	24 h	(Hadidy et al.,2022)
	(Asteraceae)							ppm 1082.13 ppm		
16.	Sphagneticola trilobata (L.) Pruski	Leaves	Hexane, Dichloromethane	Spodoptera litura Spodoptera exigua Plutella xylostella L.	Larvicidal (III) (contact	-	-	-	24 h	(Junhirun et al.,2018)
	(Asteraceae)		Ethyl acetate	Timena sylosiena E.	toxicity and anti-feedant activity)		0.88-4.2 μg/larvae (LD ₅₀)/ 0.27-2.34	-		
			Methanol (S0xhlet)				mg/ml (FI ₅₀)	-		
17.	Mimosa diplotricha Sauvalle (Asteraceae)	Leaves Root	Dried powder	Callosobruchus maculatus Fabricius	Adult	50% at 2 gm 52%	-	-	96 h	(Uyi et al.,2020)
18.	Pistia stratoides L. (Araceae)	Whole plant	95% Ethanol	Spodoptera frugiperda	Neonate larva	99 ± 1.38% surviv.	al No anti- feedant and contact toxicity.	-	4 days	(Fu et al.,2020)
		Leaves	Ethyl acetate (percolation) Nine fraction	Annopheles	Larvicidal	-	14.81 ppm (Fraction E)	-	24 h.	(Ma et al.,2019)

Note: NM: Not mentioned; -: Not studied; EO: essential oil ; FI50= median feeding deterrence; LC50= median lethal concentration; LC90= lethal concentration on which 90% mortality observed; LD50= median lethal dose

Species-wise number of studies

The species-wise number of studies revealed that Lantana camara was the most extensively studied IAPs, with 17 case studies. This was followed by Mesosphaerum sauveleons (13 studies), Chromolena odorata (10 studies), Ageratum conyzoides (6 studies), and Parthenium hysterophorus, Pontederia crassipes, and Tithonia diversifolia (each with 4 studies) (Figure 2). Other species had two or fewer than two case studies. The strong aroma and noxious nature of Lantana camara and Mesosphaerum sauveolens likely contribute to their higher number of studies. Out of 30 IAP species in Nepal, only 18 have been studied for their insecticidal potential and eleven species: Ipomoea carnea subsp. fistulosa (Mart. ex Choisy), Mikania micrantha Kunth., Alternanthera philoxeroides (Mart.) Griseb., Myriophyllum aquaticum (Vell.) Verdc., Amaranthus

spinosus L., Senna tora (L.) Roxb., Leersia hexandra Sw., Oxalis latifolia Kunth., Erigeron karvinskianus DC, Galinsoga quadriradiata Ruiz & Pav, Leucaena leucocephala (Lam.) de Wit and Spermacoce alata Aubl. were not investigated for insecticidal activity. This discrepancy may be due to researcher's preference for focusing on extensively studied medicinal plants, such as Azadirchta indica, for insecticidal properties, and prevalence of large number secondary metabolites and bioactive compounds (Kalisz et al., 2021) while common weeds are often neglected.

Parts of the IAPs used in research

Various plant parts such as leaves, stems, flowers, and roots were used singly or in combination to determine their insecticidal potential. Among these, leaves were the most frequently used, accounting

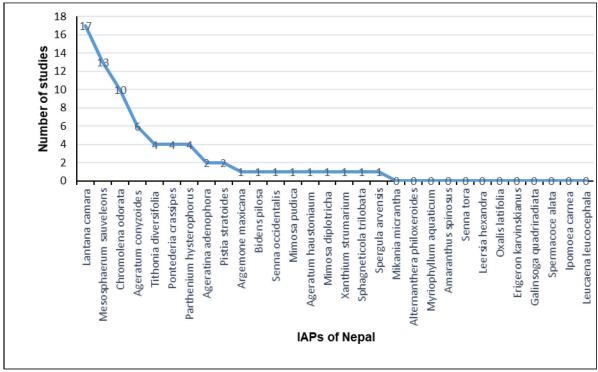


Figure 2: Number of case studies regarding insecticidal potential of different IAPs of Nepal

for more than 50% of the cases, followed by stems, roots, whole plants, and flowers (Figure 3). The frequent use of leaves can be attributed to their easy availability in different seasons and their simpler collection process compared to other plant parts. Additionally, the aromatic nature of leaves and presence of more secondary metabolites (Qaderi et al., 2023) may have attracted greater research interest. Among the reviewed cases, leaves were more frequently used part of plant with significant insecticidal effect but there were also evidences for

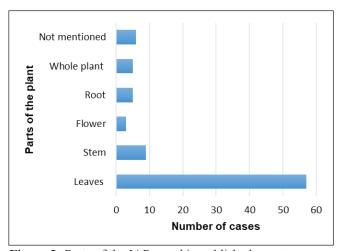


Figure 3: Parts of the IAPs used in published report

the higher insecticidal activity of stem, root and whole-body parts (Milugo et al., 2021). Thus, the review recommends the study of whole-body part of IAPs before their flowering to acquire the benefits for the management of weeds by control and utilization.

Targeted insects and their stages for the study

Among the reported data, 72% cases were studied against the mosquito species belonging to genus Annopheles, Culex and Aedes responsible for malaria, filarial disease, Japanese encephalitis, dengue, etc. whereas 28% were studied against agricultural pests and stored grain pests like Spodoptera frugiperda, Callosobruchus maculatus, Spodoptera litura, Spodoptera exigua, Plutella xylostella, Aphis craccivora, Atta cephalotes, Helicoverpa armigera, Pomacea canaliculata, Gyraulus convexiusculus, Tarebia granifera, Tribolium castaneum, Lasioderma serricorne, Callosobruchus chinensis, Sitophilus oryzae, Brevicoryne brassicae, Hellula undalis, Periplanata americana, Rhizopertha dominica, and Psoroptes lunicula (Figure 4). Regarding the stages of the targeted insects, larval stages (67%) were used in most of the cases rather than adults (28%). Some cases were also studied for pupae stage (Figure 5).

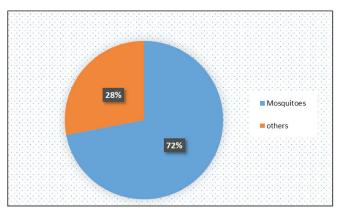


Figure 4: Targeted insects for the determination of insecticidal properties of IAPs of Nepal

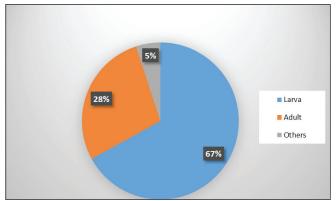


Figure 5: Stages of targeted insects for the determination of insecticidal potential of IAPs

Insecticide formulations

Various methods have been employed to formulate insecticides, including maceration, fresh juice extraction, soxhlet extraction, and hydro-distillation. These methods relied on a range of solvents from polar to non-polar. Methanol (31.66%) was the most commonly used solvent, followed by ethanol (30%). Essential oils obtained through hydro-distillation or steam distillation were also reported to be effective as insecticides. Other solvents and extracts frequently used include hexane and aqueous extracts. Petroleum ether extract was utilized against different insect larvae. Additionally, solvents such as chloroform, acetone, butanol, ethyl acetate, and dichloromethane, as well as fresh juice, and nanoparticles were used in some studies (Table 1).

The efficacy of EO from reviewed cases was found to be stronger with lower LC_{50} and LC_{90} values towards different stages of wide range of insects. This was followed by methanol extract. There

were also several evidences for the efficacy of extract formulation of other polar and non-polar solvents as well as the fresh juice. Generally, the evidences showed that petroleum ether extract was more effective to the larval stage than pupa and adult. Other evidences showed efficacy of hexane, chloroform, acetone and aqueous extract but less efficacy of ethanol extract. In order to make cost effective intervention of bio-insecticide, the use of low cost and less harmful solvent is suggested. For this purpose, review recommends the use of higher concentration of aqueous, methanol and petroleum ether extracts as insecticide but for the strongest activity EO can be appropriate. It was found that the effectiveness of the test sample depends on the concentration of the sample and post-exposure periods of sample treatment.

Efficacy of IAPs of Nepal for pest management

Based on the LC₅₀ (concentration on which 50% insects were dead) values and/or percentage mortality (Table1), each species was critically analyzed for their insecticidal potential and reported their level of insecticidal effect. Regarding the efficacy of Lantana camara, as bio-insecticide for different species of mosquito larva and adult, and agricultural pests, LC_{50} and LD_{50} value was found to be less than 100 ppm post exposure representing higher insecticidal activity. Particularly, EO or EO based nano-emulsion exhibited higher insecticidal potential with lower LC₅₀ value than that of the extract formulation with polar and non-polar solvents. Sharma et al. (2021) have reported the larvicidal activity of EO against Anopheles, Aedes and Culex mosquitoes as 94-96% mortality at 100 ppm with LC₅₀ (45.32±1.66 ppm) and LC₉₀ (96.62±1.99 ppm) at 24hrs. Udappusamy et al. (2022) reported LC₅₀ (18.18 ppm- 45.295 ppm) for different larval and pupal stages of Ades aegypti and LD50 (11.95 mg/cm²) for adult when EO loaded nano-emulsion was tested. The EO extracted from different parts of plants collected during rainy and dry season recorded LC₅₀ (45 ppm and 41.7 ppm) for larvalstage of Aedes aegypti (Luz et al., 2022). According to Aisha et al. (2024), EO on fumigant bioassay/contact toxicity showed LC₅₀ (16.70 ppm, 4.141ppm and 6.245ppm) and LD₅₀

(8.93 mg/cm², 4.82mg/cm², 6.24 mg/cm²) for the adults of agricultural pests *Tribolium castaneum*, Lasioderma serricarne and Callosobruchus chinensis, respectively. The effect of copper nanoparticles isolated from Lantana on III instar larva of *Anopheles multicolar* reported to have 100% mortality at 20ppm with LC₅₀ 12.5 ppm and LC₉₀ 18.4 ppm at 24hrs and compared to petroleum ether extract ($LC_{50} = 63.5 \text{ ppm}$ and $LC_{90} = 119.9 \text{ ppm}$), the effect was 5-6 fold higher (Abd El Hafiz Hassanain et al., 2019). Hexane extract was found to be more effective larvicide for Aedes with LC₅₀ (15.94 ppm and 20.19 ppm) than acetone extract (LC_{50} =32.62 ppm and 106.09 ppm) for sensitive and local strains, respectively. Similarly, petroleum ether extract from leaves of Lantana was reported to be more effective to larva of Aedes and Culex mosquito with the LC₅₀ value 74.93 ppm and 10.63 ppm compared to chloroform and methanol extracts (Hari & Mathew, 2018). Thus, Lantana camara could be the best alternative of synthetic insecticide for varied insects.

Regarding the efficacy of *Parthenium hysterophorus*, hexane and petroleum ether extract from root was more effective (LC₅₀=87.9 ppm and 10.7 ppm) compared to leaves and stem whereas ethanol extract of all parts showed inferior results against IV in star larva of *Anopheles arabiensis* (Tarekegn et al., 2021). In a study performed by Milugo et al. (2021), the compound parthenin and its two fractions viz ethylene glycol and azide were found to be highly effective against III instar larva of Anopheles gambiae with LC₅₀ value 154 ppm, 37 ppm and 66 ppm, respectively. They also reported that fraction of the compound parthenin had more insecticidal efficacy than the whole compound. These reported data demonstrate that, not all parts of the plants could have similar insecticidal activity but the level of effect may differ based on their solvent used for extraction, time of collection, methods of application and post exposure duration.

In the case of *Chromolena odorata*, leaves were more frequently studied for insecticidal efficacy than root and stem. In a study carried out in India by Kumar et al. (2023), petroleum ether, chloroform, and ethanol extracts of *Chromolena* against IV instar larva

of *Culex* showed strong efficacy with LC₅₀ value 0.16 ppm, 0.24 ppm and 0.643 ppm, respectively. However, in the study of Sukhthankar et al. (2014), methanol extract exhibited 100% mortality at 1000 ppm for Anopheles, 200ppm for Culex and 900 ppm for Aedes and the authors argued that methanol extract is highly effective against Culex larva than that of other species of mosquitoes. Ileke and Olabimi (2019) reported the efficacy of methanol extract against larva, pupa, and adult of Anopheles gambiae with LC₅₀ value 31.53 ppm, 53.61ppm and 296.20 ppm, respectively. The methanol extract from leaves of Chromolena when tested against tobacco eating Spodoptera litura, showed ovicidal (73±0.57%), anti-feedant (82.45±0.16%) and larvicidal (68.33±0.05%) activity at 96 hrs and less mortality was reported in acetone and aqueous extract (Gorawade et al., 2022). Along the wide cases reviewed, effects of test samples also depend upon the species of insects and their stages.

Methanol, hexane, and dichloromethane extracts of whole plant of *Spergula arvensis* were studied against IV in star larva of *Annopheles culicifiacies* for 24 hrs and reported 100% mortality at 40 μ g/ml of methanol extract revealing higher efficacy (LC₅₀= 6.84 ppm) than hexane (LC₅₀= 2265.01 ppm) and dichloromethane (LC₅₀= 1951.79 ppm) extracts (Sogan et al., 2021). Similarly, methanol extract from leaves of *Argemone maxicana* showed LC₅₀= 224.45 ppm for IV in star larva of *Anopheles subpictus* in a study by Maheshwari et al. (2023).

Regarding the insecticidal efficacy of *Ageratum conyzoides*, petroleum ether extracted from the leaves of *Ageratum* showed 78%, 77% and 73% mortality of larva of *Aedes, Anopheles* and *Culex* mosquitoes with 1.92 ppm, 1.95 ppm and 1.97 ppm (LC₅₀), respectively (Ramasamy et al.,2021) exhibiting the higher level of toxicity compared to other case studies. According to Adelaja et al. (2023), EO from leaves of this plant was effective for *Anopheles* larva (LC₅₀=190 ppm and LC₉₀= 420 ppm) but less effective for the adult. Study of aqueous extract of *Ageratum conyzoides* against pests of cabbage *Plutella xylostella*, *Brevicoryne brassicace*, *Hellula undalis* and Coccinellidae for

4 weeks reported that habitat manipulation with non-crop plants and spraying contributed to pest suppression along with improved cabbage head yield and quality (Amoabeng et al., 2020). From all of the reported studies, it can be hypothesized that the extract or EO, which is highly effective to one stage of insect, may also be ineffective to the other stage. Additionally, the effect may differ widely among different insects.

Though ethanol extract was reported to be less effective, leaves of *Bidens pilosa* showed 100% larval mortality of *Culex* mosquito at 0.6ml (Ojianwuna et al., 2021). Samuel et al. (2014) studied the potentiality of methanol, chloroform, and petroleum ether extracts from leaves of *Ageratina adenophora* on III instar larva of *Culex* and reported LC₅₀ as 509.13 ppm, 335.10 ppm and 186.98 ppm when observed for 24 hrs. Furthermore, Nong et al. (2014) reported 100% adult mortality of *Psoroptes cuniculi* when treated with 50% petroleum ether extract of leaves and stem for 4-6 hrs. Petroleum ether extract exhibited pronounced larvicidal activity compared to other extracts.

Mesosphaerum suaveolens has been reported to be highly effective against different insects attributed to its strong aroma. Obembe et al. (2024) reported 100% larvicidal, pupicidal and adulticidal effects for Anopheles gambiae on 0.8, 1 and 0.8% ethanol extracts. EO from M. suaveolens had been reported to be highly effective against III and IV instar larva and adult of *Anopheles* with LC_{50} of 3.579 ppm and 6.20 ppm, respectively (Duniya et al., 2022) but LC_{50} was 32.85 ppm for the larva of *Ades aegypti* (Moola et al., 2023) whereas Adelaja et al. (2023) reported LC₅₀ (240 ppm) for larva of *Anopheles gambiae*; and larval mortality of Spodoptera frugiperda was 6.67±0.5% (de Menezes et al., 2020). Peniche et al. (2022) studied the effect of nano-emulsion from M. suaveolenson III instar larva of Culex quinquefasciatus and reported 100% mortality at 250 ppm with LC_{50} (102.41 ppm) when observed for 24 hrs. From these cases, it can be concluded that EO or nano-emulsion can also have reduced effects than that of other extracts.

Though, *Tithonia diversifolia* was reported to have 100% mortality of different insects, the LC₅₀ value was greater than 1000 ppm which was far higher than other IAPs (Ekemezie,2021). A study by Pantoja-Pulido et al. (2020) on adulticidal effect of leaf cutter ant *Atta cephalotes* with hexane, 70% ethanol, dichloromethane, ethyl acetate butanol and aqueous extracts of *T. diversifolia* reported 70-90% mortality at 1000 ppm dichloromethane extract only within 14 days.

Pontederia crassipes was also reported to have significant insecticidal potential when tested against Culex larva and adult of Aphis craccivora. The larvae of Culex pipiens showed 100% mortality when treated with 75% ethanol and petroleum ether extract from leaves and stem of *Pontederia* whereas petroleum ether extract from root was comparatively more effective than leaves (Hasaballah, 2015). In contrast to all these, 95% ethanol extract from whole plant reported to have $98 \pm 1.38\%$ survival rate with no anti-feedant and contact toxicity on neonate larva of Spodoptera frugiperda (Fu et al.,2020). The effect of Senna occidentalis, Xanthium strumarium, Mimosa pudica, Ageratum haustonium, Sphagneticola trilobata, Mimosa diplotricha and Pistia stratoides was also significant to different insects with varied formulations.

This review investigated that the highly effective extract for larval stage can also be least effective to adult stage and vice-versa (Ileke & Olabimi, 2019; Udappusamy et al., 2022; Wangrawa et al., 2023). Also, the evidences presented that the highly effective extract against one type of insect can exhibit moderate to least effects on other types. Furthermore, the lethal dose for targeted insect can also have toxicity to non-target organisms as investigated in the review (Sogan et al., 2021). Hence, the formulation of the insecticide should be insect and stage specific and non-lethal to non-target organisms.

The availability of IAPs in Nepal posed a serious challenge in biodiversity and requires control in their growth and expansion. The potential as an insecticide would promote their use and provide a local/natural solution for insect/pest control.

Different studies were evaluated for this purpose. This review found that most of the IAPs of Nepal could have potentiality in the production of bioinsecticides against wide range of agricultural pests as well as the vector of several human diseases. Based on the above mentioned species-specific evidences representing lower LC₅₀ value but higher mortality percentage, it can be concluded that Lantana camara, Mesosphaerum suaveolens, Chromolena odorata, Ageratina adenophora, Ageratum conyzoides, Parthenium hysterophorus, Bidens pilosa, Pontederia crassipes and Tithonia diversifolia could be the potential source of bioactive compound with insecticidal activities. But Senna occidentalis, Xanthium strumarium, Mimosa pudica, Ageratum haustonium, Sphagneticola trilobata, Mimosa diplotricha and Pistia stratoides need further exploration for insecticidal activity. Though IAPs have negative impact on biodiversity and agriculture resulting in low productivity, this review recommends their utilization for the resource poor farmer to produce cheap, assessable, eco-friendly bio-insecticide instead of using hazardous synthetic insecticides.

Toxicity on non-target organism

Only a few reported studies have investigated the toxicity of plant extracts and EOs on non-target organism. Sogan et al. (2021) found that the methanol extract of Spergula arvensis was non-toxic to Poecilia reticulata Peters and Diplonychus indicus (Fabricius) at concentrations lethal to mosquito larva. Similarly, the petroleum ether extract of Ageratum conyzoides was non-toxic to the aquatic predator *Toxorhynchites splendens* (Wiedemann) at 1000 ppm, whereas the commercial pesticide temephos was toxic at 1-2 ppm (Ramasamy et al., 2021). Screening plant extracts from various species, including Lantana camara and M. suaveolens, against mosquito fish Gambusia affinins (Baird & Girard) revealed no mortality at 50 and 60 mg/L. A combination of petroleum ether extract of Lantana camara, Tecoma stans (L.) Juss.ex Kunth, M. suaveolens, and methanol extract of Nerium oleander L. in a 1:1:1:1 ratio showed a dose-dependent response above 60 mg/L after 48

hours(Hari &Mathew,2018). Abdelkhalek et al. (2022) reported no deleterious effects or toxicity in albino rats exposed to extracts from *Pontederia crassipes*. However, Luz et al. (2022) advised against using essential oils from all studied plants, including *Lantana camara*, as they were toxic to the non-target organisms *Danio rerio* (Hamilton) and *Artemia salina* (Linnaeus), with high mortality rates even at concentrations below 100μg/mL within few hrs.

Limitations

During the investigation of IAPs of Nepal for their potentiality as insecticides, there were no any published reports from Nepal till date, though concern and studies about management of IAPs is increasing. The IAPs of Nepal which are or aren't invasive to other countries were reported to have been investigated for their insecticidal activity in other countries. The effectiveness of these plants may vary among the geographical range and to the varied insects because of the variation of secondary metabolites on them (Qaderi et al., 2023). The published cases in this review reported to have several limitations and research gaps. Though the preliminary studies had been done to find out the insecticidal potential, most of the published reports lack the investigation regarding bioassay guided fractionation. Some of the studies had presented the chemical data using GC-MS analysis (Pintong et al., 2020; Ramasamy et al., 2021), however, only few studies reported bioactive compound and the insecticidal potential of pure compound and fraction of compounds(Hari & Mathew, 2018; Nong et al., 2014). Similarly, the mode of action of test samples on target organisms was also not investigated in many cases except few studies that have performed acetylcholinesterase (AChE) inhibitor activity of compound (Pantoja-Pulido et al., 2020). The effects of extract and other formulation on non-target organisms was investigated using Albino mice, Gambusia affinins, Danio rerio (zebrafish), Artemia salina, Poecilia reticulata and Diplonychus indicus in only five published reports but all other studies lack the test of non-target organisms. All of the studied cases were performed under the laboratory

conditions with either wild strain of insects or sensitive strain grown in laboratory but the efficacy under field condition was not reported. While reporting the mortality and lethal concentration, post exposure period was found to be varied ranging between 6 hours to 12 weeks though many cases conducted observation for 24 hours post exposure and the initial and residual activity of extract on target organism was rarely mentioned. The assessment of cross resistance with used bio-insecticides was also not investigated. Similarly, there is gap concerning compatibility with other pest management system; chemical profiling and bioactive compound; and challenges of using IAPs as insecticides. The studies investigated the effect of extract and negative control with solvent used for extract preparation but positive control is lacking. Future studies should assess both positive and negative controls along with the test samples so that the efficacy of the bio-insecticide can be compared with the synthetic insecticide, test conditions can be validated, resistance of insect can be detected and research can be reproducible (Vom Saal & Welshons, 2006). Furthermore, future studies regarding the sustainable management of IAPs should focus towards their utilization for peoples' livelihood and insect eradication with their active participation.

Conclusion

Among the IAPs of Nepal, 18 have been studied for their insecticidal activity and future study can be focus on all 30 IAPs of Nepal. The formulation of test sample and their effectiveness varied across different species, plant parts, and times of collection. Additionally, the insecticidal effect was found to be concentration and time-dependent. EOs and the fraction of extracts were generally more effective than simple extracts. Our study concluded that utilization of IAPs for pest management could be effective against rice weevils, cabbage beetles, stored grain pests, fall armyworms, and varieties of mosquitoes. Future research should focus on identification of bioactive compounds from those IAPs having insecticidal activity. Also the lethal and sub lethal concentration/dose of the extract.

fractions and compounds on non-target organism should be done to address the research gap. Research focusing on the comparison of positive and negative control with bio-pesticides can validate the efficacy. Mode of action of the bio-pesticide should be focused. Regarding the environmental safety, the use of non-polar and toxic solvents for the extraction and pesticide formulation should be discouraged but the use of cost-effective, less harmful, polar solvents like methanol, ethanol and water should be encouraged. As the previous researches were more targeted towards larval stage of insects, future prospect could be the study on adulticidal effect of bio-pesticide which will provide immediate solution to problematic insects. In-depth study of IAPs, their chemical profiling, biological activity, bio-active compounds can open up the dual solution of pest management and IAPs management by their utilization.

Author Contributions

T K Chhetri developed the concept and prepared original manuscript, R K Thapa critically analyzed and co-supervised the manuscript and H D Bhattarai reviewed, analyzed and supervised the manuscript preparation.

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