Insecticidal potential of seeds of *Datura metel* L., *Abrus precatorius* L., and *Diploknema butyracea* (Roxb.) H.J. Lam

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The present study aimed to assess the insecticidal efficacy of methanol extracts of the seeds of three plants: *Datura metel* L., *Abrus precatorius* L., and *Diploknema butyracea* (Roxb.) H.J. Lam. The bioactive compounds in crude extracts were analyzed using Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography-Mass Spectrometry (GC-MS). Insecticidal activities of different concentrations of extracts were evaluated against adults and the second instar larvae of *Drosophila melanogaster* by using ingestion and spraying methods. Among the three seeds, the extract of *Datura metel* L. at a concentration of 30 mg/mL consistently exhibited a higher mortality rate of 73.33±7.64% against adult insects while applying each method. However, all concentrations of the seed extracts exhibited lower mortality rates in larvae compared to adults of *D. melanogaster.* The qualitative phytochemical analysis of the seed extracts revealed the presence of detected alkaloids, phenols, flavonoids, carbohydrates, saponins, and tannins. FTIR analysis of the seeds revealed the presence of alcohols or phenols, alkanes, esters or ethers, amines, ketones, and nitro-compounds. GC-MS analysis identified a wide array of bioactive compounds. *Abrus precatorius* L. contained 1,2,3-benzenetriol, and 9-octadecenoic acid, methyl ester (E)-. Atropine, and scopolamine were abundantly detected in *Datura metel* L. Similarly, stigmasterol, 2,4,6-triaminoquinazoline, and brucine were identified in *Diploknema butyracea* (Roxb.) H.J. Lam. Since these GC-MS identified compounds are known for insecticidal properties, the seeds of all three plants particularly *Datura metel* L., can be a potential candidate for development as biopesticide against insect pests. Although field applications require further verification, our findings provide laboratory-based evidence supporting the potential of Nepalese indigenous plant-based biopesticides for reducing crop loss and enhancing food security.

Key words: GC-MS; Seed extracts; Insecticidal compounds; Pesticides; Pests.

Crop loss due to insect infestations poses a significant threat to global food security, with annual losses estimated between 20-40% (Karar et al., 2021). In the Kathmandu Valley, insect pests account for 12.5% of crop loss, with major insect pests including whiteflies, leaf miners, cyclamen mites, and caterpillars (Chudali et al., 2020). These pests adversely impact both the production and productivity of agriculture. In Nepal, the prevalence of disease severity and occurrence of new plant diseases is high. Farmers often apply insecticides indiscriminately to control

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pests, leading to environmental degradation and risks to human health (Pandey et al., 2019). Based on a report of the Government of Nepal, a total of 370208.39 kg of insecticides were imported in the fiscal year 2021/2022 and the annual consumption of insecticides is substantially higher than that of bio-pesticides, with insecticides accounting for 32.3% of the pesticide market share, compared to a mere 0.015% for bio-pesticides (PQPMC, 2022).

The negative impact of chemical insecticides has led to the exploration of alternative methods to enhance agricultural productivity. Biocontrol agents are an alternative and effective approach to managing plant pathogens and pests. These agents not only help preserve and promote human, plant and animals' health but also balance ecosystems. Various types of biocontrol agents including microorganisms such as bacteria, viruses, fungi, protozoa, nematodes, plants, and animals play an important role in improving plant health (Al-Ani et al., 2020).

Approximately 6,000 plant species have been reported as sources of biopesticides and are widely used by farmers (Jindal et al., 2013). These plants contain phytochemical compounds like flavonoids, tannins, alkaloids, phenolic acids, and saponins, that exhibit antimicrobial, insecticidal, antiviral, antioxidant, anti-inflammatory, and therapeutic properties (Zhou et al., 2023). Botanical pesticides are extracted from various plant parts including, leaves, stems, seeds, roots, bulbs, rhizomes, unripe fruits, and flower heads (Mamun & Ahmed, 2011). Botanicals with strong insecticidal properties like Neem (*Azadirachta indica*), Datura (*Datura metel*), Eucalyptus (*Eucalyptus globulus*), Ghora-neem (*Melia sempervirens*), Marigold (*Tagetes erecta*), Hijal (*Barringtonia acutangula*), Karanja (*Pongamia pinnata*), Tobacco (*Nicotiana tabacum*), Lantana (*Lantana camara*), Mahogoni (*Swietenia mahagoni*) (Mamun et al., 2015) can be easily cultivated by farmers at low cost.

Azadirachtin, a neem-based insecticide, has been widely used due to its wide range of biological activities such as repellency, antifeedancy, toxicity, and effect on growth, development, and reproduction (Kilani-Morakchi et al., 2017). *Daphne mucronata, Tagetes minuta, Calotropis procera, Boenninghausenia albiflora, Eucalyptus sideroxylon, Cinnamomum camphora,* and *Isodon rugosus* showed insecticidal activity against *D. melanogaster* (Diptera), pea aphids *Acyrthosiphon pisum* (Hemiptera), red flour beetles of *Tribolium castaneum* (Coleoptera), and armyworms of *Spodoptera exigua* (Lepidoptera) (Khan et al., 2017). Different types of plant extract preparations, such as powders, solvent extracts, essential oils, and whole plant application, have been found to possess pesticidal properties, acting as significant oviposition differences, repellents, fumigants, growth inhibitors, antifeedants, or toxic agents (Stankovic et al., 2020; Ngegba et al., 2022). Therefore, plant-derived materials could be an alternative to chemical pesticides that help to control major pests. Traditional uses of indigenous pesticidal plants offer an environmentally safe, less hazardous, and cost-effective approach to crop protection, making them an important component of an integrated pest management system (Giri et al., 2014).

The selection of indigenous plants is preferred based on their availability throughout crop-growing areas, and the users can use these plants in pest management, especially for pest control. Since these plants have toxic properties, they can be used as alternative insecticides against chemical insecticides. Several indications for the insecticidal properties of widely distributed plants like *Datura metel* L., *Abrus precatorius* L., and *Diploknema butyracea* (Roxb.) H.J. Lam are notable because of various bioactive compounds, including alkaloids, flavonoids, saponins, and terpenoids (Mamun & Ahmed, 2011; Qian et al., 2022; Uprety & Asselin, 2023). *A. precatorius* L. is a toxic plant but has pesticidal properties against a broad range of arthropods with inhibitory effects on pests like fungal plant pathogens, parasitic protozoans, and mollusks (Prasad et al., 2015). *D. metel* L. is a medicinal plant traditionally used as an insecticide to control pests like the tea mosquito bug, thrips, jassids, and aphids (Mamun & Ahmed, 2011). *D. butyracea* (Roxb.) H.J. Lam is a plant rich in tritepenic saponins and functions as an antifungal, nematicidal, pesticide, fish poison, and leech repellent (Saha et al., 2010; Upreti & Asselin, 2023; Maharjan et al., 2024; Gupta et al., 2025). It is noteworthy to delineate an array of insecticidal compounds present in the seeds of these plants.

Drosophila melanogaster, commonly known as the fruit fly, causes significant agricultural damage to vegetables and fruits, resulting in great economic loss. It is also a widely used model organism in insecticidal bioassays due to its short life cycle, rapid reproduction, and ease of handling and cultivation. If *D. melanogaster* is not controlled in time, it may cause a significant loss in fruit production and storage (Wohlenberg et al., 2009; Khan et al., 2017). Fruit flies are responsible for approximately

Figure 1: Seeds of (a) *Abrus precatorius* L., (b) *Datura metel* L., and (c) *Diploknema butyracea* (Roxb.) H.J. Lam

10-30% economic loss in cucumber production (Papadopoulos et al., 2024). The larvae, in particular cause internal damage to the fruits (Sapkota et al., 2010). In addition to direct crop damage, D. *melanogaster* acts as a vector for pathogens, transmitting diseases from one organism to another. Recent studies have demonstrated the toxic effects of *Drimia maritima* on *D. melanogaster* (Saadane et al., 2021). Moreover, microbial insecticides, weed extracts, and plant extracts have been reported as effective alternatives to synthetic insecticides for the control of *D. melanogaster* (Riaz et al., 2018; Akhtar et al., 2019).

There is an urgent need to address crop losses caused by pests and to systematically investigate botanical biopesticides for sustainable pest management. Plants are easily available, grow easily, and their extracts can be used to promote the development and application of biopesticides, which are generally safer for the environment. By exploring and utilizing these natural resources, this research could significantly contribute to developing more sustainable and environmentally friendly agricultural practices. The primary objective of this study was to evaluate the insecticidal efficacy of selected plant seed extracts against the pest *D. melanogaster*, and to identify potential insecticidal compounds through Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

Materials and methods

Chemicals and reagents

Methanol was procured from Sigma-Aldrich (USA). Spinosad; a natural chemical insecticide (Tracer Spinosad 45% SC, India), Azagro 300; a commercial biopesticide (Neem Oil-based EC containing Azadirachtin 0.03% w/w min 300 mg/L, India), and Astha; A commercial biopesticide (*Bacillus thuringiensis* 1.0% w/w, India) were used as positive control. All the chemicals/reagents used were of analytical grade.

Plant seeds and crude extraction

The collection, identification, and crude extraction of the seeds of three plant species, *viz A. precatorius* L., *D. metel* L., and *D. butyracea* (Roxb.) H.J. Lam (Figure 1) have been done following the methodology described in Maharjan et al. (2024). Briefly, seeds purchased from the local market were authenticated by a botanist. Clean, powdered seeds were subjected to methanolic extraction using a rotary evaporator.

The percentage yield of the crude extracts was calculated using the following formula (Ansari et al., 2021):

$$\text{Percent yield (\%)} = \frac{Final\ weight\ of\ extract}{Initial\ weight\ of\ raw\ material} \times 100$$

Evaluation of insecticidal activities of seed extracts

Insect rearing

Wild-type *D. melanogaster* was collected from banana peels (Ali et al., 2019). *D. melanogaster* was identified based on morphological characteristics with the help of an entomologist at the Entomology Division of the Nepal Agricultural Research Council (NARC). The adult fruit flies were reared on an artificial drosophila medium as described by Schlesener et al. (2018), at 25°C and 65% relative humidity under a photoperiod of 16 hours of light: 8 hours dark (Khan et al., 2017). Adult flies and second instar larvae were used for the bioassays.

Bioassay using adult *Drosophila*

Different concentrations (10 mg/mL, 20 mg/mL, and 30 mg/mL) of methanol extracts from the selected

seeds were prepared using dimethyl sulfoxide (DMSO) as a solvent. Three negative controls were used in the bioassays: methanol and DMSO were used as negative controls to check their toxicity against the target pest, while distilled water served as a blank control. In addition, three commercial insecticides were used as positive controls: Spinosad (a natural insecticide), *Bacillus thuringiensis* (a bacterial biopesticide), and Neem (a plant-based biopesticide). Adult bioassays were conducted using two methods: (i) ingestion through diet and (ii) spray on diet following the procedure described by Rima et al. (2021). Each treatment was tested in triplicate. Mortality of *D. melanogaster* adults was recorded at 24, 48, and 72 hours post-exposure (Khan et al., 2017). Mortality was calculated by using the following formula:

$$\text{Percentage mortality (\%)} = \frac{number\ of\ dead\ adult}{number\ of\ adult} \times 100$$

Bioassay using larval *Drosophila*

Toxicity assays were conducted on second-instar larvae of *D. melanogaster* following previously described methods (Quiroz-Carreno et al., 2020, Rima et al., 2021) with minor modifications. Crude plant extracts at concentrations of 10 mg/mL, 20 mg/mL, and 30 mg/mL were incorporated into the *Drosophila* medium. Twenty second-instar larvae were introduced into each treatment group. The negative control consisted of a diet supplemented with distilled water and methanol (solvent). Larval mortality was monitored at 24-hour intervals and continued until adult emergence. The larvae, unable to move, were considered dead. Each concentration was tested in triplicate. Larvicidal activity was assessed using two treatment methods: (i) ingestion via diet, and (ii) spray onto the diet. Larval mortality was calculated by using the following formula:

$$\text{Percentage mortality (\%)} = \frac{number\ of\ dead\ larvae}{number\ of\ larvae} \times 100$$

Analysis of seed extracts

Phytochemical screening

Preliminary phytochemical analysis of the methanol extracts of the seeds was conducted to detect the presence of various bioactive compounds using standard qualitative methods as described by Kumar and Nirmalababurao (2016). The following tests were performed: alkaloids (Hagner's test), phenols (5% ferric chloride test), flavonoids (5% sodium hydroxide test), saponins (foam test), tannins (10% sodium hydroxide test), and carbohydrates (Fehling's test).

Fourier transform infrared (FTIR) analysis

The methanolic seed extracts were analyzed using Attenuated Total Reflectance (ATR) mode on an IRAffnity-1S FTIR Spectrophotometer (Shimadzu, Japan) using transmittance mode with 40 scan and 4 cm^{-1} resolution in between 400 cm^{-1} and 4000 cm^{-1} spectral range (Chieme et al., 2022).

Gas chromatography- mass spectrometry (GC-MS) analysis

The seed extracts were prepared for GC-MS analysis following the method described by Kumari et al. (2020). Phytochemicals in the seed extracts were analyzed using a GC-MS analyzer (Agilent). The crude extracts were dissolved in methanol (GC grade) and filtered through a Whatman™ filter (0.2 µm pore size). Helium (99.99%) was used as the carrier gas, maintained at a ûow rate of 1 mL/min in the split mode (50:1). Crossbond® HP-5MS capillary columns (5% diphenyl / 95% dimethyl polysiloxane) with dimensions of 30 m length, 0.25 µm df, and 0.25 mm ID column were used to separate the fractions of compounds. A 1 µL sample was injected into the column and the injector temperature was set at 280 ºC. The start temperature of the oven 70 ºC was held for 2 min and then increased at a rate of 7 ºC per minute until it reached 310 ºC, where it was held for 1 minute. The ion source temperature was set at 250 ºC. The mass spectrum was obtained by electron ionization at 70 eV, and the detector was operated in scan mode of 30-500 Da atomic units. The total running time was 37.286 minutes, including a 3-minute solvent delay.

Identification of individual compounds was based on the comparison of mass spectra with the NIST/EPA/NIH Mass Spectral Library of the National Institute of Standards and Technology (NIST 11).

Data analysis

All experimental data were expressed as the mean ± standard deviation (SD) of three independent replicates.

Results

The seeds of the three plant species viz *Abrus precatorius* L., *Datura metel* L., and *Diploknema butyracea* (Roxb.) H.J. Lam were crushed in

powder form and subjected to methanol extraction using a Soxhlet apparatus. The total crude extracts yield obtained was 12.16% for *Abrus precatorius* L., 12.07% for *Datura metel* L., and 30.08% for *Diploknema butyracea* (Roxb.) H.J. Lam.

Adult insect mortality

Different concentrations of seed extracts with the normal diet were applied against adult and larvae of *D. melanogaster* using ingestion and spray methods. The average mortality rate indicated that Spinosad, used as a positive control, showed 100% mortality against adult fruit flies, while Neem and *Bacillus thuringiensis* were comparatively less effective. Of the three plant extracts tested, *D. metel* L. at a concentration of 30 mg/mL demonstrated consistently higher insecticidal activity, with a mortality rate of 73.33±7.64% when applied by both the ingestion and spray methods. However, *A. precatorius* L. at the same concentration displayed a mortality rate of 51.67±10.41% via ingestion and 80±10.00% via the spray method against adult *D. melanogaster* (Table 1).

Larval mortality rate

In general, the insecticidal activity of seed extracts was higher against adults compared to larvae of the targeted pest as presented in Table 1. Methanol extracts of seeds of *D. metel* L. showed the highest mortality at a concentration of 30 mg/mL with 43.33±0.58% via ingestion and 30±10.00% via spraying. At the same concentration, *Abrus precatorius* L. showed larval mortality rates of 38.33±5.77% and 26.67±7.64% through ingestion and spraying, respectively (Table 1). Preparations with lower concentrations of plant seed extracts were comparatively ineffective.

Presence of phytochemicals in plant seeds

Phytochemical screening of methanol extracts of seeds of the three plant species is shown in Table 2. The analysis revealed the presence of alkaloids, phenols, flavonoids, tannins, and carbohydrates in *A. precatorius* L. In contrast, both *D. butyracea* (Roxb.) H.J. Lam and *D. metel* L. were found to contain alkaloids and saponins only.

Fourier transform infrared (FTIR) spectral analysis of extracts

Fourier Transform Infrared (FTIR) spectroscopy analysis revealed distinct functional groups in the methanol seed extracts of the three plant species (Table 3; Figure 2).

A. precatorius L. showed stretching frequencies (ν) at 3278 cm^{-1} (O-H stretching alcohols or phenols), 2925 cm^{-1} (C-H stretching alkane), 1602 cm^{-1} (C=O

Table 1: Insecticidal activity of methanol extracts of seeds with different concentrations against *D. melanogaster*

Treatment	Ingestion in adult	Spray in adult	Ingestion in larvae	Spray in larvae
	Mean Mortality ± SD (%)	Mean Mortality ± SD (%)	Mean Mortality ± SD (%)	Mean Mortality ± SD (%)
Methanol	21.67±7.64	20.00±5.00	0.00	0.00
Spinosad	100.00	100.00	91.67±7.64	100.00
Neem	51.67±12.58	75.00±13.23	51.67±12.58	51.67±12.58
Bacillus thuringiensis	48.33±15.28	43.33±15.28	65.00±13.23	65.00±13.23
5% DMSO	3.33±2.89	5.00	0.00	0.00
A. precatorius L. 10 mg/mL	30.33±12.58	33.33±5.77	5.00	5.00
A. precatorius L. 20 mg/mL	33.33±12.58	50.00±5.00	13.33±7.64	10.00
A. precatorius L. 30 mg/mL	51.67±10.41	80.00±10.00	38.33±5.77	26.67±7.64
D. metel L. 10 mg/mL	23.33±12.58	38.00±2.89	5.00	5.00
D. metel L. 20 mg/mL	53.33±2.89	50.00±10.00	20.00±5.00	16.67±7.64
D. metel L. 30 mg/mL	73.33±7.64	73.33±7.64	43.33±2.89	30.00±10.00
D. butyracea (Roxb.) H.J. Lam 10 mg/mL	38.33±2.89	38.88±2.89	0.00	0.00
D. butyracea (Roxb.) H.J. Lam 20 mg/mL	40.00±18.03	53.33±2.89	8.33±2.89	13.33±7.64
D. butyracea (Roxb.) H.J. Lam 30 mg/mL	48.33±2.89	71.67±18.93	15.00±5.00	25.00±5.00

Table 2: Phytochemical screening of methanol extracts of seeds of the three plants

SN	Phytochemical compounds	Method of testing	*A. precatorius* L.	*D. metel* L.	*D. butyraceae* (Roxb.) H.J. Lam
1	Alkaloids	Hagner test	+	+	+
2	Phenols	(5%) $FeCl_3$ test	+	-	-
3	Flavonoids	(5%) NaOH test	+	-	-
4	Saponins	Foam test	-	+	+
5	Tannins	(10%) NaOH test	+	-	-
6	Carbohydrates	Fehling's test	+	-	-

+Present, -Absent

Table 3: FTIR analysis of methanol extracts of seeds of the three plants

SN	Frequency range (cm^{-1})	Chemical bond	Functional groups (Phyto-constituents)	The peaks shown by		
				Abrus precatorius L.	*Datura metel*. L.	*Diploknema butyracea* (Roxb.) H.J. Lam
1	3500-3200	O-H stretching	Alcohols or phenol	3278	3366	3334
2	3000-2850	C-H stretching	Alkane	2925	2921	2939
3	1750-1600	C=O stretching	Aldehyde, Ketone, Ester, or Ether	1602	1708	-
4	1650-1550	N-H bending	Secondary amine	-	-	1648
5	1360-1290	N-O stretching	Nitro compound	1351	-	-
6	1250-1020	C-N stretching	Aliphatic amine	1216	-	-
7	1320-1000	C-O stretching C-N stretching	Ester or Ether Aliphatic amine	1025	1017	1017

stretching aldehyde, ketone, ester, or ether), 1351 cm^{-1} (N-O stretching nitro compound), 1216 cm^{-1} (C-H stretching aliphatic amine), and 1025 cm^{-1} (C-O stretching ester or ether). The FTIR spectrum of *D. metel* L. showed stretching frequencies (ν) at 3366 cm^{-1} (O-H stretching alcohols or phenols), 2921 cm^{-1} (C-H stretching alkane), 1708 cm^{-1} (C=O stretching aldehydes, ketones, esters or ethers), and 1017 cm^{-1} (C-O stretching esters or ethers). Similarly, *D. butyracea* (Roxb.) H.J. Lam showed stretching frequencies (ν) at 3334 cm^{-1} (O-H stretching alcohols or phenols), 2939 cm^{-1} (C-H stretching alkane), 1648 cm^{-1} (N-H bending secondary amine), and 1017 cm^{-1} (C-O stretching ester or ether).

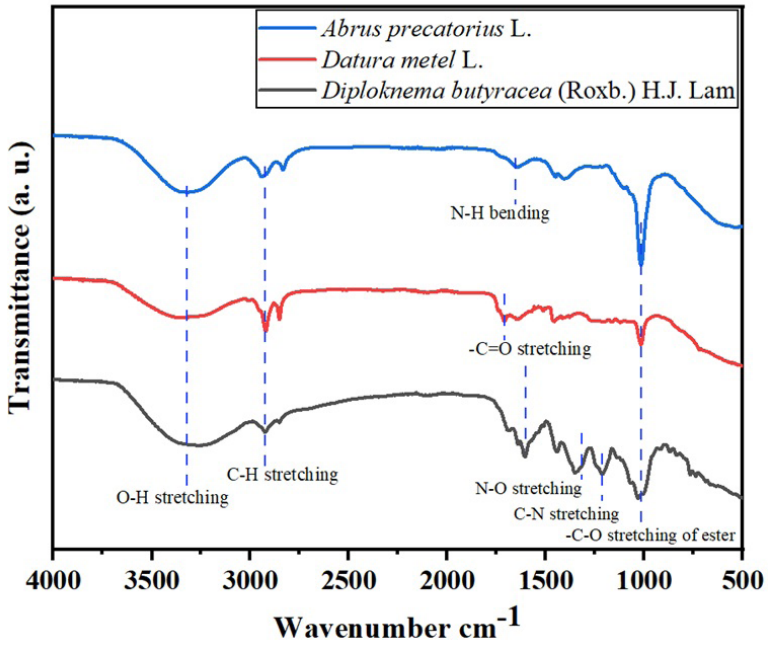

Figure 2: FTIR spectra of methanol extracts of the seeds

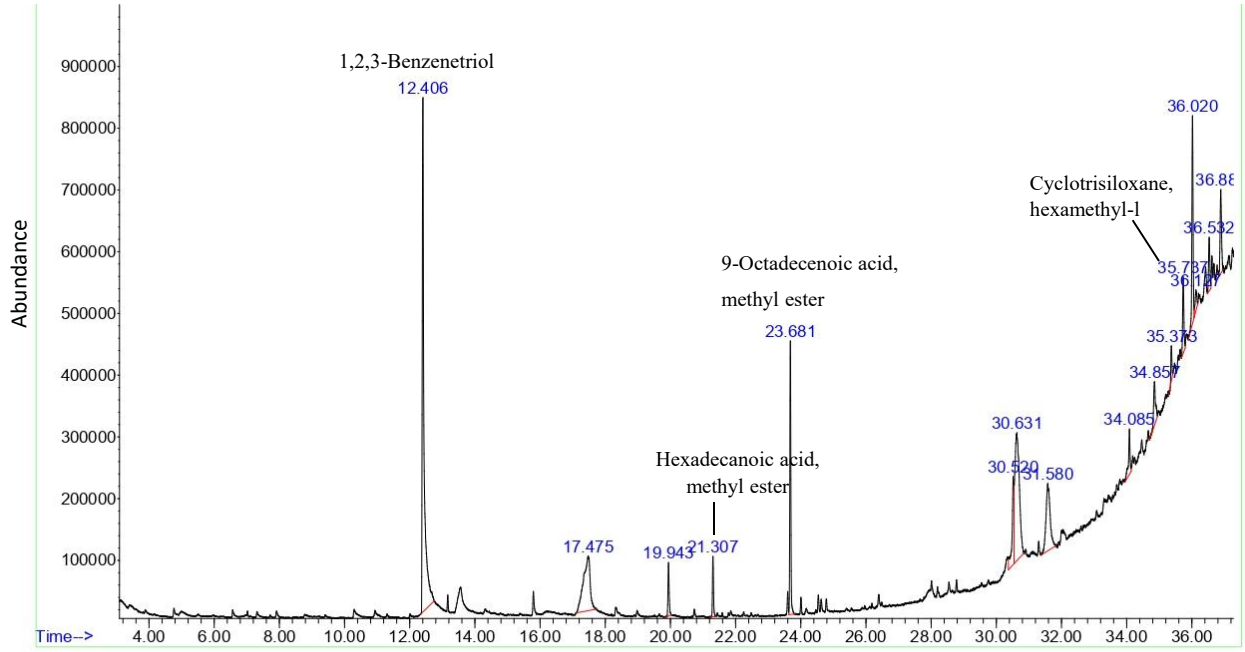

Figure 3: GC-MS chromatogram of methanol extract of seeds of *A. precatorius* L.

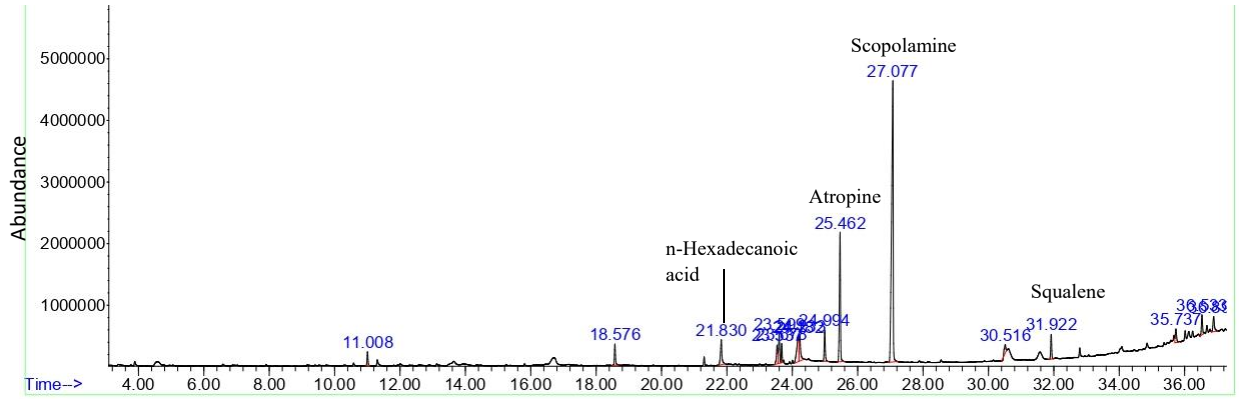

Figure 4: GC-MS chromatogram of methanol extract of seeds of *D. metel* L.

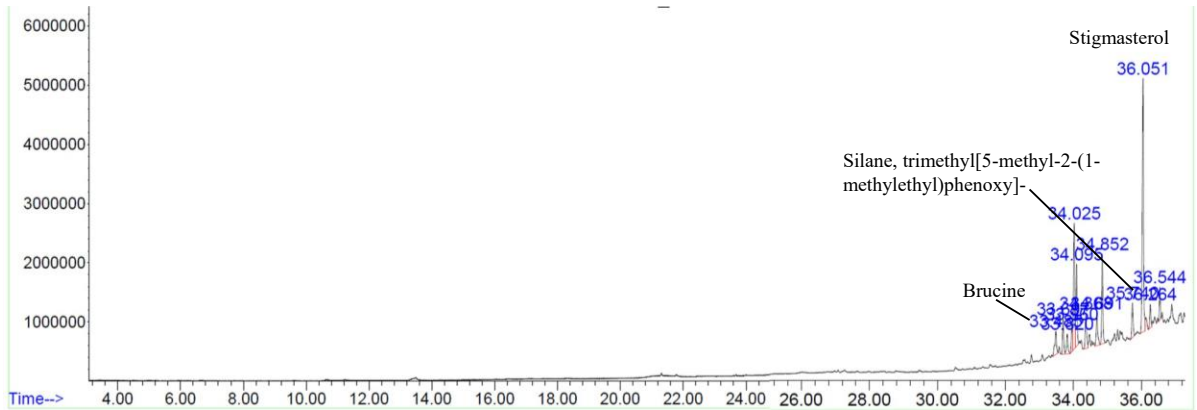

Figure 5: GC-MS chromatogram of methanol extract of seeds of *D. butyracea* (Roxb.) H.J. Lam

Insecticidal compounds detected by GC-MS

GC-MS analysis identified a diverse array of bioactive compounds, exhibiting insecticidal and toxic properties, in the methanol seed extracts of the three plant species investigated. (Figures 3-5).

GC-MS analysis revealed that the seeds of the three different plant species contained different types of insecticidal compounds. The key insecticidal and toxic compounds present in methanol extract of *A. precatorius* L. seeds (Table 4) included 1,2,3-benzenetriol (peak area, 26.95%) hexadecanoic acid, methyl ester, 9-octadecenoic acid, methyl ester (E)- and cyclotrisiloxane, hexamethyl- at retention times (RT) 12.405, 21.308, 23.679, and 35.736 minutes, respectively (Figure 3). Seed extracts of *D. metel* L. was found to have scopolamine (largest peak area, 47.29% at RT 27.077 minutes), followed by atropine, n-Hexadecanoic acid/scopoletin, 9-Octadecenoic acid (Z)-, methyl ester, and squalene (Figure 4). These compounds are known for their insecticidal toxicity as shown in Table 5. As shown in the chromatogram (Figure 5), the major insecticidal compounds identified in the seed extracts

Table 4: Key bioactive compounds identified from the methanol extract of seeds of *A. precatorius* L. using GC-MS analysis

SN	Retention Time (RT)	Area (%)	Compound Name	Function	Reference
1	12.405	26.95	1,2,3-Benzenetriol	toxic	Gomathy & Rathinam, 2016
2	21.308	1.79	Hexadecanoic acid, methyl ester	nematicide and pesticide	Krishnamoorthy & Subramaniam, 2014
3	23.679	8.03	9-Octadecenoic acid, methyl ester	Antiandrogenic, insectifuge, and anemiagenic properties	Krishnamoorthy & Subramaniam, 2014
4	35.736	2.7	Cyclotrisiloxane, hexamethyl-	biocontrol agent	Shilaluke & Moteetee, 2022

Table 5: Key bioactive compounds identified from the methanol extract of seeds of *D. metel* L. using GC-MS analysis

SN	Retention Time (RT)	Area (%)	Compound Name	Function	Reference
1	21.830	3.74	n-Hexadecanoic acid	Antioxidant, nematicide, pesticide	Elaiyaraja & Chandramohan 2016
			Scopoletin	Insecticide	Liu et al., 2023
2	23.679	1.73	9-Octadecenoic acid (Z)-, methyl ester	Antifungal, antioxidant, antimicrobial, and insectifuge	Krishnamoorthy & Subramaniam, 2014
3	25.462	14.68	Atropine	Toxic	Steenkamp et al., 2004
4	27.077	47.29	Scopolamine	Toxic	Steenkamp et al., 2004
5	31.922	2.57	Squalene	pesticide	Gomathy & Rathinam, 2016

Table 6: Key bioactive compounds identified from the methanol extract of seeds of *D. butyracea* (Roxb.) H.J. Lam using GC-MS analysis

SN	Retention Time (RT)	Area (%)	Compound Name	Function	Reference
1	33.7	4.2	Brucine	Antipathogenic, antibacterial, and toxic	Jain et al., 2023
2	34.688	4.57	2,4,6-triaminoquinazoline	Antimicrobial, antifungal, antiviral, acaricidal, and weedicide	Yaduwanshiet al., 2021
3	36.05	31.88	Stigmasterol	Larvicidal, repellent,	Gade et al., 2017
4	36.266	2.68	Silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-	Insecticide, acaricide, and animal repellent	Escobar et al., 2020

of the *D. butyraceae* (Roxb.) H.J. Lam by GC-MS analysis were brucine, 2,4,6-triaminoquinazoline, stigmasterol, and Silane, trimethyl [5-methyl-2-(1-methylethyl) phenoxy] at retention times 33.700, 34.688, 36.050, and 36.266, respectively (Table 6).

Discussion

This study primarily aims to explore the insecticidal efficacy of seeds extracts from three different plant species, with the objective of identifying suitable and effective plant-based biopesticides for managing insect pests that damage crops. Furthermore, the research investigates phytochemical compounds with insecticidal properties present in the seeds of these plant species.

In this study, Spinosad exhibited the highest mortality rates against both adult and larval stages of *Drosophila melanogaster*. Neem and *Bacillus thuringiensis* also demonstrated good insecticidal properties with moderate to high mortality rates in various modes of applications. The methanol seed extracts from the tested plants: *D. metel* L., *A. precatorius* L., and *D. butyracea* (Roxb.) H.J. Lam also showed promising insecticidal activities. All three extracts exhibited increased effectiveness with higher concentrations, suggesting a dose-dependent insecticidal response.

We found that, among the three plant seed extracts, *D. metel* L. caused significant mortality in *D. melanogaster* both in adults (73.33 ± 7.64%) and larvae (43.33 ± 2.89). Corroborating with our results, the insecticidal and insect-repellent activities of *D. metel* L. have been reported previously against various insect species (Cespedes-Mendez et al., 2021). The hexane extract of the seed kernel and leaf of *D. metel* L. was found to induce 70% mortality in three mosquito larval species (Yahaya et al., 2021). Besides seeds, the leaf extract of *D. metel* L. also exhibited insect-repellent and insecticidal effects against grasshoppers and red mites in both contact and spray application tests (Kuganathan & Ganeshalingam, 2011). Other species of *Datura* like *D. alba* were also found to be effective against *Trogoderma granarium* and *Sitophilus oryzae*, under laboratory conditions (Ali et al., 2012). These findings suggest that *D. metel* has high potential to be applied as an insecticide. However, other plant species such as *Spinacia oleracea, Ulva lactuca,* and *Drimia maritima* were also found to exhibit insecticidal activity against *D. melanogaster* through both contact and spray applications in separate studies (Rima et al., 2021; Saadane et al., 2021).

The insecticidal activity of plant extracts is dependent on their phytochemical constituents. For instance, plant alkaloids have been shown to exhibit acute and chronic insecticidal effects against *D. melanogaster* at 10 µg/mL, resulting in feeding alteration, deformations, and less development of larvae (Quiroz-Carreno et al., 2020). Qualitative phytochemical screening of the bioactive compounds revealed that alkaloids were present in all the three plant species. In addition to alkaloids, phenols, flavonoids, tannins, and carbohydrates were also detected in *A. precatorius* L. Saponins and alkaloids were found in *D. metel* L. and *D. butyraceae* (Roxb.) H.J. Lam. These findings suggest that insecticidal activity shown by extracts of seeds of these plants may be due to the active phytochemicals present in them. However, qualitative tests in this study could not detect all tested phytochemicals in the seed extracts of all the three plant species. Detection of lower or trace amounts of the phytochemicals may require extraction using different solvent systems and multiple detection methods (Shaikh & Patil, 2020). However, analytical techniques like chromatography and spectroscopy can identify and confirm the presence of specific compounds.

FTIR is an analytical technique to determine the presence of specific functional groups. Interpretation based on the frequency range indicates the presence of alcohols or phenols, alkanes, esters, and ethers in *D. metel* L., *A. precatorius* L., and *D. butyracea* (Roxb.). FTIR allows the detection of unique chemical bonds and functional groups such as hydroxyl (–OH), carbonyl (C=O), amine (–NH), and others, which are associated with different classes of phytochemicals such as alkaloids, flavonoids, and tannins, known for their pesticidal and antimicrobial properties. *D. metel* L. contains flavonoids, saponins, alkaloids, volatile oils, and steroids which are responsible for exhibiting the larvicidal properties (Yahaya et al., 2021). Tannins, terpenes, alkaloids, phenols, alcohols, and other secondary metabolites present in plants cause damage to fungal cell walls, membranes, and organelles, leading to toxicity (Lengai et al., 2020). These may be attributed to their unique pesticidal and fungicidal properties.

GC-MS has emerged as a highly effective, rapid, and relatively simple method for identifying bioactive compounds in plants. It is also widely used for the purification and structural characterization of chemical constituents. Analyzing these bioactive compounds play a significant role in advancing, updating, and maintaining the quality of herbal

formulations (Kanthal et al., 2014; Chieme et al., 2022). Recent research has identified 166 secondary metabolites with flavonoids and terpenoids exhibiting toxic properties against pest and pathogens (Qian et al., 2022). Some of the identified compounds, such as 1,2,3-benzenetriol, hexadecanoic acid, methyl ester, 9-octadecenoic acid, methyl ester, and cyclotrisiloxane, hexamethyl- have been reported to act as biocontrol agents (Krishnamoorthy & Subramaniam, 2014; Gomathy & Rathinam, 2016; Shilaluke & Moteetee, 2022).

The major chemical compounds squalene, atropine, scopolamine, scopoletin, 9-octadecenoic acid (Z)-, methyl ester, and n-hexadecanoic acid identified from seeds of *D. metel* L. were also detected in previous studies (Steenkamp et al., 2004; Krishnamoorthy & Subramaniam, 2014; Elaiyaraja & Chandramohan, 2016; Gomathy & Rathinam, 2016; Liu et al., 2023). Pratheeba et al. (2019) reported that 9,12-octadecadienoic acid (Z, Z) may be responsible for the mosquito larvicidal activity. Similarly, GC-MS analysis of *D. butyraceae* (Roxb.) H.J. Lam revealed the presence of compounds such as brucine, 2,4,6-triaminoquinazoline, stigmasterol, silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-, which are responsible for pharmacological properties such as antibacterial, antioxidant, antifungal along with the insecticidal and toxic effects (Gade et al., 2017; Escobar et al., 2020; Yaduwanshi et al., 2021; Jain et al., 2023). Triterpenic saponins present in deoiled/defatted seed cake of *D. butyraceae* have been found to exhibit feeding deterrent and insect growth regulatory effects on *Spodoptera litura* (F.) (Noctuidae: Lepidoptera). Saponins act as biological detergent due to its amphiphilic nature. When agitated in water, they produce abundant foam similar to synthetic detergents. Saponins also display hemolytic activity, which is partially responsible for toxicity (Saha et al., 2010). GC-MS profiling of *D. butyraceae* further confirmed the presence of antibacterial, antifungal, and antioxidant components. Stigmasterol derived from the *Chromolaena odorata* has been shown to induce mortality against *Culex quinquefasciatus, Aedes aegypti,* and *Chironomus riparius* through inhibition of acetylcholinesterase (Gade et al., 2017).

In this study, GC-MS profiling of seed extracts identified several bioactive compounds known for their pesticidal properties, suggesting the potential of these seeds as biocontrol agents against *D. melanogaster*. However, a key limitation is that the experiments were conducted under controlled laboratory conditions, which may not exactly reflect real field scenario. Therefore, further research involving field trials is necessary to validate the practical use of these extracts in controlling insect pests. Additionally, investigations on the key application method and mechanism of antagonism are warranted to confirm the suitable approach in agricultural practices. Nevertheless, our findings support the development of plant-based pesticides as a sustainable and environmentally friendly alternative for pest management. The use of plant biopesticides can promote the value of agricultural products and and contribute to food security.

Conclusion

The seeds of *Abrus precatorius* L., *Datura metel* L., and *Diploknema butyracea* (Roxb.) H.J. Lam demonstrated the insecticidal activities with the presence of several bioactive compounds. Our results provide laboratory-based evidence that these plant seeds have potential as biopesticides. However, the insecticidal activity of the methanolic seeds extracts varied depending on the developmental stage of the insect (i.e., adult or larval) and the method of application (ingestion or spray). Similarly, insecticidal activity increased with increasing concentration. Among the tested plants**,** *D. metel* L. exhibited showed more consistent efficacy across both ingestion and spray methods, making it a promising candidate for further evaluation as a biopesticide. Our findings demonstrated the potential of *D. metel* L. as an effective and sustainable alternative to chemical pesticides. The bioactive compounds identified through GC-MS with their toxic and insecticidal properties support the development of sustainable pest management strategies. These findings are relevant efforts to reduce the reliance on synthetic agrochemicals in countries like Nepal, where agriculture is a cornerstone of the economy and rural livelihoods.

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Author's contribution statement

E. Maharjan: Writing original draft, data acquisition, data analysis; **M. Y. Wong:** Supervision, resources, review and editing, funding acquisition; **S. K. Upadhyay:** Resources, review, and editing; **P. Panta, T. Prasai Joshi:** Data analysis, review, and editing; **R. Adhikari, D. R. Joshi:** Conceptualization, supervision, data analysis, review, and editing.

Data availability

This study offers the author's original work, which has not been published previously. All the data are embedded within the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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