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Research Article

Bioefficacy of *Roylea cinerea* leaf extract on *Plutella xylostella*: life cycle analysis and larval mortality assessment

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Background: *Plutella xylostella* (Diamondback moth), a major pest of Brassica crops, causes significant economic losses worldwide. In the present study, the larvicidal efficacy of *Roylea cinerea*, a medicinal plant native to the Western Himalayas, was evaluated for the first time against *P. xylostella* under laboratory conditions.

Methods: Leaf extracts of *R. cinerea* were prepared using two solvents: methanol and ethyl acetate, and tested at varying concentrations (0.5–3.0%) against diamond back moth larvae. In parallel, the life cycle of *P. xylostella* was observed to complete within 20–25 days under controlled conditions.

Results: At 24 hours post-treatment, the lowest mortality (10.71%) was recorded with the 0.5% methanol extract, while the highest (39.28%) was observed with the 3.0% ethyl acetate extract. After 48 hours, the methanol extract at 0.5% and 1.5% concentrations both showed 17.85% mortality, whereas the 3.0% ethyl acetate extract achieved a significantly higher mortality of 50%. By 72 hours, mortality rates increased across treatments, with the 3.0% ethyl acetate extract showing the highest efficacy (72.41%), while the lowest was again seen with 0.5% methanol extract (39.28%).

Conclusion: The study demonstrates that *R. cinerea* exhibits promising larvicidal activity against *P. xylostella*, with ethyl acetate extracts proving more effective than methanol. These findings highlight the potential of *R. cinerea* as a sustainable botanical pesticide for managing diamondback moth populations, contributing to environmentally safe pest management strategies.

Keywords: *botanicals, biopesticide, mortality, Plutella xylostella, Roylea cinerea*

Introduction

Cole vegetables like cabbage (*Brassica oleracea*) are widely grown in India, and it belong to the family Cruciferaceae. It is mostly used as a salad, boiled vegetables and possess medicinal properties, also a rich source of Ca, P, Na, K, S, Vitamin C (Gaddam et al., 2021). There has been a considerable increase in the number of *Plutella xylostella* (*P. xylostella*), commonly known as diamondback moth, attacks on cruciferous plants worldwide, especially on vegetables and oil crops. Annually, the world spends US$1.0 billion on controlling this insect because of the damage (Ahmad &

Ansari, 2012). Plant growth is reduced by this pest, resulting in 31-100% reduction in production and yield (Parajuli & Paudel, 2019). Although it originated in Europe, it has spread throughout the world due to its migratory behaviour, particularly in regions where cruciferous vegetables grow (Paul et al., 2023). About 33% of the world's cauliflower production comes from India, making it one of the top growers. A total of 84,990 lakh tonnes of the crop are produced annually on an area of 4.52 lakh hectares. The states of Bihar, Uttar Pradesh, Odisha, West Bengal, Assam, Haryana, and Maharashtra are the main producers of cauliflower in the nation (Beena & Selvi, 2022). In India, this pest is estimated to cause 16 million dollars' worth of crop losses every year (Mohan & Gujar, 2003). This pest has been reported for the first time in India on cruciferous vegetables in Uttar Pradesh, Orissa, Bihar, West Bengal, Assam, Karnataka, Maharashtra, Madhya Pradesh and Tamil Nadu (Gautam et al., 2018). There is a substantial loss of economic value for farmers due to the presence of diamond back moth. Diamond back moth (DBM) can develop and reproduce at a wide range of temperatures, despite tolerating temperature extremes (Sarfraz et al., 2005). DBM has developed resistance to synthetic insecticides, resulting in insecticide resistance, pest outbreaks, and undesirable effects on the crops. *P. xylostella* has increasingly become a pest worldwide due to a short generation time, the lack of effective natural enemies, and insecticidal resistance (Mubashir & Seram, 2022). Due to its resistance to almost every class of insecticide used in the field, *P. xylostella* has become the most challenging insect in the world to control. Larvae stage of diamondback moth is damaging stage on the crops. Larvae feeds on leaves, buds, seed flowers of the cruciferous crops. There is concern over wide-scale use of synthetic, broad-spectrum insecticides since they can have hazardous effects on humans and the environment, and insects are resistance to it (Dong et al., 2013). It has become increasingly important to develop novel insecticides that have lower risks to human health and the environment in recent years as organic agriculture demands and attention to environmental pollution have increased (Jeschke, 2016). Bioactive compounds in plants have complex combination of behavioural and physiological effects and make the insects difficult to evolve their resistance (Susmitha, 2021). Extracts of plants have been found to influence insect behaviour as well as have antifeedant and repellent properties (Charleston et al., 2005). *Melia azedarach* and the neem tree, *Azadirachta indica* was tested on the behaviour of the diamondback moth, showed great results. *Roylea cinerea* (*R. cinerea)* belongs to family Lamiaceae commonly known as Karui, is locally used against insect pests but studies regarding the insecticidal properties of this plant have not been reported yet. It is a shrub and traditionally used in Himalayan, sub- Himalaya and Nepal region to treat diabetes, malaria, skin disease, jaundice and contusions (Pundir & Mahindroo, 2019). Research on botanical extracts gains speed since they are locally and easily available, possess insecticidal properties, are cheaper to access, and are compatible with other useful insects and natural predators (Iamba & Malapa, 2020).

Materials and methods

Sampling and rearing of diamond back moth

Samples of DBM were collected from Kangra (Drammen, Shahpur, Sahora) and Mandi district (Sarkaghat). Different larval instar, pupa, and adults were collected from cabbage leaves. Then using a camel brush gently, larvae were separated from infected cabbage leaves and transferred to the freshly cabbage leaves. An insect rearing cage was used to raise the Diamondback moth in a lab environment at room temperature. Within the cage, which also functioned as a place to lay eggs, the larvae were fed their natural diet of mustard and cabbage leaves. For feeding, adult moths were given a 20% sugar and honey solution via cotton dipped in the solution and placed in a petri dish. Every two days, new leaves were added. Under a stereo microscope, eggs and larval stages on the leaves were closely examined before being moved to new leaves in the rearing cage. All of the experiment's eggs, larvae, pupae, and adults were carefully observed.

Life cycle

A pair of just emerged male and female Diamondback moths were divided and placed in a plastic container for careful examination. On the day of fertilisation, the female deposited eggs. Following that, we kept a close eye on the eggs' incubation period as well as the growth of the several larval instars, pupae, and adults. Based on variations in size, colour, and activity, the four larval instars were distinguished. After a few days, the adults emerged from the pupae, which had been divided and housed in a separate plastic container. All of their activity, including the incubation stage, was carefully monitored.

Morphometry

The life stages of egg, larval instar, pupa and adult are observed and preserved carefully in 70% alcohol. Then temporary and permanent slides of the life stages and body parts were prepared. cm scale, stereo microscope and

trinocular light compound microscope are used to study the morphometry of these life stages of the Diamondback moth.

Collection of plant material and preparation of extract

R. cinerea plant was selected for the experiment. The plant was collected in the month of February and allowed to shade dried completely. The plant *R. cinerea* were collected from a village Ropari, Sarkaghat (Mandi 31° 41' 56.2416" N and 76° 43' 56.6652" E). The leaves were selected and separated from the plant. The leaves were allowed to shade dry until all water content is lost from the leaves. The dried material is crushed and grounded using the blender. Then the powdered form is preserved for further use. Using a weighing machine, 20g of powdered plant leaves were used to make the stock solution of methanol/ethyl-acetate. Next, measuring cylinder were used to take the volume of 200ml of methanol/ethyl-acetate solvent. In a conical flask, 20g of *R. cinerea* powder was dissolved in 200ml of methanol/ethyl acetate. After ensuring complete mixing with a 5-minute stir, the solution was left undisturbed for a full day. A conical flask and Whatman filter paper No. 1 were used to filter the solution, resulting in an extract or stock solution that was ready to be used against the diamondback moth. Three different concentrations of the methanol/ethyl-acetate stock solution of 0.5%, 1.5%, and 3.0% were prepared and applied to the moth larvae.

To estimate the efficacy of the botanical extract, approximately 40 to 45 petri dishes were used. Cabbage leaves, measuring 3 to 6 cm in diameter, were thoroughly washed to avoid contamination and then placed in the petri dishes. Ten larvae of *P. xylostella* were randomly selected from the culture and added to each Petri dish. The experiment was conducted in triplicate for each concentration used, along with a control.

The mortality rate of **P. xylostella** larvae treated with the botanical extract were calculated using the following formula:

$$\text{Per cent mortality} = \frac{\text{Number of dead insects}}{\text{Total number of insects releaed}} \times 100$$

The observations recorded on mortality were corrected using Abbott's formula (Abbott, 1925) as given as

$$\text{Abbott}' \text{s corrected mortality (\%)} = \frac{\text{T} - \text{C}}{100 - \text{C}} \times 100$$

Where, T = mortality in Treatments (%)
C = mortality in Control (%)

Statistical analysis

The effective concentration of the extract against *P. xylostella* (LC50) was determined using probit analysis (Finney, 1990) in SPSS software (version 21). Statistical analyses were performed using one-way analysis of variance (ANOVA) at ($p < 0.05$), followed by Tukey's post hoc test in SPSS (version 21). Graphs were plotted in Microsoft Excel using the data analyzed in SPSS.

Results

The present study on diamondback moth was carried out in the Central University of Himachal Pradesh. In this study, biology, morphometry and efficacy of botanical extract as a biopesticide against the diamondback moth was studied. Biology of DBM- During the experiment the life cycle of the diamond back moth was observed. The life cycle of moth was observed at room temperature.

Adult

Adult diamondback moth is light brown in colour, has three triangular brown spots on its lateral side which along with the dorsal portion gives appearance of the three-diamond shaped structure giving it the name diamondback moth. On its head one pair of long setaceous antenna are present. Female possess wider wings i.e. about 5.8±0.57mm while in case of male moth it is 4.3±0.57mm. Newly emerged female can mate after 2-3 hour and eggs are laid on the same day if host plant is available. The length of the female is longer with average of 6.3±0.57 mm and male are shorter with an average of 4.6±0.57mm. Incubation period of adult male was found to be 8.5±0.70 and 11±1.41 days for female.

Eggs

Diamondback moth mate immediately after few hours of emergence. Studies revealed that males can mate two times in their life where females can mate only once in their life cycle. It is observed in studies that a female can lay up to 200-210 eggs at a time. The eggs are laid on the lower surface of the host plant leaf so as to avoid winds, rain and other environmental factors. Eggs are greenish yellow in colour and round to oval in shape. It was observed in present studies that the eggs were laid near to midrib of the leaf. The length and breadth of egg is average 0.19±0.37mm and 0.10±0.02mm. Incubation period of *P. xylostella* eggs is given in Table 1.

Table 1. Incubation period of *P. xylostella* eggs

Number of eggs	Incubation period (days)		
20	Minimum	Maximum	Average ± SD
	2	3	2.5 ± 0.70

Larvae

All the four larval instars were observed during experiment. The newly hatched larvae were very small and can merely be seen by naked eye. Each instar varied in length as they grow. Larvae change its instar by moulting process and 3 moults are observed in Diamondback moth. The first instar larvae emerge from egg and have incubation period of 3 – 4 days with an average of 3.5±0.70 days. There were sparse hairs are present on all over the body. The first instar measured 1.1± 0.63 and 0.17± 0.01mm in length and breadth respectively. It is greenish white in colour and has dark coloured head. At this stage the larvae start feeding on host plant. The head capsule length measured 140.13±4.85µm and breadth 165±9.12 µm. The second instar has yellowish green appearance and longer in size. It has light brown colour of head and exhibited incubation period ranging from 1-3 days with an average of 2± 1.41 days. Larvae were observed to be much active. The average head capsule length and breadth of second instar measured 436±122.31 µm and 367.04±144.97µm and average body length and breadth was3.25±0.5mm and 0.68±0.13mm. Third larval instar does not much different than the second instar in colour. It has light brown head and yellowish green body covered by sparse hairs. The incubation period of third instar was found to be 2-4 days with an average of 3±1.41 days. The average length and breadth of head capsule measured 627.33±24.7µm and 451±127.37µm respectively while with an average body length and breadth was 6±0.81mm and 1.07±00mm respectively. The fourth larval instar was largest in size compared to other instars. Larva has exhibited greenish colour and had dark brown head capsule. At this stage larvae feeding activity was observed to be decreased and larvae appeared sluggish. The average incubation period of 2.5±0.70 days was documented which was supported with average incubation period of 2.75±0.25 days. Recorded average head capsule length and breadth to be 698.74±88.2µm and 576±19.26µm respectively and average body length and breadth was 9.5±1.29mm and 1.19±00mm respectively. Duration of different larval instar are given in table 2 and 3.

Table 2. Durations of different larval instar of *P. xylostella*

Larval instar	Number of larvae observed			
		Minimum	**Maximum**	**Mean ± SD**
First	10	3	4	3.5± 0.70
Second	10	1	3	2.0±1.41
Third	10	2	4	3.0±1.41
Fourth	10	2	3	2.5±0.70
Total larval period		8	14	11±4.24

SD indicates the standard deviation

Pupal stage

It is the resting and non-feeding stage of *P. xylostella*. Two type of pupa stage - early and late pupa was observed during the pupal period. In early pupa, the larvae spin the cocoon around its body and undergo pupal stage in this stage the colour of the pupa remains greenish and in late pupal stage the pupa becomes blackish in colour. Average body length was7.8±1.29mm and 1.5±0.70mm breadth was observed which is in contrast with 5.09±0.46mm length and 1.20±0.09mm breadth.

Length and breadth of head capsules of different larval instars, total body length and breadth of different larval instars and total length and wings of adult *P. xylostella* are given in Table 4, 5 and 6, respectively.

Table 3. Pupal period of *P. xyllostela*

Number of larvae	Minimum	Maximum	Mean ± SD
10	3	5	4 ± 1.41

Table 4. Length and breadth of head capsules of different larval instars

Stage	Head capsule (Mean ± SD)	
	Length (µm)	Breadth (µm)
1st Instar	140.13±4.85	165±9.12
2nd Instar	436±122.31	367.04±144.97
3rd Instar	627.33±24.7	451±127.37
4th Instar	698.74±88.2	576±19.26

Table 5. Total body length and breadth of different larval stages of *P. xylostella*

Life stage	Length (mm)		Breadth (mm)	
	range	AV ± SD	range	Mean ± SD
Egg	0.16- 0.21	0.19±0.37	0.08-0.11	0.10±0.02
1st Instar	0.90- 2	1.1± 0.63	0.16-0.18	0.17±0.01
2nd Instar	3- 4	3.25±0.5	0.53-0.79	0.68±0.13
3rd Instar	5-7	6±0.81	1.07-1.08	1.07±00
4th Instar	8-11	9.5±1.29	1.19-1.20	1.19±00
Pupa	7-9	7.8±1.29	1-2	1.5±0.70

Table 6. Total length and wing span of adult *P. xylostella*

Character	Male (mm)	Female (mm)
Adult	4.6±0.57	6.3±0.57
Wing	4.3±0.57	5.8±0.57
Wing span	8.6±0.25	12.8±0.62

Bioassay

In the replicates treated solely with the solvents methanol and ethyl acetate, a mortality rate of 3.3% was observed in both cases, suggesting that the solvents themselves may contribute to toxicity. The larvae were reared on their natural food cabbage leaves. 10 larvae each was taken from the cage culture and placed in petri plates consisting different concentration of botanical extract. The experiment was conducted with in three replicates of each concentration and control. The larvae were observed after 24 hours in ethyl acetate and methanol extract it was observed that the mortality increases as the concentration increased at 24 hours interval the lowest mortality rate (Abbott's corrected mortality) was observed at 0.5% concentration of methanol extract whereas highest mortality rate observed at 3.0% concentration at 24 hours interval. Among the both plant extract of *R. cinerea*, extract ethyl acetate showed more mortality at 24 hours. At 48 hours the lowest mortality observed in methanol extract at 0.5 and 1.5% of concentration both with 17.85% of mortality rate and the highest mortality rate was observed at 3% of ethyl acetate extract with 50% and second highest mortality was observed at 1.5% of ethyl acetate concentration with 46.92%. At 72 hours the lowest mortality rate was observed in methanol extract with 0.5% concentration with 39.28% of mortality.

Table 7. Abbott's corrected mortality of *R. cinerea* ethyl-acetate extract

Treatment	% Corrected mortality at different interval		
	At 24 hours	At 48 hours	At 72 hours
0.5%	6.8	25	60.71
1.5%	10.34	46.92	64.28
3.0%	17.24	50	72.41

Table 8. Abbott's corrected mortality of *R. cinerea* methanol extract

Treatment	% Corrected mortality at different interval		
	At 24 hours	At 48 hours	At 72 hours
0.5%	3.44	17.85	39.28
1.5%	10.34	17.85	50
3.0%	13.79	39.28	57.14

The highest mortality observed at 3% of the ethyl acetate extract with 72.41% of mortality and second highest mortality was observed in 1.5% concentration of ethyl acetate extract with 64.28% of mortality. At 72 hours botanical with ethyl acetate shows better results than methanol. Abott's corrected mortality of *R. cinerea* methanolic and ethanolic extract are given in Table 7 and 8. Larvae surviving at different concentration of *R. cinerea* by using methanolic and ethanolic extract are given in table 9 and 10.

Table 9. Larvae surviving at different concentration of *R. cinerea* – methanol extract after 24, 48 and 72 hours

Methanol extract			
Treatment	**At 24 hours**	**At 48 hours**	**At 72 hours**
Control	10± 00	10 ± 00	9.67± 0.33
Solvent	9.67± 0.33	9.67± 0.33	9.33± 0.33
0.5%	10± 00	7.67±0.33$^{*@}$	5.67± 0.33$^{*@}$
1.5%	8.67± 0.33*	7.67± 0.33$^{*@}$	4.67± 0.33$^{*@}$
3%	8.33±0.33$^{*@}$	5.67± 0.33$^{*@}$	4.00± 0.33$^{*@}$

*Values are represented in MEAN ±SD followed by *and @ indicate significant difference w.r.t. control and solvent (positive control) respectively at p<0.05 post hoc Tukey's test.*

Concentrations showed the significant difference at various time intervals with respect to control and solvent. At 24 hr concentration of methanolic plant extract 1.5% and 3% showed significant difference with control. Whereas3% concentration also showed significant difference with solvent. At 48 hr 0.5%,1.5% and 3% concentration of methanolic plant extract showed significant difference with control and also0.5%,1.5% and 3% concentration showed significance difference with solvent. At 72 hr 0.5%,1.5% and 3% concentration of methanolic plant extract showed the significance difference with control and also 0.5%, 1.5% and 3% concentration showed significant difference with solvent.

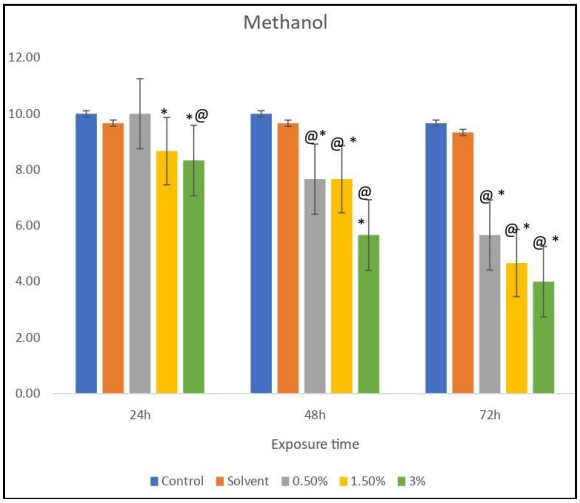

Figure 1. **Number of larvae surviving at different concentration of methanolic extract of R. cinerea after 24, 48 and 72 hr. *and @ indicates significant difference w.r.t. control and solvent (positive control) respectively at p<0.05 (One way ANOVA, post hoc Tukey's test- SPSS 21).**

Table 10. Larvae surviving at different concentration of *R. cinerea* – ethyl acetate after 24, 48 and 72 hours

Ethyl acetate			
Treatment	**At 24 hours**	**At 48 hours**	**At 72 hours**
Control	10± 00	10±00	10±00
Solvent	9.6± 0.33	9.33±0.57	9.33±0.33
0.5%	9.0± 0.57	7±1.00$^{*@}$	3.66±0.33$^{*@}$
1.5%	8.6± 0.66	5±1.00$^{*@}$	3.33±0.33$^{*@}$
3%	8.0± 1.0	4.66±0.57$^{*@}$	2.66±0.33$^{*@}$

*Values are represented in MEAN ±SD followed by *and @ indicate significant difference w.r.t. control and solvent (positive control) respectively at p<0.05 post hoc Tukey's test.*

In table 10 shows the significant difference at various time intervals with respect to control and solvent. At 24 hr concentrations showed no significance difference with solvent and control. At 48 hr concentrations of ethyl acetate

plant extract 0.5%,1.5% and 3% showed significance difference with control and also 0.5%,1.5% and 3% concentration of ethyl acetate plant extract showed significance difference with solvent. At 72 hr concentration ethyl acetate plant0.5%,1.5% and 3% showed significance difference with control and also 0.5%,1.5% and 3% concentration ethyl acetate plant shows significance difference with solvent.

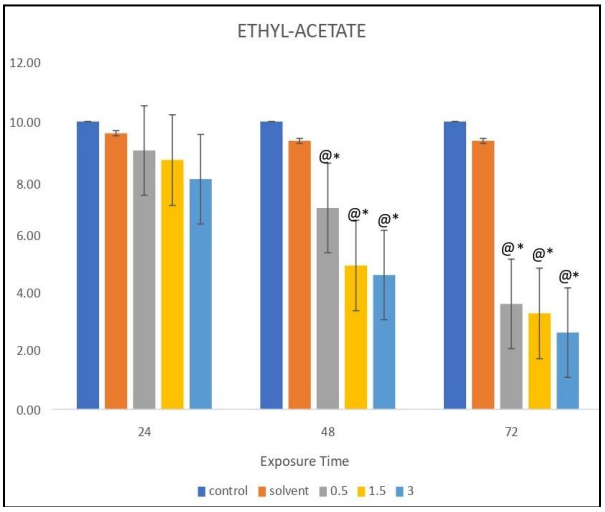

Figure 2. Number of larvae surviving at different concentration of ethyl- acetate extract of R. cinerea after 24, 48 and 72 hr. *and @ indicates significant difference w.r.t. control and solvent (positive control) respectively at p<0.05 post hoc Tukey's test. (One way ANOVA, post hoc Tukey's test- SPSS 21).

LC50 values

The value of 72 hr LC50 for extract dissolved in methanol was observed to be 1.30 gram/litre. The value of 72 hr LC50 for extract dissolved in ethyl acetate was observed to be 0.24 gram/litre. Thus, can be concluded that the ethyl acetate prepared extract possesses greater efficacy as biopesticide. Because R. cinerea contains phytochemicals such as glycosides, flavonoids, tannins, saponins, and diterpenes, it has promising anti-feeding and biopesticide properties. The plant may be a natural pest repellent because of these substances, which include tannins and saponins, which are known to decrease fitness of herbivorous insects. Furthermore, R. cinerea may be used as a biopesticide for pest control because diterpenes and phenols have insecticidal qualities. The bioactive chemicals found in the plant enhance integrated pest management tactics by providing a sustainable and environmentally friendly substitute for synthetic pesticides. The number of larvae surviving at different concentration of methanolic and ethanolic extract are given in Figure 1 and Figure 2.

Discussion

The present study demonstrates, for the first time, the larvicidal potential of *Roylea cinerea* leaf extracts against *Plutella xylostella*, a notorious pest of cruciferous crops. The results revealed that mortality increased with both concentration and exposure time, with ethyl acetate extracts showing higher toxicity than methanolic extracts. This indicates that the active compounds responsible for larvicidal activity are likely more soluble or stable in ethyl acetate, which is consistent with the polarity-dependent extraction efficiency of phytochemicals. The morphometric observations recorded in this study provide valuable baseline information on the biology of *Plutella xylostella* under controlled laboratory conditions. The complete life cycle of DBM was found to be 20–25 days, consistent with earlier reports indicating that development duration varies between 18 and 30 days depending on host plant, temperature, and relative humidity (Keerthi & Suroshe, 2024). The egg, larval, pupal, and adult stages documented here closely match the findings of previous studies, confirming that the local populations in Himachal Pradesh exhibit similar developmental traits to those reported globally (Singh et al., 2024). The larvae passed through four instars, with progressive increases in length and width at each stage. The morphometry of the 1st instar larvae revealed small size and translucent bodies, while subsequent instars showed gradual darkening, increased sclerotization, and more active feeding behavior. The pupal stage, which lasted 4–5 days, was also comparable in size and duration to published descriptions. The adult moths were small, with narrow fringed wings, measuring approximately 7–8 mm in body length, again in agreement with earlier literature (Ram et al., 2017). The significance of these morphometric results lies in providing a baseline standard for future bioassays and resistance monitoring. Since DBM populations are known to vary geographically in size, fecundity, and developmental rate, establishing such morphometric data for the Himachal

populations is crucial for tailoring local management strategies. Moreover, detailed knowledge of larval instar size is essential for timing bioassays and field applications of botanical insecticides, as susceptibility often varies with developmental stage. Integrating these findings with the larvicidal bioassays, it is noteworthy that the extracts of *Roylea cinerea* were tested against larvae that fall within the morphometric ranges documented in this study. The consistent mortality patterns across instars suggest that the extracts are effective across different larval sizes, although further work may be needed to determine instar-specific susceptibility (Javier et al., 2016). Similar findings have been reported in studies involving plant-derived pesticides. For instance, extracts from *Azadirachta indica* (neem), *Calotropis procera*, and *Lantana camara* have exhibited significant insecticidal activity against DBM larvae, supporting the potential of botanical extracts as eco-friendly alternatives to synthetic insecticides (Saxena et al., 2014). The present results are in line with these earlier reports, further emphasizing the role of phytochemicals in pest management. At 24 hours, mortality ranged from 10.71% in the lowest methanolic concentration to 39.28% in the highest ethyl acetate concentration, demonstrating the early onset of toxicity. The trend intensified over time, with maximum efficacy observed at 72 hours where 3.0% ethyl acetate extract produced 72.41% mortality compared to 39.28% with 0.5% methanol extract. These results highlight not only the strong insecticidal activity of *R. cinerea* but also the importance of exposure duration in enhancing its effectiveness. The progressive increase in mortality over 72 hours indicates that the extract may act slowly, possibly through ingestion, accumulation, or interference with larval physiological processes rather than immediate contact toxicity. Although the solvents themselves caused minor mortality (3.3% in both methanol and ethyl acetate controls), this effect was negligible compared to the treated groups, confirming that the larvicidal activity was primarily due to *R. cinerea* phytochemicals. The difference in efficacy between the two solvents reinforces the role of extraction medium, as phytochemicals such as terpenoids, alkaloids, and flavonoids - reported from *R. cinerea*—are known to possess insecticidal, growth-regulating, and feeding-deterrent properties, many of which are efficiently extracted with ethyl acetate (Susmitha et al., 2021). The efficacy levels reported in this study are comparable to or higher than those documented for other botanical extracts against *P. xylostella*. For example, *Azadirachta indica* (neem) and *Calotropis procera* extracts have shown 60–70% larval mortality under similar laboratory conditions, while *Lantana camara* achieved 55–65%. The 72.41% mortality achieved with *R. cinerea* at 3.0% concentration places it among promising candidates for botanical pest control. However, unlike synthetic insecticides, which often provide rapid and near-total mortality but lead to resistance development, botanical extracts such as *R. cinerea* may be more suitable as part of an Integrated Pest Management (IPM) approach (Ngegba et al., 2022). The high efficacy of the ethyl acetate extract also suggests potential synergistic action of multiple phytoconstituents, which could delay resistance development in pest populations. Furthermore, the moderate efficacy of methanolic extracts indicates that *R. cinerea* contains a wide range of metabolites with differential solubility, and future fractionation studies could help isolate the most active principles. Mechanistically, the observed larvicidal activity may be linked to disruption of larval midgut integrity, inhibition of digestive enzymes, interference with hormonal regulation, or neurotoxic effects (Mahmoud et al., 2019). Similar mechanisms have been suggested for plant-derived compounds such as azadirachtin, flavonoids, and terpenes, which affect insect feeding and development. The prolonged activity over 72 hours in this study supports the hypothesis of a cumulative physiological impact rather than immediate knockdown toxicity. In summary, the study establishes *R. cinerea* as an effective botanical candidate for managing *P. xylostella*. The strong larvicidal efficacy of ethyl acetate extracts, particularly at higher concentrations, highlights its potential application in developing eco-friendly biopesticides. However, further studies are required to isolate and identify the active compounds, test their efficacy under field conditions, evaluate formulation stability, and assess safety toward beneficial insects and non-target organisms. Such studies will be critical in advancing *R. cinerea* from laboratory findings to practical use in sustainable agriculture.

Conclusion

The present study establishes, for the first time, the larvicidal efficacy of *Roylea cinerea* leaf extracts against *Plutella xylostella*. Ethyl acetate extracts demonstrated greater toxicity than methanolic extracts, reflecting the influence of solvent polarity on phytochemical extraction. Larval mortality was concentration- and time-dependent, with maximum efficacy (72.41%) observed at 72 hours with 3.0% ethyl acetate extract. The morphometric observations confirmed that the Himachal populations of DBM exhibit developmental traits consistent with global reports, thereby providing valuable baseline data for resistance monitoring and bioassay standardization. The results also highlight that *R. cinerea* performs on par with or better than other botanicals such as neem, *Calotropis procera*, and *Lantana camara*. The mode of action appears to be cumulative, likely involving disruption of physiological processes rather than immediate knockdown. The bioactivity can be attributed to phytoconstituents including terpenoids, alkaloids, and flavonoids, which are efficiently extracted with ethyl acetate. These findings support the integration of *R. cinerea* into eco-friendly Integrated Pest Management programs, while also underscoring the need for further studies on compound isolation, formulation, field efficacy, and non-target safety to advance its use in sustainable agriculture.

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None.

Author contributions

Aakash Rathour: Conceptualization and methodology; Sonia: Formal analysis; Kanika Choudhary and Sarita Pathania: Visualization and validation; Aakash Rathour: Writing original draft; Sunil Kumar, Sarita Pathania and Rakesh Kumar: Reviewing and Editing. Sunil Kumar: Supervision.

Conflict of interests

The authors declare no conflict of interest.

Ethics approval

Not applicable.

AI tool usage declaration

No AI tools have been used in manuscript preparation.

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