A study on pesticidal potential of plant extract of a native species, *Boenninghausenia albiflora* (Hook) to restrain insect pests of forestry in northwest Himalayas

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 Received: 02.01.2026/Accepted: 21.03.2026

ABSTRACT

Forestry plantations in the northwest Himalayas are increasingly threatened by insect pests such as *Agrotis ipsilon* and *Plecoptera reflexa*, which cause severe defoliation and economic losses. The present study evaluated the pesticidal potential of leaf extracts of the native plant *Boenninghausenia albiflora* (Hook) against these two major forest insect pests under laboratory conditions. Leaf extracts were prepared using methanol, acetone and ethyl acetate and their insecticidal efficacy was assessed using the leaf-dip bioassay method. Among the tested solvents, the methanolic extract showed the highest extraction yield (18.58%) and insecticidal activity, causing 60.00 per cent mortality in *P reflexa* and 56.66 per cent mortality in *A ipsilon* at 1 per cent concentration after 72 h. Further bioassay of the methanolic extract at graded concentrations (0.25-1%) demonstrated a dose-dependent increase in larval mortality. The LC_{50} (%) values ranged from 0.81 to 0.89 per cent for *P reflexa* and 0.86 to 0.96 per cent for *A ipsilon*. GC-MS analysis revealed that the extract was rich in bioactive terpenoids, with α-pinene (16.56%), (+)-sylvestrene (16.27%), β-pinene (14.82%) and carvone (6.51%) as the major constituents. These compounds are known to possess insecticidal, repellent and neurotoxic effects against insect pests. The findings demonstrate that *B albiflora* leaf extract possesses significant insecticidal activity and may serve as a promising eco-friendly botanical alternative for the management of forest insect pests in the northwest Himalayan region.

Keywords: *Boenninghausenia albiflora*; Botanical pesticide; *Agrotis ipsilon*; *Plecoptera reflexa*; Insecticidal activity; GC-MS analysis; Terpenoids

INTRODUCTION

Cutworms are a well-known agricultural and garden pest that may infest a broad range of crops, greatly reducing crop productivity and *Agrotis ipsilon* is one of the most common cutworm prevailing species which belongs to the family Noctuidae (Lepidoptera) (Rodingpuia and Lalthanzara 2021). Cutworm larvae are highly destructive and aggressive feeders of leaf buds and stems; if not properly controlled, they can severely damage or even destroy the entire plant. (Fernandes et al 2013). In addition to this, *Plecoptera reflexa* Guenee (Lepidoptera: Noctuidae) is another prominent defoliator of *Dalbergia sissoo* Roxb (Fabaceae) (Roychoudhury and Mishra 2021). According to Beeson (1941), *D sissoo* plantations aged three years or older are highly susceptible to attack by these insect pests. When trees are repeatedly subjected to severe infestation, they remain defoliated for most of the growing season. Due to the substantial economic losses caused by this defoliation, poor-quality plants are often abandoned or replaced, highlighting the need to protect plantations from pest attack. To contain these pests, the use of chemicals as pesticides has been the first preference. However, with the widespread use of chemical pesticides, these insect pests have evolved resistance to a range of insecticides (Ismail 2021). The

dependability and pervasiveness of various pesticides and other organic pollutants has a devastating impact on humans due to their bioaccumulation and high toxicity (Sharma et al 2019). Subsequently, under the current concept of 'green pesticides,' several promising attempts have been made to incorporate eco-friendly substances for pest management like plant extracts, hormones, pheromones and toxins from the organic origin as well as many components of pest management, including microbiological, entomophagous nematodes, plant-derived pesticides, secondary metabolites from microorganisms, pheromones and genes used to alter crops to express insect resistance (Koul et al 2003). Plant extracts or their purified compounds exert a wide range of effects on insects, including toxicity, increased mortality, antifeedant activity, growth inhibition, suppression of reproductive behaviour and reduced fecundity and fertility (Jbilou et al 2006).

One such effect of certain plant extracts is their action on the neurotransmitter systems of pests. Analogues of plant secondary metabolites can interfere with neurotransmitter production, storage, release, binding and reuptake as well as receptor activation, receptor function and enzymes involved in signal transduction pathways (Wink 2000). According to Rattan (2010), monoterpene esters disrupt the insect nervous system by acting on voltage-dependent sodium channels and inhibiting acetylcholinesterase (AChE) activity, resulting in hyperexcitation of the central nervous system and eventual death, as illustrated in Fig 1.

The sources of novel insecticides are medicinal plants and several current medications and biopesticides are made indirectly using medicinal herbs (Ghosh et al 2012). In this view, the Rutaceae family, sometimes referred to as the Rue or Citrus family, is composed of several genera and species that have naturalised in various regions and are currently growing and being crossbred for decorative, culinary and medicinal uses (Panda et al 2019). Most species have aroma glands on their leaves or thorns which are responsible for typical aromatic odours (Yulistyarini and Hadiah 2021). *Boenninghausenia albiflora* (Hook) belongs to a monotypic genus in the family Rutaceae. This deciduous shrub flourishes in tropical and subtropical regions at elevations between 500 and 3,000 m amsl and is distributed across northern Vietnam, China, Bhutan, Nepal, Pakistan, Kashmir, India, Indonesia, the Philippines, Myanmar, Thailand, Laos and Japan (Thorne 1987). The ethnobotanical properties, the presence of essential oils and the increasing demand for natural pesticide sources that are effective, safer for humans and have minimal ecological impact with low environmental risks associated with current synthetic insecticides prompted a comprehensive investigation to be conducted on the insecticidal and repellent potential of *B albiflora* plant extract against *P reflexa* and *A ipsilon* under laboratory conditions.

MATERIAL and METHODS

Collection of plants: The plant samples of *B albiflora* were collected from the forests of Chail (30°59'47.48" N, 77°13'10.34" E), Ghanahatti (31°08'24" N, 77°05'07" E) and conifer forest of Himalayan Forest Research Institute, Panthaghati (31°04'04" N, 77°10'24" E) areas of Shimla district (elevation ranging from 300 to 6,000 m) in the northwest Himalayan region of India (Fig 2). This plant was selected due to its accessibility and its insecticidal and medicinal properties.

Extraction of plant materials: The mature and young leaves of *B albiflora* were collected and separated from the stems and branches. The leaves were then shade-dried for approximately six days at room temperature until they were completely dry. To prepare the extract from dried leaves, the maceration process was utilised using methanol, acetone and ethyl acetate as solvents (1:10% w/v) (Ingle et al 2017). The contents were frequently stirred in an orbital water Bath incubator shaker {Model: MAC-RAWS-45 (R); dimensions: 450 × 450 mm} for about 72 hours at 60°C. The extract solution was filtered using Whatman filter paper with a pore size of 11 µm. A rotary vacuum evaporator (Model: MAC-RVE-3DX) was used to remove the excess solvent from the crude extract. The concentrated extract was transferred to a sterilized amber vial and stored at 4°C in a refrigerator. The percentage yield was recorded for each solvent. Comprehensive metabolic profiling of the plant extract was performed using GC-MS analysis.

Collection and rearing of insect pests: Adult moths of *A ipsilon* were collected using light traps and maintained in containers. They were fed with 10 per cent honey solution. The eggs obtained were incubated at 25°C until hatching. The larvae were kept in separate compartments and the culture was maintained at 25°C with 60-70 per cent relative humidity. The larvae were reared on an artificial diet consisting of casein, wheat germ, dry yeast, cholesterol, agar, Wesson's salt,

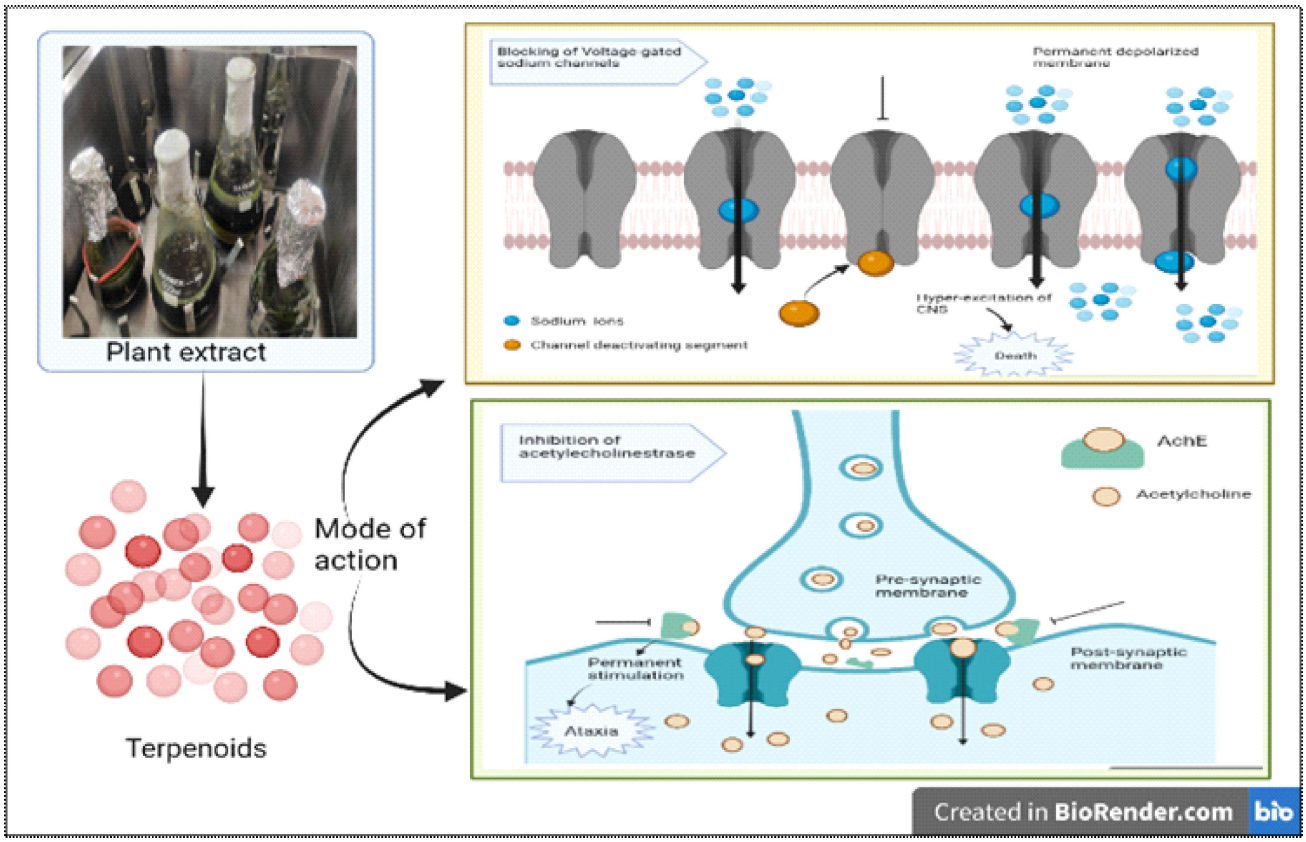

Fig 1. Mode of action of terpenoids on neurological system

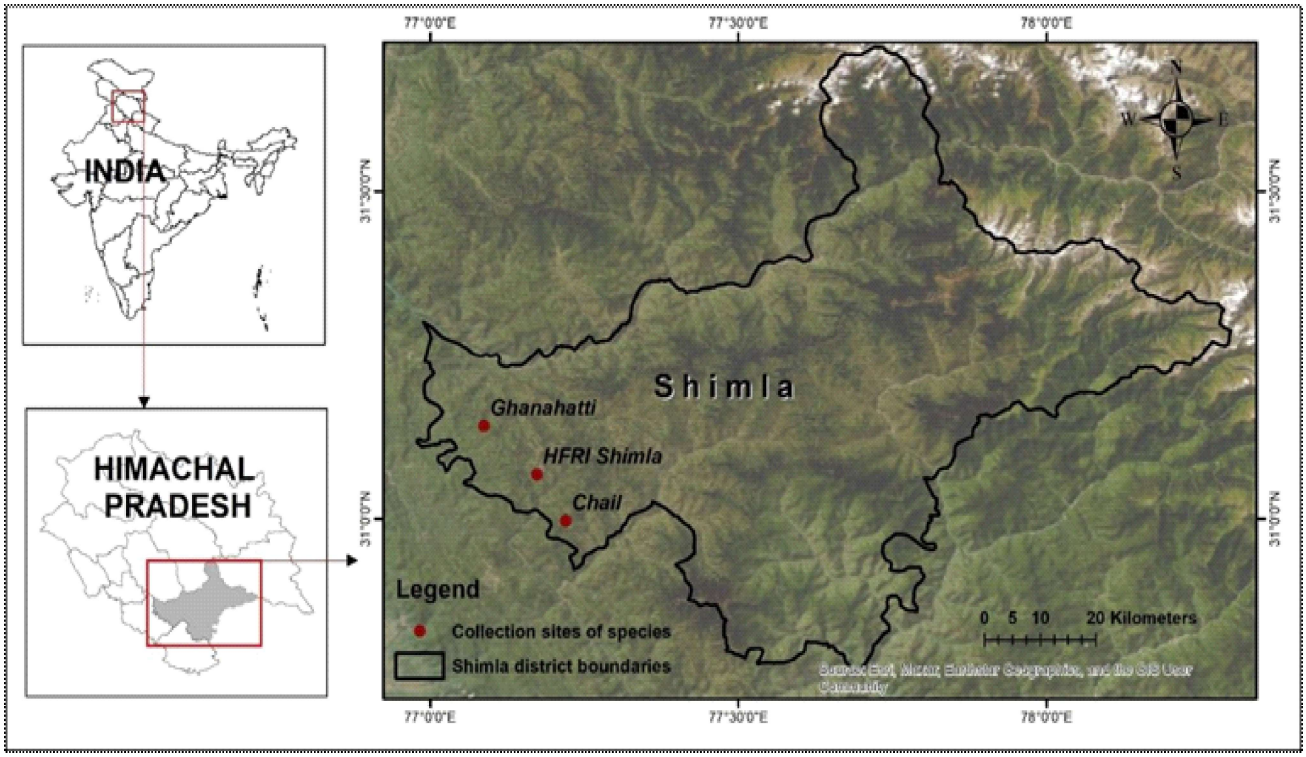

Fig 2. Map of the study area showing the native plant species collection sites

vitamins, antibiotics, fungicides and water (Hansen and Zethner 1979). *P reflexa* larvae were collected from the field and reared in wooden cages on an artificial diet containing Shisham leaf powder along with other commonly available ingredients such as agar, Kabuli gram flour, casein, yeast extract powder, Shisham leaf powder, multivitamin drops with minerals, vitamin E (Evion), ascorbic acid, streptomycin, formaldehyde (10%) and water (Prasad et al 2000). The pupae of both insect pests were separated and incubated in glass jars covered with muslin cloth until adult emergence. The emerged moths were transferred individually to rearing cages (60 × 60 × 90 cm) and provided with cotton soaked in a honey and sugar solution as food.

Bioassay study: The novel crude plant extracts formulated in different organic solvents at a 1 per cent concentration were screened against two insect pests. The leaf-dipping method described by Park et al (2002) was employed to evaluate the efficacy of the crude plant extracts against third instar larvae of *A ipsilon* and *P reflexa*. The experiment was conducted with three replications, each consisting of 10 larvae per treatment for both insect pests at a 1 per cent extract concentration. Larvae that were moribund were considered dead for the purpose of mortality assessment. Mortality observations were recorded at 24, 48 and 72 hours after treatment. Per cent mortality was calculated according to Abbott (1925):

$$\text{Abbott's corrected mortality (\%)} = \frac{\text{Mortality in treatment (\%)} - \text{Mortality in control (\%)}}{100 - \text{Mortality in control (\%)}} \times 100$$

The most susceptible crude extract was selected for bioassay study at different concentrations of plant extract against *A ipsilon* and *P reflexa*.

Plant extracts were evaluated using three organic solvents and the most effective crude extract was selected for further bioassay at lower doses. The experimental setup was similar to that used in the screening phase except for the variation in concentrations. Four concentrations (0.25, 0.50, 0.75 and 1%) of the most effective methanolic crude extract were prepared by diluting a 2 per cent stock solution and tested against each insect pest. The insecticidal activity was assessed using the leaf-dip bioassay method described by Park et al (2002). Leaf discs of the host plant measuring 6 cm in diameter were immersed in the test formulation for 3 minutes, air-dried at room temperature and placed in rearing boxes. A total of five treatments including control were applied with 10 larvae per replication and three replications comprising 30 larvae per treatment. Leaves treated with distilled water were used as the control. Larvae were introduced individually into each box and maintained at room temperature with adequate ventilation. Insecticidal activity was initially assessed after 24 hours of exposure. After the 24-hour period the treated leaf discs were replaced with fresh untreated discs and larval mortality was further monitored up to 72 hours.

Gas chromatography-mass spectrometry identification of active compounds in extracts: Crude samples were analyzed using a gas chromatography system (Thermo Fisher Scientific™ TRACE™ 1300 GC) coupled with a mass spectrometer (Thermo Fisher Scientific™ TSQ™ Duo triple quadrupole GC–MS/MS) equipped with a TriPlus RSH autosampler. A Trace TG-5MS column (40 m length × 0.15 mm internal diameter × 0.15 µm film thickness) was used for the separation of compounds. The GC analysis was performed under the following conditions: (a) injection volume of 1 µL per sample, (b) splitless injection mode, (c) injector temperature maintained at 250°C, (d) initial column temperature set at 60°C and held for 5 minutes followed by a programmed increase to 250°C at a ramp rate of 10°C per minute with a final hold of 15 minutes and (e) helium was used as the carrier gas at a flow rate of 0.7 mL per minute.

The MS analysis was carried out under the following conditions: (a) MS transfer line temperature maintained at 250°C, (b) ion source temperature set at 230°C, (c) electron impact (EI) ionization mode was used and (d) mass scanning was performed over a range of 40-600 m/z with a scan time of 4 minutes. Peak identification was achieved by comparing the obtained spectra with those in the NIST/EPA/NIH Mass Spectral Library version 2.2 (2014).

Processing of the sample for GC-MS analysis (derivatization): Derivatization was carried out using 300 µL of the extracted sample, which was first dried under nitrogen flushing. To the dried sample, 100 µL of dioxane, 10 µL of pyridine, 50 µL of BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) and 50 µL of TMCS (trimethylchlorosilane) were added. The vial

was then placed in a water bath at 70°C for 35-45 minutes with intermittent shaking to ensure proper derivatization. After incubation, the sample was allowed to cool to room temperature and 1 mL of GC-grade methanol was added.

The derivatized sample was subsequently filtered through a 0.20 µm PTFE syringe filter and used for GC-MS analysis. Freshly prepared derivatized samples were used for analysis to ensure accuracy and reliability.

Data analysis: The mortality percentages were calculated according to Abbott (1925). Mortality data from all replicates were subjected to one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test to compare the effectiveness of different plant extract concentrations against the two insect pests. In addition, probit analysis was performed following Finney (1964).

A regression analysis was conducted between the logarithm of extract concentrations and probit values to determine the LC_{50} (%) and chi-square values for larval mortality, which were expressed with 95 per cent confidence intervals including lower and upper confidence limits. Differences were considered statistically significant at $p < 0.05$. Statistical analyses were carried out using NCSS software Version 22.0.5 and Microsoft Excel Version 2301.

RESULTS

Preliminary assessment of efficacy of potent crude plant extract in different polar organic solvents against two insect pests at 1 per cent concentration: Data presented in Fig 3 demonstrate the insecticidal potential of *B albiflora* crude extracts prepared using three polar organic solvents namely acetone, ethyl acetate and methanol.

The highest average mortality of *P reflexa*, a major insect pest of Shisham and *A ipsilon* was observed with the methanolic crude extract, recording 60.00 ± 10.00 per cent and 56.66 ± 5.77 per cent mortality (mean ± standard deviation) respectively at 72 h after treatment.

In comparison, moderate mortality was observed with crude extracts prepared in acetone and ethyl acetate, which were considered less effective when compared with the control. The extraction efficiency of the solvents followed the order acetone <ethyl acetate <methanol as shown in Table 1 along with the physical properties of the plant extracts. The results indicate that methanol was the most effective solvent for extracting biologically active compounds, yielding the highest extract recovery of 18.58 per cent compared to the other solvents. The methanolic extract was translucent, seaweed green in colour with a density of 0.82 g per mL and a pH of 6.8.

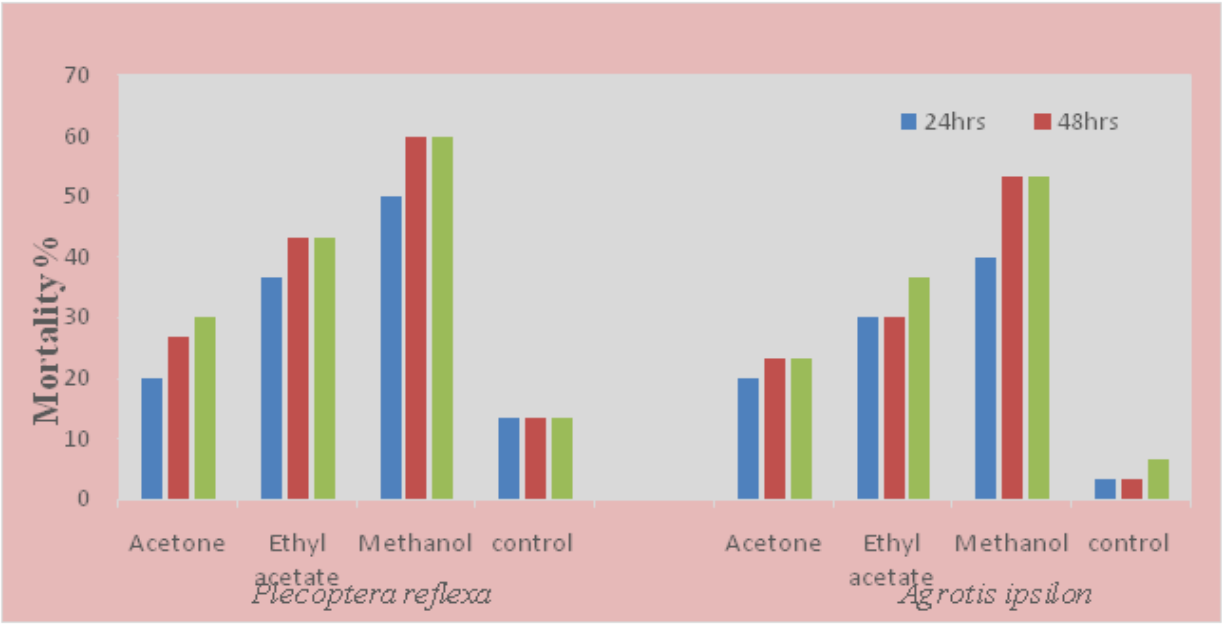

Fig 3. Screening of the novel crude plant extracts formulated in different organic solvents at 1 per cent concentration against two insect pests

Toxicity of methanolic plant extract at different concentrations against *A ipsilon* and *P reflexa*: The information on the larvicidal activity of *B albiflora* leaf extracts against third instar larvae of *P reflexa* and *A ipsilon* after 24, 48 and 72 hours is summarised in Tables 2 and 3. The analysis of responses to leaf extracts revealed that the average mortality of *P reflexa* and *A ipsilon* larvae was especially high in T_7 (0.05% cypermethrin), with 86.66 and 80.00 per cent followed by T_6 (0.5% neem) with mortality rates of 76.66 and 70.00 per cent while in T_5 (1% of plant extract) with mortality rates of 60.00 and 56.66 per cent respectively. The botanical biopesticide formulation of *B albiflora* demonstrated more than 50 per cent mortality at 1 per cent concentration in comparison to the synthetic pesticides so it can be used

Table 1. Physical properties of the *B albiflora* plant extract in different solvents

Solvent	Colour	Odour	Texture	pH	Density (g/ml)	Solubility	Sample (g)	Yield (%)
Acetone	Brown	Pungent	Translucent liquid	7	0.62	Miscible	100	16.12
Ethyl acetate	Olive green	Pungent	Translucent liquid	6.2	0.78	Miscible	100	15.66
Methanol	Seaweed green	Pungent	Translucent Liquid	6.8	0.82	Miscible	100	18.58

Table 2. Mortality rates at different concentration of *B albiflora* plant extract on 3rd instar larval stage of *P reflexa* under laboratory conditions

Treatment	Per cent mortality ± SD after (h)		
	24	48	72
T_1 Control	0.00 ± 0.00	6.66 ± 5.77	6.66 ± 5.77
T_2 (0.25% plant extract)	10.00 ± 0.00	16.66 ± 5.77	20.00 ± 10.00
T_3 (0.5% plant extract)	20.00 ± 0.00	26.66 ± 5.77	33.00 ± 5.77
T_4 (0.75% plant extract)	40.00 ± 10.00	43.33 ± 5.77	46.66 ± 5.77
T_5 (1% plant extract)	53.33 ± 5.77	56.66 ± 5.77	60.00 ± 10 .00
T_6 (0.5% nimbicidine)	66.66 ± 5.77	70.00 ± 10.00	76.66 ± 5.77
T_7 (0.05% cypermethrin)	76.66 ± 5.77	80.00 ± 0.00	86.66 ± 5.77
$CD_{0.05}$	6.29	6.29	8.9

Table 3. Mortality rates at different concentration of *B albiflora* plant extract on 3rd instar larvae of *A ipsilon* under laboratory conditions

Treatment	Per cent mortality ± SD after (h)		
	24	48	72
T_1 Control	0.00 ± 0.00	3.33 ± 5.77	3.33 ± 5.77
T_2 (0.25% plant extract)	10.00 ± 0.00	13.33 ± 5.77	16.66 ± 5.77
T_3 (0.5% plant extract)	23.33 ± 5.77	26.66 ± 5.77	30.00 ± 10.00
T_4 (0.75% plant extract)	33.33 ± 5.77	36.66 ± 5.77	40.00 ± 0.00
T_5 (1% plant extract)	50.00 ± 10.00	53.33 ± 11.54	56.66 ± 5.77
T_6 (0.5% nimbicidine)	66.66 ± 11.547	66.66 ± 11.54	70.00 ± 10.00
T_7 (0.05% cypermethrin)	73.33 ± 5.77	76.66 ± 5.77	80.00 ± 0.00
$CD_{0.05}$	8.12	9.26	7.7

as a novel biopesticide. The analysis of responses to leaf extracts revealed that the average mortality of *P reflexa* and *A ipsilon* larvae was especially high in T_7 (0.05% cypermethrin), with 86.66 and 80.00 per cent followed by T_6 (0.5% nimbicidine) with mortality rates of 76.66 and 70.00 per cent while in T_5 (1% of plant extract) with mortality rates of 60.00 and 56.66 per cent respectively. The botanical biopesticide formulation of *B albiflora* demonstrated more than 50 per cent mortality at 1 per cent concentration in comparison to the synthetic pesticides so it can be used as a novel biopesticide.

The toxicity of *B albiflora* is catalogued in Table 4. At 24, 48, and 72 h after treatment, the LC_{50} (%) values against the *P reflexa* defoliator were 0.89, 0.88 and 0.81 respectively, followed by LC_{50} (%) values of 0.93, 0.96 and 0.86 against *A ipsilon* at the corresponding time intervals, showing a significant effect at $p \leq 0.05$.

Chemical analysis: The GC-MS analysis (Fig 4) indicated that the major constituents of the leaf extract of *B albiflora* were terpenoids. In addition, other classes of compounds such as phenolics, aldehydes, ketones and benzenoid compounds were also detected as summarized in Table 5. The major terpene compounds identified based on per cent peak area were α-pinene (16.56%) and bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene (14.82%). The percentage peak

Table 4. Toxicity of *B albiflora* methanol plant extract against *P reflexa* and *A ipsilon*

Period (h)		*LC_{50} (%) (LCL-UCL)	Chi (Sig)	Regression equation	R^2
24	*P reflexa*	0.89 (1.27-3.62)	7.29 (0.006)#	2.452x+2.81	0.93
	A ipsilon	0.93 (1.01-3.59)	6.63 (0.010)#	2.304x+2.84	0.91
48	*P reflexa*	0.88 (1.42-1.94)	13.83 (0.0002)#	1.688x+3.51	0.99
	A ipsilon	0.96 (1.27-2.46)	9.46 (0.002)#	1.868x+3.28	0.97
72	*P reflexa*	0.81 (1.22-2.19)	10.82 (0.0015)#	1.712x+3.61	0.97
	A ipsilon	0.86 (1.08-2.73)	7.87 (0.005)#	1.912x+3.348	0.94

*Lethal concentration values in % (lower and upper 95% of confidence interval); #Chi values are significant at P <0.05 level

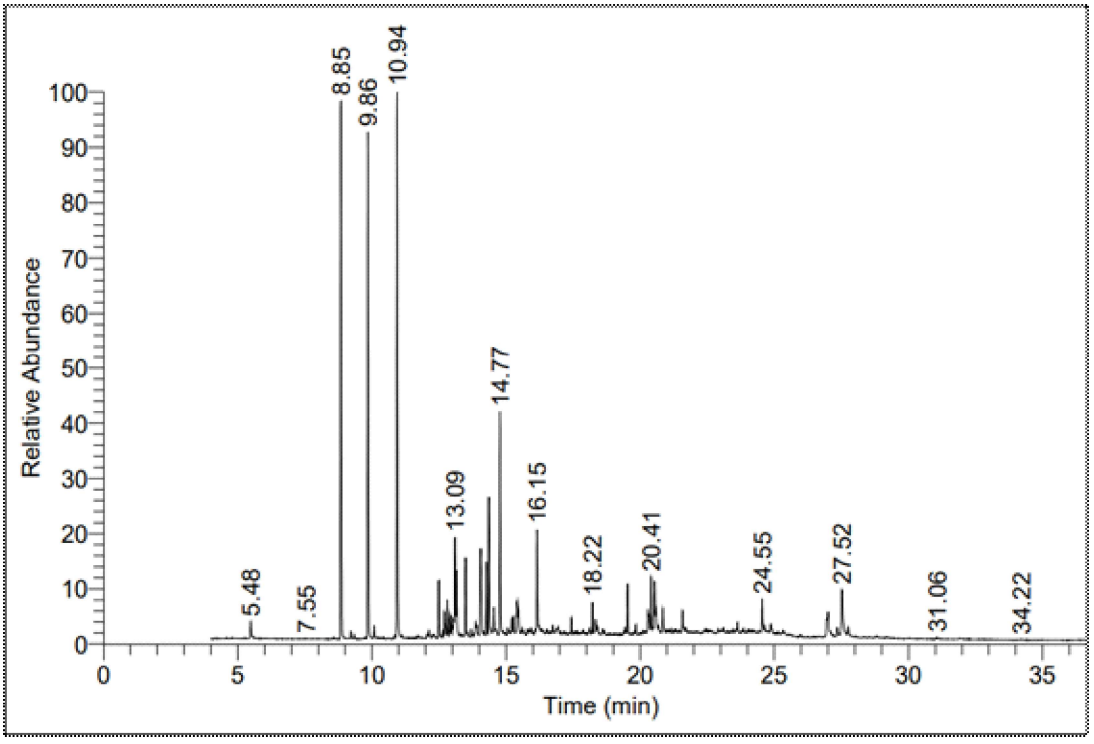

Fig 4. GC-MS chromatogram of *B albiflora* methanol leaf extract

Table 5. Chemical compounds in *B albiflora* methanol leaf extract

Compound name	Nature of compound	RT	Area (%)	Molecular formula	MW
N-Caproaldehyde	Aldehyde	5.48	0.71	$C_6H_{12}O$	100
α-Pinene	Terpene	8.85	16.56	$C_{10}H_{16}$	136
Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene (β-pinene)	Terpene	9.86	14.82	$C_{10}H_{16}$	136
(+)-Sylvestrene	Monocyclic monoterpenoids	10.94	16.27	$C_{10}H_{16}$	136
Camphenol, 6-	Alcohol	12.81	0.97	$C_{10}H_{16}O$	152
L-trans-pinocarveol	Bicyclic monoterpenoids	13.09	2.82	$C_{10}H_{16}O$	152
Trans-verbenol	Bicyclic monoterpenoids	13.16	1.67	$C_{10}H_{16}O$	152
Pinocarvone	Bicyclic monoterpenoids	13.49	2.17	$C_{10}H_{14}O$	150
Trans-p-mentha-1(7),8-dien-2-ol	Menthane monoterpenoids	13.87	0.92	$C_{10}H_{16}O$	152
2-Norpinene-2-carboxaldehyde	Bicyclic monoterpenoids	14.05	4.08	$C_{10}H_{14}O$	150
Verbenone	Bicyclic monoterpenoids	14.26	1.98	$C_{10}H_{14}O$	150
Trans-carveol	Menthane monoterpenoids	14.35	4.07	$C_{10}H_{16}O$	152
(+)-dihydrocarvone	Menthane monoterpenoids	14.55	1.08	$C_{10}H_{16}O$	152
(−) -Carvone	Menthane monoterpenoids	14.77	6.51	$C_{10}H_{14}O$	150
Carvone oxide, cis-	Menthane monoterpenoids	15.28	0.46	$C_{10}H_{14}O_2$	166
Isobornyl acetate	Bicyclic monoterpene	15.41	1.75	$C_{12}H_{20}O_2$	196
Limonen-1,2-diol	Monoterpenoid.	16.15	3.15	$C_{10}H_{18}O_2$	170
4,5-di-epi-aristolochene	Sesquiterpenoids	17.44	0.52	$C_{15}H_{24}$	204
α-copaene	Sesquiterpenoids	18.22	0.82	$C_{15}H_{24}$	204
2,4-Di-tert-butylphenol	Phenylpropanoid	18.34	0.60	$C_{14}H_{22}O$	206
Caryophyllene oxide	Sesquiterpenoid oxide	19.53	1.44	$C_{15}H_{24}O$	220
2,4,6-Tri-isopropylacetophenone	Aromatic ketone	20.41	1.48	$C_{17}H_{26}O$	246
3,4-Diethyl-1,1'-biphenyl	Benzenoids	20.54	2.60	$C_{16}H_{18}$	210
4-tert-Octyl-o-Cresol	Phenol	21.58	0.89	$C_{18}H_{30}O$	206
1-Naphthalenepropanol, 5α-ethenyldecahydro-α,5,5,8a-tetram ethyl-2-methylene-, [1S-[1α(R*),4aα,8aα]]-	Naphthalene (Bicyclic aromatic hydrocarbon)	24.55	1.13	$C_{20}H_{34}O$	290
Androstan-17-one, 3-ethyl-3-hydroxy-, (5α)-	Steroids	27.01	1.88	$C_{21}H_{34}O_2$	318
Osthole	Phenylpropanoids and polyketides	27.52	2.15	$C_{15}H_{16}O_3$	244
Hexacosane	Alkane	31.067	6.60	$C_{26}H_{54}$	366.7

RT = Retention time, MW = Molecular weight

Source: KingDraw Chemical Structure Drawing Software, KingDraw

area of monocyclic monoterpenoids was highest for (+)-sylvestrene (16.27%). Among bicyclic monoterpenoids, the identified compounds and their respective peak areas were 2-norpinene-2-carboxaldehyde (4.08%), limonen-1,2-diol (3.15%), L-trans-pinocarveol (2.82%), trans-verbenol (1.67%), pinocarvone (2.17%), verbenone (1.98%) and isobornyl acetate (1.75%). In the case of menthane monoterpenoids, the detected compounds included (–) -carvone (6.51%), trans-carveol (4.07%), (+)-dihydrocarvone (1.08%), trans-p-mentha-1(7),8-dien-2-ol (0.92%) and cis-carvone oxide (0.46%). Among the sesquiterpenoids, 4,5-di-epi-aristolochene, α-copaene and caryophyllene oxide were present with peak areas of 0.58, 0.82 and 1.44 per cent respectively. Other compounds identified in the extract included hexacosane (6.60%), osthole (2.15%), androstan-17-one, 3-ethyl-3-hydroxy-, (5α)- (1.88%), 4-tert-octyl-o-cresol (0.89%), 3,4-diethyl-1,12 -biphenyl (2.60%), 2,4,6-tri-isopropylacetophenone (1.48%), 1,12 -biphenyl, 3,4-diethyl- (1.24%), 2,4-di-tert-butylphenol (0.60%), camphenol, 6- (0.97%) and n-caproaldehyde (0.71%). The chemical structures of the major identified compounds are presented in Fig 5.

DISCUSSION

The objective of the present study was to evaluate the secondary metabolites and insecticidal efficacy of *B albiflora* plant extract. The findings obtained are consistent with those of Gahlot et al (2018), who reported that both cold and hot extraction using methanol yielded the highest extract recovery from black cutch powder. Similarly, Tandon and Mittal (2018) observed that larval mortality following treatment with *B albiflora* ranged from 33.33 to 66.67 per cent compared with 4.17 per cent in the control. They further reported that adult emergence and fecundity decreased progressively with increasing doses of the oil applied topically. A higher incidence of adult deformities was recorded at elevated doses and was significantly greater than that observed in the control. In comparison with 91.67 per cent adult emergence in the control, only 8.33 per cent emergence was recorded following topical application of 2.5 µL of *B albiflora* oil. Fecundity per female was also lowest at the highest dose, with a mean of 18.50 eggs per female for *B albiflora*. Similar results were reported by Alam et al (2011), who evaluated the toxicological effects of *B albiflora* leaf extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. The mortality rates recorded were 62.5, 60.0 and 57.5 per cent respectively. The LC_{50} (%) value of the extract was determined to be 105 ppm, indicating its significant larvicidal potential. Sharma et al (2006) also demonstrated the insecticidal efficacy of *B albiflora*, reporting effective control of insect pests at concentrations ranging from 1 to 5 per cent (w/v).

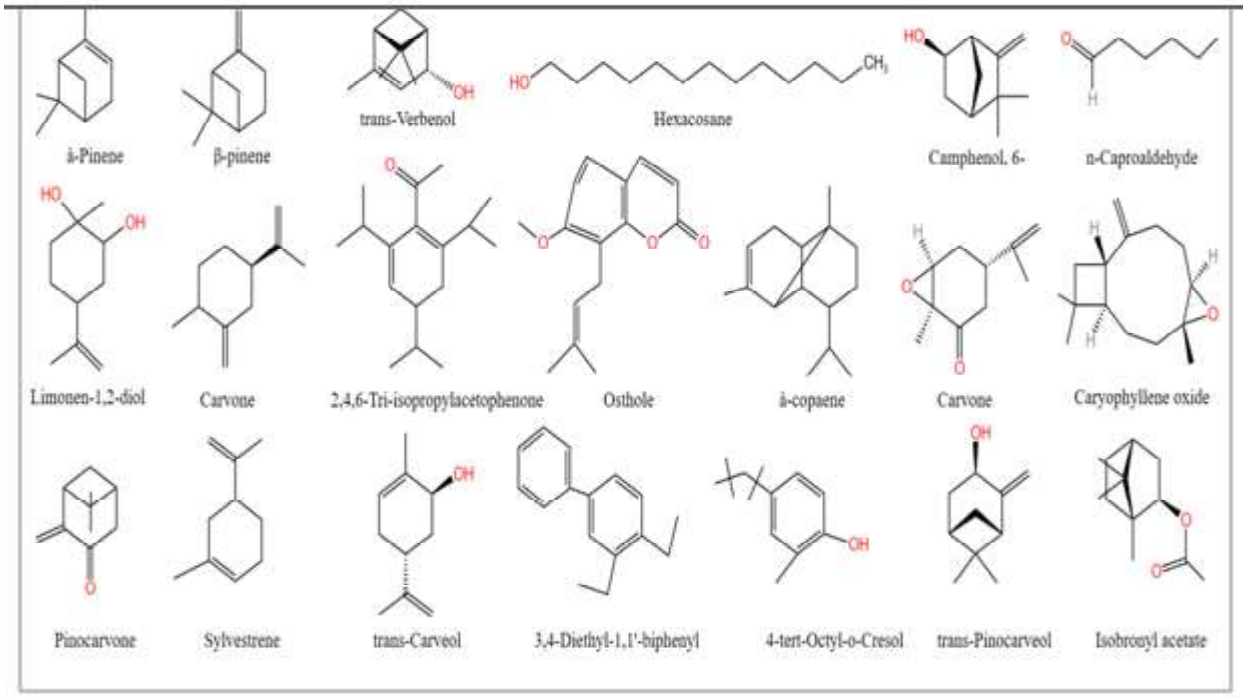

Fig 5. Chemical structures of identified compounds

Liaqat et al (2018) reported that *B albiflora* exhibits strong antibacterial properties and also observed toxic effects in Wistar rats at certain exposure levels. In a related study, Khulbe and Sati (2009) evaluated the antimicrobial activity of various organic and aqueous extracts obtained from the aerial parts of *B albiflora* against several plant pathogenic bacteria including *Bacillus subtilis*, *Staphylococcus aureus*, *Erwinia chrysanthemi*, *Escherichia coli*, *Proteus vulgaris*, *Xanthomonas phaseoli*, *X campestris* and *Agrobacterium tumefaciens*. The results demonstrated significant antibacterial activity at a concentration of 1,000 µg per mL, with zones of inhibition $\geq$10 mm observed in several cases. Furthermore, the extracts exhibited greater antibacterial activity than the standard antibiotics erythromycin (15 µg) and ampicillin (10 µg) at comparable concentrations.

Ranabhat et al (2022) reported that extracts of *Ampelocissus tomentosa* and *B albiflora* exhibited lethality against brine shrimp, with LC_{50} values of 33.11 µg per mL. Similarly, Pragyadeep et al (2017) demonstrated that the ethanolic extract of *B albiflora* possessed significant antioxidant activity with an LC_{50} value of 243.8 µg per mL. The total phenolic content and total flavonoid content were reported as 110.9 and 42.8 µg per mL respectively. The insecticidal efficacy of the extract was further evaluated at three concentrations namely 1, 5 and 10 per cent in ethanol.

Khan et al (2016) observed that methanolic extracts of *B albiflora* collected from the lower Himalayan regions of Pakistan, when tested at a 2 per cent concentration against the peach fruit fly, *Bactrocera zonata*, produced maximum repellency of 44.4 per cent in treated guava fruits. Mehmood and Shahzadi (2014) reported that the insecticidal and repellent effects of essential oils were significantly different from the control at $p \leq 0.05$, with an LC_{50} value of 12.35 µL. A significant dose-dependent response was also observed with an R^2 value of 0.803, confirming the insecticidal and repellent potential of the essential oils. These findings indicate that the compounds identified in the leaf extract possess strong insecticidal properties.

Liao et al (2017) reported that α-pinene (5.86%) present in *Melaleuca alternifolia* essential oil exhibited pronounced antifeedant activity ($AFCL_{50}$ = 8.93 mg/mL) and strong contact toxicity (LD_{50} = 50.28 µg/larva) against *Helicoverpa armigera*. Similarly, Apolinario et al (2020) identified sylvestrene (27.26%) as a major component in the essential oil of *Pilocarpus spicatus*, which demonstrated insecticidal effects on *Dysdercus peruvianus* and *Oncopeltus fasciatus*. Murillo et al (2014) identified myrtenal (2-norpinene-2-carboxaldehyde) in the essential oils of *Piper subtomentosum*, which showed insecticidal activity against second instar larvae of *Spodoptera frugiperda*, with an LC_{50} value of 13.2 µL per L of air. The present findings are also consistent with those of Marouf (2022), who reported that D-limonene (32.38%) was the major constituent in nanoemulsions of mandarin peel oil and marigold extract and caused deformities and feeding inhibition in *Spodoptera littoralis* larvae.

Yang et al (2004) reported that trans-pinocarveol present in *Eucalyptus globulus* essential oil exhibited toxic effects on the eggs and adult females of *Pediculus humanus capitis*.

Giatropoulos et al (2013) detected pinocarvone in essential oils of *Cupressus arizonica*, *Cupressus sempervirens*, *Juniperus phoenicea* and *Tetraclinis articulata*, which exhibited larvicidal and repellent activity against *Aedes albopictus*. In their study, Govindarajan et al (2012) reported that carveol (21.30%) identified in the essential oil of *Mentha spicata* exhibited significant larvicidal activity against *Anopheles stephensi* (LC_{50} = 28.50 ppm), *Aedes aegypti* (LC_{50} = 32.88 ppm) and *Culex quinquefasciatus* (LC_{50} = 35.20 ppm).

Zhang et al (2019) reported that 6-camphenol present in the essential oil of *Artemisia frigida* exhibited insecticidal activity against *Liposcelis bostrychophila*, *Lasioderma serricorne* and *Tribolium castaneum*. Similarly, In a study, Poonsri et al (2015) reported that hexacosane identified in the dichloromethane extract of *Bauhinia scandens* showed strong toxicity against second instar larvae of *Plutella xylostella*, with an LD_{50} value of 2.58 µg per larva. Caballero-Gallardo et al (2023) identified carvone as the most abundant compound in the essential oil of *Lippia alba*, which caused more than 80 per cent mortality at the lowest concentration (2 µL/mL) and exhibited strong repellent activity (RC_{50} = 2.2 µL/mL) against adults of *Ulomoides dermestoides*.

Overall, these studies support the present findings and confirm that the bioactive compounds identified in *B albiflora* leaf extract possess significant insecticidal and repellent potential.

CONCLUSION

The present study confirms that *Boenninghausenia albiflora* leaf extract possesses strong insecticidal activity against two major forest insect pests, *Agrotis ipsilon* and *Plecoptera reflexa*. Among the tested solvents, methanol proved to be the most effective for extracting biologically active compounds, resulting in the highest extract yield and larval mortality. The methanolic extract demonstrated a clear dose-dependent insecticidal effect, with LC… € values below 1 per cent, indicating high potency even at low concentrations.

GC-MS analysis revealed that the extract contains several bioactive compounds, particularly monoterpenes and sesquiterpenes such as α-pinene, sylvestrene, carvone and pinocarvone, which are known to interfere with insect nervous system function, feeding behaviour and survival. These compounds likely contribute to the observed larvicidal activity.

The results suggest that *B albiflora*, a native Himalayan plant species, has significant potential as a natural, eco-friendly botanical pesticide. Its use could help reduce dependence on synthetic insecticides, minimize environmental risks and support sustainable forest pest management. Further studies under field conditions, formulation development and evaluation of long-term ecological safety are recommended to facilitate its practical application as a biopesticide against forest plants.

ACKNOWLEDGEMENTS

The authors express their gratitude to Director General, Indian Council of Forestry Research and Education, Dehradun, Uttarakhand and Director ICFRE – Himalayan Forest Research Institute, Shimla, Himachal Pradesh for providing necessary facilities. The financial support provided by National Compensatory Afforestation Fund Management and Planning Authority (CAMPA), Ministry of Environment, Forest and Climate Change, Government of India, is highly acknowledged.

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***How to cite this article*:** Kumar P, Jaswal A, Thakur TS and Senthilkumar N 2026. A study on pesticidal potential of plant extract of a native species, *Boenninghausenia albiflora* (Hook). to restrain insect pests of forestry in northwest Himalayas. Int J Farm Sci 16(1): 50-62; doi: 10.5958/2250-0499.2026.00007.9