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Insecticidal Activity of *Berberis Royleana*: Various Extracts against *Callosobruchus Maculatus*

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Abstract

The residents of remote areas in developing countries mostly rely on traditional plants to cure different ailments. *Berberis royleana* is a rare species among the genus *Berberis* which commonly comprises 500 species distributed in Nepal, Afghanistan, Siberia, and other parts of the world. *Berberis* are used to make herbal medication to fight against infections, ocular trachoma, AIDS, and diarrhea. The current study was carried out to explore the *in vitro* insecticidal activity of *B. royleana* in various extracts by direct contact application process against the insect *Callosobruchus maculatus.* The maximum mortality rate of 60% was recorded by water fraction, whereas methanolic fraction had 50% mortality, *n*-Hexane had 40% mortality, while ethyl acetate and chloroform both had 30% mortality at 72 hours. Thus, this study indicated that from the medicinal point of view this specie is very significant, with a great potential for insecticidal activities against *C. maculatus*. It is further suggested that *B. royleana* could be used as a good insecticidal agent against dengue and malarial vectors. In addition, the side effects like toxicity could be addressed to avoid any mishaps.

1. Introduction

Plants not only generate fruits and vegetables but they also give significant secondary metabolites which constitute a significant source of therapeutics broadly utilized in pharmaceutical medications [1]. Traditional medicines are widely used globally, particularly in Pakistan, China, Japan, India, Thailand, and Sri Lanka. Approximately 12% of the medicinal plants are used, among which more than 6,000 species of higher plants are reported in Pakistan [2]. Besides these, insects are a serious concern with stored grain that destroyed the amount and quality of the grains. Therefore, higher plants and their

extracts could be utilized to control insects safely. Plant extracts are pure substances that reduce insect reproduction in different ways, including toxicity, growth inhibitors, fertility, antifeedant, and mortality suppression [3]. Beetles and other insect pests are well-known pests for both stored and field legumes around the world. It could damage stored grains, and their product amounts to 20-30% in the tropical region and 5-10% in the temperate region [4]. Synthetic pesticides have some severe flaws including pest resistance and negative impact on the environment and health concerns [5].

B. royleana is a rare species among *Berberis* which is ignored and the flower studies have yet to be identified. Smaller leaves, slender fruits, inflorescence, and pedicels distinguish it from other species in the same genus. *B. royleana* is a deciduous plant that grows up to 3m tall. The roots are quite thick and spread quickly. The stem is reddish-brown in hue with spines. The leaves are typically 7-15mm long and 6-12mm wide. The ripe fruit is oval and red, with a length of around 1cm. The fruits grow in clusters and had a harsh taste. Immature berries are black, pruinose grey, oblong, 8mm long, and 3.5mm wide. Genus *Berberis* are found in the mountainous regions of Pakistan which are about 1400 – 3500m above the sea level [6]. Steroids, tannins, saponins, phenolic alkaloids, alkaloids, flavonoids, terpenes, sugars, and glucosinolates are the active compounds mostly found in *Berberis* species. Berberine, palmatine, berbamine, malic acid, berberuin, oxyacanthine, ascorbic acid, caffeic acid, ursolic acid, beta carotene, and coumarin are some of the possibly bioactive ingredients of *Berberis* species [7-9]. *Berberis* species root's antiperiodic, antipyretic, and diaphoretic activity was comparable to quinine. The shoot has antibacterial, anti-periodic, cardiovascular, hepatoprotective, and anti-cancer properties [10].

In this regard, due to the vast biological activity of *B. royleana*, this study was designed to explore the *in vitro* insecticidal activity of *B. royleana* in various extracts of the s *C. maculatus.*

2. Materials and Methods

2.1 Sample Collection

A healthy *B. royleana* plant was collected from Azad Kashmir, Pakistan in February 2021. This plant was processed in the microbiology laboratory of Abasyn Unversity Peshawar, Pakistan.

2.2 Sample Preparation

The aerial section of *B. royleana* plant was washed with tap water and dried in the shade at room temperature. Using an electric grinder, the dried material was grounded into a powder. For subsequent processing, the powder material was kept at room temperature in polyethylene bags.

2.3 Preparation of Crude Extract of *B. royleana*

B. royleana 1000g powder was dissolved in one-liter methanol and stored at room temperature for seven days. To get the most amount of plant extract, the mixture was agitated at intervals. The Whatman filter paper was used to filter methanol-soluble components and this operation was again repeated after one week. Using a vacuum pump with a rotary evaporator at reduced pressure, the extracts were dried at 45°C. The remaining methanol was evaporated in a water bath at 45°C. The semi-solid crude extract was kept at 4°C for later usage and was kept in sterile bottles.

2.4 Fractionation

B. royleana crude extract (about 55g) was dissolved in 200ml distilled water then shaken with a magnetic shaker until the extract was entirely dissolved. The liquid was poured into a separatory funnel. Different solvents (*n*-hexane, ethyl acetate, and chloroform) were added to the mixture for further fractionation, the mixture was agitated at intervals. The filter paper was used to filter the solvents (*n*-hexane, ethyl acetate, and chloroform) and soluble components. Using a vacuum pump with a rotary evaporator at reduced pressure, the extract fractions were dried at 45°C. The remaining solvents were evaporated in a water bath at 45°C [11, 12].

2.5 Tested Insect

C. maculatus (Beetle) was collected from Nuclear Institute for Food and Agriculture (NIFA) Tarnab, Peshawar, Pakistan and was stored in sterilized plastic jars under optimum conditions for insect growth.

2.6 Screening of Insecticidal Activity against *C. maculatus* (Beetle)

Direct contact application was used to determine the insecticidal activities as described by Rehman et al. [3]. A stock solution was made by dissolving 10mg extract of each fraction (methanol, water, *n*-hexane, ethyl acetate, and chloroform) in 01ml DMSO. Then, a filter paper was cut to fit in the petri dish (09cm in diameter), and 100µg of each fraction was evenly distributed on its surface. For solvent evaporation, the filter papers were air-dried. After drying, about ten *C. maculatus* (beetle) healthy and active insects of the same size were selected and transferred to each petri dish in which the extracts were applied. In control, the same number of *C. maculatus* (beetle) was transferred to a petri dish having pure dimethyl sulfoxide (DMSO) instead of extracts. Then, *C. maculatus* insects on Petri plates were housed at room temperature with appropriate light and dark times. The plates were examined after 24, 48, and 72 hours.

2.7 Data analysis

The obtained results were analyzed by a single ANOVA test using SPSS version 23.0 software while the following formula was used to calculate the percentage of mortality rate:

Mortality % = 100 - (Number of live insects) / (Number of live insects (control)) × 100

3. Results

3.1 Obtained Extracts from *B. royleana*

The obtained, final amount of *B. royleana* crude methanol, water, *n*-Hexane, ethyl acetate, and chloroform solvent extracts after fractionation were listed in Table 1. The mean of all *B. royleana* extract fractions significantly ($p < 0.005$) killed the live *C. maculatus* insects after 24, 48, and 72 hours.

Table 1. Various Obtained Extract Fractions from *B. royleana*

S. #	Extracts	Extracted powder (g)
1.	**Crude methanol**	106
2.	**Water**	55
3.	***n*-Hexane**	0.7
4.	**Ethyl acetate**	12
5.	**Chloroform**	13

3.2 Insecticidal Activity against *C. maculatus* after 24 hours

Insecticidal activity of *B. royleana* against *C. maculatus* showed that the crude methanolic fraction extract had a greater mortality rate of 30%, water fraction extract showed 20% mortality, while *n*-hexane, ethyl acetate, and chloroform fraction extracts showed a 10% mortality rate after 24 hours, as shown in Fig. 1 and Table 2.

3.3 Insecticidal Activity against *C. maculatus* after 48 hours

After 48 hours the insecticidal activity of *B. royleana* extract against *C. maculatus* showed that the crude methanolic fraction and water fraction had a greater mortality rate (40%), while *n*-hexane had the lowest mortality rate of 20%. The other fractions such as ethyl acetate and chloroform had a 30% mortality rate as shown in Fig. 1 and Table 2.

3.4 Insecticidal Activity against *C. maculatus* after 72 hours

After 72 hours the insecticidal activity of *B. royleana* extract against *C. maculatus* showed that the water fraction had a greater mortality rate of 60% which was followed by a crude methanolic fraction which had 50% mortality rate, while *n*-Hexane had a 40% mortality rate, ethyl acetate and chloroform had 30% mortality rate. The negative control (DMSO) did not affect *C. maculatus* (Beetle) as shown in Fig. 1 and Table 2.

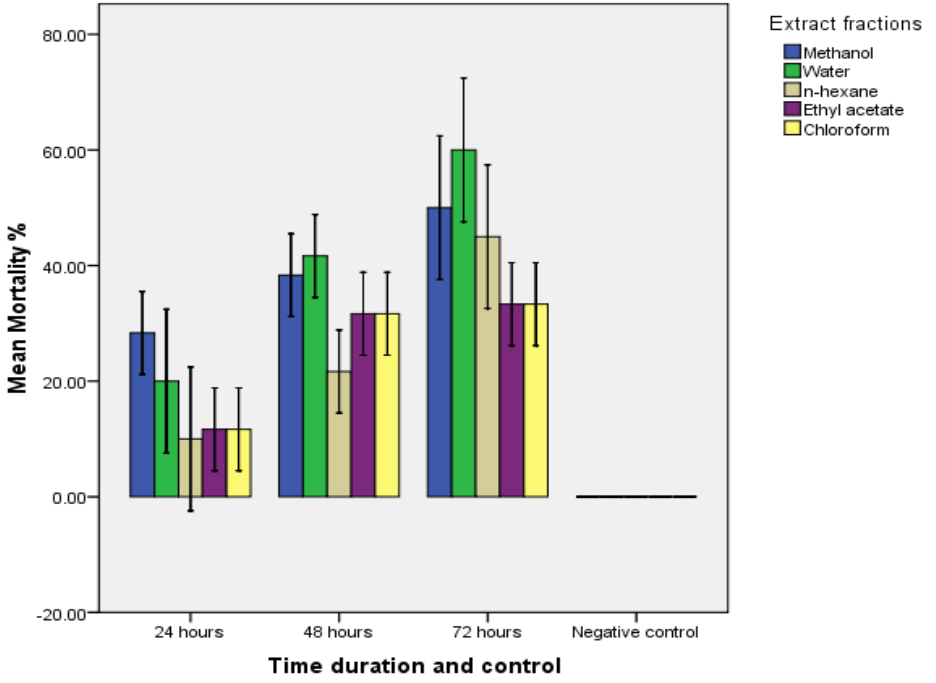

Figure 1. Insecticidal Activity of *B. royleana* against *C. maculatus* after 24, 48, and 72hours ($p < 0.005$)

Table 2. Insecticidal Activity of *B. royleana* against *C. maculatus*

Extract fractions 100 µg	Total insects	24 hours			48 hours			72 hours			Negative control (DMSO)
		Live Insects	Insects killed	Mean Mortality %	Live Insects	Insects killed	Mean Mortality %	Live Insects	Insects killed	Mean Mortality %	
Methanol	10	7	3	30	6	4	40	5	5	50	0%
Water	10	8	2	20	6	4	40	4	6	60	0%
***n*-hexane**	10	9	1	10	8	2	20	6	4	40	0%
Ethyl acetate	10	9	1	10	7	3	30	7	3	30	0%
Chloroform	10	9	1	10	7	3	30	7	3	30	0%

* Mean of all *B. royleana* extract fractions significantly ($p < 0.005$) killed the live *C. maculatus* insects

4. Discussion

Berberis spp. is usually found in the tropical regions of Pakistan and is considered one of the most essential components of both contemporary and traditional treatments [6]. Studies have already been conducted on different species of this genus. *B. baluchistanica* ethanolic extract, butanolic, and ethyl acetate fractions of the berries of this plant were evaluated for potential insecticidal activity in a prior study [13]. Hence, the potential in vitro insecticidal activity of methanolic, ethyl acetate, chloroform, *n*-hexane, and water fraction extracts of *B. royleana* against insects through direct contact was investigated in the current study.

In the present study, the insecticidal activity of different solvent fractions of *B. royleana* extracts (100µg) results are shown against *C. maculatus.* After 24, 48, and 72 hours results showed that the water fraction had the highest mortality rate of (60%) followed by a crude methanolic fraction of (50%), *n*-Hexane (40%), ethyl acetate, and chloroform rate of (30%), respectively. The negative control (DMSO) does not affect *C. maculatus.* In previous studies the ethanolic extract, butanolic, and ethyl acetate fractions of *B. baluchistanica* were no insecticidal activity against *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Trogoderma granarium,* and *Callosbruchus analis* [13]. In contrast, with the methanol extract and fractions of *Chrysophthalmum montanum*, no insecticidal activity was found in all samples against *T. castaneum* and *R. dominica* [14]. Baloch et al. [15] investigated 200 mg methanolic extract and its fractions from leaves of *Thuspeinanta brahuica*. Therefore, crude methanolic extracts showed the maximum percentage of insecticidal mortality against *T. castaneum* with a rate of (87%). Chloroform fraction showed (50%) mortality percentage while other fractions showed less activity *n*-hexane percentage of (42%), and aqueous extract percentage of (30%), whereas Ethyl acetate fraction did not show any activity [15]. Al-Kahraman et al. [16] prepared methanolic extract and its fractions of *Nepeta prateervisa.* Methanol showed maximum mortality against *T. castaneum* and *Sitophilusoryzea* with a percentage of (80%) whereas (70%) percentage was noticed against *Callosbruchus analis*, and *Rhyzopertha dominica* (60%), respectively. Chloroform against *T. castaneum* and *Sitophilusoryzea* showed (70%) percentage and other fractions *n*-Hexane, ethyl acetate, n-butanol, and aqueous showed low mortality percentage below than 50% or no activity was noticed [16]. Previous studies [13, 14] have used the same methodology, same fraction but different plant which do not had any activity but the present study had significant mortality of all fraction against *C. maculatus.* To the best of our knowledge, the current study was conducted in Pakistan for the first time on the insecticidal effects of *B. royleana* extracts against *C. maculatus*. As a result of the good insecticidal effects achieved, *B. royleana* may be investigated against dengue and malarial vectors in the current pandemic situation in Pakistan.

5. Conclusion

This study aims to investigate *B. royleana* insecticidal activity against *C.maculatus*. This current study while highlighting the insecticidal activity of extract and fraction showed the highest activity against *C. maculatus* (Beetle) after 24, 48, and 72 hours water fraction which had a greater mortality rate of 60%, the crude methanolic fraction had 50% mortality, *n*-Hexane had 40% mortality, ethyl acetate and chloroform had 30% mortality,

respectively. This study showed that the extract of *B. royleana* is good for treating insecticidal activities. Although, there are several limitations to this study, therefore, the gaps might be resolved by conducting more research to fill the gap. Due to the positive insecticidal activities, *B. royleana* might be tested against dengue and malarial vectors. Furthermore, adverse effects like toxicity could be treated to avoid any disasters.

Conflict of interest

The authors declare no conflicts of interest regarding this article.

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