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(RESEARCH ARTICLE)

Antifeedant effect of *Brassica nigra* seeds against cotton leafworm *Spodoptera littoralis* and its potential antibacterial activity

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Abstract

Water extract of mustard seeds (*Brassica nigra* L.) at 40, 20 and 10% concentrations were applied to evaluate the bioactivity of mustard seed extract against the 4th instar larvae of cotton leafworm *Spodoptera littoralis*. Increasing concentration of mustard extracts exhibited anti-feedant activity. Antibacterial potential of mustard seeds was determined using the disc diffusion test against 10 bacterial strains; Aqueous and acetone extracts showed very weak or no antibacterial activity ranging from 6.0 to 7.75 mm zone of inhibition (ZI), 6.0 mm is the diameter of the blank disc and means no activity; The methanol extract showed varied degrees of weak antibacterial activity (ranging from 6.5 to 8.75 mm ZI). *B. nigra* could be a good source of insect antifeedant agents.

Keywords: Antifeedant; *Brassica nigra*; antibacterial; *Spodoptera littoralis*

1. Introduction

The current modern life produced many negative impacts on our life style, eating habits, industry, agriculture, environment, medication and healthcare systems. Accordingly, man turned to revitalize the use of natural products in his modern life. Natural products (mostly derived from plants) are of great importance since they have a wide and diverse range of environment-friendly chemical compounds, with numerous beneficial bioactive properties and limited negative impacts or side effects [1]. Moreover, the growing interest in natural products is resulted from frequent failure of modern medicine to control many infections and disorders such as multi-drug resistant infections and immunodeficiency disorders [2], viral infections, diabetes, cancers and many more [3].

Some plants have antifeedant effects or toxicity against insects, such as the cotton leaf worm, *Spodoptera littoralis* (*S. littoralis*) belonging to family Lepidoptera (Noctuidae), one of the most important pests that attacks many economic crops such as sunflower, soybean, spinach, cucumber, barley, ghee, maize, cotton and tomato [4,5]. Using of chemical pesticides has led to many problems, such as development of pest resistance to insecticides and the impact of insecticides on the bio-enemies. Increasing of environmental risks as insecticide residues are represented a major threat to both the health of the human and the environment. Therefore, scientists encouraged to study the range of pesticides that are less dangerous and less costly. Botanical extracts can be used as an alternative method to control the pests that characterized by low toxicity to natural enemies and safe to human [6-8]. On Earth, the number of living species of plants are ranging between 250 000 to 500 000, only a small portion of these species (not more than 10%) are consumed by humans and animals [9]. This fact encourages the scientific community to investigate that largest bioactive source ever known, the plants.

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Brassica nigra (Linn.) Koch. (*B. nigra*), also known as black mustard, is a well-known wild plant of multi-purpose uses. It is belonging to family Brassicaceae, it is an annual herbaceous plant, native to the Middle East and then spread in many regions in the world where cultivated as an agriculture crop, mainly for seeds (mustard seeds) [10]. The seeds are consumed as food and enter in the production of oils, flour, mustard, flavor, vinegar and spices; besides, seeds are rich in nutrients, minerals and vitamins [11]. In traditional medicine, the black mustard seeds are used as laxative, diuretic, stimulant, emetic, appetizer, anti-asthmatic, anti-cough, anti-congestion, anti-neuralgia and many more [11]. This study aimed to investigate the potential antibacterial activity of the black mustard seeds against different bacterial strains and antifeedant activity against and the cotton leafworm, *S. littoralis*.

2. Material and methods

2.1. Plant material and extraction

Seeds of *Brassica nigra* (Figure 1) were purchased from an herbal market from Qassim. After authentication, the dried seeds were ground to a fine powder. For the testing against the larvae of *S. littoralis*, only aqueous extract was used, to avoid possible negative impacts of any alcoholic solvents used during the reconstitution of the crude. Whereas, in the antibacterial testing, aqueous, methanol and acetone was used to determine the nature of the potential antibacterial compounds. 50 grams' form *Brassica nigra (B. nigra)* powder was macerated in 500 ml of 80% methanol (v/v) and another 50 grams from the same powder was macerated in 500 ml of absolute acetone, also 50 grams was added to 500 ml boiling distilled water. Macerates were mixed well and put in a closed, well-tighten glass container and kept in the incubator at 40 °C for up to 3 days (based on the solvent) with frequent vigorous shaking (around 3 time/day). Thereafter, the macerates were filtered using a muslin cloth, followed by filtration with Whatman filter paper No.1. The filtered methanol and acetone extracts were allowed to dry in the incubator at 45 °C for another 3-6 days (based on the solvent), in order to evaporate the solvents.

2.2. Preparation of culture media and working discs

Mueller-Hinton agar was used for the antibacterial susceptibility test. 20 ml of autoclaved Mueller-Hinton agar was poured hotly in a sterile disposable Petri-dish (diameter 90 mm) and left to solidify at room temperature. Working discs were made from Whatman No.1 filter paper. 6 mm discs was punched, put inside dry well tighten Pyrex grass container and autoclaved. Methanol and aqueous extracts were yellowish sticky crude which was reconstituted in 10% DMSO (Dimethyl sulphoxide), to make 250 mg/ml. Acetone extract was yellowish oily crude which was reconstituted in absolute methanol to make two concentrations; 50% and 25% (v/v). Methanol and 10% DMSO did not show any growth inhibition for bacteria at the pre-experimental phase.

2.3. Referenced bacterial strains

Ten referenced bacterial strains were used in the current investigation, including Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus saprophyticus* ATCC 43867, *Streptococcus pneumonia* ATCC 49619 and *Bacillus cereus* ATCC 10876) and Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Proteus vulgaris* ATCC 6380, *Klebsiella pneumonia* ATCC 27736 and *Shigella flexsneri* ATCC 12022).

2.4. Antibacterial susceptibility test

The susceptibility of bacterial strains against acetone, methanol and water extracts were evaluated using agar disc diffusion method [12]. Briefly, bacterial strains were cultured for 18-24 hours in Mueller-Hinton broth, the fresh bacterial suspensions were adjusted to 0.5 McFarland standard by dilution with sterile normal saline. 20 ml of autoclaved Mueller-Hinton agar was poured in sterile Petri dish and left at room temperature until solidified, and then inoculated by transferring 100 µl of adjusted bacterial suspension to the seeded plate and spread over the agar using sterile cotton swap. Two groups of discs were impregnated with methanol and aqueous extracts and were put over the surface of the inoculated agar plates. A blank disc impregnated with chloramphenicol (5 mg/ml) was also placed on the agar plate to serve as positive control. Plates were incubated overnight at 35-37 °C. Figure 1, Summarizes the antibacterial testing of *B. nigra* seeds, all tests were performed in duplicate. The sensitivity of the bacterial strains is determined by the inhibition zone diameter (IZ), and the results were expressed according to the following criteria:

- IZ >15 mm: the extract has a high inhibitory action;
- 10 mm ≤IZ ≤15 mm: the extract has a moderate inhibitory action; and
- IZ <10 mm: the extract has a weak inhibitory action.

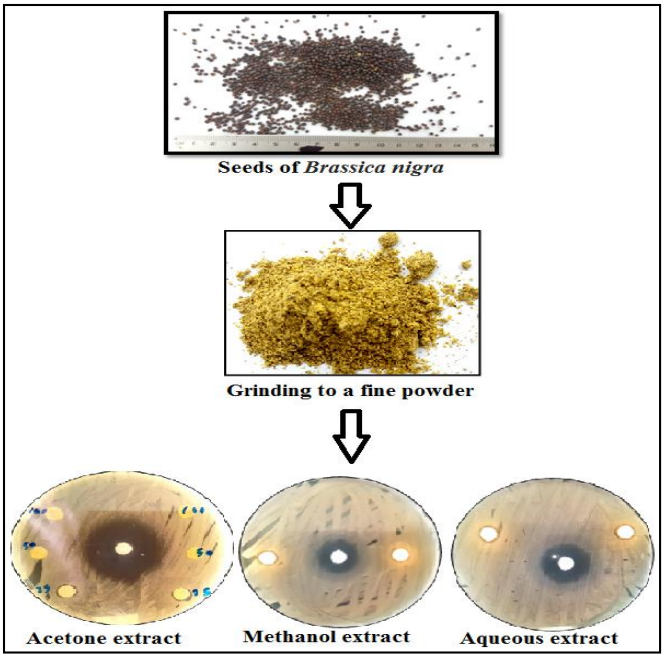

Figure 1 The antibacterial testing using disc diffusion method

2.5. Insects

The tested larvaewere obtained from a population of *S. littoralis* which fed withfresh castor leaves, *Ricinus communius* under laboratory conditions (26± 2 °C and 65±5 % R.H., with 8:16 L:D h photoperiod). This experiment was performed on newly-molted 4th instar larvae. Various concentrations were prepared (40, 20 and 10%) by dilution in distal water. Disks to which only the distal water had been applied were used as the control.

2.6. Insect treatment

250 larvae were left without food before the experiment for 3 h, then divided into 5 groups of 50 larvae each, three different concentrations of water extract (*B. nigra*), one group for the control and one group as starved larvae. Adequate discs of castor bean leaves were rinsed in different concentrations. The leaf discs sprayed with water served as control. The treated and untreated leaf disks were left at rest for 10 min to allow the solvent to evaporate. All larvae were weighted before and after treatment for 3 days. The dried leaves were placed individually in plastic Petri dishes. Afterwards, ten larvae were transferred into each petri dish and allowed to feed on the treated and untreated leaves, the starved larvae were left without feeding for 24 h. Five replicates for each treatment were performed. The starvation percentages of tested larvae were calculated [13, 14].

$$Starvation\ (\%) = \frac{C - E}{C - S} \times 100$$

Where: C = Mean weight gain of untreated larvae after 24 h; E = Mean weight gain of treated larvae for each concentration after 24 h; and S = Mean weight gain of starved untreated larvae after 24 h.

The antifeedant index (AFI) was calculated based on the formula of Sadek [15].

$$Antifeedant\ index\ (\%) = \frac{C - T}{C + T} \times 100$$

Where: C: the amount of food consumed (leaves) in the control; and T: the amount of food consumed (leaves) in the treatment.

2.7. Statistical analysis

Mean, standard error of means and graphs were performed using SPSS package, version 15. One-way analysis of variance ANOVA was used and $p < 0.05$ was used in testing the statistical significance.

3. Results and discussion

Seeds of *B. nigra* were tested for its potential antibacterial activity using disc diffusion method. Results are represented in (Table 1) and (Table 2), (Figures 2) and (Figure 3); The aqueous extract of *B. nigra* seeds revealed no antibacterial effect against most tested bacteria, except with some gram-positive bacteria, which recorded very weak effect (ranging from 7 to 7.25 mm IZ) against *Staphylococcus aureus, Staphylococcus epidermidis and Bacillus cereus,* compared with the standard antibiotic (chloramphenicol); The methanol extract was much better in antibacterial activity than the aqueous extract, although the results of the methanol extract were within the range of weak inhibitory action, the mean IZ was ranging between 6.5 to 8.75 mm. The interesting point here is these weak activities was not restricted to the gram-positive bacteria as with the aqueous extract. Acetone extract was oily in nature and recorded weak or no antibacterial activity against tested bacteria. Surprisingly, the more diluted extract (25% v/v) the more antibacterial effect. Accordingly, acetone extract at concentration 25% showed inhibitory effects more than the acetone extract at concentration 50%, although all results (IZ) were still within the range of weak inhibitory action (6 - 7.75 mm IZ) (Table 1 and Figure 2). The current results are in contradiction with many previous studies which recorded good antibacterial activity from the seeds of *B. nigra*. However, these previous studies showed noticeable variations in the antibacterial results. Tomar and Shrivastava [16] reported that the ethanol extract of *B. nigra* seeds was highly effective as antibacterial agent against *Staphylococcus aureus* (25 mm IZ) and *Escherichia coli* (20.5 mm IZ), using the cup-plate diffusion method.

Table 1 The antibacterial activity of the methanol and aqueous extracts of *Brassica nigra* seeds

Microorganism		**Tested compound**		
		Methanol extract (250 mg/ml)	**Aqueous extract (250 mg/ml)**	**Chloramphenicol (5 mg/ml)**
		Zone of inhibition in mm		
Gram-positive bacteria	Sa	8.25 ±0.25	7.0±0.0	20.0±0.0
	Se	8.5±0.5	7.25±0.25	28.5±0.5
	Ss	7.5±0.5	6.0±0.0	31.0±0.0
	Sp	8.5±0.5	6.0±0.0	19.0±1.0
	Bc	7.5±0.5	7.0±0.0	31.0±1.0
Gram-negative bacteria	Ec	7.25±0.25	6.0±0.0	19.0±1.0
	Pa	6.5±0.5	6.0±0.0	17.5±0.5
	Pv	7.5±0.5	6.0±0.0	19.0±1.0
	Kp	8.75±0.25	6.0±0.0	20.5±0.5
	Sf	7.5±0.5	6.0±0.0	20.0±0.0

*6.0 mm=No activity (Diameter of the paper disc), Sa= *Staphylococcus aureus* ATCC 29213, Se= *Staphylococcus epidermidis* ATCC 12228, Ss= *Staphylococcus saprophyticus* ATCC 43867, Sp=*Streptococcus pneumonia* ATCC 49619, Bc= *Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 25922, Pa=*Pseudomonas aeruginosa* ATCC 9027 Pv=*Proteus vulgaris* ATCC 6380, Kp=*Klebsiella pneumonia* ATCC 27736, Sf=*Shigella flexsneri* ATCC 12022.

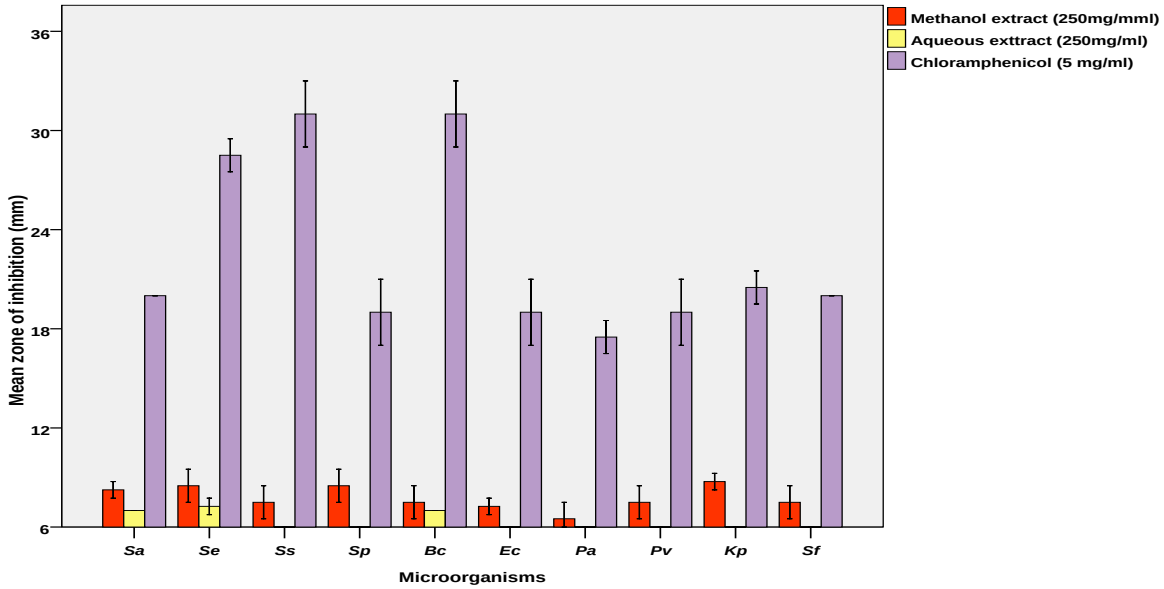

Figure 2 Comparison between antibacterial efficacy of methanol, water extracts and chloramphenicol

Table 2 The antibacterial activity of the Acetone extract of *Brassica nigra* seeds

Microorganism		**Tested compound**		
		Acetone extract (50% v/v)	**Acetone extract (25% v/v)**	**Chloramphenicol (5 mg/ml)**
		Zone of inhibition in mm		
Gram-positive bacteria	Sa	6.0±0.0	7.0±0.0	20.0±0.0
	Se	6.0±0.0	7.0±0.0	28.5±0.5
	Ss	6.5±0.0	7.5±0.5	31.0±0.0
	Sp	6.0±0.0	7.0±0.0	19.0±1.0
	Bc	6.5±0.0	7.75±0.25	31.0±1.0
Gram-negative bacteria	Ec	6.0±0.0	7.75±0.25	19.0±1.0
	Pa	6.0±0.0	6.0±0.0	17.5±0.5
	Pv	6.0±0.0	6.75±0.25	19.0±1.0
	Kp	6.0±0.0	6.75±0.25	20.5±0.5
	Sf	6.0±0.0	6.75±0.25	20.0±0.0

*6.0 mm=No activity (Diameter of the paper disc), Sa= *Staphylococcus aureus* ATCC 29213, Se=*Staphylococcus epidermidis* ATCC 12228, Ss=*Staphylococcus saprophyticus* ATCC 43867, Sp=*Streptococcus pneumonia* ATCC 49619, Bc= *Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 25922, Pa=*Pseudomonas aeruginosa* ATCC 9027 Pv=*Proteus vulgaris* ATCC 6380, Kp= *Klebsiella pneumonia* ATCC 27736, Sf=*Shigella flexsneri* ATCC 12022.

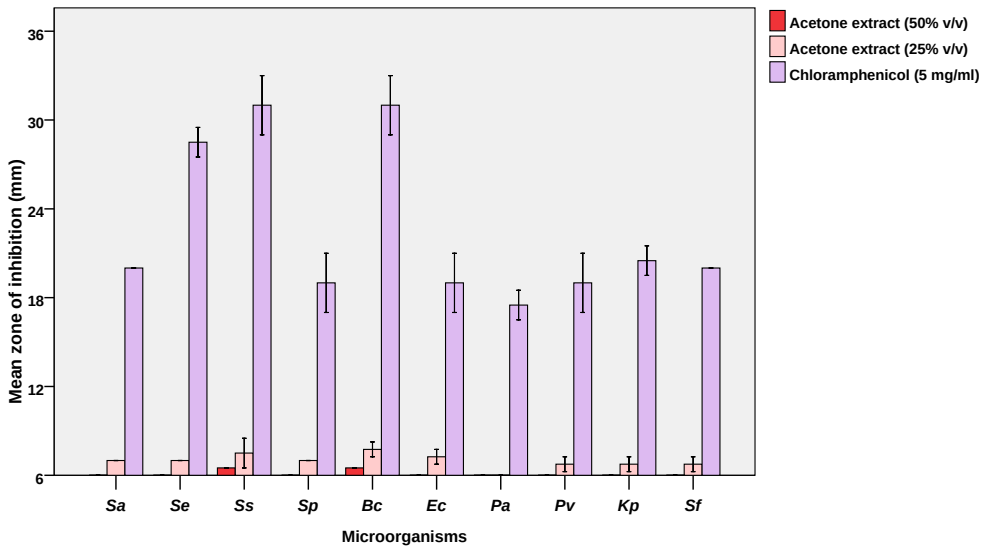

Figure 3 Comparison between antibacterial efficacy of acetone extracts and chloramphenicol

The antifeedant potential results of the aqueous extract of *B. nigra* are shown in (Table 3) and (Table 4), The antifeedant effectiveness of water extract that obtained from *B. nigra* seeds on the 4th instar larvae of *S. littoralis* was tested. Significant differences in antifeedant activity are shown in (Table 3). The antifeedant activity varying from 21.90%, 37.38%, 44.57%, at 10, 20 and 40% concentrations, respectively. Increasing in antifeedant index indicated decreased rate of feeding. Antifeedant activity was evaluated based on leaf area consumed by *S. littoralis*. Table 4 shows the starvation percentage of the 4th instar larvae of *S. littoralis* treated with the water extract of *B. nigra* seeds. The starvation percentage increased with the increasing of the concentration during 72 hours. The maximum antifeedant activity was recorded in 40 % concentration. Plants have phyto-compounds as natural products that possess plant protection properties against insect pests. Plant extracts of *A. indica*, *Citrus sinensis*, *Vitex negundo* and *Zingiber officinale* have antifeedant and growth inhibition activity against *Spodoptera litura*. Additionally, the root extracts of *Pedalium murex* showed deterrent effects [17]. The essential oils of some medicinal plants possess antifeedant property against *S. litura* [18]. An antifeedant activity of ethyl acetate leaf extract of *Pergularia daemia* against *S. litura* has been recorded [19]. Rheine that isolated from the flower of Cassia fistula showed significant antifeedant activity against lepidopteran pests *Spodoptera litura* and *Helicoverpa armigera* [20]. It was reported that the crude oil from *S. lappa* reduced leaf damage that caused by the larvae of *Spodoptera litura* [21]. The plant extract of *S. costus* may be useful for effective control of *S. littoralis* at larval stages. The present investigation suggests that usage of botanical extract may be useful as an effective strategy for controlling the economically important insect pests such as *S. littoralis* at larval stages and could possibly reduce the load of synthetic pesticides, thereby safeguarding the environment from hazards.

Table 3 Antifeedant activity of water extract of *B. nigra* seeds against 4th instar larvae of *S. littoralis*

Concentration of water extract of *B. nigra* seeds	Days post-treatment			Mean*
	1st	2nd	3rd	
	Antifeedant index (%)±SE			
10%	29.27± 4.34^{cd}	19.71± 2.26^{c}	16.73± 2.17^{bc}	21.90 %
20%	38.87± 2.26^{e}	41.07± 5.30^{a}	32.20± 4.63 ^{c}	37.38 %
40%	49.45± 5.42^{de}	45.08± 3.21^{c}	39.02± 4.09^{a}	44.57%

Data are expressed as mean ± SE(n=5), * total mean of each treatment at different time intervals, values were analysed by one-way ANOVA, where means within each column followed by different letters are significantly different (P< 0.05 by LSD).

Table 4 Starvation percentage (%) of the 4th instar larvae of *S. littoralis* treated with the water extract of *B. nigra* seeds

Treatments	Time in hour	Average weight (mg/larva)	Difference* (mg/larva)	Starvation (%)	Average
10%	0	64.01	--------	-------	24.63%
	24	66.42	+2.41	52.55	
	48	75.20	+11.91	31.92	
	72	82.02	+18.08	26.65	
20 %	0	60.33	--------	-------	47.21%
	24	62.51	+2.18	54.30	
	48	65.65	+5.32	50.15	
	72	74.21	+13.88	35.61	
40 %	0	55.80	--------	-------	45.28%
	24	58.03	+2.23	53.92	
	48	60.43	+4.63	52.06	
	72	71.11	+15.31	32.50	
Control	0	53.50	--------	-------	-------
	24	62.80	+9.30		
	48	76.95	+23.45		
	72	83.80	+30.30		
Starved larvae	0	56.02	--------	-------	-------
	24	52.21	-3.81		
	48	43.32	-12.70		
	72	40.21	-15.81		

4. Conclusion

Mustard seeds (*Brassica nigra* L.) are a famous economic plant product, with many uses, particularly in the food industry. The present study explained that among aqueous, methanol and acetone extracts, the methanol extract showed some degree of weak antibacterial activity. In contrast, the aqueous extract of mustard seeds has a good antifeedant effect on Cotton leafworm (*Spodoptera littoralis*), which suggest further future studies to isolate the bioactive antiffedant compounds as a potential natural agent for pest control.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have declared that no competing interest exists.

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