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Research Paper

Albizia odoratissima Bark has Insecticidal Activity Against the Cabbage Butterfly *Pieris brassicae*

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This study reports the toxicity of *Albizia odoratissima* (a plant used by indigenous farmers) to the 3rd instar larvae of *Pieris brassicae* – a major pest of cruciferous crops. About 53% larval mortality was recorded after 72 h exposure to 4% bark powder. Methanolic extract of the bark caused almost two times more mortality than that caused by the bark powder. The histo-pathological damage observed on the midgut of the treated insect larvae included loss of continuity of the brush border, partial or total loss of cellularity, and damage of the muscular conjunctive system covering the mid-gut epithelium and breakage of epithelium. The methanolic extract caused more acute damage than the powder.

Key Words: Biopesticide; Traditional Knowledge; Bioassay; Insect Mortality; Mid-gut; Muscular Conjunctive System

1. Introduction

There has been growing concern in recent years that pesticides (chemical) constitute a potential risk to the well being of nature and natural resources including human [1-3]. The importance to develop alternative ecofriendly pest control methods with a sense of urgency has been advocated by many [4, 5]. In this regard investigations into traditional pest management techniques and practices of yesteryears have started to gain importance [6-8]. Indigenous knowledge is the knowledge developed outside conventional laboratories by people without any formal or very low level of education. There is increasing evidence that traditional practices can contribute to development of sustainable and ecofriendly pest management methods [9, 10]. Thus, the objective of this paper is to evaluate the effectiveness of a traditional pest management practice prevalent in parts of North-East India.

Albizia odoratissima Benth (Fabaceae) is a medium-sized tree which occurs naturally in Southern China, Burma, Peninsular India, and Tropical Africa. It is mainly valued for shade and soil improvement, particularly in tea plantations [11]. Besides its known commercial uses, the powder of *A. odoratissima* bark is used as insecticide by the indigenous 'Garo' tribe of Meghalaya, India in their traditional farming [10]. The bark of *A. odoratissima* is reported to be acrid and astringent in nature [12]. However, empirical studies on the insecticidal properties of the bark are virtually absent. It is needless to mention here that several plants used in traditional pest management are reported to possess insecticidal, repellent or antifeedant properties [13, 14].

A basic requirement in determining insecticidal properties of plants has been the appraisal of the effect of plant extract on target organisms through bioassays. A series of bioassays were carried out against the cabbage white butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae) – a major pest of cabbage and other cruciferous crops. The caterpillars eat almost whole of the leaves leaving only the large veins thus causing heavy economic loss. It has been

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reported that aqueous extract of plant materials are more effective than the powder form [15, 16]. Further, available histo-pathological evidence indicates that the insect gut is one of the major sites of action of pesticides [17-19].

The present study, addressed three questions i) whether *A. odoratissima* bark is effective as insecticide, ii) if effective, which form is more effective-powder or methanolic extract, and iii) what is the nature of damage caused by the material in the insect mid-gut.

2. Materials and Methods

Insects

Laboratory colonies of the cabbage white butterfly, *P. brassicae* L. (Lepidoptera: Pieridae) were established with eggs collected from a vegetable field. The colonies were maintained on 2 week old potted seedlings of cabbage, *Brassica oleoracea* var capitata (Cruciferae). The potted plants were housed in screened cages (1 m x 1 m x 1 m) and were maintained at 17-20ºC. The taxonomy of eggs and adults were confirmed with the help of Entomology Division, Indian Council of Agricultural Research (ICAR) Research Complex for North Eastern Hill (NEH) Region, Umiam, Meghalaya.

Preparation of Bark Powder

Barks of *A. odoratissima* were cut into small pieces and air dried for 2 days. In the laboratory, the bark pieces were dried completely in a room prefumigated with 2% formalin to avoid chances of any infection. The bark was ground to powder. Prior to grinding, the bark pieces were kept overnight in an oven at 25°C, for ease in grinding. The preparation thus obtained was equivalent to the bark powder used by the original practitioners.

Preparation of Bark Extract

Methanolic extract was prepared by extracting the dry bark powder in methanol and water (4:1). Methanol is known to extract a wide range of both polar and non-polar compounds from plant materials [20]. Twenty five g dry powder was added to 250 ml of the solvent, the mixture was sonicated in an Ultrasonic Sonicator (Econo-Clean) for half an hour, left for overnight and filtered through a cotton plug. The filtrate was again subjected to filtration through filter paper (No. 1). The filtrate was evaporated to dryness by keeping in an oven at 25°C. The dry extract thus obtained was mixed with solvent to prepare a 10% (w/v) stock solution. Working solutions of the desired concentrations were prepared afresh prior to application.

Bioassay With Bark Powder (Dust Spray Method)

Fresh cabbage leaves of small size (150±2 mg) were washed thoroughly under tap water, rinsed with double distilled water and wiped with tissue paper to remove the adhered water. The leaves were then sprayed separately with 1, 2 and 4% (w/w) of *A. odoratissima* bark powder using a hand duster, just to mimic the traditional practice. There was no spray in the control leaf. The treated leaves were then placed in petri dishes (9 cm diam) and 5 freshly molted laboratory reared 3rd instar *P. brassicae* larvae were introduced on each leaf. The petri dishes containing the treated leaves were placed in an incubator at 19±2° C, 50-70% RH, and a photoperiod of 12:12 (L:D). To avoid leaf desiccation, moist cotton was wrapped around the petiole and kept moist by adding drops of double distilled water daily. Each treatment was replicated four times.

Bioassays With Bark Extract (Leaf Dip Bioassay)

Freshly collected cabbage leaves were washed thoroughly with distilled water and wiped with tissue paper to soak the water. Leaf discs of 7 cm were cut with a cork borer and were immersed for 5 min in 1, 2 and 4% (v/v) aqueous extract of *A. odoratissima* bark. These concentrations were freshly prepared from the stock solution. The control leaf discs were immersed in a mixture of only methanol and water (4:1). Each disc was placed in a petri-dish (9 cm diam.) and the edges of the discs were lined with moistened tissue paper. 5 freshly molted 3rd instar laboratory reared *P. brassicae* larvae were transferred on each disc. The petri dishes containing the treated leaves and larvae were placed in an incubator at 19±2°C, 50-70% RH, and a photoperiod of 12:12 (L:D). Each treatment was replicated four times.

Data Collection and Analysis

Evaluation of experimental treatments was made by recording larval mortality at 24 h interval over 3 days after initiation of the experiment. A larva that failed to move after repeated touching was considered dead.

The percent insect mortalities were corrected using Abbott's transformation [21]. LC_{50} (lethal concentration causing 50% mortality) was calculated using Probit analysis [22]. Estimated LC_{50} values were considered significantly different when their 95% confidence intervals (CI) did not overlap. ANOVA was performed on the arcsine square root transformed (for variance stabilization) corrected percentage mortalities and the difference between the treatment means were compared with Least Significant Difference (LSD) test to determine the significance. All the statistical calculations and analysis were done using the statistical software, SPSS, version 10.0.1(SPSS Inc., Illinois, USA). A P value < 0.05 was considered significant in all the statistical analysis.

Histopathological Studies

The effect of *A. odoratissima* bark (powder and extract) on the mid-gut structure of 3rd instar *P. brassicae* larvae was investigated using histology. At the end of each observation, the dead larvae were dissected; mid-guts were removed and fixed in Bouin's fluid. Mid-gut from the untreated larvae were also removed and fixed separately. Histological samples were prepared using routine methods [23]. After thoroughly washing under tap water, the samples were gradually dehydrated in a series of ascending grade of alcohol up to 100%, with two changes (15 min each) in each concentration. The samples were cleared of alcohol using xylene and then infiltrated with wax by passing the specimen through xylene-wax mixture in the order (3:1, 1:1, and 1:3) and lastly passed through two changes of pure wax in order to facilitate proper penetration of the wax into the tissue. Sections (6-8 µ thick) were cut from the prepared blocks using a Senior Rotary Microtome (Radical Instruments, Haryana, India).

The sections were de-waxed in xylene and gradually hydrated up to distilled water and stained with aqueous haematoxylene (5-7 min). The sections were then dehydrated up to 70% alcohol, re-stained with alcoholic eosin and dehydrated up to absolute alcohol (10 min) to remove traces of wax and de-alcoholised in xylene. The histo-pathological preparations were examined with the DMRX Image Analysis System, LEICA Q600 (Leica UK Ltd., UK) using the transmission mode.

3. Results and Discussion

The 3rd instar larvae of *P. brassicae* showed some interesting responses when exposed to *Albizia* bark (powder as well as extract) treated cabbage leaves. In the treated ones, they avoided feeding for sometime whereas in the untreated leaves, the feeding was instant. However, even in the treated leaves the larvae resumed feeding after sometime. After 48 h of exposure, the larvae displayed some peculiar behaviour. They were seen to stand on the lower part of their abdomen with the head and thorax curled. Further, rigorous lateral movement of the curled head and thorax was also observed.

Effect of Bark Powder

Exposure to *A. odoratissima* bark powder sprayed cabbage leaves resulted in some mortality in the 3rd instar larvae of the cabbage butterfly, *P. brassicae*. 1% (w/w) of the bark powder caused 21.3% mortality in 72 h, 2% powder caused 26.3% in 72 h while 4% of the powder caused 52.5% mortality in 72 h (Table 1). The larval mortality was found to increase over time and with increasing concentration and the differences in the mortality was found to be significant at P<0.05 (Table 2) in comparison to the control mortality which was only 5% at 72 HAS.

Effect of Bark Extract

In contrast to the powder, the methanolic extract caused significantly higher mortality in the larvae (Table 3). In the leaf dip bioassay, 2% of the extract caused 50% mortality while 4% of the extract caused as high as 87.5% larval mortality during a period of 72 h. In this case, the control mortality of 20% is much higher than the control mortality with the bark

Table 1: Mortality of 3rd instar *Pieris brassicae* larvae exposed to *Albizia odoratissima* bark powder in dust spray test[a]

Concentration (% w/w)	Mortality hours after spray of dust, %±SE		
	24	48	72
Control (0)	0.0±0.0 (0.0)^{aA}	0.0±0.0 (0.0)^{B}	5.0±5.0 (6.6)^{AB}
1	10.0±5.8 (13.3)^{abA}	20.0±0.0 (26.6)^{aAB}	21.3±1.3 (27.4)^{aB}
2	15.0±5.0 (19.9)^{bcA}	25.0±5.0 (29.7)^{abAB}	26.3±4.7 (30.6)^{aB}
4	25.0±5.0 (29.7)^{cA}	35.0±5.0 (36.1)^{bAB}	52.5±4.8 (46.4)^{B}

[a]Each datum represents the mean of four replicates, each set up with 5 larvae (n=20)

SE – Standard Error; values in parenthesis are arcsine transformed values; Means followed by the same letter (capital within a row and small within a column) did not differ significantly at $P<0.05$ by the Least Significance Difference (LSD) test

Table 2: Analysis of variance (ANOVA) of mortality in 3rd instar *Pieris brassicae* exposed to *Albizia odoratissima* bark powder

Time after treatment (hr)	Source	Sum of squares	df	Mean square	F	Significance level
24	Between concentrations	1867.69	3	622.56	5.51	0.013
	Within concentrations	1354.9	12	112.91		
	Total	**3222.59**	15			
48	Between concentrations	3030.12	3	1010.04	50.34	0.000
	Within concentrations	240.79	12	20.07		
	Total	**3270.92**	15			
72	Between concentrations	3212.8	3	1070.93	17.44	0.000
	Within concentrations	736.98	12	61.41		
	Total	**3949.78**	15			

Table 3: Mortality of 3rd instar *Pieris brassicae* exposed to methanolic extract of *Albizia odoratissima* bark in leaf dip bioassay[a]

Concentration (% w/w)	Mortality hours after spray of dust, %±SE		
	24	48	72
Control (0)	5.0±5.0 (6.6)^{aA}	10.0±5.8 (13.3)^{aAB}	20.0±0.0 (2662)^{aB}
1	10.0±5.8 (13.3)^{abA}	20.0±2.0 (26.5)^{abAB}	27.5±2.5 (31.6)^{abB}
2	21.2±1.2 (27.4)^{bcA}	32.5±4.3 (34.6)^{bA}	50.0±0.0 (45.0)^{b}
4	26.3±4.7 (30.6)^{c}	61.3.0±5.1 (51.6)^{A}	87.5±7.2 (69.5)^{A}

[a]Each datum represents the mean of four replicates, each set up with 5 larvae (n=20)

SE – Standard Error; values in parenthesis are arcsine transformed values; Difference in means followed by the same letter (capital within a row and small within a column) are statistically not significant ($P<0.05$; LSD)

powder (5%) suggesting the possible influence of the methanol used as solvent in the extraction process. Nevertheless, in the leaf dip bioassays also a similar pattern of mortality trend as that with bark powder was observed. In any given time, higher concentration of extracts caused significantly ($P<0.05$) high mortality and in each concentration the larval mortality was found to increase over time indicating cumulative effect of the same concentration over a period of time (Table 4).

It is evident from the findings that both the powder as well as the methanol extract of *Albizia* bark caused similar pattern of larval mortality over time and with increasing concentration. Further, mortality caused by different concentrations of each form of the bark was significantly different from their respective control mortality. However, the interesting point to note here is that the methanolic extract caused almost two-fold mortality (50% at 72 h by 2% extract) than the bark powder (26.3% at 72 h by 2% powder).

The observed better efficacy of the methanolic extract of the bark was supported by the estimated LC_{50} values (Table 5). At 72 h, the concentration for 50 % larval mortality was lower in case of the extract (1.26%) in comparison to the powder (3.68%). However, the LC_{50} for both the dry powder and the

Table 4: Analysis of variance (ANOVA) of mortality in 3rd instar *P. brassicae* exposed to methanolic extract of *A. odoratissima* bark

Time after treatment (hr)	Source	Sum of squares	df	Mean square	F	Significance level
24	Between concentrations	1558.9	3	519.63	4.62	0.023
	Within concentrations	1350.86	12	112.57		
	Total	**2909.77**	15			
48	Between concentrations	3089.26	3	1029.75	13.26	0.000
	Within concentrations	932.14	12	77.67		
	Total	**4021.4**	15			
72	Between concentrations	5678.36	3	1892.78	24.44	0.000
	Within concentrations	929.485	12	77.46		
	Total	**6607.84**	15			

Table 5: Relative toxicity of different preparations of *Albizia odoratissima* bark to 3rd instar *Pieris brassicae*[a]

Time after spray (hr)	Form of material	LC_{50}[†]	Log10 LC_{50}	95% CI		Significance (P<0.05)
				Lower	Upper	
24	Powder	18.89	1.28	0.61	586.9	Yes
	Methanolic extract	15.62	1.19	0.43	572.2	Yes
48	Powder	13.48	1.13	0.38	476.1	Yes
	Methanolic extract	2.45	0.39	1.29	17.68	Yes
72	Powder	3.68	0.57	1.66	8.17	Yes
	Methanolic extract	1.26	0.1	0.5	1.83	Yes

[a]Probit Analysis done by Finney Method (Lognormal Distribution)

[†]LC50 differ significantly where 95% CIs do not overlap

aqueous extracts decreased significantly ($P<0.05$) over time indicating the cumulative effect. This is evident from the 6-8 fold decrease in LC_{50} values from 24 h to 72 h in both forms of the bark treatment.

The mortality of the 3rd instar larvae of *P. brassicae* by a good percentage after exposure to the powder as well as methanolic extract of *A. odoratissima* bark indicates that the plant possesses insecticidal properties. It is to be mentioned here that studies based on clues from traditional practices revealed that methanol extract of yam (*Dioscorea hispida*) is effective as antifeedant and as insecticide to the larvae of the diamond black moth, *Plutella xylostella* [13].

Histo-Pathological Analysis of the Mid-gut of 3rd Instar Larvae of *P. brassicae*

Microscopical analysis of the different body parts of the target insect before and after exposure to the preparations of the plant material is essential to determine the mode of action.

Mid-gut of Control Larvae

The control larvae were from methanol: water treatment. The gut of controlled 3rd instar larvae of *P. brassicae* showed the presence of continuous brush borders (CB) which is characteristic of the mid-gut epithelium of insects. Above the brush borders, there lies a layer of columnar epithelial cells (CE). In the region next to the brush border and adjacent to the lumen, the presence of small diameter muscle fibres (SMF) was evident. Besides these, presence of small vesicles (Vs) adjacent to the lumen was also found to be clear. The mid-gut epithelial cells were closely associated with one another. The mid-gut epithelium was found to be covered by muscular conjunctive system (MCS) (Fig. 1).

Mid-gut of Larvae Treated with Bark Powder

In the larvae treated with *A. odoratissima* bark powder, continuity of the brush border was found to be disturbed and the cells were not closely associated

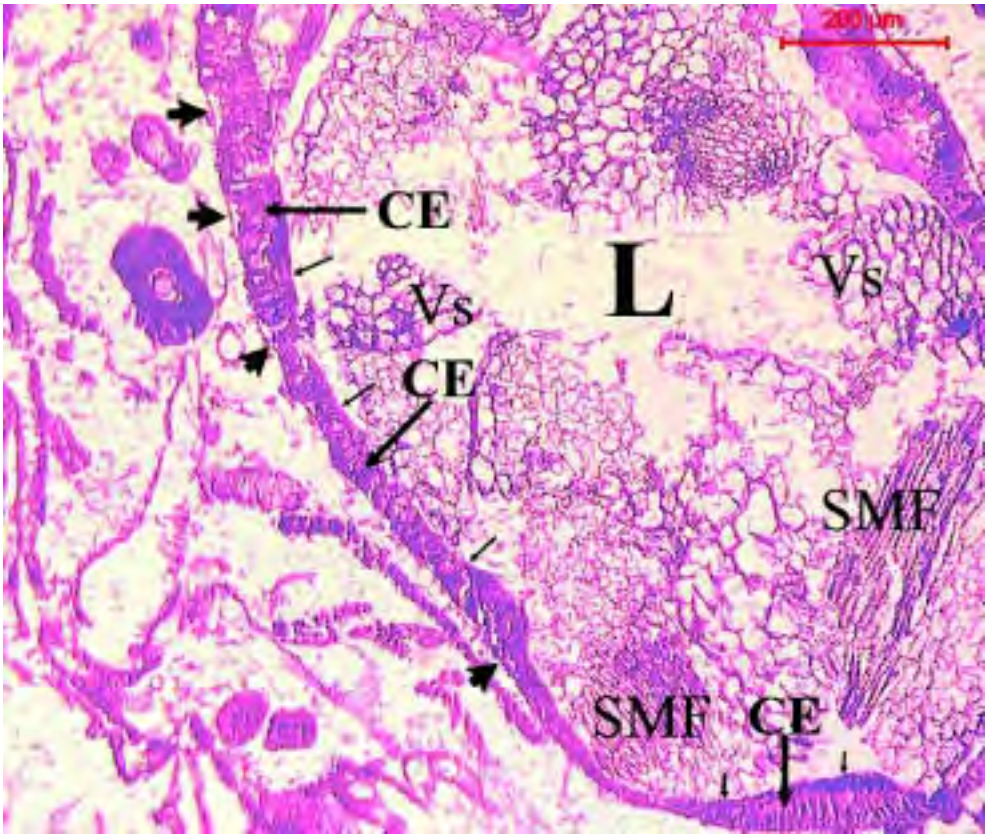

Fig. 1: Transverse section through the mid-gut of untreated 3rd instar *Pieris brassicae* larva. Thin arrows indicate brush border and thick arrows indicate muscular conjunctive system; CE=columnar epithelium; SMF=small diameter muscle fibre; Vs=vesicle; and L=lumen

with one another as in the case of control larvae. This provides evidence for damage due to the treatment of the bark powder. The small diameter muscle fibres present in the mid-gut adjacent to lumen was found to be absent. Small vesicles adjacent to the lumen as observed in the untreated larvae was also found to be absent in the larvae fed with the bark powder. The muscular conjunctive system (MCS) covering the mid-gut epithelium was also found to be damaged to some extent (Fig. 2). The columnar epithelial cells also showed swelling and breakage and vacuolization.

Mid-gut of Larvae Treated with Methanolic Extract of Bark

The representative photomicrograph of 120 sections from the gut of 12 insects with 10 replicates showed that the caterpillars treated with methanolic extract of the barks of *A. odoratissima* caused a high degree of damage to the mid-gut epithelial cells. In fact, the histological preparations were beyond recognition when compared with the micrographs prepared from the untreated larvae (Fig. 3).

Prominent histo-pathological changes in the mid-gut treated larvae substantiate the fact that the plant possesses toxic insecticidal properties. Loss of continuity of the brush border, irregularities in the spatial arrangements of gut cells, reduction in the diameter of muscle fibers, partial or total loss of cellularity, damage of the muscular conjunctive system covering the mid-gut epithelium and breakage, swelling as well as vacuolization of columnar epithelial cells in *P. brassicae* larva in response to treatment with *A. odoratissima* bark establishes the authenticity of the traditional method of using the aforementioned plant as an insecticide in general.

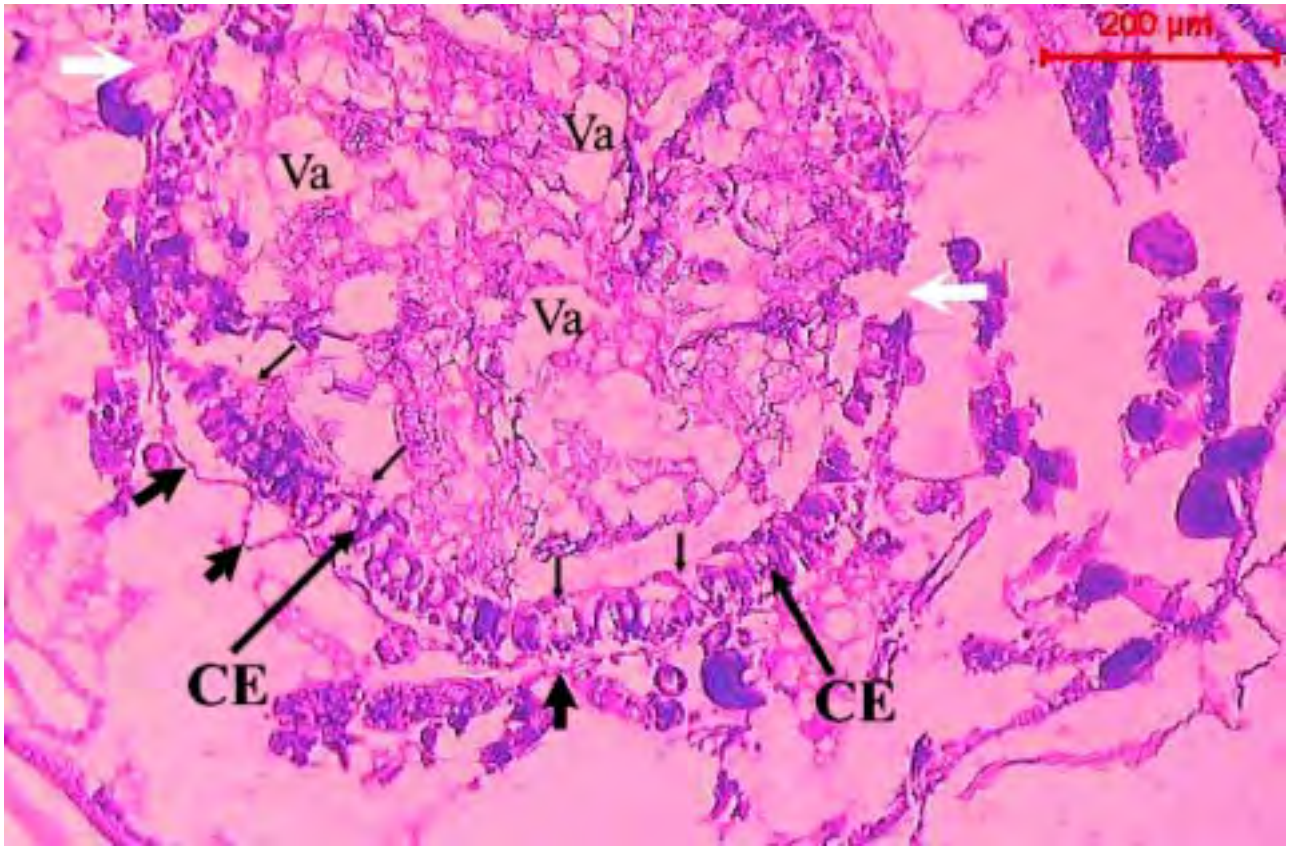

Fig. 2: Transverse section through the mid-gut of 3rd instar *Pieris brassicae* larva treated with *Albizia odoratissima* bark powder. Thin arrows indicate brush border and thick arrows indicate muscular conjunctive system. Note the breakage of mid-gut epithelium (white arrows) and enlargement of epithelial cells. CE=columnar epithelium; and Va=vacuole

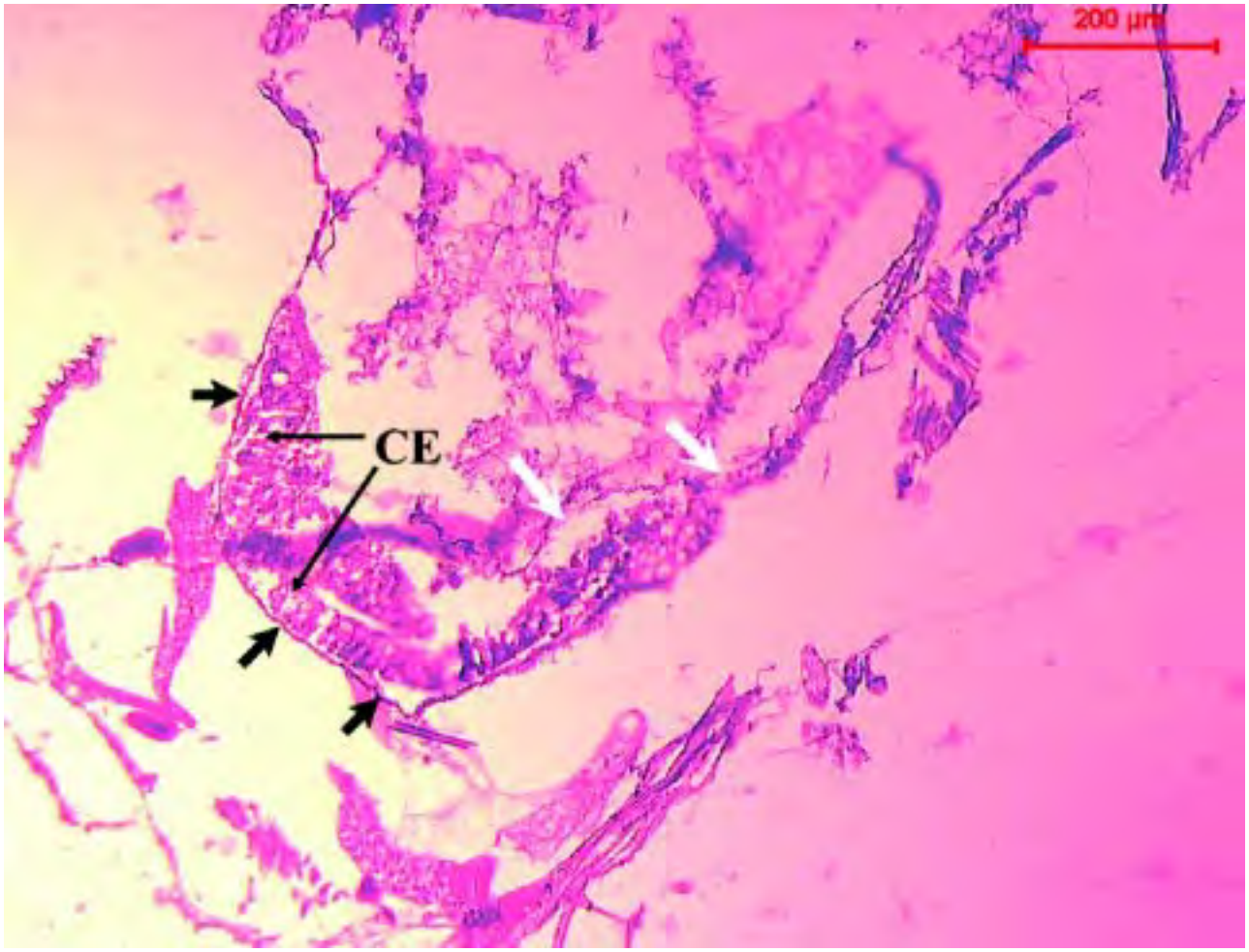

Fig. 3: Transverse section through the mid-gut of 3rd instar larva of *Pieris brassicae* treated with methanolic extract of *Albizia odoratissima* bark. Thin arrows indicate brush border and thick arrows indicate muscular conjunctive system. Note the separation of continuous brush borders (white arrows) and almost complete dialysis of the gut. CE=columnar epithelium

Severe damage to the mid-gut epithelial cells and other portions of the gut due to the treatment with methanolic extract of the bark suggest that the effectiveness of the traditional practice can, further, be enhanced through certain appropriate modifications. It is to be noted that histo-pathological studies are of general use to determine the toxic effects of pesticides in general [24]. The present study also suggests that mid-gut is one of the major site of action of the potential pesticide much in concurrence with other pesticides [17, 18] as well as botanical insecticides [19].

4. Conclusion

The present study, suggests that the bark of *A. odoratissima* may be a good source of insecticide against insect pests such as *P. brassicae*. It is to be mentioned here that methanolic extract of *A. odoratissima* bark has been reported to possess significant antimicrobial activity against Gram positive bacteria and yeast [25]. Bark of *A. odoratissima* contains steroids, phenolics, saponins, triterpenoids, flavonoids such as 7,8-dimethoxy-3',4'-methylenedioxyflavone, 7,2',4'-trimethoxy- flavone and 7,4'-dimethoxy-3'-hydroxyflavone and also

tannins such as D-catechin, isomers of leucocyanidin, melacidin and melanoxitin [25, 26, 27, 28]. Presence of such an array of secondary metabolites and toxins in the bark of *A. odortaissima* indicates its potential insecticidal application.

The chances of *A. odoratissima* bark as a potential biopesticide appears to be bright from the fact that its methanolic extract was found to have protective effects on vital tissues such as pancreas, kidney, liver, heart and spleen in albino mice, thus, indicating non-toxicity to beneficial organisms [29]. However, further extensive work is required to identify the exact anti-insect principles in the *A. odoratissima* bark towards possible developments of a synthetic biopesticide.

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