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Bioefficacy of *Artemisia capillaris* Thunb. As a botanical insecticide for the control of *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boh.)

Renuka and DR Thakur

Abstract
A study has been conducted to test the bioefficacy of *Artemisia capillaris* Thunb. for the control of *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boh.) under laboratory conditions. Different concentration of *Ar. capillaris* in methanol and acetone at 2, 4, 6 and 8% including control (0%) were tested against both the pest species. Methanol extract of *A. capillaris* was more effective against both the bruchid pests than acetone. It was observed that mortality of adult bruchid was directly proportional to the concentration levels and the exposure time of the pest. The plant extract at different concentration levels significantly ($p \leq 0.001$) reduced the fecundity of both the bruchids as compared to control. Like fecundity, F_1 progenies of bruchid pests also reduced significantly both in methanol and acetone extract as compared to control. It has been therefore, concluded that *A. capillaris* could play an important role in the management of both the pest species in storage.

Keywords: *Artemisia capillaris*, botanical insecticide, *Acanthoscelides obtectus*, *Zabrotes subfasciatus*, control

1. Introduction
Pulses constitute an important source of dietary protein for the developing nations like India per capita consumption of animal protein is low. This legume family fixes the atmospheric nitrogen and thus improves the soil fertility. Therefore, legume crops are generally cultivated in rotation to keep soil fertile. *Phaseolus vulgaris* is widely consumed throughout the world as it is the major source of proteins, calories, vitamins and minerals and it also contributes in targeting diseases like cancer, diabetes and heart diseases [1].

Two bruchid species, *Acanthoscelides obtectus* (Say) commonly known as bean weevil and *Zabrotes subfasciatus* (Boh.) commonly called Mexican bean weevil have been recognized as major pests of been seeds worldwide. *Ac. obtectus* is a pest of cooler areas and starts infestation in the field and continues in the storage, whereas *Za. subfasciatus* is confined to warmer areas and exclusively a storage pest [2, 3, 4]. Both the pests are cosmopolitan and cause great damage to stored grains thus affecting the quality of beans both for cooking and planting [5].

Control of bruchid with plant extracts is a novel approach to avoid the problem of residual toxicity, insect resistance, photo-toxicity, vertebrate toxicity, toxic residues in food grains and environmental hazards raised by the increased use of synthetic insecticides [6]. Plant materials based insecticides are target specific, non-toxic to organisms, biodegradable and cheap.

Artemisia capillaris Thunb. belongs to family Asteraceae, commonly known as wormwood is an aromatic, perennial, bushy and woody shrub. It has antipyretic, anti-inflammatory, antimicrobial, antimalarial properties [7, 8]. This plant also stimulates the production of bile in the liver. Artemisinin chemical produced by this plant is very effective against the malaria causing parasite, *Plasmodium falciparum*.

The objective of this study was to investigate the insecticidal potency of *Ar. capillaris* against *Ac. obtectus* and *Za. subfasciatus* and found effective in controlling both pests thus recommended for the management of bruchids attacking stored kidney beans. The insecticidal efficacy of *Ar. capillaris* against bruchids has been evaluated for the first time and promising results have been observed to control the bruchid pests in storage.

2. Materials and Methods

2.1 Sample Collection

This study was carried out from May 2012 to October 2012. The infested and uninfested seeds of *P. vulgaris* were collected from the different areas of the Himachal Pradesh. After emergence of the adult bruchids from infested samples, cultures were initiated and sublet under controlled condition of temperature and relative humidity. Cultures were propagated in different petri-dishes (90 mm diameter, Tarsons and 105 mm diameter, Borosil) and wire mesh cages ($12\times10\times10^2$ cm) along with host seeds. Adults thus emerged were identified as *Za. subfasciatus* and *Ac. obtectus* by running them in the dichotomous key developed by Arora, 1977, Johnson, 1990 and Kingsolver, 2004 [9, 10, 11].

2.2 Maintenance of the Culture: Reserve culture of *Za. subfasciatus* and *Ac. obtectus* both were maintained in the laboratory in 500 ml glass jars covered with muslin cloth. All alive and dead insects were discarded after fifteen days of introduction. Insects were counted as dead when they failed to move any part of their body. The treated and control seeds were kept until emergence of F_1 progeny. The number of F_1 progeny was counted both in treated and control seeds.

2.3 Collection and identification of the plant: *Ac. capillaris* was collected from District, Lahaul & Spiti (Himachal Pradesh) and identified by taxonomic key developed by Eichler (1883) [12] and also with the help of Plant taxonomist, Prof. M. K. Seth, Department of Biosciences, Himachal Pradesh University, Shimla (H. P). The plant materials were washed and air dried in the shade before use.

Aerial parts of the plant were used in the present study. Plant extract was prepared in methanol and acetone according to the method of Talukdar and Howse (1993) [13] with a few modifications. Twenty grams of ground leaf powder of plant was mixed with 100 ml of solvents, stirred for 30 minute using a magnetic stirrer and then left undisturbed for 24 hours. The mixture was then filtered through Whatman # 1 paper, and the solids were stirred again for 15 minutes with the same solvent and filtrates were combined. The solvent from the pooled filtered solution was evaporated in a water bath by setting the temperature of water bath according to the boiling point of the solvent used. After complete evaporation of the solvents, final crude extracts were dissolved in solvents before use. Different concentration levels, 2, 4, 6, 8% were prepared and 1ml of each concentration was applied to the filter papers placed in the petri-dishes and allowed to air dried until the complete evaporation of solvents. Concentrations used were determined after conducting preliminary experiments to standardize the doses and then used for assessing their insecticidal and seed protective effects.

Methanol and acetone treated filter paper placed in petri-dishes were taken as control and both of them found nontoxic to the pests. Then 50 healthy seeds of *P. vulgaris* were placed on the extract treated filter papers inside the petri-dishes. Five pairs of freshly emerged adults of *Ac. obtectus* and *Za. subfasciatus* were released in different petri-dishes and covered for the next 7 days for observation. The number of dead insects in each petri-dish was counted after 24, 48, 72 and 96 hrs and also up to 100% mortality of pests. The experiment was designed in a completely randomized design in three replications each.

2.4 Data Analysis: Percentage insect mortality was calculated by using Abbott's formula (Abbott, 1925) [14] as follows:

Correct % mortality= $1 - C_n - C_T / C_T \times 100$

Where, n- number of insects, C-control and T-treatment.

Mortality data in different concentrations levels were subjected to Multivariate ANOVA using SPSS-16 software. Percent data were arcsine transformed by using formula:

(ASIN ((value/100)^0.5)*57.32484)

Duncan's Multiple Range Tests (DMRT) was applied to all the means using SPSS-16 software.

Efficacy of *Ac. capillaris* as an insecticide against bruchid was studied for the three following parameters, viz. mortality response of adult bruchids, eggs deterrence and F_1 progeny reduction.

3. Results

3.1 Mortality response of adult bruchids: Methanol and acetone extract of *Ar. capillaris* was found toxic to *Ac. obtectus* and *Za. subfasciatus* and significantly brought the mortality of adults as compared to the control. Methanolic extract was more effective than acetone against both the pests. Mortality of adults was directly proportional to concentration of extract and exposure duration. Methanol extract at 6 and 8% concentration levels was effective than lower concentration and gave 100% mortality on 6.66±0.66 and 6.33±0.33 days of treatment both in *Ac. obtectus* and *Za. subfasciatus* respectively with statistically ($p\leq0.001$) almost similar values. The acetone extract at 8% concentration level was most effective giving 100% mortality of *Ac. obtectus* and *Za. subfasciatus* on 7.66±0.88 and 7.33±0.66 days of treatment respectively. Acetone extract of *Ac. capillaris* at 4 and 6% concentration both gave 100% mortality on 9.66±0.88 days after treatment in *Ac. obtectus*, whereas same concentrations resulted 100% mortality on 10.66±0.66 and 9.33±0.66 days respectively in *Za. subfasciatus*. Methanol and acetone extracts of *Ar. capillaris* at different concentration were statistically different to the control for both the pest species (Fig. 1, 2, 3, 4 and Table 1, 2).

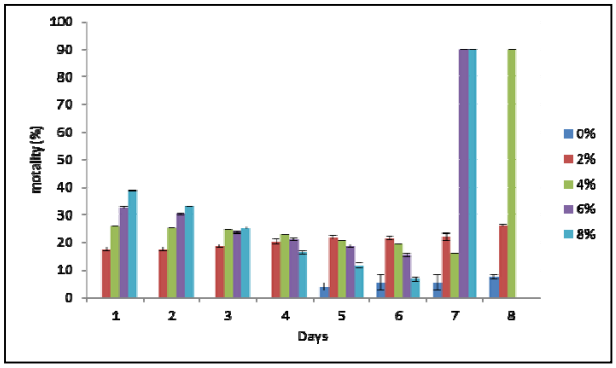

Fig 1: Adult mortality of *A. obtectus* resulted from different concentrations of *A. capillaris* in methanol extract.

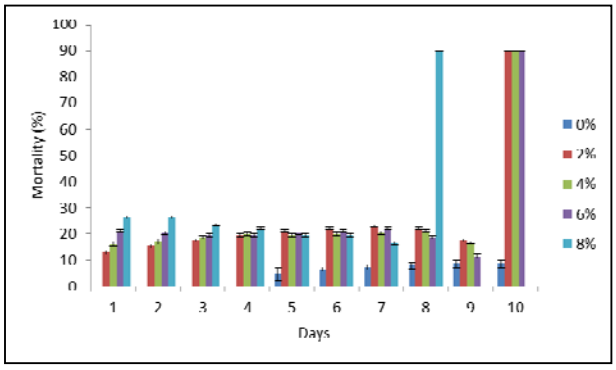

Fig 2: Adult mortality of *A. obtectus* resulted from different concentrations of *A. capillaris* in acetone extract.

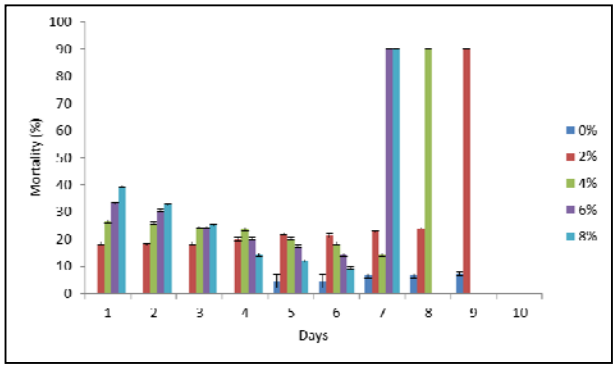

Fig 3: Adult mortality of *Z. subfasciatus* resulted from different concentrations of *A. capillaris* in methanol extract.

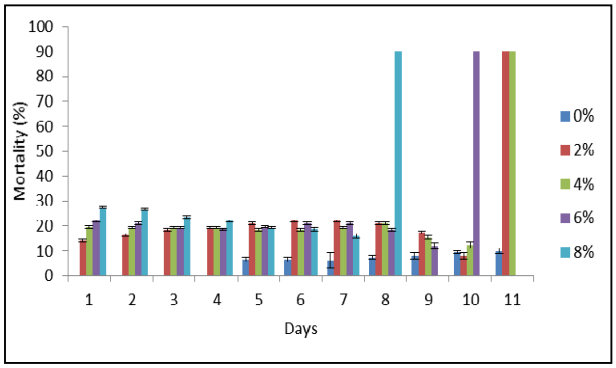

Fig 4: Adult mortality of *Z. subfasciatus* resulted from different concentrations of *A. capillaris* in acetone extract.

Table 1: Adult mortality of *A. obtectus* treated with different concentration of *A. capillaris* in methanol and acetone extracts

Extract	Dose (%)	Insect mortality (%)									
		1st D	2nd D	3rd D	4th D	5th D	6th D	7th D	8th D	9th D	10th D
Methanol	0	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{d}	3.82±1.41^{c}	5.42±2.71^{d}	5.42±2.71^{d}	7.33±0.79^{c}	7.94±1.22^{b}	
	2	17.44±0.58^{d}	17.44±0.58^{d}	18.42±0.55^{b}	20.23 ±1.00^{b}	21.68±0.74^{a}	21.40±0.73^{a}	21.91±1.29^{b}	25.84±0.42^{b}	90.04±0.00^{a}	
	4	25.84±0.42^{c}	25.10 ±0.43^{c}	24.35 ±0.43^{a}	22.78 ±0.46^{a}	20.54±0.76^{ab}	19.36±0.52^{a}	16.02±0.96^{c}	90.04±0.00^{a}		
	6	32.59±0.36^{b}	30.00±0.38^{b}	23.58±0.45^{a}	21.13 ±0.49^{ab}	18.42±0.55^{b}	15.31±0.64^{b}	90.04±0.00^{a}			
	8	38.66±0.34^{a}	33.01 ±0.21^{a}	25.10 ±0.43^{a}	16.41 ±0.61^{c}	11.47±0.85^{c}	6.53 ±0.79^{c}	90.04±0.00^{a}			
Acetone	0	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{c}	4.62±2.41^{c}	6.53±0.79^{d}	7.33±0.79^{c}	7.94±1.22^{b}	8.56±1.41^{e}	8.56±1.41^{b}
	2	12.88±0.76^{d}	15.31±0.64^{c}	17.44±0.58^{c}	19.36±0.52^{b}	21.13±0.49^{a}	21.97±0.47^{a}	22.78±0.46^{a}	21.97±0.47^{b}	17.44±0.58^{b}	90.04±0.00^{a}
	4	16.02±0.96^{c}	17.08±0.91^{c}	18.42±0.55^{bc}	19.95±0.79^{b}	19.36±0.52^{a}	19.95±0.79^{b}	20.26±0.51^{b}	21.13±0.49^{b}	16.77±0.34^{b}	90.04±0.00^{a}
	6	21.13±0.49^{b}	20.26±0.51^{b}	19.36±0.52^{b}	19.36±0.52^{b}	20.26±0.51^{a}	21.13±0.49^{ab}	21.97±0.47^{a}	18.42±0.55^{c}	90.04±0.00^{a}	
	8	26.56±0.41^{a}	26.56±0.41^{a}	23.58±0.45^{a}	21.97±0.47^{a}	19.26±0.52^{a}	19.36±0.52^{b}	16.41±0.61^{c}	90.04±0.00^{a}		

All values are mean ±SE of three replicates. The data original mortality of *A. obtectus* were corrected by Abbott's formula and then transformed into arcsin √percentage values before statistical analysis. Values followed by different letters within a column are significantly different at the 5% level of probability (Duncan's multiple range tests). *90.04 represent 100% mortality of pests. *D (day).

Table 2: Adult mortality of *Z. subfasciatus* treated with different concentration of *A. capillaris* in methanol and acetone extracts.

Extract	Dose (%)	Insect mortality (%)									
		1st D	2nd D	3rd D	4th D	5th D	6th D	7th D	8th D	9th D	10th D	11th D
Methanol	0	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{d}	4.62±2.41^{d}	4.62±2.41^{d}	6.53±0.79^{d}	6.53±0.79^{c}	7.33±0.79^{b}		
	2	18.42±0.55^{d}	18.11±0.32^{d}	18.42±0.55^{b}	19.95±0.79^{b}	21.97±0.47^{a}	21.40±0.73^{a}	22.75±0.43^{b}	23.84±0.25^{b}	90.04±0.00^{a}		
	4	26.56±0.41^{c}	25.84±0.42^{c}	24.35±0.43^{a}	23.68±0.46^{a}	20.26±0.51^{ab}	18.42±0.55^{a}	14.14±0.69^{c}	90.04±0.00^{a}			
	6	33.22±0.36^{b}	30.66±0.37^{b}	24.35±0.43^{a}	20.26±0.51^{b}	17.44±0.58^{b}	14.14±0.69^{b}	90.04±0.00^{a}				
	8	39.24±0.33^{a}	32.59±0.36^{a}	25.10±0.43^{a}	14.14±0.69^{c}	12.00±0.46^{c}	9.35±0.61^{c}	90.04±0.00^{a}				
Acetone	0	0.00±000^{e}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{c}	6.53±0.79^{c}	6.53±0.79^{c}	6.03±3.06^{c}	7.33±0.79^{d}	7.94±1.22^{c}	9.35±0.61^{b}	9.88±0.98^{b}
	2	14.14±0.69^{d}	16.41±0.61^{d}	18.42±0.55^{b}	19.36±0.52^{b}	21.13±0.49^{a}	21.97±0.47^{a}	21.97±0.47^{a}	21.13±0.49^{b}	17.44±0.58^{b}	7.94±1.22^{b}	90.04±0.00^{a}
	4	19.66±0.61^{c}	19.36±0.52^{c}	19.36±0.52^{b}	19.36±0.52^{b}	18.42±0.55^{b}	18.42±0.55^{b}	19.36±0.52^{ab}	21.13±0.49^{b}	15.31±0.64^{b}	12.35±1.24^{a}	90.04±0.00^{a}
	6	21.97±0.47^{b}	21.13±0.49^{b}	19.36±0.52^{b}	18.75±0.31^{b}	19.67±0.30^{ab}	21.13±0.49^{a}	21.13±0.49^{a}	18.42±0.55^{bc}	90.04±0.00^{a}		
	8	27.27±0.40^{a}	26.56±0.41^{a}	23.58±0.45^{a}	21.97±0.47^{a}	19.36±0.52^{ab}	18.72±0.82^{b}	16.02±0.96^{b}	90.04±0.00^{a}			

All values are mean ±SE of three replicates. The data original mortality of *A. obtectus* were corrected by Abbott's formula and then transformed into arcsin √percentage values before statistical analysis. Values followed by different letters within a column are significantly different at the 5% level of probability (Duncan's multiple range tests). *90.04 represent 100% mortality of pests. *D (day).

3.2 Eggs deterrence: The plant extract at different concentration significantly reduced the fecundity of both the bruchids as compared to control where maximum 142.67±4.48 and 134.33±2.33 eggs were laid by *Ac. obtectus* and *Za. subfasciatus* respectively. The numbers of egg laid on *P. vulgaris* seeds treated with 8% concentration of *Ac. capillaris* extract in methanol and acetone were 52.66±1.20 and 56.33±0.88 by *Ac. obtectus* and 50.33±1.20 and 54.33±1.20 by *Za. subfasciatus* respectively. Methanol and acetone extract of *Ar. capillaris* at 2 and 4% concentration levels were least effective than 6 and 8% but even significantly better to reduce the biotic as compared to control (Table 3).

Table 3: Numbers of eggs and F_1 adults of *A. obtectus* and *Z. subfasciatus* treated with methanol and acetone extracts of *A. capillaris* at different concentrations.

Plant extracts	Dose (%)	*A. obtectus*		*Z. subfasciatus*	
		No. of eggs	No. of adults	No of eggs	No of adults
Methanol	0	139.67±0.88^{a}	106.00±2.08^{a}	141.00±2.08^{a}	109.67±1.76^{a}
	2	97.66±1.45^{b}	73.00±2.64^{b}	94.66±2.02^{b}	74.33±1.76^{b}
	4	75.33±1.45^{c}	52.00±2.08^{c}	74.66±2.02^{c}	53.33±1.45^{c}
	6	65.00±1.73^{d}	41.00±2.08^{d}	62.00±1.73^{d}	43.66±2.02^{d}
	8	52.66±1.20^{e}	35.00±2.30^{d}	50.33±1.20^{e}	32.33±1.45^{e}
	F	619.68***	164.07***	372.49***	318.89***
Acetone	0	132.33±1.45^{a}	107.67±1.45^{a}	133.33±1.16^{a}	104.67±2.02^{a}
	2	99.33±1.20^{b}	73.00±2.64^{b}	97.33±1.45^{b}	104.67±2.02^{a}

	4	78.33±0.88^{c}	55.66±2.33^{c}	77.66±1.45^{c}	52.00±1.73^{c}
	6	67.66±1.45^{d}	51.66±2.02^{c}	67.33±1.76^{d}	43.66±1.76^{d}
	8	56.33±0.88^{e}	36.33±2.02^{d}	54.00±1.53^{e}	32.66±1.76^{e}
	F	622.44***	163.04***	382.77***	253.53***

All values are mean ± SE of three replicates. Values followed by different letters within a column are significantly different at $p \leq 0.05$ (Duncan's multiple range tests). F values followed by asterisks are significant at $p \leq 0.001$ (One-way ANOVA test)

3.3 F_1 progeny reduction: Like fecundity, F_1 progenies of bruchid pests also reduced significantly at different concentration of plant extract both in methanol and acetone as compared to control where 114.67±2.60 and 107.67±4.05 adults of *Ac. obtectus* and *Za. subfasciatus* respectively emerged successfully. It is evident from the Table 3 that 8% concentration of *Ac. capillaris* in methanol and acetone resulted in production of 35.33±2.02 and 36.33±2.02 adults of *Ac. obtectus* and 32.33±1.45 and 32.66±1.76 of *Za. subfasciatus* respectively. The emergence of F_1 progenies were also less among the seeds treated with 2, 4 and 6% concentration of plant extract in methanol and acetone as compared to control (Table 3).

4. Discussion

The present study unveiled that the plant extract of *Ar. capillaris* in both methanol and acetone proved effective in causing mortality of adult bruchid, reducing fecundity and F_1 adult emergence. However, methanol extract was more effective against both *Ac. obtectus* and *Za. subfasciatus* and both 6 and 8% concentration gave 100% mortality of both pests after 6.66±0.66 and 6.33±0.33 days respectively. The acetone extract at 8% concentration was most effective to bring 100% mortality of *Ac. obtectus* and *Za. subfasciatus* on 7.66±0.88 and 7.33±0.66 days of treatment respectively. Whereas no mortality was observed up to 5th day at control both in *Ac. obtectus* and *Za. subfasciatus* respectively. It was observed that mortality of adult bruchids was directly proportional to the concentration levels and exposure. Higher doses and longer exposure resulted in appreciable mortality of bruchids and those observations are in accordance with the study of Patole (2009) [15] and Ziaee and Moharramipour (2013) [16]. Diwan and Saxena (2010) [17] showed that more than 90% mortality of *Callosobruchus maculatus* with in 24 hours while studying the insecticidal property of flavonoid isolated from *Tephrosia purpuria*. Significant differences between mortality recorded in the extract treated and controls shows that there might be some active compounds are embedded in plant extract responsible for the mortality of the insects. The results of the present investigation are similar to the observation of Asawalam *et al*. (2007) [18] who reported that insecticidal activity of any plant is due to active constituents of the plant concerned. Mean oviposition decreased significantly ($p \leq 0.001$) with increase in treatment dosage to 52.66±1.20 and 56.33±0.88 eggs per female of *Ac. obtectus* and 50.33±1.20 and 54.33±1.20 eggs per female of *Za. subfasciatus* at 8% methanol and acetone concentration as compared 142.67±4.48 and 134.33±2.33 eggs per female of *Ac. obtectus* and *Za. subfasciatus* respectively at control. Similarly emergence of F_1 adults also decreased significantly from a high value 114.67±2.60 and 107.67±4.05 at control to a comparatively low value of 35.33±2.02 and 32.33±1.45 F_1 adults of *Ac. obtectus* and *Za. subfasciatus* respectively at 8% methanol extract and 36.33±2.02 and 32.66±1.76 F_1 adults in same concentration of extract in acetone. Yankanchi and Lendi (2009) [19] found that oviposition deterrence of *C. chinensis* was 96.8 and 92.6% in seeds treated with 20 mg/gm *Withania somnifera* and *Tridax procumbens* powder respectively. Authors also recorded 100% ovicidal activity at 5mg/gm of *W. somnifera* and *T. procumbens* and 20 mg/gm of *Pongamia pinnata* and *Gliricidia maculata* leaf powder suppressed 100% F_1 adult's emergence of the same pest. The observed adult mortality, eggs deterrence and reduced F_1 generation, after the application of the extracts may be due to the active components of the plant species extracted by the solvent used.

5. Conclusion

From the present study it has been concluded that *Ar. capillaris* possesses insecticidal properties and methanol and acetone extract of the plant even at very low concentrations significantly increased the adult mortality and reduced the fecundity and F_1 adult emergence of both the pests. However, methanol extract of the tested plant was more effective against both the bruchid pests. Significant differences in mortality oviposition and adult emergence of pest species in different solvents shows the presence of active compounds of plants that were responsible for insecticidal activities of plants against insect pests. The use of *Ar. capillaris* as an alternative to synthetic insecticides for the management of stored grain pests has been advocated through the study.

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