Effect of cold water extracts of *Acacia modesta* Wall. and *Glycyrrhiza glabra* Linn. on *Tribolium castaneum* and *Lemna minor*

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Abstract: The aim of the present study was to introduce an alternative way for insects control through biodegradable plants materials. The different cold water extracts dilutions of *Acacia modesta* and *Glycyrrhiza glabra* were tested against *Tribolium castaneum*. The extracts dilutions of both plants caused mortality of the *Tribolium castaneum.* ANOVA revealed that dilutions and plants were highly significant. The interaction between plants and dilutions was also significant at $P < 0.05$. Phytotoxic activity showed that dilutions of *Acacia modesta* and *Glycyrrhiza glabra* extracts significantly inhibited the growth of *Lemna minor*. ANOVA showed that dilutions of both plants extracts were significant at $P < 0.05$.

Keywords: *Tribolium castaneum, Lemna minor, Acacia modesta* and *Glycyrrhiza glabra*

INTRODUCTION

The struggle to make the environment comfortable and friendly by reducing the pollution rate through degradable materials isolated from bio-source especially the plants is good attempt to overcome certain problems such as insects control. The insects are controlled from the last century by different synthetic chemicals which are harmful for other living organism in same environment. Most of these toxic chemicals inter the food chain and cause pollution of the environment. Scientists and researchers are in continues struggle to introduce biodegradable plants materials for insects control to reduce the pollution (Malau & James, 2008). The applications of medicinal plants extracts on insects show the action or property of the medicinal plants. Insects mortality rate showed the toxicity of the plants extracts which are useful in the pharmacognostic study of medicinal plants as well. Insecticidal activity is also useful for the evaluation of drugs which could be used against many microbial and viral diseases (Othira *et al*., 2009). It has been proved by scientists to control the insects by those materials which are derived from plants. These plants materials are less harmful against other living organisms in the same environment. Plants insects have been resisting to certain chemicals used from the last 15-20 years (Isman 2000). Throughout history of human plants materials are used in different important products such as medicines, cosmetics, insecticides and medicines. Most of plants essential oils is good source of bioactive components (Lahlou *et al*., 2001; Cetin *et al*., 2004). Herbicides are of great importance and are considered safe as compared to synthetic products. People are getting great interest in natural products they think it is less harmful and environment friendly (Joy *et al*., 2001).

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MATERIALS AND METHODS

Insecticidal activity

Collection and Preservation of plants

The fresh specimens of *Acacia modesta* and *Glycyrrhiza glabra* were collected in April, 2010 from Botany Department, University of Peshawar and Pakistan Forest Institute (PFI), Peshawar. The specimens were identified at Botany Department, University of Peshawar. Each specimen was cleaned, washed, separated and dried in air. The whole plants of *A. modesta* (stem+bark, root, leaves and seeds) and *G. glabra* (rhizome, leaves and stem) were ground by electric grinder.

Preparation of the extracts

Twenty grams of ground plant material were soaked in distilled water for 48 hrs and were filtered using standard filter paper. Different dilutions of the plant extracts were made 20%, 15%, 10% and 5% using following formula.

$M1C1=M2C2$

M1: Known volume of standard solution.
C1: Concentration (%) of standard solution.
M2: Wanted volume to be made.
C2: Wanted concentration (%) to be made (Khan *et al*., 2008)

The insecticidal activity was carried out at Nuclear Institute For Food and Agriculture, (NIFA), Tarnab, Peshawar.

Requirements

Test insects (*Tribolium castaneum*), distilled water, methyl alcohol, Petri plates (9 cm diameter), micropipette (1000 µl), growth chamber at 27°C, oven, test sample, filter paper, glass vials, brush, flasks with aluminum foil etc.

Procedure

Day-1

[1]The filter papers were cut according to the size of Petri plate (9 cm) and put them in the plates.

[2]Loaded the whole sample over the filter paper with the help of micropipette.

[3]The plates were left for 24 hrs to evaporate the solvent completely.

Day-2

[1]Next day (after the evaporation of solvent) put 10 insects of each species in each plate (test and control) with the help of a clean brush. The healthy and active insects of same size and age were selected.

[2]Incubated the plates at 27 ^{o}C for 24 hours with 60% relative humidity in growth chamber.

Day-3

[1]Assessed the survival of the insects (counted the number of survivals of each species)

[2]Calculated the percentage inhibition or percentage mortality with the help of following formula:

$$\left[\text{Percentage mortality} = 100\frac{\text{- No of insects alive in test}}{\text{No of insects alive in control}} \times 100\right]$$

STATISTICAL ANALYSIS

The data on mortality (%) were subjected to two way analysis of variance (ANOVA) and differences between samples were determined by F-test using the Statistical Analysis System (SAS, 1999) program. Values of $P < 0.05$ were considered as significant (Khan *et al*., 2008).

Phytotoxic activity

Requirements

Lemna minor, plant specimens, distilled water, Petri plates (9cm diameter), micropipette (1000µl), test sample, filter paper, oven, flasks, E-medium, glass vials, brush etc.

Collection and preservation of plants

The fresh specimens of *A. modesta* and *G. glabra* were collected in April, 2010 from Botany Department, University of Peshawar and Botanical garden, Pakistan Forest Institute (PFI), Peshawar. The specimens were identified at Botany Department, University of Peshawar. Each specimen was cleaned, washed and separated and was dried in air for ten days. The whole plants of *A. modesta* (stems, roots, leaves and seeds) and *G. glabra* (rhizome, leaves and stems) were ground by electric grinder.

Preparation of the extracts

Twenty (20g) of plant materials were taken for first and ten (10g) for second concentration solution of *A. modesta* and *G. glabra* as whole plants in 80 ml and 90 ml two standard solutions of water in flasks. The flasks were covered with aluminum foil and allowed to stand for 48 hrs. The extracts were filtered by Whatman filter paper. The aqueous extracts were diluted further. Serial dilutions of extracts (10 ml + 90 ml, 30 ml+70 ml, 50 ml+50 ml, 90 ml +10 ml) were made of the stock solutions and tested for phytotoxicity against *Lemna minor*.

Media

E-medium was made from different salts with different amounts which provide mineral nutrients for the *Lemna minor* plant. The salts were first weighed at particular amount given in the following table and then were dissolved in distillated water and volume was made up to 1000 ml.

Procedure

Thirty (ml) of E-medium was added in each Petri dish. Four (ml) of each dilution of standard solution of *A. modesta* and *G. glabra* extracts was applied on the *Lemna minor*. Ten plants of *Lemna minor* were taken in E-medium and each plant had three fronds. Then, the Petri dishes were kept at normal room temperature for a week and the growth inhibition was recorded.

The results were statistically analyzed using (ANOVA) and F-test. The corrected (%) for inhibition of the fronds was determined by the following formula (Khan *et al*., 2008; Malau & James, 2008; Hussain *et al*., 2010).

Formula for the inhibition % of Lemna minor fronds

$$\text{Inhibition \%} = \frac{\left(100 - \text{Number of fronds in test sample}\right)}{\text{Number of fronds in control}} \times 100$$

Composition of E-medium

S.No	Chemical Name	g/L
1.	Zinc sulfate ($ZnSO_4.5H2O$)	0.00022
2.	Calcium nitrate ($Ca(NO_2)_2.4H_2O$)	1.180
3.	Potassium dihydrogen phosphate (KH_2PO_4)	0.68
4.	Ferric chloride ($FeCl_3.6H2O$)	0.00540
5.	Potassium nitrate(KNO_3)	1.515
6.	Copper sulfate ($CuSO_4.5H_2O$)	0.00022
7.	Sodium molybdate($Na_2MO_4.2H_2O$)	0.00012
8.	Ethylene diamino tetra acetic acid (EDTA)	0.01120
9.	Magnesium sulfate ($MgSO_4.7H_2O$)	0.492
10.	Boric acid (H_3BO_3)	0.00286
11.	Manganous chloride ($MnCl_2.4H_2O$)	0.00362

STATISTICAL ANALYSIS

The phytotoxic results were subjected to two way analysis of variance (ANOVA) and differences between samples were determined by F-test using the Statistical Analysis System (SAS, 1999) program. Values of $P < 0.05$ were considered as significant (Khan *et al*., 2008).

RESULTS

Insecticidal activity

In the present insecticidal activity it was observed that various dilutions of extracts of *A. modesta* and *G. glabra* caused significant mortality of *Tribolium castaneum.* In the present study insects percentage mortality showed the toxicity of both plants extracts on store insect *T. castaneum*. This property of *A. modesta* and *G. glabra* is useful for insects control and pharmacognostic study.

The mean mortality of *T. castaneum* was (9.6) for *A. modesta* and (6.80) for *G. glabra* as compared to control (0.00) after 24 hrs. ANOVA showed that plants, dilutions and plant and dilution interaction were significant after 24hrs (table 1). The mean mortality of *T. castaneum* was (20.80) for *A. modesta* and (13.20) for *G. glabra* as compared to control (0.00) after 48 hrs. ANOVA revealed that plants, dilutions and plant and dilution interaction were significant after 48hrs (table 2). The mean mortality of *T. castaneum* was (32. 50) for *A. modesta* and (20.80) for *G. glabra* as compared to control (0.00) after 72 hrs. ANOVA showed that plants, dilutions and plant and dilution interaction were significant after 72hrs (table 3). The mortality values were significantly different at $P<0.05$. After 24 hrs the mean mortality (%) value at 5% was (5.00), at 10% it was (10.00), at 15% it was (11.00) and at 20% it was (15.00) (table 1). The mean mortality (%) value recorded after 48 hrs was (14.00) at 5%, (20.00) at 10%, it was (24.00) at 15% and it was (27.00) at 20% (table 2). The mean mortality (%) value was (19.00) at 5%, (29.00) at 10%, (39.00) at 15%, and (47.00) at 20% after 72 hrs, and the highest mean mortality (%) was recorded at 20% after 72 hrs and lowest mean mortality (%) was observed at 5% after 24 hrs as compared to control**.** Maximum mortality caused by *A. modesta* was 56% and 38% by *G. glabra* at 20% after 72 (table 3). The mortality (%) of *Tribolium castaneum* increased with the increase in concentration of *A. modesta* and *G. glabra* extracts. It also suggested that the effect on *Tribolium castaneum* increased with the increase in concentration of the plants extract and exposure time and the effect decreased when the concentration and exposure time decreased.

Table 1: Effect of different dilutions of *Acacia modesta* Wall. and *Glycyrrhiza glabra* Linn. against *Tribolum castaneum* after 24 hrs

Dilutions	Mortality (%)		Dilutions Means
	Acacia modesta	*Glycyrrhiza glabra*	
20%	20.00 a	10.00bc	15.00a
15%	12.00b	10.00bc	11.00b
10%	10.00bc	10.00bc	10.00b
5%	6.00cd	4.00de	5.00c
Control	0.00e	0.00e	0.00d
Plants Means	9.6a	6.80b	--------

Mean values followed by similar letters are insignificant . ANOVA values are significant (for plants, dilutions and P×D). LSD value for plants = 2.061, LSD value for dilutions = 3.259, LSD value for interaction P×D = 4.609 at ($P < 0.05$) level of probability

Table 2: Effect of different dilutions of *Acacia modesta* Wall. and *Glycyrrhiza glabra* Linn. against *Tribolum castaneum* after 48 hrs

Dilutions	Mortality (%)		Dilutions Means
	Acacia modesta	*Glycyrrhiza glabra*	
20%	36.00a	18.00cd	27.00a
15%	26.00b	22.00bc	24.00b
10%	26.00b	14.00d	20.00b
5%	16.00cd	12.00d	14.00e
Control	0.00e	0.00e	0.00d
Plants Means	20.80a	13.20b	--------

Mean values followed by similar letters are insignificant. ANOVA values are significant for (plants, dilutions and P×D) (LSD value for plants = 3.430, LSD value for dilutions = 5.423, LSD value for interaction P×D = 7.669) at ($P < 0.05$) level of probability

Phytotoxic activity

The present phytotoxic activity of *A. modesta* and *G. glabra* against *Lemna minor* showed that dilutions of these plants extracts caused growth inhibition of *Lemna minor* fronds when observed after one week. The *A. modesta* caused maximum 21.667% while *G.. glabra* caused 14.667% inhibition at 10+90 (distilled water + standard solution) of 20g plants extracts (table 5).

The F-test and analysis of variance (ANOVA) showed that the plants extracts dilutions were significant while the plants were insignificant with each other. The mean growth inhibition percentage for (10g) and (20g) plants extracts were (7.167, 4.000, 3.00, 1.000) and (18.167, 11.833, 8.667, 6.667) for different plants extracts dilutions as compared to control (0.000) (tables 4, 5).

The total mean growth inhibition for (10g) and (20g) plants extracts was (2.867) and (7.933) by *A. modesta* and (3.200) and (10.200) by *G.. glabra* as compared to control (0.000) after one week. The present study showed that growth inhibition caused by *G.. glabra* is higher than *A. modesta* at both the concentrations of the plants extracts. ANOVA revealed that the plants were insignificant. The dilutions of the plants extracts affected the growth inhibition percentage (tables 4 and 5).

DISCUSSION

Insecticidal activity

The present study revealed that different extracts dilutions of *A. modesta* and *G. glabra* caused mortality of *Tribolium castaneum* and mortality values were significantly different at $P < 0.05$. ANOVA showed that both the plants

Table 3: Effect of different dilutions of *A. modesta* Wall. and *G. glabra* Linn. against *T. castaneum* after 72 hrs

Dilutions	Mortality (%)		Dilutions Means
	Acacia modesta	*Glycyrrhiza glabra*	
20%	56.00a	38.00b	47.00a
15%	48.00a	30.00bc	39.00b
10%	36.00b	22.00cd	29.00c
5%	24.00c	14.00d	19.00d
Control	0.00e	0.00e	0.00e
Plants Means	32.50a	20.80b	--------

Mean values followed by similar letters are insignificant. ANOVA values are significant for (plants, dilutions and P×D) (LSD value for plants = 3.430, LSD value for dilutions = 5.423, LSD value for interaction P×D = 7.669 at (P < 0.05) level of probability

Table 4: Effect of different dilutions of *A. modesta* Wall. and *G. glabra* Linn. (10g) extracts against *L. minor*

S. No.	Dilutions (ml)	Growth inhibition (%)		Dilutions Means
		Glycyrrhiza glabra	*Acacia modesta*	
1	10+90	7.000 a	7.333 a	7.167 a
2	30+70	5.000 ab	3.000 b	4.000 ab
3	50+50	3.000 bc	3.000 b	3.000 bc
4	90+10	1.000 c	1.000 c	1.000 c
5	0	0.000 d	0.000 d	0.000 d
Plants Means		3.200	2.867	--------

Mean values followed by similar letters indicating insignificant results. Plants are insignificant while dilutions are significant. LSD value = 4.331 at (P < 0.05) level of probability

Table 5: Effect of different dilutions of *A. modesta* Wall. and *G. glabra* Linn. (20g) extracts against *L. minor*

S. No.	Dilutions (ml)	Growth inhibition (%)		Dilutions Means
		Glycyrrhiza glabra	*Acacia modesta*	
1	10+90	21.667 a	14.667 a	18.167 a
2	30+70	11.333 b	12.333 b	11.833 ab
3	50+50	10.000 bc	7.333 bc	8.667 b
4	90+10	8.000 bc	5.333 bc	6.667 bc
5	0	0.000 c	0.000 c	0.000 c
Plants Means		10.200	7.933	----------

Mean values followed by similar letters indicating insignificant results. Plants are insignificant while dilutions are significant. LSD value = 6.908 at (P< 0.05) level of probability.

extracts revealed that the mean mortality (%) was maximum after 72 hrs and minimum after 24 hrs as compared to control which showed direct proportionality with time. Maximum mortality (56.0%) was caused by *A. modesta* while *G. glabra* caused (38.0%) mortality at 20% dilution after 72hrs (table 3). This is in agreement with the findings of Kundu *et al*. (2007) and Khani and Asghari (2012). Ahmad *et al*., (2011) reported that the crude methanolic extract and various fractions were inactive against *T. castaneum.* The mortality (%) of *Tribolium castaneum* increased with the increase in concentration of both plants extracts. The mortality (%) was also directly proportional to concentration. This agrees with the findings of (Kundu *et al*., 2007; Jbilou *et al*., 2006) who worked on the medicinal plants for insecticidal activity using *Tribolium castaneum*. It was evident from the results that both the plants are potential sources of botanical insecticides against *T. castaneum.* Their toxic effects are time and concentration dependent.The major chemical constituents reported in rhizomes of *Glycyrrhiza glabra* are glycyrrhizin, glycyrrhizinic acid, glabin A and B, coumarines, triterpenes sterols and glabridin (Rabbits *et al*., 2007; Gantait *et al.*, 2010).These compounds could be responsible for the death of insects.

Phytotoxic activity

The dilutions of *A. modesta* and *G. glabra* were significantly phytotoxic against *Lemna minor.* The *A. modesta* caused maximum inhibition of *Lemna minor* as compare to *G. glabra* at (10+90) for 20g plants material. The total mean growth inhibition for (10g) and (20g) plants extracts was (2.867) and (7.933) by *A. modesta* and (3.200) and (10.200) by *G. glabra* as compared to control (0.000). It was observed that growth inhibition caused by *G. glabra* was higher than *A. modesta* at both the concentrations of the plants extracts. ANOVA indicated that the plants were insignificant while the dilutions of the plants extracts significantly ($P < 0.05$) inhibited the growth of the *Lemna* plants in the present investigation. Saponins have allelopathic property (Chaieb, 2010; Lee *et al*., 2004). They have been reported in *G. glabra* and they might be the cause of the inhibition of the *Lemna* plants in the present study.

CONCLUSION

In insecticidal activity both the plants showed significant mortality of *Tribolium castaneum* and these plants are useful in future for the control of *T. castaneum* population. Botanical pesticides reveal broad-spectrum activity, and are specific in their mode of action, easy to process, and safe for environment. They are cost effective and may abate the environmental pollution and health hazards. These plants could be potential sources of new phytotoxic agents that could be used for the control of weeds. Research should be directed towards isolation of insecticidal and phytotoxic bioactive compounds as well as further field trials can be carried out to confirm the present findings.

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