

PHYTOTOXIC AND INSECTICIDAL ACTIVITY OF PLANTS OF FAMILY ZYGOPHYLLACEAE AND EUPHORBIACEAE

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ABSTRACT

The statistical analysis revealed that 10g and 20g extracts of all the tested plants significantly inhibited the growth of *Lemna minor* with significant differences. The plants means were non-significant while the interaction between extracts and plants was significant. *Fagonia cretica*, *Peganum harmala*, *Tribulus terrestris*, *Chrozophora tinctoria* and *Ricinus communis* caused significant growth inhibition of *Lemna minor* in all the dilutions. The interaction between plants and dilution was non-significant. Among the three plants extracts, *Peganum harmala* showed the highest insects mortality followed by *Fagonia cretica* at the same dose. *Tribulus terrestris* showed the lowest mortality of *Tribolium castaneum*. All the doses (5 to 20%) of the plant extracts showed significant differences of mortality of *T. castaneum* as compared to control. The highest dose mean (12.8%) was shown by *P. harmala*, while the lowest dose mean was shown by *T. terrestris*.

Key Words: Allelopathy, Phytotoxic Activity, Zygophyllaceae, Euphorbiaceae

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INTRODUCTION

Phytotoxic natural products may be utilized either directly or as lead compounds for the development of herbicides (Morimoto *et al.*, 2009). A number of secondary metabolites in plants can act as allelochemicals to other plants. Under certain conditions, these compounds are released into the environment by exudation from living plant tissues and decomposition of plant material may affect the growth of the neighboring or successional plants Rice (1984) and Einhellig (1996). Using the *Lemna* assay, it is observed that natural antitumour compounds can inhibit *Lemna* growth. In addition, it was also discovered that some substances stimulate frond proliferation, and the assay may be useful to detect new plant growth stimulants. The commercial need for such natural, biodegradable, herbicides and plant growth stimulants may someday be filled with natural products detected by the simple and convenient *Lemna* bioassay (Atta-ur-Rahman, 1991).

Several workers reported the phytotoxic activity of various medicinal plants such as Khan *et al.* (2002) reported that *Abroma augusta* seed oil inhibited the growth of *Lemna aequinoctialis*. Allan and Adkins (2005) reported that *Chamaesyce hyssopifolia*, *Melaleuca quinquenervia*, *Acacia farnesiana*, *Ageratum conyzoides* and *Alphitonia excelsa* showed phytotoxicity against *Lemna aequinoctialis*. Hussain *et al.* (2009) reported that n-hexane, n-butanol, chloroform and water fractions of *Nepeta juncea* showed insignificant phytotoxic effect against *L. minor*. Khuda *et al.* (2012) reported the phytotoxic activity of chloroform fractions from *Achyranthes aspera* and ethyl acetate fraction from *Duchesnea indica*.

Over the past 15 years, interest in botanical insecticides has increased as a result of environmental concerns and insect populations becoming resistant to conventional chemicals (Siddiqui, *et al.*, 2002; Zaidi *et al.*, 2006). Botanical insecticides are naturally occurring insecticides that are derived from plants (Isman, 2000). Many alternatives have been tested to replace methyl bromide fumigation for stored products and quarantine uses. There is an urgent need to develop safe alternatives which have the potential to replace the toxic fumigants, yet are effective, economical and convenient to use (Ayvaz *et al.*, 2010). Different scientists have worked on the insecticidal activity of plants such as *Saussurea lappa*, *Peganum harmala* and *Valeriana officinalis* (Kanvil *et al.*, 2006), *Tribulus terrestris* (Singh *et al.* 2008), *Peganum harmala* (Jbilou *et al.*, 2006), *Melia azadarach*, *Myrtus communis*, *Mentha longifolia*, *Peganum harmala* and *Cymbopogon citrates* (Saljoqi *et al.* 2006). No reference is available regarding the phytotoxic and insecticidal activity of *Fagonia cretica*, *Chrozophora tinctoria* and *Ricinus communis*.

MATERIALS AND METHODS

Lemna minor was used as a test organism. The experimental conditions included light intensity (9000 Lux), photoperiod (12 hours), incubation condition (28° C, 56 ± 1% rh). Ten plants per flask were used. Number of plants per dose = Single with a rosette of three fronds. Phytotoxic activity of the extracts was carried out against the *L. minor* following McLaughlin *et al.* (1991) and Nisar *et al.* (2011). Ten grams of whole plant of *F. cretica*, *P. harmala*, *T. terrestris*, *C. tinctoria* and *R. communis* were soaked in 90 ml of distilled water and twenty grams of the same plants were soaked in 80 ml of distilled water as two standard solutions of distilled water in conical flasks. The flasks were covered with aluminum foil and allowed to stand at 25 ° C for 48 hrs. The extracts were filtered by Whatman filter paper. The aqueous extracts were further diluted by diluting the initial concentration by a factor of

twenty e.g., (10 ml + 90 ml, 30 ml+70 ml, 50 ml+50 ml, 90 ml +10 ml) plants extracts + distilled water, respectively. The various dilutions were tested for phytotoxicity against *L. minor*.

E-medium was prepared by mixing various constituents in 1000 ml distilled water and pH was adjusted between 5.5-6.0 by adding KOH pellets. The constituents and their respective proportions are: 1. Potassium dihydrogen phosphate (0.68gm/L). 2. Potassium nitrate (1.515gm/L). 3. Calcium nitrate (1.180gm/L). 4. Magnesium sulphate (0.492gm/L). 5. Boric acid (0.00286gm/L). 6. Magnesium chloride (0.00362gm/L). 7. Ferric chloride (0.00540gm/L). 8. Zinc sulphate (0.00022gm/L). 9. Copper sulphate (0.00022gm/L) 10. Sodium molybdate. 11. Ethylene diamino tetracetic acid. The E-medium was autoclaved at 121 °C for 15 min. Fifteen (15 mg) of extract was dissolved in 1.5 ml of solvent (Methanol/n-hexane) serving as stock solution. Three sterilized flasks were incubated with 10 µl, 100 µl and 1000 µl of solution pipetted from the stock solution and added to the flasks containing *Lemna* plants. Twenty ml of E-medium was added to each flask. Other flasks were supplemented with E-medium only as control. Plants were examined daily during incubation. Flasks were placed in growth cabinet for 7 days. The number of fronds / flask were counted and recorded on seventh day. Results were analysed as growth regulation in terms of percentage as described by (Nisar *et al.*, 2011).

Insecticidal Activity of Tribolium castaneum

Twenty grams (20g) of ground plant material was soaked in 80 ml of distilled water for 48 hrs. They were filtered using standard filter paper. Different dilutions of the plant extracts were made (20%, 15%, 10% and 5%) from the stock solution. Each extract was then used for insecticidal activity against *Tribolium castaneum*, stored insect at NIFA (Nuclear Institute for Food and Agriculture) Tarnab, Peshawar. Day-1 : The filter papers were cut according to the size of petri plate (9 cm or 90 mm) and were placed in the plates. The test sample was poured over the filter paper with the help of micropipette. The plates were left for 24 hrs at 25 °C to evaporate the solvent completely. Day-2: Next day (after the evaporation of solvent) *Tribolium castaneum* (10 healthy insects) were kept 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. The plates were incubated at 27 °C for 24 hours with 60% relative humidity in growth chamber. Day-3: The number of the living insects was counted. The mortality percentage was calculated with the help of following formula:

$$\text{Mortality (\%)} = 100 - \frac{\text{Number of insects alive in test}}{\text{Number of insects alive in control}} \times 100$$

The insecticidal activity was done following Khan *et al.* (2008).

Phytochemical Screening

The fresh specimens of *F. cretica*, *P. harmala*, *T. terrestris*, *C. tinctoria* and *R. communis* were collected from Peshawar and Attock Hills. The plant samples were washed, cleaned, and air-dried for about one week and crushed by grinding machine and powdered samples were used for phytochemical screening. Chemical tests were carried out on the aqueous, methanolic and n-hexane extracts to detect alkaloids, mucilage, anthraquinone, saponins, oil, tannin, flavonoids, steroids, terpenoids, glycosides, phlobatannins. Similarly, the chemical group tests namely phenolic, carboxylic acid, alcohol, hydroxyl group, aldehyde and/or ketone groups were carried out following the methods of Harborne (1973), Ngoci *et al.* (2011), Edeoga *et al.* (2005) and Evans (2009).

RESULTS AND DISCUSSION

Phytotoxic Activity

The ANOVA values (Tables 1-2) showed that 10g and 20g extracts of all the tested plants significantly inhibited the growth of *Lemna minor* with significant differences. The plants means were non-significant. The interaction between extracts and plants was also significant. *Fagonia cretica*, *Peganum harmala*, *Tribulus terrestris*, *Chrozophora tinctoria* and *Ricinus communis* caused significant growth inhibition of *L. minor* in all the dilutions. The interaction between plants and dilution was non-significant (Tables 1-2). Allelopathy is an important ecological factor in the plant kingdom including phytotoxic medicinal plants. Flavonoids reportedly act as allelochemicals (Samanta *et al.*, 2011). They were found in the methanolic and n-hexane extract of all the tested plants. These findings are in agreement with many workers (Jain *et al.*, 2010, Jain *et al.*, 2010; Alamdari 2011; Ahmad *et al.*, 2011; Rajkala *et al.*, 2011; Hussain *et al.*, 2011; Raja and Venkataraman, 2011. It was found that some members of Euphorbiaceae family have the potential allelopathic effect (Sisodia and Siddiqui, 2010; Gilani *et al.* 2010; Silva *et al.*, 2006; Ma, *et al.*, 2011) and these findings support the present results. The variations in the inhibition of *Lemna* by plants might be due to solvent and plant material. Allelopathic effects are related to plants, their parts and extraction procedures (Begum and Hussain, 1980).

The present study showed significant interaction between concentration and plants. The concentration and plants interaction mean value was the highest in *F. cretica* and the lowest in *T. terrestris* (Table 1). The

phytochemical screening of these plants showed that oils were found in stems and fruits of *F. cretica*, in the stems and leaves of *P. harmala*, in the roots, leaves and fruits of *T. terrestris*. Glycosides were present in the methanolic extract of *F. cretica*, *P. harmala* and in the n-hexane extract of *F. cretica* and *Tribulus terrestris*. Terpenoids were found in the methanolic extract of *P. harmala* and in the n-hexane extract of all tested plants. All these phytochemicals have the capability to exhibit phytotoxicity. This is in agreement with the work of Jha *et al.* (2010), Abrosca *et al.* (2005), Mancini *et al.* (2009), Hegazy and Farrag (2007) and Hale *et al.* (2004), who also observed phytotoxicity in plants. Steroids were present in the methanolic extract of *P. harmala* and *T. terrestris* and in the n-hexane extract of *F. cretica*, *P. harmala*. Amino acids were found in the methanolic extract of *F. cretica* and *P. harmala* and in the n-hexane extract of *P. harmala* and *T. terrestris*. Several workers reported the allelopathic effect of amino acids against plants (Uma *et al.*, 2009; Williams and Hoagland, 2007). Allelochemicals inhibit electron transport in mitochondria (Kapoor, 2011). Reducing sugars were present in the methanolic extract of *F. cretica*, *P. harmala* and *T. terrestris*. Phenolic compounds and their derivatives are potential inhibitors of germination and seedling growth and have allelopathic applications in agriculture and forestry as herbicides (Li *et al.*, 2010). Phenolics were found in the methanolic extract of *P. harmala* and in the n-hexane extract of *T. terrestris*. Anthraquinones were present in the stems of *P. harmala*, *F. cretica*, in leaves of *P. harmala*. These findings are in contrast with the findings of Hussain *et al.* (2011). The active principles detected in the above plants could be responsible for the inhibition of *Lemna* plants in the present study. Romero-Romero *et al.* (2002) stated that phytotoxins affect membrane permeability, ion uptake, electron transport in photosynthesis and respiratory chains, enzymatic activity, and cell division. In the present study the concentration and dilution interaction was also significant (Table 1).

Zygophyllaceae

The ANOVA values indicated that methanolic extracts of *F. cretica* significantly inhibited the growth of *L. minor* with significant differences among concentrations. *Fagonia cretica* showed 5.57, 12.22 and 50.0 % growth inhibition of *L. minor* while *P. harmala*, and *T. terrestris* did not show the significant differences and their results were statistically non-significant (Table 3). The ANOVA values showed that n-hexane extract of *F. cretica*, *P. harmala*, and *T. terrestris* showed non-significant results (Table 4). It was seen that methanolic extract of *F. cretica* showed the maximum growth inhibition at 1000 μ l as compare to the n-hexane extract of *F. cretica* at 100 μ l (Table 3). The n-hexane extract of *P. harmala* showed the maximum growth inhibition at 1000 μ l as compare to the methanolic extract at the same concentration (Table 4). These findings are in agreement with Nisar *et al.* (2010) who reported similar findings for *Impatiens bicolor*. The methanolic extract of *T. terrestris* showed the maximum growth inhibition at 100 μ l as compare to its n-hexane extract at 10 μ l (Tables 3-4). Rahman *et al.* (2011) reported similar results and observed that n-hexane extract of stem bark of *Pistacia integerrima* showed the least phytotoxic effect as compared to other solvents. Reason for this differential activity might be the solubility of phytochemicals.

Saponins have allelopathic property (Chaieb, 2010; Lee *et al.*, 2004). Saponins were detected in stems, leaves and fruits of *F. cretica* and *P. harmala* also in roots, stems and leaves of *T. terrestris* and in stems of *C. tinctoria*. The present findings agree with Kianbakht and Jahanianian (2003). The methanolic extract of *F. cretica*, *P. harmala*, *C. tinctoria* also had saponins. Shao-Lin *et al.* (2004) stated that phytotoxins activity varies with plant tissues. The leaves, stems, roots and seeds can release allelochemicals which differ in different parts or tissues of some plants. Stem, leaves and fruits of *F. cretica* and *P. harmala* had alkaloids. They were also found in roots, stem and leaves of *T. terrestris*. The work of Raja and Venkataraman (2011) supported the present findings as they reported alkaloids in *T. terrestris*. The relatively greater growth inhibition caused by *F. cretica* as compared to other plants was as a result of the active constituents in the plant acting in a more synergistic manner. Mucilage was present in stems and fruits of *F. cretica*. It was also found in roots, leaves and fruits of *T. terrestris*. The study concludes that *F. cretica* might be useful for weed control.

Euphorbiaceae

The ANOVA values showed that methanolic extracts of *R. communis* significantly inhibited the growth of *L. minor* with significant differences among concentrations. The *C. tinctoria* results were statistically non-significant (Table 3). The ANOVA values showed that n-hexane extracts of *C. tinctoria* and *R. communis* did not significantly inhibit the growth of *L. minor* (Table 4). The methanolic extract of *C. tinctoria* showed the maximum growth inhibition at 100 μ l as compare to the n-hexane extract of *C. tinctoria* at 10 μ l (Table 3). These results are supported by Dzoyem *et al.* (2011) who reported similar results from methanolic extract of *Diospyros canaliculata*. The methanolic extract of *R. communis* had the maximum growth inhibition at 1000 μ l as compare to the n-hexane extract of *R. communis* at 1000 μ l (Table 3 and 4). These results agree with Ahmed *et al.* (2003) who reported that methanolic extract of *Chenopodium murale* caused excellent inhibition of *Lemna* plants. Several researchers reported the inhibition of *Lemna* plants mostly at the high concentration (Khan *et al.*, 2005; Zaidi *et al.*, 2006; Ateeq-ur-Rehman *et al.*, 2009).

Oils were detected in roots, stems and fruits of *C. tinctoria* and in stems and leaves of *R. communis*. Saponins were found in stems of *C. tinctoria* and in roots and stems of *R. communis*. Alkaloids were found in leaves

of *Chrozophora tinctoria* and in the stems and leaves of *R. communis*. This agrees with Kensa and Yasmin (2011) who detected alkaloids in *R. communis*. Alkaloids interfere with cell division and presence of these substances might be responsible for the inhibition of *L. minor* in the present study. The toxic phytochemicals may either damage the DNA or inhibit protein synthesis, photosynthesis and plant growth (Shao-Lin *et al.*, 2004). Several workers reported the inhibitory role of alkaloids in plants (Jha *et al.*, 2010; Hagazy and Farrag, 2007; Sodaeizadeh and Damme, 2009). Present work supports these findings. Steroids were present in the methanolic extract of *C. tinctoria*. Amino acids were detected in the n-hexane extract of *C. tinctoria*. Phenolics were found in the methanolic and n-hexane extract of *R. communis*. Anthraquinones were present in the stems of *C. tinctoria* and *R. communis*. Mucilage was found in leaves of *C. tinctoria* and in the stems and leaves of *R. communis*. Tannins are allelopathic and inhibitory to most test organisms. Tannins were noted in roots, leaves of *C. tinctoria* and in all parts of *R. communis*. Jha *et al.* (2010) and Kensa and Yasmin (2011) strongly supported these findings. The methanolic extract of *C. tinctoria* and *R. communis* had tannins. Phlobatannins were present in roots, leaves and fruits of *C. tinctoria*. The presence of these phytochemicals detected in the investigated plants suggest that the inhibition of *Lemna* plants might be due to these compounds as they are very heterogenous mixtures of a single substance acting in a synergistic way or antagonistic manner. Phytotoxins activity varied with solvent extraction method as several workers have reported allelopathic potentials with n-hexane, acetone and water extracts of *Evolvulus alsinoides* and *Melissa officinalis*, with water extraction having the strongest allelopathic activity (Shao-Lin *et al.*, 2004).

The n-hexane extract of all the investigated plants showed the absence of saponins, alkaloids, tannins, phlobatannins and anthraquinones with the exception of flavonoids and this is in agreement with Srinivasan *et al.* (2007) who reported similar behaviour. In the present study methanolic extract of *F. cretica*, *P. harmala*, *T. terrestris*, *C. tinctoria* and *R. communis* revealed the presence of hydroxyl, carboxylic and phenolic groups while methanolic and n-hexane extract of *F. cretica*, *P. harmala*, *T. terrestris*, *C. tinctoria* and *R. communis* showed the absence of aldehyde and ketone group. The toxicity depends upon the part assayed and solvent system. This agrees with other workers (Hussain *et al.*, 2010; Hussain *et al.*, 2011; Ladhari *et al.*, 2011; Lopez *et al.*, 2009; Shao-Lin *et al.*, 2004), who reported phytotoxicity to be part and species specific and had an independent effect on various physiological processes. Toxicity is specie related and it plays important role in the plant distribution. In the present study the growth inhibition of *Lemna* plants increased with increasing concentration of extracts (Tables 3-4). These findings are in accordance with Rashid *et al.* (2009) and Nisar *et al.* (2011). Björkman (2011) stated that variation in plant species, developmental stage, plant organ and competition, season; climatic factors, light (intensity, quality, duration) and CO₂ significantly affect content and profile of phytochemicals. In addition, release and activity of allelo-chemicals may be affected by soil moisture, soil pH, soil organic matter content or soil salinity (Farooq *et al.*, 2011).

Table 1. ANOVA mean values for the effect of plants extracts concentration of some members of family Zygophyllaceae and Euphorbiaceae on growth inhibition of *Lemna minor* L.

Conc	Species	Dilutions (ml)					Control	CXP
		CXPXD						
		10+90	30+70	50+50	90+10			
10 (g)	<i>Fagonia cretica</i> L.	49.9	14.9	12.67	10.0	0.0	17.52 a	
	<i>Peganum harmala</i> L.	54.0	16.5	5.3	7.3	0.0	16.65 ab	
	<i>Tribulus terrestris</i> L.	31.3	9.6	9.9	6.6	0.0	11.53 bcd	
	<i>Chrozophora tinctoria</i> (L.) Raf	34.6	4.0	13.9	6.6	0.0	11.88 bcd	
	<i>Ricinus communis</i> L.	50.0	6.3	4.6	7.9	0.0	13.8 abc	
20 (g)	<i>Fagonia cretica</i> L.	13.21	5.3	5.3	10.6	0.0	6.9 d	
	<i>Peganum harmala</i> L.	12.8	12.0	7.9	7.3	0.0	8.0 d	
	<i>Tribulus terrestris</i> L.	12.54	10.6	7.3	14.0	0.0	8.9 cd	
	<i>Chrozophora tinctoria</i> (L.) Raf	21.3	16.6	7.3	5.9	0.0	10.2 cd	
	<i>Ricinus communis</i> L.	20.1	14.6	14.0	8.6	0.0	11.49 bcd	
PXD	<i>Fagonia cretica</i> L.	31.6	10.1	9.0	10.3	0.0	NS	
	<i>Peganum harmala</i> L.	33.4	14.2	6.6	7.3	0.0	NS	
	<i>Tribulus terrestris</i> L.	21.9	10.1	8.6	10.3	0.0	NS	
	<i>Chrozophora tinctoria</i> (L.) Raf	27.9	10.3	10.6	6.3	0.0	NS	
	<i>Ricinus communis</i> L.	35	10.4	9.3	8.3	0.0	NS	
10(g)		43.9 a	10.3 c	9.3c	7.7c	0.0d	CXD	
20(g)		16.0 b	11.8 bc	8.3 c	9.3 c	0.0 d		
Dilution Mean		30.0 a	11.0 b	8.8 b	8.5 b	0.0 c		
Plant Mean		12.2	12.3	10.2	11.0	12.6	NS	

LSD for Conc= 2.34 ; LSD for CXP= 5.24; LSD for dilution= 3.708; LSD for CXD=5.244 ; Coefficient of variation = 80.37% . Mean followed by different letters in the respective columns are significantly different at 5 % probability level according to LSD test.

Key: P=Plants; C=Concentration; D=Dilution; NS=Non-significant

Allelo-chemicals, inhibiting weeds growth, become favorable choice for natural pesticides. Many allelo-chemicals can be used to produce natural pesticides and much remains to be done in order to transform the laboratory studies into commercial products. In short, diversity of allelo-chemicals means that they can be used in multiple purposes. Synthesizing these compounds has important ecological significance and economic potentials. It can be predicted that allelo-chemicals will become an important impetus for eco-agricultural development. Allelopathy can help to explain the inhibitory effects or toxicity in the processes of rotation, intercrop and such studies can help avoid wasting billions of dollars in worldwide agricultural practices (Shao-Lin *et al.*, 2004).

Table 2. Mean squares values for the effect of various concentration of plants extracts of some members of family Zygophyllaceae and Euphorbiaceae on growth inhibition of *Lemna minor* L.

K Value	Source	Degree of freedom	Sum of square	Mean square	F value	Probability	Result
2	Conc (C)	1	1659.202	1659.202	18.7674	0.0000	*
4	Plants (P)	4	208.325	52.081	0.5891	-	NS
6	CXP	4	860.138	215.035	2.4323	0.0488	*
8	Dilution (D)	4	24522.300	6130.575	69.3436	0.0000	*
10	CXD	4	8204.223	2051.056	23.1997	0.0000	*
12	PXD	16	1222.116	76.382	0.8640	-	NS
14	CXPXD	16	1942.172	121.386	1.3730	0.1579	NS
-15	Error	200	17681.725	88.409			
	Total	249	56300.201				

Key: * = Significant; NS = Non-significant

Table 3. Effect of different concentrations of methanolic plant extracts of some members of family Zygophyllaceae and Euphorbiaceae on growth inhibition of *Lemna minor* L.

Conc (µL)	Zygophyllaceae			Euphorbiaceae		
	<i>Fagonia cretica</i> L.	<i>Peganum harmala</i> L.	<i>Tribulus terrestris</i> L.	<i>Chrozophora tinctoria</i> (L.) Raf	<i>Ricinus communis</i> L	
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	
Control	10.0 b ± 4.68	10.0 ± 5.18	10.0 ± 9.63	10.0 ± 5.47	10.0b ± 8.74	
10	5.57 b ± 4.68	13.3 ± 5.18	26.5 ± 9.63	13.3 ± 5.47	25.55b ± 8.74	
100	12.2 b ± 4.68	6.6 ± 5.18	34.4 ± 9.63	23.33 ± 5.47	33.33b ± 8.74	
1000	50.0 a ± 4.68	20.0 ± 5.18	26.6 ± 9.63	17.77 ± 5.47	61.09b ± 8.74	
Conc Means	19.44	12.5	24.41	16.11	32.49	
COV	41.70%	71.89%	68.28%	58.83%	46.59%	
LSD at α=0.05	15.27	Nil	Nil	Nil	28.50	

Key: SE = Standard Error; COV = Coefficient of variation, LSD = Least significant difference.

Table 4. Effect of different concentrations of n-hexane plant extracts of some members of family Zygophyllaceae and Euphorbiaceae on growth inhibition of *Lemna minor* L.

Conc (µL)	Zygophyllaceae			Euphorbiaceae	
	<i>Fagonia cretica</i> L.	<i>Peganum harmala</i> L.	<i>Tribulus terrestris</i> L.	<i>Chrozophora tinctoria</i> (L.) Raf	<i>Ricinus communis</i> L
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Control	10.0 ± 12.07	10.0 ± 10.86	10.0 ± 5.3	10.0 ± 3.09	10.0 ± 11.95
10	0.0 ± 12.07	16.6 ± 10.86	14.0 ± 5.3	7.7 ± 3.09	25.55 ± 11.9
100	43.0 ± 12.07	10.0 ± 10.86	3.3 ± 5.3	0.0 ± 3.09	33.33 ± 11.9
1000	1.0 ± 12.07	51.0 ± 10.86	4.4 ± 5.3	0.0 ± 3.09	61.09 ± 11.9
Conc Means	13.61	21.95	8.05	4.44	22.78
COV	153.61%	85.70%	114.57%	120.56%	90.9%
LSD at α=0.05	Nil	Nil	Nil	Nil	Nil

Key: SE= Standard Error; COV=Coefficient of variation, LSD=Least significant difference

Effect on mortality of *Tribolium castaneum*

Zygophyllaceae

Among the three plants extracts, *P. harmala* showed the highest insects mortality (21.0%), followed by *F. cretica* (9.0%) at the same dose (Table 5). *Tribulus terrestris* showed the lowest (2.6%) mortality. All the doses (5 to 20%) of the plant extracts showed significant differences of mortality of *T. castaneum* as compared to control. The highest dose mean (12.8%) was shown by *P. harmala*, while the lowest dose mean was shown by *Tribulus terrestris* (Tables 5). This is in agreement with the findings of Jbilou *et al.* (2006). Several workers reported the insecticidal activities of *P. harmala* (Jbilou *et al.*, 2006; Nateghpour, *et al.*, 2006;

Khattak, *et al.*, 2005; Shonouda, *et al.*, 2008; Ramya, *et al.*, 2008) and these findings strengthened the present results. Many workers have reported the biological activities of *P. harmala* (Edziri *et al.*, 2010; Sarpeleh, *et al.*, 2009 and Takhi, *et al.*, 2011) and suggested that active principles present in *Peganum harmala* might be responsible for the mortality of *T. castaneum* as in the present study. ANOVA for the effect of aqueous extract of *F. cretica*, *P. harmala* and *T. terrestris* on the mortality of insects revealed highly significant differences (Table 5).

Earlier workers reported various phytochemicals in members of Family Euphorbiaceae (Nwokocha *et al.*, 2011; Daniyan *et al.*, 2011; Edeoga *et al.*, 2005; James and Friday, 2010; Reuben *et al.*, 2008; Sule and Sani 2008; Mughal *et al.*, 2010; Singh *et al.*, 2010; Kensa and Yasmin, 2011 and Verma *et al.*, 2011). The presence of these phytochemicals may explain the toxic effects on *T. castaneum*. The variation in the mortality of *T. castaneum* might be due to the change in the solvents used. The mortality of *T. castaneum* increased significantly with the increasing concentration of extracts from 5 to 20% of extracts of all the tested plants (Table 5). These findings are supported by Khanna *et al.* (2003); Hameed *et al.* (2012); Abbasipour *et al.* (2010) and Hussain *et al.* (2010).

Euphorbiaceae

Among the two plants, *C. tinctoria* showed the maximum insects mortality (40.0%) while *R. communis* showed the lowest (5.0%) mortality at the same dose (Table 5). In the case of *C. tinctoria* the 5% and 10 % doses were ineffective showing insignificant mortality compared to the higher doses. In the case of *R. communis* all the doses (5% to 20%) showed significant ($p < 0.05$) differences in the mortality of *T. castaneum* compared to control. The highest dose mean (15.75%) was revealed by *C. tinctoria* followed by (3.5%) by *R. communis* (Table 5). ANOVA for the effect of aqueous extract of *C. tinctoria* and *R. communis* on the mortality of *T. castaneum* showed that doses were significant (Table 5).

Table 5. Effect of extracts of some plants on mortality (%) of *Tribolium castaneum*

Doses	Zygophyllaceae			Euphorbiaceae	
	<i>Fagonia cretica</i> L.	<i>Peganum harmala</i> L.	<i>Tribulus terrestris</i> L.	<i>Chrozophora tinctoria</i> (L.) Raf	<i>Ricinus communis</i> L
	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE
Control	0.8 c \pm 0.87	0.6d \pm 1.22	1.0 c \pm 0.45	0.75 a \pm 0.38	1.58 c \pm 0.83
5%	4.0 b \pm 0.87	7.3 c \pm 1.22	1.0 c \pm 0.45	0.0 c \pm 0.38	2.33 c \pm 0.83
10%	6.6 ab \pm 0.87	16.6b \pm 1.22	7.7 a \pm 0.45	0.0 c \pm 0.38	7.6 a \pm 0.83
15%	7.0 a \pm 0.87	18.3a b \pm 1.22	2.6b \pm 0.45	38.0b \pm 0.38	1.0c \pm 0.83
20%	4.0 b \pm 0.87	21.0a \pm 1.22	2.6b \pm 0.45	40.0a \pm 0.38	5.0b \pm 0.83
Plant Means	4.5	12.8	3.01	15.75	3.5
COV	33.58%	16.48%	25.77%	4.20%	40.71%
LSD	2.75	3.8	1.4	1.2	2.6

Key: SE= Standard Error; COV=Coefficient of variation, LSD=Least significant Difference.

CONCLUSION AND RECOMMENDATIONS

In insecticidal activity all the plants showed significant mortality of *Tribolium castaneum* as compared to control and these plants can be used in the control of *T. castaneum* population with integrated pest management which seems to be economically feasible and ecologically sound and investigated plants had toxic principles with significant insecticidal effect and could be a potential grain protectant against *T. castaneum*. These plants could be used for the isolation of active constituents as plants are promising source of pest control compounds that have generated extraordinary interest in the recent years. Botanical pesticides reveal broad-spectrum activity, and are specific in their mode of action, easy to process, produce, use 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 and insecticidal agents. As effective insecticidal activities were observed, more research should be directed towards isolation of insecticidal bioactive compounds as well as further field trials can be carried out to confirm the present findings.

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