Phytotoxicity Study of *Euphorbia granulata* Forssk against *Lemna minor* and Radish Seeds

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Abstract: The phytotoxic effect of *Euphorbia granulata* Forssk was investigating using *Lemna minor* growth, radish seed germination and roots length determination. No lethality was recorded at 0.01 and 1.0mg/ml concentrations however, at 0.1mg/ml of plant extracts the number of fronds increased by 21%. The lower toxic concentration (LTC) and the upper toxic concentrations (UTC) determined were 5.0mg/ml and 55.0mg/ml respectively while the LC_{50} was 33.88mg/ml. In case of radish seeds, 92.7, 95.3 and 94% germination take place with negative control, 1.0mg/ml, and 7.5mg/ml concentrations while the roots sizes at day five were 5.1, 5.4 and 5.5cm with negative control, 1.0 and 10mg/ml dose of extract respectively. The results indicated no inhibitory but a slight stimulatory effect of the plant extract.

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Introduction

Plants have biomechanisms to save from hostile plants and animals (e.g. insects). They produce certain metabolites intheir environments that not only save them from offensive organisms (plants, animals, microbes) but also eliminate the hostile one (Inderjit & Callaway. 2003; Yasmin *et al*., 2011). These metabolites are called phytochemicals and may be present in all of their parts. In hostile plants these phytochemicals induce chlorosis, wilting or death (Lungu *et* al., 2011). Some have stimulatory effect on non-hostile or friendly plants also (Yasmin *et al*., 2011). The phytochemicals that produces inhibitory or stimulatory impact on hostile or friendly plants are called allelochemicals and the phenomenon is called as allelopathy while the toxicity to other plants is called as phytotoxicity (Lungu*et* al., 2011; Khan *et al*., 2012; Gilani *et al*., 2010). Lemna bioassay an easy to perform method can be used to check plants for it toxic effect on other plants (Ateeq-ur-Rehman *et al*., 2009). Studies have shown that natural antitumor products inhibit lemna growth but other stimulates its growth. Therefore lemna phytotoxicity assay can be a rapid, easy, economical and simple bioassay and a tool for searching antitumor, phytotoxic and plant growth stimulant constituents (Hussain *et al*., 2010; Ayatollahi *et al*., 2010). Selective phytotoxicity of plant extracts can also be used to develop herbicides.

Materials and Methods

Phytotoxicity was evaluated on Lemna growth, radish seeds germination and radish roots length determination.

Lemna bioassay

Atta-ur-Rahman & Choudhary (2005) were followed for lemna bioassay. *Lemna minor*from local pond having three and two fronds were collected for this experiment. The pond water from where lemna was collected was filtered sterilized in autoclave and used as growth media. From stock solution (100mg/ml) of *E. granulata* three dilutions of 5000 µg, 500 µg and 50 µg/ml were prepared in DMSO. From each of the above samples 4ml were added to 50ml beaker and allowed to evaporate till dryness. Into each beaker 15 ml of growth media were added. The pH was adjusted to 5.5 and 10 *lemna minor* each having three fronds were added with the help of a forceps. The final volume of media was raised to 20 ml with growth media so that it contained1000, 100 and 10 µg/ml of extract sample. Glyphosate was positive control while growth media negative control. All the beakers were covered with aluminum foil to minimize evaporation. The experiment was performed in five replicate and was left at room temperature. The plants were observed daily for one week. The lemna fronds were counted in all beakers and recorded for toxicity calculations.

$$\text{Mortality\%} = \left[\frac{100 - \text{Number of fronds in test samples}}{\text{Number of fronds in negative control}} \right] \times 100$$

Radish seeds phytotoxicity

Radish seed germination: McLaughlin & Rogers (1998) technique with some adjustments was adapted to check phytotoxicity of *E. granulata.* From stock solutions of extract a two dilutions of 7.5mg/ml and 1mg/ml were prepared in DMSO. Filter paper discs of 9 cm were soaked with 5ml of each extract sample and were dried in an electric oven at 50^0C. They were placed in 9cm petridishes. The discs were soaked again with 5ml of sterilized (autoclaved) tape water. Fifty radish seeds (surface sterilized with 0.1%HgCl3) were distributed at equal distance from each other in each plate. To avoid contamination the plates were sealed using parafilm and let to grow at room temperature. The seeds were checked and noted daily for five days. For negative control DMSO were used. The experiment was performed in triplicate and the percentage inhibitions of seeds growth were calculated at the end of experiment.

Roots length measurement: Extract concentration of 10mg/ml and 1mg/ml were used in this experiment. The set up was repeated for determining roots length. Here the increases in rootlengths were monitored daily. Fifteen seeds were used in each experiment and the experiment was performed in triplicate. At the end of experiment the average growth of roots in each sample and negative control were compared.

Results and Discussions

Lemna bioassay: Inhibitory effects of *E. granulata* extract on the growth *Lemna minor* are presented in Table 1. The results revealed that all the fronds survived (100%) in DMSO while 100% inhibition was noted for glyphosate. At 0.01 mg/ml of extracts, no inhibition occurred while at 0.1 mg/ml the number of fronds increased by 21% from 45 to 57. Only one frond wilted (2%) at 1.0 mg/ml concentration (Table 1). In self-defense plants produces certain compounds which directly inhibit growth of other plants. They may create repulsive smell and taste to protect from herbivores. These compounds are not always toxic but may be neutral or stimulatory for certain organisms (Inderjit & Callaway. 2003; Yasmin *et al*., 1999). Many biological molecules are high profile drugs. Natural products that show inhibitory effects on tumor growth have also shown inhibitory effect on lemna growth. Therefore, lemna bioassay is considered as one of the initial screening test for searching antitumor drugs (Hussain *et al*., 2010; Ayatollahi *et al*., 2010; Ahmad *et al*., 2011). The present study revealed no inhibitory effect at 0.01 and 1.0 mg/ml on the growth of *Lemna minor*. At these concentrations a a neutral impact was observed. However, at 0.1 mg/ml concentration the number of lemna fronds increase from 45 to 57, showing a significant increase of +21% in the number of fronds. This indicates that the extract had stimulatory effect at this specific dose. The toxic levels and LC_{50} were also determined (Fig. 24). The LC_{50} was calculated using probit values versus log of doses used (Tables 2, 3). At 5mg/ml strength no frond growth showed inhibition while the 100% inhibition was noted at 55 mg/ml (Table 2). The high toxicity value of 55mg/ml and smallest safe value of 5 mg/ml led to high LC_{50} value. These values suggest that the plant is not harmful at ordinary dose and had no activity against weeds growth. Previously, the plant was checked for antitumor potential and the present results show a complete correlation in two activities. In case of antitumor activity stimulatory effect was noted for *Agrobacterium tumefaciens* by slightly increasing the number of tumors produced. Terpenes and phenols are considered as importantallelochemicals (Sarkar *et al*., 2012). In preliminaryphytochemical detection tests, terpenes and perpenoids were not detected in *E. granulata*. This shows an agreement between the phytochemistry and present investigations of this plant. Growth hormones like auxin and cytokinin are important growth hormones. The present stimulant effect of extract might be due to some compounds having growth hormones like effect (Shahid-ud-daula & Basher, 2009).

Literature shows different observations using different plants extracts. Lungu *et al,* (2011) observed that aqueous and alcoholic extracts of *Melia azedarach L,*had inhibitory effects on germination and growth of *Lactuca sativa* L. Ahmad *et al*, (2011) reported ethyl acetate fraction of the extract of *Zizyphus jujuba* moderately phytotoxic to*Lemna minor* at 1000 µg/ml. Ateeq-ur-Rehman *et al*, (2009) found *Thymus serpyllum* L. with no phytotoxicity against *Lemna minor* or radish seeds. Poor phytotoxic activity of different extracts of *Nepeta juncea* even at higher doses was reported by Hussain *et al*, (2009).Khan *et al*, (2008) used ethanolic extracts of *Trichodesma indicum, Aconitum leave* and *Sauromatum guttatum* against *Lemna minor* and found them highly effective in inhibiting the plant growth at only 500 pg/ ml concentration. Islam *et al,* (2009) reported that *Oldenlandia diffusa* (Willd.) Roxb significantly inhibited seed germination and root growth of radish seeds.

Radish seeds germination and growth method

The effect of extracts on seeds germination and radical elongation of radish are given in Tables 3 and 4, respectively.

Effect on seeds germination: At 7.5 mg/ml extract, out of 150 seeds 141 germinated. This showed 94% germination. At 1.0 mg/ml 143 seeds germinated (95%). In negative control 139 seeds germinated (93%). Percentage inhibition was 6%at 7.5 mg/ml ,

4.7%at 1.0 mg/ml while 7.3% was inhibition noted in negative control.

The standard criteria use for percent inhibition was, 0 to 39% represent low or poor activity, 40 to 59% moderate activity, 60 to 69% is good and above 70% is considered as significant activity (Ayatollahi *et al*., 2010).When comparing to these standards, it appeared that the extract had no inhibitory effect against radish seeds germination. Studies shows that mono terpenoids and sesquiterpenoids plays important role in inhibiting the growth of seedlings by inhibiting cell division and inducing structural breaks in roots (Mancini *et al*., 2011). Not only during the phytochemical investigations of this plant these phytochemicals were not detected but our present results also suggest that *E. granulata* lacks these allelopathic compounds.

Effect on seeds radical growth: At 10 mg/ml of extract the roots mean length was 1.4 cm (±0.41) on day one that increased in length to 5.5 cm (±0.892) on day 5. Similarly the mean root length recorded for 1.0 mg/ml on day 1 was 1.8 cm (±0.262) thatattained maximum length of 5.4 cm (±0.448) on 5th day of experiment. In negative control the growth recorded was 1.3 (±0.401) on day 1 which attained 5.1 cm length (±1.16).

The mean growth in all the three sets was almost equal that suggests no impact on overall growth. However, comparing the increases in length from day onward gives some indication of impact. The mean length of radical on day 1for 10 mg/ml concentration was 1.4 cm. From this day onward the average increases in lengths were 293%. The root length for 1.0 mg/ml from 1.8 cm on day 1 to 5.4 cm on day 5 was a 200% increase in the mean roots length. Furthermore, the length of radical in negative control increased from 1.3 to 5.1 cm, a 292% increase. Carefully looking at these results shows that the increases in roots length for 10 mg and negative control are similar. This suggests two possibilities: In the first case the 1 mg dose looks inhibitory when compared to negative control. However, comparing the results of 1.0 and 10 mg doses, a stimulatory effect was observed with increase dose of extract.

Table 1. Effect of ethanolic extract of *Euphorbia granulata* on Lemna minor growth.

Parameters	NC	Extract concentration used			PC
	DMSO	0.01mg/ml	0.1 mg/ml	1.0mg/ml	Glyphosate
No of fronds used	45	45	45	45	45
Fronds survived	45	45	57	44	0
Dead fronds	0	0	(- 12)	1	45
LC_{50} (mg/ml)	-	33.88	33.88	33.88	-
UTC (mg/ml)	-	55.0	55.0	55.0	-
LTC (mg/ml)	-	5.0	5.0	5.0	-

NC = negative control, PC = positive control, UTC = upper toxic concentration, LTC = lower toxic concentration,
Note: The negative values represent the increase in fronds number.

Table 2. Calculating LC_{50} of *Euphorbia granulata* for Lemna minor.

Groups	Dose (mg)	Log of dose	Total fronds	Dead fronds	Percent dead	Corrected percentage	Probit value
1	5.0	0.70	45	0	0	2.5	3.04
2	17.0	1.23	45	3	6.7	6.7	3.49
3	29.0	1.46	45	17	37.8	37.8	4.68
4	41.0	1.61	45	34	75.5	75.5	5.69
5	55.0	1.74	45	45	100	97.5	6.96

Note. Log value against probit 5 was 1.53 and its antilog value was 33.88 (LD50)

Table 3. Conversion table from percentage to probit value.

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

Table 4. Impact of *Euphorbia granulata* extract on radish seeds germination.

Concentration mg / ml	Seeds used	Seeds germinated	Percent germination
7.5	150	141	94
1.0	150	143	95.3
Negative control	150	139	92.7

Table 5. Impact of *Euphorbia granulata*extract on radical elongation of radish seeds.

Concentration mg / ml	Radical length with ±SD				
	D1	D2	D3	D4	D5
10.0	1.4 ± 0.41	2.6 ± 0.576	3.5 ± 0.656	4.1 ± 0.719	5.5 ± 0.892
1.0	1.8 ± 0.262	2.4 ± 0.251	3.7 ± 0.428	4.7 ± 0.405	5.4 ± 0.448
Negative control	1.3 ± 0.401	2.1 ± 0.558	3.4 ± 0.853	4.3 ± 1.023	5.1 ± 1.16

D1 = day 1st, D2 = day 2nd, D3 = day 3rd, D4 = day 4th, D = day 5th.

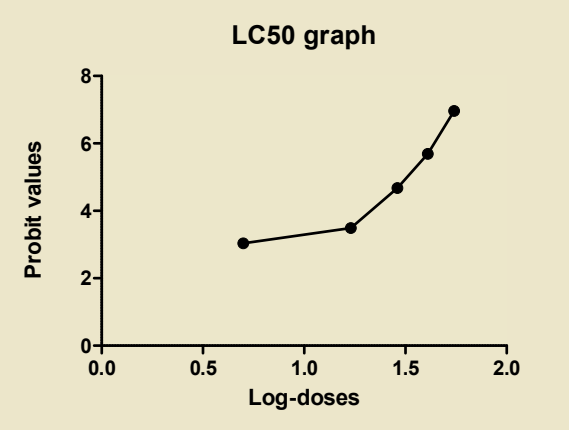

Fig. 1. LC_{50} determination for EG extract against *Lemna minor.*

Conclusion

It can be concluded that at 0.1 mg/ml dose *E. granulata*extracts enhanced the lemna growth. However neither inhibitory nor stimulatory effect were noted on seed germination or seedling growth at any of the extract doses.The effect on radical elongation was somewhat ambiguous. The extract was inhibitory against *Lemna minor*only at high concentration of 55.0mg/ml and LC_{50} value of 33.88mg/ml.

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