INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–2–240–242
http://www.ijab.org

Toxicity of Four Synthetic Plant Hormones IAA, NAA, 2,4-D and GA against *Artemia salina* (Leach)

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ABSTRACT

Due to their wide spread distribution and toxic nature on biological system, plant hormones were screened for their cytotoxic assessment in *Artemia salina* Leach. A simple zoological organism *Artemia salina* was used for cytotoxicological evaluation and probit analyses was used to calculate median lethal concentrations (LC_{50}) values. IAA was found to have most cytotoxic with LC_{50} of 4.39 ppm among the plant hormones. NAA exhibited almost similar LC_{50} value (5.75 ppm) of IAA. Other two 2,4-D and GA gave the values at 16.63 and 20.13 ppm, respectively. The rank order of toxicity of the four plant hormones against *Artemia salina* was IAA > NAA > 2,4-D > GA.

Key Words: Plant hormones; Cytotoxicity; *Artemia salina* (Leach)

INTRODUCTION

In the *in vivo* bioscreening methods of cytotoxicity measurement, brine shrimp lethality bioassay is a comparatively simple and widely acceptable technique. A simple zoological organism *Artemia salina* Leach is used for conventional monitoring the toxicity of tested bioactive compound(s) (McLauglin *et al*., 1991). The shrimp lethality assay was proposed by Michael *et al.* (1959) and later developed by Vanhaecke *et al.* (1981) and Sleet and Brendel (1983). It is based on the ability to kill laboratory-cultured brine shrimp (*Artemia* nauplii). The assay is considered a useful tool for preliminary assessment of toxicity (Solis *et al*., 1993) and it has been used for the detection of fungal toxins (Harwig & Scott, 1971), plant extract toxicity (McLauglin *et al*., 1991), heavy metals (Martinez *et al*., 1998), cyanobacterial toxins (Jaki *et al*., 1999), pesticides (Barahona & Sánchez- Fortún, 1999), and cytotoxicity testing of dental materials (Pelka *et al*., 2000). The plant hormones, IAA, NAA, 2-4-D and Gibberellic acid (GA) were found to have excellent role as plant growth promoters but they also reported to have various important uses in different commercial aspects like 2-4-D used as herbicide (Raven *et al*., 1976; Audesirk & Audesirk, 1986). NAA was reported to reduce fruit drop (Raven *et al*., 1976) and GA along with IAA were reported to produce larger fruits (Raven *et al*., 1976). Other important bioactive properties have been studied with these compounds e.g., anticancer property has been reported for the compound indoleacetic acid (IAA) and its different derivatives (Folkes *et al*., 2002, 2003; Rossiter *et al*., 2002). Diverse mechanisms have also been reported associated with their activities (Folkes *et al*., 1999, 2003; Greco *et al*., 2000). IAA has antifungal property against some plant fungi (Yue *et al*., 2000; Pal *et al*., 2001; Pedras & Montaut, 2003). Plant hormones are widely used throughout the world as agricultural purposes and diverse activities of these bioactive synthetic hormones encouraged us to investigate their cytotoxic characteristics. Toxic nature of compounds against *Artemia salina* often indicates toxicity to other normal cells and to assess the toxic properties of these plant hormones we studied their effects in different concentration against brine shrimp.

MATERIALS AND METHODS

Preparation of simulated seawater. 38 g of sea-salt (non ionized NaCl) was weighed accurately, dissolved in one liter of sterilized distilled water and then filtered off to get clear solution. The pH of the seawater was maintained between 8 and 9 using $NaHCO_3$ solution.

Hatching of brine shrimp eggs. *Artemia salina* Leach (brine shrimp eggs) collected from the pet shop was used as the test organism. Simulated seawater was taken in the small tank and the shrimp eggs (1.5 g L^{-1}) were added to one side of the tank and this side was covered. The shrimps were allowed for two days to hatch and matured as nauplii (larvae). Constant oxygen supply was carried out during the hatching time. The hatched shrimps were attracted to the lamp light on the other side of the divided tank through the perforated dam. These nauplii were taken for this bioassay.

Preparation of the test sample solution. The plant hormones were collected as the reagent grade (Fluka, Germany). 1.0 mg of each of the tested compounds (IAA, NAA, 2,4-D and GA) and antimicrobial agent ampicillin trihydrate were accurately weighed and dissolved in 0.1 mL of DMSO (dimethyl sulfooxide). Thus a concentration of 10 μg μL^{-1} was obtained which was used as stock solution.

From the stock solutions 2.5, 5.0, 7.5, 10, 20, 40 and 80 µL were placed in seven different vials making the volume up to 5 mL by artificial sea water (3.8% NaCl solution). The final concentration of the samples, in the vials became 2.5, 5.0, 7.5, 10, 20, 40 and 80 µg mL^{-1}, respectively (Sheikh *et al*., 2004).

Application of brine shrimp nauplii. With the help of the Pasteur pipette 10 living nauplii were added to each of the vials containing 5 mL of simulated sea-water. A magnifying glass was used for convenient counting of the nauplii. For the control test of each vial, one vial containing the same volume of DMSO plus water up to 5 mL was used and 10 living nauplii were also taken in these control vials. Precautions were taken to avoid eggs during taking the shrimps in the vials.

Counting of nauplii. After 24 h of incubation, the vials were observed using a magnifying glass and the number of survived nauplii in each vial were counted and recorded. From this data, the percentage of mortality of the nauplii was calculated for each concentration of the sample.

Statistical analysis. Probit analysis was used to analyze data from bioassay experiments (Finney, 1971). In toxicity study on *Artemia salina* the brine shrimp nauplii were typically exposed to several concentrations of a tested compound to determine the concentration that will kill 50% of shrimps within a given time span. In the present study, we also used probit analysis in IBM compatible computer to calculate LC_{50} values of the tested compounds IAA, NAA, 2,4-D and GA.

RESULTS AND DISCUSSION

Results of toxicity study on *Artemia salina* were obtained after probit transformation of the resulting mortality data for the compounds IAA, NAA, 2,4-D, GA and ampicillin trihydrate were cited in the Table I and II. LC_{50} values of the compounds IAA and NAA were found to be 4.39 and 5.75 ppm, respectively which were almost similar to the value of 5.14 ppm for the standard ampicillin trihydrate (Table II). In the previous literature, Gallic acid was found to have LC_{50} value of 4.53 (Sarkar *et al*., 1988) and which was used as standard for to assess cytotoxicity of plant extract. In the present investigation, we used ampicillin trihydrate as standard and it was interesting finding that the LC_{50} value of this standard (5.14 ppm) was

Table I. Probit mortality data of IAA, NAA, 2,4-D, GA and Ampicillin trihydrate against *Artemia salina*

Test samples	Dose	Logdose	Number	Kill	Kill %	Corr %	Emp probit	Expt probit	Wrk probit	Weight	Final probit
NAA	2.5	0.39793	10	2.66	26.6	27	4.39	4.19318	4.436	4.71	4.19827
	5	0.69896	10	3.66	36.6	37	4.67	4.86476	4.682	6.27	4.86434
	7.5	0.87505	10	5.33	53.3	53	5.08	5.25760	5.098	6.27	5.25396
	10	0.99998	10	7.33	73.3	73	5.61	5.53633	5.584	5.81	5.53040
	20	1.30101	10	9.33	93.3	93	6.48	6.20791	6.383	3.7	6.19647
IAA	2.5	0.39793	10	3.33	33.3	33	4.56	4.45994	4.57	5.58	4.45067
	5	0.69896	10	5.66	56.6	57	5.18	5.13861	5.165	6.34	5.12629
	7.5	0.87505	10	6	60	60	5.25	5.53560	5.22	5.81	5.52150
	10	0.99998	10	8	80	80	5.85	5.81727	5.8	5.03	5.80190
	20	1.30101	10	9.66	96.6	97	6.88	6.49594	6.759	3.02	6.47752
2,4-D	5	0.69896	10	1.66	16.6	17	4.05	3.98440	4.062	4.05	3.98365
	7.5	0.87505	10	2	20	20	4.16	4.3289	4.17	5.32	4.32652
	10	0.99998	10	3.66	36.6	37	4.67	4.57332	4.656	5.81	4.56979
	20	1.30101	10	5.66	56.6	57	5.18	5.16223	5.165	6.34	5.15593
	40	1.60204	10	7.66	76.6	77	5.74	5.75114	5.734	5.32	5.74206
GA	5	0.69896	10	1	10	10	3.72	3.78399	3.72	3.36	3.82835
	10	0.99998	10	3	30	30	4.48	4.39199	4.49	5.32	4.41142
	20	1.30101	10	5.33	53.3	53	5.08	5.00	5.075	6.37	4.99449
	40	1.60204	10	6.66	66.6	67	5.44	5.60800	5.43	5.58	5.57756
	80	1.90307	10	9	90	90	6.28	6.21600	6.23	3.7	6.16063
Ampicillin trihydra	1	0	10	2	20	20	4.16	4.11949	4.17	4.71	4.12434
	2	0.30102	10	3	30	30	4.48	4.49726	4.48	5.58	4.49503
	5	0.69896	10	5	50	50	5	4.99664	4.99	6.34	4.98507
	10	0.99998	10	6	60	60	5.25	5.37441	5.24	6.16	5.35577
	20	1.30101	10	8	80	80	5.85	5.75218	5.83	5.32	5.72646

Table II. LC50 values of compounds IAA, NAA, 2,4-D, GA and Ampicillin trihydrate and reference value of standard Gallic acid and anticancer agent Bleomycin.

Compounds	LC_{50} (ppm)	95% Confidence limit (ppm)		Regression Equation	λ^2	df
		Lower	Upper			
NAA	5.75	3.82	8.66	Y = 3.32 + 2.21 X	0.77	3
IAA	4.39	2.77	6.94	Y = 3.55 + 2.24 X	0.85	3
2,4-D	16.63	10.38	26.62	Y = 2.62 + 1.94 X	0.19	3
GA	20.13	12.55	32.28	Y = 2.47 + 1.93 X	0.25	3
Ampicillin	5.14	2.57	10.28	Y = 4.12 + 1.23 X	0.15	3
Bleomycin	0.41	0.27	0.62	Y = 3.16 + 2.99 X	0.62	2
Gallic acid	4.53	3.33	6.15	Y = 3.93 + 1.62 X	1.25	2

almost similar to the standard cytotoxic agent Gallic acid (4.53 ppm). This will support our study of evaluating cytotoxicity of the plant hormones. The LC_{50} value of anticancer agent bleomycin in brine shrimp lethality bioassay was reported at 0.41 ppm (Sheikh *et al*., 2004). By correlating this data of bleomycin with the tested compounds IAA and NAA, it was indicative that cytotoxicity and its mechanism of the two tested compounds were significantly different than the bleomycin. IAA and NAA were found to have less harmful agricultural compounds as toxicity of these agents against brine shrimp was much lower than the standard cytotoxic agent bleomycin.

Other two tested compounds 2,4-D and GA showed LC_{50} values of 16.63 and 20.13 ppm, respectively which was indicative of their much lower toxicity against brine shrimp than the IAA and NAA. As higher LC_{50} value indicates lower toxicity so, these results indicated that the two agricultural agents 2,4-D and GA were comparatively less toxic to *Artemia salina*. From the results, it was also clear that 2,4-D and GA exhibited different cytotoxic property than IAA and NAA against the brine shrimp nauplii which indicated their different mechanism of cytotoxic actions.

CONCLUSION

In the present study we observed almost similar toxic character of naphthaleneacetic acid (NAA) with indoleacetic acid (IAA) which was an interesting finding. IAA and its derivatives were well reputed to have cytotoxic properties (Folkes *et al*., 2002, 2003; Rossiter *et al*., 2002) so, we could propose that NAA might have similar anticancer characteristics as IAA and further investigations might help to explore NAA or its derivatives as potent anticancer agent(s). Though the cytotoxic potency of NAA was found almost similar to IAA so, it indicated that they might have similar mechanism of cytotoxicity. Though this is primary study so, further study will led the scientists to find the exact mechanism of toxicity effect of NAA against *Artemia salina* which could be a valuable tool for to explore new type(s) of cytotoxic agent(s).

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(Received 28 November 2004; Accepted 20 January 2005)