African Journal of Biotechnology Vol. 8 (18), pp. 4465-4468, 15 September, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

The environmental toxicity of *Dicerothamnus rhinocerotis* and *Galenia africana*

E. J. Pool*, J. A. Klaasen and Y. P. Shoko

Department of Medical Bioscience, the University of the Western Cape, Private bag X17, Bellvile, 7535, South Africa.

Accepted 22 June, 2009

Over the years the use of pesticides has greatly increased. This in turn has lead to concern about the adverse effects that the pesticides may have on non-target organisms in the environment. Due to increasing awareness there is great pressure to reduce the use of synthetic pesticides. An alternative to the use of synthetic pesticides is the exploitation of natural botanical products with pesticidal potential. *Dicerothamnus rhinocerotis* and *Galenia africana* are plants, indigenous to South Africa, with fungicidal properties against a fungal pathogen that causes grey-mould rot on a wide range of agricultural produce. In this study a series of acute toxicity tests were conducted to estimate the potential environmental effect of *D. rhinocerotis* and *G. africana*. The acute toxicities of the plants were determined using the species *Daphnia pulex*, *Selenastrum capricornutum*, *Vibrio fischeri* and *Poecilia reticulata* as bio-indicators. Results obtained showed that *G. africana* had higher toxicity units than *D. rhinocerotis*, thus showing that *G. africana* is more toxic to the aquatic environment compared to *D. rhinocerotis*.

Key words: Pesticides, *Galenia africana*, *Dicerothamnus rhinocerotis*, acute toxicity.

INTRODUCTION

Pesticides are used at a global scale for pest control thus allowing the maintenance of agricultural produce and contribute to economic growth and stability (Arias-Estevez et al., 2008). The wide use of pesticides has however become a pervasive threat to natural ecosystems. There is increasing awareness of potential environmental and health problems associated with pesticide use (Carbone et al., 2007; Caserta et al., 2007; Kodavanti et al., 2008). This has resulted in pressure to reduce the use of synthetic pesticides. An emerging alternative to synthetic pesticides is the study and exploitation of naturally occurring products with pesticidal properties (Isman, 2006 and Regnault-Roger and Philogene, 2008). In order to evaluate the effects of new pesticides, there is need for toxicological data on organisms' representative of the various ecosystems.

Dicerothamnus rhinocerotis (L.f.) (= *Elytropappus rhinocerotis* (L.f.), popularly known as rhenoster bush ("renosterbos" in vernacular) or rhinoceros bush, is a bush shrub of about 1 - 2 m in height, with small grayish-green leaves and tiny flower heads which are almost inconspicuous (Levyns, 1935). *D. rhinocerotis* is of the family asteraceae and is native to Southern Africa (Van et al., 1997).

Galenia africana, commonly known in vernacular as "kraalbos" or "geelbos", is a perennial shrub belonging to the family aizoaceae (Van der Lugt et al., 1992). *G. africana* was most common in the Namaqualand region of South Africa but has recently become more widespread in the western and southern Karoo (Kellerman et al., 1988). *G. africana* is an aromatic, woody sub-shrub that grows to 0.5 - 1 m in height (Van der Lugt et al., 1992). Its green leaves are oppositely arranged with small yellow flowers at the end of the twigs (Kock, 1928; Kellerman et al., 1988).

Whole plant extracts of D. *rhinocerotis* and *G. africana* exhibit antifungal properties against *Botrytis cinerea*. *B. cinerea* is a fungal pathogen that causes grey mould/gray mold rot, on a large number of economically important agricultural and horticultural crops such as fruits, flowers and green tissue (Jarvis, 1997).

Due to the antifungal properties of the two plants they may have potential use on agricultural produce as fungi-

*Corresponding author. E-mail: epool@uwc.ac.za. Tel.: +2721 959 3535. Fax: +2721 959 3125.

Table 1. EC_{50} values obtained for *D. rhinocerotis* and *G. africana* when using *S. capricornutum* and *V. fischeri.*

Test	Exposure period	EC_{50} values for *D. rhinocerotis* (µg/ml)	EC_{50} values for *G. africana* (µg/ml)
V. fischeri bioluminiscent test	15 min	110	1
V. fischeri bioluminiscent test	30 min	100	0.7
S. capricornutum test	72 h	390	100

cides. Ecotoxicity studies are required to determine safety of the plant extracts before they can be used commercially. In this study the acute toxicity of *D. rhinocerotis* and *G. africana* was determined by using several aquatic species. LD_{50} and EC_{50} levels obtained from this study will be used to set maximum allowed environmental levels of these plant extracts.

MATERIALS AND METHODS

Plant extractions

Two medicinal plant species, *G. africana* and *D. rhinocerotis,* used in traditional medicine practices in South Africa were obtained and prepared for analysis. 20% (w/v) extracts were prepared in 94.4% ethanol by Parceval (Pty) Ltd pharmaceuticals (South Africa). Leaves of the plants were milled (sieve size~ 2 - 3 mm) after which the leaves were mixed with 94.4% ethanol at 20 g leaves per 100 ml ethanol. The mixture was shaken for brief periods during overnight extraction. The extracts were then pressed to separate the tincture and the milled leaves. Filtration was carried out to clean tincture from plant debris and the extracts were then stored at 20°C. The extracts were then air dried and resuspended in DMSO to obtain 50% (w/v) extract. For this study the extracts were further diluted to 1 mg/ml using deionised water, which was used as the highest concentration.

Test species

The test species used in this study are important components of many aquatic communities and therefore can be adversely affected by aquatic pollutants. *Poecilia reticulata* (guppy fish, also known as the million fish) at 7 - 21 day post-hatch and 24 h old *Daphnia pulex* (water flea) were obtained from in-house cultures at Golder Associates Research Laboratories. *Selenastrum capricornutum*, (Printz algae beads, CCAP 27814, Cambridge UK) and lyophilised *Vibrio fischeri* luminiscent bacteria (NRRL B-11177) were also used for this study.

General test conditions

All toxicity tests were conducted in appropriate environmentally controlled rooms using standard techniques. The tests were conducted at Golder Associates Research Laboratories.

V. fischeri bioluminiscent test

The methods employed for the toxicity testing of *G. africana* and *D. rhinocerotis* on *Vibrio fischeri* followed those outlined in EN ISO (International Organisation for Standardisation) 11348-3, 1998. Test chambers used were polystyrene cuvettes for Luminoskan TL luminometer (Hygiene Monitoring System). *V. fischeri* were exposed at dilutions of plant extracts for 15 and 30 min. Test endpoints included percentage growth inhibition relative to control and EC_{50} values. Two replicates were carried out for each of the samples and statistical analysis was done using bio orbit software.

Selenastrum capricornutum growth inhibition test

The methods carried out for the toxicity testing of *G. africana* and *D. rhinocerotis* on *S. capricornutum* followed the Organisation for Economic Cooperation and Development (OECD) guideline 201, 1984. Test chambers used were 10 cm path length long cells. Exposure period was 72 h. Test endpoints included percentage growth inhibition relative to control and EC_{50} values. Two replicates were carried out for each of the samples and statistical analysis was done using regression analyses.

Daphnia pulex acute toxicity test

The methods used for the toxicity screening of *G. africana* and *D. rhinocerotis* on D. *pulex* followed those outlined by the United States Environmental Protection Agency (US EPA),1993 (600/4-90/027F). Test chambers used were 50 ml disposable polystyrene cups. Exposure periods were 24 and 48 h. Test endpoints included percentage mortality and LC_{50} values. 4 replicates, each with 5 test organisms per chamber, were used to test each sample. Statistical analysis was carried out using probit software/TSK.

Poecilia reticulata acute toxicity test

The methods employed for the toxicity testing of *G. africana* and *D. rhinocerotis* on *P. reticulata* was according to that outlined by the US EPA, 1996 (712-C-96-118). Test chambers used were 250 ml disposable polystyrene cups. Exposure period was 96 h. Test endpoints included percentage mortality and LC_{50} values. 2 replicates, each with 5 test organisms per chamber, were used to test each sample. Statistical analysis was carried out using probit software/ TSK.

RESULTS

The term EC_{50} refers to the concentration of a drug which induces a response halfway between the baseline and maximum. It is often used as a measure of the potency of a substance, therefore the lower the EC_{50} value the more potent the substance. In this study the EC_{50} values for the 2 plant extracts were obtained using *S. capricornutum* and *V. fischeri*. Table 1 shows the concentrations of the 2 plant extracts where 50% of the population exhibits a response.

Table 2. LC_{50} values obtained for *D. rhinocerotis* and *G. africana* when using *P. reticulata* and *D. pulex.*

Test	Exposure period (h)	LC_{50} values for *D. rhinocerotis* (ug/ml)	LC_{50} values for *G. africana* (ug/ml)
D. pulex acute toxicity test	24	340	40
D. pulex acute toxicity test	48	240	30
P. reticulata test	96	210	20

Table 3. Toxicity units and the measure of toxicity.

Toxicity unit	Conclusion
< 1	Limited to not acutely toxic
1 - 2	Negligibly acute toxic
2 - 10	Mildly acute toxic
10 - 100	Acutely toxic
> 100	Highly acutely toxic

Table 4. Toxicity units obtained for *D. rhinocerotis and G. africana.*

Test species	Toxicity units for *D. rhinocerotis*	Toxicity units for *G. africana*
V. fischeri	10	1429
S. capricornutum	2.6	10
D. pulex	4.2	33
P. reticulata	5	50

LC_{50} is the concentration of a substance which kills 50% of test animals exposed in a given time. In this study the LC_{50} values for the 2 plant extracts were obtained using *P. reticulata* and *D. pulex*. Table 2 thus shows the concentrations of the 2 plant extracts which kills 50% of the population of test animals.

The results in Tables 1 and 2 show that *D. rhinocerotis* has higher EC_{50} and LC_{50} values as compared to *G. africana*, thus showing that *G. africana* is more toxic to the aquatic species as compared to *D. rhinocerotis*.

From the above results toxicity units were then calculated for each of the plant extracts. For each test performed the toxicity unit was calculated as 100% (full strength effluent expressed as percentage) divided by the EC_{50} or LC_{50} values. Toxicity units were used as a measure of acute toxicity, Table 3.

The toxicity units obtained when the different species were exposed to *D. rhinocerotis* and *G. africana* are shown in Table 4. *G. africana* had higher toxicity units than *D. rhinocerotis*, thus showing that *G. africana* is more toxic to the aquatic species as compared to *D. rhinocerotis*. *D. rhinocerotis* is mildly acutely toxic to all the species used in this study. On the other hand *G. africana* is mildly toxic to *S. capricornutum*, acutely toxic to *D. pulex* and *P. reticulata* and highly acutely toxic to *V. fischeri*.

DISCUSSION

A major consideration in approving pesticides for commercial use is whether they pose an unreasonable risk to humans and to the environment. In order to evaluate environmentally safe levels of chemicals toxicological data on organisms, representative of the various ecosystems are required. Such data is often unavailable or inadequate. The aim of this study was to determine the ecological risks associated with using extracts of *D. rhinocerotis* and G*. africana*. A set of toxicological data for organisms' representative of the aquatic environment (algae, water flea, fish and bacteria) was obtained.

The study showed that *D. rhinocerotis* has higher EC_{50} and LC_{50} values compared to *G. africana*. From the toxicity units calculated, *D. rhinocerotis* showed mild acute toxicity to all the species used in this study while *G. africana* ranged from mildly toxic to highly acutely toxic. The set of toxicological data on organisms representative of the aquatic environment (algae, water flea, fish and bacteria) obtained in this study therefore shows that *G. africana* is more toxic to the aquatic environment as compared to *D. rhinocerotis*.

G. africana and *D. rhinocerotis* extracts showed inhibition of fungal growth at concentrations greater than 31.25 and 125 mg/ml, respectively (Knowles, 2005). This showed that the 2 plant extracts are effective as fungicides. From the results obtained in this current study, it can be concluded that at concentrations greater than 31.25 mg/ml (*G. africana*) and 125 mg/ml (*D. rhinocerotis*), the extracts will be toxic to the aquatic environment.

In this current study both *G. africana* and *D. rhinocerotis* extracts showed effectiveness against the bacteria *V. fischeri*. Further studies may be conducted with different bacteria to ascertain if the plant extracts may be used as disinfectants.

Studies have shown that *D. rhinocerotis and G. africana* extracts are more effective against the grey mould causing fungi, *B. cinerea*, when used in combination with synthetic fungicides (Vries, 2007). Complete inhibition of the fungi *B. cinerea* was observed for *D. rhinocerotis and G. africana* concentrations greater than at 0.95 mg/ml, when used in combination with kresoxim-methyl (a synthetic pesticide currently used on fungi) (Knowles, 2005; Vries, 2007). Ecotoxicity studies can therefore be carried out for the combination of kresoxim-

methyl and the botanical extracts at these low concentrations.

Once a pesticide is introduced into the environment it is influenced by many processes which determine its ultimate fate. Pesticides thus not only affect aquatic environments, but other environments are also affected. Further studies on other environments are therefore necessary for risk assessment of the plant extracts if they are to be used as pesticides.

ACKNOWLEDGEMENTS

Special thanks to the Innovation Fund for financial support and the University of the Western Cape for infrastructure.

REFERENCES

Arias-Estévez M, López-Periago E, Martínez-Carballo E, Simal-Gándara J, Mejuto J, García-Río L (2008). The mobility and degradation of pesticides in soils and the pollution of ground water source. Agric. Ecosyst. Environ. 123: 247-260.

Carbone P, Giardano F, Nori F, Mantovani A, Taruscio D, Lauria L, Figa-Talamana I (2007). The possible role of endocrine disrupting chemicals in the aetiology of cryptochidism and hypospadias: a population-based case-control study in rural Sicily. Int. J. Androl. 30: 3-13.

Caserta D, Maranghi L, Mantovani A, Marci R, Maranghi F, Moscarini M (2007). Impact of endocrine disruptor chemicals in gynecology. Human Reproduction Update, 4: 59-72.

De Kock G (1928). Disease of sheep in relation to the pasture under South African conditions. J. South Afr. Vet. Med. Association, 1: 9-38.

Isman MB (2006). Botanical insecticides, deterrents and repellants in modern agriculture and an increasingly regulated world. Ann. Rev. Entomol. 51: 45-66

Jarvis WR (1997). *Botryotinia* and *Botrytis* species, Taxonomy, Physiology and Pathology. A Guide to the Literature p. 15.

Kellerman TS, Coetzer JAW, Naude TW (1988). Plant poisoning and mycotoxicoses of live stock in South Africa. Oxford University Press Cape Town, pp. 18-20.

Knowles C (2005). Synergistic effects of mixtures of the kresoxim-methyl fungicide and medicinal plant extracts *in vitro* and *in vivo* against *Botrytis cinerea*. Masters dissertation, University of the Western Cape, South Africa.

Kodavanti PRS, Senthil Kumar K, Loganathan BG (2008). Organohalogen pollutants and human health. Int. Encyclopedia Public Health, pp. 686-693.

Levyns MR (1935). A revision of *Elytropappus* Cass. J. South Afr. Bot. 1: 89-103.

Regnault-Roger C, Philogene BJR (2008). Past and current prospects for use of botanicals and plant allelochemicals in intergrated pest management. Pharmaceut. Biol. 46: 41-52.

Van der Lugt JJ, Schultz RA, Fourie N, Hon LJ, Jordaan P, Labuschagne L (1992). *Galenia Africana* L. poisoning in sheep and goats: hepatic and cardiac changes. Onderstepoort J. Vet. Res. 59: 323-333.

Van Wyk BE, van Oudtshoorn B, Gericke N (1997). Medical plants of South Africa. Briza Publishers, Pretoria, South Africa, pp. 246-247.

Vries FA (2007). Synergistic effects of mixtures of fungicides and medicinal plant extracts against Botrytis cinerea. PhD dissertation, University of the Western Cape, South Africa.