CARYOLOGIA Vol. 64, no. 1: 117-120, 2011

Antimitotic effects of the biopesticide oxymatrine

Duygu Akdeniz and Ali Özmen*

Adnan Menderes Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Aydın, Turkey

Abstract — Biopesticides offer a more sustainable solution to pest control than synthetic alternatives. Naturally plant products, called secondary metabolites include thousands of alkaloids, terpenoids, phenolics and minor secondary chemicals. *Sophora flavescens* Aiton (Leguminosae) is an ancient Chinese herb that grows wild in China. About two dozen alkaloids have been identified in Ku Shen, with matrine and oxymatrine being the major compounds. Ku Shen extracts have been formulated as pesticides either alone or in mixtures with conventional synthetic pesticides. In this study we have analyzed the probable cytotoxic and genotoxic effects of oxymatrine on plant test system for determining the safety of use as biopesticide. This alkaloid has reduced the mitotic index in Allium root meristem in higher concentrations but hasn't show distinct genotoxic effects. In conclusion oxymatrine can be proposed as safe at this end point for using as biopesticide while the plant physiological system can ruled out certain effects.

Key words: alkaloid, Allium test, biopesticide, oxymatrine.

INTRODUCTION

Biopesticides offer a more sustainable solution to pest control than synthetic alternatives. Chemical pesticides are withdrawn owing to resistance problems. Biopesticides do not feature residue problems. However, biopesticides are not as effective as chemicals. Compatibility with synthetic pesticides varies, and shelf life is often shorter (Coloma *et al.* 2010). Currently the development of biological or non-chemical pesticides as replacements for conventional pesticides is an important strategy. Hence the plants are a great source for solving this problem. Naturally plant products, called secondary metabolites include thousands of alkaloids, terpenoids, phenolics and minor secondary chemicals.

Sophora flavescens Aiton (Leguminosae) is an ancient Chinese herb that grows wild in China. The dry root of the plant is commonly called as Ku Shen. About two dozen alkaloids have been identified in Ku Shen, with matrine and oxymatrine being the major compounds (Mao and Henderson 2007). Ku Shen extracts have been formulated as pesticides either alone or in mixtures with conventional synthetic pesticides to manage populations of various insect pests, fungal and bacterial diseases, and nematodes in vegetable, fruit, flower, and tea production in China (Lou *et al.* 1997; Zheng *et al.* 2000; Fu *et al.* 2005; Mao and Henderson 2007). It has shown that matrine and oxymatrine has a strong antifeedant effect against Formosan subterranean termite (Mao and Henderson 2007; Verma *et al*. 2009). In a screening study of Chinese medicinal herbs against two stored-grain insects the methanol extract of *Sophora flavescens* has represented contact toxicity and feeding-deterrent activities (Liu *et al.* 2007). Honey bees come into contact with various pollutants during their foraging activity and are considered as an environmental indicator of high sensitivity. Honey bees may be constantly exposed to pesticides whenever their colonies are sited in agricultural areas. The result of a study indicates that oxymatrine has weak effects on honey bees in acetylcholinesterase (AChE) and adenosine triphosphatase (ATPase) activities as biochemical indicators (Rabea *et al.* 2010).

In this study we have analyzed the probable cytotoxic and genotoxic effects of oxymatrine on

*Corresponding author: phone: +90 256 2182000-1869; fax: +90 256 2135379; e-mail: aozmen@adu.edu.tr

plant roots by considering the field applications. Application of a biopesticide on soil or directly on the plant can cause diffuse in plant tissues or organs. In such a case it is important to define the applied compound as safe in plant tissue and cell machineries. Therefore we have planned an Allium test for evaluating cytotoxicity since Allium test fits well in a test battery composed of prokaryotes and /or other eukaryotes (FISKESJÖ 1993).

MATERIALS AND METHODS

Test materials - Oxymatrine were purchased from Shangai Xinma Bio-Tech Co, Ltd., in %98 purity. It was derived from *Sophora flavescens*. Onions for Allium test were purchased from commercially available markets.

Preparing of oxymatrine stock solution and concentrations - Stock solution was prepared from 10 g pure oxymatrine by solving it in 1 L water. It has been applied in 3 concentrations (1 g/L, 5 g/L and 10 g/L) in Allium test and the concentrations were prepared by dilution of the stock solution.

Allium test - Allium cepa has been used for evaluating cytotoxic properties since the early 1920's (GRANT 1982). Small onion bulbs are carefully unscaled and cultivated on top of test tubes filled with different concentrations of oxymatrine. Tap water was used as control. The test tubes were kept in an incubator at 24±2^{0}C. After 72 h the roots were counted and their lengths were measured for each onion. The emerged roots has been fixed with glacial acetic acid/ absolute alcohol (1/3 v/v). For evaluation, the root tips were put into aceto-orcein dye. Well-stained root tips were prepared for microscopic observation by squashing on a slide. MI was expressed in terms of divided cells/total cells.

Statistical analysis - A statistical analysis was performed on the collected data by using Graph Pad Prism 5.0. The means of the control and oxymatrine obtained from descriptive analysis and one way ANOVA test has been performed to obtain P values.

Growth inhibition of oxymatrine on Allium roots - After 72 h treatment, root lengths and numbers have been determined for control and for each concentration of oxymatrine. The collected data are presented in Table 1. Oxymatrine has inhibited the outputs of Allium roots and reduced their growth in concentration dependent manner (Table 1). Otherwise oxymatrine has stimulated the output and growth of roots in used minimal concentration 1 g/l. But this effect was meaningless statistically (Figure 1a, 1b). Even so it can be suggested that oxymatrine stimulates the growth of onions in the minimal concentration.

Cytotoxic and genotoxic effects - In Figure 2 the mitotic indexes are presented for control and for treatment concentrations of oxymatrine. It is evident that oxymatrine reduced the mitotic index significantly (Figure 2). The MI was reduced up to %50 of control. That means oxymatrine has anti-mitotic properties that can stop the mitosis in anywhere of the cell cycle. During the microscopic observations chromosome analysis have been made but the results did not show induction of chromosome or chromatid type of aberration in the treated cells. Only a few changes in cell poles, C-mitosis (colchicine like mitosis) and duplications of chromosomal sets have been observed and the numbers of this were meaningless statistically.

TABLE 1 — The average root lengths and numbers in control and in treatment concentrations after 72 h.

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Tap Water	RN	34	35	21	32	36	33	34	36	30	33
	ARL (mm)	20	20	15	20	10	21	17	18	22	19
Oxymatrine (1 g/l)	RN	37	32	35	39	48	36	38	37	40	35
	ARL (mm)	22	25	20	25	15	20	17	16	19	18
Oxymatrine (5 g/l)	RN	27	28	20	21	24	26	20	22	21	25
	ARL (mm)	17	21	13	10	12	14	15	14	16	13
Oxymatrine (10 g/l)	RN	27	18	26	26	30	27	28	28	23	26
	ARL (mm)	15	10	10	18	18	11	12	15	13	14

RN: root number, ARL: average root length.

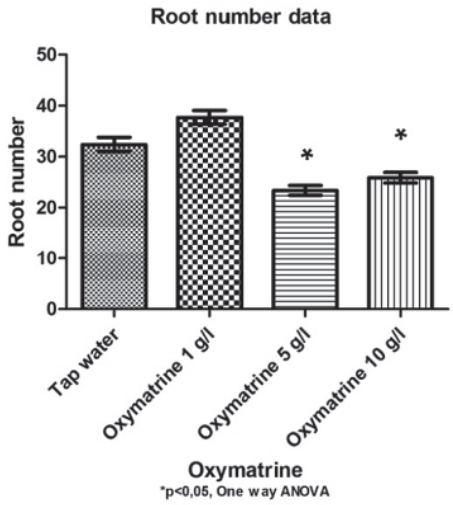

Fig. 1a — The average root numbers in control and in treatment concentrations after 72 h.

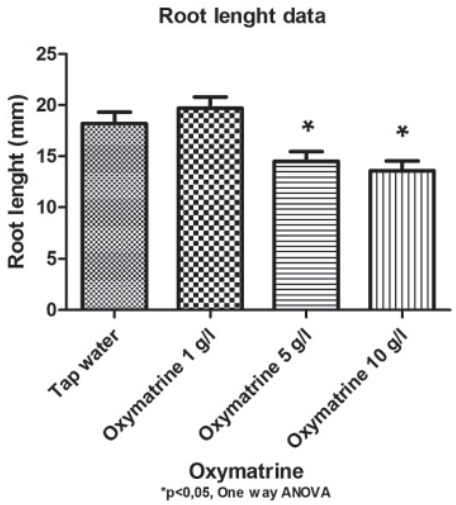

Fig. 1b — The average root lengths in control and in treatment concentrations after 72 h.

TABLE 2 — Number of roots and analyzed cells

	Used onions	Evaluated roots	Evaluated cells	Dividing cells
Tap Water	10	100	10000	2878
Oxymatrine (1 g/l)	10	100	10000	2588
Oxymatrine (5 g/l)	10	100	10000	1787
Oxymatrine (10 g/l)	10	100	10000	1376

DISCUSSION

Some secondary metabolites are considered as metabolic waste products, for example, alkaloids may function as nitrogen waste products. However, a significant portion of the products derived from secondary pathways serve either as protective agents against various pathogens (e.g. insects, fungi or bacteria) or growth regulatory molecules (e.g. hormone-like substances that stimulate or inhibit cell division). Due to these physiological functions, secondary metabolites are potential anticancer drugs, since either direct cytotoxicity is affected on cancer cells or the course of tumor development is modulated, and eventually inhibited. Administration of these compounds at low concentrations may be lethal for microorganisms and small animals but in larger organisms they may specifically affect the fastest growing tissues (KINTZIOS and BARBERAKI 2004). Hence in this study the mitotic index has been reduced at %14 levels by cytotoxic effects of oxymatrine (Figure 2). Reduction in the mitotic activity could be due to inhibition of DNA synthesis or blocking in the G2 phase of the cell cycle, preventing the cell from entering mitosis (TÜRKOĞLU 2008). Furthermore oxymatrine can affect the cytoskeleton by tubulin polymerization or degradation.

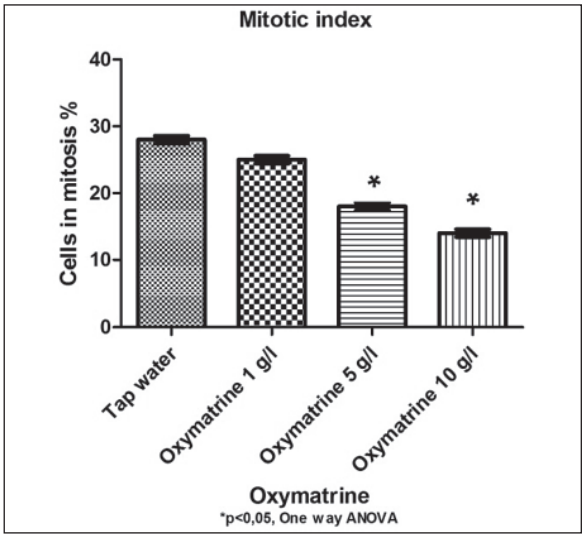

Fig. 2 — Mitotic indexes (MI) in control and in treatment concentrations after 72 h.

The species *A. cepa* also presents other advantages with suitable chromosomal features; this plant bears large and few chromosomes ($2n$ = 16) what facilitates the evaluation of chromosome damages or disturbances in cell division cycle. This test have proved to be of great value, since combine a high sensibility to detect mutagens in different environments and a great capacity to evaluate distinct genetic endpoints, from point mutations to chromosomal aberrations (LEME and MORALES 2008). Chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal material. Most of the chromosomal aberrations observed in cells are lethal, but there are many corresponding aberrations that are viable and can cause genetic effects, either somatic or inherited (AKINBORO and BAKARE 2007). My results did not show induction of chromosome or chromatid type of aberration in the treated cells. Only a few changes in cell poles and duplications of chromosomal sets have been observed.

C-mitosis indicated that the chemical inhibited spindle formation similar to the effect of colchicine (BADR 1983), and induction of C-mitosis commonly associated with spindle poisons, indicating turbogenic effect (SHAHIN and EL-AMOODI 1991). ODEIGAH *et al.* (1997) describes the presence of C-mitoses as a possibly reversible effect (weak toxic effect). In this study colchicine like mitosis has been observed by microscopic analysis of Allium roots. Data are not given because the number of C-mitosis in this study was not important statistically.

In respect of this results, oxymatrine can stop the mitosis in anywhere of the cell cycle. This endpoint indicates the cytotoxic effects of oxymatrine. This effect is only acceptable for higher concentrations of oxymatrine in laboratory tests, after 72 h direct applications. Otherwise oxymatrine has no distinctive genotoxic effects. If the applied concentrations in the field, degradation of biopesticides in field applications and the intake amounts of the plants from applied substances are considered, oxymatrine can be proposed as safe for using as biopesticide while the plant physiological system can ruled out certain effects.

Acnowledgment — The authors are indebted to Shangai Xinma Bio-Tech Co, Ltd.

REFERENCES

AKINBORO A. and BAKARE A.A., 2007 — *Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on Allium cepa Linn.* Journal of Ethnopharmacology, 112: 470-475.

BADR A., 1983 — *Mitodepressive and chromotoxic activities of two herbicides in A. cepa.* Cytologia, 48: 451-457.

COLOMA A.G., REINA M., DIAZ C.E. and FRAGA B.M., 2010 — *Natural Product-Based Biopesticides for Insect Control.* Comprehensive Natural Products II, 2010, Chapter 3.09, Pages 237-268.

FISKESJÖ G., 1993 — *Allium Test 1:A 2-3 day plant test for toxicity assesment by measuring the mean root growth of onions (Allium cepa L.).* Environmental Toxicology and Water Quality, 8: 461-470.

FU, Y., WANG C. and YE F., 2005 — *The application of Sophora flavescens Ait. alkaloids in China.* Pestic. Sci. Admin. 26: 30-33.

GRANT W.F., 1982 — *Chromosome aberration assays in Allium.* Mutation Research, 99: 273-291.

KINTZIOS S.E. and BARBERAKI M.G., 2004 — *Plants That Fight Cancer.* By CRC Press LLC, USA.

LEME D.M. and MARIN-MORALES M.A., 2008 — *Chromosome aberration and micronucleus frequencies in Allium cepa cells exposed to petroleum polluted water - A case study.* Mutation Research, 650: 80-86.

LIU Z.L., GOH S.H. and HO S.H., 2007 — *Screening of Chinese medicinal herbs for bioactivity against Sitophilus zeamais Motschulsky and Tribolium castaneum (Herbst).* Journal of Stored Products Research, 43: 290-296.

LOU W.-C., MU L.Y. and LI Y.S., 1997 — *Toxic effects of alkoloids from plants against insect pests.* Pesticides, 36: 11-15.

MAO L. and HENDERSON G., 2007 — *Antifeedant Activity and Acute and Residual Toxicity of Alkaloids from Sophora flavescens (Leguminosae) Against Formosan Subterranean Termites (Isoptera: Rhinotermitidae).* J. Econ. Entomol., 100(3): 866-870.

ODEIGAH P.G.C., NURUDEEN O. and AMUND O.O., 1997 — *Genotoxicity of oil field wastewater in Nigeria.* Hereditas, 126: 161-167.

RABEA E.I., NASR H.M. and BADAWY M.E.I., 2010 — *Toxic Effect and Biochemical Study of Chlorfluazuron, Oxymatrine, and Spinosad on Honey Bees (Apis mellifera).* Arch. Environ. Contam. Toxicol., 58: 722-732.

SHAHIN S.A. and EL-AMOODI K.H.H., 1991 — *Induction of numerical chromosomal aberrations during DNA synthesis using the fungicides nimrod and rubigan-4 in root tips of Vicia faba L.* Mutat. Res., 261: 169-176.

TÜRKOĞLU Ş., 2008 — *Evaluation of genotoxic effects of sodium propionate, calcium propionate and potassium propionate on the root meristem cells of Allium cepa.* Food and Chemical Toxicology, 46: 2035-2041.

VERMA M., SHARMA S. and PRASAD R., 2009 — *Biological alternatives for termite control: A review.* International Biodeterioration & Biodegradation, 63: 959-972.

ZHENG, Y. Q., YAO J.R. and SHAO X.D., 2000 — *Review on the constituents and agricultural application of Sophora flavescens Ait.* Pestic. Sci. Admin., 21: 24-26.

Received October 20th 2010; accepted December 9th 2010