

Clastogenic and Cytotoxic Effects of Four Pesticides Used to Control Insect Pests of Stored Products on Root Meristems of *Allium cepa*

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Abstract

While the use of pesticides of stored product has increased productivity in agriculture, there is concern about their use because some are mutagens and/or carcinogens, harm non-target organisms or cause pest resistance. In this study Grain Treat (GT) (Mercaptothion, 10 g kg⁻¹, Permethrin 1.5 g kg⁻¹); QuickPhos (QP) (Aluminium Phosphide, 560 g kg⁻¹); Nuvan Profi (NP) (Dichlorvos, 124 g kg⁻¹) and *Eriocephalus punctulatus* plant smoke condensate (EPSC) were evaluated for cytotoxicity and the induction of genotoxicity in the onion (*Allium cepa*) test. Onion seeds were germinated and exposed to pesticides (mg mL⁻¹), GT (12.5, 25, 50); QP (0.75, 1.5, 3.0); NP (0.064, 0.128, 0.256); EPSC (0.0025, 0.0049, 0.0098) for 24 hours. For each concentration, three root tips were transferred to three microscope slides, stained with aceto-carmine, covered with cover slip, squashed and observed microscopically. The cytotoxicity and genotoxicity induced by each pesticide concentration was compared with the value for the concomitant negative control using t-test. Only QP (3.0 mg mL⁻¹) and NP (0.256 mg mL⁻¹) significantly depressed the MI, i.e. cytotoxic ($P < 0.05$). Genotoxicity was determined by examining, 100 anaphase and telophase cells on each of three slides per concentration for chromosome aberration (CA). The three concentrations of each pesticide induced CAs ($P < 0.05$) in the following general order of frequency; stickiness > multipolarity > c-mitosis > anaphase and telophase bridges > chromosome Laggards. The induction of sticky chromosomes indicated that the pesticides caused abnormal DNA condensation, abnormal chromosome coiling and inactivated the spindles. Because abnormalities of the cell division process results from the genotoxic effects of environmental chemicals, the four pesticides have the potential to cause aneuploidy in exposed organisms and adverse human health and environmental effects.

Keywords: *Allium cepa*, chromosome aberration, cytotoxicity, pesticides, smoke

1. Introduction

More than 55% of Africans, according to Nukenine (2010), earn their livelihood from agriculture. However, both in the field and during storage, the products are threatened by insects, rodents, birds and other pests (Hayma, 2003). It was suggested by Raja et al. (2001), that insect damage in stored grains and other durable commodities may amount to 10-40% in developing countries, where modern storage technologies have not been introduced. In order to reduce post-harvest grain losses caused by insect-pests, mainly grain weevils, grain borers, grain beetles and grain moths and other bio-agents, insecticides have been used extensively to control infestations of these insect-pests (Jackai, 1998). Insecticides are used worldwide because, by acting against pests both during storage of crops and in the field, they have greatly improved agricultural yield (Taylor et al., 1997). In addition to the use of chemical pesticides, there are traditional post-harvest pest management methods. Blum & Bekele (2002) mentioned the use of *Euphorbia tirucalli*, *Phytolacca dodecandra*, *Tagetes minuta* and *Capsicum frutescens* as plants with pesticidal effects, which are used by farmers in Ethiopia, to protect stored grains. According to Raja et al. (2001) plant oils and their bioactive chemical constituents, due to their low mammalian toxicity and low persistence in the environment, are also used for the protection of agricultural products. In Southern Africa, including Lesotho, smoke from burning the stem and leaves of wild rosemary (*Eriocephalus punctulatus*) plant is used to control insect-pests that attack stored grains and other durable commodities (Samie & Nefefe, 2012). In the Northeast Uplands of India, pulse, maize, onion and other crops, are kept in the kitchen around 2-3 meters above the ground at an angle of 45° from the cooking place, for the kitchen smoke to drive away insects and reduce fungal attack (Sinha, 2010).

Many different pesticides are used in agriculture in Lesotho to protect stored food products from damage and thereby increase productivity including the following formulations, Quickphos (QP), Grain Treat (GT), Nuvar Profi (NP) and smoke condensate from burnt *Eriocephalus punctulatus* plant (EPSC). The active ingredient in QuickPhos tablets is aluminium phosphide. The phosphine that is generated from the aluminium phosphide is a fumigant (Beckett et al., 2007). Grain Treat and Nuvar are contact insecticides (Snelson, 1987). The active ingredients in Grain Treat are the organophosphate, mercaptothion also known as malathion, carbophos or maldison (EXTOXNET, 1993) and the pyrethroid, permethrin (Snelson, 1987). The active ingredient in Nuvar Profi, from the product label, is the organophosphate, dichlorvos. *Eriocephalus punctulatus* is an aromatic plant and the genus *Eriocephalus* commonly known as Cape chamomile and wild rosemary, is a member of the family *Asteraceae* (tribe Anthemideae) which is endemic to Southern Africa. The plants contain volatile oils and the extracts of the genus *E. punctulatus* possess antimicrobial, anti-inflammatory, and antioxidant activities, and also inhibited acetylcholinesterase (Njenga & Viljoen, 2006; Samie & Nefefe, 2012). The fumes of the burning fresh *E. punctulatus* are used to disinfect the houses (Samie & Nefefe, 2012). Smoke is known to impart certain chemicals on smoked foods which enable them to resist microbial spoilage (Draudt, 1963). Chemically, the essential oils of plants are composed of terpenoids and aromatic polypropanoids (Simon, 1990).

Despite all these benefits however, residues of pesticides are known to remain in soil (Subbarao, 1999), water (Medina et al., 1999) and also in vegetables and fruits (Ahouangninou et al., 2012; Osman et al., 2010) which constitute a risk for human health. According to Pimentel et al. (1998) the mutagenicity of pesticides for non-target organisms and their effects on ecosystems are of concern worldwide. In a joint report of WHO and UNEP, cited in Richter (2002) human pesticide poisonings worldwide was put at over 26 million with about 220,000 deaths per year. Other reported cases of adverse effects to health caused by pesticides include damage to the nervous system (Kamel et al., 2007), respiratory and lung disorders (Hoppin et al., 2008), damage to reproductive organs (Hileman, 1994) and birth defects (Rojas et al., 2000).

It is well known that plants are direct recipients of agrottoxins and the *Allium cepa* assay is one of the plant assay systems used widely to study the genotoxic effects of pesticides. Many of such studies have demonstrated the induction of chromosomal aberrations by pesticides (Fernandes et al., 2007). Plants have been shown to be valid alternatives to animal testing because chemicals which induce chromosomal aberration (CA) in plant cells frequently induce identical chromosome aberrations in animal cell cultures (Grant, 1978). Gap exists in the available data on the genotoxicity of some of the pesticides used in Lesotho in the *Allium cepa* assay. While Dichlorvos, malathion and/or mixtures containing them have been tested in the *Vicia faba* meristem cells for chromosomal aberrations (Amer & Ali, 1986) and the *Allium cepa* assay (Asita & Makhalemele, 2009), we are not aware of genotoxicity tests of Phosphine, permethrin and smoke condensate using the *Allium cepa* assay system.

The objective of this study was to investigate the genotoxic effects of some pesticides used in Lesotho to control stored-product insect pests namely, Quickphos (QP), Grain Treat (GT), Nuvar Profi (NP) and smoke condensate from burnt *Eriocephalus punctulatus* plant (EPSC) using the *Allium cepa* anaphase-telophase chromosome aberration assay.

2. Materials and Methods

Test organism: *Allium cepa* (onion) seeds of the Texas Grano 502 P.R.R variety used in this study was, obtained from Sakata seeds, Republic of South Africa.

Pesticides: Grain Treat (GT) [Mercaptothion, 10 g kg⁻¹, Permethrin 1.5 g kg⁻¹] was manufactured by Kombat (Pty) Ltd, Republic of South Africa; QuickPhos (QP) tablets [Aluminium phosphide, 560 g kg⁻¹] was obtained from United Phosphorus (Pty) Limited of South Africa; Nuvar Profi (NP) [Dichlorvos 124 g kg⁻¹] was a product of AVIMA (Pty) Ltd. Republic of South Africa; the plant, *Eriocephalus punctulatus* was collected from Mohale's Hoek, Lesotho. The smoke condensate (EPSC) was obtained by burning the aerial parts of the plant in an open top metal tin, in accordance with the method of Asita and Campbell (1990) with some modification. The smoke condensed in an inverted glass funnel which was placed above the open top. The condensate was scraped from the funnel into a watch glass with a metal spatula.

2.1 Experiments to Select Concentrations of Pesticide to Use

Preliminary experiments were conducted to determine the concentrations of each pesticide to be used in the actual genotoxicity experiments. The pesticides were dissolved in distilled water while the smoke condensate was dissolved in dimethyl sulphoxide (DMSO) (Merck, Germany) and serially diluted with distilled water. One hundred *Allium cepa* seeds were broadcast on filter paper moistened with different concentrations of each pesticide or with water (negative control), in separate Petri dishes, for 72 hours at 22± 2 °C. The effective

concentration (EC_{50}) ($mg\ ml^{-1}$) was the concentration that inhibited the germination of 50% of the seeds or the effective concentration for 50% growth inhibition for relative reduction of root length when onion bulbs were used (Asita & Makhalemele, 2009; Yildiz & Arıkan, 2008). The EC_{50} s ($mg\ ml^{-1}$) values for the four pesticides were approximately GT (>200); QP (9); NP (3.58) and EPSC (0.164).

With the exception of GT which did not inhibit germination even at the limit of solubility, the EC_{50} s were too toxic in trial experiments and not enough cells in division stages could be observed. Therefore, in the genotoxicity experiments, the highest concentration in each case was lower than the EC_{50} . The highest concentration of the smoke condensate contained about $0.98\ mg\ ml^{-1}$ (0.098%) DMSO which, alone was neither cytotoxic nor genotoxic. The following concentrations ($mg\ ml^{-1}$) of the pesticides were used in the experiments: GT (12.5, 25, 50); QP (0.75, 1.5, 3.0); NP (0.064, 0.128, 0.256); EPSC (0.0025, 0.0049, 0.0098).

2.2 Genotoxicity Assay

The method of Matsumoto et al. (2006) was used with modification. Briefly, onion seeds were spread on water moistened filter paper in a Petri dish, at room temperature ($22\pm 2\ ^\circ C$), until they germinated (about 72 to 96 hours) and the radicles reached about 2 cm. Ten germinated seeds were placed on separate filter papers moistened with either of 3 different concentrations of each pesticide for 24 hours at room temperature, in a Petri dish. For the concurrent negative control, seeds were placed on filter paper moistened with distilled water also for 24 hours, i.e. (acute treatment) after which some of the rooted seeds

were collected at random and assessed (Matsumoto et al., 2006).

2.3 Root Harvest and Slide Preparation

After the 24 hours exposure, three root tips from three seeds per concentration were collected at random and assessed. Root tips, 1-2 cm long, were cut from the treated germinated seeds and fixed in alcohol-acetic acid (ethanol: glacial acetic acid in 3:1 ratio) for 24 hours in a refrigerator at $4-6\ ^\circ C$. The root tips were washed twice in ice cold water for 10 minutes each and allowed to dry. A solution of 1N HCl at $60\ ^\circ C$ (Matsumoto et al. (2006) was added to the root tips for 10 minutes. The root tips were again washed with distilled water. The HCl treatment was repeated. For each concentration of pesticide, three root tips were transferred individually to a clean microscope slide and cut about 2 mm from the growing tip. The tips were kept while the rest was discarded. Each root tip was cover in aceto-carmin stain (Carolina Biological Supply Company, USA) for 10 minutes. A glass cover slip was placed on the root tip and tapped gently to spread the cells evenly to form a monolayer using a pencil eraser. The formation of a monolayer was to facilitate the scoring process for normal and aberrant cells in the different stages of the cell cycle. The slides were coded and viewed under the light microscope (Olympus CX 21). The cells were scored blind under oil immersion at 1000 x magnification. The cells with the most representative of each class of structural aberration were photographed with a Canon camera model Power Shot A640 mounted on a Zeiss PrimoStar microscope.

2.4 Scoring of Slides and Data Analysis

2.4.1 Cytotoxicity

A total of 2000 cells were scored on each slide, three slides ($n = 3$) for each concentration, and classified as interphase or one of the division staged (Prophase, Metaphase, Anaphase or telophase). A total of 6000 cells were thus scored for each treatment group and the control group.

The mitotic index (MI) was expressed as the number of cells in the division stages per 1000 cells scored. The MI of each treatment group was compared with the negative control group MI using t-test at 0.05, level of significance using the SPSS for windows software version 11.0. Mitotic index is considered a parameter that allows one to estimate the frequency of cellular division (Marcano et al., 2004) and the reduction of mitotic activities has been used frequently to trace substances that are cytotoxic (Linnainmaa et al., 1978; Smaka-Kincl et al., 1996).

2.4.2 Genotoxicity

One hundred dividing cells in anaphase and telophase stages in each of three slides for each concentration of pesticide were examined for the presence of chromosome aberration, that is, 300 anaphase and telophase cells for each concentration. The percentage of anaphase and telophase cells with aberrations among the 300 cells examined in each treatment group was compared with the percentage of aberrant anaphase and telophase cells in the concomitant negative control group using t-test.

3. Results

3.1 Chromosome Aberrations

Photographs of the most representative pictures of normal mitotic cells and cells containing the different types of chromosome aberrations that were observed and scored are presented in Figure 1. The following aberrations, adapted from Parry et al. (1985) were observed and scored in anaphase-telophase cells as endpoints for determination of cytogenetic effects of the pesticides: sticky chromosomes (S), Multipolar anaphases and telophases (Multipolar), c-mitosis (C-Mit), anaphase and telophase bridges (A.B), chromosome largards (L).

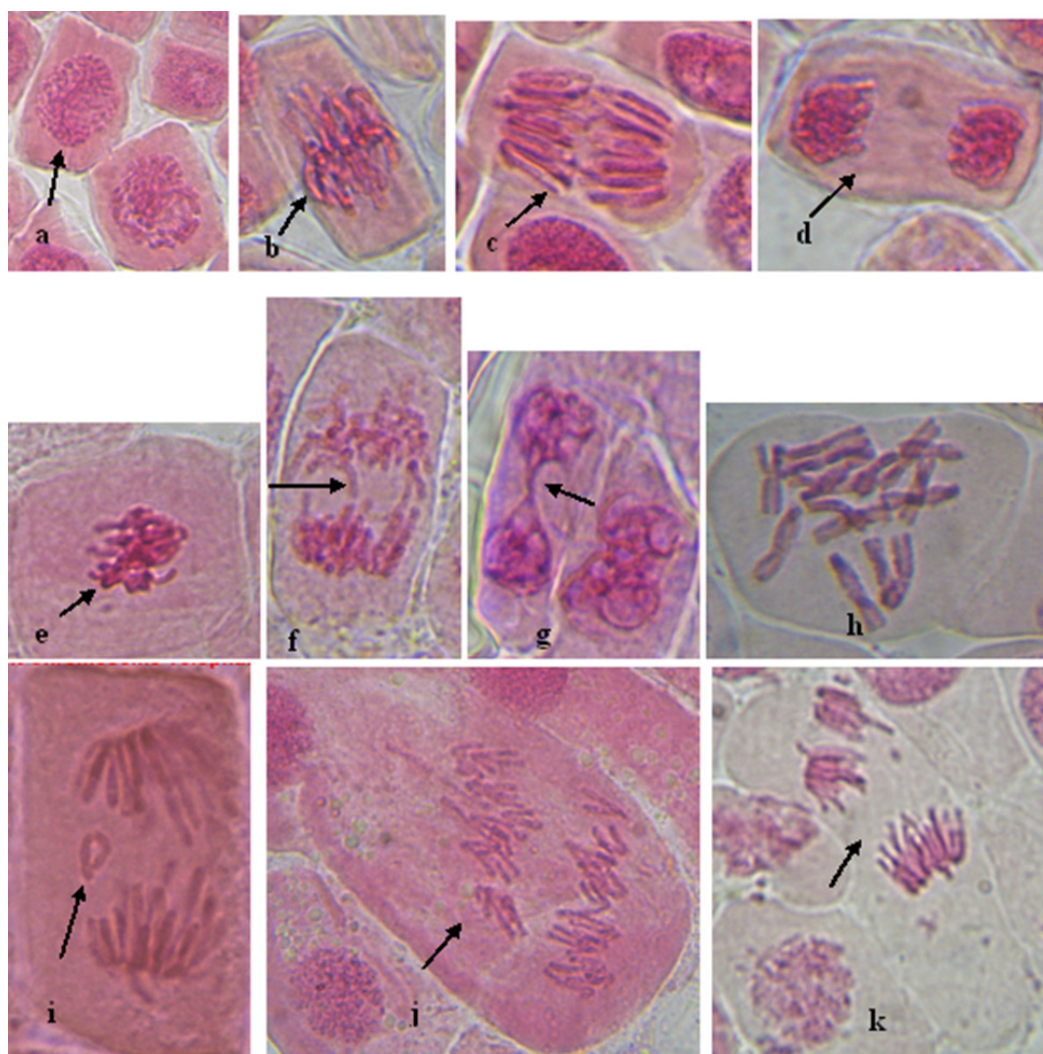


Figure 1. Photographs of cells of *Allium cepa* showing untreated cells in normal division stages and pesticides treated cells with the different types of chromosomal abnormalities. Magnification is 1000 X

(a) Normal prophase cell (b) Normal metaphase (c) Normal anaphase (d) Normal telophase (e) Sticky metaphase (f) Chromosome bridges at telophase (g) Sticky telophase bridge (h) C-mitosis (i) Vagrant or laggard chromosome (j) Multipolar anaphase (k) Multipolar late anaphase.

- Sticky chromosomes (S) - chromosomes that fail to condense completely at metaphase. Chromatin masses which are undistinguishable as chromosomes are seen as clumps in extreme cases. Cells having sticky chromosomes lack spindle fibres.
- Multipolar anaphases and telophases (Multipolar) - these are cells in anaphase and telophase stages that have more than two spindle poles instead of the normal two.

- Anaphase or Telophase bridge (A.B) - Dicentric chromosomes that form a bridge between both poles at anaphase or telophase caused mainly by the breakage and fusion of chromosomes.
- C-Mitosis (C-Mit) - Mitotic cells that lack spindle fibres with unattached whole chromosomes lying scattered throughout the cell. The effect is commonly produced in cells treated with the spindle poisons, colchicines or colcemid, hence C-Mitosis.
- Laggard (L) - These are whole chromosomes that fail to migrate to either pole at anaphase because of possible damage to the kinetochore.

3.2 Cytotoxicity

The results of the effects on the mitotic index (as a measure of cytotoxicity) of exposing root tip cells of onion to the different concentrations of the pesticides are presented in Table 1. Only the highest concentration of QP (3.0 mg mL⁻¹) and NP (0.256 mg mL⁻¹) induced significant cytotoxic effects ($P < 0.05$) in treated root tip cells, i.e. had significantly reduced mitotic indices, compared to the concomitant negative control.

Table 1. The effects of pesticides treatment on the different cell cycle stages

TC	Statistics	Cell in interph	Cells in division stages					Total	Total cells scored	MI	
			Proph	Metaph	Anaph	Teloph					
Water	Control	Mean	1815	103	33	24	25	185	2000	93	
		SD	46	20	10	9	8	46	0	23	
	12.5	Mean	1848	93	26	15	22	156	2000	78	
		SD	6	15	6	2	3	9	0	4	
	25	Mean	1790	131	36	24	19	210	2000	105	
		SD	49	34	22	9	8	49	0	24	
	50	Mean	1838	103	25	17	21	166	2000	83	
		SD	12	4	7	3	4	9	0	5	
	GT (mg mL ⁻¹)	0.75	Mean	1876	57	39	12	16	124	2000	62
			SD	27	23	3	6	3	27	0	13
		1.5	Mean	1900	63	18	11	10	102	2000	51
			SD	32	32	16	7	6	58	0	29
3		Mean	1916	37	18	12	17	84	2000	42‡	
		SD	36	21	4	6	13	36	0	18	

TC	Statistics	Cell in interph	Cells in division stages					Total	Total cells scored	MI
			Proph	Metaph	Anaph	Teloph				
NP (mg mL ⁻¹)	0.064	Mean	1638	197	50	45	70	362	2000	181
		SD	151	81	28	10	32	151	0	76
	0.128	Mean	1819	103	29	25	24	181	2000	90
		SD	12	20	11	13	4	12	0	6
	0.256	Mean	1961	25	9	2	7	43	2000	22‡
		SD	22	16	6	2	5	24	0	12
EPSC (mg mL ⁻¹)	0.0025	Mean	1833	88	29	23	27	167	2000	84
		SD	45	26	10	12	6	45	0	23
	0.0049	Mean	1861	78	24	12	25	139	2000	69
		SD	32	10	5	10	10	32	0	16
	0.0098	Mean	1880	57	28	13	22	120	2000	60
		SD	45	13	16	9	14	45	0	23

TC = Test Compound; SD = Standard deviation; GT = Grain Treat insecticide; QP = Quickphos ; NP = Nuvan Profi; EPSC = Eriocephalu punctulatus smoke condensate; MI = Mitotic Index; Interph = Interphase; Proph = Prophase; Metaph = Metaphase; Anaph = Anaphase; Teloph = Telophase; ‡ = Significant difference from negative control at $P < 0.05$ in the t-test, $n = 3$.

3.3 Genotoxic Effects of the Pesticides

The results of the genotoxicity tests of the pesticides on *Allium cepa* root tip meristem cells are presented in Table 2. All the three concentrations of GT, QP, NP and EPSC that were tested induced significant genotoxic effects ($P < 0.05$). Grain Treat induced c-mitosis, multipolar cells and sticky chromosomes. QuickPhos induced anaphase and telophase bridges, c-mitosis, multipolar cells and sticky chromosomes. Nuvan Profi induced anaphase and telophase bridges, Lagards, c-mitosis, multipolar cells and sticky chromosomes. *Eriocephalus punctulatus* smoke condensate (EPSC) induced multipolar cells and sticky chromosomes.

Table 2. Genotoxic effects of the pesticides in the onion root tip chromosome aberration assay

TC		Statistics	A&T cells Scored	Cells with aberrations in 100 anaphase and telophase cells scored					
				A.B %	L %	C-Mit %	Multipolar %	S %	Total CA %
Water	Control	Mean	100	0	0	0	0	0	0
		SD	0	0	0	0	0	0	0
	12.5	Mean	100	0	0	26	1	56	83*
		SD	0	0	0	6	1	6.24	4.36
	25	Mean	100	0	0	22.33	6	62.67	91*
		SD	0	0	0	6.66	2	13.32	11.27
	50	Mean	100	0	0	24	5.67	53	82.67*
		SD	0	0	0	3.46	3.06	10.54	13.43
	0.75	Mean	100	1	0	7.67	9.67	50	68.33*
		SD	0	1.73	0	9.29	7.09	15.62	1.15
	1.5	Mean	100	0.33	0	9.33	5.33	49.33	64.33*
		SD	0	0.58	0	1.15	1.53	14.19	12.5
QP (mg mL ⁻¹)	3	Mean	100	1.33	0	20.33	0	60.33	82*
		SD	0	2.31	0	12.66	0	20.79	10.44
TC		Statistics	A&T cells Scored	Cells with aberrations in 100 anaphase and telophase cells scored					
				A.B %	L %	C-Mit %	Multipolar %	S %	Total CA %
NP (mg mL ⁻¹)	0.064	Mean	100	1.67	0.67	2.33	2.67	52.67	60*
		SD	0	0.58	0.58	2.52	2.89	11.02	12.77
	0.128	Mean	100	0	0	1	8.33	33.33	42.67*
		SD	0	0	0	1.73	4.04	11.15	13.61
	0.256	Mean	100	0	0	0	7.33	12.33	19.67*
		SD	0	0	0	0	4.16	1.53	4.51
EPSC (mg mL ⁻¹)	0.0025	Mean	100	0.67	0.33	0	8	17	26*
		SD	0	0.58	0.58	0	3.61	6.56	4.36
	0.0049	Mean	100	0	0	0	0.67	7.67	8.33*
		SD	0	0	0	0	0.58	1.53	1.53
	0.0098	Mean	100	0	0	0	3.33	25.33	28.67*
		SD	0	0	0	0	3.06	4.16	2.31

TC = Test Compound; SD = Standard deviation; GT = Grain Treat insecticide; QP = Quickphos; NP = Nuvan Profi; EPSC = *Eriocephalu punctulatus* smoke condensate; A&T = Anaphase and Telophase cells scored; CA % = Cells with chromosome aberration as % of A&T cells examined; C-Mit = C-Mitosis; A.B = Anaphase or Telophase bridge; S = Sticky; L = Laggard; * Significant difference at $P < 0.05$ in the t-test, $n = 3$.

The order of prevalence of the different types of aberrations that were induced is presented in Table 3. The order of frequency was: stickiness ($74.83\% \pm 10.04$) > multipolarity ($12.42\% \pm 11.53$) > c-mitosis ($11.83\% \pm 12.57$) > anaphase and telophase bridges ($0.42\% \pm 0.61$) > chromosome laggards ($0.08\% \pm 0.21$). The sticky chromosomes, multipolar and C-mitosis aberrations together constituted between 96 to 100% of the aberrations induced by any of the pesticides.

Table 3. The frequency of induction of the different types of aberrations by some pesticides

	TC	Total CA %	A.B as % of total CA	L as % of total CA	C-Mit as % of total CA	Multipolar as % of total CA	S as % of total CA	C-Mit + Multipolar + S as % of total CA	C-Mit + S as % of total CA
Water	Control	0	0	0	0	0	0	0	0
GT (mg mL ⁻¹)	12.5	83	0	0	31	1	67	100	99
	25	91	0	0	25	7	69	100	93
	50	82.67	0	0	29	7	64	100	93
	0.75	68.33	1	0	11	14	73	99	84
QP (mg mL ⁻¹)	1.5	64.33	0.33	0	15	8	77	99	91
	3	82	1.33	0	25	0	74	98	98
	0.064	60	1.67	0.67	4	4	88	96	92
NP (mg mL ⁻¹)	0.128	42.67	0	0	2	20	78	100	80
	0.256	19.67	0	0	0	37	63	100	63
	0.0025	26	0.67	0.33	0	31	65	96	65
EPSC (mg mL ⁻¹)	0.0049	8.33	0	0	0	8	92	100	92
	0.0098	28.67	0	0	0	12	88	100	88
	Sum		5	1	142	149	898	1188	1038
	Average		0.42	0.08	11.83	12.42	74.83	99	86.5
	S.D		0.61	0.21	12.57	11.53	10.04		

TC = Test Compound; S.D = Standard Deviation; GT = Grain Treat insecticide; QP = Quickphos; NP = Nuvan Profi; EPSC = Erioccephalu punctulatus smoke condensate; CA % = Cells with chromosome aberration as % of A&T cells examined; C-Mit = C-Mitosis; A.B = Anaphase or Telophase bridge; S = Sticky; L = Laggard.

4. Discussion

In the present study, only the highest concentration of QP (3.0 mg mL⁻¹) and NP (0.256 mg mL⁻¹) were cytotoxic ($P < 0.05$) i.e. significantly reduced the mitotic index (below 50%), when compared to that of the concomitant negative control. The EC₅₀ values (mg mL⁻¹), determined in the germination inhibition experiments for the test compounds were: GT (> 200); QP (9); NP (3.58) and EPSC (0.164). According to Rank and Nielsen (1977), the mitotic index will never be below half of the control if the EC₅₀ value is used as the highest concentration for the genotoxicity test. The decline of MI below 22% of that of the negative control can have lethal impact on the organism (Antonsie-wiez, 1990). In the present study however, the highest concentrations of the pesticides tested were only fractions of their respective EC₅₀ values as follows; QP (1/3), NP (1/14), GT (EC₅₀ not determined) and EPSC (1/16). The failure of most of the concentrations to reduce the MI to below 50% of the control was therefore not surprising and agrees with the results of Rank and Nielsen (1997). However, the mitotic index was reduced to below 50% of the control by concentration of QP and NP that were below their respective EC₅₀s in the present study which suggested that, for some compounds, even concentrations below the EC₅₀ can reduce the MI below 50% of the control. The observation demonstrates that the MI is a more sensitive end point than the effective concentration that inhibits the germination of 50% of seeds (EC₅₀).

In the present study, none of the concentrations (mg mL⁻¹) of GT tested (12.5, 25, 50) was cytotoxic. However, in the AlamarBlue cytotoxicity assay, malathion (50 µM malathion) prompted cytotoxicity in murine splenocytes *in vitro* (Rabideau, 2001). The lack of cytotoxicity observed in the present study when compared to the positive result for malathion in the AlamarBlue cytotoxicity assay could be due to several possible reasons. Whereas the present study used a plant *in vivo* assay system the AlamarBlue cytotoxicity assay used animal cells *in vitro*. Secondly whereas pure malathion was tested in the AlamarBlue assay, GT is a mixture of malathion and permethrin and pesticides in mixtures are known to interact in complex ways including synergism or antagonism (Cloyd et al., 2007). Only the highest concentration of QP was toxic. Phosphine, which is the active ingredient in QP induced a significant increase in chromosomal aberrations and reduced mitotic indices (MIs) in the bone marrow cells of male Sprague Dawley rats after 6 hours of inhalation exposure, at the highest dose tested, HDT, (19 ppm) (U.S EPA, 1999). In the present study, only the highest concentration of NP was toxic. Dichlorvos, the active ingredient in NP was shown to decrease the mitotic index of *Vicia faba* root tip meristem cells treated with it (Amer & Ali, 1986; Kontek et al., 2007).

Erioccephalus punctulatus is an aromatic plant that contains volatile oils (Samie & Nefefe, 2012), which

comprise of hydrocarbons, mainly terpenes (monoterpenes, sesquiterpenes and diterpenes), oxygenated terpenes, alcohols, aldehydes, carboxylic acids, esters, fatty acids and ketones (Simon, 1990). In the present study, none of the concentrations of the smoke condensate of *Eriocephalus punctulatus* tested significantly decreased the mitotic index of *Vicia faba* root tip meristem cells, as shown in Table 2.

Burning wood smoke contains over 300 different compounds, mainly phenols, carbonyls, acids, furans, alcohols, esters, lactones and polycyclic aromatic hydrocarbons, PAHs, (Hamm, 1977; Mohler, 1978). Antimicrobial effect of smoke condensates from different woods against *Saccharomyces cerevisiae*, *Staphylococcus aureus* and *Escherichia coli* has been demonstrated (Asita & Campbell, 1990). However, the extract of *Eriocephalus punctulatus* has been shown to possess antimicrobial, anti-inflammatory, and antioxidant activities, and also inhibit acetylcholinesterase (Njenga & Viljoen, 2006; Samie & Nefefe, 2012).

Mitotic index is considered a parameter that allows one to estimate the frequency of cellular division (Marcano et al., 2004) and the reduction of mitotic activities has been used frequently to trace substances that are cytotoxic (Linnainmaa et al., 1978; Smaka-Kincl et al., 1996). Many investigators have recorded a depression of the mitotic index following the treatment of test organisms with pesticides (Panda & Sahu, 1985; Amer & Farah, 1974). In trial experiments in the present study, the EC₅₀s of the pesticides tested reduced the MI of the onion root tip meristem cells to the extent that not enough cells in division stages could be observed. However, in the actual genotoxicity tests, only the highest concentration of QP (3.0 mg ml⁻¹) and NP (0.256 mg ml⁻¹) induced significant cytotoxic effects ($P < 0.05$) which was indicative of a mitodepressive effect.

When root-tip meristem cells of *Allium cepa* were treated with the pesticides screened in this work, one or more of the concentrations of each pesticide caused the following five cytological abnormalities summarized in Table 3: GT (c-mit, multipolar, and sticky chromosomes), QP (AB, c-mit, multipolar, and sticky chromosomes), NP (AB, laggard, c-mit, multipolar, sticky chromosomes), and EPSC (AB, laggard, c-mit., multipolar and sticky chromosomes). The lowest concentration of Nuvan Profi (0.064 mg ml⁻¹) induced all five types of chromosomal aberrations. In all cases, the most prevalent type was the sticky chromosomes. When the sum of each type of aberration induced by the three concentrations of the four pesticides was taken, the following order of prevalence was observed: stickiness (74.83% ± 10.04) > multipolarity (12.42% ± 11.53) > c-mitosis (11.83% ± 12.57) > anaphase and telophase bridges (0.42% ± 0.61) > chromosome laggards (0.08% ± 0.21). The stick chromosomes, multipolar and c-mitosis aberrations together constituted between 96 to 100% of the aberrations in all cases (Table 3.). The results showed that cell division was not blocked by the concentrations of the pesticides that were tested.

Malathion (mercaptothion), one of the active ingredients in GT and its metabolite, malaoxon, did not induce genotoxic effects in tests with *S. typhimurium* TA97, TA98, and TA100 at doses of 10-1000 µg/plate (Imamura & Talcott, 1985). However, permethrin which was the second active ingredient in GT caused an increase in chromosomal aberrations in human lymphocytes cultures exposed to it (Barrueco et al., 1992). Another permethrin-containing insecticide called Ambush caused increases in sex-linked lethal mutations during larval development in fruit flies (Kale et al., 1995). In an *in vivo* study that involved 31 phosphine (active ingredient in QP) fumigators and 21 controls no significant differences was found between fumigators and controls in the incidence of micronuclei in lymphocytes in peripheral blood and extracts of urine of fumigators were not mutagenic in two strains of *Salmonella typhimurium* (TA100 and TA98) (Barbosa & Bonin, 1994). In the present study with *Allium cepa* root tip meristem cells however, the three concentrations of GT tested induced genotoxicity thus corroborating the findings of the studies with cultures of human lymphocytes but not the *in vitro* studies with *Salmonella typhimurium* cited above.

Phosphine has consistently produced negative results in the Ames test with *S. typhimurium* sp. TA98, TA100, TA1535, TA1537 and *E. coli* sp. WP2UVRA, at concentrations of 0.02-0.5%, in the presence or absence of metabolic activation (COM, 1997). However, human lymphocyte cells treated with phosphine exhibited concentration-related chromosomal damage (deletions, gaps and strand breaks) similar to the genotoxic effects observed in blood cells of some farm workers who were exposed to phosphine as they administered pesticides (US EPA, 2003). The three concentrations of QuickPhos (Aluminium Phosphide, 560 g/kg) tested in the present study induced genotoxicity in meristem cells of onion root tip, which was contrary to the negative results of the Ames test with *S. typhimurium* sp but agreed with the study involving human lymphocytes cited above. The present study provides additional information on the genotoxic effects of phosphine in plant cells.

Dichlorvos, the active ingredient in NP, used at concentrations of 32-250 ppm (i.e. 32-250 µg ml⁻¹) was shown to decrease the MI and to increase chromosomal aberrations in *Vicia faba* meristem cells (Amer & Ali, 1986). Dichlorvos was mutagenic in the Ames *salmonella* mutagenicity assay and other tests using bacteria or animal

cell cultures (US EPA, 1988). In studies conducted in live animals, no mutagenic evidence was observed and this was attributed to the fact that in live animals it is metabolized rapidly and excreted (Gallo & Lawryk, 1991). Results of the present study showed that the three concentrations of NP tested induced genotoxicity thus corroborating the findings of the 1986 study by Amer and Ali with *Vicia faba*. The concentration of dichlorvos in the NP pesticide tested in the present study was about 124 g kg^{-1} , i.e. 12.4%. Therefore, dichlorvos in the concentrations of NP that were tested in the present study was about 7.936, 15.82 and 32.24 ppm, which compared only with the lowest concentration of dichlorvos that was tested in the 1986 study of Amer and Ali, (1986) and suggested that *Allium cepa* root meristem cells are more sensitive than *Vicia faba* meristem. In a previous study by Asita and Matobole (2010), *Allium cepa* root meristem cells were shown to be more sensitive than *Vicia faba* meristem in detecting genotoxic compounds. The observation that lower concentrations of dichlorvos induced genotoxic effects in *Allium cepa* root meristem cells just as the higher concentrations used in studies with *Vicia faba* root meristem cells could also suggest that dichlorvos in the NP mixture was more potent than the pure product. Synergistic interaction or potentiation between or among pesticides in mixtures is a well-known phenomenon (Cloyd et al., 2007). For instance, glyphosate alone rarely caused genetic damage in laboratory tests whereas roundup, a glyphosate product, was mutagenic (Rank et al., 1993). The mutagenic effects of the condensates of wood smoke have been demonstrated in *S. typhimurium* reverse mutation assays (Nagao et al., 1977; Kamens et al., 1984; Asita et al., 1991) and mutagenic polycyclic aromatic hydrocarbons (PAHs) were detected in African smoked fish (Mossanda et al., 1979). All the three concentrations of *Eriocephalus punctulatus* smoke condensate (EPSC) tested in the present study with *Allium cepa* root tip meristem cells induced genotoxicity. Water-soluble extract of tobacco smoke condensate (TSC) induced cytological abnormalities in root-tip meristem cells of *Allium cepa*. The cytological abnormalities included stickiness, chromosome breaks during metaphase and chromosome bridges and lagging during anaphase (Sabharwal et al., 1975). Genotoxicity of the essential oils which is the source of the aroma of *Eriocephalus punctulatus* (Simon, 1990) was not tested in the present study. However, in the literature, mutagenicity tests of oils have given different results. While the oils from leaves/ twigs of Manuka (*Leptospermum scoparium*) for instance, were not mutagenic in the Ames test, fennel, bitter (*Foeniculum vulgare vulgare*) seed oil induced genotoxic effect in the *B. subtilis* DNA-repair test and Estragole, present in the volatile oil, was tumorigenic in animals (Dweck, 2009).

Stickiness of chromosomes was attributed to abnormal DNA condensation (Österberg et al., 1984) and the entanglement of inter-chromosomal chromatin fibers (Patil & Bhat, 1992). The most prevalent type of aberration which was induced by the four pesticides in the present study was chromosome stickiness. It could therefore be concluded that GT, QP, NP and EPSC caused abnormal DNA condensation, abnormal chromosome coiling and entanglement of inter-chromosomal chromatin fibers. Levan (1938) described colchicine mitosis (c-metaphase or c-anaphase) as an inactivation of the spindle followed by a random scattering of the condensed chromosomes in the cell. It has also been suggested by Yildiz and Arikan (2008) that large number of laggard chromosomes and c-anaphases indicated that a test compound acted as a strong spindle inhibitor. According to Elghamery et al. (2003) the induction of vagrant chromosomes leads to the production of daughter cells with unequal number of chromosomes in their nuclei which, as a consequence are unequal in size or shaped irregularly at interphase. Badr et al. (1992) attributed the induction of anaphase/telophase bridges to chromosome breaks, stickiness and breakage and reunion of the broken ends of chromosomes. Therefore the observed anaphase/telophase bridges in the present study suggested a clastogenic effect of QP, NP and EPSC. The four pesticides tested thus caused abnormal DNA condensation, abnormal chromosome coiling and inactivation of the spindle. In addition, QP, NP and EPSC were also clastogenic. According to Norppa (2004), most adverse effects on health, caused by genotoxins, result from genetic damage in somatic as well as germinal cells. It has also been suggested that any genotoxic effects of environmental chemicals, is likely to result from abnormalities of the cell division process (Parry et al., 1999). The four pesticides therefore, have the potential to cause aneuploidy in exposed organisms and adverse human health and environmental effects.

5. Conclusion

Root-tip meristem cells of *Allium cepa* were exposure to concentrations (mg ml^{-1}) of GT (12.5, 25, 50); QP (0.75, 1.5, 3.0); NP (0.064, 0.128, 0.256) and EPSC (0.0025, 0.0049, 0.0098) for 24 h and assessed for cytotoxicity and genotoxicity. Only QP (3.0 mg ml^{-1}) and NP (0.256 mg ml^{-1}) caused a significant reduction ($P < 0.05$) in the mitotic index compared with the negative control, which was indicative of mitodepressive effect. The following types of chromosome aberrations were observed and recorded in anaphase-telophase cells: anaphase or telophase bridge (A.B), laggard (L), c-mitosis (C-Mit), multipolar anaphases and telophases (Multipolar) and sticky chromosomes (S). When the sum of each type of aberration induced by the three concentrations of the four

pesticides was taken, the following order of prevalence was observed: stickiness ($74.83\% \pm 10.04$) > multipolarity ($12.42\% \pm 11.53$) > c-mitosis ($11.83\% \pm 12.57$) > anaphase and telophase bridges ($0.42\% \pm 0.61$) > chromosome largards ($0.08\% \pm 0.21$). The induction of sticky chromosomes indicated that the pesticides caused abnormal DNA condensation, abnormal chromosome coiling and inactivated the spindles. Most adverse effects on health by genotoxins are the result of genetic damage and the genetic activity of chemicals is most likely to result from abnormalities of the cell division process. Because modifications of cell division process often results in the production of daughter cells with abnormal chromosome numbers and aneuploid or polyploidy karyotypes, these pesticides have the potential to cause adverse effects to both human health and the environment.

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