International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 2(K); February 2018; Page No. 10338-10342
DOI: http://dx.doi.org/10.24327/ijcar.2018.10342.1746

Research Article

COMPARATIVE ANALYSIS OF THE TOXICITY LEVELS OF COMMERCIAL COILS AND NATURAL CAMPHOR USED AS MOSQUITO REPELLENTS

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A R T I C L E I N F O

Article History:

Received 18th November, 2017
Received in revised form 27th December, 2017
Accepted 14th January, 2018
Published online 28th February, 2018

Key words:

Allium cepa; Camphor; Chromosome aberrations;Cytotoxicity; Genotoxicity; Mosquito coil

A B S T R A C T

The aim of the study was to compare toxicity levels of two commonly used mosquito repellents - camphor and commercial mosquito coils on a scientific basis. The *Allium cepa* assay, one of the most efficient approaches to assess toxic effects of environmental chemicals was chosen for the study. Phytotoxic, cytotoxic and genotoxic parameters were assessed to identify potential risks in using these repellents. Studies showed a reduction in root growth rate with increasing concentrations for both camphor and mosquito coil extracts. Root growth inhibition was very less for camphor when compared with negative control, distilled water. Mitotic index also showed a reduction but the abnormality percentage was on a high with increasing concentration for both extracts. *Allium* grown in mosquito coil extracts had low mitotic index and high frequency of aberrations compared to camphor and negative control. Major aberrations observed include chromosome stickiness, binucleate cell, chromosome bridges, misorientations, c-metaphases and stellate ana/telophases. Binucleate cells were only confined to mosquito coil extract and positive control (methyl parathion) treated cells. All values were statistically significant at $p<0.05\%$. The results indicated that camphor is safer and effective for protection against mosquitoes than commercial mosquito coils as far as risk on health is concerned.

INTRODUCTION

Mosquitoes have always been a source of epidemics all over the world. To overcome the menace, several products with mosquitocidal properties are being developed but its side effects and safety have not been the major concern. This has led to major health problems in developing countries as they are the main consumers of commercial products like mosquito coils. Thus, the demand for a natural remedy to control mosquitoes has always been a herculean task for authorities.

Mosquito serves as crucial vector for a number of arboviruses and parasites that are maintained in nature through biological transmission between susceptible vertebrate hosts by blood feeding arthropods responsible for inflammation/encephalitis, dengue, malaria, rift valley fever, yellow fever and others. Vector control is by far the most successful method for reducing incidences of mosquito borne diseases, but the emergence of widespread insecticide resistance and the potential environmental issues associated with some synthetic insecticides (such as DDT) has indicated that additional approaches to control the proliferation of mosquito population would be an urgent priority research (Kishore *et al*., 2011). The World Health Organization points to the fact that the range of mosquitoes is being expanded due to global warming. Even malaria mosquitoes are appearing in upland areas where they've never been seen before (Patel *et al*., 2012). Female mosquitoes are attracted by the carbon dioxide, excretory products and lactic acid present in sweat in warm blooded animals. The odour perception is through chemoreceptors present in the antennae. The mosquito repellents block the lactic acid receptor which destroys the upwind flight and as a result the mosquito loses its contact with the host (Elissa *et al*., 2004; Sah *et al*., 2010). Mosquito coil, one of most commonly used mosquito repellent, is a repelling incense usually shaped into spiral, and typically made from a dried paste of pyrethrum powder. Mosquito coils are widely used in Asia, Africa, and South America (Liu *et al*., 2003). India is a large consumer of mosquito coils as compared to the electric products like mats and liquid vaporizers (Phal *et al*., 2012). When a mosquito coil is burned, the insecticide evaporates with the smoke, which repels the mosquitoes from entering the room (Lee *et al*., 1999). The smoke emitted from the burning mosquito coil consists of submicron particles (diameter less than 1 micron) coated with a considerable amount of heavy metals, allethrin and a wide range of organic vapours, such as phenol and o-cresol. Therefore, a lengthy exposure to this smoke will cause adverse effects on the consumers (Liu *et al*., 1987).

An effective remedy against mosquito which has not received considerable attention is camphor. Camphor is a natural mosquito repellent without side effects. It is extracted from the

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wood of camphor laurel (*Cinnamomum camphora*), an evergreen tree growing in various parts of Asia. Sulphur repels mosquitoes and the same ingredient is found in camphor. It is a good analgesic and known to improve the quality of air.

Despite these facts and that mosquito coil smoke have many potential adverse health effects, large populations in developing countries still use mosquito coils in their daily lives. Thus, to make informative recommendations to consumers about the effects of various mosquito repellents, toxicity levels of commercial mosquito coils and natural camphor were analyzed using *Allium cepa* assay. The *A. cepa* assay is an efficient test for chemical screening and *in situ* monitoring for genotoxicity of environmental contaminants. It has also been widely used to study genotoxicity of many pesticides revealing that these compounds can induce chromosomal aberrations in root meristems of *A. cepa* (Ma *et al*., 1994; Fernandes *et al*., 2007). In addition to this, *Allium* test has been found to have high correlation with other test system (MIT-217 cell test with mice, rats or humans *in vivo*) and could be used as an alternative to laboratory animal in toxicological research (Fiskesjo and Levan, 1993).

Thus, this study aims at revealing the toxicity levels of mosquito coil and camphor using phytotoxic, cytotoxic and genotoxic effects on *A. cepa* as an alert system.

MATERIALS AND METHODS

Sample material and Control

Commercial mosquito coils and camphor were purchased from the local market. Fresh aqueous extracts of mosquito coil and camphor was made with the help of pestle and mortar. Lowest concentrations of both the extract *viz.,* 0.1, 0.05, 0.01, and 0.005% (w/v) were chosen after preliminary toxicity analysis as the test solutions.

Distilled water and an organophosphorus pesticide, methyl parathion (0.01%), was taken as negative and positive control respectively.

Phytotoxicity assay

Onion bulbs free from agricultural pesticides and growth inhibitors were grown by suspending the bulbs on cylindrical glass bottles containing control as well as test solutions. Root growth was determined by measuring the length of adventitious roots per bulbs every 24h for 72h. Test and control solutions were daily changed. The experiment was conducted in triplicate. Apart from root length, other macroscopic parameters like root consistency and colour, the presence of tumours, hook roots and twisted roots were also examined.

Cytotoxicity and Genotoxicity assay

Germinated bulbs with healthy roots from the control and test solutions were collected at peak mitotic period (9am-10am) after 72h, washed in distilled water and immediately fixed in modified Carnoy's fluid for 1h. Mitotic squash preparation was done with the help of improved techniques (Sharma and Sharma, 1990). The root tips were hydrolysed in 1N HCl for 5-10 minutes and stained with 2% aceto-carmine for 4 hours. Mitotic index and abnormality percentage were calculated by counting the mitotic cells and aberrant cells respectively out of the total cells scored. Aberrant cells were determined by scoring cells with binucleate cell, c-metaphases, chromosome bridges, stickiness, misorientations and stellate ana/telophases. All the slides were scanned, tabulated and photomicrographs were taken with Leica ICC 50 digital camera attached to LEICA DM 500 research microscope.

Statistical analysis

Data obtained on root length, mitotic index and abnormality percentage were statistically analysed. One way ANOVA and tukey's-b test was performed to determine mean separation and significance of treatments using SPSS version 20, SPSS Inc., Chicago, USA. Each data point represents the arithmetic mean ± standard error of at least three independent experiments.

RESULTS AND DISCUSSION

The study involving comparison of phytotoxic, cytotoxic and genotoxic effects of mosquito coil and camphor extract at varying concentration on *A. cepa* as test system yielded significant results when compared with the control. Toxicity levels were higher for mosquito coil extracts and also increased with increasing concentrations. Camphor showed less toxicity and was found to be safer for use when compared to commercial coils since they are natural in origin.

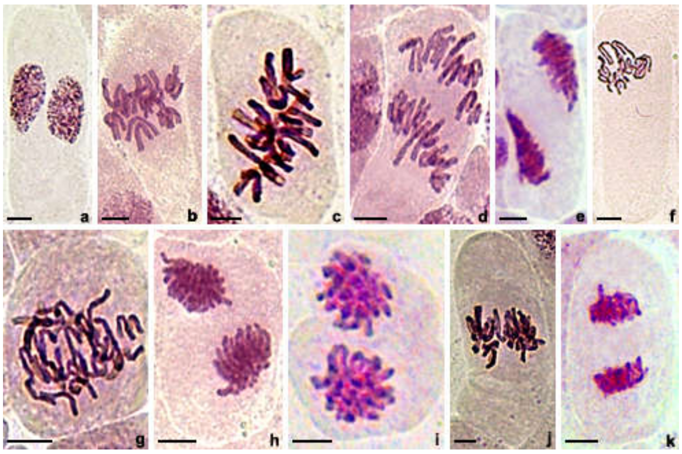

Fig. 1 Chromosome aberrations in mosquito coil extract treated *Allium cepa* root tip cells

a Binucleate cell; b C-metaphase; c Diagonal metaphase; d Diagonal anaphase; e Diagonal telophase; f Misorientation at metaphase, g Multiple bridges at anaphase; h Shift in microtubule organizing centre; i Stellate anaphase; j Sticky metaphase; k Sticky anaphase. Bar represents 5µm

Phytotoxic assay

The effect of the test and control solutions on root growth is summarized in Table 1.

Table 1 Root growth of *Allium cepa* roots exposed to control and test solutions

Extract	Concentration (%)	Root length at different time durations (mean ±SE) (cm)		
		24 h	48 h	72 h
NC	-	0.93±0.07^{a}	1.50±0.06^{a}	2.53±0.14^{a}
PC	0.01	0.10±0.00^{c}	0.17±0.03^{d}	0.23±0.03^{f}
Camphor	0.005	0.53±0.03^{b}	1.33±0.11^{a}	1.97±0.12^{b}
	0.01	0.37±0.03^{b}	1.07±0.09^{b}	1.60±0.11^{b,c}
	0.05	0.17±0.03^{c}	0.77±0.03^{c}	1.23±0.03^{c,d}
	0.1	0.10±0.00^{c}	0.27±0.03^{d}	0.80±0.07^{e}
Mosquito coil	0.005	0.17±0.03^{c}	0.83±0.03^{c}	1.20±0.06^{c,d}
	0.01	0.13±0.03^{c}	0.77±0.03^{c}	1.00±0.06^{d,e}
	0.05	0.13±0.03^{c}	0.73±0.03^{c}	0.93±0.03^{d,e}
	0.1	0.00±0.00^{c}	0.37±0.03^{d}	0.73±0.03^{e}

SE–standard error. . NC – Negative control (Distilled water), PC – Positive control (Methyl parathion). Means within a column followed by the same letters are not significantly different ($p<0.05$) as determined by tukey's-b test

As compared to negative control, the extracts showed significant effect on root growth of *A. cepa*. Root growth was found to be low for higher concentrations for both camphor and mosquito coil extracts but camphor showed relevant root growth at lower concentrations. Moreover, macroscopic parameters studied revealed that roots grown in camphor extract were stout and healthy as in negative control while those in mosquito coil extract were slender and weak as in positive control. Roots grown in camphor extract had a length of 1.97±0.12 while those of coil extract showed 1.20±0.06 at lowest concentration of 0.005% after 72h. All values were significant at $p<0.05$ when compared to control. No other macroscopic changes were evident in growing roots under any experimental condition.

Sustained root growth is regulated by the combined activities of cell division in the mitotically active meristematic zone and cell elongation that occurs subsequently in the more proximal regions of the root tip (Shishkova *et al*., 2008). It is well known that growth rates can be affected by inhibition or disruption of any of these processes, which involve independent events (Obroucheva, 2008). The phytotoxic assay revealed significant reduction in length of roots grown in mosquito coil extract. It may be due to interruption in cellular activities which is evident from reduced mitotic indices. Moreover, inhibition of root growth may result mainly from impaired cell elongation as suggested by Herrero *et al*. (2012). This is explained by the increased number of chromosome aberrations in roots grown in mosquito coil extract. Thus, phytotoxic studies emphasize the fact that camphor is safer for use than commercial coils.

Cytotoxic assay

Table 2 shows the comparison among mitotic indices of positive and negative control with varying concentrations of camphor and mosquito coil extracts. Significant difference in mitotic index was not observed in camphor extract at low concentrations when compared to negative control while mosquito coil showed attentive decrease both at low and high concentrations.

All values were significant at $p<0.05$ when compared to control. Mitotic index is a parameter that allows estimating 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 (Smaka-Kincl *et al*., 1996). Compared to distilled water, mosquito coil showed decreased mitotic index than camphor in a dose dependent manner. Since mitotic index is a quantitative estimation of the mitotic activities in an organism or a particular organ, the reduction in mitotic index is due to the constituents in the extracts that in turn have cytotoxic effects (Sreeranjini and Siril, 2011). Hence, it may be concluded that the constituents in commercial mosquito coils are hazardous than those of camphor which resulted in low mitotic index.

Genotoxic assay

The test solutions and positive control induced genotoxic effects on root tips which are estimated by the number and type of chromosome aberrations. The genotoxic effects on *A. cepa* roots caused by camphor and mosquito coil extracts are depicted in Table 2. The abnormality percentage of mosquito coil was very high (25.18±0.69) and more than the positive control at the highest concentration. Camphor had comparatively very less genotoxic effect as evident from its abnormality percentage. All values were statistically significant at $p<0.05$ when compared to control and the most common abnormality observed were chromosome stickiness and misorientations. The other frequently observed aberrations included binucleate cell, c-metaphases, chromosome bridges, and stellate ana/telophases (Figure 1). Binucleate cell was only observed with extracts of mosquito coil and positive control which indicated its severe genotoxic effect.

The chromosome aberration assay is one of the few direct methods capable of measuring mutations in systems exposed to putative mutagenic or carcinogenic substances (Rank *et al*., 2002; Leme *et al*., 2008). Several chromosome aberrations were detected in roots treated with test solutions and positive control which indicated that the fate of most cells was cell death.

Table 2 Mitotic index and abnormality percentage of *Allium cepa* roots exposed to control and test solutions

Extract	Concentration (%)	Total no. of cells ±SE	Aberrations±SE						Mitotic index ±SE	Abnormality (%) ±SE
			Binucleate cell	C-metaphase	Chromosome Bridge	Stickiness	Misorientation	Stellate arrangement		
NC	-	368±2.03	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	85.05±0.94^{a}	0.00±0.00^{f}
PC	0.01	396±5.69	15±0.58	4±0.33	20±0.88	16±0.33	26±0.33	18±0.58	28.03±0.70^{g}	24.97±0.53^{a}
	0.005	458±3.28	0±0.00	4±0.33	3±0.00	7±0.33	12±0.58	2±0.33	83.84±0.51^{a,b}	6.11±0.28^{e}
	0.01	450±2.08	0±0.00	5±0.33	4±0.33	10±0.33	15±0.58	3±0.67	81.33±0.63^{b}	8.22±0.12^{d,e}
Camphor	0.05	483±4.62	0±0.00	8±0.33	4±0.33	13±0.33	18±0.58	4±0.33	73.50±0.21^{c}	9.73±0.14^{d}
	0.1	492±5.51	0±0.00	12±0.58	8±0.33	17±0.88	22±0.33	5±0.33	67.28±1.10^{d}	13.01±0.73^{c}
	0.005	417±1.15	5±0.33	10±0.33	5±0.33	13±0.33	18±0.58	10±0.33	67.62±0.15^{d}	14.63±0.92^{c}
	0.01	394±1.76	6±0.58	13±0.33	9±0.00	18±0.58	20±0.67	13±0.67	64.72±0.45^{d,e}	20.05±0.18^{b}
Mosquito coil	0.05	389±1.76	7±0.33	14±0.33	8±0.33	22±0.33	22±0.33	14±0.67	62.72±0.62^{c}	22.36±1.05^{a,b}
	0.1	409±2.60	8±0.33	17±0.33	12±0.00	23±0.33	26±0.33	17±0.33	58.92±0.41^{f}	25.18±0.69^{a}

SE – standard error. NC – Negative control (Distilled water), PC – Positive control (Methyl parathion). Means within a column followed by the same letters are not significantly different

Chromosome stickiness is regarded as a physiological effect caused by the affected proteins of the chromosome. It may cause incomplete separation of daughter chromosomes as a result of the cross-linkage of chromoproteins (Kong and Ma, 1999; Tkalec *et al*., 2009). Liu *et al*. (1992) suggested that sticky chromosomes reflect a highly toxic effect, usually of an irreversible type, and probably lead to cell death. Frequency of chromosome stickiness was found to be significantly high in root tips treated with mosquito coil extracts than those with camphor indicating the high genotoxic effect. Chromosome bridges may be due to chromosomal stickiness and the subsequent failure of free anaphase separation. These bridges are usually formed by joined sister chromatids that stay together until late anaphase or telophase (Gömürgen, 2005; Türkoglu, 2008).

Binucleate cells usually arises as a result of prevention of cytokinesis or cell plate formation (Celik and Aslanturk, 2010), which might be due to the suppression of phragmoplast formation in the early telophase (Soliman, 2001). Abu and Mba (2011) suggested that cytokinetic failure leading to multinucleate cells is a severe deleterious effect that can lead to cancerous cells in tissues. It could only be observed in positive control and mosquito coil extract treated root meristems during the present study. C-mitotic effect is a common sign of spindle inhibition. The presence of c-metaphase indicates effects on the organization of chromatin, which may be related to an imbalance of the proteins responsible for the structure of nuclear chromatin (Kurás *et al*., 2006). Misorientations can be due to a distortion of the spindle apparatus, a tilt in the equatorial organization of metaphase chromosomes or a change in the direction of movement of daughter chromosomes during anaphase (Selim *et al*., 1981). Stellate arrangement may be due to the clumping of daughter chromosomes into star like structures near the polar region of the cell. Thus, the chromosome aberrations observed indicates a high risk of genotoxicity. Camphor extract treated cells when compared with positive control and mosquito coil extracts are safer for use as they have comparatively less frequency of aberrations.

Comparative account on commercial coils and camphor

From the studies conducted, it is found that mosquito coils have high cytotoxic and genotoxic effect when compared to camphor. The major active ingredients of most mosquito coils are pyrethrins, accounting for about 0.3 – 0.4% of the coil mass (Lukwa and Chandiwana, 1998). The remaining components of mosquito coils include organic fillers, binders, dyes and other additives capable of burning well without flame. The combustion of these remaining components generates large amounts of submicrometer particles and gaseous pollutants such as acenaphthene, penanthrene and benzo(a)pyrene (Liu *et al*., 2003). Liu and Sun (1988) investigated emissions of organic compounds from mosquito coil smoke by using gas chromatography–mass spectrometry (GC–MS). The major identified volatile organic compounds constituted allethrin, phenol, benzene, toluene and xylene, as well as aromatic and aliphatic hydrocarbons. According to a study by UC Riverside scientists, many mosquito coils - most notably those manufactured in Asia - often contain upto one percent BCME (bis[chloromethyl]ether. BCME has been described as the most potent lung cancer chemical ever discovered. Camphor ($C_{10}H_{16}O$; 1,7,7-trimethylbicyclo[2.2.1]heptan-3-one) on the other hand are naturally occuring bicyclic monoterpenes.

Chromosome aberration studies on *A. cepa* have also revealed the potent risk in the use of mosquito coils. The results of higher plant bioassays should not be neglected, considering that a chemical able to induce chromosome damages in plants can also offer risk to other groups of living organisms, since the damaged material is DNA, which is common to all organisms (Leme and Marin-Morales, 2009). Thus, it may be concluded from the present study that for a healthy protection from mosquitoes, it is recommended to use natural camphor over mosquito coils.

Acknowledgements

First author kindly acknowledge Kerala State Council for Science, Technology & Environment for providing financial assistance through KSCSTE fellowship.

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How to cite this article:

Sandhya Vincent Neelamkavil and John E. Thoppil (2018) 'Comparative Analysis of the Toxicity Levels of Commercial Coils And Natural Camphor Used as Mosquito Repellents', *International Journal of Current Advanced Research,* 07(2), pp. 10338-10342. DOI: http://dx.doi.org/10.24327/ijcar.2018.10342.1746

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