



Cytogenotoxic assessment of the aqueous extract of *Citrullus lanatus* (*Cucurbitaceae*) leaves using the spermatogonial germ-line cells of *Zonocerus variegatus* L. (Orthoptera: Pyrgomorphidae)

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Abstract

The popularity of watermelon, *Citrullus lanatus* (Cucurbitaceae) has increased in recent years in Cameroon due to the important nutrients it contains and health benefits it presents. Inspired by the ethno botanical and ethno medicinal uses of watermelon, this study was designed to evaluate its cytogenotoxic properties using the spermatogonial germ-line cells of the grasshopper *Zonocerus variegatus* L. (Orthoptera: Pyrgomorphidae). Fifth instar *Z. variegatus* individuals were treated to different concentrations of (0µg/l, 5µg/l, 10µg/l, 20µg/l, 30µg/l and 50µg/l) of aqueous extract of watermelon, *C. lanatus* leaves by injection into the abdomen respectively. Distilled water (0 µg/ml) was used as the control. Results revealed that *C. lanatus* leaf extracts exhibited cytogenotoxic effects and these were concentration dependent. Among the extracts, the 50 µg/ml recorded the highest percent aberrant cells while the 5 µg/ml showed the lowest. As regards genotoxicity, all the extract-treated cells showed different chromosomal aberrations that included bridges, laggards, vagrant chromosomes and sticky chromosomes. The 50 µg/ml extract significantly produced the highest incidences (25.83%) while the 5 µg/ml extract had the lowest incidence (1.57%) of chromosomal aberrations. The control induced only one laggard (0.95%). In general the different concentrations induced low amounts of cytogenotoxic aberrations. These results led us to conclude that *C. lanatus* exhibits some cytogenotoxic properties at high concentrations thus emphasizing that the fruit should be consumed by humans with caution.

Keywords: *Citrullus lanatus*, leaf extract, cytogenotoxic effects, chromosomal aberrations, *Zonocerus variegatus*

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1. Introduction

Watermelon, *C. lanatus*, is a member of the family Cucurbitaceae (Edwards et al., 2003). It is an annual plant with a long weak trailing stem, and green leaves that are sometimes deeply lobed and pinnate in form, measuring

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60 to 200 mm long and 40 to 150 mm wide (Mabberley, 2008). This plant produces a large fruit that is often oval to spherical in shape, with a dark green and smooth rind that sometimes shows irregular areas of pale green color. The mesoderm is sweet, reddish to yellowish in color, and is a juicy refreshing flesh. It contains small black seeds. Watermelon is presently among the most widely grown vegetable crops in Cameroon and the world in general (MINADER, 2018; Gusmini and Raleigh, 2003; and Wehner, 2008). The fruit is therefore commonly consumed in several parts of Cameroon and its popularity has in recent years increased because of its rich nutritional profile and the several health benefits presented. Watermelon contains very high water content and bioactive components such as cucurbitacin, triterpenes, sterols, alkaloids, vitamins and minerals (Erhirhie and Ekene, 2013; Oseni and Okoye, 2013; Aruna et al., 2014; Naz et al., 2014; Islam et al., 2015; Maoto et al., 2019). It is used in traditional medicine as a purgative, vermifuge, demulcent, diuretic and tonic (Erhirhie and Ekene, 2013). It has also been found to be effective in the reduction of the extent of cancer insurgence, cardiovascular disorder, diabetes and macular diseases (Naz et al., 2014; and Maoto et al., 2019). The consumption of *C. lanatus* is also credited with significant reduction of body weight, Body Mass Index (BMI), systolic blood pressure and waist-to-hip ratio (Lum et al., 2019). A review of the medicinal values of *C. lanatus* revealed a wide range of pharmacological and biological activities that include; antimicrobial, antioxidant, antiplasmodial, anti-inflammatory, antiprotastic hyperplasia activity, analgesic properties, effect on the histology of the kidney, antisecretory, antidiabetic, laxative, antinulcerogenesis and hepatoprotective activities (Chumsard et al., 2019). The watermelon fruit is also eaten for its antihypertensive, antidiarrhoeal properties and cardioprotective values (Ikeda et al., 2000; and Erhirhie and Ekene, 2013). The high lycopene levels in watermelon have been linked to heart health, bone health and prostate cancer prevention (Edwards et al., 2003; Naz et al., 2014, Allerton et al., 2018; and Dammak et al., 2019). Watermelon has been shown to have aphrodisiac properties (Enema et al., 2018). The aphrodisiac properties of watermelon is supported by the fact that flesh extract has been reported to cause a significant increase in mounting, intromission and ejaculatory frequency in laboratory rats (Munglue et al., 2014). Watermelon has also been reported to contain a high level of the amino acid L-citrulline which has been linked to the stimulation of blood flow to the penis (Cormio et al., 2011).

In spite of these numerous benefits of eating watermelon, adverse effects have also been reported. High consumption of watermelon, resulting in more than 30 mg of lycopene daily, could potentially cause nausea, diarrhea, indigestion, irregular heart beat and bloating (Rana, 2018). It has also been shown that much consumption of watermelon is linked to the risk of developing liver inflammation and the causation of cardiovascular problems (Picincu, 2019).

Inspired by these numerous important nutritional and ethno-medicinal attributes as well as the adverse effects recorded on consumption of watermelon (*C. lanatus*) this study was designed to evaluate the possible cytogenotoxic properties of the plant. This was done with the help of aqueous leaf extract and the *Z. variegatus* test assay.

2. Materials and methods

Sundried leaves of *C. lanatus* were ground into powder and 100 g of the powder infused with distilled water for 24 h before filtering. The filtrate was next concentrated at 45 °C in a hot oven for 48 h. The aqueous solution obtained 28.8% of total dry weight of the leaves of *C. lanatus* was then used to prepare 0µg/l, 5µg/l, 10µg/l, 20µg/l, 30µg/l and 50µg/l solutions used in this study.

Forty (40) male *Z. variegatus* individuals in the 5th instar stage were placed into five groups and labeled A, B, C, D and E. Individuals in the five groups were respectively administered 0µg/l, 5µg/l, 10µg/l, 20µg/l, 30µg/l and 50µg/l of *C. lanatus* leaf extract. Individuals in group A were used as the control. The grasshoppers were next incubated in the laboratory for 72 h, killed in chloroform and dissected for the testes in insect saline (68% Sodium Chloride). The testes were placed in labeled vials containing Canoy's solution (3:1 Ethanol: Acetic acid fixative) and stored in a refrigerator at 4 °C until needed.

Testes thus obtained were used to prepare chromosome smears by the method of Seino et al (2007). These chromosome smears were examined using a Fisher binocular microscope with the 10X and 40X objectives while taking note of the number of cells per treatment with chromosome aberrations and the number of the different types of chromosomal aberrations per treatment present. Photographs of interesting chromosome aberrations present were made using a ITEL A51 phone mounted with a 5.0 pixa lens. Data on the number of different types of chromosome aberrations were subjected to the one-way ANOVA at $p < 0.05$ level of significance.

3. Results and discussion

A wide range of assays are in use for screening cytogenotoxic effects of substances that include plant extracts. The most common ones in use are: onion (*Allium cepa*), laboratory rats (rodents), Chinese hamster cells and *Vicia faba* (Haroun and Al Shehri, 2001; Wang and Xie, 2007; Bhattacharya and Haldar, 2012; Asthana and Kumar, 2014; Bakare AA et al., 2013; Alimba and Bakare, 2016; Datta et al., 2018; and Wijeyaratne and Wadasinghe, 2019). Though the use of grasshopper chromosomes to evaluate genotoxic effect of substances dates as far back as the early 1950s, only a few are readily available in literature (Pandey and Banerjee, 1985; Seino et al., 2013a; Seino et al., 2014; and Meena et al., 2015). Grasshoppers are suited for cytogenotoxic assessment because of the large sizes of their chromosomes, the relatively few number of chromosomes present in the karyotype and the ease with which these chromosomes can be prepared (Camacho, 2014). In addition, grasshoppers present similar chromosome aberrations that are comparable to those in mammals (humans). The pest grasshopper *Z. variegatus* assay was chosen for this study because of the above reasons. It has also been used variously to evaluate cytogenotoxic properties of *Annona muricata* and *Capsicum frutescens* (Seino et al., 2013a; and Seino et al., 2014). The male karyotype of *Z. variegatus* used in the control for this study (individuals treated with distilled water) was observed to be 19 and the sex determining mechanism, OX ($2n=19XO B\delta$). There were therefore nine pairs of autosomes and one sex chromosome present. All of these chromosomes were acrocentric in morphology. This is the standard Pyrgomorphidae karyotype (Faluyi and Olorode, 1988; Williams and Ogunbiyi, 1995; and Seino et al., 2013b). Also the meiotic process recorded in these control individuals revealed Prophase -1 (Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis), Metpahse -1, Anaphase -1, Telophase-1, Metaphase -2, Anaphase -2 and Telophase -2 stages (Figure 1). Accordingly to the criteria of Faluyi (2011) and Seino et al (2013b), the meiotic process in the control individuals was judged to be normal.

Several studies have been carried out on the cytogenotoxic effects of extracts of plants but none has been done for watermelon, *C. lanatus*, which is one of the most popular fruits in Cameroon and worldwide. Unlike in the control, the effect of treatment with different concentrations of aqueous leaf extract on meiotic chromosomes in *Z. variegatus* resulted in some cytogenotoxic abnormalities (Figure 1). The abnormalities recorded included the formation of bridges, laggards, vagrant chromosomes and sticky chromosomes. Bridges were common in Anaphase -1, laggard chromosomes in Anaphase -1, Telophase -1 and Anaphase -2; vagrant chromosomes in Anaphase -1, while sticky chromosomes were common to Prophase -1 (Diplotene), Metaphase -1 and Anaphase 1 stages. Similar cytogenotoxic abnormalities have been reported in *Vicia faba* (Haroun and Shehri, 2001), the root tip cells of *Allium cepa* (Olorunfemi et al., 2012; and Pandey et al., 2014) and in grasshopper germ lines (Seino et al., 2013a; and Seino et al., 2014).

The data recorded on the frequencies of these meiotic abnormalities are summarized in Table 1. The data revealed that sticky chromosomes (2.31%) were the most induced chromosomal abnormality for all

Table 1: Frequency of meiotic chromosome abnormalities induced by aqueous extract of *C. lanatus* on germ line cells of male *Z. variegatus* L.

Conc. of leaf extract	No. of cells examined	Bridges		Laggards		Vagrant chromosomes		Sticky chromosomes		Disturbed spindle		Total of meiotic abnormalities	
		N0	%	N0	%	N0	%	N0	%	N0	%	N0	%
0 $\mu\text{g/ml}$	105	0	0	1	0	0	0	0	0	0	0	1	0.95
5 $\mu\text{g/ml}$	191	0	0	1	0.52	1	0.52	1	0.52	0	0	3	1.57
10 $\mu\text{g/ml}$	103	1	0.97	0	0	1	0.97	1	0.97	0	0	5	4.85
20 $\mu\text{g/ml}$	123	1	0.81	1	0.81	1	0.81	1	0.81	1	0.81	11	8.94
30 $\mu\text{g/ml}$	122	3	2.46	3	2.46	2	1.64	3	2.46	1	0.82	23	18.85
50 $\mu\text{g/ml}$	120	5	4.17	6	0.05	4	3.33	4	3.33	2	1.67	31	25.83
Total	644	10	1.55	12	1.86	9	0.29	13	2.31	4	0.62	43	6.68

concentrations of the extract while vagrant chromosomes (0.29%) were the least. The percentage chromosomal abnormalities induced by the aqueous leaf extract of *C. lanatus* were in the series: Sticky chromosomes > Laggards > Bridges > Disturbed Anaphases > Vagrant chromosomes. Table 1 also revealed that there was a corresponding increase in each of the chromosomal abnormalities recorded with increase in extract concentration (Figure 2). Total percentage of cytogenotoxic abnormalities recorded on treatment with aqueous extract of the leaves of *C. lanatus* was also observed to increase significantly ($p > 0.05$) with concentration of extract. Table 1 further shows that there was a significant difference ($p > 0.05$) in terms of chromosomal abnormalities between the control (0.95%) and treatment with 50 $\mu\text{g}/\text{ml}$ of extract. Since the number of abnormalities resulting from these treatment reached a maximum of 25.83%, our results have therefore indicated that the aqueous extract prepared from the leaves of *C. lanatus* has a low cytogenotoxic effect on the germ cells of *Z. variegatus*.

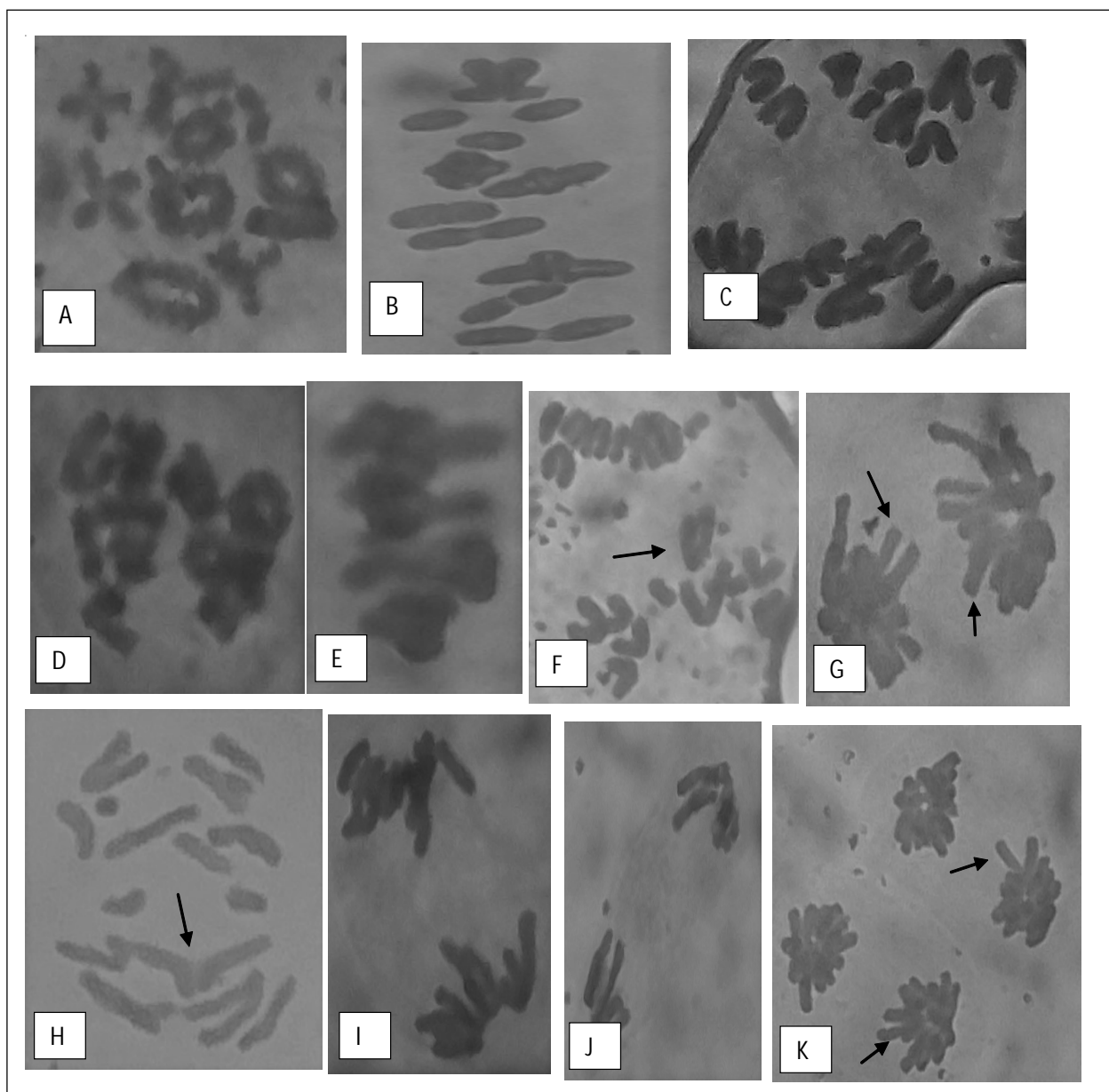


Figure 1: Meiotic chromosome abnormalities induced in *Z. variegatus* treated with aqueous leaf extracts of watermelon (*C. lanatus*): (A) = Normal Diplotene; (B) = Normal Metaphase - 1 (side view); (C) = Normal Anaphase - 1; (D) = Sticky Diplotene (Prophase-1); (E) = Sticky Metaphase - 1; (F) = Anaphase - 1 (Arrow indicating laggard); (G) = Early Telophase - 1 (Arrow indicating laggards); (H) = Anaphase - 2 (Arrow indicating bridge); (I) = Sticky Anaphase - 2; (J) = Anaphase - 2 (Chromosome loss); (K) = Telophase - 2 (Arrows indicating laggards)

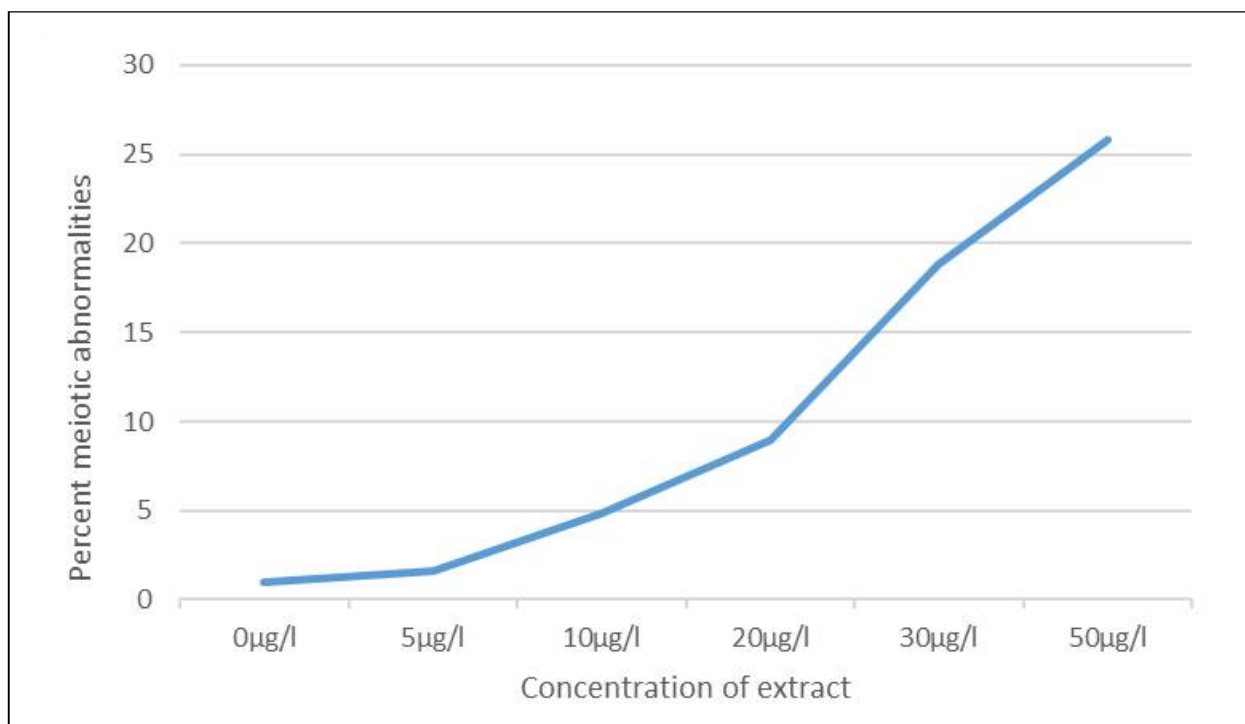


Figure 2: Relationship between different concentrations of aqueous leaf extract of watermelon (*C. lanatus*) and meiotic chromosome abnormalities induced in *Z. variegatus* L

4. Conclusion

This study revealed that aqueous extract of the leaves of *C. lanatus*, affected meiotic activities in *Z. variegatus*. Cytogenotoxic abnormalities induced in *Z. variegatus* by treatment with various concentrations of aqueous extract of the leaves of *C. lanatus* included; bridges, laggards, vagrant chromosomes, sticky chromosomes and spindle disturbances. Though increasing the concentrations of extract brought about significant increases in cytogenotoxic abnormalities, the maximum of 25.83% obtained for treatment with 50 µg/ml of extract was relatively very low, this however indicated that *C. lanatus* possess some cytogenotoxic potentials upon *Z. variegatus* cells. Therefore on the basis of these results, it is suggested that watermelon, *C. lanatus*, can be consumed by humans with caution.

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