Original ResearchArticle

Cytogenetic Toxicity and Infertile Properties of the Aqueous Extract of *Cucumis melo* Seeds (Cucurbitaceae) in Male *Zonocerus variegatus*Cell Model (Orthoptera: Pyrgomorphidae)

Abstract.

Background: The Muskmelon, a variety of *Cucumis melo* L, is popular in Cameroon for its high nutritional and medicinal values. However, the cytogenotoxicand infertile properties of this species is yet to be evaluated. During this study, phytochemical analysis by colorimetric dosage of dry extracts of *C. melo* was carried out and the cytogenetic toxicity properties evaluated. **Results**: Qualitative analysis revealed the seeds of the species to contain alkaloids, phenols, flavonoids, triterpenoids, tannins, saponins, anthocyanins and sterols while quantitative analysis on the other hand indicated 46 ± 0.71 Gallic Acid Equivalent, 29.44 ± 0.87 Tannic Acid Equivalent and 5.87 ± 0.67 Quercetin Equivalent respectively for total phenols, for total tannins and for total flavonoid. The treatment of male individuals of *Zonocerus variegatus* with various concentration of the aqueous extracts of the seeds of *C. melo* revealed that increasing concentrations (0, 5, 10, 20, 30 and 40 µg/ml) of the aqueous extract resulted in significant and proportional regression of the meiotic index and chiasma frequency. Chromosomal abnormalities were also induced in Anaphase-1.**Conclusions**: These results indicate that the aqueous extract of *C. melo* seeds can be used to selectively eliminate orthopteran pests.It could be used in the fight against gonadal cancer, as the extract has been shown to be more effective in reducing the pool of dividing gonadal cells. Consumers of *C. melo* seeds are advised to use this plant with caution, as it can alter spermatogenesis and other processes.

KeywordsPhytochemistry, *Cucumis melo* seed, meiotic index, infertility, *Zonocerus variegatus*model

1. Introduction

The therapeutic use of medicinal plants is very common, especially in developing countries [1]. Many medicinal plant extracts and their active principles have been described and used as therapeutic molecules [2, 3]. However, there is considerable interest in the determination of health risks that these molecules may produce. Indeed, many of these plants contain phytocompounds known for their cytogenotoxic characteristics [4, 5, 6]. Therefore, an evaluation of the anti-meiotic potential is necessary to ensure a relatively safe use of these medicinal plants [7]. One of such medicinal plant with important nutritional and therapeutic values is the Melon (*Cucumis melo* L.). *C. melo* L. is an angiosperm of the order Cucurbitales, family Cucurbitaceae, genus Cucumis. It is an annual, herbaceous plant grown in all warm regions of the world [1]. Each part of this plant is used either as food or as medicines against various diseases in humans. Secondary metabolites including alkaloids, flavonoids, phytosterols, glycosides, saponins and terpenes have been isolated

in this plant [2, 8]. Moreover, extracts of *C. melo*have shown antioxidant [9, 10], anti-ulcer [11], anticancer [12, 13], antidiabetic [14], antimicrobials and anthelmintics [15, 16] properties. Ethnomedicine also reports that the seeds and their oils can be used against dysuria and gout or as purgatives and dewormers[1, 11, 17]. The seeds are consumed mainly in the form of pistachio dish. In view of its chemical composition above all in terpenoids and cucurbitacins, the aqueous extracts of the seeds of Cantaloupensis variety of *C. melo*could interfere with cell division and therefore spermatogenesis in the division model observed in *Z. variegatus*. Indeed, the cytogenetic effects of spermatogenesis are in fact characterized by alterations in the process of meiosis resulting in the appearance of chromosomal abnormalities (structure and number) likely to hinder or delay the transmission of information, genetics and reproduction of the species [18, 7]. Hence, an evaluation of the cytogenotoxic potential is necessary to ensure a relatively safe use of this medicinal plant [19, 20]. It is known that cytogenotoxic bioassays on plants or certain animals have a good correlation with mammalian cells [21, 22]. Thus, the aqueous extract of the seeds of *C. melo* was administered to *Zonocerus variegatus* assay in order to access cytogenetic properties during prophase-1 and anaphase-1 of meiosis-1, since it is representative of the whole process [23, 24, 21]. Various concentrations of *C. melo*seed extracts were studied to verify whether they presented a cytogenotoxic risk. Pest *Z. variegatus* L. (2n=19), family Pyrgomorphidaewas found interesting to study cytogenotoxic effects due to their large and visible chromosomes which is easy to stain and available at all seasons of the year [23, 24]. *C. melo* seeds are shown to possess important medicinal and economic properties but also devastating effects on reproductive health are reported. The seeds of *C. melo* are of an economic importance. And no study has been carried out for its growing consumption notwithstanding. The general objective of the study is to highlight the cytotoxic and genotoxic effects of the seeds of this plant. It will consist firstly on the qualitative and quantitative analysis and secondly on the demonstration of the infertile properties of the aqueous extract of this plant through chromosomal damage during anaphase-1 of meiosis. This could be beneficial for drug therapists as well as producers, sellers and consumers.

2. Methods

2.1. Collection of plant seeds and preparation of extract

The ripe melon fruit samples were harvested in May 2022 from a field in the Penka Michel Health District in the Menoua Division, West Region of Cameroon. This site is located at latitude 5°30’2’’N and longitude 10°14’39’’E (1.463 m above sea level). After the harvest (start 6:13 am and stop 7: am), the samples of the fresh ripe fruits were sent to the National Herbarium of Cameroon, where the plant was authenticated as *Cucumis melo* L. of the Cantaloupensis Americano variety by comparison with the material from Letouzey R n° 7220 of the specimen of Herbarium collection Number 8073/SRF/Cam authenticated by Dr. NGANSOP Eric. The harvested fruits were then sanitized and the extracted seeds were dried at room temperature and then reduced to powder using an electric grinder. The powder obtained was used for the preparation of the aqueous extracts according to the method of [20]. Four hundred (400) grams of powder from the seeds of *C. melo* was weighed and introduced into a container of 2 L of distilled water then the mixture was allowed to stand for 36 hours at room temperature. The mixture was stirred once a day using a spatula for better extraction of the active principles of the plant. The macerate was first filtered using a sieve with a mesh diameter of 150 µm, then successively with cotton and Wattman No. 1 type filter paper placed in a glass funnel resting on a one-liter graduated beaker. After 2 hours, the filtrate obtained was divided into 5 stainless steel dishes and placed in an oven at 40°C for 48 hours to allow the water to evaporate. Subsequently, the dry extract powder was obtained. According to the protocol of [25], five (05) graduate-concentrations were prepared (5 µg/ml, 10 µg/ml, 20 µg/ml, 30 µg/ml, and 40 µg/ml) by successive dilution.

2.2.Qualitative analysis of the aqueous extract of *C. melo* seeds

The determination of the classes of compounds present in the seeds of *C. melo* was carried out according to the standard methods described by [26].

2.2.1. Flavonoid examination (Shinoda test)

The flavonoid test was done by Shinoda test: 0.01 g of extract was dissolved in 3 ml of methanol. The mixture was treated with 0.05 g of magnesium shavings and 3 drops of concentrated HCl. The flavonoids were identified by the presence of the following colorations:

orange for flavones; red for xanthones and pink for flavonols. For chalcones and aurones, 0.1 g of extract was mixed with 3 ml of concentrated sulfuric acid and then stirred for 5 minutes. The presence of the chalcones was characterized by the red color and the aurones by the blue color.

2.2.2. Analysis of alkaloids (Meyer's test)

Meyer's test was used for the detection of alkaloids. To do this, 0.01 g of extract was placed in a test tube in the presence of 3 ml of an aqueous solution of hydrochloric acid (50% V/V). The mixture was treated with 3 drops of "Meyer's reagent". The formation of a white or yellowish precipitate indicated the presence of alkaloids.

2.2.3. Analysis of saponins

For the detection of saponins, 0.01 g of extract was dissolved in 5 ml of distilled water, then boiled for 5 minutes. After cooling, the contents of each 15x160 mm test tube were shaken vigorously for 30 seconds, then allowed to stand. The appearance of a persistent foam with a height of more than one cm characterizes the presence of saponins.

2.2.4. Analysis of triterpenes and steroids (Liebermann-Burchard test)

The Liebermann-Burchard test was used for the detection of steroids and triterpenes. To do this, 0.01 g of extract was dissolved in 3 ml of chloroform, then 3 ml of acetic anhydride was added and the mixture was cooled in ice for 3 minutes. Finally, a drop of concentrated sulfuric acid was added. The presence of triterpenes was confirmed by the appearance of a purplish red color and that of steroids by the successive appearance of blue, green, red or orange colors.

2.2.5. Analysis of anthraquinones

For the detection of anthraquinones, 0.01 g of extract was dissolved in a mixture of 4 ml of ether-chloroform (1:1 v/v). The solution thus obtained was treated with 4 ml of 10% (W/V) sodium hydroxide. Quinones were identified by the presence of a red coloration.

2.2.6. Analysis of phenols

For the detection of phenols, 0.01 g of extract was dissolved in 3 ml of ethanol, then the mixture received 3 drops of 10% (V/V) iron III chloride. The appearance of the blue-purple or greenish color indicated the presence of phenols.

2.2.7. Analysis of polyphenols

For the detection of polyphenols, 0.01 g of extract was heated in the presence of 5 ml of ethanol, then 3 drops of ferric cyanide were added to this solution. The presence of polyphenols was marked by the appearance of the blue-green color.

2.2.8. Analysis of tannins

For the determination of tannins, 0.01 g of extract was boiled for 5 minutes in a tube containing 5 ml of water. The mixture after cooling received 5 ml of 2% NaCl (W/V) and 5 ml of 1% gelatin (W/V). The appearance of a precipitate confirmed the presence of tannins.

2.2.9. Analysis of anthocyanins

For the determination of anthraquinones, 0.01 g of extract was boiled in the presence of 5 ml of an aqueous solution of HCl (1% V/V). The color change has been noted. The presence of anthocyanins was marked by the orange color.

2.3. Quantitative analysis of the aqueous extract of *C. melo* seeds

2.3.1. Determination of total phenols in the aqueous extract of *C. melo* seeds

The total phenol content was determined by the method described by [27]. The reagent consists of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). It is reduced, during the oxidation of phenols, to a mixture of blue oxides of tungsten and molybdenum. These blue pigments have a maximum absorption which varies according to the qualitative and/or quantitative composition of the phenolic mixtures in addition to the pH of the solutions, generally obtained by adding sodium carbonate. The reaction mixture in this test consisted of 20 µL of extract (2 mg/mL), 100 µL of Folin-Ciocalteu's reagent (diluted 10 times in water) and 80 µL of a solution of sodium carbonate at 20%. The mixture was stirred and incubated in a water bath at 20°C for 30 minutes, then the absorbance was measured on a spectrophotometer at 765 nm. The extract was replaced with distilled water in the white tubes. A standard curve was drawn using gallic acid (gallic acid concentration ranged from 0.015 to 2 mg/mL). The results were expressed in milligram equivalent of gallic acid per gram of extract. (**$y = 1963.4x$; $R^2 = 0.9926$**).

2.3.2. Determination of total flavonoids from the aqueous extract of *C. melo* seeds

The total flavonoid content of the extracts was determined using the aluminum chloride colorimetric method [28]. A volume of 100 µL of extract (2 mg/mL) was mixed with 50 µL of aluminum chloride (1.2%), then 50 µL of potassium acetate (120 mM) was added. The mixture was incubated for 30 minutes at room temperature and the absorbance was measured on a spectrophotometer at 415 nm. Total flavonoid content was calculated using the quercetin standard curve (quercetin concentration ranged from 0.015 to 2 mg/mL) and results were expressed as milligram equivalent of quercetin per gram of extract **($y = 300.75x$; $R^2 = 0.0897$).**

2.3.3. Quantification of total tannins in the aqueous extract of *C. melo* seeds

The tannin content was determined by the Folin-Ciocalteu method as described by [29]. Briefly, the reaction mixture in this test consisted of 100 µL of extracts (2 mg/mL), 500 µL of Folin-Ciocalteu's reagent (diluted 10 times in water), 1000 µL of a carbonate solution of 35% sodium and 8.4 mL of distilled water. The mixture was stirred and incubated at room temperature for 30 min, then the absorbance was measured on a spectrophotometer at 700 nm. The extracts were replaced with distilled water in the white tubes. A standard curve was drawn using tannic acid (tannic acid concentration ranged from 100, 200, 300, 400, 500 µg/mL). The results were expressed in milligram equivalent of tannic acid per gram of extract (**$y = 0.0005x + 0.017$; $R^2 = 0.9873$**).

2.4. Repartition and treatments of grasshoppers Model

2.4.1. Justification of processingparameters

First of all, as the 50 µg/ml concentration was lethal for more than 50% (LD50= 17.62 µg/mg) of the locusts tested during the screening, the concentrations (40, 30, 20, 10 and 5 µg/ml) were retained for this study. Similarly, the dose (0.05g / kg), for example 50µg/mg of body weight was administered to grasshoppers. This dose is obtained after extrapolating from previous work in the research team [15]. Then, the work carried out by [15, 23] led to the adoption of the intraperitoneal route of administration in *Z. variegatus* since the extract is placed directly in the hemocoel. Finally, a meiotic cycle corresponds to 24 hours, that is to say one generation of spermatozoa [23]. Studying three successive generations (F0, F1 and F3) of spermatozoa requires 96 hours knowing that an additional 24 hours is necessary for the extract to be metabolized by the cells of the grasshopper. Eighty (80) adult male locusts were divided into six groups of five (05) individuals each. The six

groups are then labeled G0, G1, G2, G3, G4, and G5 and given respective increasing concentrations of 0 µ (distilled water), 5 µg/ml, 10 µg/ml, 20 µg/ml, 30 µg/ ml, and 40 µg/ml of plant extract. The 5 individuals of group G0 were used as controls. The grasshoppers were killed in the death chamber containing the chloroform smoke. Dissection was performed in insect saline solution (68% sodium chloride). The follicles obtained were placed in labeled vials containing Carnoy's solution (ethanol 3:1: acetic acid fixative) and stored in the refrigerator at 40°C to prepare chromosome smears. The capture and killing of the grasshoppers were carried out in accordance with the ethical prescriptions of the Department of Animal Biology of the University of Dschang relating to invertebrates.

2.5. Meaning and importance of cytogenetic parameters

2.5.1. Preparation and examinations of smears

Meiotic chromosome smears were prepared by the method of [23] and examined using a FISHER binocular microscope with 40X and 100X objectives using Cedar wood immersion oil. Counting and examination of normal and/or abnormal parameters were performed on 250 follicles as described by [15], taking into account prophase-1 and anaphase-1 in meiotic division.

2.5.2. Meiosis one and meiotic index

Meiosis one is studied in this work because all the major and relevant events such as genetic mixings take place there at this time. Intrachromosomic shuffling or crossing-over, for example, takes place in prophase one of meiosis one [30, 31]. Interchromosmic shuffling also takes place during meiosis one, precisely in metaphase and anaphase one [32]. The meiotic index (MI) expresses the percentage of germ cells in meiotic division out of the total number of cells observed. This index was calculated according to the formula of [30] as follows: MI (%) = Number of cells in (P-1, M-1, A-1 or T-1)/Total number of cells examined×100. Where P-1=Prophase-1; M-1=Metaphase-1; A-1=Anaphase-1; T-1=Telophase-1. The meiotic index is a relevant parameter of meiosis, it is used to measure the cytotoxicity of the extract, since it has been demonstrated that a meiotic index lower than 12.25±0.75% is a sign of cytotoxic inhibition whereas chromosomal abnormalities reflect genotoxicity [34, 31]. The percentage of abnormalities in anaphase-1 (AI) is calculated according to the formula of [21] as follows: AI (%) = (AS, AR, MA, IA or AB)/Total number of cells analyzed in anaphase-1×100, withAS=Anaphase with stars; AR=Anaphase with stem chromosomes; MA=Multipolar Anaphase; IA=Anaphase with reversed poles; AB=Anaphase with chromosomal breaks. Percentage of abnormalities in anaphase will serve as an indicator of inhibition of intrachromosomal shuffling, while that recorded in prophase provides information on errors in intra-chromosomal shuffling [35].

2.5.3. Zonocerus *variegatus*Model

The *Z. variegatus* pest was used because it satisfies the necessary conditions for an effective biological model. Chromosomes are acrocentric, few (2n=19), and large (16 µm). The process of meiosis is normal and chiasmatized[33]. The staining of the chromosomes is solid with lactopropionic orcein, this allows easy detection of gaps and morphological and structural deviations of the chromosomes. According to [23, 24, 32] no other insect provides such convenient and complete collections of stages or phases of meiotic division; most complex cell division. An extrapolation of the results to mammalian cells is possible, since the locust is also equipped with enzymes involved in the biotoxification and biodetoxification of xenobiotics comparable to those of liver cells [30, 32]. Moreover, locusts are available during all seasons [15]. Photographs of interesting chromosomes present at prophase-1 and anaphase-1 were made using a Techno, Camon 16 phone mounted with a 48M AIQUAD Camera.

2.5.4. Statistical analysis of the data

The Python 3.1 statistical software Pandas package was used for this analysis. The mean value of each group of ten slides prepared per concentration of the tested extract and per grasshopper

wassubjected to the one-way ANOVA test followed by the Tukey post hoc test (HSD) at the level of significance of $p<0.5$ [31].

3. Results

3.1. Qualitative analysis of *C. melo* seeds

Qualitative phytochemistry reveals that this extract contains six (06) classes of the following compounds: alkaloids, phenols, flavonoids, sterol, triterpenoids, tannins, saponins and anthocyanins (Table 1). Quantitative phytochemical analysis of this extract shows that it contains a high proportion of total phenols followed by total tannins and finally a low rate of flavonoids (Table 2).

Table 1: Qualitative analysis of phytochemical compounds of aqueous extract of *C. melo*

Phytochemicalcompounds	Seeds
Alkaloids	+
Phenols	+
Flavonoids	+
Sterols	+
Triterpenoids	+
Tannins	+
Saponins	+
Anthocyanins	+
Anthraquinones	−

Where + = Detectable; − = Not Detectable.

3.1.1. Quantitative analysis of *C. melo* seeds

Table 2: summarizes the level of secondary metabolites from the quantitative analysis of the aqueous extract of *C. melo* seeds.

Table 2: Quantificative rate of total phenols, flavonoids and tannins in the aqueous extract seeds of *C. melo*

Rate of secondarymetabolites	Rate of secondarymetabolites		
	Total Phenols (mgEAG/g extract)	Total Flavonoids (mgEQ/g extract)	Total Tannins (mgEAT/g extract)
Seeds	46 ± 0.71	5.87 ± 0.67	29.44 ± 0.87
Butylhydroxytoluene	445.85 ± 0.14	67.49 ± 0.37	56.23 ± 0.61

Where EAG=Gallic Acid Equivalent; EQ= Quercetin Equivalent; EAT= Tannic Acid Equivalent.

3.1.2. Cytogenetic analysis

In comparison with the control (11.70±1.74%), it appears from Table 3 that the aqueous extract of *C. melo* seeds significantly ($p<0.01$-0.001) decreased the average frequency of chiasmata from 10.17±1.43% to 07.84 ±2.14% at concentrations 10 and 40 µg/ml respectively. The Mean frequency of bivalents with one chiasma only (Fig.1-i) decreased with high concentrations (30 and 40 µg/ml). On the other hand, the Mean frequency of bivalents with two or more chiasmata (Fig.1-ii-iii) were profoundly affected even by concentrations below 30 µg/ml. The extract significantly reduced the meiotic index by more than 10.11±4.10% on average between the control (14.25±4.69%) and the 40 µg/ml treatment (4.75±2.17%) (Table 4). A significant increase ($p<0.05$-0.01) in the percentage of anaphase-1 abnormalities in *Z. variegatus* was recorded after exposure with the aqueous extract of

the seeds compared to the group with distilled water. However, two groups of anaphase-1 abnormalities stand out: majority abnormalities with 9.8±1.87% anaphase with stars [AS] (Fig.2-B); 6.86±0.16% of anaphases with chromosome breaks [AB] (Fig.2-F) and two less represented minority abnormalities where an average percentage of (2.83±0.21%) of anaphase with stem chromosomes [AR] was recorded (Fig.2-C) and finally a summed percentage of 0.84±0.22% of multipolar anaphase and anaphase with reversed poles [AM+IA] (Fig.2-D-E).

Table 3: Chiasma frequencies at prophase-1 of *Z. variegatus* treated with *C. melo*

Prophase-1 reproduction parameters			
Treatments (µg/ml)	**Mean chiasma frequency**	**Mean frequency of bivalents with one chiasma**	**Mean frequency of bivalents with two or more chiasmata**
Control (0 µg/ml)	11.70±1.74^{a}	60.44±.0.29^{a}	24.31±0.65^{a}
5	11.02±1.10^{a}	59.89±0.41^{a}	24.03±0.51^{a}
10	10.17±1.43^{b}	58.98±0.10^{a}	21.32±1.03^{b}
20	09.75±1.89^{c}	58.60±1.02^{a}	18.09±1.50^{c}
30	08.43±2.01^{d}	57.53±1.00^{b}	16.61±1.87^{d}
40	07.84±2.14^{e}	51.02±1.28^{c}	12.11±2.13^{e}

Number of trials n=5. Groups that have no letters in common in column differ significantly from the control group (Distilled water), **p<0.01, α= 0.05.**

Table 4: Anaphase one abnormalities percentages of *Z. variegatus* treated with *C. melo*

Meiotic germ lines cells activity		**Anaphase-1 abnormalities** (250 cells per individual)				
Treatments (µg/ml)	TDC	MI (%)	AS (%)	AR (%)	MA+IA (%)	AB (%)
Control (0 µg/ml)	57	14.25±4.69^{a}	0^{a}	0^{a}	0^{a}	0^{a}
5	51	12.75±3.94^{b}	1.61 ± 0.13^{b}	1.44±0.14^{b}	0.31± 0.9^{b}	2.38±0.13^{b}
10	47	11.75±3.8^{c}	2.55 ±0.19^{c}	1.89±0.18^{c}	0.38± 0.3^{b}	3.52±0.12^{c}
20	44	11.00±4.08^{d}	4.25 ±0.39^{d}	2.97±0.14^{d}	0.58±0.22^{c}	4.65±0.14^{d}
30	32	8.00±3.39^{e}	5.25 ±1.14^{e}	2.69±0.22^{e}	0.7± 0.2^{de}	6.76±0.13^{e}
40	19	4.75±2.17^{f}	9.8 ±1.87^{f}	2.83±0.21^{f}	0.84±0.22^{f}	6.86±0.16^{f}

Number of trials n=5, Groups that have no letters in common in column differ significantly from the control group (Distilled water), **p<0.001, α= 0.05.** TDC- Total Dividing Cells, MI- Meiotic Index, AS-Anaphase with Stars; AR-Anaphase with stem chromosomes; MA- Multipolar Anaphase; IA-Anaphase with reversed poles; AB-Anaphase with chromosomal breaks.

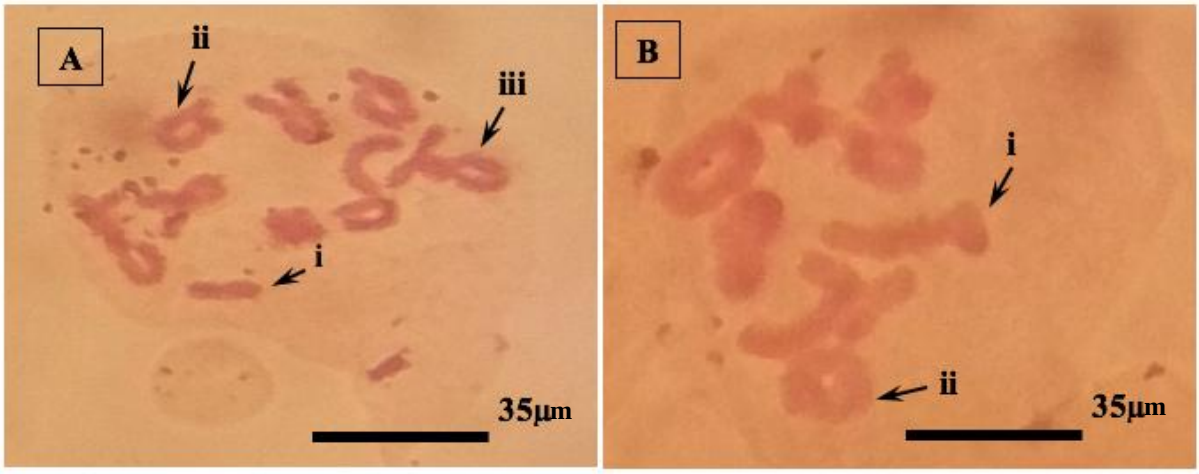

Fig.1: Composite diplotene figures in *Z. Variegatus* treated with tap water and seed extract of *C. melo*. **(A: Composite shape bivalents figures in *Z. Variegatus* treated with distilled water. B: Composite shape bivalents figures in *Z. Variegatus* treated with seeds extract of *C. melo*.) i= Rod shape bivalents (1-chiasma); ii= Ring shape bivalents (2-chiasmata); iii= double ring shape bivalents (3-chiasmata).**

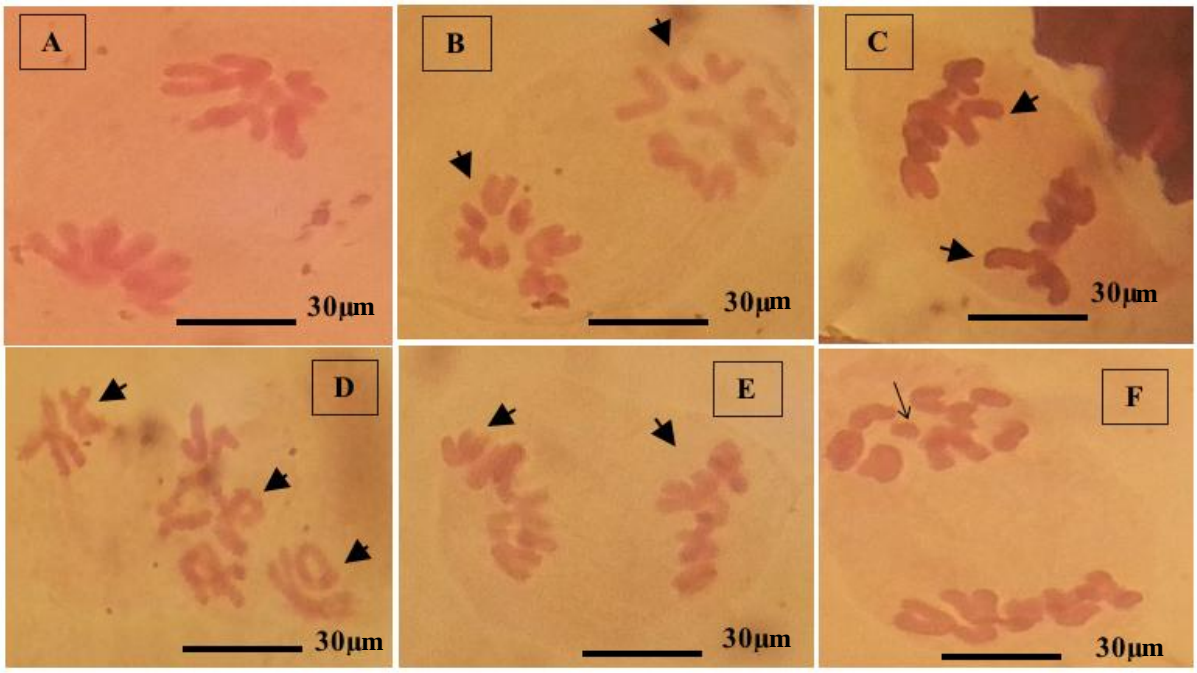

Fig.2: Types of Anaphase-1 abnormalities in treated and untreated grasshoppers

A: Normal anaphase in *Z. variegatus* treated with distilled water; B: Anaphase with stars; C: Anaphase with stem chromosomes; D: Multipolar anaphase; E: Anaphase with reversed poles; F: Anaphase with chromosomal breaks.

4. Discussion

The normal and harmonious course of the meiotic process, in particular a good segregation of chromosomes, is directly reflected in the fertility of sexually reproducing organisms. A typical meiosis can alter chromosomal mechanisms, lack of pairing of homologous chromosomes, lack of recombination, unbalanced chromosomal segregation, and a drastic drop in chiasma frequency can affect gametic viability, leading to reproductive problems [32]. Chiasmas and their frequencies are very essential for the fixation of homologous chromosomes in diplotene-paired bivalents of

prophase-1 and therefore the subsequent segregation of these homologs at the cell poles during anaphase-1 of meiosis. Similarly, the meiotic index is a reliable indicator of the progress of spermatogenesis according to [33]. According to [30], the meiotic index and the chiasmatic frequency are two parameters determining the success of reproduction in a population. Therefore, these two indices become essential for the production of normal and genetically balanced sperm. Authors have demonstrated that in panmictic reproduction in the natural habitat, reduced chiasmal frequencies indicate a population that is less stable and therefore less able to adapt to sudden ecological changes in its habitat, unlike a population with high chiasmata frequencies [34, 35]. The sperm produced by each individual belonging to a population with high chiasma frequencies means that the genes they contain have also achieved a high rate of allelic mixing by crossing over to adaptive phenotypes. On the other hand, sperm from a population with low chiasma frequencies have potentially less reproductive success. In the current study the extract significantly reduced the average frequency of chiasmata so a majority of gametes are likely to be infertile. There was a regression and preferential disappearance of the frequency of bivalents in double rings responsible for more than two chiasmas (Fig.1-B-iii). This disappearance has a consequence on the separation of homologs in anaphase-1 of meiosis. These results supportthe report of [36] who recorded an increase in the average frequency of chiasmas in *Z. variegatus* treated with aqueous extracts of *Annona muricata* (soursop). In reality, once in the hemocoel, the extract is drained by the hemolymph to the testicular follicles and by facilitated diffusion, it is absorbed into the cytosol of the follicle cells of Z. Variegatus via the biological membrane [37]. The stored extract is in the cytosol, and after their metabolisms by the cells, the metabolites formed electrophilic compounds. Being very reactive, terpenoids, especially cucurbitacins, become alkylating and therefore likely to form new methyl groups which are at the origin of the covalent bonds between the two strands of DNA called adducts [19, 32]. The position, time and number of adducts formed on the DNA molecule are the cause of multifaceted chromosomal abnormalities [38]. The inhibitory effect of the extract can also be attributed to the diversity of anaphase-1 abnormalities such as [AS]; the [AB]; the [AR] and finally the minority [AM+IA]. [AS] could result from the persistence of bridges during meiotic inhibition with the inability of chromosomes to move towards one of the poles. [37] showed that potassium sulfite and potassium nitrate used as food preservatives caused aberrant outcrossing and then anaphases in *Allium cepa*. Accumulation of stellate anaphases evolves into multipolar anaphase or pole-reversed anaphase [AM+IA] in the absence of DNA self-repair [5, 6]. Anaphases with chromosomal breaks [AB] and anaphases with stem chromosomes [AR], on the other hand, are persistent anomalies. Thus, the induction of chromosomal breaks by *C. melo* extract may be independent of its effect on the amount of DNA. Therefore, further studies involving the effect of aqueous extract of *C. melo* on DNA and RNA are needed. According to [12], any disturbance in spindle formation can lead to uneven distribution of chromosomes and therefore to chromosomal breaks. These abnormalities reflect highly genotoxic effects, generally of the irreversible type, which can probably lead to cell death and therefore to infertility. These results are consistent with the results of many research groups that have examined the effects of different chemicals on different materials. [38] demonstrated that *Ocimumgratissimum* (Lamiaceae), a food condiment consumed in Africa and Asia, is spermatotoxic, cytotoxic and genotoxic because the aqueous extract of this plant induces chromosomal aberrations as well as abnormal spermatozoa in albino mice. Abnormal sperm had either an aneuploid or a polyploid due to poor homolog separation at anaphase-1. In the same context, the authors [39, 22, 40, 41, 42] proved the infertile effect of the cytogenotoxicity of two aqueous extracts.

5. Conclusions

This first cytogenotoxicity study of *C. melo* revealed that the aqueous extract of *C. melo* seeds is anti-meiotic at the concentrations tested. The anti-meiotic effects are illustrated by the inhibition of meiosis with drastic decrease in the frequency of chiasmas as well as the marked emergence of various anaphase-1 abnormalities in the forming spermatozoa, thus highly pro-infertile. The extract

can be used to target orthopterans (Pyrgomorphidae), which are significant crop pests in sub-Saharan Africa in general and Cameroon in particular. It may be effective in combating gonadal cancer, as the extract has been shown to reduce the meiotic index (the extract being highly concentrated in total phenols) [35]. Researchers should use the *Z. variegatus* assay for rapid cytogenetic studies. Consumers of *C. melo* seeds should use this plant with caution, as it can alter spermatogenesis, among other things.

List of abbreviations

Not used

REFERENCES

[1] P. Milind and S.Kulwant, "Musk melon is eat-must melon," *International Research Journal of Pharmacy*, vol. 2, n°18, pp. 52-57, 2011.

[2] S. Mallek-Ayadi, N. Bahloul et N. Kechaou, " Chemical composition and bioactive compounds of Cucumis melo L. seeds: Potential source for new trends of plant oils," *Process Safety and Environmental Protection*, vol. 113, pp. 68-77, 2018.

[3] V. Vishwakarma, J. K. Gupta and P. K. Upadhyay, "Pharmacological importance of Cucumis melo L. An overview," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 10, n°13, pp. 11-21, 2017.

[4] O. Timothy, M. Idu, D. I. Olorunfemi and O. Ovuakporie-Uvo, "Cytotoxic and genotoxic properties of leaf extract of Icacina trichantha Oliv.," *South African Journal of Botany*, vol. 1, n°19, p. 71– 74, 2014.

[5] A. B. Akinboro and A. A. Bakare, " Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on Allium cepa," *Journal of Ethnopharmacology*, vol. 112, pp. 470-475, 2007.

[6] I. Oyeyemi and A. Bakare, " Genotoxic and antigenotoxic effect of aqueous extract of Spondias mombin L., Nymphia lutos L. and Luffa cylindrica L. On Allium cepa root tip cells," *Caryologia*, vol. 66, pp. 360- 367, 2013.

[7] M. Shraddha, S. Hatangadi, S. More, B. Ghag, N. Bhatia et M. Indap, "Anti-mitotic and Cytotoxic Potential of Methanolic Extract from Lingula anatina," *International Journal of Science and Research*, vol. 2020, pp. 78-89, 2020.

[8] N. Başaran, D. Paslı and A. A. Başaran, " Unpredictable adverse effects of herbal products," *Food and Chemical Toxicology*, vol. 159, pp. 112-762, 2022.

[9] F. Yasar, K. Sebnem and E. Sebnem, " Determination of anti-oxidant activities in some melon (Cucumis melo L.) varieties and cultivars under salt stress," *The Journal of Horticultural Science and Biotechnology*, vol. 4, n°184, pp. 627-630, 2006.

[10] C. I. Ismail, K. C. Chan, A. A. Mariod and M. Ismail, " Phenolic content and antioxidant activity of cantaloupe (Cucumis melo L.) methanolic extracts," *Food Chemestry*, vol. 11, n°19, pp. 643-647, 2010.

[11] N. S. Gill, J. Bajwa, P. Sharma, S. Sood, P. Sharma and M. Bali, " Evaluation of therapeutic potential of traditionally consumed Cucumis melo seeds," *Asian Journal of Plant Sciences*, vol. 10, n°11, pp. 86-91, 2011.

[12] X. Chen, J. G. Bao, Q. Ding, J. Lu, M. Huang and Y. Wang, " Biological activities and potential molecular targets of cucurbitacins: a focus on cancer," *Anti-cancer drugs*, vol. 23, n°18, pp. 777-787, 2012.

[13] R. Agata and O. Beata, " Vegetables from the Cucurbitaceae family and their products: Positive effect on human health, " *Nutrition*, vol. 17, p. 110788, 2020.

[14] L. Chen et H. Y. Kang, "Anti-inflammatory and antioxidant activities of red pepper (Capsicum annuum L.) stalk extracts: Comparison of pericarp and placenta extracts," *Journal of Functional Foods*, pp. 1724-1731, 2013.

[15] T. Dongmo, R. Seino and M. Yacouba, " Insecticidal Activity of Ageratum conyzoides (Asteraceae) Aqueous Extracts against the Grasshopper Zonocerus variegatus (Orthoptera: Pyrgomorphidae)," *Journal of Agriculture and Ecology Research International*, vol. 21, n°18, pp. 29-36, 2020.

[16] A. S. Shubha, S. M. Devaraju, V. Srinivasa, C. Kantharaj, C. S. Ravi, A. Akshay and A. Shanwaz, " Medicinal and nutritional importance of bitter melon (Momordica charantia L): A review article," *Journal of Pharmacognosy and Phytochemistry*, vol. 4, pp. 297-300, 2018.

[17] S. Kale, B. Matthäus, F. Aljuhaimi, I. Ö. M. M. Ahmed, K. Ghafoor and H. A. Alqah, "A comparative study of the properties of 10 variety melon seeds and seed oils, " *Journal of Food Processing and Preservation*, vol. 4, n°12, pp. 314-463, 2020.

[18] Y. He, J. Li, L. Peng, Q. Li, Y. Chu, Q. Lin and S. Ju, " Effects of Ran-GTP/importin β inhibition on the meiotic division of porcine oocytes," *Histochemistry and Cell Biology*, pp. 1-12, 2022.

[19] X. Wang, M. Tanaka, H. S. Peixoto and M. Wink, " Cucurbitacins: elucidation of their interactions with the cytoskeleton," *Peer J*, vol. 5, pp. 33-57, 2017.

[20] V. A. Houmènou, " Phytochemical and cytotoxicity study of some plants used in the treatment of female sterility in southern Benin," *European Scientific Journal*, vol. 14, n°16, pp. 156-171, 2018.

[21] J. Rank, " The method of Allium anaphase-telophase chromosome aberration assay, " *Ekologija*, vol. 3, pp. 38-42, 2003.

[22] H. Sven, E. Lennard, J. Jörgen and G. Fiskesjö, " Analogy Models for Prediction of Human Toxicity," *Alternatives to Laboratory Animals*, vol. 18, n°11, pp. 16-23, 1990.

[23] J. O. Faluyi, " Possible models for autocidal control based on the meiotic dynamics of *Zonocerus variegatus* L.(Orthoptera: Pyrgomorphidae)," *International Journal of Tropical Insect Science*, vol. 9, n°12, pp. 205-209, 1988.

[24] D. L. Pittman, J. Cobb, J. Kerry, A. Lawriston, M. Deborah, E. Cooper and A. J. S. C. Mary, " Meiotic Prophase Arrest with Failure of Chromosome Synapsis in Mice Deficient for Dmc1, a Germline-Specific RecA Homolog," *Heredity*, vol. 3, pp. 78-89, 2019.

[25] A. Seino R, I. Dongmo Tonleu and D. Shambo, " First description of chromosomes in *Pyrgomorpha vignaudii* (Orthoptera: Pyrgomorphidae) collected from Cameroon," *Journal of Zoological and Bioscience*, vol. 2, n°14, pp. 13-16, 2013a.

[26] G. O. Williams and B. Ogunbiyi, "Chromosome morphology and meiosis in *Zonocerus variegatus* L. (Orthoptera, Pyrgomorphidae)," *Cytologia*, vol. 60, pp. 111-116, 1995.

[27] J. K. Grover and S. P. Yadav, " Pharmacological actions and potential uses of *Momordica charantia*: a review," *Journal of Ethnopharmacology*, vol. 93, n°13, pp. 123-132, 2004.

[28] J. B. Harborne, Phenolic compounds, Dordrecht: Springer, 1973, pp. 33-88.

[29] Ramde-Tiendrebeogo, A., Tibiri, A., Hilou, A., Lompo, M., Millogo-Kone, H., Nacoulma, O. G., and Guissou, I. P." Antioxidative and antibacterial activities of phenolic compounds from Ficus sue Forssk," *International Journal of Biological and Chemical Sciences*, vol. 6, n°11, pp. 328-336, 2012.

[30] C.-C. Chang, M.-H. Yang, H.-M. Wen and J.-C. Chern, " Estimation of total flavonoid content in propolis by two complementary colometric methods," *Journal of Food and Drug Analysis*, vol. 10, n°13, pp. 3-18, 2002.

[31] M. Govindappa, S. Naga-Sravya, M. Poojashri, T. Sadananda and C. Chandrappa, " Antimicrobial, Antioxidant and in virto Anti-inflammatory activity of Ethanol extract and Active Phytochemical screening of Wedelia trilobata (L.) Hitchc," *Journal of Pharmacognosy and Phytochemistry*, vol. 3, n°13, pp. 43-51, 2011.

[32] H. An, M. Hu, P. Li, G. Geng, Q. Zhang and S. Zhang, " Chromosomal behavior during meiosis in the progeny of *Triticum timopheevii* × hexaploid wild oat," *PLoS One*, vol. 10, n°15, pp. 126-138, 2015.

[33] W. GO and O. BI, " Chromosome morphology and meiosis in *Zonocerus variegatus* L.(Orthoptera, Pyrgomorphidae).," *Cytologia*, vol. 60, n°12, pp. 111-116, 1995.

[34] Y. Li, M. Zhang, S. Li, R. Lv, P. Chen, R. Liu, G. Liang and L. Yin, " The Use of the Nematode Caenorhabditis elegans to evaluate the Adverse Effects of Epoxiconazole Exposure on Spermatogenesis, " *International Journal of Environmental Research Public Health*, vol. 2016, n°1993, p. 13, 2016.

[35] L. Oliveira and M. Patrícia, " Meiotic index and palynology of *Eugenia involucrata* DC-Myrtaceae / Índice meiótico e palinologia de cerejeira-do-mato (Eugenia involucrata DC-Myrtaceae)," *R. Ci. agrovet.*, vol. 17, n°14, pp. 481-490, 2018.

[36] C. F. Silvana, E. Usandizaga, M. Martínez, A. I. H. and A. A. Carlos, " Mode of reproduction and meiotic chromosome behavior in Acroceras macrum, " *Reproduction*, 2020.

[37] K. Nongthombam et D. Sharma, " Data Analysis using Python, " *International Journal of Engineering Research and Technology*, vol. 3, pp. 6-18, 2021.

[38] P. Parra-Nunez, M. Pradillo and J. L. Santos, " Competition for chiasma formation between identical and homologous (but not identical) chromosomes in synthetic autotetraploids of Arabidopsis thaliana," *Frontiers in Plant Science*, vol. 9, p. 1924, 2019.

[39] L. Paweenuch, A. Kesara and C. Ploenpit, " Chromosome Evolution Based on Variation in Chromosome Number and Chiasma Frequency in the Genus Ocimum L. from Thailand," *Cytologia*, vol. 84, n°13, pp. 199-206, 2019.

[40] R. Seino, T. I. Dongmo, R. N. Chifon and D. Shambo, "Possible Cytogenetic Effect of *Capsicum frutescens* (Solanaceae) Extracts on Meiosis in the Grasshopper Taphronota thaelephora Stal. Orthoptera: Pyrgomorphidae," *Enliven*, vol. 1, n°11, p. 002, 2014.

[41] W. F. Grant, "Chromosome aberration assays in allium: A report of the U.S. environmental protection agency gene-tox program,"*Mutation Research/Reviews in Genetic Toxicology*, vol. 99, n°13, pp. 273-291, 1982.

[42] A. Akinboro and A. Bakare, " Spermatotoxic, cytotoxic and genotoxic evaluation of aqueous extract of Ocimum gratissimum in albino mice," *Open Access Journal of Medicinal and Aromatic Plants,* vol. 4, n°11, pp. 10-14, 2013.

[43] L. M. Granato, L. C. d. S. Ramos and C. A. F. Pinto-Maglio, " Relationship between meiotic instability and fertility in F2 generation Arabusta coffee plants," *Bragantia,* vol. 78, n°12, p. 215–228, 2019.

[44] Z. J. Sabrina, A. J., M. K. Cindy, K. M. Saskia, A. M. M. and R. Sjoerd, " Distinct prophase arrest mechanisms in human male meiosis," *Development,* vol. 145, n°116, p. 160614, 2018.

[45] F. C. Foong, A. Mohammad and S. J. Ichwan, " Biological properties of cucumber (*Cucumis sativus* L.) extracts," *Malaysian Journal of Analytical Sciences,* vol. 19, n°116, pp. 218-1222, 2015.

[46] M. Miroslava and M. D. Sava, "Low chiasma frequency as an aetiological factor in male infertility, " *Reproduction,* p. 15, 1982.

[47] S. Ibrahim, " New 2-(2-phenylethyl) chromone derivatives from the seeds of *Cucumis melo* L var. reticulatus," *Natural Product Commun,* vol. 5, n°13, 2010.

[48] S. Kale, B. Matthäus, F. Aljuhaimi, I. A. Ahmed, M. M. Özcan, K. Ghafoor and H. A. Alqah, "A comparative study of the properties of 10 variety melon seeds and seed oils," *Journal of Food Processing and Preservation,* vol. 4, pp. 314-463, 2020.

[49] R. I. Seino and T. Dongmo, " The effect of the dry and wet seasons on chiasma frequency in male Taphronota thaelephora Stal. 1873 and Zonocerus variegatus L. 1753 (Orthoptera: Pyrgomorphidae) in Cameroon," *International Journal of Biosciences,* vol. 3, n°14, pp. 1-7, 2013b.

[50] S. Wakid and H. A. Harun, "Antioxidant activity of melon fruit peel extracts," *Journal of Academia,* vol. 8, n°11, pp. 53-57, 2020.