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Anti-meiotic potential of aqueous extracts of Annona muricata (Annonaceae) and Cymbopogon citratus (Poaceae) as an alternative to chemical insecticides, tested on the Zonocerus variegatus (Pyrgomorphidae) model

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Abstract

Annona muricata and Cymbopogon citratus are two medicinal plants used in traditional medicine, cosmetics and the agri-food industry. Phytochemical analysis of their leaves harvested in West Cameroon reveals a high content of phenolic compounds, terpenoids and flavonoids. This study aimed to evaluate the potential cytogenotoxic effects of these leaves on chromosomal behaviour during meiosis-I in the pest grasshopper Zonocerus variegatus.

Aqueous extracts of A. muricata and C. citratus were prepared by maceration and aqueous infusion, respectively. Fifty grasshoppers acclimatized for 72 hours were divided into 6 groups (5 individuals per group and per plant). Concentrations of 5, 10, 25, 50 and 100 µg/ml were tested. Each specimen received 0.01 ml of treatment by a single intraperitoneal inoculation. After 96 h of incubation, the grasshoppers were sacrificed and then dissected in order to isolate the testicular follicles for the preparation of chromosome smears.

The meiotic index (MI) and the frequency of chromosomal abnormalities were analysed. The results indicate a significant ($p < 0.05$) and dose-dependent decrease in MI for all extracts compared with the control (14.19 ± 0.00%). The inhibitory effect proved more marked with C. citratus (4.11 ± 1.43%) than with A. muricata (7.82 ± 1.15%), i.e. respective reductions of 71% and 45%. Moreover, the extracts significantly increased ($p < 0.05$) the frequency of abnormalities, with C. citratus proving the most incriminated. The predominant alterations in metaphase-I and anaphase-I are agglutinations (4.11%) and chromosome bridges (2.58%).

These aqueous extracts show clear cytogenotoxic effects in Z. variegatus. Their proven bioinsecticidal properties constitute a promising alternative for controlling this major crop pest in Central, West and East Africa.

Keywords: Cytogenotoxic effects, meiotic index, crude aqueous extracts, meiotic chromosomes, Biological control.

Introduction

Plant diversity is indispensable to humanity, feeding the agri-food, cosmetic and medical sectors (Rakotomanana, 2013). As a permanent source of bioactive molecules, plants are used by more than 80% of the world's population, particularly in developing countries (UN, 2023; Paola et al., 2022). For example, *Petroselinum crispum* (1000 mg/kg) shows an anti-anaemic effect (Atto et al., 2023), while infusions of *Acacia albida* show activity against hepatitis B and C (Guinnin et al., 2015). However, the use of certain medicinal plants can cause deleterious effects (Seino et al., 2020; Ngnaniyyi et al., 2022). Thus, the aqueous extract of *Cola verticillata* (40 µg/ml) slows meiosis and increases the frequency of chromosomal abnormalities in *Zonocerus variegatus* (Tagne, 2024). These toxic properties find a strategic application in the protection of staple crops (maize, bean, cassava) against pests (Heumou et al., 2022; Seino et al., 2022; Ngnaniyyi, 2024). In East Africa and Central Africa respectively, the desert locust *Shistocerca gregaria* and the migratory locust *Locusta migratoria* are known to be the most destructive to crops (Dongmo *et al*., 2020; Wandji *et al*., 2021). To this list is added the grasshopper *Zonocerus variegatus* ranked third among pests after mealybugs and borers in Cameroon, showing significantly harmful effects on crops (Kekeunou *et al*., 2014; Ngnaniyyi *et al*., 2024). According to a FAO report (2022), fall armyworm caterpillars in the Far North Region of Cameroon caused a cereal deficit of 74560 tonnes in 2021, compared with only 15560 tonnes in 2020, leading producers to abandon pastures. Likewise, another recent study conducted in the Far North of Cameroon on wheat crops recorded nearly 5729 insects responsible for losses of 49.98% in rice yield (Ismael., 2023).

Although the introduction of natural predators is applied in temperate zones, classical biological control remains difficult to transpose to the tropical environment (Parent, 2023). Faced with these threats, including *Shistocerca gregaria*, *Locusta migratoria* and *Z. variegatus* (Dongmo et al., 2020; Wandji et al., 2021; Ngnaniyyi et al., 2024), acridian control has long relied on synthetic insecticides (Heumou et al., 2022). Yet more than 98% of the volumes sprayed reach unintended targets (FAO, 2020). Residues of organochlorine pesticides (DDT,

aldrin, endosulfan) frequently exceed the thresholds tolerated by the FAO, the WHO and the EU in soils (0.18–160 µg/kg) and waters (0.02–0.40 µg/L) (FAO, 2020, 2024; Zongo et al., 2015).

Hence, the use of botanical extracts stands out as an ecological, effective and biodegradable alternative for regulating insect populations without altering environmental health (Seino et al., 2013b; Dongmo et al., 2020; Abdoul et al., 2024). *Annona muricata* (Annonaceae) and *Cymbopogon citratus* (Poaceae) are two medicinal plants widely used throughout the world for their therapeutic virtues. Previous work has established their respective cytotoxic and lethal properties (Seino et al., 2013a; Lloyd et al., 2019). In *A. muricata*, phytochemical characterization highlights various secondary metabolites, notably alkaloids, acetogenins, terpenoids, polyphenols, flavonoids, saponins and caffeic acids (Machraoui et al., 2018; Kazman et al., 2022). The application of 28 µl of ethanolic extract of its dried seeds disrupts meiosis in *Zonocerus variegatus*, by increasing the frequency of chiasmata and inducing chromosomal aberrations such as agglutinations, lagging chromosomes or abnormal anaphases (Seino et al., 2013a). Moreover, at a concentration of 1 g/ml, the aqueous extracts of these two species exert a lethal effect on brine shrimp (Lloyd et al., 2019). This study aims to deepen the toxicological profile of these two plants by providing new cytogenotoxic data, in order to develop biological control solutions against the pest grasshopper *Z. variegatus*. The main objective was thus to evaluate the cytotoxicity and genotoxicity of aqueous leaf extracts of *A. muricata* and *C. citratus* on the meiotic dynamics of *Z. variegatus*, targeting in particular the meiotic index and the rate of chromosomal abnormalities.

II. Materials and methods

II.1. Plant material

II.1.1. Location and collection of the plants.

The leaves of *Annona muricata* and of *Cymbopogon citratus* constituted the plant material of this study. They were harvested in Ngui village and Foto village respectively on 30 November 2023 and 01 December 2023 in the morning, at coordinates N 05°26'272'' E 010°03'202'' and N 05°26'676'' E 010°03'703''. Ngui village and Foto village are located in the Dschang Subdivision, administrative seat of the Menoua Division (West Region of Cameroon).

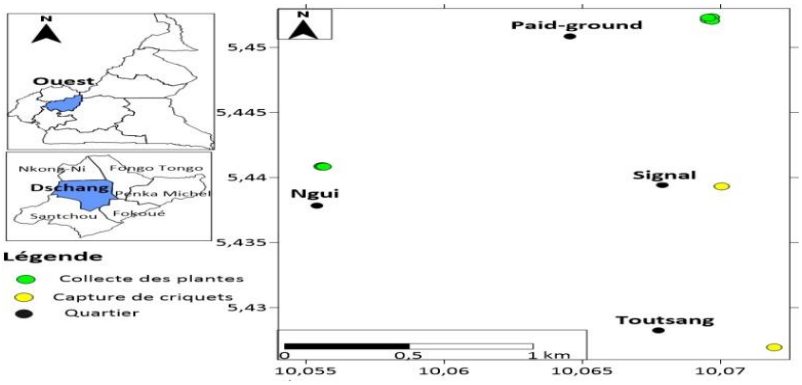


Fig. 1 Location map of the study areas

II.1.2. Authentication of the plants *Annona muricata* and *Cymbopogon citratus*

Freshly harvested samples (leaf, stem, fruit) of the same day, the plants were packed separately in A4-format papers which were sent in sealed envelopes to the National Herbarium of Cameroon for authentication. (Appendix 3).

The plants were authenticated by Mr NGANSOP T. Eric. The plants were authenticated as *Annona muricata* L. (Annonaceae) (Thonn.), by comparison with the original material of the National Herbarium No. 18681/SRFCam collected by Dang D. under No. 162 and *Cymbopogon citratus* (DC.) Stapf (Poaceae) identified by comparison with the original material of the National Herbarium No. 18628/SRFCam collected by Dang D. under No. 202.

II.1.3. Preparation of the aqueous extracts

II.1.3.1. Drying and obtaining dry powders of *A. muricata* and of *C. citratus*

Five kilograms (5) of fresh leaves of each plant were harvested. Once harvested, the leaves were washed, then dried away from the sun at room temperature and then crushed using an electric grinder

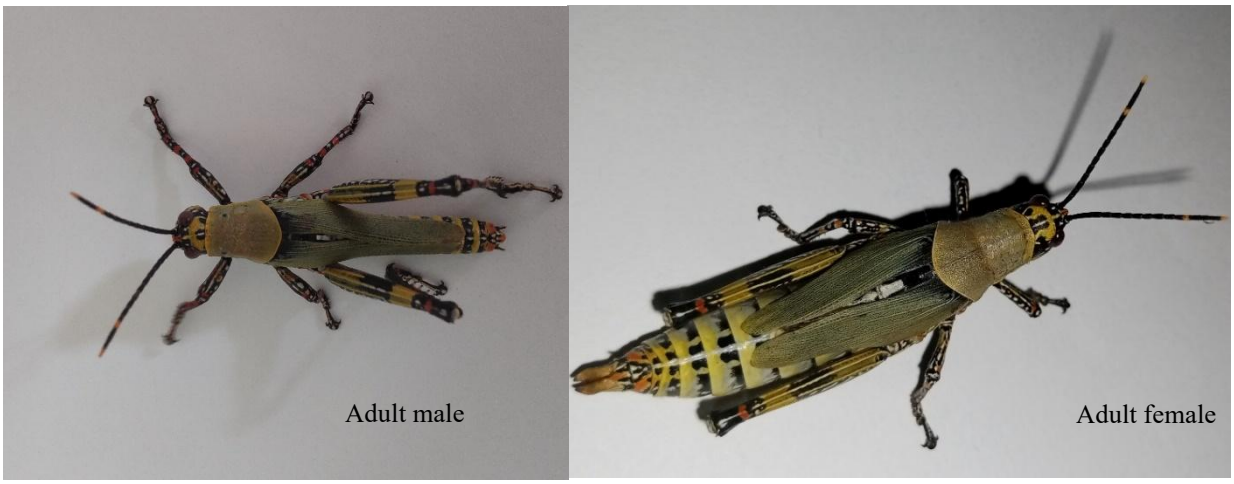
II.1.3.2. Preparation of the dry extract of the leaves of *Cymbopogon citratus*

For the preparation of the aqueous extracts, 250 g of the powders of *Annona muricata* and of *Cymbopogon citratus* were macerated and infused, respectively. The solutions obtained were successively filtered through Whatman paper No. 4 and No. 1. The filtrates obtained, dark and sticky for *A. muricata* and green, dark and pasty for *C. citratus* were distributed into stainless-steel plates and then placed in an oven for drying at a temperature of 45°C for 72 h. The dry extract of *A. muricata* was sticky in nature, crystal-shaped and foul-smelling, whereas that of *C. citratus* obtained was dark red, sticky, aromatic and shiny. The extraction yields were 8.97% and 9.72% respectively for *A. muricata* and *C. citratus.* Then, using an electric balance, 10 g of each dry extract obtained were weighed and then introduced into a beaker containing 50 ml of distilled water. Once the mixture was homogeneous, an additional 50 ml of distilled water was added. In this way the solution of extract of *A. muricata* and of *C. citratus* at a concentration of 100 µg/ml was obtained (stock solution). By dilution, the other solutions at concentrations of 50, 25, 10 and 5 µg/ml were obtained (Lloyd *et al.,* 2019).

II.2. Animal material

II.2.1. Identification, authentication and selection of adult male *Zonocerus variegatus*

The animal material of this work consisted of pest grasshoppers (*Zonocerus variegatus*). As the captures were carried out in the same locality as the plants, the pedological conditions are identical. The relief is characterized by high plateaus, with a temperate Sudano-Guinean type climate. The collection area in particular is characterized by food-crop vegetation such as cassava, maize, plantain and bean. The grasshoppers were captured using a sweep net for active individuals able to fly and by direct hand collection for individuals that were less active or poorly adapted to flight. Specimens were identified in the field using a magnifying glass and the identification key of Mestre (1988). Description and measurements were carried out on the individuals. Sexual dimorphism was necessary for identification. Generally, the size of male grasshoppers (36.32 mm) is smaller than that of females (38.42 mm) and their body is slimmer. The antennae and wings of the males are more developed, allowing them respectively to detect



the pheromones emitted by the females and to be capable of long-distance flights (Kekeunou et al. 2014). The hoof-shaped abdominal extremity constitutes the subgenital plate, which frequently elongates and becomes conical and pointed or flattens into a knife blade, sometimes very long. In the male of this species, the subgenital plate has a conical shape.

Fig. 2 photograph of *Zonocerus variegatus* a) adult male b) adult female

II.2.2. Making of cage bottles and acclimatization of the grasshoppers

Once selected, the grasshoppers were introduced into cage-bottles made according to the technique of Popov (1990). These cages were made from one-and-a-half-litre plastic bottles. Popov's technique consists of perforating the bottles with a pointed-tip soldering iron longitudinally and parallel to one another, thus allowing good ventilation (Fig. 6). The latter reproduce a low-stress environment like the natural environment (Popov., 1990). Once at the laboratory of the Applied Biology and Ecology Research Unit of the University of Dschang (URBEA), the grasshoppers were acclimatized. The principle of acclimatization consists in

cancelling the stress linked to the capture and transport of the grasshoppers to the laboratory. Seventy-two hours (72 h) are necessary according to Camacho *et al.* (2015). During this period, the grasshoppers were fed every day at precisely 9 a.m. Feeding consisted of introducing 10 g of fresh leaves of *Manihot esculenta* picked *in situ* (this quantity should cover the energy needs of 5 grasshoppers according to Olodore *et al*. (2018). Before acclimatization, the grasshoppers were divided into 11 groups of five individuals.

II.2.3. Distribution and treatment of the grasshoppers

In total, 55 grasshoppers were treated. Five (5) grasshoppers received distilled water (neutral control), and 50 grasshoppers received aqueous extracts of *Annona muricata* and of *Cymbopogon citratus* (25 individuals for each extract and 5 individuals per concentration). Group 1 or neutral control group consisted of 5 individuals treated with distilled water. Groups 2, 3, 4, 5, 6 consisted of 5 individuals receiving separately the two aqueous extracts at the respective concentrations of 5, 10, 25, 50 and 100 µg/ml. Using an insulin syringe, each grasshopper received a single injection of 0.01 ml/kg of distilled water or aqueous extract.

II.2.4. Periods, routes of administration of the extracts and incubation of the grasshoppers

According to the work of Seino *et al*. (2013b, 2020), the time required to incubate *Z. variegatus* treated with the extracts is 96 h. This time proved to be ideal for an evaluation of anti-meiotic effects using the *Z. variegatus model,* because in 96 h, the extracts will be totally metabolized by the follicular cells constituting the target in this study. Likewise, this period is necessary to observe 3 successive meiotic cycles.

The extracts were administered by the intraperitoneal route. This route allows the extracts to reach directly the haemocoel of *Z. variegatus.* The haemocoel contains the haemolymph in which the organs of the grasshopper bathe and consequently, the extracts will be assimilated by the testicular follicles (Shashi *et al*., 2015).

II.2.5. Killing of the grasshoppers, dissection and preparation of chromosome smears

Once the 96 h of incubation had elapsed, the grasshoppers were killed using a smoker into which cotton soaked in ethyl acetate had been introduced. Once the grasshoppers were killed, they were dissected as follows: First, the 2 pairs of wings of the grasshoppers were cut and removed with a pair of scissors in order to expose the dorsal part of the abdomen and thorax. Then, the last metamere of the abdomen was cut to facilitate access to the abdominal organs. Finally, again using a pair of scissors, the dorsal midline of the abdomen was cut, and the follicular cells were detached from the other organs, then isolated and stored in vials containing

beforehand a fixative (mixture of glacial acetic acid and absolute ethanol in volume ratios of 1V: 3V). The vials containing the testicular follicles were immediately stored at 4°C. Then, chromosome smears were prepared according to the lacto-acetic orcein spreading method of Seino (2007), which can be described in three essential steps:

Step 1: On each microscope slide, 2 testicular follicles were placed and also covered with 50 µl of 45% acetic acid (2 drops) for 12 min. The acetic acid softens the follicular tissue, causing the swelling of the follicles, which will thus be more permeable to the stain.

Step 2: After this time (12 min), the residual acetic acid was blotted off using an 8 mm piece of filter paper (Whatman paper No. 4) and the follicles were covered again with 50 µl of stain for 18 min.

Step 3: Finally, a coverslip was placed over the preparation and firmly held in place using the thumb and index finger. Spreading of the follicles between the slide and the coverslip was facilitated by gently tapping the slide with the rounded end of a dissecting needle, which causes the cells to dissociate so that they form a monolayer. Clear nail polish was applied all around the preparation to prevent the cells from drying out. The smears were examined at the 40X objective.

II.3. Evaluation of the meiotic index in *Zonocerus variegatus*

The counting of dividing cells was done using the technique described by Shweta *et al*. (2013). This technique consists in counting only the dividing cells present in the left (l), right (r), top (t) and bottom (b) fields over the total number of cells counted, whether dividing or not. The meiotic index was calculated as follows (Fig. 13):

Equation 1*: *Evaluation of the meiotic index:

$$\mathrm{MI}(\%) = \frac{\text{number of dividing cells}}{\text{total number of cells observed}} \text{ x } 100$$

In general, 2 testicular follicles comprise 480 cells. As the number of individuals per treatment is 5, the total number of cells observed is 2400 per treatment.

II.4. Evaluation of chromosomal abnormalities in *Zonocerus variegatus*

According to Seino *et al*. (2007), chromosomal abnormalities (structures) were identified by comparison with the standard chromosomes of Seino *et al.* (2020). The abnormalities retained are: chromosome bridges, lagging chromosomes (LC), sticky chromosomes (SC), chromosome breaks (CB), disturbed anaphases (DA) and micronuclei (MN)

II.5. Statistical analysis of the data

The abnormalities were photographed using an AQUOS brand phone; 12-megapixel camera. These images were processed with the photo software Paint 3D (version 20.1.0) on the Mac iOS operating system. These micrographs were useful in the description of the meiotic parameters (Ngnanyyi *et al*., 2022).

Analysis of variance (ANOVA) tests and Tukey's post hoc test (HSD) were used for normally distributed data and the Wilcoxon-Mann-Whitney test for non-normally distributed data. Data collection and preprocessing were carried out using Python version 3.2.1 on the Mac OS X operating system (Rinc., Chicago, IL., United States), as well as with the Excel spreadsheet of the Microsoft Office 2019 suite (Microsoft Corporation, Redmond, WA, United States) and R software version 4.2.2, in accordance with the conceptual data analysis plan of Sherrer (2007). The results were expressed as mean ± standard error of the mean (SEM). Differences were considered significant at a probability threshold of ($p<0.05$).

III. Results

III.1. Effects of the aqueous extracts of *Annona muricata* and *Cymbopogon citratus* on the meiotic index of *Zonocerus variegatus*

Individuals that received distilled water show a normal meiotic process, unlike individuals treated with the plant extract. Table 1 and Fig. 3 present the results of the evolution of the meiotic index (MI) in *Zonocerus variegatus* treated for 96 h. The MI of the grasshoppers of the control group (0 µg/ml) is 14.19 ± 0.00% on average. This normal value significantly decreased ($p<0.05$) from 10 µg/ml for the aqueous extract of *C. citratus* and 5 µg/ml for the aqueous extract of *A. muricata*. At concentrations between 10 and 25 µg/ml, the two aqueous extracts (AE) have a weak MI-reducing effect. However, the AE of *C. citratus* has effects greater than those of *A. muricata.* The same trend is observed for the highest concentrations between 50 and 100 µg/ml. The two AE decreased respectively from 14.19 ± 0.00% to 7.82 ± 1.15 and from 14.19 ± 0.00% to 4.11 ± 1.43, i.e. a reduction rate of 45% for the AE of *A. muricata* and of 71% for the AE of *C. citratus.*

III.2. Effects of the aqueous extracts of *Annona muricata* and of *Cymbopogon citratus* on the frequency of chromosomal abnormalities in *Zonocerus variegatus*

Fig. 3 presents the evolution of all the chromosomal abnormalities of *Z. variegatus* treated with the AE of the leaves of *A. muricata* and of *C. citratus* for 4 days. It emerges that chromosomal

abnormalities such as sticky chromosomes (Fig. 7) and bridges (Fig. 4) had the highest frequencies, at 4.11 and 2.58% respectively. Next, lagging chromosomes (Fig. 5) and disturbed anaphases (Fig. 6) had a similar frequency of 1.05 and 1.00% respectively. Chromosome breaks (Table 2) showed the lowest frequency, which is 0.51%.

IV. Discussion

IV.1. Effects of the extracts on the meiotic index of *Z. variegatus*

Several studies have shown that the meiotic index (MI) is a fairly relevant parameter for examining the toxicity of xenobiotics at the cellular scale (Elena *et al*., 2018; Ngnaniyyi *et al*., 2023). These authors specify that the MI makes it possible to appreciate the proportions of gonadal cells that enter meiosis to give spermatozoa. The study revealed a significant concentration-dependent decrease in MI, the latter being more pronounced with the AE of *C. citratus* than with that of *A. muricata*. This decrease in MI can be explained overall by the arrest of the meiotic process of the cells involved. In reality, the arrest of the meiotic process corresponds to an inhibitory effect of the extract. Studies have shown that several classes of secondary metabolites contained in the extracts have inhibited cell lines in *Z. variegatus* (Seino *et al*., 2022; Ngnaniyyi *et al*., 2024). The more pronounced decrease in MI observed with the AE of *C. citratus* at high concentrations could be explained by the high content of this extract in alkaloids, flavonoids, saponins, caffeic acids and terpenoids (Machraoui *et al*., 2018). Our results are similar to those of Ngnaniyyi *et al*. (2024) when they demonstrated that the cucurbitacins, alkaloids, phenols, terpenoids, flavonoids, among others, contained in melon seeds (*Cucumus melo*) inhibited MI by more than 12.15% in *Z. variegatus*. This result testifies to the high toxicity of the AE of *C. citratus* compared with that of *A. muricata* and also corroborates the study of Lloyd *et al*. (2019) which showed a high toxicity of the AE of *C. citratus* (LC50= 0.49) compared with *A. muricata* (LC50= 0.87) on the lethality of brine shrimp.

Table 1: Effects of the aqueous extracts of *Annona muricata* and of *Cymbopogon citratus* on the meiotic index in *Zonocerus variegatus*

Concentration(µg/ml)	*A. muricata*(%)	*C. citratus*(%)	*P*-value
0	14.19±0.00[a]	14.19±0.00[a]	0.060131
5	14.02±1.06[a]	12.10±1.95[b]	0.000113
10	12.35±1.01[b]	10.25±1.18[c]	0.000418
25	10.73±0.87[c]	10.02±1.33[c]	0.000315
50	8.68±0.71[d]	6.62±1.01[d]	0.000710
100	7.82±0.44[d]	4.11±0.40[e]	0.000493

Values are means ± standard error. Number of trials n=5. Statistical test used: one-way ANOVA. a; b; c; d; e indicate significant differences between concentrations.

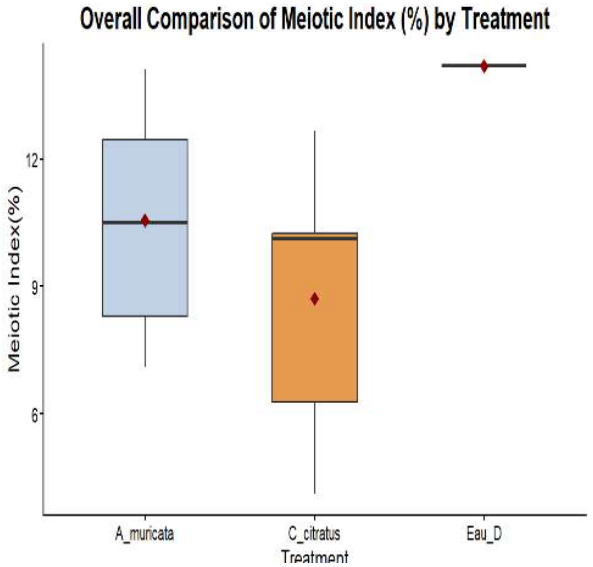


Fig. 1 Illustration of the variations of the meiotic index according to different treatments

IV.2. Effects of the extracts on the frequency of chromosomal abnormalities in *Z. variegatus*

Six (06) types of chromosomal abnormalities were identified in *Z. variegatus* treated with the aqueous extracts of *A. muricata* and of *C. citratus*. These abnormalities are: chromosome bridges (CBR), lagging chromosomes (LC), sticky chromosomes, chromosome breaks, disturbed anaphases and micronuclei (MN).

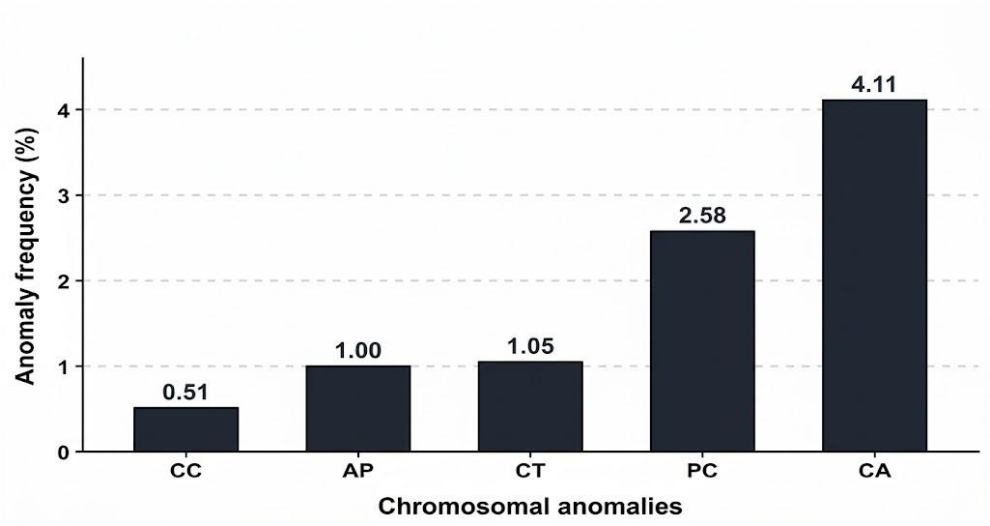


Fig. 2 Evolution of chromosomal abnormalities

Once in the haemocoel, the aqueous extracts (Annona muricata and Cymbopogon citratus) are carried by the haemolymph and metabolized by the gonadal cells. Their secondary metabolites interact with the DNA of the meiotic cells, forming adducts that give rise to several chromosomal abnormalities: chromosome bridges (CBR), chromosome breaks (CB), lagging chromosomes (LC), disturbed anaphases (DA), sticky chromosomes (SC) and micronuclei (MN).

a. Chromosome bridges (CBR)

Caused by the abnormal binding of chromosomes, CBR make up 28.5 ± 1.2% of the abnormalities in *Z. variegatus*. Their frequency increases in a concentration-dependent manner, particularly with *C. citratus*, which is richer in saponins. These abnormalities result from a dysfunction of the achromatic spindle (replication defects or alteration of tubulin) induced by aneugenic and clastogenic effects of phenolic compounds (Tagne, 2023). These results corroborate those of Dongmo et al. (2022) on *Ageratum conyzoides* and *Rauvolfia vomitoria*. The persistence of CBR can subsequently give rise to chromosome breaks (Dongmo et al., 2020).

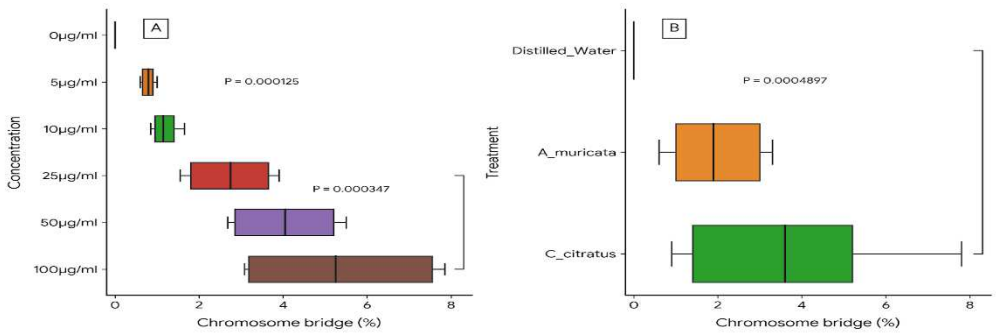


Fig. 3 Effects of the aqueous extracts of *Annona muricata* and of *Cymbopogon citratus* on chromosome bridges in *Zonocerus variegatus*

b. Chromosome breaks (CB) and Micronuclei (MN)

Corresponding to the fragmentation of chromosomes, CB testify to a strong irreversible cytogenotoxicity capable of leading to cell death and infertility (Ngnaniyyi et al., 2024). They result from poor spindle formation and unequal distribution of chromosomes (Chen et al., 2012). Our observations agree with Dongmo et al. (2020), but diverge from Seino et al. (2013a), whose ethanolic extract of *A. muricata* seeds caused no CB. This difference is explained by the nature of the solvent and the composition of the organs extracted (leaves vs seeds). Prolonged CB lead to the formation of micronucleated cells (MN).

Table 2: Effects of the aqueous extracts of *Annona muricata* and of *Cymbopogon citratus* on chromosome breaks and micronuclei in *Zonocerus variegatus*

Concentration(µg/ml)	*A. muricata* (%)	*C. citratus* (%)	*P-value*
0	0±0.00	0±0.00	0.060131
5	2.06±0.21	1.07±0.11	0.000113
10	3.47±0.21	0.58±0.25	0.000418
25	4.12±0.61	1.03±0.55	0.000315
50	5.90±1.40	1.81±0.75	0.000710
100	7.06±1.31	1.99±0.81	0.000493

Values are means ± standard error. Number of trials n=5. Statistical test used: one-way ANOVA.

c. Lagging chromosomes (LC)

LC are chromosomes lagging in anaphase whose ascent is slowed by chemical compounds binding to motor proteins. They disturb genomic stability and lead to the loss of genetic material (Gordon et al., 2012; Pampalana et al., 2016). *A. muricata* and *C. citratus* induce LC at respective frequencies of 2.9% and 1%, which is consistent with the work of Seino et al. (2013a) on the concentration-dependent effect of *A. muricata*.

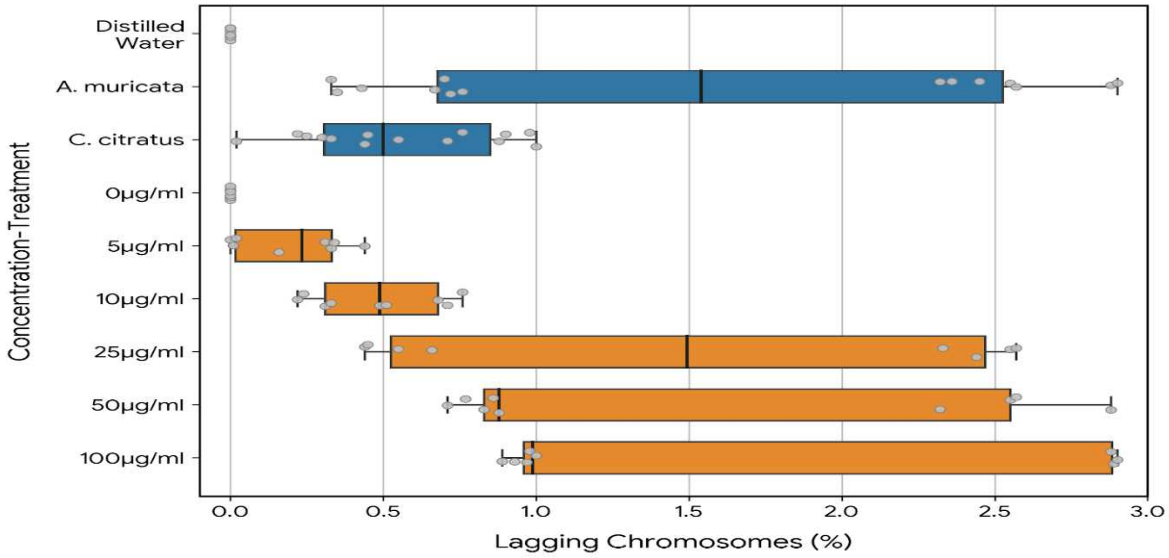


Fig. 4 Effects of the aqueous extracts of *Annona muricata* and of *Cymbopogon citratus* on lagging chromosomes in *Zonocerus variegatus*

d. Disturbed anaphases (DA)

Characterized by an absence or partiality of polar ascent, DA reflect the toxicity of the extracts on the genome (Ngnaniyyi et al., 2024). A significant ($p < 0.05$) and concentration-dependent increase in their frequency is observed, more marked for *C. citratus* (2.9%) than for *A. muricata* (1.3%). They result from the alteration of microtubules and spindle protein kinases by the secondary metabolites. These disturbances take various forms (star-shaped, multipolar, reversed-pole or with breaks) and agree with the mean frequency of 3.33% reported by Seino et al. (2013a).

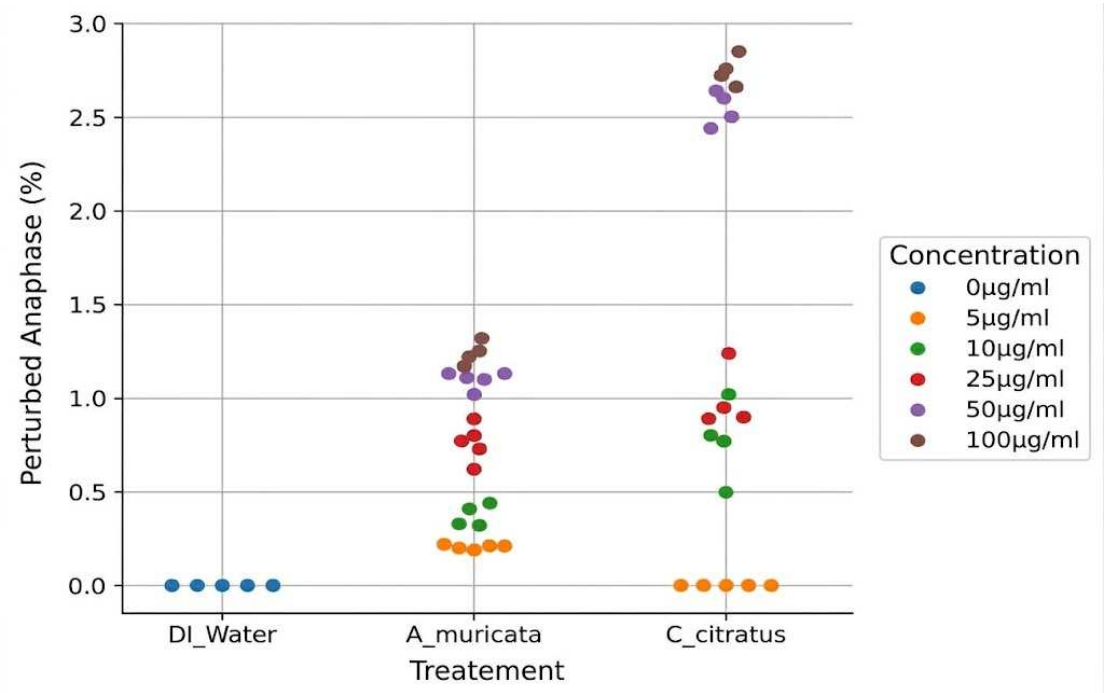


Fig. 5 Effects of the aqueous extracts of Annona muricata and of Cymbopogon citratus on Disturbed anaphases in Zonocerus variegatus

e. Sticky chromosomes (SC)

Agglutination results from the deactivation of division proteins, forming abnormal chromosomal aggregates at all meiotic phases (Dongmo et al., 2020; Seino et al., 2020). The weaker effect of *A. muricata* compared with *C. citratus* reflects its lower content of active secondary metabolites. This result agrees with Tagne et al. (2024) on *Cola verticillata*, but differs from Seino et al. (2013a), a divergence again attributed to the mode of extraction (aqueous versus ethanolic).

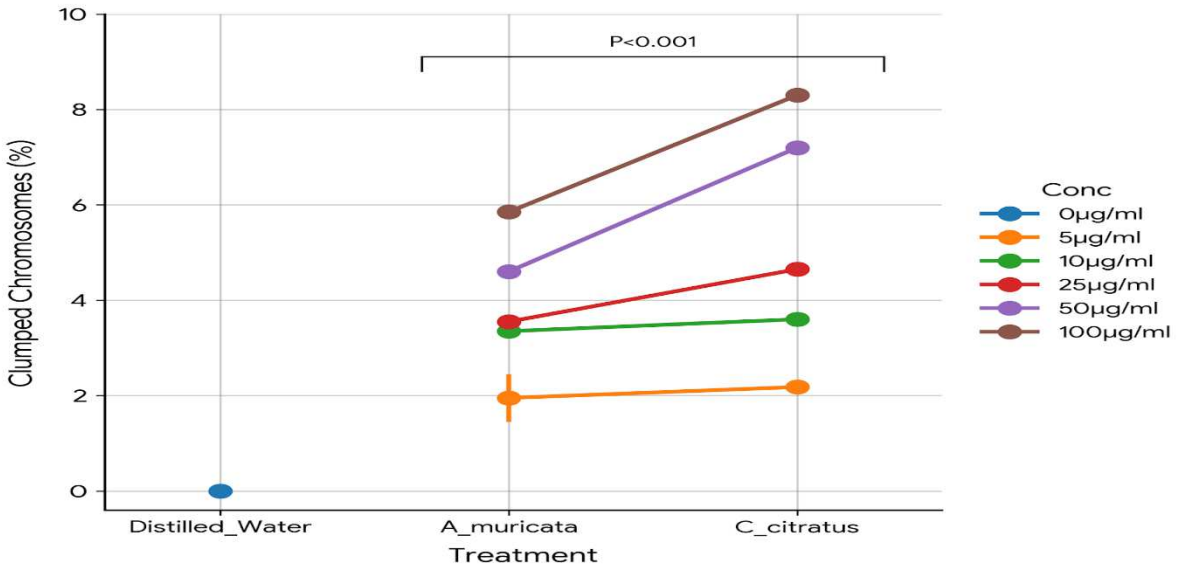


Fig. 7 Effects of the aqueous extracts of *Annona muricata* and of *Cymbopogon citratus* on Sticky chromosomes in *Zonocerus variegatus*

f.* Illustration of the evolution of some chromosomal abnormalities in *Zonocerus variegatus

Some chromosomal abnormalities induced in the germ cells of Z. variegatus by the extracts were photographed and illustrated in Fig. 10 below:

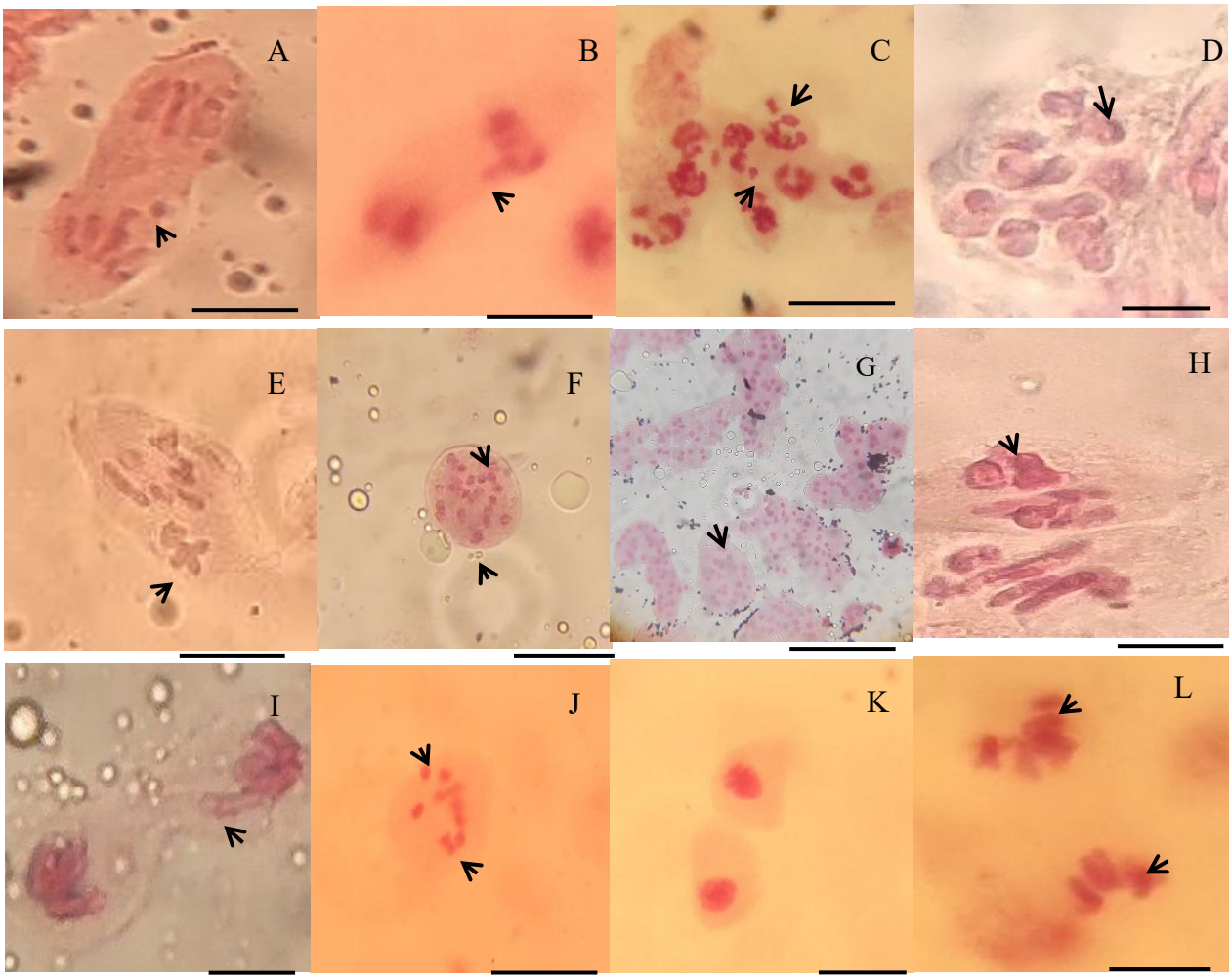


Fig. 8 Micrographs of chromosomal abnormalities in *Zonocerus variegatus*

A: Anaphase-1 with chromosome break induced at 50 µg/ml of the AE of *C. citratus*;
B: Anaphase with lagging chromosome induced at 25 µg/ml of the AE of *C. citratus*;
C: Anaphase with chromosome break induced at 100 µg/ml of the AE of *A. muricata*;
D: Metaphase-1 with poorly organized chromosomes induced at 50 µg/ml of the AE of *C. citratus*;
E: Metaphase-1 with chromosome bridge induced at 25 µg/ml of the AE of *C. citratus*;
F: Cell with micronuclei induced at 100 µg/ml of the AE of *A. muricata*;
G: Cells with micronuclei induced at 50 µg/ml of the AE of *C. citratus*;
H: Metaphase with sticky chromosome at 50 µg/ml of the AE of *A. muricata*;
I: Anaphase with lagging chromosome induced at 50 µg/ml of the AE of *A. muricata ;*
J: Metaphase-1 with sticky chromosome induced at 25 µg/ml of the AE of *C. citratus*.
K: Telophase-2 with sticky chromosome induced at 50 µg/ml of the AE of *C. citratus* ;
L: Anaphase-1 with sticky chromosome induced at 5 µg/ml of the AE of *C. citratus*.

Conclusion

The richness of the flora in secondary metabolites, used by more than 80% of the world's population (including 60% in traditional medicine), motivates the constant search for new molecules. These compounds may be therapeutic or induce cellular and genetic toxicities, a property that can be exploited in the fight against insect pests.

This study evaluated the cytogenotoxic effects of the aqueous extracts of *Annona muricata* and of *Cymbopogon citratus* on the meiosis of the pest grasshopper *Zonocerus variegatus*. The main results indicate that:

- The aqueous extracts of both plants decreased the meiotic index by reducing the number of dividing cells.
- They induced a significant increase in the frequency of chromosomal abnormalities.
- The extract of *A. muricata* shows a lower cytogenotoxicity than that of C. citratus.

In conclusion, the maceration of *A. muricata* and the infusion of *C. citratus* alter the course of meiosis in *Z. variegatus*. These cytogenotoxic properties offer promising prospects for the design of bio-insecticides intended for the control of acridian populations and the protection of the environment.

Declaration

Conflict of interest

All authors certify that they have no affiliation with or involvement in any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

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Inteligence artificial

The authors declare that they used the AI assistant to improve readability and style, and to ensure the absence of grammar, spelling and punctuation mistakes.

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