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Cytogenetic Effects of Ethanol Extract of Sun Dried Seeds of Soursop (*Annona muricata*) on The Male Germ Line Cells of The African Pest Grasshopper *Zonocerus variegatus* L.

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This study was carried out to investigate the effect of Ethanol extract of the seeds of *Annona muricata* on some meiotic parameters in the pest grasshopper *Zonocerus variegatus*. Adult grasshoppers were treated with 28µl of the Ethanol extract of sun dry seeds of *Annona muricata and* absolute ethanol (control) for 48 hours and all the parameters (chiasma frequency, formation of rod and ring shaped bivalents and meiotic anomalies) were affected by the extract. Chiasma formation was observed to significantly ($P<0.05$) increase following treatment and this was correlated by a significant increase in the formation of ring shaped bivalents. The incidences of meiotic anomalies such as laggards and bridges at first and second meiotic Anaphases, second meiotic Metaphase as well as in first and second meiotic Telophases were observed to increase when insects were treated with the ethanol extract. These observations indicated that the Ethanol extract of the seeds of *A. muricata* contain substances that could induce cytotoxicity. The importance of such property in the formulation of grasshopper pest control measures cannot be overemphasized.

Keyword: Ethanol Extract, Sundried Seeds, *Annona muricata*, Cytogentic Effect, *Zonocerus variegatus.*

1. Introduction

Biopesticides also referred to botanical insecticides are naturally occurring insect toxins extracted from plants. These extract are non-synthetic and can be used in the control of insect pests. Several such chemicals have been proposed for use in insect pest control. They include extracts from the neem plant that have been shown to be effective in the control of grasshopper pests [1, 2] wood ash from eucalyptus has been shown in the laboratory to be a good anti-feedants for the African pest grasshopper *Zonocerus variegatus* [3]. Bioinsecticides act quickly, causing immediate paralysis, death, or cessation of feeding [4]. Many but not all, are less toxic than synthetic pesticides to insects, mammals and plants. They tend to break down rapidly in the environment and are therefore environment friendly. Plant extracts and their essential oils are one of several non-synthetic chemical control options that have recently received attention for controlling grasshopper pest species and plant diseases [5, 6, 7].

Annona muricata (Annonaceae) is a small tree/shrub commonly cultivated in Cameroon, tropical America, Asia and Africa. This species thrives well in places where there is a clear division between the rainy and dry seasons and

generally prefer dry sites [8]. This species has been implicated in ovicidal, insecticidal, repellent, antifeedant, antinematode / nematocidal activities for several field pests. It is a contact poison to many field insect pests. The effective ingredients are found in the unripe fruit and in the seeds, leaves and roots[9]. The powder from the seeds of *A. muricata* causes painful irritation when in contact with eyes of humans. The effective ingredient is found in unripe fruits, seeds, leaves and roots and the oil content of the seeds amounts to 42 – 45% [10, 11].

It is well known that chiasma frequency at meiosis varies with different environments and there is considerable literature describing the influence of temperature changes[12], chemical treatments [13,14] and ionizing radiation treatments[15] on chiasma formation. Almost all the tested synthetic chemicals used in agriculture have shown harmful cytogenetic effects [14]. It is believed that natural compounds that have insecticidal properties could affect the cytogenetics of the insects too. It has been recently reported that *Kocha indica* extract to cause various types of meiotic abnormalities in *Vicia faba* [16]. Amongst these abnormalities included the presence of univalents and multivalents, laggards and bridges. The *Kocha indica* extract also negatively affected chiasma frequency in *V. faba*. Similarly the extracts of *Trigonella foenum graecum* L. have been reported to have genotoxic effects in *Pisum sativum* seeds [4].

Extracts of *A. muricata* have shown to possess insecticidal properties [10] and is therefore expected to affect the cytogenetics of *Z. variegatus*. Extracts of *A. muricata* have shown such insecticidal properties [10] and is therefore expected to affect the cytogenetics of *Z. variegatus*. The present investigation was undertaken to study the cytogenetic activities of the ethanol extract of sun dried seeds of *A. muricata* in the testes of the African pest grasshopper *Zonocerus variegatus* L.

2. Materials and Methods

2.1 Biological Material

The biological material used in this study consisted of twenty adult male individuals of the short-horn pest grasshopper, *Z. variegatus* (Orthoptera: Pyrgomorphidae). This species has been shown to have a diploid chromosome complement of 2n =19 XO♂ [17]. Each adult male grasshopper weighed an average 2.5grams.

Table 1: Mean Diplotene chiasma frequency from five ells in each of 10 treated and 10 control individuals of *Z. variegatus*

Individual	Control	Treated with 28µl of extract
1	12.60±0.89	14.60±0.49
2	15.40±0.89	15.00±1.09
3	13.60±0.89	14.20±1.17
4	13.60±0.55	14.40±1.02
5	14.00±0.00	15.40±1.72
6	13.20±1.79	15.40±1.74
7	12.60±1.14	14.40±1.02
8	12.60±1.14	14.00±1.09
9	14.80±1.64	15.20±0.98
10	13.60±0.55	14.60±0.49
Total	**13.40±0.29**	**14.70±0.18**

2.2 Preparation and administration of the Ethanol extract

Ethanol extract of sour sop, *A. muricata* seeds were prepared following the method of [10] Seven hundred grams (700g) of powder obtained by grinding sun dry seeds of *A. muricta* were mixed in 300ml of absolute Ethanol. The mixture was covered and allowed to stand for 48 hours. After complete infusion of the active ingredient had taken place the solvent was decanted, filtered into a beaker and then evaporated for five hours using a rotative evaporator. A dark brown unpleasant smelling extract (28µl for Ethanol extract) was obtained and used for this study.

One group of insects made up of 10 male grasshoppers was administered 0.05ml of the extract via peritoneal injection while the second group of 10 grasshoppers that served as the control was administered 0.05ml of absolute Ethanol. After administration of the extract, the insects were allowed for 72 hours. The testes were then removed after vivisection, fixed and

preserved in acetic acid – ethanol (3:1) at 4°C until use. Lactic – Propionic Orcein squash technique[19] was used to prepare meiotic cells (chromosome smears).

Table 2: F ratios for the comparison of chiasma frequency in treated and control individuals of *Z. variegatus*

Source	Sum of squares	df	Mean square	F	Significance
Corrected Model	111.250a	9	12.361	4.978	0.000
Intercept	19740.250	1	19740.250	7.949E3	0.000
Cell	3.200	4	0.800	0.322	0.862
Treatment	42.250	1	42.250	17.013	0.000
Cell * Treatment	65.800	4	16.450	6.624	0.000
Error	223.500	90	2.483	-	-
Total	20075.000	100	-	-	-
Corrected Total	334.750	99	-	-	-

a. R squared = 0.332 (Adjusted R Squared = 0.266)

2.3 Cytogenetic survey

The cytogenetic survey carried out included the determination of Diplotene chiasma frequency and percentages of rod and ring shaped bivalents. Meiotic abnormalities such laggards and bridges observed in meiotic stages (Anaphase-1, Telophase-1, Metaphase-2, Anaphase-2) in both treated and control individuals were recorded.

2.4 Statistical analysis

Data on chiasma frequencies, frequencies of rod and ring shaped bivalence were reported as mean values and standard errors. SPSS computer soft ware was used to estimate significance differences at $p<0.05$ for variance, Duncan's Multiple Range Test and Student t-test.

3. Results and Discussion

3.1 Chiasma frequency

Chiasma frequencies obtained for control and treated individuals are given in Table 1. The results indicated mean chiasma frequency was higher in treated (14.70±0.18) than control (13.40±0.29) individuals. These results indicated difference between individuals treated with ethanol extract of *A. muricata* and those in the control. The data in Table 1 was subjected to ANOVA and the F value obtained (17.013) (Table 2) indicated that the mean chiasma frequency in treated individuals was significantly higher ($P<<0.05$) than mean chiasma frequency in the control individuals. Data on chiasma frequencies indicated that the extract strongly affected genetic recombination in male individuals of *Z. variegatus*. It is a known fact that chiasma formation involves chromosome breakage and reunion. The position of the chiasmata and frequency of formation are regulated by both genetic and environmental factors [19]. Since the ethanol extract of the seeds of *A. muricata* increased genetic recombination, this indicated that the extract modified the genetic environment or the internal environment so as to change the expression of polygenes that regulate chiasma formation.

3.2 Frequency of rod and ring bivalents

Information on the frequency of rod and ring shaped bivalents in Diplotene cells of treated and control individuals are given in Table 3. The results indicated that the mean frequency of rod shaped bivalents was higher in control individuals (71.22%) than in individuals treated with ethanol extract of *A. muricata* (69.87%). However, Student's t values calculated indicated that the observed differences were not significant ($P>0.05$). On the other hand, mean frequency of ring shaped bivalents was higher in individuals treated with ethanol extract of *A. muricata* (30.22%) than control individuals (28.77%). Student's t values calculated indicated that the observed differences were significant ($P>0.05$). The results therefore indicated that the extract did not favour the formation of the rod but favoured the formation of ring shaped bivalents. This result confirms the observation that the extract modified the internal environment which resulted in increased chiasma formation since increase chiasma has been linked to increase formation of ring bivalents [20]. Elsewhere, increase in chiasma frequency has been attributed to increase in number of ring bivalents in male germ line cells in the plant *Festuca pratensis* Huds treated with phosphate [14].

Table 3: Frequency (%) of rod and ring shaped bivalents in five male meiotic cells per treated and control individual of *Z. variegatus*

Individuals	Diplotene Cells scored	Rod shaped bivalents		Ring shaped bivalents	
		Treated	Control	Treated	Control
1	20	7.8	6.7	1.2	2.3
2	20	6.6	5.1	2.4	3.9
3	20	6.2	6.7	2.8	2.3
4	20	6.0	6.7	3.0	2.3
5	20	6.0	6.7	3.0	2.3
6	20	6.0	6.4	3.0	2.6
7	20	6.0	6.9	3.0	2.1
8	20	6.0	6.9	3.0	2.1
9	20	6.0	5.3	3.0	3.7
10	20	6.2	6.7	2.8	2.3
Total	200	62.8	64.1	27.2	25.9
Mean	20	6.28	6.41	2.72	2.59
Mean %	-	**69.87**	**71.22**	**30.22**	**28.77**

3.3 Meiotic abnormalities

Anaphase, Metaphase and Telophase Laggards and bridges were some of the meiotic abnormalities encountered in both treated and control individuals. The incidences of these abnormalities and the stages in which they were identified are given in Table 4. This table revealed more laggards in Anaphase I, Telophase 1, and Anaphase II of treated than control individuals. It was also similarly observed that there were more bridges formed in Anaphase II cells of treated than control individuals. Lagging chromosomes was the more frequent meiotic abnormality recorded during the study than formation of bridges. Available information has revealed that a low incidence of meiotic abnormalities spontaneously occur in the male germ line cells of several acridids such as *Poekilocerus pictus*[13]. This indicates that in nature, there is no absolutely normal meiotic cycle without abnormalities in chromosome structure and behaviour. These errors introduce

Table 4: Frequency of male meiotic anomalies in five cells of treated and control individuals of *Z. variegatus*

Indiv.	A_1 - Laggards		A_1 - Bridges		T_1 - Laggards		M_2 - Laggards		A_2 - Laggards		A_2 - Bridges		T_2 - Laggards		TOTAL	
	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	1	0	0	0	1	0	0	0	0	0	1	0	0	0	3	0
3	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
6	1	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0
7	1	1	0	0	0	0	0	0	1	1	0	0	1	1	3	3
8	1	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0
9	0	0	0	0	1	0	1	0	0	0	0	0	0	0	2	0
10	0	0	0	0	0	0	0	0	1	0	0	0	1	1	2	1
Total	4	1	0	0	3	0	3	0	2	1	2	0	2	2	16	4
Mean	0.4	0.1	0	0	0.3	0	0.3	0	0.2	0.1	0.2	0	0.2	0.2	1.6	0.4
Mean %	**8%**	**2%**	0%	0%	**6%**	**0**	**6%**	**0**	**4%**	**2%**	4%	0%	**4%**	**4%**	16%	4%

A_1 = Anaphase -1, T_1= Telophase -1, M_2 = Metaphase -2, A2 = Anaphase -2, T_2= Telophase- 2, C= Control, T= Treated

genetic variation which may be beneficial or detrimental to the species carrying them. The errors are subject to natural selection which helps to create new genotypes that may be adaptive and hence beneficial to the species. Our results revealed that exposure of acridid meiotic cells to the extract of *A. muricata* brought about a significant increase in the incidence of these meiotic abnormalities (compare total percentage of anomalies in Table 4). It can therefore be concluded from this study that sufficiently increasing the concentration of the *A. muricata* extract can result in adverse effects on the meiotic process of acridid grasshoppers and may therefore lead to decrease fertility. It would not be unreasonable to suggest that entomologists should take advantage of the effects of the extract of *A. muricata* in altering chiasma frequency and hence genetic recombination in acridid pest grasshoppers.

From the aforesaid result, it becomes clear that the Ethanol extract of sun dried seeds of soursop, *Annona muricata* is genotoxic and is able to produce meiotic aberrations in the African pest grasshopper *Zonocerus variegatus*.

4. References

1. Hill SD. Agricultural insect pests of the Tropics and their control. Cambridge University press, London, 1983, 746.
2. Jiliani G, Su HCF. Laboratory studies on several plant materials as repellants for the protection of cereal grain. Journal of economic Entomology 1983; 79(1):154–156.
3. Seino RA, Focho DA. The antifeedant activitiy of cow urine and woodash to the pest grasshopper *Zonocerus variegatus* L. (Orthoptera: Pyrgomorphidae). African Journal of Natural Sciences 2001; 4:56–59.
4. Mohamed FI, El-Ashry ZM. Cytogenetic effect of Allelochemicals *Brassica nigra* L. extracts on Pisum sativum L. World Applied Sciences Journal 2012; 20(3):344–353.
5. Khater HF. Biocontrol of some insects. Ph.D Thesis, Faculty of Veterinary Medicine, Moshtohor, Zagazig university, Benha Branch, 2003.
6. Soylu EM, Tok FM, Soylu S, Kaya AD, Gulsun. Antifungal activities of the essential oils on post-harvest disease agent Penicillin digitatum. Pakistan Journal of Biological sciences 2005; 8: 25–29.
7. Abad M, Garcia JC, Sprecher DJ, Friendship CRM, Buhr M, irkwood RN. Effect of insernation-ovulation interval and addition of seminal plasma on sow fertility to insemination of cyopreserved sperm. Reproduction of Domestic Animals 2007; 42:418–422.
8. Burkill HM. The useful plants of West Tropical Africa. Ed 2, Vol. 1, Families A-D, Kew Royal Botanic Gardens, UK, 1985.
9. Nayar SL. Vegetable insecticides. Bulletin Inst Science India 1955; 4:137-145.
10. Shambo DN. Antifeedant and insecticidal activities of aqueous extract of Annona muricata (Annonaceae) to the African pest grasshopper *Zonocerus variegatus* L. (Orthoptera: Pyrgomorphidae). Maitrise dissertation, Departemnt of Animal Biology, University of Dschang, Cameroon, 2000, 128.
11. Stoll G. Natural crop protection in the tropics. Margraf Verlag, Second and revised enlarged edition. CTA, 2000.
12. Henderson SA. Temperature and chiasma formation in *Schistocerca gregaria* II. Cytological effects of 400C and the Mechanism for heat induced univalence. Chromosoma (Berl.) 1962; 13:437–463.
13. Gururaj E, Uma O. Cytogenetic effects of Liv. 52 on the male germ line cells of Poeilocerus pictus (Acrdiddae: Orthoptera). Journal of Mysore University: section 1993; 33:181–188.
14. Deniz B, Tufan A. The effects of phosphate treatments on chromosome pairing in Diploid and autotetraploid meadow fescue (Festuca pratensis Huds). Tr J of Agriculture and Forestry 1998; 22:349–356.
15. Lawrence CW. The effect of the irradiation of different stages in microsporongenesis on chiasma frequency. Heredity 1961; 16:83-89.
16. Haroun SA. Mutagenic effects of Kochia indica extract on *Vicia faba* L. Journal of American Science 2010; 6(7):292-297.
17. Faluyi JO, Olorode O. Mitotic chromosomes studies in nymphal and adult populations of *Zonocerus variegatus* L. (Orthoptera: Pyrgomorphidae). Journal of African Zoology 1988; 102:475–480.
18. Seino RA, Tongleu ID, Manjeli Y. Cytogenetic characterisation of *Taphronota thaelephora* Stal. 1873. (Orthoptera: Pyrgomorphidae) from Cameroon II. Description of mitotic

chromosomes. International Journal of Biological and Chemical Sciences 2012; 6(4):1624–1632
19. Verma PS, Agarwal VK. Cell Biology, Molecular Biology, Evolution and Ecology. S Chand & Company Ltd, New Delhi, 2005.
20. Mahabal R. Fundamentals of Cytognetics and Genetics. Asoke K Gosh, HI Learning Private Limited, Connaught Circus, New Delhi, 2010.