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Possible Cytogenetic Effect of *Capsicum frutescens* (Solanaceae) Extracts on Meiosis in the Grasshopper *Taphronota thaelephora* Stal. (Orthoptera: Pyrgomorphidae)

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Abstract

Pepper, *Capsicum frutescens* is an important Solanaceae species used as a common spice for food in many countries and in particular Cameroonian households. The aim of this study was to investigate the cytogenotoxic effect of the aqueous extract from *C. frutescens* fruits on the pest grasshopper *Taphronota thaelephora* (Orthoptera: Pyrgomorphidae) germ line cells by cytogenetic studies that have never been done before for pepper extracts. For this, adult individuals of *T. thaelephora* were treated for 72 hours with different concentrations of pepper extracts (0%, 10%, 25%, 40%, 50% & 60%). Chromosome number and the meiotic behaviour of chromosomes were analyzed. The results revealed that the extract did not induce chromosome breaks but could induce laggards and bridges in meiotic anaphases 1 &2 and metaphase -2 in the male germ cells of *T. thaelephora.* Increasing the concentration of the extract also significantly increased ($P<0.05$) meiotic abnormalities in the germ line of *T. thaelephora.* Chiasma frequency significantly increased when the concentration of the aqueous extract of *C. Frutescens* was increased.

Keywords

Capsicum frutescens; Aqueous extract; Cytogenetic effect; Taphronota thaelephora

Introduction

The cytogenetic effects of many plants extracts on grasshopper pest species are still unevaluated even though cytogenetic assays have been widely used in genotoxicity assessments to test compounds under both in vitro and in vivo conditions. A review of available literature revealed that studies have been carried out regarding the cytogenetic effects of plant extracts on other plants [1], fungi [2-4], on maize stem borers [5] and one on grasshoppers [6]. These investigations revealed that plant extracts induced marked decrease in mitotic index accompanied by considerable percentage chromosomal aberrations, meiotic abnormalities such as stickiness, disturbed chromosomes, bridges and lagging chromosomes [1,7-9]. The degree of cytological aberrations in either mitosis or meiosis is regarded as one of the dependable criteria for estimating the effect of a mutagen [10]. Mutagen induced chromosome abnormalities are the primary basis of genetic change therefore investigation of chromosome breakage, type of aberrations and their genetic consequences form an integral part of most of the mutation studies [11].

Capsicum frutescens (Solanaceae) commonly referred as pepper in Cameroon is widely distributed in the tropics and subtropics. It is a common spice for food in many Cameroonian households. It is eaten cooked or uncooked. Peppers (Chillies) originated in South America where they have been cultivated since ancient times. They were introduced to the rest of the world by Spaniards and the Portuguese. The ripe fruit has insecticidal properties and the effective substances are highest in the skin and in the seeds [12,13]. Active ingredients function as stomach poisons to target pests [13]. Extracts from *C. frutescens* have proofed good antifeedants for the African pest grasshopper *Taphronota thaelephora* [14].

Considering the potential insecticidal use of *C. frutescens* extract, and the lack of data about its cytogenotoxicity, this investigation was carried out to study the effect of *C. frutescens* on meiosis in the pest grasshopper *T. thaelephora.*

Materials and Methods

Preparation of Extract

Dried yellow pepper (*Capsicum frutescens*) fruits were obtained from the local market in Dschang. 100 grams of the finely ground dried fruits were stirred in 1 litre of distilled water and allowed to stand overnight. The filtrate (stock solution) was used to prepare five concentrations of 0%, 10%, 25%, 40%, 50% and 60% of pepper (*Capsicum frutescens*) (Table 1) and used for the study.

Administration of Extract

Sixty adult male grasshoppers of *Taphronota thaelephora* were separated into 6 groups of 10 grasshoppers each. The groups were labelled A, B, C, D, E & F and were respectively administered 0%, 10%, 25%, 40%, 50% & 60% of extract. 1 ml of the extract was injected into the peritoneum of each grasshopper and incubated for 72 hours. The grasshoppers were then dissected, the testes removed and fixed in Canoy's solution (3:1 Ethanol – acetic acid) then stored in a refrigerator at 4°C until used.

Cytogenetic examination

Meiotic chromosomes were obtained using the classical lactic-propionic-orcein squash technique [15]. Slides were prepared for individuals treated with the six concentrations under study and examined for chromosome number, precocious movements of chromosomes in meiotic Anaphases (Laggards) and formation of bridges in first and second meiotic Anaphases and second meiotic Metaphases. The chiasma frequencies in 25 Diplotene cells for each of 10 individuals per treatment were recorded.

Results and Discussion

Chromosome Number

Diplotene and Metaphase 1 cells obtained from individuals subjected to the different treatments revealed 9 bivalents and one univalent giving a chromosome complement of 2n=19. The univalent was the X- chromosome because it exhibited the reversal type of heteropycnosis a characteristic of Orthoptera grasshoppers [16]. In all the cells examined, chromosomes were acrocentric in morphology. No telocentric, metacentric or sub-metacentric chromosomes were present. Chromosomes have been described as acrocentric in *T. Thaelephora* [17]. Since telocentrics, metacentric and submetacentrics were absent, this indicated that treatment of *T. thaelephora* did not result in chromosome breakages (structural aberrations) and hence mutation. Since these were absent in treated and control individuals, it is conclusive that the extract of *C. frutescent* was not mutagenic.

Meiotic Studies

Chromosome aberrations are important cytogenetic endpoints that are routinely used in cytotoxicity and genotoxicity evaluations [18-21]. In the present studies, Diplotene (Prohase-1), Metaphase-1, Anaphase-1, Metaphase -2 and Anaphase-2 stages were recorded in both treated and control individuals of *T. thaelephora*. Treatment with aqueous extract *of C. frutescens* did not affect the sequence of the meiotic process in the individuals of *T. thaelephora.* However, the *extract* induced some aberrations that included laggards and bridges in Anaphase-1, Metaphase -2 and anaphase -2. Table 2 revealed that 0 and 10% of extract did not induce any laggards and bridges (meiotic abnormalities) in this grasshopper. Meiotic abnormalities were recorded with treatment of concentrations of the extract that were above 25%. Laggards were more commonly observed than bridges in Anaphase-1, Metaphase- 2 and Anaphase -2 cells. The highest percentage of laggards (14.29%) was recorded with treatment of 60% of *C. frutescens* extract. The highest percentage of bridges (7.79%) was recorded with a treatment of 60% of extract. More abnormalities were observed in Anaphase -1 cells than in the other meiotic stages considered. Therefore the meiotic abnormalities increased progressively with increase in concentration of extract. Different types of mutagenic alterations detected by cytogenetic techniques are thought to have aneugenic (chromosome lagging and effects on spindle) effects while chromosome aberrations are thought to arise from chromosome breakage and exchange [1,20,21]. We observed aneugenic effects in our study mainly when we applied concentrations higher than 25%of the extract.

Chiasma Frequency

A few studies have been carried out to determine the effect of plant extracts on the meiotic process in animals and in particular pest grasshoppers. One of such studies revealed that the aqueous extract of the seeds of *Annona muricata* affected chiasma formation in the pest grasshopper *Zonocerus variegatus* L. by changing the intracellular environment of germ cells [6]. During this study, mean chiasma frequency was observed to increase progressively with increase in concentration of aqueous extract of *C. frutescens* (Table 3). Mean chiasma frequencies were subjected to the Student-Newman – Keulsone wayvariance analysis. This analysis revealed mean chiasma frequencies recorded for all test concentrations were significantly higher ($p<0.5$) than for the control. However, there was no significant change in mean chiasma frequencies with subsequent increase in concentration of *C. frustescens* extract (Table 4). These results indicated that 10% of the aqueous extract of *C. frutescens* sufficiently modified the internal environment of the germ cells in *T. thaelephora* as to induce a change in chiasma formation. Therefore, extracts of *C. frutescens* possess some genotoxic properties which could produce meiotic aberrations in the pest grasshopper *T. thaelephora.*

Table 1: Preparation of aqueous solutions of pepper (*C. frutescens*) used in the study

Concentration (W/V) of dry pepper (C. frutescens)	Weight of dry pepper (C. frutescens) powder (grams)	Volume of distilled water (litre)
0%	00.0	1.00
10%	100	1.00
25%	250	1.00
40%	400	1.00
50%	500	1.00
60%	600	1.00

Table 2: Percentage (%) frequency of male meiotic aberrations in Capsicum frutescens treated T. thaelephora

Treatment Dose	Total number of cells scored	Anaphase – 1		Metaphase – 2		Anaphase – 2		Total abnormality %
		Laggards	Bridges	Laggards	Bridges	Laggards	Bridges	
Control	150	–	–	–	–	–	–	–
10%	200	–	–	–	–	–	–	–
25%	170	–	–	–	–	–	0.10	5.88
40%	188	0.12	0.13	0.13	0.10	0.12	0.13	38.83
50%	175	0.12	0.13	0.13	0.10	0.12	0.13	28.00
60%	154	0.12	0.13	0.13	0.10	0.12	0.13	47.40

Table 3:Mean chiasma frequency in Capsicum frutescens treated T. thaelephora
Means with the same letter are not significantly different at $P \leq 0.05$ (Student-Newman-Keuls Test)
Means were compared with analysis of variance (ANOVA) using Student-Newman-Keuls Test at $P \leq 0.05$ SPSS 15 for Window

Treatment	Total number of cells scored	Individual											Mean
		1	2	3	4	5	6	7	8	9	10	Total	
Control	250	11	11	10	11	15	15	10	11	11	11	116	11.60±1.74a
10%	250	12	12	14	11	12	12	12	14	11	12	122	12.20±0.98ab
25%	250	14	12	11	14	12	11	12	14	14	12	126	12.60±1.20ab
40%	250	15	13	12	11	13	13	12	11	13	15	128	12.80±1.33ab
50%	250	12	14	12	13	14	12	12	13	14	14	130	13.00±0.89ab
60%	250	15	13	14	13	13	13	13	13	15	14	132	13.60±0.80b
Total	1500	–	–	–	–	–	–	–	–	–	–	754	–

Table 4: ANOVA for male mean chiasma frequencies in Capsicum frutescens treated T. thaelephora

ONEWAY ANOVA

Control	Sum of squares	df	Mean Square	F	Significance
Between Groups Within Groups Total	23.533 86.400 109.933	5 54 59	4.707 1.600	2.942	.020

Reference

1. Zeinab El-ashry MMFI (2012) Determination of the genotoxic effects of *Trigonellafoenum graecum* L. extract in stored *Pisum sativum* seeds. Asian Journal of Agricultural Sciences 4: 264-269.
2. Ali-Shtayeh MS, Abu Ghdeib SI (1999) Antifungal activities of plant extracts against dermatophytes. Mycoses 42: 665-672.
3. Telezhenetskaya MV, D'yakonov AL (2004) Alkaloids of Penganum harmala. Unusual reaction of penganine and vasicinone. Chem Nat Comp 27: 471-474.
4. Olli S, Kirti PB (2006) Cloning, characterization and antifungal activity of defensin Tfgdi *Trigonella foenum-graecum* L. J Biochem Mol Biol 39: 278-283.
5. Ande AT, Wahedi JA, Fatoba PO (2010) Biocidal Activities of some tropical moss extracts against Maize stem borers. Ethnobotanical Leaflets 14: 479-490.
6. Seino RA, Shambo DN, DongmoTI, ChiffonRN (2013) 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. Journal of Entomology and Zoology Studies 1: 41-45.
7. Adam ZM, Mikhael E, El-ashry ZM, Ehsan NO, Ali RT (2008) Cytogenetic and biochemical studies on Vicia faba plants treated with two insecticidal active plant extracts. J Genet Engin Biotech 6: 19 -27.
8. Teerarak M, Laosinwattana C, Charoenying P (2010) Evaluation of allelopathic, decomposition and cytogenetic activities of *Jasminum officinale* L. f. Var. *grandiflorum* (L.) Kob. On bioassay plants. Bioresour Technol 101: 5677-5684.
9. Mohamed FI, El-ashry MZ (2012) Cytogenetic effect of Allelochemicals *Brassica nigra* L. Extract on *Pisum sativum* L. World Applied Sciences Journal 20: 344-353.
10. Jafri IF, Khan AH, Gulfishan M (2011) Genotoxic effects of 5-bromouracil on cytomorphological characters of *Cichorium intybus* L. African Journal of Biotechnology 10: 10595-10599.
11. Bhat TA, Sharma M, Anis M (2007) Comparative analysis of meiotic aberrations induced by diethylsulphonate and sodium azide in broad bean (*Vicia faba* L). Asian Journal of Plant Sciences 6: 1051-1057.
12. Spicket RGW (1955) The chemistry of some lesser known insecticides of plant origin. Colonial Plant and Animal products 5: 288-303.
13. Stoll G (2000) Natural crop protection in the tropics. Second and enlarged edition. Margraf Verlag. 97985 Weikersheim 375.
14. Nembot E (1999) Food Preferences in the African pest grasshopper Taphronota thaelephora Stal. (Orthoptera: Pyrgomorphidae) collected in the Menoua Division of Cameroon. Maitrise Memoire, University of Dschang, Cameroon.
15. Seino RA, Dongmo TI (2013) 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 3: 1-7.
16. Hewitt GM (1979) Grasshoppers and Crickets. Animal cytogenetics. Insecta, I. Orthoptera. Vol 3 Grebruger Borntraeger Ed. Berlin Stuttgart.
17. White MJD (1973) Animal cytology and Evolution. 3rd ed Cambridge Univsersity Press. 961.
18. Chacon DR, Dalla-Libera AN, Cintra DEC, Carvalho JCT, Oliveira GA, et al. (2002) Absence of genotoxic and antigenotoxic effects of a standardized extract of the medicinal plant *Solanum melongena* on peripheral blood and bone marrow cells of Wistar rats. Cytologia 67: 417-422.
19. Ferreira L, Carvalho JCT, Maistro EL (2003) Standardized *Solanum melongena* extract presents protective effects against chromosomal aberrations induced by Doxorubicin in Wistar rat bone marrow cells. Cytologia 68: 177-181.
20. Zanoni FD, Andrade SF, Bastos JK, Maistro EL (2005) Clastogenicity of the Austropienckia populnea (Celastraceae) bark wood extract in Wistar rat bone marrow cells. Cytologia 70: 303-308.
21. Pugliesi GC, Andrade SF, Bastos JK, Maistro EL (2007) In vivo clastogenicity assessment of Autroplenckia populnea (Celastraceae) leaves extract using micronucleus and chromosome aberration assay. Cytologia 72: 1-6.
22. Sousa MS, Silva PS, Viccini LF (2010) Cytogenotoxicity of *Chymbopogon citrates* (DC) Stapf (Lemon grass) aqueous extract in vegetal test systems. An Acad Bras Ciene 82: 305-311.