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Cytogenotoxic and antimicrobial effects of *Nezara viridula* (L.) (Hemiptera: Heteroptera: Pentatomidae) alcoholic extracts

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Abstract. Due to their multifunctionality and the numerous fields of applicability, insects are extensively studied today for both their biomedical and nutritional properties. In the current study the cytogenotoxic and antimicrobial potential of ethanol and methanol extracts of *Nezara viridula* (Linnaeus 1758) was evaluated using the *Allium* test, respectively the disk diffusion test. A mitostimulatory effect of the extracts of *N. viridula* and a variation of the cytogenotoxic activity of the extracts in a gender-dependent manner was noticed. As well, significant variations of the mitotic index were determined through the type of solvent used and the concentration of the extracts. High frequency chromosomal aberrations and mitotic abnormalities were recorded with high concentration ethanolic extracts. Following the testing of four standard bacterial strains and two standard yeast strains, a slightly antimicrobial activity was observed when compared to control. The use of invasive species in such studies opens up new perspectives on the potential of organisms considered harmful.

Keywords: insect, gender, extracts, bioactivity, mitotic index.

INTRODUCTION

Throughout time, despite their numbers and their significant therapeutic properties, insects had a minor role in traditional medicine and healthcare practices or in the synthesis of modern drugs as compared to plants. In traditional medicine the use of insect species has been recorded in regions of eastern Asia (e.g. China, India, Korea, and Japan), Africa, South and Central America (Costa-Neto 2002; Figueirêdo et al. 2015; Meyer-Rochow 2017; Bairagi 2019; Zhang et al. 2023; Yong et al. 2023).

According to Feng et al. (2009), in China, over 100 insect species have been used for their medicinal potential since ancient times. Namba et al. (1988) showed that 54 types of crude drugs derived from insects are men-

tioned in a Chinese manuscript from the beginning of the 7th century. Stink bugs are among the insect species mentioned by traditional medicine. *Aspongopus chinensis* (Hemiptera: Pentatomidae), common in China and known in traditional medicine for its analgesic effects and for its role in the treatment of nephropathy, was investigated recently for its antitumor properties (Luo et al. 2012; Tan et al. 2019). Syrup, powder, wax, oils, and tea obtained from the eggs, larvae or adults of the insects from the families Formicidae, Belastomatidae, Termitidae, Cicadidae, Gryllotalpidae, Asilidae, Pompilidae, Pentatomidae, etc. are used in north-east of Brazil for therapeutic purposes (Costa-Neto 2002).

In the European culture, the use of insects as a source of food or for therapeutic purposes is rarely mentioned (Ulicsni et al. 2016). Cantharidin, known in European traditional medicine especially for its high toxicity to human body, has anti-tumor properties (Rauh et al. 2007). Maggot therapy, simple and effective, has been used in Europe in the treatment of chronic wounds, such as diabetic food wounds or postoperative infections (Sherman et al. 2000). The use of the products provided by *Apis mellifera*, such as honey, venom, royal jelly, and propolis, was discussed in recent, comprehensive reviews (Pasupuleti et al. 2017; Wehbe et al. 2019).

The reviews by Zhou et al. (2005) and Park and Kim (2010) systematized the notable applications of chitin and its derivatives, which due to their biocompatibility and non-toxic nature were thoroughly studied to document their biological and biomedical properties. Furthermore, an increasing number of authors have observed the progress made during the last decades in the treatment of different conditions through the use of compounds obtained from insects and other arthropods. They support the development of insect-based biotechnologies and biotesting using insects in order to obtain new products for modern medicine (Ratcliffe et al. 2014; Ejiofor 2016; Seabrooks and Hu 2017). Ratcliffe et al. (2011) recommended the use of insects as models in the study of the immune response to human pathogens.

Moreover, in a world facing an alarming increase in bacterial and fungal resistance to antimicrobials, the identification of new substances or complex mixtures with antimicrobial properties has become a priority. For instance, strains of *Staphylococcus aureus* have a great variety in their resistance to antibiotics, often through horizontal gene transfer of genetic elements (Foster 2017), so new therapeutic solutions are required. *Candida albicans* and *C. parapsilosis* are human pathogens and also a normal commensal; the frequency of *C. parapsilosis* infections is higher in immunocompromised patients (Trofa et al. 2008) and they need an alternative solution for treatment.

The green stink bug *N. viridula* presents a remarkable polyphagia, with over 150 plant species identified as hosts; however, they prefer leguminous and brassicaceous plants and they cause serious damage to these plants (Oho and Kiritani 1960; Panizzi et al. 2000; Panizzi 2004). The very efficient secretory / defensive system developed by this species was probably one of the most important factors that led to their worldwide range expansion. A series of research studies was focused on the composition and the role of secretions in stink bugs (Gilby and Waterhouse 1965; Aldrich et al. 1978; Lockwood and Story 1987; Borges and Aldrich 1992; Pavis et al. 1994; Sturaro et al. 1994).

Starting from the wide distribution of the species *N. viridula* and the fact that it is an invasive species with specific defensive secretions, in the current paper we set out to investigate the cytogenotoxic and antimicrobial effects of alcoholic extracts of *N. viridula* as a platform for further applications. From our knowledge, this is the first paper to present the bioactivity of alcoholic extracts of *N. viridula*.

MATERIALS AND METHODS

Preparation of insect extracts

The biological material consisted of larvae and adults of *N. viridula* collected in the period July-October from cucumbers of the Cornichon variety from an organic culture located in the Cotmeana Plateau in southern Romania, from site N 44.98482°, E 024.72828°, altitude 348 m a.s.l. The larvae were reared in laboratory conditions until the emergence of adults; they were fed with organic cucumber leaves. The two genders were separated considering the phenotypic differences and the biological material was kept in the freezer at -18 °C until the preparation of extracts. The alcoholic extracts of *N. viridula* were obtained by grinding and macerating 5 g of each gender, females and males respectively, in 100 ml ethyl alcohol 96° and methyl alcohol 96°, respectively, for 48 hours, at room temperature (18-20 °C). The extracts were filtered using Whatman no. 1 filter paper.

Evaluation of genotoxic activity of male and female extracts

The cytogenotoxic effects of the *N. viridula* extracts were evaluated with the *Allium* test. The bulbs of *A. cepa* (a local variety) with a diameter of 3-4 cm were taken from a private farm. No phytosanitary treatments were applied to obtain the bulbs. Before use they were macro-

Table 1. Encoding of the samples.

Encoding	Male/Female (M/F)	Ethanol/Methanol (Et/Met)
NEM 5% / 15% / 25%	M	Et
NMM 5% / 15% / 25%	M	Met
NEF 5% / 15% / 25%	F	Et
NMF 5% / 15% / 25%	F	Met

scopically inspected to be free from pests. Root primordia were carefully exposed and immediately afterwards the bulbs were suspended in 30 ml containers with the discoid stem in contact with distilled water for 48 h. The treatment with ethanol and methanol female and male extracts of *N. viridula* with concentrations of 5%, 15% and 25% (Table 1) was applied for the next 48 h. The negative sample was represented by the onion roots obtained by suspending the bulbs with the discoid stem in contact with distilled water for 96 hours. Three identical samples were prepared for each specific sample configuration. Rhizogenesis was stimulated by keeping the containers in the dark at room temperature (20-22 °C).

After 96 hours of semi-static exposure (Rank 2003), the roots with a length of 5-10 mm were cut from the base with a sharp razor blade and immersed into Framer's fixative (absolute ethanol: glacial acetic acid, 3:1 v/v), over the night, at 4 °C, to preserve cell integrity. The fixed roots were hydrolysed in HCl 1N, at 60 °C, for 15 minutes by partial dissolution of pectic substances and stained with orcein-acetic solution 1%, for 15 minutes, at 60 °C. Microscopic slides were obtained through the squash technique. To prevent the quick drying of the microscopic slides the edges of the cover slips were sealed with nail varnish (Grant 1982).

Microscopic slides were analysed using an Olympus CX 31 microscope at a magnification of 400× (ocular - objective 10×40). The representative images of the different phases of mitosis, as well as of the chromosomal aberrations were captured using Color View I CCD digital camera.

For each experimental sample we analysed approximately 3 000 cells in different phases of the cell cycle. The mitotic index (MI) was determined as the percentage ratio of the total number of mitotic cells to the total number of cells examined in the microscopic preparation. The frequency of mitotic phases was determined by calculating the percentage ratio of the number of cells in a certain mitotic phase (prophase, metaphase, anaphase or telophase) to the total number of cells examined in the microscopic slides.

Evaluation of antimicrobial effect of insect extracts

To estimate the antimicrobial effect of insect extracts we used four standard bacterial strains (both Gram positive and Gram negative): *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* A Group ATCC 19615, *Bacillus subtilis* subsp. *spizizenii* ISM 68/53 (equivalent ATCC 6633) and *Escherichia coli* ATCC 25922. Two standard yeast strains of *Candida albicans* ATCC 10231 and *C. parapsilosis* ATCC 22019 were also used.

In vitro assessment of antimicrobial effects of *N. viridula* extracts were performed by disk diffusion test (Ma et al. 2019; Balouiri et al. 2016). Specific culture medium was used to test the sensitivity of standard bacterial strains: Mueller Hinton agar (MHA) for *S. aureus*, *B. subtilis* and E. *coli* strains, Mueller Hinton agar supplemented with 5% for *S. pyogenes* strain and Sabouraud agar for *Candida* strains (Graso Biotech).

The bacterial or yeast suspensions (0.5 McFarland) were inoculated on the sterile medium, then sterile filter paper disks (6 mm Ø) were placed onto medium surface. Each paper disk was impregnated with 10 µL of undiluted insect extracts. Ethanol 96° (E) and methanol 99.8% (M) were used as negative controls. Gentamicin 10 µg per disk (Tody Laboratories) and Fluconazole 25 µg per disk (Oxoid) were used as positive controls to test bacteria (ATB), and yeasts (AM), respectively. The experimental variants were coded according to Table 1. After 18-20 h at appropriate temperature (37 °C), the diameter of inhibition zones was measured and the average of those three values was compared. The yeast strains were incubated for 48 h to reveal the sensitivity to the extracts.

Statistical analysis

Each experimental variant comprised 3 trials. For the processing and valorisation of the data we used the statistical analysis program SPSS for Windows (Statistical Package for Social Science), version 20.0 (2010), applying the One-Way ANOVA model, and the Duncan's test for multiple comparison, respectively. The significance of the differences between the effects of the variables or the interaction between them, for which the calculated F had significant values at a level of confidence of 95%, was noted in small letters. The relationship between an interval variable and a categorical variable was determined by Eta correlation ratio. The results are presented as mean ± SE for n=3 bulbs/ sample.

RESULTS

Cytogenotoxic activity of male and female extracts of N. viridula

After 48 hours from the exposure to the alcoholic extracts, the cells in different mitotic phases were evaluated using the optical microscope; results are shown in Figure 1 as percentage of cells in mitosis. The MI determined for the control sample (5.96%) was not significantly different from the MIs calculated for the samples defined by the concentration 5% of the extracts of *N. viridula*. Similarly, compared to the control, the extracts of *N. viridula* with a concentration of 15% produced an insignificant increase of the MI, except for NEF 15%. However, increasing the concentration of the extracts to 25% was associated with a significant increase of MI in meristematic root cells, irrespective of the solvent used.

Regarding the correlation between the dependent and the nominal variables, the Eta coefficient of 0.638 indicates that 63% of the IM variation may be attributed to the independent variable (concentration), while the Eta coefficient of 0.09 indicates that there is a very slight positive correlation between gender and IM, with only 0.09% of the IM variation being attributed to gender (Table 2).

Figure 2 shows the results regarding the distribution of the phases of mitotic division in the root meristem cells of *A. cepa* exposed to the action of ethanol and methanol female and male extracts of *N. viridula.*

Table 2. Influence of nominal variables on the mitotic index in the root meristem cells of *A. cepa* (interpretation of the correlation between the dependent and the categorical variables using the Eta coefficient).

Directional measures		Value
Nominal by Interval Eta	Concentration Dependent	1.000
	IM Dependent	0.638
	Gender Dependent	0.957
	IM Dependent	0.098

In the control roots the values of the indices were of 75.4% for the prophase, 11.2% for the metaphase, 4.7% for the anaphase, and 8.6% for the telophase. Keeping the roots for 48 hours in ethanol and methanol extracts of *N. viridula* with a concentration of 5% led to a significant increase in the percentage of prophases associated with a significant decrease of metaphases, except for the sample NMM 5%.

The prophase index with a high value was associated with decreased or zero metaphase index. A significant increased metaphase index was noticed for NMM 5%, 15% and 25%. Compared with the control, the highest values of the anaphase index were recorded in the experimental samples NEF 15%, NEM 25% and NMM 25% (Figure 2).

The genotoxic effects of the alcoholic extracts of *N. viridula* were assessed by registering the chromosomal aberrations in the meristematic root cells of *A. cepa* (Table 3). Sticky chromosomes, anaphase bridges,

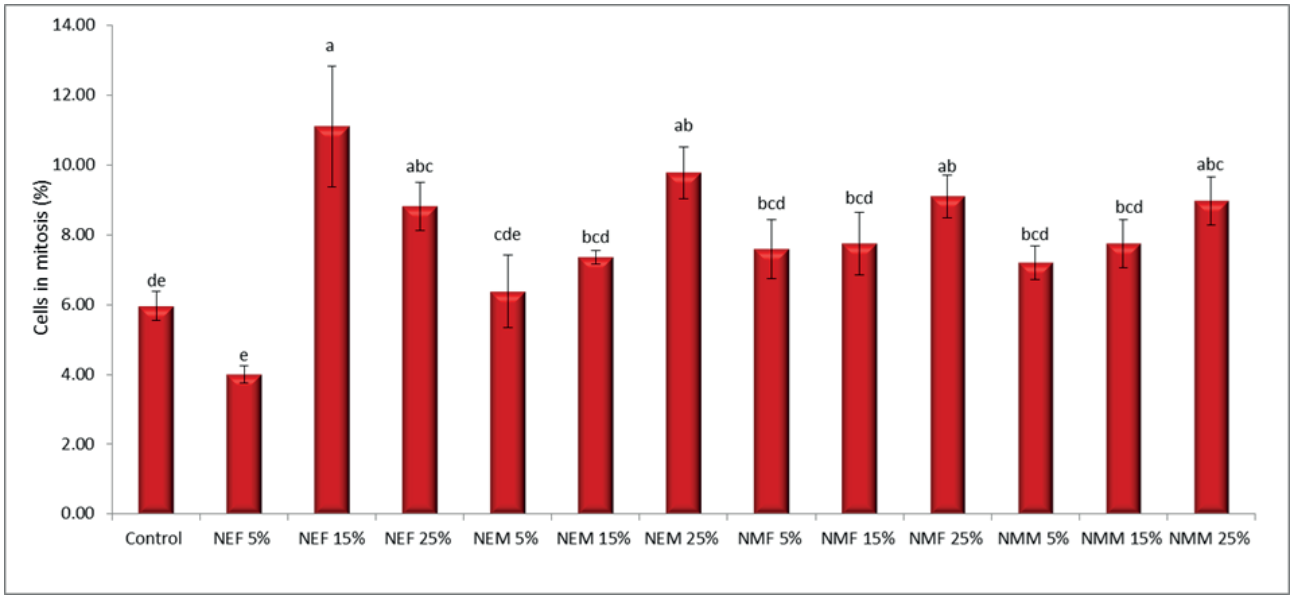

Figure 1. Influence of ethanol and methanol female and male extracts of *N. viridula* on the mitotic index in the meristematic root cells of *A. cepa* (a, b, c, d, e: interpretation of significant differences using Duncan's test, $p<0.05$).

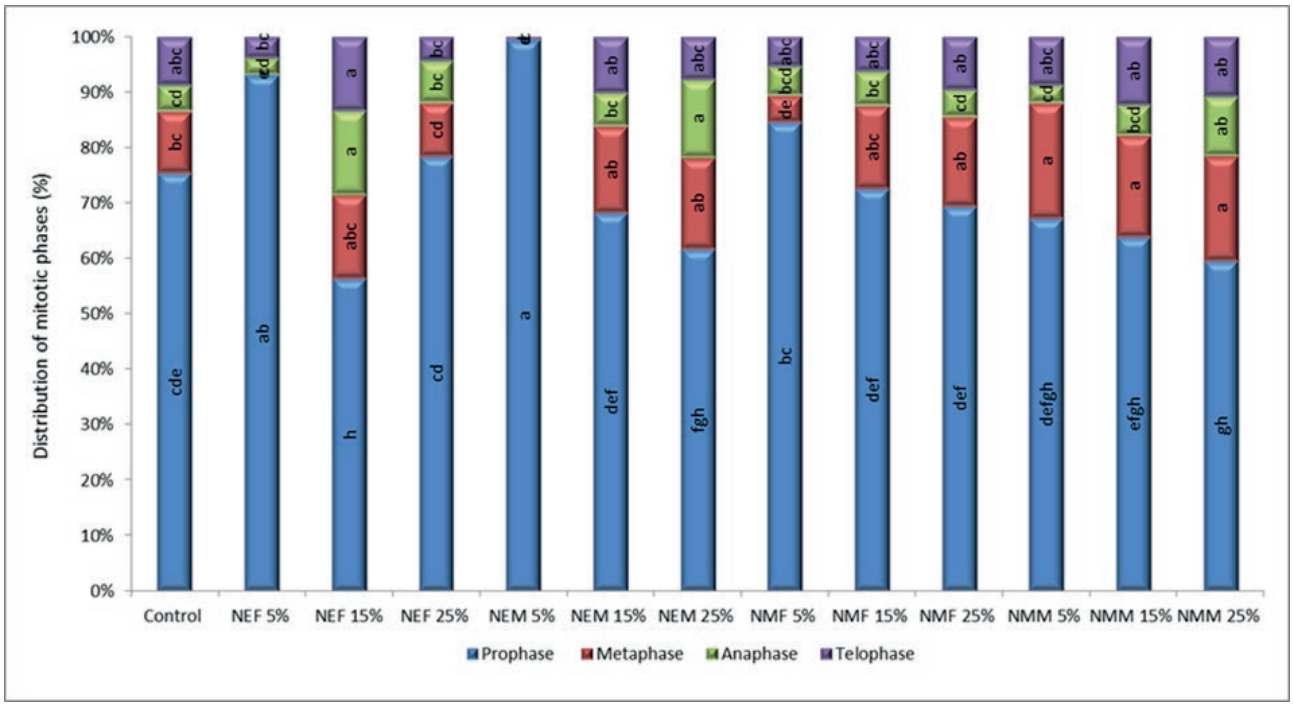

Figure 2. Influence of alcoholic extracts of *N. viridula* on the distribution of the phases of mitotic division in the root meristem cells of *A. cepa* (a, b, c, d, e, f, g, h: interpretation of significant differences, using Duncan's test, $p<0.05$).

C-mitoses, vagrant and laggard chromosomes, polyploidy, fragments of chromosomes, but also some mitotic anomalies such as micronuclei, binucleate cells or nucleoplasmic bridges (Figure 3) were observed with a variable frequency in root tip cells. The incubation of onion roots in extracts of *N. viridula* did not produce significant differences in the total frequency of chromosomal and mitotic aberrations, except for NEF 25%, the sample with the highest frequency.

Of the interphase anomalies, micronuclei were observed in the experimental samples defined by extracts at 15% concentration, and the nucleoplasmic bridges were more frequently identified in the cells treated with extracts of *N. viridula* female, irrespective of the solvent. Laggards, stickies, anaphase bridges and multipolar anaphases were predominant in the tested samples. The frequency of sticky chromosomes was ranging between 20.06% in the sample NMM 5% and 90.47% in the sample NEF 15%, and the anaphase bridges varied between 5.55% in the NMM 5% and 91.67% in NMF 15%.

Antimicrobial activity of male and female extracts of N. viridula

The antimicrobial effect of *N. viridula* extracts is presented in Table 4.

The largest zones of growth inhibition were observed for *Candida albicans* (between 11.33 mm and 15 mm) and *C. parapsilosis* (between 10.66 mm and 12.66 mm), under the action of ethanol extracts. While the same inhibition zone was induced by extracts and negative control in *C. albicans*, *C. parapsilosis* was sensitive to ethanol extracts.

The inhibitory effect produced by insect extracts on *B. subtilis* had almost the same value with the one for *C. parapsilosis*. Both ethanol and methanol extracts had a greater impact against bacteria than negative controls (an exception occurred for methanol extracts from females of *N. viridula*). With smaller values for the diameter of the inhibition zones than *B. subtilis*, but bigger than the negative controls, the *E. coli* strains demonstrated a sensitivity to insect extracts.

However, *S. aureus*, in correlation with its well-known resistance to many antimicrobial substances (Foster 2017), and *S. pyogenes*, revealed the smallest zones of growth inhibition. The *S. aureus* strain had a slight sensitivity to the methanol extract of males of *N. viridula*, and *S. pyogenes* demonstrated a slight sensitivity to the ethanol extract of males of *N. viridula*; the other values were smaller than those to negative controls.

Certain bacteria were influenced by insect extracts, depending on the gender of insects. For instance, for

Table 3. Frequency of chromosomal aberrations and mitotic anomalies induced by the action of alcoholic extracts of *N. viridula* in the root meristems of *A. cepa* (a, b, c, d, e, f, g interpretation of significant differences using Duncan's test, $p<0.05$).

Exposure variants	Micro-nucleus	Binucleate cells	Nucleoplasmic brisges	Vagrant forms	C-mitoze	Sticky chromosomes	Anphase bridges	Multipolar anaphase	Telophase bridges	Laggards forms	Others	Total
Control	-	0.2±0.03ab	-	-	-	35.74±12.16abc	14.09±5.9de	0.32±0.32b	-	0.32±0.32b	0.41±0.41a	1.26±0.41b
NEF 5%	0.65±0.21a	0.22±0.05ab	0.33±0.33b	-	-	-	-	0.27±0.27b	2.08±2.08 ab	0.69±0.12a	0.17±0.06a	2,5±0.32b
NEF 15%	-	0.45±0.45a	16.66±9.62a	-	-	90.47±4.76a	-	-	-	-	0.14±0.1a	3.32±0.76b
NEF 25%	-	-	3.45±1.03b	-	-	77.5±11.46ab	76.39±6.06abc	-	-	-	0.03±0.03a	9.48±2.39a
NEM 5%	0.96±0.22a	0.18±0.06ab	-	-	-	-	-	0.33±0.33b	-	0.33±0.33 b	0.64±0.27a	2.03±0.51b
NEM 15%	-	-	-	1.75±1.75a	-	85.67±7.69ab	77.78±14.7ab	0.06±0.06c	-	1.45±1.45 a	-	1.43±0.26b
NEM 25%	-	-	-	-	-	86.75±6.88ab	78.71±7ab	-	-	-	-	2.46±0.22b
NMF 5%	0.81±0.45a	-	0.04±0.04b	-	-	73.81±14.48ab	35.55±19.37cd	0.11±0.11c	-	0.11±0.11b	0.05±0.03a	1.57±0.35b
NMF 15%	-	0.04±0.04ab	0.1±0.1b	-	-	61.52±6.24abc	91.67±8.33a	0.96±0.53a	-	0.96±0.53 b	-	2.55±0.43b
NMF 25%	-	0.08±0.04ab	0.69±0.35b	-	-	37.61±1.81abc	41.66±12.73cd	0.19±0.09c	-	0.19±0.09b	0.03±0.03a	1.74±0.37b
NMM 5%	0.15±0.06b	0.03±0.03ab	0.18±0.14b	2.22±2.22a	68.53±11.88a	20.06±0.77bc	5.55±5.55e	0.1±0.06c	9.52±9.52 a	2.56±2.56a	4.76±4.76a	3.41±0.7b
NMM 15%	-	-	-	-	1.19±1.19b	65.28±63.65abc	61.11±13.89abc	-	-	-	-	2.21±0.48b
NMM 25%	-	-	-	-	6.95±3.56b	64.68±4.57abc	50.53±3.68bc	-	-	-	-	1.57±0.13b

Figure 3. Chromosomal and mitotic aberrations identified in the root tips of *A. cepa* exposed to the extracts of *N. viridula*: (a) sticky chromosomes – NEM 15%; (b) anaphase bridges – NEM 25%; (c) C-Mitosis – NMM 15%; (d) giant cell – NEF 25%; (e) vagrants – NEM 15%; (f) micronucleus – NMF 15%; (g) binucleate – NEM 5%; (h) polyploidy – NMM 15%; (i) telophase bridge and chromosome fragment – NEF 25%; (j) laggard – NMF 15%; (k) multipolar telophase – NMM 15%; (l) star polar anaphase – NEM25%; (m) multipolar anaphase – NMM 15%; (n) nucleoplasmic bridges – NEF 5%; (o); (p) apoptotic bodies – NMF5%.

Figure 4. The inhibition zones induced by alcoholic extracts of *Nezara viridula* (L.): (a) Gram negative bacteria - bacillus; (b) Gram positive bacteria - bacillus; (c) Gram positive bacteria - coccus; (d) Eukariotic microorganism - yeast.

B. subtilis the inhibition zones had higher diameters by the ethanol extracts from females of *N. viridula* and the methanol extracts of males of *N. viridula*. At the same

Table 4. The diameter of inhibition zones induced by alcoholic extracts of *N. viridula*.

Experimental variants	Inhibition zone (mm) *S. aureus*	Inhibition zone (mm) *S. pyogenes*	Inhibition zone (mm) *B. subtilis*	Inhibition zone (mm) *E. coli*	Inhibition zone (mm) *C. parapsilosis*	Inhibition zone (mm) *C. albicans*
NEM	8.66	9.33	12.66	11.66	12.66	15
NEF	8.66	8.66	13.33	11.66	12.33	15
NMM	9.33	7.33	11.66	9	10.66	12
NMF	7.66	7.66	9	9	11.33	11.33
Ethanol (E)	8.66	9	11.66	10	12	15
Methanol (M)	8.66	8	9	8	11.33	14
Gentamicin 10 μg (ATB)	25	28	31	25	-	-
Fluconazole 25 μg (AM)	-	-	-	-	37	25

time, *Escherichia coli* were equally inhibited by the extracts from males or females of *N. viridula*.

All the values obtained for the growth inhibition zones under the action of insect extracts were smaller than the values for positive controls (either antibiotic or antifungal agents).

DISCUSSIONS

Statistical analysis of the data revealed differences and significant differences of the cytogenotoxic endpoints under research. Aldrich et al. (1978) found that the repugnant defensive secretion from both males and females of *N. viridula* contains (*E*)-2-hexenal, hexanal, 1-hexanol, and *n*-tridecane. Although the above authors found the *n*-tridecane content was three times more than males, in our study mitosis progression was influenced by gender only by ethanolic samples which induced the highest and the lowest MI. One of the main mechanisms involved in cytotoxicity and genotoxicity is the overproduction of reactive oxygen species (ROS) that can induce reversible and irreversible changes in proteins and cause DNA damage (Zhu et al. 2013; Choudhury et al. 2016; Tanaka and Hadwiger 2017). In this context, it is worth mentioning that aldehydes, such as hexanal can act as secondary messengers of oxidative stress controlling cell proliferation, cell differentiation and cell death (Barerra et al. 2008; Barrera 2012). The cytotoxicity of extracts, which was dependent on concentration, could be attributed to epoxides (Marshall and Caldwell 1996), which are the main constituents of pheromones in the males of *N. viridula* (Brézot et al. 1994).

The cytotoxic effect of the *N. viridula* extracts was manifested through an increase in prophase frequency and a decrease in metaphase frequency, suggesting that cells underwent mitosis, but were arrested during prophase. Inhibition of an early mitotic stage could be due to the alteration of the chromosome condensation mechanism or the inhibition of the microtubule assembly mechanism, which leads to prometaphase arrest (Oliva et al. 2002). NMM, irrespective of their concentration, induced a significant metaphase arrest in root tip cells suggesting the disturbed spindle function, which produced C-mitosis and polyploidy (Figure 3).

Chromosomal aberrations and micronuclei are biomarkers of genotoxicity and chromosomal instability determined by mutagenic agents (Bonciu et al. 2018). As complex mixtures, the extracts act as clastogenic agents inducing the formation of ana-telophase bridges and micronuclei, but also as aneugenic agents by inducing delays, adherence and multipolarity (Leme and Marin-Morales 2009). However, analysing the total frequency of chromosomal and mitotic aberrations it was noticed that NEF 25% induced the highest genotoxicity. The analytical characterization of the extracts could add a clue regarding the high genotoxicity, especially for the ethanol female extracts of *N. viridula*. Until now, literature analysis provides scarce information on the cytogenotoxic effects of extracts obtained from insects, in terms of investigating their therapeutic potential. A series of studies noticed the absence of cytogenotoxic effects in the case of extracts obtained from edible insect species, including *Zonocerus variegatus*, *Oryctes boas* (Memiş et al. 2013), *Onitis* spp., *Caelifera* spp. and *Gryllotalpa* spp. (Koc et al 2014), *Locusta migratoria* (Turkez et al. 2014), confirming the safety of their consumption by humans. Several recent papers presented the results of research on the genotoxic effects of extracts obtained from other invertebrate animals. Jayathilake and Jayewardena (2021) investigated with the *Allium* test aqueous extracts from the sea cucumber, *Bohadschia vitiensis*, known for certain biological activities and found reduced genotoxic effects consisting of 0.1-0.2% chromosomal aberrations

including chromosomal bridges, c-mitosis, chromosomal breaks, and vagrants. By using the *Allium* bioassay, the mitodepressive effect of the marine sponge extracts *Luffariella herdmani* was highlighted, the results obtained suggesting the antitumor potential of the substances contained (Kuruppuarachchi et al. 2023).

In our study, the antimicrobial effect of insect extracts was noted especially for ethanol extracts of *N. viridula* against those microorganisms which usually are opportunistic pathogens.

The bacteria and yeast with higher pathogenic properties (*S. aureus*, *S. pyogenes* and *C. albicans*) are less sensitive to the action of extracts, although other studies emphasized that Methicillin-resistant *S. aureus* can be inhibited by extracts from insects used in traditional Chinese medicine (Ma et al. 2019) or that *Candida albicans* growth can be inhibited by peptides from supermeal worm, *Zophobas morio* (Fabricius) (Faruck et al. 2017).

Some studies found the antimicrobial activity of certain edible insects to their microbiota, particularly in the case of antimicrobial peptides that can be used for developing new drugs against multidrug-resistant pathogens (Mudalungu et al. 2021). Antimicrobial peptides (AMPs) from insect sources were also used to reduce biofilm associated *S. aureus* and *E. coli*; the authors found out the AMPs combined with antibiotics may be a better alternative than antibiotics alone (Sahoo et al. 2021).

The effect of ethanol as a negative control was mostly higher than methanol and the diameter of inhibition zones was the largest for positive control (antibiotic or antifungal agent). Neither bacteria nor yeasts were affected by insect extracts more than by standard antimicrobials (Figure 4). The results revealed some differences between extracts according to the gender of insects, but no obvious correspondence was established.

CONCLUSION

The mitotic index recorded after the exposure of the onion roots to the action of alcoholic extracts of *N. viridula* indicated specific and significant variations in relation to solvent and dilution. The ethanolic extracts determined the widest variation of the mitotic index, regardless of the tested concentration, while the methanolic extracts had significant mitostimulatory effects only in higher concentrations. The lower concentration extracts were associated with a blocking of cells in prophase, while the extracts obtained from *N. viridula* males determined the increase in the frequency of metaphases.

The antimicrobial effect of alcoholic extracts from *N. viridula* was obvious against bacterial strains *Escherichia coli* and *Bacillus subtilis*; *Staphylococcus aureus* and *Streptococcus pyogenes* presented only a slight sensitivity to the insect extracts. The differences between male or female insect extracts regarding their antimicrobial activity were unsteady, requiring further investigation into the production and specific application of insect extracts.

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