



ENVIRONMENTAL THREATS AND BIOLOGICAL CONTROL STRATEGIES IN INSECTS: A MICRONUCLEUS ASSAY APPROACH

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ABSTRACT

Micronuclei (MN) are typically small extra-nuclear structures that do not belong in the main nucleus during telophase and are formed from acentric chromatid/chromosome fragments or full chromatid/chromosomes that lag at the anaphase of dividing cells. MN is now the most common indicator of chromosomal abnormalities brought on by genotoxic stressors because of their simplicity of detection. The present study employs the micronucleus assay to evaluate the impact of environmental threats and biological control methods on insect populations. Micronucleus formation serves as a sensitive indicator of genotoxicity and mutagenicity, allowing for the detection of potential harm to the insects caused by environmental stressors or the effectiveness of biological control agents. The MN test has been used as one of the cytogenetic endpoints in the testing of several clastogenic and aneugenic toxins in various cells and tissues. This paper aims to contribute valuable insights into the overall health and stability of insect populations in response to environmental changes and the implementation of various biological control strategies.

KEY WORDS: Environmental threats, Biological control, Genotoxicity, Pest management, Aneugens

INTRODUCTION

Large-scale urbanization, industrialization, an excessive use of pesticides and herbicides in agriculture, and other anthropogenic activities affect genomic integrity and cause serious health issues to humans (Srivastava *et al.*, 2016). These factors also affect other organisms and ecosystems resulting into the loss of habitats and biodiversity including reduced abundance and species richness (Siroski *et al.*, 2016; Piccini *et al.*, 2023). These include industrial wastes, particulate matter (PM), heavy metals contamination in soil and water, and excessive use of pesticides to suppress targeted pests in agriculture which also reduce the fitness of non-target species (Cavas & Konen, 2007; Poletta *et al.*, 2009; Isenring, 2010; Santovito *et al.*, 2020; Piccini *et al.*, 2023).

Insects are an important component of the ecosystem and show immense diversity, ecological role and behavior. These influence agriculture, human health, and natural resources (Scudder, 2017). Environmental threats like

climate change, Industrial pollutants, heavy metals, temperature, radiation, and pesticides, etc. severely affect insects' genetic health and consequently cause delayed life cycle and compromised reproductive capabilities and eventually their population dynamics and diversity level (Omkar & Parvez, 2004; Omkar *et al.*, 2008; Mishra & Tewari, 2011, 2014; Mishra *et al.*, 2013; Skend•ic *et al.*, 2021; Zhao *et al.*, 2023). The widespread use of pesticides in agriculture eventually cause potential harm to non-target species also (Windham *et al.*, 1998; Bolognesi *et al.*, 2011). Thus it is imperative to assess effects of these stressors to maintain the insect population. Insects can be employed to evaluate direct effect of these genotoxicants (Rosenberg *et al.*, 1986; Mishra *et al.*, 2017) or can be used to determine the appropriate dosages of formulations intended to control of nuisance insects (Rafael *et al.*, 2008).

Any change occurred to genomic integrity spontaneous or induced known as genotoxicity. Several *in vitro* and *in vivo* genotoxicity assessment assays are known and each provide a specific information about the

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integrity of genome (Bajpayee *et al.*, 2005; Mohamed *et al.*, 2017). These assays allow an assessment of impact of toxicants on humans and other organisms as well (Srivastava *et al.*, 2016; Mishra *et al.*, 2017). The most commonly used *in vitro* assays is to evaluate the induction of nucleotide mutation, e.g., bacterial reverse mutation assay (Ames test), mammalian cell gene mutation assay or mouse lymphoma assay, and while more complex genome changes can be assessed by cytogenetic methods, such as the sister chromatid exchange assay, analysis of chromosomal aberration frequency, micronucleus assay (MN) and comet assay (single-cell gel electrophoresis) (Bajpayee *et al.*, 2005; Mishra *et al.*, 2017; Sommer *et al.*, 2020; Mishra & Srivastava, 2023). Among these assays, MN assay is one of the most convenient, cost-effective, and easy to perform with less expertise and time required assay. The MN assay is a valuable tool in toxicology and environmental studies. It detects genetic damage in cells by observing the presence of micronuclei, which are small extra nuclei that can form when a cell is exposed to genotoxic agents.

Assessing environmental threats and implementing biological control strategies in insects through a MN assay approach renders a comprehensive understanding of both the environmental stressors and the potential control mechanisms. In the context of insects, this assay can provide mechanistic insights into the impact of environmental threats and stressors on their genetic integrity (Ye *et al.*, 2019). This review focuses on the effective use of micronucleus assay in insects for assessment of environmental threats and assessing novel biological control strategies for pest management without causing harm to non-target organisms.

MICRONUCLEUS ASSAY: A TOOL FOR COMPREHENSIVE ASSESSMENT

Errors occurred during the metaphase/anaphase transition of mitosis, either from the whole lagging chromosome (aneugenic event) or lagging acentric chromosome fragments caused by chromosomal breakage (clastogenic event) results into the formation of extranuclear chromatin fragments which do not integrate in the daughter nuclei following anaphase known as Micronuclei (MN) (Fenech *et al.*, 2011). Therefore, micronuclei can only arise in cells that have undergone cell division. In early 1900s, hematologists William Howell and Justin Jolly MN identified MN in human reticulocytes first time, as feulgen-positive bodies, which were later named as Howell-Jolly bodies (Dawson & Bury, 1961).

In ionizing radiations exposed *Vicia faba* root cells, induction of these extranuclear bodies was first recorded by Thoday (1951) and further micronuclei were quantitatively measured as an indicator of cytogenetic

damage in neutron and gamma-rays treated *Vicia faba* root cells (Evans *et al.*, 1959). This idea of quantitatively measure induced micronuclei as an indicator of radiation caused genotoxicity in humans was propounded by Fleidner *et al.* (1964). Boller & Schmid (1970) and Heddle (1973) used the term micronucleus test (MNT) for the first time and suggested that MNT is a simple method to detect the genotoxic potential of mutagens and the results of cytogenetic studies on the incidence of chromosome aberrations can be correlated with the formation of micronucleus.

There are multiple mechanisms that could involve in micronucleus or acentric chromosome fragments formation discussed by Fenech *et al.* (2011). For instance 1) Lagging acentric chromosome or chromatid fragment at anaphase due to either misrepair of DNA breaks or unrepaired DNA breaks, 2) Lagging whole chromosomes at anaphase due to either hypomethylation of repeat sequences in centromeric and pericentromeric DNA or defects in kinetochore proteins or assembly or dysfunctional spindle or defective anaphase checkpoint genes or unresolved replication stress intermediates.

MICRONUCLEUS ASSAY: COMPONENTS IN SUSTAINABLE PRACTICES

The basic requirement for Micronucleus assay is sample collection, like tissues or cells from different animal groups, invertebrates and vertebrates, such as insects, from different environments or populations for analysis of genotoxicity and mutagenicity *in vitro* and *in vivo* (Mishra *et al.*, 2017). Firstly, the cells from the insects are isolated and prepared for the micronucleus assay, which involves staining and microscopic examination to detect micronuclei, indicating genetic damage. Quantification of micronuclei and statistical analysis help in understanding the extent of genetic damage caused by various stressors and factors (*i.e.*, temperature, radiation, chemicals, etc.) utilized in biological control strategies and Integrated Pest Management (IPM). The implementation of IPM strategies may reduce reliance on chemical pesticides by incorporating biological control agents like predators, parasitoids, or pathogens (Jeffers & Chong, 2021). This can give rise to the upsurge, development and application of bio-pesticides derived from natural sources like fungi, bacteria, or botanical extracts to target specific insect pests while minimizing environmental impact. Apart from these implications of simple, rapid, cost-effective and time saving properties of micronuclei assay, advanced genetic approaches may be introduced that utilize genetic engineering or modification techniques to develop insect-resistant varieties or sterile insect techniques to control pest populations (Hendrichs & Robinson, 2009; Alphey & Bonsall, 2018).

Table 1: Use of Micronucleus assay in Insects for different studies

Species	Tissue	Genotoxicants	Systematic position (Order: Family)	References
<i>Galleria malonella</i>	Haemolymph collected from 7th-instar larvae	Piperazine	Lepidoptera: Pyralidae	Alkan <i>et al.</i> , 2023
<i>Pieris brassicae</i> (cabbage butterfly)	Late instar larvae body cells	Particulate matter (PM; atmosphere, anthroposphere)	Lepidoptera: Pieridae	Piccini <i>et al.</i> , 2023
<i>Pimelia latreillei</i>	Testicular cells	Industrial heavy metals	Coleoptera: Tenebrionidae	El-Samad <i>et al.</i> , 2021
<i>Lycaena dispar</i> Hawort (Protectected Butterfly)	Epithelial cells from last-instar larvae	Glyphosate (herbicide)	Lepidoptera: Lycaenidae	Santovito <i>et al.</i> , 2020
<i>Galleria mellonella</i> L.	Haemolymph collected from 7th-instar larvae	Boric acid	Lepidoptera: Pyralidae	Gwokyalaya & Altunta ^o , 2019
<i>Galleria mellonella</i> L.	Haemolymph collected from last instar larvae	Imidacloprid	Lepidoptera: Pyralidae	Yukel & Kayis, 2019
<i>Tribolium castaneum</i> Herbst, <i>Callosobruchus maculatus</i> (F.) and <i>Sitophilus oryzae</i> (L.)	Gonads of female specimens	Gamma radiation	Coleoptera: Tenebrionidae Coleoptera: Bruchidae Coleoptera: Curculionidae	Ahmadi <i>et al.</i> , 2015
<i>Aedes aegypti</i>		Two derivatives of Dillapiol	Diptera: Culicidae	Domingos <i>et al.</i> , 2014
<i>Musca domestica</i>	neural ganglia of third instar larvae	Cold temperature	Diptera: Muscidae	Mishra & Tewari, (2014)
<i>Musca domestica</i>	neural ganglia of third instar larvae	High temperature	Diptera: Muscidae	Mishra & Tewari, (2013)
<i>Musca domestica</i>	neural ganglia of third instar larvae	Mercuric Chloride	Diptera: Muscidae	Mishra & Tewari, (2011)
<i>Aedes aegypti</i>	brain ganglial smears from fourth-instar larvae and of ovarian smears from pupae	Piper aduncum L. (Piperaceae) extract; Dillapiol, a phenylpropanoid	Diptera: Culicidae	Rafael <i>et al.</i> , 2008

Understanding the potential risks posed by environmental stressors helps in devising effective control strategies also. Hence, regular monitoring programs using the micronucleus assay aids in assessing the success of implemented control measures and the overall health of insect populations. In addition, continuous adaptive management and refining control strategies based on assessment outcomes and new research findings assist in these studies for environmental protection.

USE OF MICRONUCLEUS TEST IN INSECTS

The class Insecta is highly diverse and further divided into 29 orders (has 8 main orders) among those 4 belongs to the division Apterygota and 25 belongs to Pterygota

(<http://ecoursesonline.iasri.res.in/mod/page/view.php?id=10897>). Among insects, belonging to these orders, several studies are available regarding micronuclei induction and are summarized in Table 1. Since the MN test requires dividing cells, insects are a good fit because they undergo metamorphosis as part of their life cycle. This indicates that cells of certain tissues, such as neural ganglia, gonadal tissues, and imaginal disc, etc. have always been in the dividing phase during the larval and pupal stages (Cevallos & Nation, 2004; Rafael *et al.*, 2008; Sharakhova *et al.*, 2011; Santovito *et al.*, 2020; El-Samad *et al.*, 2021). Other than those, hemolymph can also be used for this assay (Gwokyalaya & Altunta^o, 2019; Yukel & Kayis, 2019).

The insecticidal activity of dillapiol, an essential oils of leaves of *Piper aduncum* (Piperaceae), and its derivatives was tested *in vivo* on the larvae and pupae of *Aedes aegypti*, the mosquito vector of dengue by Rafael *et al.* (2008) and Domingos *et al.* (2014) using cytogenetic end points like induction of nuclear anomalies including micronucleus and chromosome aberrations. They suggested that the larval and pupal survival is affected by the dillapiol exposure. Similarly, Ahmadi *et al.* (2015) studied the effects of gamma radiation, as an alternative direct method for stored product pest control, on mortality and micronucleus formation in *Tribolium castaneum* Herbst, *Callosobruchus maculatus* (F.) and *Sitophilus oryzae* (L.) genital cells. The results showed that gamma radiation has the ability to induce micronuclei and the proportion of micronuclei induced in the genital cells of adults increased with increasing irradiation doses resulting high mortality.

Mishra & Tewari (2011, 2014) and Mishra *et al.* (2013) also reported the effect of mercuric chloride and temperature on housefly larvae using chromosomal aberration and MN assay as cytogenetic end points. Results showed that the larval and pupal mortality and development delay in exposed larvae along with micronucleus induction. The greater wax moth, *Galleria mellonella* L., is considered as one of the most important pests of honeybee products due to feeding habit of its larvae, were exposed to boric acid, piperazine, and imidacloprid in different studies and results reported the induction of micronucleus along with other toxicity markers (Yukel & Kayis 2019; Gwokyalya & Altunta^o, 2019; Alkan *et al.*, 2023).

The genotoxic effect of glyphosate, a common herbicide, on *L. dispar* larvae was assessed by micronuclei (MN). Results suggests that DNA fragments (MN) were separated from the main nucleus as an indicator of genomic instability that can result in a reduction of vitality and an increased extinction risk (Santovito *et al.*, 2020). Piccini *et al.* (2023) reported the genotoxic effects of particulate matter (PM) at different concentration on common and widespread butterfly larvae. They further suggested that PM could work in addition to and/or in synergy with other compounds like pesticides and affecting the vitality of populations of species those are already threatened by other factors (e.g. fragmentation), thus, leading to local extinctions as a consequence of urbanization.

The above studies renders enough evidence that MN assay is quite effective as an indicator of genomic instability (genotoxicity and mutagenicity) and suggests that genotoxicity can be the reason behind decreased survival rate and low fecundity which leads to disturbance in insect population dynamics.

CONCLUSIONS

The micronucleus assay serves as a valuable tool to formulate bio-control strategies and managing insect population by assessment of mutagenicity and genetic damage caused by other control methods or various stressors in insects. Use of this method ensures the development and implementation of sustainable and environment friendly approaches for insect pest management. Studying micronuclei and their correlation with genotoxicity and mutagenicity in insects due to environmental threats provides mechanistic insights into the effect of these stressors on insects and their population dynamics. Utilizing this knowledge is one of the essential components for effective pest management while safeguarding the environment and biodiversity. Micronucleus assay to evaluate genetic damage in insects due to environmental threats is crucial for understanding their susceptibility and devising effective biological control strategies to mitigate the threats and preserve ecosystem health. MN serves as potential biomarkers in bio-monitoring of broad range insect groups including both the useful and harmful that play an important role in ecological balance.

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