



GENOTOXIC EFFECTS OF THIAMETHOXAM (ACTARA) ON SPATHOSTERNUMPRASINIFERUM: ASSESSING DNA DAMAGE AND CHROMOSOMAL ABERRATIONS IN GRASSHOPPER SPECIES

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Abstract: The widespread use of thiamethoxam, a neonicotinoid insecticide, has raised concerns about its potential genotoxic effects on non-target organisms. This study aimed to investigate the genotoxicity of thiamethoxam on the grasshopper species *SpathosternumPrasiniferum*. We assessed DNA damage and chromosomal aberrations in grasshopper individuals exposed to sublethal concentrations of thiamethoxam using the alkaline comet assay and micronucleus test, respectively. Our results demonstrate a significant increase in DNA damage and the formation of micronuclei in grasshoppers exposed to thiamethoxam compared to the control group, indicating genotoxic effects.

Keywords: thiamethoxam, genotoxic effects, *SpathosternumPrasiniferum*, grasshoppers, DNA damage, chromosomal aberrations, neonicotinoid insecticide, ecological risks, non-target organisms, pesticide risk assessment, genetic integrity, population dynamics, ecosystem health, biodiversity.

Introduction:

Thiamethoxam is a neonicotinoid pesticide that has been widely used. Its potential impact on organisms other than the target species, however, has caused environmental concerns. Neonicotinoids, including thiamethoxam, are systemic insecticides that are extensively applied in agricultural practices to control a wide range of pests such as aphids, leafhoppers, and beetles. Although their efficacy against target insects is well-established, there is growing apprehension about the unintended consequences on non-target species and ecosystem health.

The genotoxic effects of pesticides have gained significant attention in recent years, as they pose potential risks to the genetic integrity of exposed organisms. Genotoxicity refers to the capacity of a substance to induce damage to DNA and chromosomes, leading to alterations in the genetic material. The alterations may manifest themselves as DNA breaks, chromosomal abnormalities, or the formation of microscopic nuclei. Genotoxic damage can have severe consequences, including mutations, cell death, impaired reproduction, and overall fitness reduction in affected individuals.

Spathosternumprasiniferum is a species of grasshopper that inhabits various ecosystems. It serves as an ecological indicator, and has a vital role to play in the dynamics of ecosystems. Due to their susceptibility and feeding behavior, grasshoppers can be exposed to pesticides residues on agricultural landscapes despite their importance. Thus, assessing

the genotoxic effects of pesticides, such as thiamethoxam, on grasshoppers is essential to understand the potential ecological risks associated with their use.

Studies examining the genotoxic effects of thiamethoxam on non-target organisms have primarily focused on pollinators such as honeybees and butterflies. There has only been limited research on thiamethoxam's genotoxic effect on grasshopper species. It is therefore important to evaluate the ecological effects of thiamethoxam and to contribute to the understanding of pesticide induced genotoxicity.

This study aims to assess the genotoxic effects of thiamethoxam on *SpathosternumPrasiniferum* grasshoppers. Specifically, we will examine DNA damage using the alkaline comet assay, which allows the detection of single- and double-strand breaks in DNA. We will also evaluate chromosomal abnormalities using the micronucleus assay, which allows the identification of both structural and numerical changes to chromosomes. By examining both DNA damage and chromosomal aberrations, we aim to provide a comprehensive understanding of the genotoxic effects of thiamethoxam on this grasshopper species.

This study's findings will add to our understanding of the ecological effects of thiamethoxam, and help us develop effective risk management strategies. Understanding the genotoxic effects of thiamethoxam on grasshoppers is vital for safeguarding ecosystem health and maintaining the balance of agricultural landscapes.

Literature Review

Thiamethoxam, a widely used neonicotinoid insecticide, has raised concerns due to its potential genotoxic effects on non-target organisms. In recent years, studies have focused on assessing the genotoxicity of thiamethoxam in various species to understand its ecological risks. This literature review provides an overview of the current knowledge regarding the genotoxic effects of thiamethoxam on *SpathosternumPrasiniferum* grasshoppers.

Several studies have demonstrated the genotoxic potential of thiamethoxam in *SpathosternumPrasiniferum* grasshoppers. Anderson et al. (2023) conducted a study assessing DNA damage and chromosomal aberrations in thiamethoxam-exposed grasshoppers. They found a marked increase in micronuclei and DNA damage, which indicated genotoxicity. Similarly, Kumar et al. (2022) conducted a study using the alkaline comet assay and reported significant DNA damage in grasshoppers exposed to thiamethoxam.

The genotoxic effects observed in *SpathosternumPrasiniferum* grasshoppers highlight the importance of considering the potential ecological risks associated with thiamethoxam. Smith et. al. The impact of DNA damage on fitness and reproduction success in grasshopper populations was highlighted by (2023). They suggested that genotoxicity induced by thiamethoxam can have cascading effects on population dynamics and ecosystem functioning.

The concerns about the genotoxic effect of neonicotinoids on non-target organisms are similar to those raised by thiamethoxam's effects in grasshoppers. Previous research has primarily focused on the genotoxic effects of neonicotinoids on pollinators, such as bees and butterflies. However, Gupta et al. (2023) highlighted the importance of evaluating the genotoxic potential of neonicotinoids in grasshoppers as they play significant roles in ecosystem processes and serve as ecological indicators.

The regulatory frameworks used to assess pesticide risks are primarily focused on acute toxicities, and genotoxicity is often neglected. Patel et al. (2022) stressed the importance of incorporating genotoxicity evaluations into pesticide registration and reevaluation

processes to ensure a comprehensive understanding of their ecological impacts. The group stressed the importance of a precautionary strategy and effective mitigation strategies in order to protect non-target species.

In the future, research should be focused on understanding the mechanisms that cause thiamethoxam to induce genotoxicity among *SpathosternumPrasiniferum* Grasshoppers. Johnson et al. 2022 suggested that specific pathways or cellular processes affected thiamethoxam in order to understand the molecular base of its genotoxic effect should be investigated. Moreover, Sharma et al. The study of the effects of genetic damage to grasshopper populations on **dynamics of communities and ecosystem** was emphasized by Sharma et al.

Methods

The collection of grasshoppers and their maintenance is crucial to the viability and health of experimental subjects. This is a detailed explanation of the methods for collecting and maintaining grasshoppers:

1. **Collection of Grasshoppers:** *SpathosternumPrasiniferum* was collected in areas free from pesticides and with habitats similar to their natural environments. Care was taken to choose collection sites that had minimal or no exposure to agricultural pesticides, ensuring that the grasshoppers used in the study were representative of non-exposed populations. The adult grasshoppers were gently captured using entomological nets. The nets were carefully maneuvered to minimize harm or stress to the captured individuals.
2. **Transfer to the Laboratory:** After collection, the grasshoppers were transferred to the laboratory in well-ventilated containers or cages. Containers were created to mimic their habitat as closely as possible. It is essential to maintain appropriate temperature, humidity, and lighting conditions during the transfer to prevent any undue stress or physiological changes in the grasshoppers.
3. **Acclimation Period:** The grasshoppers received an acclimation time upon arrival in the lab. This period allows the grasshoppers to adjust to the new laboratory conditions gradually. The acclimation period typically lasted for a defined duration, during which the grasshoppers were exposed to controlled environmental conditions. *SpathosternumPrasiniferum* thrives in temperatures between 25+-2degC. The relative humidity level was 60+-5% to ensure adequate moisture for grasshoppers. To simulate the natural cycle of day and night, a regular 12-hour photoperiod was set up.
4. **Diet:** The grasshoppers received a diet closely matching their preferences for natural vegetation during the acclimation phase and the entire duration of the experiment. As food, fresh vegetation was offered, such as grasses and leaves. The diet composition was based on prior knowledge of the grasshoppers' natural diet, ensuring that their nutritional requirements were met. The food was regularly replenished to maintain a constant supply of fresh and appropriate vegetation for the grasshoppers.
5. During the experimental and acclimation periods, grasshoppers are regularly checked for any signs of illness or injury. The grasshoppers were observed to make sure they exhibited normal behaviors, eating patterns and reproduction activities. Individuals exhibiting signs of stress or illness were either isolated or given the necessary veterinary treatment. Temperature, humidity and lighting were monitored closely and adjusted as needed in order to keep grasshoppers healthy.

By following these procedures for grasshopper collection and maintenance, it ensured that the experimental subjects were healthy, adapted to the laboratory conditions, and provided with suitable nutrition, thus minimizing any confounding factors that could potentially affect the subsequent genotoxicity assessments.

Results:

The DNA damage and chromosomal abnormalities of *SpathosternumPrasiniferum* were measured using both the micronucleus assay, which measures chromosomal anomalies using an alkaline-comet test. Results showed significant genotoxic reactions in the grasshoppers treated with thiamethoxam as compared to control groups.

1. **DNA Analysis:** An alkaline-comet test was used to measure DNA damage among grasshoppers exposed to thiamethoxam. Comet assays showed that the DNA damage was significantly higher in treated groups than in control groups. Parameters such as comet tail length, tail intensity, and olive tail moment were significantly higher in the thiamethoxam-exposed groups, indicating the induction of DNA strand breaks and genetic damage. The results suggest that grasshoppers are genotoxic after thiamethoxam treatment.
2. **Chromosomal Abberation Analysis:** A micronucleus analysis was performed to assess chromosomal abnormalities in grasshoppers that were exposed to thiamethoxam. Results showed a dose dependent increase in micronuclei formation in grasshoppers that were exposed to thiamethoxam. The micronuclei, which are extranuclear small bodies, indicate that chromosomal loss or damage occurred during cell division. The higher frequency of micronuclei observed in the treated groups compared to the control group suggests the presence of chromosomal aberrations induced by thiamethoxam exposure.

Overall, the results of both the DNA damage analysis and chromosomal aberration analysis provide strong evidence of the genotoxic effects of thiamethoxam on *SpathosternumPrasiniferum* grasshoppers. Increased DNA damage, as well as the presence of small nuclei in grasshoppers' cells, indicate that exposure to thiamethoxam can cause genetic changes and disruptions.

These genotoxic effects have significant implications for the health and survival of grasshopper populations. Genetic damage can lead to reduced reproductive success, impaired development, and decreased population fitness. The potential transmission of genetic changes to future generations could have a long-term effect on *SpathosternumPrasiniferum*'s population dynamics and ecological interactions in the ecosystem.

It is crucial to consider these genotoxic effects when assessing the overall ecological risks associated with thiamethoxam use. The findings of this study highlight the need for comprehensive evaluations of pesticide genotoxicity on non-target organisms and the implementation of effective risk assessment and management strategies to mitigate potential harm to ecosystems and biodiversity.

Further research is warranted to investigate the underlying mechanisms of thiamethoxam-induced genotoxicity in grasshoppers and to assess the broader ecological consequences of such genetic damage on population dynamics, community interactions, and ecosystem functioning.

Discussion:

This study demonstrates the genotoxic effect of thiamethoxam in *SpathosternumPrasiniferum*, which provides valuable insight into potential environmental risks related to the use of neonicotinoid pesticides. The significant increase in DNA damage observed in the grasshoppers exposed to thiamethoxam, as indicated by the alkaline comet assay, indicates that this pesticide induces genotoxic effects by causing DNA strand breaks. The higher number of micronuclei in the groups exposed to thiamethoxam suggests that there are chromosomal abnormalities. This confirms the potential genotoxic effects of the insecticide.

The genotoxic effects observed in this study have important implications for the health and survival of *SpathosternumPrasiniferum* grasshopper populations. DNA damage and chromosomal aberrations can lead to reduced reproductive success, impaired development, and decreased overall fitness. These effects can have cascading impacts on population dynamics, potentially leading to population decline or local extinctions. Furthermore, the transmission of genetic damage to subsequent generations can perpetuate the negative impacts of thiamethoxam exposure over time.

This study's findings are in line with the growing concern about ecological hazards associated with neonicotinoid pesticides. Previous research has primarily focused on the impacts of neonicotinoids on pollinators such as bees and butterflies. Our study, however, highlights the importance of considering the effects of pesticides such as neonicotinoids on non-target species, like grasshoppers. These organisms play an important role in ecosystem function and are indicators of the health of the environment.

This study's findings on the genotoxic properties of thiamethoxam highlight the need to take precautions to reduce the risk of harming non-target organisms. In order to develop effective risk assessments, it is important that they consider both the short-term toxicity and the long-term potential genetic effects of pesticides. These findings should also be considered by regulatory agencies and decision-makers when making decisions about pesticides and adopting appropriate mitigation measures for non-targeted species.

Further research is needed to deepen our understanding of the underlying mechanisms of thiamethoxam-induced genotoxicity in grasshoppers. Investigating the specific pathways and cellular processes affected by this pesticide can provide valuable insights into the molecular basis of its genotoxic effects. Furthermore, studies examining the broader ecological consequences of genetic damage on grasshopper populations, community dynamics, and ecosystem functioning will contribute to a comprehensive assessment of the impacts of thiamethoxam and other neonicotinoids.

In conclusion, this study demonstrates the genotoxic effects of thiamethoxam on *SpathosternumPrasiniferum* grasshoppers, highlighting the importance of considering the potential ecological risks associated with the use of this neonicotinoid insecticide. These DNA damage patterns and chromosomal abnormalities highlight the importance of robust risk assessments and effective management strategies to protect non-target organisms while maintaining ecosystem health.

Conclusion:

In conclusion, this study provides compelling evidence of the genotoxic effects of thiamethoxam on *SpathosternumPrasiniferum* grasshoppers. The results demonstrate a significant increase in DNA damage and chromosomal aberrations in the grasshoppers exposed to thiamethoxam, indicating the genotoxic potential of this neonicotinoid insecticide. The findings show the environmental risks of using thiamethoxam. They also highlight the need to consider the long-term effects of the insecticide on organisms other than the target species.

Genotoxic effects observed in grasshopper populations of *SpathosternumPrasiniferum* have a significant impact on their health and viability. DNA damage can cause chromosomal abnormalities and reduced fitness. This can affect population dynamics, ecological interactions and the species's ability to reproduce. Transmission of genetic damage from previous generations to future generations is a concern for the genetic integrity of grasshopper populations that have been exposed to thiamethoxam.

This study highlights the need to incorporate genotoxicity assessment into risk assessments of pesticides and regulatory frameworks. Current pesticide evaluations primarily focus on acute toxicity and short-term effects, but the genotoxic potential of pesticides should be

considered to ensure comprehensive and accurate risk assessments. To protect ecosystems and biodiversity, it is important to assess the environmental impacts of pesticides.

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