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Inorganic and synthetic base fertilizers affect the virulence of entomopathogenic nematode Oscheius tipulae

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Abstract

Entomopathogenic nematodes (EPNs) are promising biological control agents for soil-dwelling insect pests, but their performance may be affected by agricultural inputs commonly applied in crop production systems. This study evaluated the compatibility of three fertilizer sources (urea, potassium sulphate, and potassium chloride) with an isolate of *Oscheius tipulae*. Three independent laboratory bioassays were conducted in a completely randomized design, using fertilizer solutions at 1%, 5%, and 10%, with distilled water as the control. After 48 h of exposure, infective juvenile viability, infectivity against *Tenebrio molitor* larvae, reproduction, second-generation viability, and second-generation infectivity were assessed. All fertilizers negatively affected *O. tipulae*, but the magnitude of the effect differed among products. Urea was the most aggressive treatment, strongly reducing viability, completely suppressing second infectivity, and causing 100% reduction in reproduction at 5% and 10% solutions. Potassium sulphate showed intermediate toxicity, mainly reducing viability and reproduction, especially at 10% concentration. Potassium chloride reduced initial viability but had a lower overall impact because infectivity and reproduction were largely preserved. These findings indicate that concentrated fertilizer solutions may impair the persistence and biological activity of *O. tipulae*, particularly when urea is used.

Introduction

Biological control has emerged as an important and sustainable strategy for managing insect pests in agricultural systems, particularly as part of integrated pest management programs that aim to reduce reliance on synthetic insecticides (Nepomoceno et al. 2023; Matuska-Łyżwa et al. 2024). Among the biological agents used for this purpose, entomopathogenic nematodes (EPNs) are especially relevant because they naturally occur in soils and actively search for insect hosts. The most widely studied and commercially used EPNs belong to the genera *Steinernema* and *Heterorhabditis*, which are associated with the symbiotic bacteria *Xenorhabdus* and *Photorhabdus*, respectively (Půža & Machado 2024). Infective juveniles enter the insect host through natural openings or directly through the cuticle, release their bacterial symbionts into the hemocoel, and cause rapid host death through septicemia, toxins, and immune suppression, often within 24–48 h (Půža & Machado 2024). After host death, the nematodes reproduce inside the cadaver and new infective juveniles emerge to search for additional hosts, allowing them to recycle within pest populations under suitable environmental conditions. Because of these characteristics, EPNs have been used or investigated for the control of several pest groups, including Diptera, Coleoptera, Lepidoptera, and Thysanoptera, in agricultural and horticultural crops (Matuska-Łyżwa et al. 2024).

In addition to *Steinernema* and *Heterorhabditis*, other nematode taxa have shown insecticidal potential and may broaden the range of biological control agents available for pest management. Species of the genus *Oscheius* have attracted increasing attention because several isolates are capable of infecting, killing, and reproducing in insects (Tabassum et al. 2016; Loulou et al. 2022; Abdisa et al. 2024). Approximately 30 species are currently recognized within *Oscheius*, and more than 13 have been reported to show insecticidal activity (Tabassum et al. 2016). For example, *Oscheius tipulae* isolates

have demonstrated potential against *Ceratitis capitata*, affecting eggs, larvae, and pupae, and have also reproduced successfully in *Galleria mellonella* larvae (Loulou et al. 2022). More recently, Abdisa et al. (2024) reported that an *O. tipulae* isolate exhibited pathogenicity against insects from different orders, including lepidopteran, coleopteran, and dipteran hosts. These findings indicate that *Oscheius* species may complement the commercially available *Steinernema* and *Heterorhabditis* nematodes used in biological control programs.

Because soil is one of the natural habitats of EPNs, these organisms have considerable potential for controlling soil-dwelling and cryptic insect pests. In the soil environment, EPNs benefit from partial protection against ultraviolet radiation and desiccation, while their mobility allows infective juveniles to locate susceptible hosts belowground. However, the persistence and efficacy of EPNs are strongly influenced by environmental and management-related factors, including soil texture, moisture, temperature, pH, host availability, irrigation, tillage, pesticide use, and fertilization (Shehata et al. 2021; Matuska-Łyżwa et al. 2024). Fertilizers are especially relevant because they are routinely applied in productive agricultural areas and may alter soil chemical conditions, osmotic stress, salinity, and nutrient availability, all of which can influence nematode survival, movement, infectivity, and reproduction (Matuska-Łyżwa et al. 2024).

Globally, the use of synthetic and inorganic fertilizers has increased substantially over recent decades and has been closely associated with gains in crop production and agricultural productivity, particularly through the improved supply of essential nutrients such as nitrogen, phosphorus, and potassium (FAO 2024; Ludemann et al. 2024; Nguyen et al. 2024). Global datasets show that chemical and mineral fertilizers have become key inputs in modern agriculture, with nitrogen, phosphorus, and potassium application rates monitored across countries, regions, and crop production systems (FAO 2024; Nguyen et al. 2024). Although fertilizers contribute to food production, their excessive or unbalanced use can affect soil quality and biological communities, creating a need to evaluate their compatibility with beneficial soil organisms used in biological control (Ludemann et al. 2024; Matuska-Łyżwa et al. 2024; Nguyen et al. 2024).

Given the widespread use of inorganic and synthetic fertilizers in agriculture worldwide, the compatibility between EPNs and these fertilizers remains insufficiently studied, particularly for emerging biocontrol taxa such as *Oscheius*. Therefore, this study aimed to determine whether different inorganic and synthetic fertilizers affect the viability, infectivity, and reproduction of the EPN *O. tipulae*.

Material and Methods

Experimental design

Three independent single-factor experiments were conducted using a completely randomized design. The experiments followed the protocol recommended by the International Organization for Biological and Integrated Control – West Palaearctic Regional Section (IOBC/WPRS), as proposed by Vainio (1992),

with adaptations for the present study. Each experiment evaluated the compatibility of *Oscheius tipulae* with one inorganic or synthetic fertilizer: urea, potassium chloride, or potassium sulfate. For each fertilizer, three concentrations were tested: 1%, 5%, and 10%. Each treatment consisted of five replicates, and distilled water was used as the control treatment.

Entomopathogenic nematode isolate and multiplication

The entomopathogenic nematode used in the experiments is an isolate of *O. tipulae*, collected from a soybean field located in Xaxim, Santa Catarina, Brazil. The isolate was first identified by molecular identification using the ribosomal DNA sequencing containing partial 18S rRNA, ITS1, 5.8 rRNA, ITS2, and partial 28S rRNA sequences (GenBank accession number KT728758.1). Thereafter, the isolate was multiplied in larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), following the methodology described by Potrich et al. (2007). Infective juveniles (IJs) obtained from the multiplication process were used in the bioassays.

Fertilizers and preparation of solutions

Three inorganic and synthetic fertilizers commonly used in agricultural production systems were evaluated: urea, potassium sulfate, and potassium chloride. Each fertilizer was diluted in 1 L of distilled water to obtain solutions at concentrations of 1%, 5%, and 10%. The main nutritional components and solubility characteristics of the fertilizers are presented in Table 1.

Table 1
List and description of the inorganic and synthetic fertilizers tested.

Fertilizer name	Characteristic	Nutrient content	Solubility
Urea	Synthetic and organic	45% N	High (1.2 kg L^{-1} at 25°C)
Potassium sulphate	Natural and inorganic	50% K_2O; 18% S	Low (0.12 kg L^{-1} at 25°C)
Potassium chloride	Natural and inorganic	60% K_2O; 47% Cl	High (0.35 kg L^{-1} at 25°C)

Preparation of nematode suspensions and exposure to fertilizers

Before the establishment of each experiment, the initial viability of *O. tipulae* infective juveniles was determined. The viability values recorded on the dates when the experiments were established are shown in Table 2.

Table 2
Initial viability of *Oscheius tipulae* on the dates when the experiments were established.

Experiment	Fertilizer used	Viability of *O. tipulae* used in the experiment
01	Urea	94.31%
02	Potassium sulphate	98.34%
03	Potassium chloride	98.30%

For each replicate, approximately 1,500 infective juveniles were suspended in 2 mL of distilled water. The suspension was transferred to tubes containing 13 mL of the respective fertilizer solution, resulting in a final volume of 15 mL per tube. In the control treatment, tubes contained 13 mL of distilled water and 2 mL of the IJ suspension. The tubes were maintained in a biochemical oxygen demand (BOD) chamber at 24 ± 1°C and 70 ± 10% relative humidity, in the dark, for 48 h.

Viability assessment after fertilizer exposure

After 48 h of exposure, IJ viability was assessed by transferring 0.1 mL aliquots from each tube into 10 wells of an ELISA plate. Viable and non-viable infective juveniles were counted under a stereomicroscope for each replicate. Live nematodes were identified by their characteristic whiplash movement, whereas dead nematodes were recognized by their immobile and straight body posture.

Washing procedure and infectivity assay

After the viability assessment, the nematodes from each treatment were washed three times to remove fertilizer residues before the infectivity assay. In each washing step, 7.5 mL of distilled water was added to each tube, and the tubes were kept in a BOD chamber for 30 min to allow the nematodes to settle. After sedimentation, 7.5 mL of the supernatant was carefully discarded. This procedure was repeated two additional times. Following the washing procedure, inoculation chambers were prepared in Petri dishes. Each dish received two sheets of filter paper, 10 larvae of *T. molitor*, and 2 mL of the corresponding nematode suspension. The dishes were maintained under the same controlled conditions described above. After two days, dead larvae were transferred, together with one sheet of filter paper, to Petri dishes used as dry chambers. These dishes were kept in the BOD chamber for an additional five days.

Reproduction assay and second-generation evaluations

After the dry chamber period, White traps were prepared to recover infective juveniles emerging from the cadavers. Nematode emergence was monitored, and the number of IJs produced per treatment was

recorded to determine reproduction. When sufficient emergence occurred, the recovered IJs were used to repeat the procedure in order to determine second viability and second infectivity.

Data analysis and compatibility classification

Prior to statistical analysis, viability and infectivity datasets were examined for outliers and assessed for compliance with the assumptions of normality and homogeneity of variances. When these assumptions were met, data were analyzed by analysis of variance (ANOVA), and treatment means were compared using Tukey's Honest Significant Difference (HSD) test at the 5% significance level ($p \leq 0.05$). For variables that did not satisfy the assumptions required for ANOVA, non-parametric analyses were applied. Specifically, the Kruskal–Wallis test was conducted at the 5% significance level, followed, when appropriate, by pairwise comparisons using the Steel–Dwass procedure. The statistical method used for each dataset is indicated in the corresponding table or figure caption to ensure transparency, consistency, and reproducibility of the results. All statistical analyses were conducted using JMP® Pro 17.0 software.

Nematode mortality was corrected using Abbott's formula (Abbott 1925):

$$Mc\ (\%) = [(Mo - Mt) \div (100 - Mt)]\ \times\ \ 100$$

where *Mc* is the corrected mortality, *Mo* is the observed mortality in the treatment, and *Mt* is the mortality observed in the control treatment.

Infectivity was expressed as the percentage of dead *T. molitor* larvae. The reduction in infectivity caused by each treatment was calculated according to Vainio (1992):

$$Rinf\ (\%) = [1 - (It \div Ic)]\ \times\ \ 100$$

where *Rinf* is the reduction in infectivity, *It* is the infectivity observed in the treatment, and *Ic* is the infectivity observed in the control treatment.

Reproduction was determined by counting the infective juveniles that emerged from *T. molitor* larvae in White traps. The reduction in reproduction caused by each treatment was calculated according to Vainio (1992):

$$Rfec\ (\%) = [1 - (Ft \div Fc)]\ \times\ \ 100$$

where *Rfec* is the reduction in reproduction, *Ft* is the reproduction observed in the treatment, and *Fc* is the reproduction observed in the control treatment.

The total insecticidal effect of the treatments was calculated using the formula modified by Peters and Poullot (2004), following IOBC/WPRS standards:

$$E\ (\%) = 100 - ((100\% - Mc\%) \times\ (100\% - Rinf\%) \times\ (100\% - Rfec\%))$$

where *E* represents the overall insecticidal effect of the treatment on the nematodes, considering corrected mortality, reduction in infectivity, and reduction in reproduction.

Based on IOBC/WPRS classification criteria, treatments were classified as harmless when $E < 30\%$, slightly harmful when $E = 30–79\%$, moderately harmful when $E = 80–99\%$, and harmful when $E > 99\%$.

Results

Exposure to the inorganic and synthetic fertilizers markedly affected the performance of *O. tipulae*, although the magnitude and pattern of response differed among fertilizers and concentrations. Urea caused a strong reduction in the first viability of *O. tipulae* at all tested concentrations (Table 3). In the control treatment, first viability was 92.0%, whereas viability decreased to 39.9%, 52.7%, and 48.2% after exposure to urea at 1%, 5%, and 10%, respectively. Although urea at 5% resulted in slightly higher viability than urea at 1%, all urea treatments remained substantially below the control. First infectivity was also affected, especially at the highest concentration. While the control caused 100% mortality of *T. molitor* larvae, infectivity remained high at 1% and 5% urea, with 98.0% and 96.0%, respectively, but declined sharply to 70.0% at 10% urea (Table 3). The strongest impact of urea was observed in the second-generation evaluations. Second viability fell to 19.9% at 1% urea and reached 0.0% at both 5% and 10%. Likewise, second infectivity was completely suppressed in all urea treatments, with 0.0% larval mortality, compared with 98.0% in the control (Table 3). These results show that urea severely compromised the persistence and biological activity of the nematode after the initial exposure.

Table 3
Compatibility of *Oscheius tipulae* after exposure to different concentrations of urea, potassium sulphate (PS) and potassium chloride (PC).

Treatment	1st Viability (%)	1st Infectivity (%)	2nd Viability (%)	2nd Infectivity (%)
Control	92.0 ± 0.98 [a]	100.0 ± 0.00 [a]	98.0 ± 0.83 [a]	98.0 ± 2.00 [a]
Urea 1%	39.9 ± 2.93 [c]	98.0 ± 2.00 [a]	19.9 ± 19.9 [ab]	0.0 ± 0.00 [b]
Urea 5%	52.7 ± 3.57 [b]	96.0 ± 4.00 [a]	0.0 ± 0.00 [b]	0.0 ± 0.00 [b]
Urea 10%	48.2 ± 2.67 [bc]	70.0 ± 17.88 [b]	0.0 ± 0.00 [b]	0.0 ± 0.00 [b]
CV (%)	36.5	24.9	156.7	177.9
ChiSq	0.0032	0.0335	0.0032	0.0003
Control	97.5 ± 0.98 [a]	98.0 ± 2.00 [a]	96.9 ± 0.83 [a]	100.0 ± 0.00 [*ns]
PS 1%	63.0 ± 2.93 [b]	88.0 ± 4.89 [ab]	67.7 ± 19.9 [b]	100.0 ± 0.00
PS 5%	65.8 ± 3.57 [b]	86.0 ± 9.79 [ab]	60.4 ± 0.00 [bc]	100.0 ± 0.00
PS 10%	50.2 ± 2.67 [c]	76.0 ± 2.44 [b]	52.6 ± 0.00 [c]	100.0 ± 0.00
CV (%)	26.7	16.3	25.8	0.00
ChiSq	0.0021	0.0410	0.0016	1.0000
Control	98.3 ± 0.19 [a]	92.0 ± 4.89 [*ns]	98.5 ± 0.32 [a]	100.0 ± 0.00 [*ns]
PC 1%	36.2 ± 1.57 [b]	100.0 ± 0.00	91.2 ± 0.27 [b]	100.0 ± 0.00
PC 5%	33.1 ± 1.89 [b]	96.0 ± 2.44	86.3 ± 0.73 [c]	100.0 ± 0.00
PC 10%	21.9 ± 1.16 [c]	84.0 ± 8.12	85.8 ± 0.25 [c]	100.0 ± 0.00
CV (%)	64.9	12.6	5.86	0.00
ChiSq	0.0010	0.2180	0.0011	1.0000

Means followed by the same letter in each column do not differ statistically according to Steel-Dwass test at 5%. [*ns]: not significant. Values are means ± standard deviation.

The corrected mortality and reproductive reduction data confirmed the high aggressiveness of urea (Table 4). Urea caused corrected mortality between 33.3% and 49.3%, while reduction in reproduction reached 100.0% at both 5% and 10%. Consequently, urea at 5% and 10% was classified as harmful, whereas urea at 1% was only slightly harmful because reproduction was reduced by 25.8%. In terms of insecticidal effect, urea produced the highest overall values among the fertilizers, with the 5% and 10%

concentrations reaching approximately 100% effect (Fig. 1). The overall comparison also confirmed urea as the most aggressive fertilizer, with the highest mean insecticidal effect and a moderately harmful classification according to the IOBC classification (Fig. 2).

Table 4
Corrected mortality (*Mc%*), reduced infectivity (*Rinf%*), reduced reproduction (*Rfec%*), and insecticidal effect (*E%*) observed after exposure of *Oscheius tipulae* to different concentrations of urea, potassium sulphate (PS) and potassium chloride (PC).

Treatment	***Mc (%)***	***Rinf (%)***	***Rfec (%)***	**IOBC Classification**
Control	0.00	0.0	0.0	Harmless
Urea 1%	49.3	2.0	25.8	Slightly harmful
Urea 5%	33.3	4.0	100.0	Harmful
Urea 10%	38.7	30.0	100.0	Harmful
Control	0.00	0.0	0.0	Harmless
PS 1%	38.6	10.4	53.1	Slightly harmful
PS 5%	42.4	12.2	51.1	Slightly harmful
PS 10%	45.3	22.2	60.2	Moderately harmful
Control	0.00	0.0	0.0	Harmless
PC 1%	68.8	0.00	0.0	Slightly harmful
PC 5%	53.9	4.00	0.0	Slightly harmful
PC 10%	61.1	13.0	0.0	Slightly harmful

Potassium sulphate produced an intermediate level of toxicity to *O. tipulae*. First viability decreased from 97.5% in the control to 63.0%, 65.8%, and 50.2% at 1%, 5%, and 10%, respectively (Table 3). This indicates a concentration-dependent tendency, with the strongest reduction occurring at 10%. First infectivity also declined progressively with increasing concentration, from 98.0% in the control to 88.0%, 86.0%, and 76.0% at 1%, 5%, and 10%, respectively. Second viability followed the same pattern, with values of 67.7%, 60.4%, and 52.6%, compared with 96.9% in the control. However, second infectivity remained at 100.0% in all potassium sulphate treatments, suggesting that the surviving nematodes retained infective capacity after reproduction (Table 3). Despite this, potassium sulphate caused considerable reproductive reduction, ranging from 51.1% to 60.2%, and the 10% concentration was classified as moderately harmful (Table 4). The insecticidal effect increased with concentration, with 10% potassium sulphate producing the highest value within this fertilizer group (Fig. 1).

Potassium chloride showed a different response pattern. It caused the greatest immediate reduction in first viability among the potassium fertilizers, decreasing from 98.3% in the control to 36.2%, 33.1%, and

21.9% at 1%, 5%, and 10%, respectively (Table 3). However, first infectivity was less consistently affected, remaining statistically non-significant and ranging from 84.0% to 100.0%. Second viability was reduced but remained relatively high, with values of 91.2%, 86.3%, and 85.8%, and second infectivity remained 100.0% in all concentrations (Table 3). Unlike urea and potassium sulphate, potassium chloride caused no reduction in reproduction, and all concentrations were classified as slightly harmful (Table 4). Accordingly, although potassium chloride reduced initial viability, its overall insecticidal effect was lower than that of urea and potassium sulphate (Figs. 1 and 2).

Taken together, the results demonstrate that all fertilizers negatively affected *O. tipulae*, but with clear differences in intensity. Urea was the most aggressive product, mainly because it eliminated second infectivity and strongly reduced reproduction. Potassium sulphate ranked second, showing moderate reductions in viability, infectivity, and reproduction, especially at 10%. Potassium chloride was the least aggressive overall, despite reducing first viability, because nematode infectivity and reproduction were largely preserved.

Discussion

The present study demonstrates that inorganic and synthetic base fertilizers can markedly compromise the biological performance of *O. tipulae*, although the intensity of this effect depended on the fertilizer source and concentration. Under the laboratory conditions used here, urea was the most aggressive product, followed by potassium sulphate and potassium chloride. This ranking was mainly associated with the combined responses of viability, infectivity, reproduction, and the calculated insecticidal effect. These results reinforce that EPN compatibility cannot be inferred only from immediate survival, because sublethal effects on infectivity and reproduction may strongly reduce field persistence and biocontrol potential.

The high aggressiveness of urea was evident from the sharp reduction in first viability, the complete suppression of second infectivity, and the 100% reduction in reproduction at 5% and 10%. This response is biologically coherent because urea rapidly hydrolyses in soil and aqueous environments, generating ammonium and potentially ammonia, depending on pH, temperature, urease activity, and buffering capacity (Motasim et al. 2024). Ammonia and high ammonium concentrations may be toxic to soft-bodied soil organisms and can alter osmotic balance, membrane integrity, and metabolic activity. For EPNs, the infective juvenile stage is adapted for persistence but remains sensitive to osmotic stress, pH shifts, and dissolved ions (Georgis and Gaugler 1991; Matuska-Łyżwa et al. 2024). The strong loss of second-generation infectivity suggests that urea did not only kill nematodes directly, but also impaired physiological quality among surviving juveniles, reducing their ability to recycle through the host. Similar concerns have been reported for inorganic fertilizers affecting the virulence of *Steinernema glaseri* (Shehata et al. 2021), and recent work shows that nitrogen addition can restructure soil nematode communities and alter their metabolic footprint (Li et al. 2024; Atira and Kakouli-Duarte 2025).

Potassium sulphate produced an intermediate response. Although it was less aggressive than urea, it consistently reduced first viability, first infectivity, second viability, and reproduction, especially at 10%. Potassium sulphate has lower solubility than urea and potassium chloride, but once dissolved it releases potassium and sulphate ions that may increase electrical conductivity and osmotic pressure. These effects can restrict nematode movement, reduce host-finding ability, and interfere with water balance. The fact that second infectivity remained high among recovered nematodes indicates that potassium sulphate did not eliminate infective capacity in the surviving population; however, the reduction in reproduction indicates that fewer or less vigorous infective juveniles were produced. This is important because EPN efficacy in soil depends not only on initial host mortality but also on recycling within cadavers (Rahoo et al. 2017; Loulou et al. 2022). The moderate harmful classification of potassium sulphate at 10% therefore reflects a biologically meaningful impairment of population renewal.

Potassium chloride caused the strongest immediate reduction in first viability among the potassium fertilizers, but its overall effect was lower because infectivity and reproduction were largely preserved. The high solubility of potassium chloride and the chloride load may explain the initial mortality. Chloride salts can increase salinity and osmotic stress, and excessive soluble ions may negatively affect microbial activity and other soil organisms (Silva et al. 2001; Malal et al. 2025). However, in this study the surviving nematodes maintained high infectivity, and reproduction was not reduced. This suggests that potassium chloride caused an acute filtering effect, eliminating a portion of the exposed juveniles but leaving a remaining population able to infect and reproduce. From a biological control perspective, this is still relevant because a reduced viable population may lower the probability of host encounter under field conditions, even when surviving juveniles remain pathogenic.

The concentrations tested here should be interpreted carefully in relation to commercial fertilizer use. The solutions of 1%, 5%, and 10% correspond to approximately 10, 50, and 100 g of fertilizer per liter, respectively. Assuming one hectare contains approximately 2,000,000 L of soil in the biologically active layer (up to 20 cm depth), these concentrations would be equivalent to about 20,000, 100,000, and 200,000 kg ha^{-1} if uniformly distributed through that soil volume. These values are far above normal field applications. For example, a high urea application of 500 kg ha^{-1} corresponds to 0.25 g L^{-1}, or approximately 0.025%, meaning that the lowest tested concentration, 1%, was about 40 times higher than this commercial maximum. For potassium fertilizers, if 200–300 kg ha^{-1} of product is applied, the equivalent concentration is approximately 0.01–0.015%, making the 1% treatment about 67–100 times higher.

Therefore, the laboratory concentrations used in this study represent a high-exposure compatibility scenario rather than direct simulation of uniform field dilution. Nevertheless, these results remain relevant because fertilizers are not instantly and uniformly diluted in soil. Localized fertilizer bands, granule dissolution zones, fertigation pulses, and low soil moisture may temporarily create concentrated microsites with high electrical conductivity, ammonium, ammonia, potassium, sulphate, or chloride levels. These microsites may be particularly harmful if EPNs are applied simultaneously or shortly after fertilization. Recent fertilizer datasets confirm that nutrient application varies widely among crops and

regions (Nguyen et al. 2024), and studies using field rates such as 0–270 kg N ha^{-1}, 90–120 kg P_2O_5 ha^{-1} or 0–290 kg K_2O ha^{-1} show that even agronomic doses can alter soil biological communities (Cheng and Wang 2023; Li et al. 2024; Ni et al. 2024).

Conclusions

These results indicate that urea, potassium sulphate, and potassium chloride may be incompatible with *O. tipulae* when nematodes are exposed to concentrated fertilizer solutions. Urea presents the greatest risk because it impaired both immediate and subsequent biological activity. Potassium sulphate showed intermediate risk through reductions in viability and reproduction, whereas potassium chloride mainly affected initial viability. For practical use, EPN applications should preferably be separated from fertilizer applications, especially urea and concentrated potassium sources, and future studies should evaluate lower, field-equivalent concentrations, soil-buffered systems, different soil moisture levels, and time intervals between fertilization and nematode release.

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Author Contribution

MAT designed the experiments. PEP and SPT conducted the trials and performed data collection. RDBD carried out the statistical analysis. RDBD and JLM wrote the manuscript. MAT and SMS revised the manuscript.

References

1. Abbott WS. (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18:265-266, 1925. https://doi.org/10.1093/jee/18.2.265a

2. Abdisa, E., Esmaeily, M., Kwon, J., Jin, G., & Kim, Y. (2024). A nematode isolate, *Oscheius tipulae*, exhibiting a wide pathogenic spectrum and its application to control dipteran insect pests. Archives of Insect Biochemistry and Physiology 117:e22152. https://doi.org/10.1002/arch.22152
3. Atira LS, Kakouli-Duarte T. (2025). Implications of Fertilisation on Soil Nematode Community Structure and Nematode-Mediated Nutrient Cycling. Crops, 5:50. https://doi.org/10.3390/crops5040050
4. Cheng, L, Wang H. (2023). Responses of Soil Bacterial Communities and Enzyme Activities to Straw Return and Potassium Fertilization with Two Soils Under Soil Potassium Balance Condition in Rice–Wheat System. Journal of Soil Science and Plant Nutrition, 23:1845–1859. https://doi.org/10.1007/s42729-023-01142-5
5. Food and Agriculture Organization of the United Nations – FAO. (2024). Fertilizers by nutrient (Global – National – Annual) – FAOSTAT. Available at: https://data.apps.fao.org/catalog/dataset/fertilizers-by-nutrient-rfn. Accessed in June 2026.
6. Georgis R, Gaugler R. (1991). Predictability in biological control using entomopathogenic nematodes. Journal of Economic Entomology, 84:713-720. https://doi.org/10.1093/jee/84.3.713
7. Li L, Luo Z, Nian L, Li L, Niu Y, Prasath BB, Haider FU, Zhang Y, He R, Liu J. (2024). Response of soil nematode community structure and metabolic footprint to nitrogen addition in alfalfa fields on the Loess Plateau. Scientific Reports, 14:17640. https://doi.org/10.1038/s41598-024-68393-9
8. Loulou A, Guerfali MM, Muller A, Bhat AH, Abolafia J, Machado RAR, Kallel S. (2022). Potential of *Oscheius tipulae* nematodes as biological control agents against *Ceratitis capitata*. *PLOS ONE, 17:* e0269106. https://doi.org/10.1371/journal.pone.0269106
9. Ludemann CI, Wanner N, Chivenge P, Dobermann A, Einarsson R, Grassini P, Gruere A, Jackson K, Lassaletta L, Maggi F, Onli-Laryea G, van Ittersum MK, Vishwakarma S, Zhang X, Tubiello FN. (2024). A global FAOSTAT reference database of cropland nutrient budgets and nutrient use efficiency (1921-2020): Nitrogen, phosphorus and potassium. Earth System Science Data, 16:525–541. https://doi.org/10.5194/essd-16-525-2024
10. Malal H, Garcia JA, Marrs A, Ait Hamza M, Emerson C, Nocco M, Lakhtar H, Lazcano C. (2025). Organic and inorganic fertilizers modulate the response of the soil microbiome to salinity stress. Frontiers in Microbiology, 16:1551586. https://doi.org/10.3389/fmicb.2025.1551586
11. Matuska-Łyżwa J, Duda S, Nowak D, Kaca W. (2024). Impact of abiotic and biotic environmental conditions on the development and infectivity of entomopathogenic nematodes in agricultural soils. Insects, 15:421. https://doi.org/10.3390/insects15060421
12. Motasim AM, Samsuri AW, Nabayi A, Akter A, Haque MA, Sukor ASA, Adibah AM. (2024). Urea application in soil: processes, losses, and alternatives—a review. Discover Agriculture, 2:42. https://doi.org/10.1007/s44279-024-00060-z
13. Nepomoceno TAR, Plein C, Carniatto I. (2023). Sustainable development in family farming: implications for the biological control of insect pests. International Journal of Environmental Resilience Research and Science, 5:1-19. https://doi.org/10.48075/ijerrs.v5i1.30797

14. Nguyen TH, Tang FHM, Conchedda G, Casse L, Obli-Laryea G, Tubiello FN, Maggi F. (2024). NPKGRIDS: A global georeferenced dataset of N, P_2O_5, and K_2O fertilizer application rates for 173 crops. Scientific Data 11:1179. https://doi.org/10.1038/s41597-024-04030-4
15. Ni X, Zhu X, Feng Q, Zhao D, Huang W, Pan F. (2024). Effect of Application Rates of N and P Fertilizers on Soil Nematode Community Structure in Mollisols. Agronomy, 14:507. https://doi.org/10.3390/agronomy14030507
16. Peters A, Poullot D. (2004). Side effects of surfactants and pesticides on entomopathogenic nematodes assessed using advanced IOBC guidelines. IOBC/WPRS Bulletin, 27(6):67-72.
17. Potrich TD, Lorini I, Voss M, Steffens MCS, Pavani DP. (2007). Methodology for rearing *Tenebrio molitor* in the laboratory to obtain larvae. Passo Fundo: EMBRAPA Trigo. 35p.
18. Půža V, Machado RAR. (2024). Systematics and phylogeny of the entomopathogenic nematobacterial complexes *Steinernema–Xenorhabdus* and *Heterorhabditis–Photorhabdus*. Zoological Letters, 10:13. https://doi.org/10.1186/s40851-024-00235-y
19. Rahoo AM, Mukhtar T, Gowen SR, Rahoo RK, Abro SI. (2017). Reproductive potential and host-searching ability of the entomopathogenic nematode *Steinernema feltiae*. Pakistan Journal of Zoology, 49:229-234. https://doi.org/10.17582/journal.pjz/2017.49.1.229.234
20. Shehata IE, Hammam MMA, Abd-Elgawad MMM. (2021). Effects of inorganic fertilizers on virulence of the entomopathogenic nematode *Steinernema glaseri* and peanut germination under field conditions. Agronomy, 11(5):945. https://doi.org/10.3390/agronomy11050945
21. Silva MAG, Boaretto AE, Fernandes HG, Boaretto R, Melo AMT, Scivittaro WB. (2001). Chemical characteristics of an oxisol fertilized with urea and potassium chloride in a protected environment. Scientia Agricola, 58:561-566. https://doi.org/10.1590/S0103-90162001000300019
22. Tabassum K A, Shahina F, Nasira K, Erum Y. (2016). Description of six new species of *Oscheius andrassy*, 1976 (Nematoda: Rhabditida) from Pakistan with a key and diagnostic compendium to species of the genus. Pakistan Journal of Nematology 34:109–161. https://doi.org/10.18681/pjn.v34.i02.p109
23. Vainio A. (1992). Guideline for laboratory testing of the side-effects of pesticides on entomophagous nematodes *Steinernema* spp. IOBC/WPRS Bulletin, 15(3):145-147.

Figures

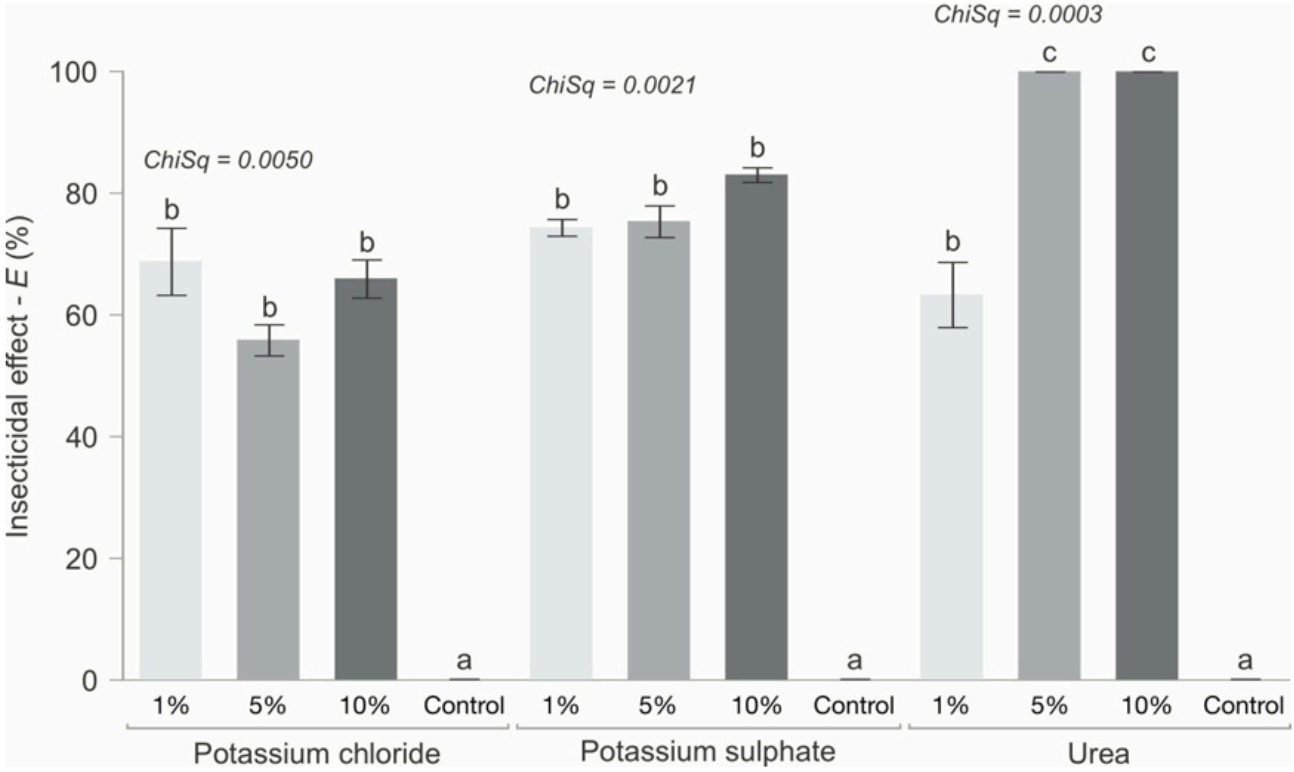


Figure 1

Insecticidal effect of different concentrations of urea, potassium sulphate and potassium chloride on *Oscheius tipulae.* Bars followed by the same letter within each fertilizer do not differ statistically according to Steel-Dwass test at 5%. ^{*ns}: not significant. Error bars represent the standard error.

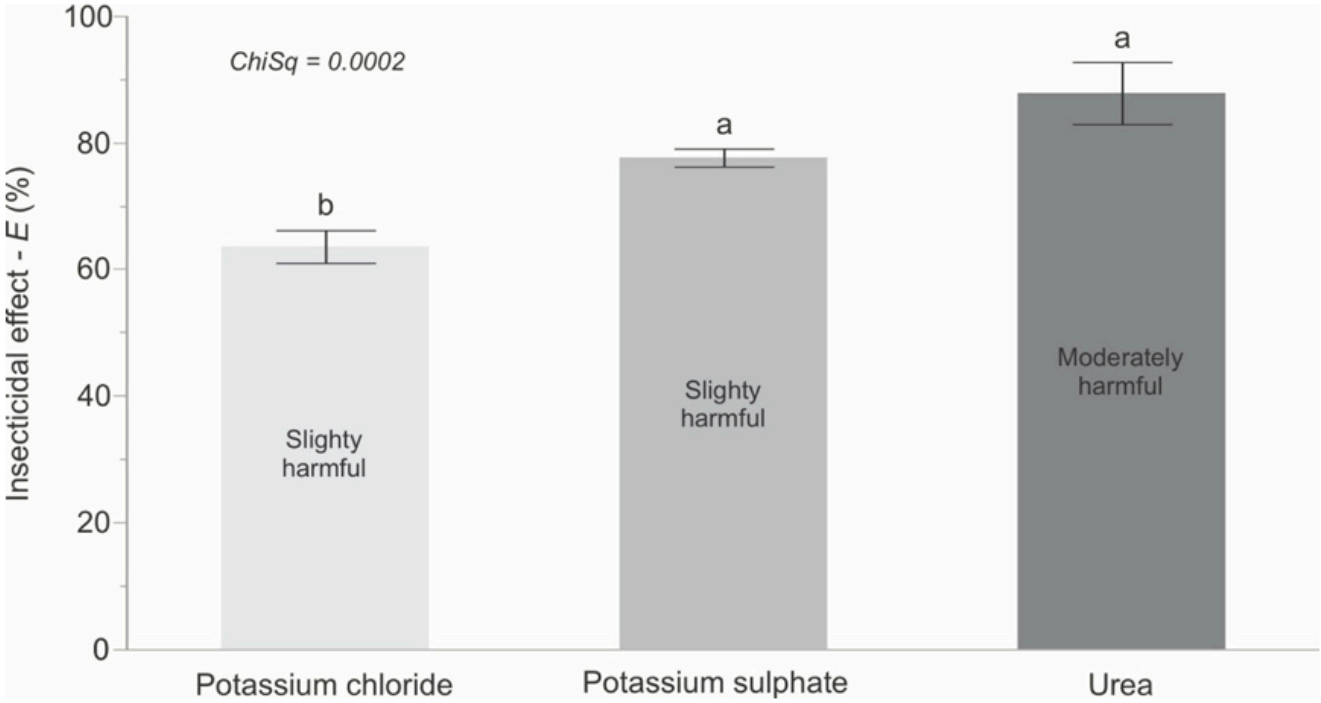


Figure 2

Overall insecticidal effect of urea, potassium sulphate and potassium chloride on *Oscheius tipulae*. Bars followed by the same letter within each fertilizer do not differ statistically according to Steel-Dwass test at 5%. ^{*ns}: not significant. Error bars represent the standard error.