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Determination of concentration thresholds (EC_{10}, EC_{20}, EC_{50}) of mineral fertilisers for *Aedes aegypti* (Linnaeus, 1762) mosquito larvae in a laboratory test

Определение концентрационных порогов (EC_{10}, EC_{20}, EC_{50}) минеральных удобрений для личинок комаров *Aedes aegypti* (Linnaeus, 1762) в лабораторном тесте

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Key words: *Aedes aegypti*, mosquito larvae, mineral fertilisers, ammophos, double superphosphate, calcium nitrate, sublethal toxicity, $EC_{50.}$

Ключевые слова: *Aedes aegypti*, личинки комаров, минеральные удобрения, аммофос, двойной суперфосфат, кальциевая селитра, сублетальная токсичность, $EC_{50.}$

Abstract. In a 20-day chronic laboratory test, concentration thresholds (EC_{10}, EC_{20}, EC_{50}) were determined for three mineral fertilisers — ammophos (AM), double superphosphate (BSP), and calcium nitrate (CN) — on larvae of the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae). Calculations were performed using probit analysis with a three-parameter log-logistic model (LL.3). For *A. aegypti*, BSP was the most toxic (EC_{50} = 4.1 mg/mL), followed by CN (EC_{50} = 5.8 mg/mL); AM was the least toxic (EC_{50} = 12.8 mg/mL). The EC_{20} BSP, CN, and AM for values were 2.4, 4.5, and 8.9 mg/mL, and the EC_{10} values were 1.8, 3.9, and 7.2 mg/mL, respectively. All fitted models showed excellent agreement with the data (pseudo-R^2 = 0.87–0.91; χ^2 test, $p > 0.05$). The obtained sublethal concentrations (EC_{10}–EC_{20}) can be used for planning multigeneration selection experiments on mosquitoes to study evolutionary adaptation to chronic anthropogenic stress, as well as in ecological and epidemiological monitoring and in risk assessment related to the contamination of aquatic ecosystems by agrochemicals.

Резюме. В 20-ти суточном лабораторном тесте определены концентрационные пороги (EC_{10}, EC_{20}, EC_{50}) для трёх минеральных удобрений: аммофоса (AM), двойного суперфосфата (BSP) и кальциевой селитры (CN), на личинках комаров *Aedes aegypti* (L.) (Diptera: Culicidae). Расчёты выполнены методом пробит анализа с трёхпараметрической логистической моделью (LL.3). Наиболее токсичным для *A. aegypti* оказался BSP (EC_{50} = 4,1 мг/мл), за ним следовала CN (EC_{50} = 5,8 мг/мл); наименее токсичным оказался AM (EC_{50} = 12,8 мг/мл). Значения EC_{20} для BSP, CN и AM составили 2,4, 4,5 и 8,9 мг/мл и EC_{10} — 1,8; 3,9 и 7,2 мг/мл соответственно. Все построенные модели продемонстрировали высокое качество аппроксимации (псевдо-R^2 = 0,87–0,91; χ^2 тест, $p > 0,05$). Полученные сублетальные концентрации (EC_{10}–EC_{20}) могут быть использованы при планировании экспериментов по селекции комаров для изучения эволюционной адаптации к антропогенному стрессу, а также в эколого-эпидемиологическом мониторинге и при оценке рисков, связанных с загрязнением водных экосистем агрохимикатами.

Introduction

Mosquitoes (Diptera: Culicidae) are among the most significant groups of animals due to their role as vectors of pathogens causing vector-borne diseases in humans and animals [Mathiarasan et al., 2025]. The larval stages of mosquitoes develop in water, often in temporary water bodies, which are particularly vulnerable to anthropogenic pollution [Ambadiang et al., 2023].

The widespread use of nitrogen, particularly in the form of mineral fertilisers, leads to a cascade of environmental consequences. Nitrogen pollution is now considered one of the most serious threats to biodiversity, exerting a pronounced impact on aquatic ecosystems and their biota [Galloway et al., 2008; Díaz-Álvarez et al., 2018; Payne et al., 2017]. In regions with intensive agriculture, significant amounts of mineral fertilisers, ammophos, superphosphate, calcium nitrate and their analogues, enter water bodies [Leon et al., 2024; Ahmed, Hayes, 2025]. Although the eutrophying effect of fertilisers is well known [Dodds, Smith, 2016], their direct toxic effect on mosquito larvae remains poorly studied [Gomez Isaza et al., 2020].

Of particular interest are sublethal concentrations of such pollutants (causing no more than 20–30 % mortality), as these allow for the study of physiological and evolutionary adaptations of insects to continuous

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stress [Kibuthu et al., 2016; Sial et al., 2018]. Unlike lethal doses, sublethal concentrations trigger specific physiological responses, including increased activity of antioxidant and detoxification enzymes, hormesis, and transgenerational plasticity. However, targeted studies on the effects of sublethal stress doses of fertilisers on the enzyme systems and hormesis mechanisms in mosquito larvae remain poorly understood compared to the extensive research on the effects of pesticides [Victor, Reuben, 2000; Andreazza et al., 2021].

Nitrate accumulation is one of the most significant global threats to aquatic organisms [Jenkinson, 2001]. Under natural conditions, nitrate concentrations are low (~0–2 mg/L), but anthropogenic pollution has increased them 10- to 100-fold [Galloway et al., 2008], reaching 25–100 mg/L in regions with intensive agriculture [Camargo, Alonso, 2006]. In addition, substantial amounts of nutrients are released from aquaculture operations [Cao et al., 2007; Verdegem, 2013]. As a result of surface runoff and soil leaching, significant quantities of ammonium, phosphate, and nitrate compounds enter freshwater ecosystems, where they can affect non-target aquatic organisms [Flores et al., 2020].

Several early studies examined the acute toxicity of mineral fertilisers to mosquitoes; however, most of them date back to the 1980s–1990s. For example, Fedorova [1989] reported lethal concentrations of superphosphate, ammophos (AM), and nitrophoska for *Culex pipiens* Linnaeus, 1758 larvae under laboratory conditions. Since then, fertiliser compositions have changed, and the concept of sublethal effects has gained considerable importance in ecotoxicology, making it necessary to revise and refine threshold concentrations for common mosquito species.

For the present study, we selected three widely used mineral fertilisers with different ionic compositions: AM (NH_4^+ and $H_2PO_4^-$), double superphosphate (BSP; Ca^{2+} and $H_2PO_4^-$), and calcium nitrate (CN; Ca^{2+} and NO_3^-). Despite their agronomic importance, systematic data on the acute toxicity of these fertilisers to mosquito larvae are lacking in the modern literature, and estimates of EC_{10} and EC_{20} (concentrations causing 10 and 20 % mortality, respectively) have not been previously published.

The aim of this study was to determine sublethal concentration ranges (EC_{10} and EC_{20}) for AM, BSP, and AM in *Aedes aegypti* (Linnaeus, 1762) mosquito larvae under standardized laboratory conditions. These values can serve as a reference basis for subsequent multigeneration selection experiments aimed at characterising the biochemical and evolutionary responses to sublethal stress induced by mineral fertilisers. In addition, we compared the toxicity of the three fertilisers to identify which ionic component poses the greatest hazard to mosquito larvae.

Material and methods

Study object

Laboratory-bred *Aedes aegypti* larvae of the III–IV instar from the collection of the Institute of Systematics and Ecology of Animals, Siberian Branch of the Russian Academy of Sciences (Novosibirsk, Russia) were used in the experiments. The insects were maintained under controlled conditions: air temperature 26 ± 1°C, relative humidity 60 ± 10 % RH, photoperiod 12:12 (light/dark), and fed daily with finely ground fish food (Cichlid Colour, Germany). Prior to the experiments, the larvae were acclimated to the rearing conditions for 7 days.

Fertilisers and solution preparation

The study used commercial mineral fertilisers of various grades: AM (12 % NH_4^+, 52 % P_2O_5; Terra Master LLC, Russia), BSP (25 % P_2O_5; Buysky Chemical Works, JSC, Russia), and CN (15.5 % CaO, 15.5 % N–NO_3, 0.7 % N–NH_4; Sredneuralsk Copper Smelter, JSC, Russia). Solutions were prepared immediately before each experiment using dechlorinated tap water.

Experimental design

Chronic toxicity tests were conducted in a 10-day static regime (without solution renewal). For each fertiliser, a series of concentrations was used, selected in preliminary experiments to cover a mortality range from 0 to 100 %: for AM — 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16 mg/mL; for BSP — 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0 mg/mL; for CN — 0.06, 0.125, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 mg/mL. Larvae in the control group were kept in dechlorinated water without any fertiliser addition. Each experimental variant comprised three biological replicates (1 replicate = 10–15 larvae). Mortality was recorded daily; individuals showing no visible movement upon gentle mechanical stimulation were considered dead. The maintenance conditions were consistent with those for stock mosquito larvae.

Statistical analysis

Effective concentrations (EC_{10}, EC_{20}, EC_{50}) and their 95 % confidence intervals were calculated using probit analysis with a three-parameter logistic model (LL.3). Modeling was performed in the R environment using the drc package [Ritz et al., 2015] with binomial regression and weights proportional to sample size. Confidence intervals were estimated using the delta method. Model goodness-of-fit was assessed using McFadden's pseudo-R^2 (values > 0.8 were considered excellent) and the χ^2 goodness-of-fit test ($p > 0.05$ indicated an adequate model).

The supplementary Table S1: Raw data for acute toxicity tests (cumulative mortality on day 20 for each concentration and replicate) is presented in the Appendix.

Results and discussion

In the present study, a first comparative quantitative assessment of the sublethal toxicity of three widely used mineral fertilisers (CN, BSP, CN) to mosquito larvae has been conducted in a 10-day test. The obtained EC_{50} values allow the fertilisers to be ranked by toxicity: BSP > CN > AM. These data significantly complement the scarce and largely outdated information on the toxicity of fertilisers to aquatic invertebrates [Fedorova, 1989] and provide, for the first time, the EC_{10} and EC_{20} values required for planning sublethal selection experiments.

In the 10-day chronic laboratory test on mosquito larvae, effective concentrations (EC_{10}, EC_{20}, and EC_{50}) were determined for CN, BSP, and AM (Table 1). All fitted models showed excellent agreement with the data: McFadden's pseudo-R^2 values ranged from 0.87 to 0.91. The χ^2 goodness-of-fit test confirmed that the model adequately described the data for all three fertilisers ($p > 0.05$): $\chi^2 = 4.53$, $df = 10$, p = 0.60 (CN); $\chi^2 = 6.17$, $df = 9$, $p = 0.72$ (BSP); $\chi^2 = 8.24$, $df = 10$, $p = 0.61$ (AM).

The three mineral fertilisers differed markedly in their toxicity to mosquito larvae (Fig. 1). The most toxic fertiliser was BSP (EC_{50} = 4.1 mg/mL, 95 % CI: 3.5–4.8 mg/mL), followed by CN (EC_{50} = 5.8 mg/mL, 95 % CI: 5.1–6.6 mg/mL), while AM was the least toxic (EC_{50} = 12.8 mg/mL, 95 % CI: 11.4–14.5 mg/mL). The EC_{20} values were 2.4 mg/mL (BSP), 4.5 mg/mL (CN), and 8.9 mg/mL (AM), whereas the EC10 values were 1.8, 3.9, and 7.2 mg/mL, respectively.

Differences in toxicity to mosquito larvae among the applied fertilisers can be explained by their different ionic compositions and mechanisms of action. The most toxic component of AM is the ammonium ion (NH_4^+), which at sublethal concentrations can disrupt the functioning of Na^+/K^+-ATPase, leading to ATP depletion and neurological disturbances [Dasarathy et al., 2017]. Calcium nitrate proved to be moderately toxic; nitrate (NO_3^-) on its own is relatively low in toxicity; however, under conditions of prolonged exposure (10 days), its bacterial reduction to nitrite (NO_2^-) is possible, which causes methemoglobinemia in vertebrates, including humans [Kross et al., 1992]. It should be noted that our tests were performed under static conditions without renewal of the test solutions; therefore, the actual nitrate concentrations could have decreased due to microbial

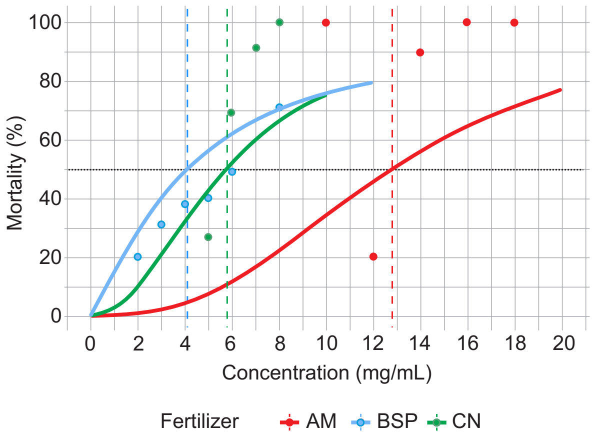

Fig. 1. Dose-response curves for calcium nitrate (CN), double superphosphate (BSP), and ammophos (AM) for *Aedes aegypti* larvae. Designations: Solid lines represent fitted three-parameter log-logistic models (LL.3); points show observed mortality (symbol size proportional to sample size); vertical dashed lines indicate EC_{50} values for each fertiliser; the horizontal dotted line marks 50 % mortality.

Рис. 1. Кривые «доза-эффект» трёх минеральных удобрений: кальциевой селитры (CN), двойного суперфосфата (BSP) и аммофоса (AM) для личинок *Aedes aegypti*. Обозначения: сплошные линии соответствуют подобранным трёхпараметрическим логистическим моделям зависимости от логарифма дозы (LL.3), точками показана наблюдаемая смертность (размер символа пропорционален объёму выборки), вертикальные пунктирные линии обозначают значения EC_{50} для каждого удобрения, горизонтальная пунктирная линия отмечает 50 % смертность.

degradation; consequently, the obtained EC_{50} values are conservative estimates.

The steepest slope was observed for AM (6.2 ± 1.1), indicating a sharper increase in mortality with increasing concentration compared to CN and BSP. In contrast, the slope for BSP was the shallowest (2.9 ± 0.4), suggesting a more gradual development of the toxic effect.

The most unexpected finding was the high toxicity of BSP, which is likely due to a combination of several factors: the acidic nature of the solutions (pH 3–4), an excess of Ca^{2+} ions disrupting cellular signaling, and the direct damaging effect of phosphates on the cuticle and intestinal epithelium [Orrenius et al., 1992]. However, we did not control the pH or use buffer systems, so the contribution of the acidic component to the overall

Table 1. Effective concentrations and parameters of the logistic (LL.3) model for three mineral fertilisers
Таблица 1. Эффективные концентрации и параметры логистических моделей (LL.3) для трёх минеральных удобрений

Parameter	Calcium nitrate (CN)	Double superphosphate (BSP)	Ammophos (AM)
EC_{10}	3.9 (3.1–4.6)	1.8 (1.2–2.3)	7.2 (5.8–8.9)
EC_{20}	4.5 (3.7–5.2)	2.4 (1.8–3.0)	8.9 (7.4–10.7)
EC_{50}	5.8 (5.1–6.6)	4.1 (3.5–4.8)	12.8 (11.4–14.5)
Slope ± SE	4.8 ± 0.7	2.9 ± 0.4	6.2 ± 1.1
Pseudo-R^2 (McFadden)	0.89	0.91	0.87
χ^2 (*df*)	4.53 (10)	6.17 (9)	8.24 (10)
p (χ^2)	0.60	0.72	0.61

toxicity of superphosphate remains not fully separated from the ionic action of Ca^{2+} and PO_4^{3-}. Overall, our data complement the current understanding that conventional phosphorus fertilisers, having a high leaching potential, pose long-term risks to water quality not only through eutrophication but also through direct toxicity to aquatic organisms [Leon et al., 2024]. This necessitates the implementation of effective nutrient capture methods from diffuse runoff before they enter fishery water bodies [Ahmed, Hayes, 2025].

Comparison with published data shows that our 10-day EC_{50} values for *A. aegypti* (4.1–12.8 mg/mL) are comparable to the 24-h LC_{50} values reported by Fedorova [Fedorova, 1989] for *Culex pipiens* (5–15 g/L; note that 1 g/L = 1 mg/mL). Given that our exposure duration was 10-fold longer, this similarity suggests that the toxic effects of mineral fertilisers on mosquito larvae manifest rapidly, within the first 24 hours, with no substantial cumulative toxicity over longer periods under static test conditions. The slight differences observed may be attributed to species specificity (*A. aegypti* vs *C. pipiens*), larval instar, or water chemistry. Our results highlight the importance of considering both exposure duration and species identity when interpreting ecotoxicological data for fertilisers [Gomez Isaza et al., 2020].

Certain sublethal concentrations (EC_{10}–EC_{20}) identified in this study are of ecological significance. Although these levels cause mortality of only 10–20 % of individuals, they can serve as drivers of microevolutionary changes in mosquito populations under long-term anthropogenic pressure [Kibuthu et al., 2016]. The differences in the slopes of the dose-response curves (ranging from 2.9 for BSP to 6.2 for AM) point to different mechanisms of toxic action of the fertilisers: gradual, multifactorial for BSP and threshold-type, likely associated with ammonium toxicosis, for AM.

In real agroecosystems, mosquito larvae are exposed not to a single stressor but to a complex mixture of pollutants (pesticides, fertilisers, heavy metals), which can lead to synergistic or antagonistic effects [Kibuthu et al., 2016; Ambadiang et al., 2023]. Even non-lethal concentrations can redirect the body's energetic resources toward the stress response, reducing fecundity and survival at different stages of metamorphosis [Kibuthu et al., 2016]. The question arises: does exposure to sublethal concentrations of fertilisers lead to similar processes, creating a risk of cross-resistance? In this context, the threshold concentrations we obtained for individual fertilisers are a necessary baseline for further research on the combined effects of agrochemicals and can be used in the planning of selection experiments, in ecological and epidemiological monitoring, and in risk assessment related to the contamination of aquatic ecosystems by agrochemicals.

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Table S1. Raw data for acute toxicity tests (cumulative mortality on day 20 for each concentration and replicate)

Таблица S1. Исходные данные тестов на острую токсичность (кумулятивная смертность на 20-й день для каждой концентрации и повторности

Fertiliser	Concentration (mg/mL)	Replicate	N	Dead (day 20)
AM	16	1	30	30
AM	16	2	30	30
AM	16	3	30	30
AM	14	1	30	24
AM	14	2	30	27
AM	14	3	30	28
AM	12	1	30	7
AM	12	2	30	6
AM	12	3	30	8
AM	10	1	30	6
AM	10	2	30	5
AM	10	3	30	7
AM	9	1	45	4
AM	9	2	45	3
AM	9	3	45	5
AM	8	1	45	3
AM	8	2	45	2
AM	8	3	45	3
AM	7	1	45	3
AM	7	2	45	2
AM	7	3	45	4
AM	6	1	45	2
AM	6	2	45	1
AM	6	3	45	3
AM	5	1	30	1
AM	5	2	30	1
AM	5	3	30	1
AM	4	1	45	2
AM	4	2	45	2
AM	4	3	45	2
AM	3	1	45	1
AM	3	2	45	1
AM	3	3	45	1
AM	2	1	30	1
AM	2	2	30	0
AM	2	3	30	2
AM	1.5	1	30	0
AM	1.5	2	30	0
AM	1.5	3	30	0
AM	1	1	30	0
AM	1	2	30	0
AM	1	3	30	0
AM	0.5	1	45	0
AM	0.5	2	45	0
AM	0.5	3	45	0
AM	0.25	1	45	0
AM	0.25	2	45	0
AM	0.25	3	45	0
AM	0.125	1	45	0
AM	0.125	2	45	0
AM	0.125	3	45	0
AM	0.06	1	45	0
AM	0.06	2	45	0
AM	0.06	3	45	0
BSP	10	1	45	45
BSP	10	2	45	45
BSP	10	3	45	45
BSP	8	1	45	32
BSP	8	2	45	30
BSP	8	3	45	34
BSP	7	1	45	26
BSP	7	2	45	24
BSP	7	3	45	28
BSP	6	1	45	22
BSP	6	2	45	20
BSP	6	3	45	23
BSP	5	1	30	12
BSP	5	2	30	11
BSP	5	3	30	12
BSP	4	1	45	17
BSP	4	2	45	15
BSP	4	3	45	16
BSP	3	1	45	14
BSP	3	2	45	12
BSP	3	3	45	14
BSP	2	1	45	9
BSP	2	2	45	8
BSP	2	3	45	10
BSP	1.5	1	30	0
BSP	1.5	2	30	0
BSP	1.5	3	30	0
BSP	1	1	30	0
BSP	1	2	30	0
BSP	1	3	30	0
BSP	0.5	1	45	1
BSP	0.5	2	45	0
BSP	0.5	3	45	2
BSP	0.25	1	45	1
BSP	0.25	2	45	0
BSP	0.25	3	45	2
BSP	0.125	1	45	0
BSP	0.125	2	45	0
BSP	0.125	3	45	0
BSP	0.6	1	45	0