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Assessing the potential of mosquito larval rearing water for enhanced tomato seedling establishment

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Abstract

Vigorous seedlings guarantee satisfactory production in the forward stages of the vegetation period. This study aimed to evaluate the impact of bio-based rearing water of two mosquito species (*Culiseta* sp. and *Culex* sp.) on tomato germination, emergence, and seedling quality. For this purpose, two distinct larval-rearing waters (LRW)(with diverse larval densities), and fry food-applied water were used as bio-priming agents. The findings revealed that using bio-based rearing water could enhance the vigor of tomato seeds. All *Culex* sp. derived LRWs had a shorter mean germination time than the control group. One *Culex* sp. derived larval rearing water treatment resulted in the shortest mean germination time (4.35 days), whereas one *Culiseta* sp. derived larval rearing water treatment resulted in the longest (6.20 days). There were no statistically significant differences in stem length but significant differences in plant length. Plant length was shorter in larval rearing water and fry food-applied water than in the control. The stem diameters of plants primed with larval rearing water were generally wider than the control. According to analyses of the plant length, stem length, and stem diameter measurements, the larval rearing water and fry food-applied water treatments may have had a reductive influence on plant length but provided significant support for thicker seedlings, which are more beneficial for seedlings. Other germination and growth characteristics (vigor index of germination, emergence percentage, mean time of emergence, vigor index of emergence, plant length, stem length, leaf width, leaf length, stem fresh weight, stem dry weight, root dry weight) did not show significant variation among treatments. Using larval rearing water as a bio-priming agent in agriculture offers several benefits. Larval rearing water enhances seed germination and vigor due to its possibly rich nutrient content and bioactive compounds, promoting faster and more uniform germination. It is an eco-friendly and cost-effective alternative to chemical treatments, supporting sustainable agricultural practices.

Keywords: Priming, Tomato, Seedling, *Culex* sp., *Culiseta* sp.

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INTRODUCTION

Tomato is the most important and producing crop among vegetables in the world. In 2021, just 189 million tons of tomatoes were harvested in a 5 million ha (FAOSTAT, 2023). Using seedling in tomato production is commonly practices. Satisfactory plant growth and high yield can only obtain with healthy, uniform and vigour seedlings (Çelebi, 2019). In seedling production, a high emergence rate and healthy and homogeneous seedlings in a short time is very important (Arın & Arabacı, 2019). Therefore, some pre-sowing seed treatments are widely used to improve seed quality. One of them is priming which initiate the physiological process of germination with limited water intake under controlled conditions of seeds before sowing, to obtain high and homogeneous seed germination and emergence. Various compounds or treatments, such as salts, chemical solutions, bio compounds, etc., can be used for this purpose (Alan & İlbi, 2018; Azarmi et al., 2011; Farooq et al., 2005; Hernández et al., 2021; Hernández-Herrera et al., 2014; Li et al., 2023; Nakaune et al., 2012; Nawaz et al., 2011). Using bio-based or organic compounds for such applications may be more environmentally and safer than chemical compounds (Chakraborti et al., 2022; Righini et al., 2021). Other hand, recycling biological wastes can reduce environmental pollution and convert waste into valuable agricultural inputs. This recycling process contributes to sustainability by promoting a circular economy, enhancing resource efficiency, and reducing the reliance on synthetic chemicals. These applications not only increase the vigor of seedlings but also can accelerate germination and provide resistance to unfavorable environmental conditions, such as extreme temperatures or humidity (Singh et al., 2020; Toribio et al., 2021).

Mosquitoes belonging to the *Culex* spp. family are prevalent and can be found in tropical and temperate climate zones across all continents except Antarctica (Diaz-Badillo et al., 2011). *Culiseta* sp. larvae are commonly found in rock pools or artificial containers, often associated with *Culex* sp. However, seeing them in natural water bodies such as pools or ditches is rare (Becker et al., 2020). Only a few studies have been undertaken to investigate the effects of mosquito larvae on rearing water, focusing on the problem from a microbiological standpoint. These studies revealed that the presence of mosquito larvae can alter the microorganismal composition of water and can increase, decrease, or have no effect on total bacterial abundance depending on the species and environmental conditions (Kaufman et al., 1999; Muturi et al., 2020; Paradise & Dunson, 1998; Walker et al., 1991). Most relevant studies have concentrated on a few mosquito species, including *Aedes triseriatus* (Kaufman et al., 1999; Paradise & Dunson, 1998; Walker et al., 1991), *Culex nigripalpus* (Duguma et al., 2017), and *Culex restuans* (Muturi et al., 2020). The trophic cascade has been used to describe the impacts of larvae on rearing water (Duguma et al., 2017; Muturi et al., 2020). However, it has been underlined that mosquito larvae can play a vital role in structuring microbial food sources, and the larvae's release of some nitrogenous wastes may be one of the critical consequences of this phenomenon (Muturi et al., 2020).

In our previous study, we saw that priming with LRW of *Culex pipiens* improved germination, emergence of cabbage seeds and seedling quality (Şahin et al., 2022). This time, we have evaluated the effect of bio-based larval rearing water of mosquito species Culiseta sp., and Culex sp. on germination and emergence of tomato seeds and also, on seedling characteristics.

MATERIALS AND METHODS

The experiments were conducted using UGT-281824 tomato seeds supplied by The United Genetics Seed Company. In the study, two distinct larval-rearing waters (LRW) and fry food-applied water (FFW) were used as agents, while distilled water served as the control (C). The first LRW was derived from water containers including *Culiseta sp.* with five distinct densities (Cs 1; 1 instar larva, Cs 3; 3 instar larva, Cs 4; 4 instar larva, Cs 7; 7 instar larva, and Cs 8; 8 instar larva), and the second LRW was derived from water containers including *Culex sp.* with seven distinct densities (4X; 4 instar larva, 8X; 8 instar larva, 16X; 16 instar larva, 32X; 32 instar larva, 64X; 64 instar larva, 128X; 128 instar larva, and 256X; 256 instar larva) of the first instar larva.

The germination tests were conducted for ten days, in a Plant Growth Chamber (ALC 800) set to 25±1°C, 65±5 % RH, and complete darkness. Fifty seeds were planted in each 9 cm ∅ plexiglass Petri dish, and 5 ml of LRW were used for moistening (distilled water for control). When radicle emergence was ≥2mm, it was considered to be a germinated seed and counted daily. The emergence tests were carried out on growth shelves equipped with daylight fluorescents. Seeds were sown in 72 cell seedling trays with 0.1 ml volume. As a growing medium, peat recommended for vegetable seedling production was used (Klassmann Potground-H, DoktorTarsa Inc., Antalya, Türkiye). It possessed a pH of 6.0 and an EC-value of 0.40 dS m^{-1} and added amount of fertiliser (NPK fertiliser 14:10:18): 1.5 kg m^{-3}.When the emergence began, seedlings were exposed to 195-210 µmol $m^{-2}s^{-1}$ PPFD (photosynthetic photon flux density) for 14 h/day. The counts were done every day and the seedling with fully opened cotyledons were recorded as emergenced. During the emergence test, average minimum and maximum relative humidity (RH%) and temperature (°C) values were recorded as 48 RH%, 54 RH%, and 21°C, 27°C, respectively.

The germination (GP) and emergence (EP) percentages were transformed arcsine square root before statistical analyses, but real values of germination/emergence percentages were presented in Tables. Mean germination time (MT_G) and mean emergence time (MT_E) were calculated according to Equation 1. Vigor indexes of germination (VI_G) and emergence (VI_E) were calculated according to (Mereddy et al.) (Equation 2).

$$MT = \frac{\sum n.t}{\sum n} \quad \text{(Eq.1)}$$

n = number of newly emerging seedlings/germinated seeds at a time t, t = days from sowing, and Σ n = total emergence seedling/germinated seed.

$$VI = \left(\frac{G1}{D1}\right) + \left(\frac{G2}{D2}\right) + \cdots + \left(\frac{GL}{DL}\right) \quad \text{(Eq.2)}$$

G1 = number of emerged/germinated seeds (first count), D1 = number of days to first count, GL = number of emerged/germinated seeds (last count), and DL = number of days to last count.

Seedling and stem length (cm), stem diameter (mm), leaf length and width (cm), leaf number per seedling, and stem and root fresh and dry weight (g) in randomly selected seedlings that have reached planting size were determined. Stem and root weight loss (g) are calculated from the differences in fresh and dry weights of seedlings.

The study conducted germination and emergence tests using a randomized blocks experimental design with three replications. All data were subjected to analysis of variance (ANOVA), and the mean value was compared with the LSD test. Statistical analyses are conducted using R statistical analysis software version 4.1.0. (Core, 2013) and Agricolae library (de Mendiburu & de Mendiburu, 2019).

RESULTS AND DISCUSSION

According to the results of the germination test, the germination percentage ranged from 100.00% to 86.67% and was statistically insignificant (Table 1). The mean germination time (MTG) among treatments varied between 6.20 and 4.35 days. All *Culex* sp. derived LRWs had a shorter MTG than the control. The 256X treatment of *Culex* sp. had the shortest MTG, while the seeds of CS8 treatment germinated for the longest time. All *Culiseta* sp. treatments had a longer MTG than the control (except for CS3 in the same group as the control). In contrast, most *Culiseta* sp. treatments extended the main germination time, implying slower germination. Yet, all *Culiseta* sp. treatments, except for CS3, were still faster than the control, pointing to their potential utility in enhancing germination efficiency, albeit to a lesser extent than *Culex* sp. derived treatments.

Table 1. The characteristics of LRWs-treated tomato seeds regard germination and emergence.

Treatment	GP	MTG	VIG	EP	MTE	VIE
CS1	100.00	5.96 de	4.58	100.00	13.68	1.51
CS3	97.33	5.28 abcde	5.19	95.00	13.74	1.47
CS4	96.00	5.41 bcde	4.83	100.00	13.62	1.50
CS7	97.33	5.63 cde	4.63	100.00	13.29	1.55
CS8	100.00	6.20 e	4.51	98.33	13.25	1.54
4X	96.00	5.02 abcd	5.32	100.00	13.25	1.59
8X	86.67	4.76 abc	4.80	100.00	13.48	1.51
16X	96.00	5.18 abcd	5.06	100.00	13.85	1.52
32X	98.67	4.83 abc	5.83	100.00	13.71	1.51
64X	96.00	4.62 ab	5.52	95.00	13.77	1.43
128X	98.67	4.82 abc	5.42	98.33	13.58	1.50
256X	93.33	4.35 a	5.56	100.00	13.33	1.55
C	97.33	5.36 abcde	4.88	100.00	13.33	1.53
FFW	96.00	4.89 abc	5.38	96.67	13.23	1.54

GP; Germination Percentage (%), MTG; Mean Time of Germination (days), VIG; Vigor Index of Germination, EP; Emergence Percentage (%), MTE; Mean Time of Emergence (days), VIE; Vigor Index of Emergence

There were no statistically significant differences in the vigor index of germination, emergence percentage, mean time of emergence, or vigor index of emergence. The vigor index of germination ranged from 4.51 (CS8) to 5.83 (32X), and the control was recorded as 4.88. Emergence percentage was generally high and ranged from 100.00 % to 95.00 %; control was recorded as 100.00 %. The mean time of emergence ranged from 13.85 (16X) to 13.23 (FFW) days, and the meantime of all groupswas 13.50 days. The vigor index of emergence ranged from 1.59 (4X) to 1.43 (64X).

The longest plant length was observed in the control group plants (34.74 cm), and the shortest was in the 128X group (28.05 cm). Even though all groups showed less plant length than the control, the *Culiseta* treatment groups were more like the control group. The 256X group that showed the best result respecting the mean germination time (4.35 days) also recorded shorter seedlings (28.59 cm) than the mean of all *Culex*-derived treatment groups (29.86 cm) (Table 2).

The stem length ranged from 10.31 cm to 11.19 cm, the leaf width was 4.41 cm to 6.46 cm, and the leaf length was 4.67 cm to 5.68 cm. There were no statistically significant differences among treatments concerning these three characteristics, but differences in the number of leaves per seedlings were important, and the highest and lowest values were observed in 4X with 3.63 and in CS1 with 2.77, respectively. The stem diameter for the average of all treatments was 2.71 mm and the largest stem diameter was 2.86 mm (128X), the smallest stem diameter was 2.51 mm (4X), and the control group was 2.76 mm (Figure 1).

Although the difference among the treatments is not significant, the recorded highest stem fresh weight was 1.24 g, the lowest was 0.93 g, and the average was 1.09 g. There were statistically significant variations between groups in root fresh weight. The FFW treatment group had the highest RFW (0.81 g), whereas the 64X treatment group had the lowest (0.64 g). Both stem and root dry weight parameters had no statistically significant differences. The stem dry weight varied from 0.18 g to 0.13 g and averaged 0.15 g, whereas the root dry weight ranged from 0.10 g to 0.05 g and averaged 0.06 g (Table 3).

Significant differences were found among seven distinct statistical groups when considering both stem and root weight loss. Regarding stem and root, the 4X treatment group showed the highest weight loss, with values of 1.06 g and 0.75 g, respectively. On the other hand, the 64X treatment group displayed the lowest weight loss in both stem and root, with recorded values of 0.78 g and 0.57 g, respectively. The average stem weight loss was determined to be 0.93 g, and the average root weight loss was 0.64 g. These values were similar to the weight losses observed in both stem and root within the control group (Figure 2).

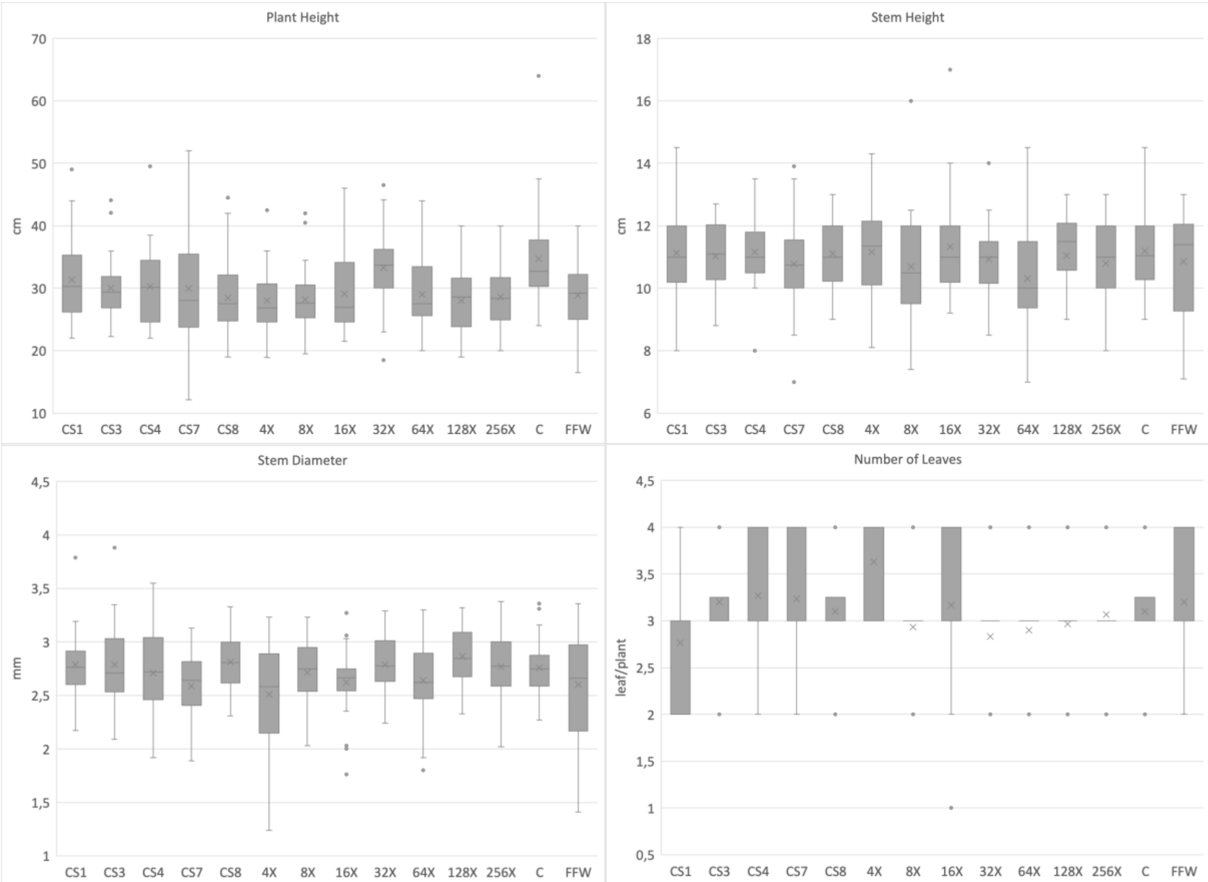

Figure 1. Changing the tomato seedling characteristics according to treatments

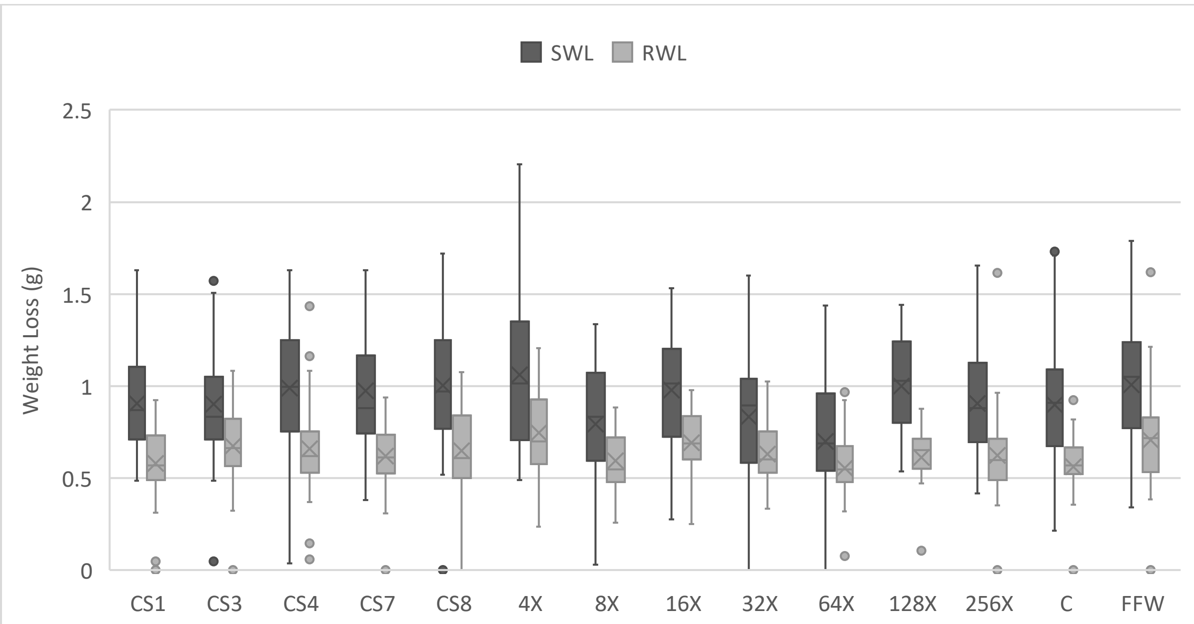

Figure 2. Changing of root and stem weight loss According to different treatments (SWL; stem weight loss, RWL; root weight loss)

Table 2. The effect of different LRWs on some characteristics of tomato seedlings

Treatment	PL	SL	LW	LL	NL	SD
CS1	31.41 abc	11.12	4.88	4.92	2.77 c	2.79 ab
CS3	30.06 abc	11.03	5.01	4.91	3.20 abc	2.79 ab
CS4	30.27 abc	11.16	5.03	5.14	3.27 ab	2.71 abc
CS7	29.98 abc	10.77	4.56	4.69	3.23 ab	2.59 bc
CS8	28.45 c	11.11	4.82	5.41	3.10 bc	2.81 ab
4X	28.05 c	11.16	4.79	4.79	3.63 a	2.51 c
8X	28.21 c	10.70	4.73	5.68	2.93 bc	2.71 abc
16X	29.16 bc	11.34	5.13	4.92	3.17 bc	2.62 abc
32X	33.31 ab	10.93	4.65	4.90	2.83 bc	2.79 ab
64X	29.01 bc	10.31	4.41	4.67	2.90 bc	2.64 abc
128X	28.05 c	11.05	5.06	5.21	2.97 bc	2.86 a
256X	28.59 bc	10.80	4.87	4.80	3.07 bc	2.77 ab
C	34.74 a	11.19	6.46	5.02	3.10 bc	2.76 abc
FFW	28.83 bc	10.86	4.73	5.01	3.20 abc	2.60 bc

PL; Plant Length (cm), SL; Stem Length (cm), LW; Leaf Width (cm), LL; Leaf Length (cm), NL; Number of Leaves, SD; Stem Diameter (mm)

Table 3. The effect of different LRWs on fresh and dry weights of tomato seedlings

Treatment	SFW	RFW	SDW	RDW	SWL	RWL
CS1	1.06	0.65 bc	0.15	0.05	0.91 abcd	0.60 ab
CS3	1.08	0.77 abc	0.18	0.08	0.90 abcd	0.70 bcd
CS4	1.16	0.75 abc	0.17	0.09	0.99 bcd	0.66 abcd
CS7	1.11	0.69 abc	0.13	0.05	0.97 abcd	0.64 abcd
CS8	1.16	0.72 abc	0.15	0.06	1.04 cd	0.67 abcd
4X	1.24	0.80 ab	0.18	0.06	1.06 d	0.75 d
8X	0.93	0.66 abc	0.13	0.06	0.79 ab	0.59 ab
16X	1.13	0.77 abc	0.15	0.08	0.98 bcd	0.69 abcd
32X	1.03	0.68 abc	0.16	0.05	0.86 abc	0.63 abcd
64X	0.96	0.64 c	0.15	0.07	0.78 a	0.57 a
128X	1.19	0.71 abc	0.15	0.10	1.03 cd	0.61 abc
256X	1.05	0.70 abc	0.15	0.06	0.90 abcd	0.65 abcd
C	1.11	0.67 abc	0.17	0.05	0.93 abcd	0.60 ab
FFW	1.14	0.81 a	0.14	0.07	1.01 cd	0.73 cd

SFW; Stem Fresh Weight (g), RFW; Root Fresh Weight (g) SDW; Stem Dry Weight (g), RDW; Root Dry Weight (g), SWL; Stem Weight Loss (g), RWL; Root Weight Loss (g)

Some bio-compounds can improve seedling features such as height, diameter, or weight. For example, a study investigated the effects of *Trichoderma* isolates on tomato seedlings and showed that applications of the isolates significantly increased shoot height and diameter, as shoot and root fresh and dry weight in seedlings (Azarmi et al., 2011). In our study, there were no statistically significant differences in stem length, but LRW and FFW showed shorter seedling lengths than the control (Table 2). The stem diameters of seedlings obtained from priming seeds with two distinct larval-rearing waters with diverse larval densities, and fry food-applied water has considerably varied, and generally, LRW treatments showed more wide stem diameters than the control. According to the plant length, stem length, and stem diameter results, LRW and FFW treatments had a negative effect on root development but good support for thicker plants in tomatoes which are more favourable for seedlings (Azarmi et al., 2011). Considering seedling production, quality, and transportation, thick seedlings that are not tall are generally preferred. In this regard, 128X could be suggested because it was better than others (including control) as a priming treatment.

All bio-compounds don't show similar effects. For instance, irrigation with cyanobacteria-contaminated waters caused biomass loss in tomatoes, especially root biomass (Levizou et al., 2017). Another study found several plant and microbial metabolites can reduce emergence and negatively influence root and shoot fresh weight in tomato seedlings (Jung et al., 1999). In our research, applications with various sourced LRWs had varying results. *Culiseta* sp. derived LRW resulted in the same or more weight reduction than the control in aspects of the stem and root weight loss. On the other hand, *Culex* sp. 64X treatment gave the best result in these two aspects.

Another study found no variations in germination percentage but substantial differences in germination vigor index when wastewater was reused in broad bean irrigation (Shannag et al., 2021). There were no statistical differences in germination and emergence characteristics, except in the mean germination time. However, there were significant differences in plant length, number of leaves, stem diameter, root fresh weight, stem and root weight loss.

It is possible that the using the wastewater obtained from fish farming in vegetable seedling production. In a study dealing with it's using, it has demostrated that it can be used as irrigation water of *Tabebuia aurea* seedlings by diluting 25-50 % (Pinto et al., 2016). However, it has been seen that the higher concentration results in adverse effects. In our study, there was no linear relationship between concentrations (each concentration has its unique effect)

CONCLUSION

The results of this study suggest that *Culex* and *Culiseta* sp. derived LRWs can significantly influence the mean time of germination of seeds and seedling growth characteristics of tomato seedlings. While the germination percentage was found to be statistically insignificant, the mean germination time varied considerably among the treatments. Interestingly, *Culex* sp. derived LRWs were found to reduce the mean germination time, thus promoting faster germination. This finding is noteworthy as rapid germination is generally associated with improved seedling establishment and could be advantageous in both field and seedling growing conditions. Most other germination and growth characteristics did not vary significantly among treatments, suggesting that while LRW treatments can influence germination time, their impact on other early growth aspects may be limited. However, we also had good results like 128X treatment, which led to shorter but thicker seedlings, which are favourable seedling characteristics.

This study aligns with previous research indicating that LRWs affect seed germination and seedling growth, while demonstrating that effects can vary significantly depending on the LRW source. Importantly, the recycling of biological waste like LRW not only reduces environmental pollution but also transforms waste into valuable agricultural inputs. The recycling of biological waste process is crucial for sustainability, promoting a circular economy, enhancing resource efficiency, and reducing reliance on synthetic chemicals. These advantages of recycling underline the potential of LRW to contribute significantly to sustainable agriculture and improved productivity. Future research should focus on confirming these findings and investigating the underlying mechanisms, particularly the role of different compounds in *Culex* and *Culiseta* sp. derived LRWs and their physiological impacts on seed germination and seedling growth.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare no conflicts of interest.

Author contribution

NS: Methodology, Investigation, Data analysis, Writing-Original Draft, Review & Editing; LA: Supervision, Funding acquisition, Conceptualization, Methodology, Review & Editing; EB: Methodology, Investigation, Data Collecting; EU: Methodology, Investigation, Data Collecting

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