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A Comparative Analysis of Botanical Extracts from *Rosmarinus officinalis, Nigella sativa, Lippia citriodora*, and *Origanum vulgare* for Optimizing Rotifer (*Brachionus plicatilis*) Culture

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Abstract

Despite progress in artificial diets, live rotifers (*Brachionus plicatilis*) remain essential for larval fish in marine hatcheries due to their superior digestibility, requiring additives to ensure a healthy culture. This study investigated the effects of water-based extracts from *Rosmarinus officinalis* (rosemary), *Nigella sativa* (black cumin), *Lippia citriodora* (lemon grass), and *Origanum vulgare* (thyme) on rotifer culture performance. Rotifers were cultured for 29 days with extracts at 0.25, 0.50, and 1.00 ppm and compared to a control group. All variables (extract, concentration, and time) significantly affected performance ($P<0.05$). The 1.00 ppm concentrations of *R. officinalis* and *N. sativa* proved most effective, yielding the highest rotifer densities (2.4×10^5 individuals L^{-1}). These groups also exhibited superior reproductive performance, measured by the proportion of egg-carrying females, and showed significant dose-dependent changes in swimming patterns. These findings demonstrate that 1.00 ppm extracts of rosemary and black cumin are viable, natural additives for optimizing rotifer productivity. This approach offers a sustainable method to enhance live feed quality, potentially providing significant advantages for the early larval feeding stages in marine aquaculture.

Introduction

Marine fish larvae exhibit exceptionally high mortality rates during early-life stages, with wild populations experiencing losses exceeding 99% (China & Holzman, 2014; Arevalo et al., 2023) and hatchery-reared larvae facing mortality rates ≥80% (Sales, 2011; Langdon, 2018). This vulnerability aligns with the critical-period hypothesis, which identifies the transition from endogenous yolk-sac dependence to exogenous feeding as a developmental bottleneck (Tiedemann & Bils, 2016). During this phase, rapid physiological restructuring occurs, and mortality peaks, emphasizing the decisive role of feed accessibility and nutritional quality in survival (Yúfera & Darias, 2007; Benini et al., 2022). Overcoming this challenge is a key priority for a sustainable "Blue Economy," which relies on robust and efficient aquaculture production (Seyhan et al., 2025).

Despite advances in hatchery technology, formulated microdiets often yield inferior growth and survival rates for marine fish larvae compared to live prey, such as rotifers and *Artemia* (Rathore et al., 2016). The small particle size of microdiets (<200 µm) limits effective nutrient enrichment, while larvae's rapid growth and continuous foraging necessitate exceptionally high dietary intake (Howell, 1973; Dhert et al., 2001; Lubzens et al., 2001; Langdon, 2018). Temperature-driven digestive kinetics further exacerbate this demand, as gastric evacuation rates increase exponentially with temperature, necessitating

more frequent feeding to sustain growth (Basçinar et al., 2016; 2017; Mazlum et al., 2020; Dürrani, 2025). A meta-analysis of 27 freshwater species revealed that larvae fed compound diets face a 2.5-fold higher mortality risk (95% CI: 2.09–2.89) than those provided live feeds (Sales, 2011). Consequently, live prey – notably the rotifer *Brachionus plicatilis* – remains indispensable in larviculture due to its optimal size (50–200 µm), neutral buoyancy, rapid reproduction, and adaptability to intensive culture systems (Fukusho, 1989; A. Hagiwara et al., 2001; Sterzelecki et al., 2021; Kuo et al., 2022). Rotifers' inherent docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) content further supports larval development (Lubzens et al., 2001; Atsushi Hagiwara et al., 2007; Kandathil Radhakrishnan et al., 2020).

Optimizing rotifer production and enrichment protocols is critical to maximizing their nutritional value. Decades of research have established that rotifers can be biochemically enhanced through short-term enrichment with commercial products like Selco, yeasts, and various oil emulsions to boost lipids, protein, and n-3 highly unsaturated fatty acid (HUFA) levels (Fernández-Reiriz et al., 1993; Eryalcin, 2018). However, this optimization is complex, with performance being highly dependent on the specific rotifer species, morphotype, and the precise feed or enrichment product used (Ajah, 2008; Waqalevu et al., 2019; Özdogan & Savaş, 2022). While these methods are effective, they often rely on costly synthetic or variable biological products, steering research towards more sustainable and stable additives.

Herbal extracts, particularly those from *Rosmarinus officinalis* (rosemary), *Nigella sativa* (black cumin), *Lippia citriodora* (lemon verbena), and *Origanum vulgare* (oregano), represent promising natural alternatives to synthetic aquaculture additives (Hernández-Contreras & Hernández, 2020; Dawood et al., 2021). This approach is validated by recent studies showing plant-based additives improve growth and immunity in finfish (Habib et al., 2024; Afzal et al., 2024; Ujan et al., 2025a, 2025b). The bioactive compounds in these plants, such as carnosic acid (antioxidant), thymoquinone (antimicrobial), and carvacrol (immunomodulatory), are known to enhance growth, stress resilience, and pathogen resistance in species like shrimp and seabass (Reverter et al., 2017; Karataş et al., 2020; Cakir & Karatas, 2024). Despite these benefits in finfish, their application directly to rotifer culture remains underexplored, particularly in terms of optimal dosage, compound stability, and long-term impacts on rotifer viability and nutritional value. Consequently, this study evaluated the effects of the *L. citriodora*, *N. sativa*, *O. vulgare*, and *R. officinalis* extracts (0.25–1.00 ppm) on rotifer performance by analyzing growth rates, survival, reproductive output, and behavioral responses. By identifying optimal dosing strategies, this study aims to improve sustainable rotifer production systems, enhance larval nutrition, and reduce reliance on conventional feeds. These findings will inform eco-friendly strategies for addressing economic and health constraints in marine larviculture and fostering resource-efficient aquaculture practices.

Materials and Methods

Plant Extracts

Water-based extracts of *R. officinalis*, *N. sativa*, *L. citriodora*, and *O. vulgare* were selected due to their established bioactivity and regional availability. The extracts, derived from Mediterranean-grown plants, were supplied by Talya Herbal Products (Antalya, Türkiye) and produced in accordance with food and feed additive standards. They exhibited a characteristic yellowish hue and were stored at +4°C in dark conditions to preserve their chemical and biological integrity.

Total polyphenol content was measured using a modified Folin-Ciocalteu method (Singleton & Rossi, 1965). Absorbance of the resulting blue chromophore was quantified at ~760 nm using a BioTek Synergy HTX plate reader at Anadolu University. The results, expressed as gallic acid equivalents (GAE), were as follows (mean±s.d.): *O. vulgare* 272.65±9.52 µg GAE mL^{-1}, *R. officinalis* 49.86±1.29 µg GAE mL^{-1}, *L. citriodora* 40.16±0.65 µg GAE mL^{-1}, and *N. sativa* 18.88±3.60 µg GAE mL^{-1}.

Rotifer Culture and Experimental Setup

Stock populations of *Brachionus* sp. rotifers were obtained from the Zooplankton Unit of the Central Fisheries Research Institute (SUMAE). They were cultured in an incubator at 24±1°C under a 12:12 h light-dark cycle to mimic natural environmental rhythms to promote optimal growth and reproduction. Daily feedings of *Nannochloropsis oculata* microalgae (1×10^6 cells mL^{-1}) cultured in-house were provided.

The culture volume was scaled progressively from 10 mL test tubes to 5 L and finally to 6 L flasks to meet experimental needs. Corresponding phytoplankton feeding volumes were accordingly increased: 100 mL day^{-1} for cultures up to 1 L, 150 mL day^{-1} for 1–3 L, and 200 mL day^{-1} for 3–6 L cultures.

The effects of the four plant extracts were tested at three concentrations (0.25, 0.5, and 1.00 ppm). Each treatment group and control were replicated three times. The 29-day experiment was initiated by stocking each culture vessel at an initial population density of 50 individuals mL^{-1}. The control group was fed only phytoplankton (*N. oculata*) with no plant extract added. The specified dose of each plant extract was added simultaneously with the daily phytoplankton feeding, with volumes adjusted to match the increasing culture size to ensure stable concentrations throughout the 29-day experimental period.

Throughout the experiment, water quality parameters were regularly monitored to maintain

optimal conditions for *B. plicatilis* cultures. The temperature remained stable at 24±1°C and the average pH measured at 8.6. While pH values remained stable in the *R. officinalis* and *N. sativa* groups, a gradual decrease to 7.3–7.6 was observed in the *O. vulgare* and *L. citriodora* groups, coinciding with reduced culture stability and increased rotifer mortality.

Growth and Survival Assessment

To assess rotifer population dynamics, 1 mL subsamples were collected daily from each replicate. Counts of individuals with and without eggs and total rotifers were performed using a Sedgewick-Rafter counting chamber. During this daily microscopic analysis, qualitative observations of rotifer swimming behavior (e.g., normal activity vs. sluggish or erratic movement) were systematically recorded as a standardized indicator of culture health.

Population growth was quantified as the Population as a Percentage of Initial, calculated using the formula:

$$\text{Population growth} = \left({}^{N_t}/_{N_0}\right) \times 100$$

where N_0 is the initial density and N_t is the density at time t. This metric is distinct from the survival rate, which refers only to the proportion of living individuals and cannot exceed 100%.

Statistical Analysis

The effects of treatment, dose (ppm), and day on rotifer counts (with egg, without egg, and total) were tested using a factorial analysis of variance (ANOVA). Subsequently, post hoc pairwise comparisons of estimated marginal means (EMMs) were conducted with Tukey's HSD correction for multiple comparisons. Statistical significance was determined using the *F*-test (P<0.05). All statistical analyses were performed using R statistical software (v. 4.3.3; R Core Team, 2024) within the RStudio interface (v. 2024.12.0; RStudio Team, 2020).

Results

Model Diagnostics

Diagnostic plots confirmed the assumptions for factorial ANOVA: the Q–Q plot showed that residuals were approximately normally distributed, and the scale–location plot indicated homoscedasticity (constant variance) across the fitted values (Figure 1). Although a few high-leverage observations were identified, Cook's distance analysis revealed that no individual data point exerted an undue influence on the model. These findings support the suitability of the analysis

Effects of Treatment, Dose, and Time on Rotifer Abundance

Interaction Effects of Treatment, Dose, and Treatment Time

A factorial ANOVA was conducted to assess the effects of treatment (group), dose (ppm), day, and their interactions on rotifer abundance, with counts recorded as individuals with eggs, without eggs, and as total counts. Significant main effects were observed for treatment, dose, and day (Table 1). Specifically, treatment significantly affected rotifers without eggs [$F_{(4,994)}$= 29.82, P<0.01], with similar significant effects for rotifers with eggs [$F_{(4,994)}$= 14.01, P<0.01] and total counts [$F_{(4,994)}$= 34.16, P<0.01]. Dose exerted strong effects across all measures, and day significantly influenced counts for rotifers without eggs and total counts [$F_{(1,994)}$= 231.46 and 177.21, respectively, both P<0.01], but not for rotifers with eggs [$F_{(1,994)}$= 0.00, P=0.99]. In addition, significant interactions (treatment × dose, dose × day, and the three-way interaction) suggest that the impact of treatment varies with both dose and time (Table 1).

Dose Comparisons Among Treatments

Pairwise comparisons within each treatment group indicated that dose effects varied among the plant extracts (Table 2). The comparison between 0.25 and 1.00 ppm was statistically significant in *L. citriodora*, whereas *N. sativa* and *O. vulgare* showed clear dose-dependent reductions in rotifer abundance at 1.00 ppm (P<0.01). *R. officinalis* exhibited the most pronounced dose effects, with all pairwise comparisons reaching high significance (P<0.01).

Group Comparisons at Each Dose

Post–hoc pairwise comparisons at each dose further revealed differences rotifer counts among the experimental groups (Table 3). At 0.25 ppm, no significant differences were observed among the plant extracts, suggesting a uniform impact at lower concentrations (Figure 2). At 0.50 ppm, however, the *R. officinalis* group exhibited significantly higher counts than the *L. citriodora*, *O. vulgare*, and *N. sativa groups* (P<0.01). At 1.00 ppm, the significant differences between groups were most pronounced: *R. officinalis* exhibited the highest rotifer counts, while *L. citriodora* maintained the lowest counts across all doses (Figure 2).

Observed Versus Predicted Rotifer Total Counts

A comparison of the observed and predicted total rotifer counts illustrates that the predictive model tended to underestimate rotifer abundance at higher observed counts (Figure 3). Notably, the *R. officinalis* and *N. sativa* groups exhibited the highest observed

counts at 0.50 and 1.00 ppm, suggesting that these extracts have the most pronounced effects on rotifer abundance.

Population Growth, Reproduction, and Behavior

Population Growth and Density

Treatments with *R. officinalis* and *N. sativa* yielded significantly higher rotifer densities compared to the control group ($P<0.05$). Peak densities reached 2.4×10^5 individuals L^{-1} in the *R. officinalis* group, 2.1×10^5 individuals L^{-1} in the *N. sativa* group, and 1.4×10^5 individuals L^{-1} in the *L. citriodora* group, compared to 7.6×10^4 individuals L^{-1} in the control group. Moreover, population growth was most favorable at the highest extract doses (Figure 4). The average population increase (calculated as a percentage of initial density) reached 400% in the *R. officinalis* group and 280% in the *N. sativa* group, compared with 131% in the control. *O. vulgare* and *L. citriodora* exhibited intermediate rates (200% and 270%, respectively).

Reproductive Success

Reproductive success was assessed by quantifying the proportion of egg-carrying females. Across the tested doses (0.25, 0.50, and 1.00 ppm), reproductive outcomes were consistently superior to the control. The most marked enhancements in egg production were observed in the *R. officinalis* and *N. sativa* treatments, which maintained a higher proportion of egg-carrying females.

Behavioral Observations

Behavioral responses were evaluated by monitoring the swimming patterns and movement speeds of rotifers under different extract conditions. Daily microscopic observations revealed that rotifers exposed to *R. officinalis* showed no significant changes in swimming speed across the tested doses (0.25, 0.50, and 1.00 ppm), whereas those treated with *N. sativa* exhibited decreased mobility at the highest dose, including occasional collapses. In contrast, exposure to

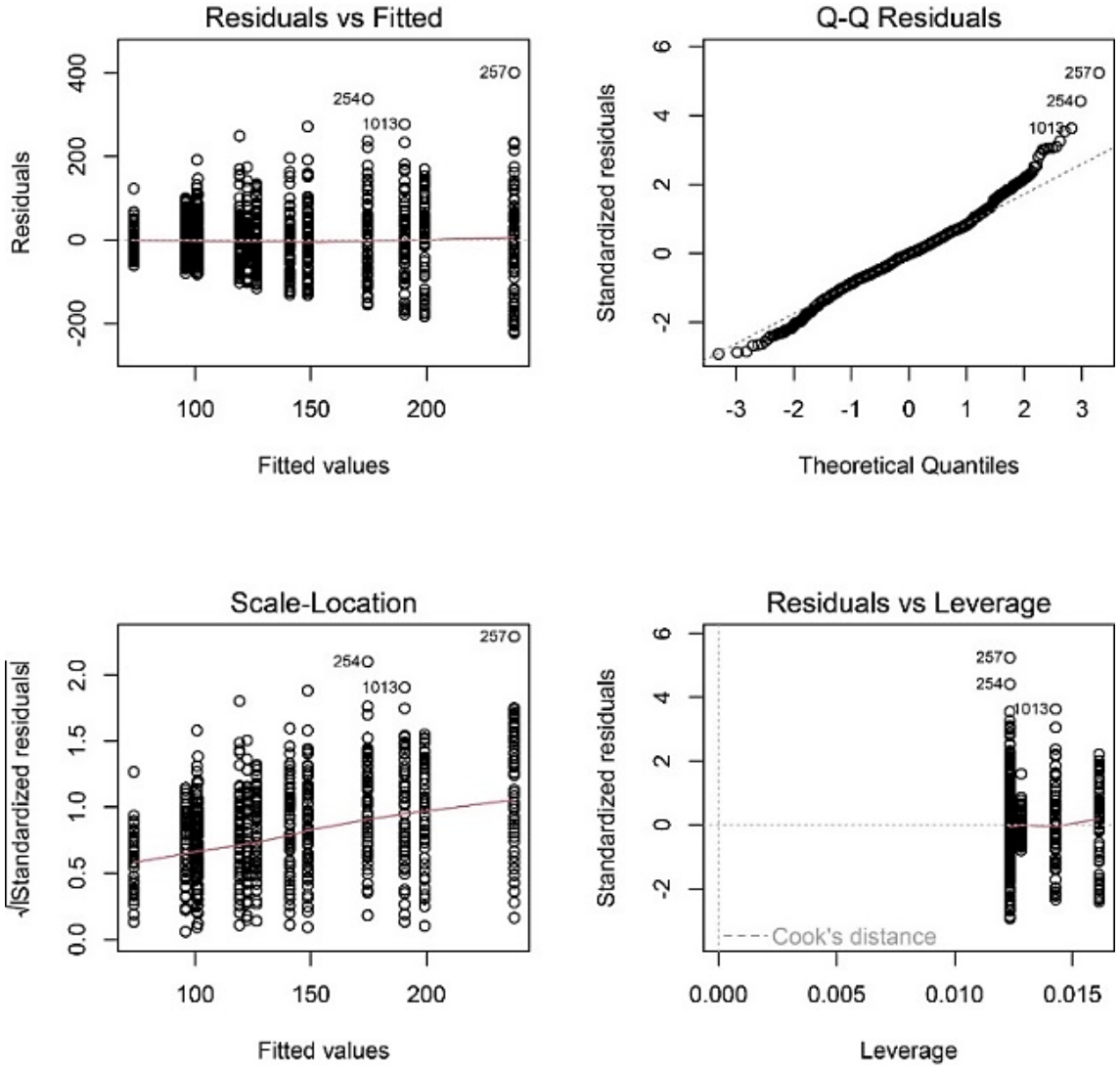

Figure 1. Diagnostic plots of the linear model (*model total*), including Residuals vs. Fitted values (top-left), Q-Q plot of residuals (top-right), Scale-Location plot (bottom-left), and Residuals vs. Leverage plot (bottom-right).

Table 1. Results of factorial ANOVA showing the effects of Group, Dose (ppm), Day, and their interactions on rotifer counts (without egg, with egg, and total). Significant p-values indicate factors significantly affecting rotifer abundance.

	Response: Rotifer without eggs					Response: Rotifer with egg					Response: Rotifer (with + without egg)				
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	4	437976	109494	29.82	<0.01	4	31371	7842.7	14.01	0.00	4	657709	164427	34.16	<0.01
Dose(ppm)	2	738396	369198	100.54	<0.01	2	57909	28954.7	51.73	<0.01	2	1211370	605685	125.85	<0.01
Day	1	849982	849982	231.46	<0.01	1	0	0.1	0.00	0.99	1	852912	852912	177.21	<0.01
Treatment:Dose(ppm)	6	145456	24243	6.60	0.00	6	15487	2581.1	4.61	0.00	6	240276	40046	8.32	0.00
Treatment:Day	4	48665	12166	3.31	0.01	4	2862	715.6	1.28	0.28	4	61763	15441	3.21	0.01
Dose_ppm:Day	2	157077	78539	21.39	0.00	2	3468	1733.9	3.10	0.05	2	195584	97792	20.32	0.00
Treatment:Dose(ppm):Day	6	58991	9832	2.68	0.01	6	6554	1092.4	1.95	0.07	6	65320	10887	2.26	0.04
Residuals	994	3650174	3672			994	556323	559.7			994	4784062	4813		

Table 2. Pairwise comparisons of the effect of Dose (ppm) within each treatment Group, based on estimated marginal means. P-values are Tukey-adjusted for multiple comparisons

Contrast Dose (ppm)	*Lippia citriodora*					*Nigella sativa*					*Origanum vulgare*					*Rosmarinus officinalis*				
	estimate	SE	df	t.ratio	p.value	estimate	SE	df	t.ratio	p.value	estimate	SE	df	t.ratio	p.value	estimate	SE	df	t.ratio	p.value
0.25-0.50	-21.3	12.1	1007	-1.8	0.183	-14.4	12.1	1007	-1.2	0.459	-18.5	12.1	1007	-1.5	0.278	-78.7	12.1	1007	-6.5	<0001
0.25–1.00	-47.6	12.1	1007	-3.9	0.001	-72.7	13.0	1007	-5.6	<0001	-90	12.6	1007	-7.2	<0001	-142.2	12.1	1007	-11.8	<0001
0.50–1.00	-26.3	12.1	1007	-2.2	0.076	-58.3	13.0	1007	-4.5	<0001	-71.5	12.6	1007	-5.7	<0001	-63.5	12.1	1007	-5.3	<0001

Table 3. Post-hoc pairwise comparisons of estimated marginal means (EMMs) for rotifer total counts between experimental groups (*Rosmarinus officinalis, Nigella sativa, Lippia citriodora*, and *Origanum vulgare*) within each dose concentration (Dose ppm) using emmeans. Pairwise contrasts were adjusted using Tukey's method, showing significant differences in rotifer total counts between groups at different dose levels

CONTRAST	Dose: 0.25 ppm					Dose: 0.50 ppm					Dose: 1.00 ppm				
	estimate	SE	df	t.ratio	p.value	estimate	SE	df	t.ratio	p.value	estimate	SE	df	t.ratio	p.value
L. citriodora-N. sativa	-25.49	12.10	1007	-2.11	0.151	-18.57	12.1	1007	-1.535	0.417	-50.58	13.00	1007	-3.90	0.001
L. citriodora-O. vulgare	0.42	12.10	1007	0.04	1.000	3.26	12.1	1007	0.27	0.993	-42.02	12.60	1007	-3.35	0.005
L. citriodora-R. officinalis	5.31	12.10	1007	0.44	0.972	-52.11	12.1	1007	-4.309	0.000	-89.32	12.10	1007	-7.39	<0001
N. sativa-O. vulgare	25.91	12.10	1007	2.14	0.140	21.83	12.1	1007	1.805	0.272	8.57	13.40	1007	0.64	0.920
N. sativa-R. officinalis	30.8	12.10	1007	2.55	0.054	-33.54	12.1	1007	-2.774	0.029	-38.74	13.00	1007	-2.98	0.016
O. vulgare-R. officinalis	4.89	12.10	1007	0.40	0.978	-55.37	12.1	1007	-4.579	<0001	-47.31	12.60	1007	-3.77	0.001

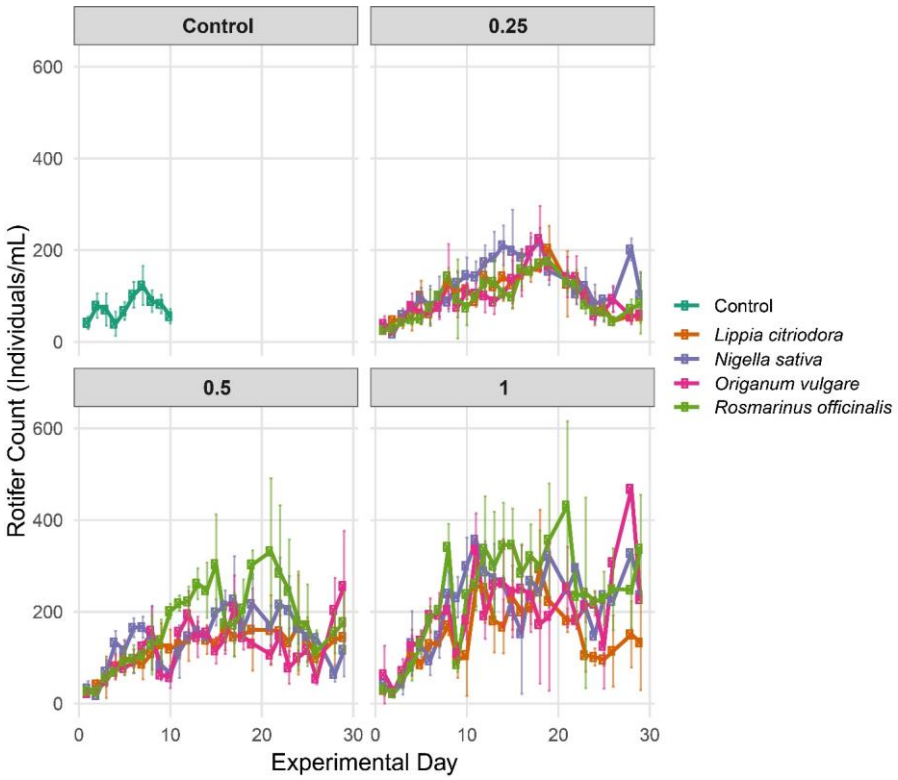

Figure 2. Mean total rotifer counts over time among different treatment groups and extract concentrations (Mean±SE). Post-hoc comparisons (Tukey's HSD) revealed significant differences between treatment groups, particularly at the 0.50 ppm and 1.00 ppm doses (P<0.05). Full details of these pairwise comparisons are presented in Table 3.

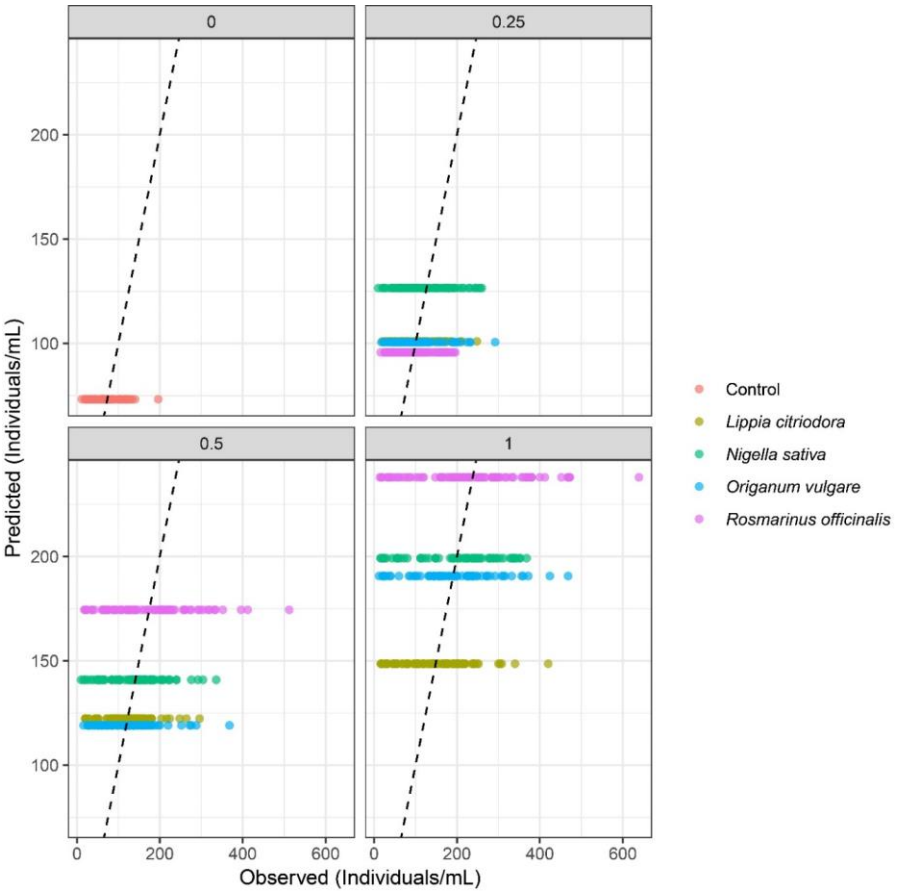

Figure 3. Observed versus predicted total rotifer counts in different treatment groups and extract concentrations. The dashed diagonal line represents the 1:1 relationship. The factorial ANOVA model used for these predictions was highly significant (P<0.01); full statistical results are presented in Table 1.

L. citriodora resulted in a modest reduction in swimming speed at 1.00 ppm, although the overall behavior remained comparable to the control. *O. vulgare* consistently induced reductions in swimming speed across all concentrations. These results indicate that while *O. vulgare* exerted the most consistent negative behavioral effect, *R. officinalis* was the most stable extract, showing no adverse impacts on rotifer behavior.

Discussion

This study demonstrated the promising potential of botanical extracts namely *R. officinalis*, *N. sativa*, *L. citriodora*, and *O. vulgare*–to enhance rotifer production, a critical advancement for improving live-feed quality in larval rearing systems, which is a key priority for an industry that continually faces significant challenges with fish mortality events and other economic losses (Aydın et al., 2024; Bal & Dürrani, 2025). Although plant-derived supplements are well-documented for improving growth, immunity, and stress resistance in finfish, this field continues to expand with recent evidence on *Withania* (Habib et al., 2024; Afzal et al., 2024), *Chenopodium* (Ujan et al., 2025a), and *Urtica* (Ujan et al., 2025b). This existing and growing body of literature (Hernández-Contreras & Hernández, 2020; Karataş et al., 2020; Dawood et al., 2021) contrasts with the limited application of these extracts in rotifer cultivation. Previous studies on rotifer enrichment have predominantly focused on commercial lipid emulsions (e.g., DHA-Gold) and microalgae (Eryalcin, 2018; Waqalevu et al., 2019; Özdogan & Savaş, 2022), leaving plant-based alternatives largely unexamined. This study addresses this gap by providing a systematic evaluation of these four extracts on rotifer population dynamics.

Through a concentration-gradient experiment (0.25, 0.50, and 1.00 ppm), this study observed distinct dose-response patterns. At the low dose of 0.25 ppm, all extracts significantly enhanced rotifer abundance compared with the control. However, effects diverged significantly at higher concentrations. The 1.00 ppm

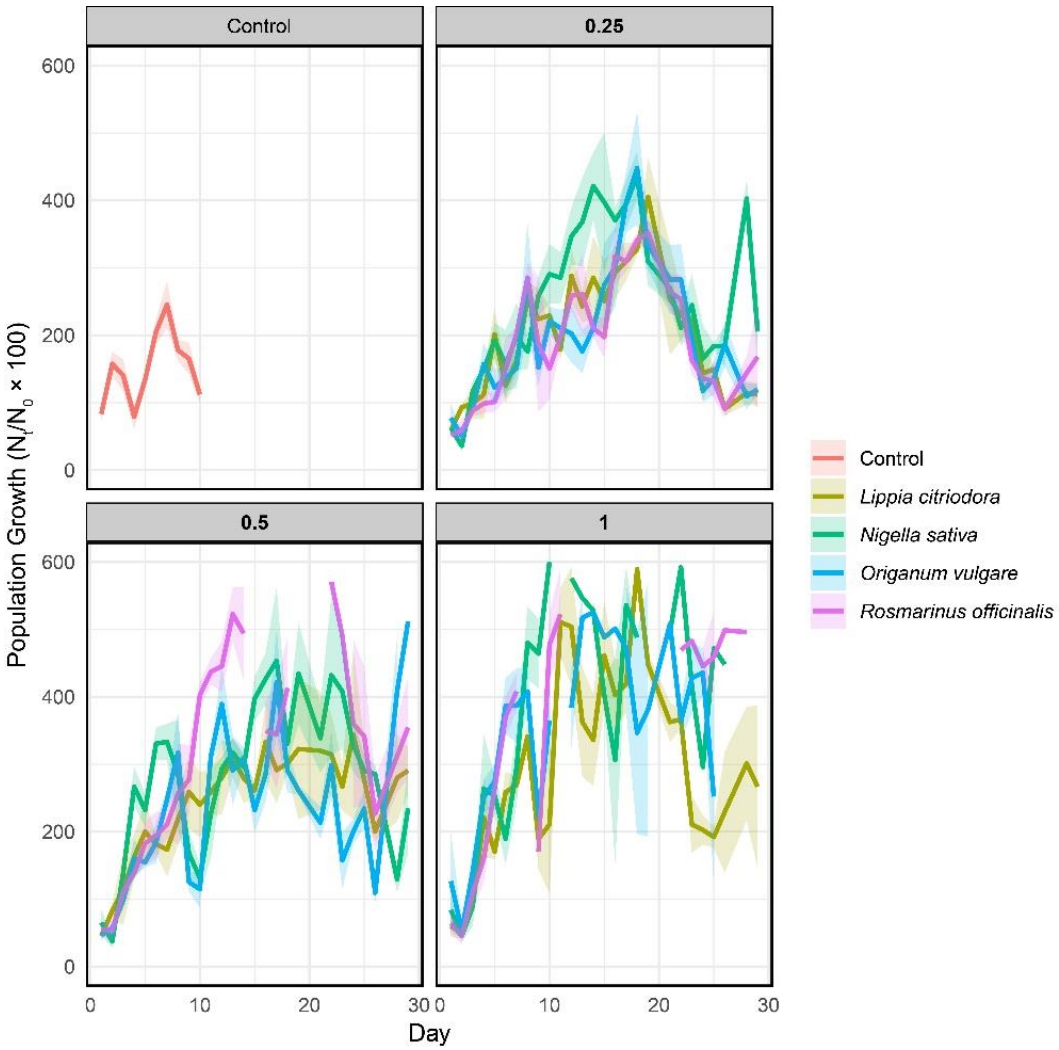

Figure 4. Mean population growth of rotifers over time for different extract treatments and concentrations. The Y-axis represents the population density at a given day (N_t) as a percentage of the mean initial population density (N_0), calculated as (N_t/N_0×100). Error bars (shaded regions) represent the standard error (SE) of the mean. ANOVA confirmed that the effects of treatment, dose, and day (as well as their interactions) were statistically significant ($P<0.01$), as detailed in Table 1.

concentrations of *R. officinalis* and *N. sativa* were the most successful, yielding the highest population densities and superior reproductive performance, as seen in the proportion of egg-carrying females. This suggests a potent, positive dose-response for these two extracts. In sharp contrast, *L. citriodora* and *O. vulgare* exhibited concentration-dependent trade-offs: lower doses (≤0.50 ppm) were beneficial, but higher concentrations reduced population growth and inhibited motility.

The superior performance of *R. officinalis* is likely attributable to its principal phenolic constituents, rosmarinic and carnosic acids, which are known for potent antioxidant and antimicrobial activities (Nieto et al., 2018; Cakir & Karatas, 2024). These compounds protect lipids from peroxidation and scavenge reactive oxygen species (Martínez et al., 2019; Park et al., 2019). This antioxidant mechanism is particularly valuable in rotifer culture, as it can protect the high content of essential fatty acids (EFAs) from degradation–a critical factor for maintaining the nutritional value and supporting the high fecundity of rotifers (Waqalevu et al., 2019; Osmanoğlu et al., 2024). The benefits of these antioxidant pathways are well-established in finfish, where they are proven to improve oxidative status, confer stress resistance, and support growth under challenge conditions (Naiel et al., 2020; Karataş et al., 2020; Xu et al., 2024).

Similarly, the strong performance of *N. sativa* extract, especially at 1.00 ppm, is attributed to its primary bioactive compound, thymoquinone. Thymoquinone is renowned for its potent antimicrobial, anti-inflammatory, and immunostimulant properties (Dawood et al., 2021). Its presence in the culture water likely suppressed harmful bacteria, reducing the pathogenic load and improving the microbial environment. This “water-conditioning” effect would directly benefit rotifer health, survival, and reproductive output by reducing biological stressors.

In contrast, the inhibitory effects observed for *L. citriodora* and *O. vulgare* at higher doses also align with their known phytochemical profiles. *O. vulgare* is rich in carvacrol and thymol, compounds widely used for their strong antiparasitic and antimicrobial actions in fish (Alagawany et al., 2020; Ghafarifarsani et al., 2021). While beneficial at low doses, these compounds can become toxic or inhibitory at higher concentrations. Likewise, the reduced performance of *L. citriodora* at 1.00 ppm suggests a species-specific metabolic limit for rotifers, a finding that contrasts with its growth-enhancing effects in larger species like carp (Gholipourkanani et al., 2017).

Conclusions

This study demonstrates that plant-derived extracts, particularly *R. officinalis* and *N. sativa*, can sustainably and significantly enhance the production and reproductive performance of the rotifer *B. plicatilis*. The 1.00 ppm concentrations of these two extracts yielded the most significant population growth, highlighting their potential as viable, eco-friendly additives in aquaculture. In contrast, the reduced efficacy of *L. citriodora* and *O. vulgare* at higher doses suggests inhibitory effects from compounds like carvacrol and thymol, underscoring the critical importance of dosage precision. The findings of this study open two clear directions for future research. First, as this study used whole extracts, subsequent work should focus on isolating the key bioactive compounds (e.g., carnosic acid from *R. officinalis* and thymoquinone from *N. sativa*) to pinpoint the precise mechanisms driving the enhancement in rotifer performance. Second, and most critically, the ultimate goal of this research must be validated. A follow-up study is essential to assess the growth, survival, and immune response of marine fish larvae fed the rotifers enriched with *R. officinalis* and *N. sativa*. This step is necessary to confirm that the benefits observed in the live feed are successfully transferred up the food chain, which would confirm their value as a sustainable and effective strategy in marine larviculture.

Ethical Statement

All procedures and experimental protocols were approved by the Animal Experiments Local Ethics Committee of the Central Fisheries Research Institute (SUMAE) (Approval No: 3325.04.02-19), under the supervision of the General Directorate of Agricultural Research and Policies.

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Author Contribution

Ayça ALTUNTAŞ: Conceptualization, Funding acquisition, Project administration, Methodology, Formal analysis, Investigation, Writing – Review & Editing. İlker Zeki KURTOĞLU: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – Original Draft, Writing – Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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