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RESEARCH ARTICLE

Nigella sativa-Based Extender Enhances Nilem (*Osteochilus vittatus*) Sperm Quality: Implications for Sustainable Aquaculture Practices

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Yuni Astuti ^{1,2}, Abinawanto . ¹, RRS. Pudji Sinarni Dewi³, Astari Dwiranti¹, Retno Lestari¹, Fadhillah .¹, Suci Lestari²

¹Departement of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, West Java, Indonesia²Education of Biology, FKIP, Universitas Muhammadiyah Prof DR. Hamka, East Jakarta, Special Capital Region of Jakarta, Indonesia³Research Institute for Freshwater Aquaculture and Fisheries Extension, Ministry of Marine Affairs, Bogor, West Java, Indonesia

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Abstract

Abstract*

Background and Aim

Preservation of Nilem (*Osteochilus vittatus*) spermatozoa is essential for sustainable aquaculture and the development of reproductive biotechnology. This study evaluated the effectiveness of a commercial Habbatussauda (*Nigella sativa*) extract as a natural antioxidant-based extender for maintaining sperm quality during 48 h cold storage at 4 °C.

Materials and Methods

Sperm samples were supplemented with commercial habbatussauda extract at concentrations of 1% (P₁), 2% (P₂), 3% (P₃), and 4% (P₄). Untreated sperm served as a negative control (P₀), while sperm treated with 0.005% pure thymoquinone acted as a positive control (P_{TQ}). All samples were stored at 4 °C for 48 hours with six replicates per treatment. Post-storage assessments included sperm viability, motility, and morphological abnormalities. Ultrastructural integrity of spermatozoa was qualitatively evaluated using Cryogenic Scanning Electron Microscopy (CRYO-SEM).

Results

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1. Siti Maulida , Universitas Syiah Kuala, Banda Aceh, Indonesia
2. Muhammad Fauzi , Riau University, Pekanbaru, Indonesia
3. Md. Moshir Rahman , University of California Davis, Davis, USA

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The 2% habbatussauda extract (P₂) yielded the highest sperm viability ($84.80 \pm 5.54\%$) and motility ($73.25 \pm 10.82\%$) and significantly reduced abnormality rates compared to the untreated control. CRYO-SEM analysis revealed severe ultrastructural damage in untreated spermatozoa, including plasma membrane disruption, head deformation, and flagellar breakage. In contrast, spermatozoa preserved with 1–2% extract, particularly P₂, maintained intact plasma membranes, normal head morphology, and well-preserved flagella. Higher extract concentrations (3–4%) induced mild ultrastructural alterations.

Conclusion

A 2% commercial habbatussauda (*Nigella sativa*) extract effectively preserves Nile sperm quality and ultrastructure during short-term cold storage, supporting its application as a natural antioxidant protectant in aquaculture.

Keywords

Fish sperm preservation, *Osteochilus vittatus*, *Nigella sativa*, Cold storage, Antioxidant protectant, CRYO-SEM, Aquaculture sustainability

Corresponding author: Abinawanto . (abinawanto.ms@sci.ui.ac.id)

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Introduction

Nilem (*Osteochilus vittatus*) is a freshwater fish species native to Southeast Asia and plays an important role in local aquaculture due to its high nutritional value and economic potential. In recent years, the demand for Nilem has increased, reaching approximately 13 tons in 2023. However, production remains relatively low, at around 2 tons per year, mainly due to constraints in broodstock management and seed production.¹

Successful reproduction in Nilem aquaculture largely depends on the availability of high-quality sperm. One commonly applied approach to maintain sperm quality is low-temperature storage at 4°C or cryogenic preservation at -196°C. Despite its effectiveness, low-temperature preservation can induce cellular damage through protein denaturation, alterations in cell volume such as shrinkage or swelling, and ice crystal formation. These process may trigger oxidative stress mediated by reactive oxygen species (ROS), ultimately reducing sperm viability and fertilization potential.²

To minimize cryo-induced damage, sperm extenders are widely used during preservation. Synthetic cryoprotectants such as dimethyl sulfoxide (DMSO), methanol, and glycerol have been shown to improve sperm survival; however, their application may be limited by cytotoxic effect.^{3,4} Consequently, the use of natural ingredients as alternative extender components has gained increasing attention. Natural extenders containing antioxidants compounds-including egg yolk, honey, coconut water, green tea, melon, and habbatussauda (*Nigella sativa*)-have demonstrated promising results in maintaining sperm quality during storage.^{5,6} Commercial *Nigella sativa* products, available in the form of oils or powders, are widely accessible; however, their effectiveness as components of sperm extenders but not been fully explored. Examples include Habbatussauda Kurma ajwa, Habbatussauda Oil Extra Propolis Trigona, Habbasyi garlic formulations.

Sperm quality is commonly evaluated based on functional parameters such as motility, viability, and morphological abnormality. While these indicators are informative, they do not fully represent the underlying ultrastructural integrity of spermatozoa. Ultrastructural analysis through electron microscopy, including scanning and transmission electron microscopy, has been used to describe the detailed morphology of fish spermatozoa and to assess structural changes post-preservation in various teleost species, revealing damage to membranes, mitochondria, and flagella following cryo-treatment.^{7,8} Cryo-Scanning Electron Microscopy (CRYO-SEM) further enables visualization of cells in their frozen state with minimal preparation artifacts, providing insight into structural integrity at low temperatures that cannot be detected by light microscopy alone.

Therefore, this study aimed to develop and evaluate sperm extender based on commercial *Nigella sativa* formulations for preserving Nilem sperm at 4°C for 48 hours. The effects on sperm viability, motility, abnormality, and ultrastructural integrity were assessed using conventional sperm quality analysis and CRYO-SEM. The findings of this study are expected to contribute to the development of sustainable aquaculture practices through improved reproductive preservation technologies.

Methods

The research location was the Mina Eduwisata for Domesticated Freshwater Fish, under the Agency for Marine and Fisheries Human Resources Counseling and Development, Bogor, West Java; Bioimaging laboratory at FMIPA UI; and Genomics laboratory at BRIN, Cibinong. It was conducted from January to September 2025.

Ethical approval

This investigation forms part of a longitudinal research initiative. The ethical approval number 1024/UN2.F1/ETIK/PPM.00.02/2025 refers to authorization for animal experimentation granted by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia–Cipto Mangunkusumo Hospital.⁹

Extraction and analysis of commercial habbatussauda

The present study did not use raw *Nigella sativa* seeds directly. Instead, a commercially processed *Nigella sativa* seed product marketed as Habbatussauda cap Kurma Ajwa in powdered form was used. Habbatussauda Cap Kurma Ajwa [Vicomas Internasional Indonesia; registration number POM TR 073367991] was administered at a total amount of four tablets (3 gr). As the material was a standardized commercial product, botanical identification by a qualified botanist and voucher specimen deposition were not applicable.

Four tablets (3 gr) were accurately weighed, and ground into a fine powder, which was then transferred into a beaker. Distilled water was added as the extraction solvent at a ratio of 2:1 (v/w) relative to the powder weight. Aqueous extraction was performed using the infusion method by heating the mixture in a water bath at 90 °C for 15 minutes with intermittent stirring to facilitate the release of water-soluble bioactive compounds. The extract was subsequently filtered through Whatman No. 1 filter paper to remove insoluble residues.¹⁰

The chemical composition and antioxidant activity of the commercial *Nigella sativa* extract used in this study had been previously characterized and reported in a conference prosiding.¹¹ Using Gas Chromatography-Mass Spectrometry (GC-MS), the extract was shown to contain several bioactive compounds commonly associated with *Nigella sativa*, including antioxidant-related constituents. In addition, antioxidant capacity assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay demonstrated notable free-radical inhibition activity.¹² These findings indicate that the extract possesses antioxidant properties that may contribute to its protective effects during low-temperature sperm preservation.

Semen collection

All animal procedures were conducted in accordance with the study was approved under ethical approval number 1024/UN2.F1/ETIK/PPM.00.02/2025. The Nile tilapia (*Osteochilus vittatus*) were not euthanized for sperm collection. The fish were subjected to light anesthesia using MS-222 (Arwana Stabilizer), buffered with sodium bicarbonate at a 1:1 ratio, in accordance with the guidelines of the Ethics Committee of the Faculty of Medicine, Universitas Indonesia–Cipto Mangunkusumo Hospital for animal experimentation and the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.¹³ Twelve hours post-Ovaprim administration, sperm was collected from the male Nile tilapia (*Osteochilus vittatus*) by applying gentle pressure along the abdomen toward the urogenital opening.¹⁴ The milt was aspirated using a 3 mL syringe and immediately transferred into a microtube. Samples were then stored in an ice-cooled container to preserve their viability and prevent degradation.

The preservation process was conducted at 4°C for 28 hours

Semen samples were diluted at a ratio of 1:10 using treatment solutions containing *Nigella sativa* (habbatussauda) extract at concentrations of 0%, 1%, 2%, 3%, and 4%. The diluent was prepared using Fish Ringer's solution, composed of 1.625 g NaCl [Merck, catalogue no. 1.06404.1000], 0.0625 g KCl [Merck, catalogue no. 104938], 0.0875 g CaCl₂·2H₂O [Merck, catalogue no. 102382], and 0.05 g NaHCO₃ [Merck, catalogue no. 1.06329.0500] dissolved in 250 mL of distilled water.¹⁵ A positive control was prepared by dissolving pure thymoquinone (P_{TQ}) [98%, Merck, catalogue no. 274666] at a concentration of 0.005% in distilled water.¹⁶ Following dilution, all semen samples were transferred into sterile microtubes and stored at refrigeration temperature (4 °C) for a duration of 48 hours. Sperm viability was assessed post-storage to evaluate the efficacy of each treatment in preserving semen quality.

Evaluation of post-thawed sperm quality

Viability

A 10 µL aliquot of sperm, previously diluted at a 1:100 ratio, was deposited onto a glass slide for viability evaluation. Subsequently, 10 µL of Eosin-Y solution [Merck, catalogue no. E4382] was added, and the preparation was carefully covered with a coverslip. The sample was examined microscopically at multiple fields. Viable spermatozoa remained unstained, whereas non-viable cells absorbed the dye, enabling their distinction during microscopic analysis. Microscopic evaluation of sperm viability was conducted under a light microscope [Leica DM500, Germany] at 400× magnification at room temperature (25–27 °C), with images captured at multiple observation fields.

Motility

Sperm motility assessment required a 400-fold dilution to facilitate counting by enumerating both total and motile spermatozoa. The initial 10-fold dilution was prepared by mixing 10 µL of sperm with 90 µL of fish Ringer's solution. Subsequently, 10 µL of this mixture was added to 390 µL of activator solution to achieve the final 400-fold dilution. After thorough homogenization, 10 µL of the diluted sample was pipetted onto a glass slide and covered with a 20 × 20 mm coverslip. Motility observations were conducted using a light microscope [Leica DM500, Germany] at 400× magnification under room temperature conditions (25–27 °C), with videos recorded at five distinct fields. Total sperm count was calculated as the sum of motile and immotile spermatozoa.

Abnormality

Spermatozoa abnormalities were assessed using Giemsa staining, which consists of a mixture of eosin and methylene blue. Eosin selectively stains the cytoplasm, while methylene blue targets the nuclei of the spermatozoa. A 100-fold dilution of the sperm sample was prepared by mixing 10 µL of sperm with 90 µL of Fish Ringer's solution. From this dilution, 10 µL of the sperm suspension was placed at the upper edge of a clean glass slide. The sample was then smeared using the edge of another glass slide held at a 45° angle, by drawing it across the sample to the lower edge of the slide. The smear was air-dried and fixed in 99.9% pro-analysis methanol for 3 minutes. Fixed smears were subsequently immersed in Giemsa stain [Thermo Scientific Chemicals, catalogue no. B21172.18] for 30 minutes,¹⁷ followed by gentle rinsing

under running water and air-drying.¹⁵ Microscopic evaluation of sperm abnormalities was conducted under a light microscope [Leica DM500, Germany] at 400× magnification at room temperature (25–27 °C), with images captured at multiple observation fields. Abnormal spermatozoa were identified by characteristic morphological defects, such as headless tails, tailless heads, coiled tails, or double heads.

CRYO-SEM imaging of sperm ultrastructure following preservation treatments

Ultrastructural analysis of Nile (*Osteochilus vittatus*) spermatozoa was performed using Cryogenic Scanning Electron Microscopy (CRYO-SEM). Sperm samples were collected after preservation at 4 °C for 48 hours according to the experimental treatments, while fresh sperma samples were used as unpreserved control. The experimental groups included: P₀ (sperm post-preservation without any protectant/negative control); P₁-P₄ (sperm preserved with commercial Habbatussauda extract at concentrations of 1% (P₁), 2% (P₂), 3% (P₃), and 4% (P₄)); P_{TQ} (positive control, sperma preserved with 0,005% pure Thymoquinone).

For CRYO-SEM preparation, 50 µL of sperm from each treatment was mixed with 500 µL glutaraldehyde solution [2.5% v/v; Electron Microscopy Sciences, USA; catalog no. 50-190-1193] as a fixation agent and gently homogenized. The mixture was incubated at 4 °C for 2 hours to ensure adequate fixation prior to cryogenic precessing. An aliquot of the fixed sperm sample was placed onto a pre-cooled aluminium cryo-sample holder and rapidly frozen by plunge-freezing in liquid nitrogen slush to preserve the native ultrastructure and minimize ice crystal formation. The frozen samples were transferred under vacuum to the cryogenic preparation system. Cryogenic preparation and imaging were conducted using a cryo-electron microscopy system operated in SEM mode [Aquilos 2, Thermo Fisher Scientific, Netherlands]. The focused ion beam (FIB) function was not used in this study. Surface ice contamination was reduced by controlled sublimation (etching) at approximately -90 °C for several minutes. Samples were subsequently coated with a thin conductive layer of platinum using the integrated sputter-coating system to prevent charging during imaging.

Ultrastructure observations were performed under high-vacuum conditions at an accelerating voltage of 10 Kv. Representative micrographs were acquired for each treatment group. Observations focused on qualitative assessment of sperm morphology, including head shape and integrity, midpiece structure, plasma membrane condition, and flagellum morphology.

Data analysis

Prior to statistical analyses, data were examined for normal distribution using the Shapiro–Wilk test, and the assumption of homogeneity of variances was evaluated using Levene’s test. When both assumptions were satisfied, group means were compared using one-way analysis of variance (ANOVA). In instances where the homogeneity of variances assumption was not met, Welch’s ANOVA was employed as an alternative. For post hoc analysis, Tukey’s Honestly Significant Difference (HSD) test was applied to determine specific group differences. A p-value of ≤ 0.05 was considered statistically significant. All statistical procedures were carried out using SPSS software [version 23; IBM Corp., Armonk, NY, USA]. CRYO-SEM micrographs were analyzed visually and descriptively. Morphological features were compared across treatment groups to identify structural alterations induced by preservation or protection with habbatussauda commercial extract or Thymoquinone. Descriptions of observed abnormalities (e.g., membrane disruption, head deformation, midpiece swelling, or flagellum bending) were documented. Statistical analysis was not applied, as the focus was on qualitative evaluation.

Results

Sperm viability, motility, and abnormality

Figure 1 illustrates the differentiation between viable and non-viable spermatozoa as identified through Eosin-Y staining.

Figure 1 shows that sperm cells remained unstained, appearing clear due to their ability to exclude the dye, while non-viable cells took up the stain, displaying a red coloration in the head region. The original micrographs corresponding to this analysis are also available in the Figshare repository at <https://doi.org/10.6084/m9.figshare.30230881>. Sperm motility was assessed by observing the patterns of movement and categorized into three groups: progressive motility, indicated by forward linear movement; non-progressive motility, defined by movement without forward progression; and immotility, characterized by a complete absence of movement. The highest average motility level was observed in treatment group P₂, with a mean value of 73.25 ± 10.82 .

Figure 2 shows Nile spermatozoa stained with Giemsa. Label 'a' indicates normal spermatozoa, while label b, c, d, and e illustrate various morphological abnormalities, including tail defect (b), microcephali (c), macrocephali (d), and coiled tail (e). The data are available in Figshare at <https://doi.org/10.6084/m9.figshare.30230941>.

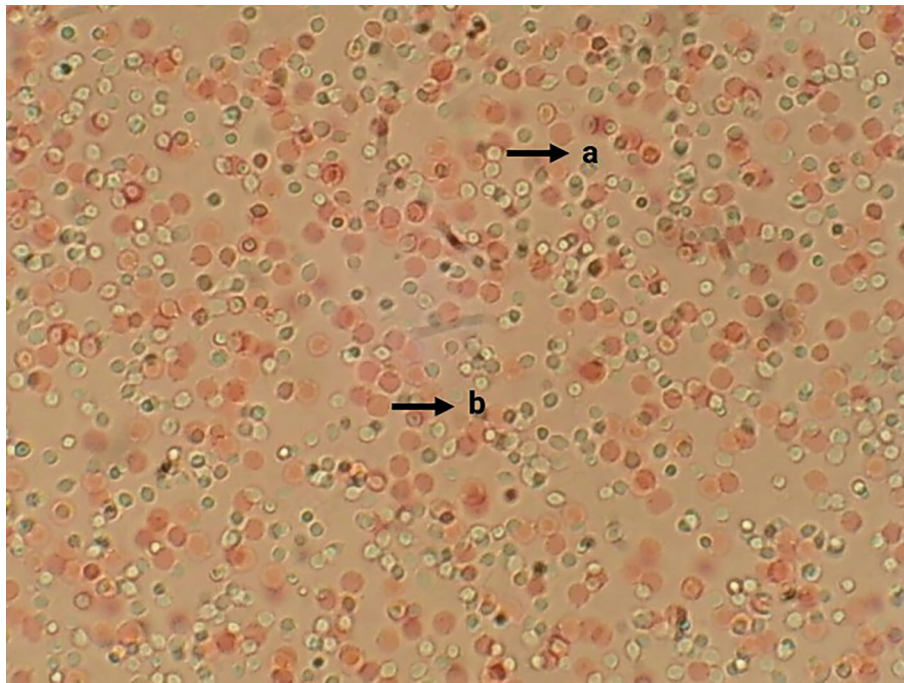


Figure 1. Microscopic visualization of Nilem spermatozoa following Eosin-Y staining for viability assessment. Label (a) indicates viable spermatozoa, while label (b) denotes non-viable spermatozoa.

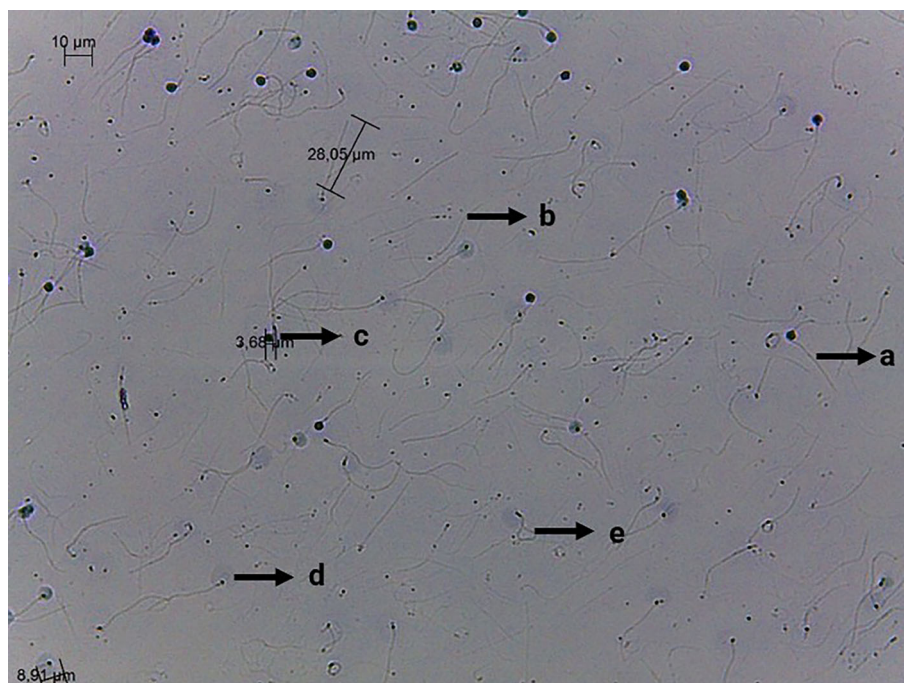


Figure 2. Microscopic visualization of Nilem spermatozoa following Giemsa staining for abnormality assessment. Label (a) indicates normal spermatozoa, while label (b,c,d,e) denotes abnormal spermatozoa (tail defect, small head, large head, and coiled tail).

Table 1 presents the statistical analysis results for viability, motility, and abnormality of Nilem spermatozoa. The data underlying this study are available in Figshare at <https://doi.org/10.6084/m9.figshare.30231094>. The addition of 2% commercial habbatussauda extract as an extender significantly improved sperm viability (84.80 ± 5.54) and motility

Table 1. The viability, motility, and abnormality levels of Nile spermatozoa after preservation at 4°C for 48 hours.

Treatment	Viability (%)	Motility (%)	Abnormality (%)
P ₀ (0% Hb)	67.60±5.32 ^a	12.70±8.25 ^a	88.99±5.17 ^b
P ₁ (1% Hb)	89.00±3.00 ^{bc}	27.77±13.75 ^{ac}	59.89±13.59 ^a
P ₂ (2% Hb)	84.80±5.54 ^{bc}	73.25±10.82 ^b	46.46±12.10 ^a
P ₃ (3% Hb)	77.20±9.31 ^{ac}	69.69±9.78 ^{bd}	56.56±6.41 ^a
P ₄ (4% Hb)	66.00±19.17 ^{ac}	48.80±12.48 ^{de}	80.33±5.35 ^b
P _{TQ} (0.005% TQ)	90.00±3.08 ^b	35.34±9.81 ^c	45.01±4.96 ^a

P₀: Nile semen in fish ring without extract habbatussauda (Hb) commercial; P₁, P₂, P₃, P₄: Nile semen in fish ring added with 1, 2, 3, 4% extract habbatussauda commercial; P_{TQ}: Nile semen in fish ring added with 0.005% pure Thymoquinone (TQ). Different superscript in the same column show significant differences ($p < 0.05$).

(73.25 ± 10.82) compared to the control group (67.60 ± 5.32 and 12.70 ± 8.25, respectively), while significantly reducing sperm abnormality (46.46 ± 12.10) relative to the control (88.99 ± 5.17) after 48 hours of preservation at 4 °C ($p < 0.05$). The Welch test showed a significance value of 0.000 (< 0.05), indicating a statistically significant difference in spermatozoa viability between the commercial habbatussauda extract treatments and the control group. According to Games Howell post hoc test, the 1% commercial habbatussauda extract treatment (P₁) yielded the highest sperm viability (89.00 ± 3.00). Additionally, the P_{TQ} treatment containing 0.005% pure Thymoquinone preserved sperm viability at 90 ± 3.08, comparable to that of fresh Nile spermatozoa. The one-way ANOVA revealed a significant difference in Nile spermatozoa motility among treatment groups, with a p -value of 0.000 (< 0.05). Subsequent Tukey's post hoc test indicated that the addition of 2% (P₂) commercial habbatussauda extract (73.25±10.82^b) provided the most effective protection of sperm motility during cold preservation. One-way ANOVA analysis indicated a statistically significant difference in spermatozoa abnormality among the treatment groups ($p = 0.000$, < 0.05). Further Tukey's post hoc test revealed that treatment with 2% commercial habbatussauda extract (P₂) significantly decreased spermatozoa abnormality (46.46 ± 12.10^a) relative to other treatments during cold storage. The use of pure thymoquinone as a positive control showed no significant differences in sperm viability, motility, and abnormality compared to the 2% commercial habbatussauda extract (P₂), suggesting that thymoquinone in the commercial extract has a similar effect to the pure compound.

CRYO-SEM observations

CRYO-SEM analysis provided qualitative confirmation of the effect of preservation and protectant treatment on sperm ultrastructure (Figure 3), the data are available in Figshare at <https://doi.org/10.6084/m9.figshare.31083916>.

Fresh spermatozoa exhibited rounded heads with smooth plasma membranes, small midpieces, and long, straight flagella, indicating intact and well-preserved morphology. In contrast, sperm preserved without protectant (P₀) showed marked structural deterioration, characterized by enlarged or damaged heads, broken flagella, and perforated plasma membranes, reflecting severe preservation-induced damage. Treatment with 1% commercial habbatussauda extract (P₁) maintained largely intact heads, midpieces, and flagella, although some spermatozoa displayed coiled or shortened flagella and slightly wavy membranes. Spermatozoa preserved with 2% habbatussauda extract (P₂) predominantly exhibited normal, rounded head morphology with gently wavy or locally thickened plasma membranes, while flagella were generally long, with occasional coiling. At higher extract concentration, structural abnormalities became more evident; the 3% treatment (P₃) revealed spermatozoa lacking flagella or possessing shortened and coiled tails, whereas the 4% treatment (P₄) showed small heads with localized swellings and flagella frequently wrapped around the head. The positive control group treated with 0.005% pure Thymoquinone (P_{TQ}) preserved sperm ultrastructure comparable to fresh sperm, with normal head size, slightly wavy yet smooth plasma membranes, and long, straight flagella. Overall, the CRYO-SEM observations qualitatively supported the quantitative findings, indicating that a 2% concentration of commercial habbatussauda extract effectively preserved sperm ultrastructure during cold storage.

Discussion

The results (Table 1) showed that spermatozoa viability (84.00 ± 5.54), motility (73.25 ± 10.82), and abnormality (46.46 ± 12.10) in the 2% habbatussauda extract (P₂) group indicated a high level of preservation quality. These values suggest that the addition of commercial habbatussauda extract effectively maintained membrane integrity and motility while reducing morphological damage during cold storage. Similarly, the positive control group using pure thymoquinone (P_{TQ}) also showed high viability (90.00 ± 3.08) and low abnormality (45.01 ± 4.96), although its motility was

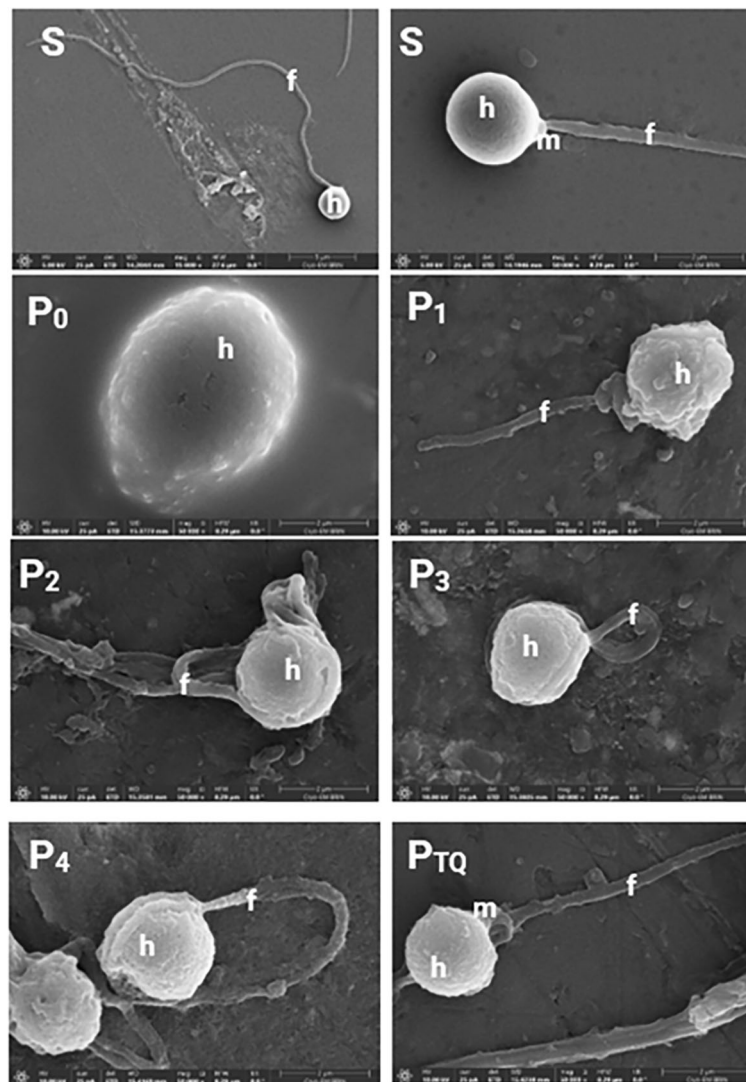


Figure 3. Cryogenic Scanning Electron Micrograph (CRYO-SEM) morphology of nilem (*Osteochilus vittatus*) spermatozoa under different treatments, scale bar 2 μ m. S= fresh sperm (untreated), P₀= negative control 0% habbatussauda extract, P₁= 1% habbatussauda extract, P₂= 2% habbatussauda extract, P₃= 3% habbatussauda extract, P₄= 4% habbatussauda extract, P_{TQ}= 0.005% pure Thymoquinone, h= head, m= midpiece, f= flagellum.

notably lower (35.34 ± 9.81) compared to P₂. In contrast, the control group without commercial habbatussauda extract exhibited significantly lower viability (67.60 ± 5.32), reduced motility (12.70 ± 8.25), and higher abnormality (88.99 ± 5.17), indicating the absence of protective effects during cold storage. These findings highlight the potential of commercial habbatussauda extract, particularly at 2%, to serve as an effective natural cryoprotectant, likely due to its thymoquinone content.

The present findings demonstrate that supplementing nilem (*Osteochilus vittatus*) sperm extender with 2% commercial habbatussauda extract (P₂) significantly improved post-preservation sperm quality, as evidenced by higher viability, enhanced motility, and reduced abnormality compares to untreated controls. These improvements likely reflect the extract's ability to counteract cold stress-induced damage, which is known to involve oxidative stress and Reactive Oxygen Species (ROS) accumulation during preservation and cold storage.^{6,18} Excessive ROS production can disrupt plasma membranes, damage mitochondria, and impair ATP synthesis, leading to reduce motility and viability and increased morphological defect in nilem spermatozoa.^{19–24} Such oxidative mechanisms have been implicated broadly in fish sperm cryodamage, where imbalance between ROS and endogenous antioxidant defenses results in lipid peroxidation, protein oxidation, and ultrastructural alterations.^{25,26}

GC-MS profiling of the commercial habbatussauda extract revealed the presence of multiple antioxidant compounds, including Thymoquinone,²⁷ p-cymene-2,5-diol,²² Cyclodecasiloxane, Eicosamethyl,²⁸ Phenol 2,4-bis(1,1-dimethylethyl)-, phosphite, and Tris(2,4-di-tert-butylphenyl) phosphate—as well as propylene glycol, consistent with previous reports of bioactive constituents in *Nigella sativa*.¹¹ The molecular weights of these antioxidant compounds exceed 146 g/mol, preventing them from crossing the spermatozoa membrane; thus, they function as extracellular protectants.²⁷ In contrast, propylene glycol, with a molecular weight of 76 g/mol, is capable of penetrating the sperm membrane and acts as an intracellular protectant.²⁹ The extract also exhibited high free-radical scavenging capacity in the DPPH assay (IC₅₀ = 6.61 mg/mL), confirming robust antioxidant potential. These biochemical properties provide a plausible explanation for the improved membrane integrity and motility observed in the P₂ group, as antioxidants can neutralize ROS before they initiate lipid peroxidation and membrane destabilization. This mechanistic role is supported by studies showing that *Nigella sativa* extract reduce ROS and improve functional sperm parameters, including membrane integrity and mitochondrial potential, in other species.^{18,26}

Ultrastructural evidence from CRYO-SEM qualitatively corroborated these functional benefits. Spermatozoa in the P₂ group retained head shape, plasma membrane continuity, and flagellar integrity closely resembling that of the fresh sperm, whereas untreated nilem sperm exhibited severe membrane disruptions and structural breakdown. These observations align with the notion that antioxidant supplementation preserves fine ultrastructure by preventing oxidative disruption of lipid bilayers and cytoskeleton elements.^{30,31}

Interestingly, although pure Thymoquinone (P_{TQ}) also conferred protective effect, motility in P_{TQ} group was lower than in the P₂ group, suggesting that the combined action of multiple antioxidant compounds in the commercial extract may offer a synergistic advantage over a single isolated molecule. Such synergy could enhance membrane and mitochondrial protection beyond the capacity of Thymoquinone alone.

At higher extract concentrations (P₃ and P₄), sperm quality deteriorated, implying a concentration-dependent threshold beyond which antioxidant components may no longer confer benefit and may even disturb osmotic or redox balance. Similar dose-dependent antioxidant effect have been in other reproductive studies, where low to moderated antioxidant supplementation was beneficial, but excessive doses did not yield additional improvements and occasionally exerted pro-oxidant effect.

Conclusion

Commercial habbatussauda (*Nigella sativa*) extract effectively improved the quality of nilem (*Osteochilus vittatus*) spermatozoa during cold storage at 4° C. The 2% extract concentration provided the optimal protective effect, resulting in higher sperm viability and motility, lower abnormality rates, and better-preserved ultrastructure after 48 hours of preservation. CRYO-SEM analysis confirmed that spermatozoa treated with 2% habbatussauda extract retained intact plasma membranes and flagellar structural damage. These protective effects are attributed to the strong antioxidant compounds identified by GC-MS and its high free-radical scavenging activity in the DPPH assay. Overall, commercial habbatussauda extract at 2% represents a promising natural protectant for short-term nilem sperm preservation and has potential applications in sustainable aquaculture.

Ethical considerations

As part of a longitudinal research initiative, this study was conducted with ethical clearance number 1024/UN2.F1/ETIK/PPM.00.02/2025, granted by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia–Cipto Mangunkusumo Hospital, which approved all animal experimental procedures. The data available in Figshare at DOI: <https://doi.org/10.6084/m9.figshare.30231112>.⁹

Data availability

The data underlying this study are available in Figshare at DOI: <https://doi.org/10.6084/m9.figshare.30231094>,³² under an open CC BY 4.0.

Reporting guidelines

The ARRIVE checklist. Figshare. DOI: <https://doi.org/10.6084/m9.figshare.30231361>³³ under an open CC BY 4.0

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References

1. kabupaten bogor badan pusat statistik: **Tabel Terkait Subjek. Produksi ikan konsumsi**. 2024.
[Reference Source](#)
2. El-mahdy MA, Zhu Q, Wang Q, *et al.*: **Thymoquinone Induces Apoptosis through Activation of Caspase-8 and Mitochondrial Events in p53-null Myeloblastic Leukemia HL-60 Cells**. *Int. J. Cancer*. 2005; **117**(3): 409–417.
[PubMed Abstract](#) | [Publisher Full Text](#)
3. Muthmainnah CR, Eriani K, Hasri I, *et al.*: **Theriogenology Effect of Glutathione on Sperm Quality after Short-term Cryopreservation in Seurukan Fish Osteochilus vittatus (Cyprinidae)**. *Theriogenology*. 2018; **122**: 30–34.
[PubMed Abstract](#) | [Publisher Full Text](#)
4. Setiyono P, Raharjo E: **Profil Spermatozoa Ikan Nilem (Osteochilus vittatus) yang Disimpan dalam Larutan Ringer-Gliserin**. *Prosiding Semnas Biologi ke-9*. FMIPA Universitas Negeri Semarang; 2021; pp. 178–184.
[Reference Source](#)
5. Lestari S: **Madu sebagai Agen Antioksidan pada Spermatozoa Ikan Dewa (Tor soro) Pascapreservasi 48 Jam**. Universitas Indonesia; 2022.
6. Inanç ME, *et al.*: **Thymoquinone Improves Motility, Plasma Membrane Integrity and DNA Integrity of Frozen – Thawed Ram Semen**. *Andrologia*. 2022; **54**: e14547–e14510.
[PubMed Abstract](#) | [Publisher Full Text](#)
7. Zhang Q, He Y, Zhu S, *et al.*: **Effects of Cryopreservation on The Ultrastructure and Enzyme Activities of Rare Minnow (Gobiocypris rarus) Sperm**. *Aquac. Reports*. 2025; **45**(103213).
[Publisher Full Text](#)
8. Zhu Z, *et al.*: **Developing Efficient Methods of Sperm Cryopreservation for Three Fish Species (Cyprinus carpio L., Schizothorax prenanti, Glyptosternum maculatum)**. *Int. J. Mol. Sci.* 2025; **26**(4648): 1–21.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Astuti Y, *et al.*: **Dataset for: Ethical Approval for animal**. *Figshare*. 2025.
[Publisher Full Text](#)
10. Sariyem S, Sunarjo L, Haniyati M: **Efektifitas Ekstrak Daun Sukun Hasil Perebusan terhadap Pertumbuhan Koloni Bakteri Streptococcus Mutans**. *J. Kesehat. Gigi*. 2015; **2**(2): 104–109.
[Publisher Full Text](#) | [Reference Source](#)
11. Astuti Y, *et al.*: **Identification of Bioactive Compounds in Habbatussauda (Nigella sativa), and their Potential as Sperm Protectants in Nilem Fish (Osteochilus vittatus)**. *Postpreservation*. *IOP Conf. Ser. Earth Environ. Sci.* 2025; **1550**(012037).
[Publisher Full Text](#)
12. Lykholat VV, Khromykh NO, Liashenko OV, *et al.*: **Phytochemical Profiles and Antimicrobial Activity of The Inflorescences of Sorbus domestica, S. aucuparia, and S. torminalis**. *Biosyst. Divers.* 2023; **31**(3): 290–296.
[Publisher Full Text](#)
13. Leary S, *et al.*: **AVMA Guidelines for the Euthanasia of Animals: 2020 Edition**. the American Veterinary Medical Association; 2020; vol. **202**(2).
[Publisher Full Text](#)
14. Nugraha A, Hendra: **Teknik Pembuatan Pakan, Pemeliharaan Induk, dan Pemijahan Ikan Nilem (Osteochilus hasselti)**. *Bul. Tek. Litkayasa Akuakultur*. 2017; **15**(2): 77–81.
[Publisher Full Text](#)
15. Lestari S, Abinawanto A, Bowolaksono RG, *et al.*: **The Percentage of embryo viability after 48h sperm cryopreservation: effect of various natural cryoprotectant**. *The Percentage of embryo viability after 48h sperm cryopreservation: effect of various natural cryoprotectant*. *IOP Conference Series: Earth and Environmental Science*. IOP Publishing; 2020; pp. 3–8.
[Publisher Full Text](#)
16. Abdul-Rahman S, Kamarzaman: **Prophylactic Effects of Nigella sativa Extract and Thymoquinone against Cyclophosphamide-induced Sperm Head Abnormalities and Chromatin Instability in Mice**. *Prophylactic Effects of Nigella sativa Extract and Thymoquinone against Cyclophosphamide-induced Sp*. *Bull. Environ. Pharmacol. Life Sci.* 2017; **16**(10): 5–16.
[Publisher Full Text](#)
17. Puasa R: **Studi Perbandingan Jumlah Parasit Malaria Menggunakan Variasi Waktu Pewarnaan pada Konsentrasi Giemsa 3% di Laboratorium RSUD Dr. H. Chasan Boesoirie Ternate**. *J. Ris. Kesehat*. 2017; **6**(2): 23–27.
[Publisher Full Text](#)
18. Khosravi T, Reza A, Dastjerdi V, *et al.*: **Investigation of The Effects of Thymoquinone (TQ) and Polyvinylpyrrolidone (PVP) on The Motility, Viability, and Chromatin Status of Sperm in Normozoospermic Semen Samples**. *Ann. Med. Surg.* 2024; **86**(2): 826–830.
[Publisher Full Text](#)
19. Rurangwa E, Kime DE, Ollevier F, *et al.*: **The Measurement of Sperm Motility and Factors Affecting Sperm Quality in Cultured Fish**. *Aquaculture*. 2003; **234**(2004): 1–28.
[Publisher Full Text](#)
20. Pšenicka M, Rodina M, Flajšhans M, *et al.*: **Structural Abnormalities of Common Carp Cyprinus carpio Spermatozoa**. *Fish Physiol. Biochem.* 2009; **35**(4): 591–597.
[PubMed Abstract](#) | [Publisher Full Text](#)
21. Abinawanto A, Yimastria S, Pertiwi: **Sperm Analysis of Lukas Fish (Puntius bramoides): Motility, Viability and Abnormality**. *AIP Conf. Proc.* 2025; **020133**(2008): 1–6.
[Publisher Full Text](#)
22. Barkah NN, Wiryawan IKG, Retnani Y, *et al.*: **Evaluasi Rekalitas Ekstraksi Biji, Minyak, dan Limbah Habbatussauda (Nigella sativa) terhadap Komponen Bioaktif dan Kemampuan Penghambatan Bakteri**. *J. Ilmu Nutr. dan Teknol. Pakan*. 2025; **23**(1): 64–73.
[Publisher Full Text](#)
23. Marshalita N, Risky JG, Jasmadi RN: **Pengobatan Nabi: Efek Jinten Hitam (Nigella sativa) untuk Meningkatkan Kualitas Sperm**. *J. Ilm. Kesehat*. 2020; **9**(1): 1–6.
[Publisher Full Text](#) | [Reference Source](#)
24. Figueroa E, Lee-estevez M, Valdebenito I, *et al.*: **Effects of Cryopreservation on Mitochondrial Function and Sperm Quality in Fish**. *Aquaculture*. 2019; **511**(February): 634190.
[Publisher Full Text](#)
25. Nasiri Z, Ghorbani F, Seify M, *et al.*: **Effect of Aqueous Nigella Sativa Extract on The Functional Parameters of Post-Thaw Human Spermatozoa During Vitrification**. *Korean Soc. Reprod. Med.* 2022; **49**(2): 110–116.
[Publisher Full Text](#) | [Reference Source](#)
26. Akhter MAAS, Qadeer AAS: **Antioxidant Activity of Nigella sativa Seeds Aqueous Extract and Its Use For Cryopreservation of Buffalo Spermatozoa**. *Andrologia*. 2018; **50**(6): e13020–e13027.
[PubMed Abstract](#) | [Publisher Full Text](#)
27. Ezzati M, Shanehbandi D, Hamdi K, *et al.*: **Influence of Cryopreservation on Structure and Function of Mammalian Spermatozoa: An Overview**. *Cell Tissue Bank*. 2020; **21**(1): 1–15.
[PubMed Abstract](#) | [Publisher Full Text](#)
28. Rasyid A, Putra MY: **Antibacterial and Antioxidant Activity of Sea Cucumber Extracts Collected From Lampung Waters, Indonesia**. *Kuwait J. Sci.* 2023; **50**(4): 615–621.
[Publisher Full Text](#)
29. Da KP, BY, TL, *et al.*: **Micro encapsulation of Bovine Spermatozoa: Cryopreservation of Microencapsulation Sperm using Glycerol**. *Indones. J. Anim. Vet. Sci.* 20(4): 233–241.
[Publisher Full Text](#) | [Reference Source](#)
30. Mujahidurrohman M, Yulianti E, Lukman HY: **Ability of Melon (Cucumis melo. L) Fruit Juices Based Tris Diluent on The Quality of Frozen Spermatozoa of Bali Cattle After Thawing**. *J. Biol. Trop.* 2023; **23**(3): 450–463.
[Publisher Full Text](#) | [Reference Source](#)
31. Mustofa I, Susilowati S, Suprayogi TW, *et al.*: **Epigallocatechin-3-gallate chitosan Nanoparticles in An Extender Improve the Antioxidant Capacity and Post-thawed Quality of Kacang Goat Semen**. *F1000Res*. 2023; **12**: 1–17.
[Publisher Full Text](#)
32. Astuti Y, *et al.*: **Dataset: Data analysis of fresh & post preservation sperm**. *Figshare*. 2025.
[Publisher Full Text](#)
33. Astuti Y, *et al.*: **Dataset: The ARRIVE guidelines 2.0: author checklist**. *Figshare*. 2025.
[Publisher Full Text](#)
34. Astuti Y, *et al.*: **Dataset: grant information NKB_365.pdf**. *Figshare*. 2025.
[Publisher Full Text](#)
35. Astuti Y, *et al.*: **Dataset: Approval letter**. *Figshare*. 2025.
[Publisher Full Text](#)

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Reviewer Report 18 May 2026

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Md. Moshir Rahman 

Department of Biology and Agricultural Engineering, University of California Davis, Davis, California, USA

The manuscript entitled “*Nigella sativa*-Based Extender Enhances Nilem (*Osteochilus vittatus*) Sperm Quality: Implications for Sustainable Aquaculture Practices” addresses an interesting topic related to natural antioxidant-based sperm preservation in fish aquaculture. The use of commercial *Nigella sativa* extract and CRYO-SEM observations provides potentially useful preliminary information for short-term sperm preservation. However, the manuscript currently contains major methodological, analytical, interpretative, and writing-related weaknesses that substantially limit the scientific reliability of the findings. In particular, the procedures used for sperm motility assessment, sperm counting, abnormality evaluation, replication structure, and statistical analyses require significant clarification and improvement. Several methodological descriptions are incomplete or inconsistent with standard sperm quality assessment protocols commonly used in fish reproductive biology. Therefore, major revision is required before the manuscript can be considered for indexing in a reputed journal.

Major Comments

1. The Introduction contains many grammatical and scientific wording issues that reduce clarity and readability. Several examples include “sperma quality,” “dinethyl sulfoxide,” “fertilization potential,” “Cincecually,” and “cryprotectants.” The manuscript requires careful English revision throughout the entire text.
2. The manuscript discusses both cryopreservation at -196°C and chilled storage at 4°C together, although the present study only evaluates refrigerated preservation. These are fundamentally different preservation methods with different mechanisms of cellular injury. The Introduction should clearly distinguish chilled storage from cryopreservation.
3. The study objective is mentioned, but the hypothesis is not clearly stated. Including a clear hypothesis would improve the scientific framework of the study.
4. The production statistics presented for Nilem appear questionable and require updated references and clarification regarding the geographic scale of the reported production data.
5. The description of the commercial *Nigella sativa* product is insufficient for reproducibility.

The manuscript does not provide the actual concentration of active ingredients such as thymoquinone in the commercial tablets used for extraction. Since commercial herbal products can vary substantially in composition, this information is essential.

6. The extraction procedure lacks important methodological details, including extraction yield, storage condition of the extract, final extract concentration, and whether the extract was freshly prepared or stored before use.
7. The manuscript relies heavily on antioxidant characterization from a conference proceeding rather than presenting sufficient supporting data within the current study. Key GC-MS and antioxidant information should be summarized more completely in this manuscript.
8. The semen collection section requires major clarification. The number of males used, body weight, reproductive condition, and whether semen samples were pooled are not described. These factors strongly influence sperm quality and are essential for interpreting the results.
9. Ovaprim dosage, route of administration, and exact latency period details are missing. These details are necessary for reproducibility.
10. The manuscript does not explain how contamination by urine, feces, blood, or water was prevented during stripping. Such contamination can strongly affect fish sperm quality and motility.
11. There is a major inconsistency regarding storage duration. One section states preservation for 28 hours, whereas the remainder of the manuscript repeatedly states 48 hours. This inconsistency must be corrected throughout the manuscript.
12. The manuscript does not report sperm concentration standardization before dilution. Initial sperm concentration can strongly influence motility and viability measurements. Standard sperm counting methods such as hemocytometer counting or CASA-integrated concentration analysis should be considered.

1. The viability assessment method is incomplete. The manuscript does not specify how many sperm cells were counted per replicate or how viability percentages were calculated. Counting procedures should be standardized clearly.
2. The sperm motility assessment method is a major weakness of the manuscript. Motility was evaluated manually using standard microscopy rather than Computer-Assisted Sperm Analysis (CASA), which is currently considered a standard and more reliable method in fish sperm biology. The authors should acknowledge this important limitation clearly in the Discussion.
3. The activator solution used for motility assessment is not properly described. Fish sperm motility is highly sensitive to activating medium composition, osmolarity, ionic concentration, and pH. Full details should be provided.
4. Important motility assessment details are missing, including activation time, observation duration, frame rate, and whether motility was measured immediately after activation. These are critical parameters in fish sperm motility studies.
5. The abnormality assessment method lacks standardization details. Please specify how many spermatozoa were evaluated per slide and per replicate.
6. The abnormality classification system is oversimplified. Fish sperm abnormalities are commonly categorized systematically into head, midpiece, and flagellar defects. A more standardized classification approach would improve scientific quality.
7. The CRYO-SEM observations are entirely qualitative. No quantitative scoring system or blinded evaluation approach was used. Therefore, the ultrastructural conclusions remain somewhat subjective.
8. The statistical workflow requires substantial clarification. The manuscript mentions Shapiro-

Wilk, Levene's test, ANOVA, Welch ANOVA, Tukey HSD, and Games-Howell tests, but it is unclear which variables failed assumptions and which exact tests were applied to each parameter.

1. The manuscript states that six replicates were used per treatment, but it is unclear whether these were true biological replicates from different males or technical replicates derived from pooled semen. This is a very important issue because pseudoreplication could substantially affect statistical validity.
2. The Results section lacks sufficient quantitative detail regarding sperm counting procedures. It remains unclear how motility percentages were determined and whether actual sperm counts or visual estimates were used.
3. The manuscript states that P2 produced the best overall results, but later the text reports higher viability values in P1 and PTQ groups. These interpretations should be revised for consistency.
4. Table 1 contains confusing superscript lettering patterns that make interpretation difficult. Please revise the statistical grouping format for clarity.
1. The extremely low motility value in the untreated control group requires further discussion. The authors should explain whether storage conditions or activation procedures may have contributed to this sharp decline.
2. The CRYO-SEM interpretations appear overstated in several places. Since the observations are qualitative and based on representative images only, statements such as "effectively preserved sperm ultrastructure" should be expressed more cautiously.
3. 27. The Discussion repeatedly attributes sperm protection to antioxidant activity and ROS reduction, yet ROS, lipid peroxidation, antioxidant enzymes, mitochondrial function, or membrane integrity biomarkers were not directly measured in this study. Therefore, these mechanistic explanations remain speculative and should be discussed more cautiously.
1. The statement suggesting that compounds larger than 146 g/mol cannot cross sperm membranes is oversimplified and requires stronger scientific support. Membrane permeability depends on multiple physicochemical properties, not only molecular weight.
2. The discussion of synergistic antioxidant effects between multiple compounds remains speculative because no direct mechanistic comparison was conducted in this study.
3. The conclusion overgeneralizes the aquaculture implications of the findings. Since fertilization success, hatching rate, larval quality, and long-term reproductive performance were not evaluated, broader conclusions regarding sustainable aquaculture should be expressed more cautiously.

Minor Comments

1. "sperma quality" should be corrected to "sperm quality."
2. "fertilization protential" should be corrected to "fertilization potential."
3. "dinethyl sulfoxide" should be corrected to "dimethyl sulfoxide."
4. "Cincecuently" should be corrected to "Consequently."
5. "cryprotectants" should be corrected to "cryoprotectants."
6. "conference prosiding" should be corrected to "conference proceeding."
7. "ground into ai fine powder" should be corrected to "ground into a fine powder."
8. "20 20 mm coverslip" should be written as "20 × 20 mm coverslip."
9. Several grammatical errors remain throughout the Methods section and require careful

editing.

10. "colomn" in Table 1 should be corrected to "columnn."
11. The table formatting should be improved for readability.
12. Several typographical errors remain throughout the Discussion section, including "defferent," "qualitively," "compares," and "initiatite." Please revise the manuscript carefully for language accuracy.

Overall Recommendation

Although the manuscript addresses a relevant topic in fish reproductive biotechnology, the current study contains major methodological and analytical weaknesses that substantially limit confidence in the findings and conclusions. The lack of standardized sperm quality assessment methods, insufficient methodological detail, unclear replication structure, limited statistical clarity, and overinterpretation of mechanistic effects represent serious scientific concerns. Therefore, in its current form, I do not recommend the manuscript for indexing.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I have strong expertise in fish reproductive biology, aquaculture, sperm physiology, and cryopreservation. My research includes sperm quality assessment, reproductive performance, microscopy-based analyses, and statistical evaluation of aquaculture-related experiments, with multiple peer-reviewed publications in these fields.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 13 May 2026

<https://doi.org/10.5256/f1000research.189026.r480150>

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Muhammad Fauzi

Faculty of Fishery and Marine Science, Riau University, Pekanbaru, Indonesia

1. Concern:

- The manuscript contains multiple **grammatical errors and typographical inconsistencies**, which reduce readability and professionalism.
- Terminology is inconsistent (e.g., "sperma" vs "sperm").
- Some methodological descriptions are unclear or contradictory, particularly regarding storage duration.
- Literature is cited, but **critical engagement with recent high-impact studies is limited**, especially regarding:
 - Mechanisms of oxidative stress in sperm preservation
 - Dose-dependent antioxidant effects

Recommendations:

- Conduct professional language editing.
- Standardize terminology throughout the manuscript.
- Expand literature discussion to include more recent and mechanistic studies.
- Improve clarity in methodological descriptions.

2. Concern:

In conclusion, the study demonstrates a fundamentally sound experimental design through the inclusion of appropriate control groups, adequate replication (n=6), and the use of standard sperm quality assessments. Nevertheless, several critical limitations reduce the technical robustness and scientific reliability of the findings. The most significant concern is the absence of extract standardization, particularly regarding the quantification of thymoquinone and the characterization of active compound variability in the *Nigella sativa* preparation, which substantially limits reproducibility and comparability across studies. In addition, methodological inconsistency regarding the sperm storage duration between the Methods and Results sections requires clarification to ensure data reliability. The proposed antioxidant mechanism also remains speculative due to the lack of biochemical validation, including measurements of reactive oxygen species (ROS), lipid peroxidation markers such as malondialdehyde (MDA), and mitochondrial or ATP activity. Furthermore, the CRYO-SEM analysis is limited to qualitative descriptions without quantitative scoring or comparative evaluation, thereby weakening the strength of the ultrastructural conclusions. Therefore, further methodological refinement, extract standardization, biochemical validation, and quantitative ultrastructural analysis are necessary to enhance the scientific rigor and overall impact of the study.

Recommendations:

- Quantify active compounds (e.g., HPLC/GC-MS)
- Correct methodological inconsistencies

- Include biochemical assays or limit mechanistic claims
- Add semi-quantitative analysis for SEM observations

3. Concern:

Several additional concerns related to the preparation and characterization of the extract also need to be addressed to strengthen the methodological rigor of the study. The study does not provide a detailed characterization of the extract composition, limiting the ability to identify and correlate specific bioactive compounds with the observed biological effects. Furthermore, the extraction procedure lacks sufficient scientific justification, particularly the use of a high extraction temperature (90°C), which may potentially degrade thermolabile bioactive constituents and alter extract stability. Important physicochemical parameters, including pH and osmolarity, were also not reported, despite their potential influence on sperm viability and preservation outcomes. In addition, the manuscript lacks clarity regarding the dilution procedures and the consistency of extract preparation, making it difficult to assess reproducibility and standardization across experimental replicates. These limitations should be carefully addressed to improve the reliability, transparency, and reproducibility of the study findings.

Recommendations:

- Provide full chemical characterization of the extract
- Justify extraction conditions with references
- Report physicochemical parameters (pH, osmolarity)
- Clarify all preparation steps

4. Concern:

The statistical analysis applied in this study is generally appropriate and methodologically acceptable, as demonstrated by the use of normality testing (Shapiro–Wilk test), homogeneity testing (Levene’s test), followed by ANOVA and post hoc analyses for group comparisons. These approaches support the validity of the statistical interpretation of the data. However, several minor issues should be clarified to improve transparency and reporting quality. In particular, Table 1 does not specify whether the presented values are expressed as standard deviation (SD) or standard error (SE), which may lead to ambiguity in data interpretation. Additionally, the specific type of post hoc test employed should be explicitly stated in the table legend to ensure methodological clarity and reproducibility. Addressing these points would strengthen the overall presentation and statistical reporting of the study.

Recommendations:

- Clarify statistical reporting format
- Improve transparency in tables

5. Concern:

Several limitations related to data availability and reporting should also be acknowledged. The raw data supporting the SEM analysis and image quantification are not presented in sufficient detail, limiting the transparency and reproducibility of the ultrastructural evaluation. In particular, the absence of comprehensive quantitative image analysis restricts independent verification of the reported observations. Additionally, no biochemical validation data are available, as oxidative stress markers and related biochemical parameters were not measured in the study.

Consequently, the proposed mechanistic interpretations regarding antioxidant activity remain unsupported by direct experimental evidence. Providing more detailed raw datasets and incorporating biochemical validation in future studies would substantially improve the reliability and interpretability of the findings.

Recommendations:

- Provide more comprehensive datasets
- Include raw imaging data and metadata where possible

6. Concern:

Several concerns related to data interpretation and scientific claims should be carefully addressed. The mechanistic conclusions regarding the antioxidant effects of the *Nigella sativa* extract are not directly supported by experimental evidence, as no biochemical assays or oxidative stress markers were evaluated in the present study. Consequently, the proposed antioxidant mechanism remains inferential rather than experimentally validated. In addition, the discussion tends to overgeneralize the potential application of the findings within the context of sustainable aquaculture. Given the study's current scope and limitations, broader claims regarding large-scale applicability, sustainability benefits, or industrial implementation should be presented more cautiously and supported by additional in vivo, long-term, and field-based investigations. A more balanced interpretation would improve the scientific accuracy and credibility of the manuscript.

Recommendations:

- Limit conclusions to observed results
- Clearly state limitations
- Avoid overinterpretation of mechanisms

Mandatory Revisions

Several mandatory revisions are required before the manuscript can be considered scientifically rigorous and suitable for indexing. First, the *Nigella sativa* extract must be properly standardized through the quantification of major bioactive compounds, particularly thymoquinone, to ensure reproducibility and comparability of the findings. Second, the methodological inconsistency regarding the storage duration (28 vs 48 hours) must be clearly resolved to avoid ambiguity in data interpretation. Third, methodological transparency should be improved by reporting essential physicochemical parameters, including pH and osmolarity, which may influence sperm preservation outcomes. In addition, the discussion section should be revised to avoid unsupported mechanistic claims related to antioxidant activity unless supported by direct biochemical evidence. Statistical reporting also requires clarification, particularly regarding whether the data are presented as standard deviation (SD) or standard error (SE), as well as explicit identification of the post hoc test used. Finally, comprehensive language and formatting revisions are necessary to improve clarity, readability, and overall professional presentation of the manuscript.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Aquatic resources management; Fishires Biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 15 April 2026

<https://doi.org/10.5256/f1000research.189026.r472527>

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Siti Maulida 

Department of Aquaculture, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh, Aceh, Indonesia

Manuscript: Nigella sativa-Based Extender Enhances Nilem (*Osteochilus vittatus*) Sperm Quality: Implications for Sustainable Aquaculture Practices

Reviewer: Siti Maulida, Universitas Syiah Kuala

Recommendation: Approved

Summary

This manuscript evaluates the efficacy of Nigella sativa extract as a natural additive in extenders to maintain the sperm quality of Nile fish (*Osteochilus vittatus*) during cold storage. The topic is highly relevant to sustainable aquaculture and is supported by the use of a positive control (Thymoquinone) and ultrastructural analysis (CRYO-SEM). Overall, the study provides interesting insights; however, certain technical aspects of the methodology and the depth of the discussion require clarification to enhance the scientific rigor and replicability of the article.

Strengths

1. **Methodological Validity:** The authors correctly performed statistical assumption tests (normality and homogeneity) prior to ANOVA, ensuring the validity of the data interpretation.
2. **Ultrastructural Visualization:** The use of CRYO-SEM provides high-quality visual evidence of sperm morphological integrity, which effectively complements the functional motility data.
3. **Experimental Design:** The inclusion of a positive control (Thymoquinone) allows for a clear comparison between the crude extract and its primary active compound.

Suggestions for Improvement

1. Methods

- **Extract Standardization:** As a commercial powder was utilized, the authors should provide further details regarding the concentration of the primary active compound (specifically Thymoquinone) in the product. This information, based either on the product label or relevant literature associated with the specific brand, is vital for global replicability.
- **Justification of Extraction Procedure:** The infusion method was conducted at 90°C for 15 minutes. Please provide a scientific rationale or supporting citations for the selection of this temperature, considering the potential for thermal degradation of certain antioxidant compounds within *Nigella sativa*.

2. Results

- **Statistical Clarity:** In Table 1, clearly specify whether the values after the "±" symbol represent Standard Deviation (SD) or Standard Error. Additionally, include a footnote to the table specifying the post-hoc test used (e.g., Duncan's or Tukey's) to generate the significance notations (superscripts).
- **Comparative SEM Description:** Enhance the narrative by providing a more detailed comparative description of the P2 group (optimal treatment) versus the controls, highlighting specific morphological improvements as shown in the SEM images.
- **Abnormality Characterization:** For the high abnormality rate in the P4 group (80.33%), the authors should briefly describe the dominant types of morphological defects observed (e.g., coiled tails or head deformities).

3. Discussion

- **Analysis of Failure at High Dosages:** The authors should deepen the analysis regarding the significant decline in sperm quality at the 4% dosage (P4). The discussion should address whether this resulted from a loss of protective effects or from potential additional physicochemical stress (e.g., pH or osmolarity changes) induced by the extract at high concentrations.
- **Structure-Function Relationship:** The discussion would be strengthened by specifically linking the observed ultrastructural damage (e.g., plasma membrane and flagella integrity) to the failure of biochemical mechanisms that trigger the decline in sperm motility duration.
- **Practical Implications:** In alignment with the title's reference to "Sustainable Aquaculture," the authors are suggested to provide a brief overview of the cost-effectiveness of using this natural extract compared to purified synthetic antioxidants for mass-scale hatchery operations.

Conclusion

This article makes a valuable contribution to the preservation technology of local fish sperm. By clarifying the methodological aspects regarding media characteristics and providing a more robust discussion on dosage effects, this study will serve as a strong reference for the aquaculture community.

Recommendation Status: Approved

References

1. Ahmad A., *et al.*: A review on therapeutic potential of *Nigella sativa*: A miracle herb. *Asian Pacific Journal of Tropical Biomedicine*, 3(5), 337–352. 2013.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: My research area: aquaculture, biotechnology, cryopreservation

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 08 Jun 2026

Yuni Astuti

We sincerely thank the reviewer for the careful review of our manuscript and for the constructive comments and valuable suggestions. We highly appreciate the time and expertise invested in evaluating our work. The comments provided are insightful and will be very helpful in improving the quality, clarity, and scientific rigor of the manuscript. We acknowledge all comments and suggestions raised by the reviewer and will revise the manuscript accordingly. The recommended improvements regarding methodological details, statistical presentation, interpretation of results, and discussion of the findings will be carefully addressed in the revised version. We believe that these revisions will strengthen the manuscript and enhance its contribution to the field of sustainable aquaculture.

Competing Interests: The authors declare that they have no competing interests related to this work.

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