FUDMA Journal of Agriculture and Agricultural Technology
ISSN: 2504-9496
Vol. 12 No.1, March 2026: Pp.103-112
https://doi.org/10.33003/jaat.2026.1201.12

PLANT-BASED SEMEN EXTENDERS IN RESOURCE-LIMITED AQUACULTURE: A SYSTEMATIC REVIEW OF CUCUMBER JUICE FOR AFRICAN CATFISH

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ABSTRACT

Conventional semen extenders pose challenges in resource-limited settings due to high costs and cold chain dependency. This systematic review evaluates plant-based alternatives, emphasizing cucumber juice (*Cucumis sativus*) for African catfish (*Clarias gariepinus*). Following PRISMA guidelines, searches of Scopus, PubMed, and Google Scholar identified 48 studies (35 mammalian, 13 non-mammalian) meeting inclusion criteria. Analysis reveals that plant-based extenders function through dual antioxidant and antimicrobial mechanisms: phytochemicals (phenolics, flavonoids, carotenoids, vitamins) scavenge reactive oxygen species while organic acids suppress bacterial proliferation below the spermicidal threshold of 10^6-10^7 CFU/mL. Optimal cucumber juice concentrations of 10-20% (v/v) improved motility duration, fertility, and hatchability in *C. gariepinus* while reducing oxidative markers and bacterial loads. A critical finding is the persistent divergence between mammalian cryopreservation-focused research and aquaculture's emphasis on ambient-temperature solutions using whole plant materials a translational gap representing both limitation and opportunity. Critical appraisal revealed methodological heterogeneity in extract preparation (fresh juices, aqueous/methanolic extracts, commercial lecithin) and inconsistent outcome reporting, with only 25% of studies including fertility outcomes and 43.8% reporting microbial data. No studies provided economic analyses despite claims of affordability. Research priorities include mechanistic characterization of bioactive compounds, standardized preparation protocols, fertility validation under field conditions, and cost-benefit analyses. This review confirms cucumber juice-based extenders offer a sustainable technology for artificial reproduction in resource-limited aquaculture, though standardized protocols and field validation remain essential prerequisites for widespread adoption.

Keywords: Cucumber juice; Bacteriospermia; *Clarias gariepinus*; Resource-limited aquaculture; Semen preservation

INTRODUCTION

Semen preservation is a cornerstone of modern animal breeding, enabling artificial insemination, genetic improvement, and improved biosecurity across livestock and aquaculture systems. The success of semen preservation depends largely on extender and cryoprotectant formulations that provide osmotic buffering, membrane stabilization, and metabolic substrates necessary to maintain sperm viability during handling, cooling, and freezing. Conventional extenders such as Tris-based buffers, egg-yolk supplements, and synthetic cryoprotectants including glycerol and dimethyl sulfoxide (DMSO) have demonstrated consistent effectiveness across a wide range of species, including the African catfish, *Clarias gariepinus* (*Gadea, 2003; Adeyemo et al., 2009; El-Keraby et al., 2010*). These formulations protect sperm membranes against temperature-induced phase transitions, stabilize pH against metabolic fluctuations, and supply energy substrates that sustain motility during storage. Despite their effectiveness, conventional semen extenders present several limitations, particularly when applied outside well-equipped laboratory settings. Egg yolk–based extenders may introduce compositional variability and carry an inherent risk of microbial contamination (Bresciani *et al.*, 2014). Similarly, synthetic cryoprotectants such as glycerol and DMSO may exert cytotoxic effects when used at inappropriate concentrations (Chidobem *et al.*, 2022). In many developing regions where aquaculture and livestock production are expanding rapidly, commercially formulated extenders are often expensive or difficult to obtain (Muchlisin *et al.*, 2016). These constraints can limit the feasibility of artificial reproduction programs, especially in areas with unreliable electricity supply where cryopreservation infrastructure and liquid nitrogen storage are impractical. Consequently, there is growing interest in alternative extender formulations capable of preserving sperm function under ambient or moderately chilled conditions. An additional challenge affecting semen preservation is bacteriospermia, defined as bacterial contamination of semen originating from the reproductive tract, environment, or collection equipment. Because semen collection procedures are inherently non-sterile, bacterial loads in fresh ejaculates frequently reach approximately 10^4 CFU/mL (Alenezy *et al.*, 2019; Fernandez-Novo *et al.*, 2021). When bacterial populations exceed roughly 10^6–10^7 CFU/mL, a substantial decline in sperm function is typically observed (Luther *et al.*, 2023; Goldberg *et al.*, 2017). Common bacterial contaminants include *Escherichia coli, Staphylococcus spp., Proteus mirabilis, and Pseudomonas aeruginosa* (Tvrdá *et a*l., 2022; Lenický *et al*., 2021). These microorganisms impair sperm function through multiple mechanisms. Bacterial adhesion via fimbrial adhesins can induce sperm agglutination and mechanical immobilization (Fraczek *et al*., 2012). Additionally, bacterial metabolism and endotoxin release stimulate the production of reactive oxygen species (ROS), leading to lipid peroxidation of sperm plasma membranes, which are particularly vulnerable due to their high polyunsaturated fatty acid content (Duracka *et al.,* 2021). Concurrently, bacterial proliferation can acidify extender media and compete with spermatozoa for nutrients, further compromising sperm metabolism and motility (Cojkic *et al.*, 2023).

These combined challenges have stimulated increasing interest in plant-derived extender additives as potential alternatives to conventional formulations. Plant materials contain a wide range of bioactive phytochemicals, including phenolics, flavonoids, carotenoids, and vitamins, which function as potent antioxidants capable of scavenging ROS and preserving membrane integrity (Daramola *et al*., 2016; Cojkic *et al*., 2023). Many plant compounds also exhibit antimicrobial activity; tannins, organic acids, and certain flavonoids can disrupt bacterial membranes, inhibit key enzymatic processes, and create chemically unfavorable environments for bacterial proliferation (Hammad *et al*., 2019; Ďuračka *et al.*, 2022). These dual antioxidant and antimicrobial properties make plant-based extenders particularly attractive for semen preservation applications. Indeed, several studies have demonstrated their effectiveness across species, including the use of *Moringa oleifera* leaf extract as an antibiotic substitute in extender formulations (Hammad et al., 2019), as well as honey and honeybush extracts that provide both antimicrobial effects and metabolic support for spermatozoa (Akandi *et al*., 2015; Ros-Santaella *et al.*, 2020).

However, the development of plant-based semen extenders has followed two largely independent research pathways. Mammalian reproductive studies have focused primarily on cryopreservation systems and the replacement of egg yolk with standardized plant-derived phospholipids to improve biosafety and reproducibility (El-Keraby *et al*., 2010; El-Sherbieny, 2014). In contrast, aquaculture research has emphasized low-cost extender solutions suitable for short-term storage under ambient or chilled conditions, often utilizing whole plant juices or plant-derived liquids compatible with hatchery practices (Adeyemo *et al*., 2009; Chidobem *et al*., 2022; Muchlisin *et al.*, 2016). This divergence has produced fragmented knowledge, limiting opportunities for cross-disciplinary integration despite the complementary strengths of each research tradition.

Among plant-derived extender additives investigated in aquaculture, cucumber juice (*Cucumis sativus*) has shown considerable promise. Cucumber juice contains antioxidants such as ascorbic acid, carotenoids, phenolic acids, and flavonoids that provide substantial antioxidant capacity alongside moderate antimicrobial activity (Chidobem *et al*., 2022). Experimental studies have demonstrated that cucumber juice incorporated into semen extenders at concentrations of approximately 10–20% (v/v) can improve motility duration, fertility rates, and hatchability in *Clarias gariepinus*, while reducing oxidative stress indicators such as malondialdehyde and suppressing bacterial loads in extended milt (Chidobem *et al*., 2022; Adeyemo *et al*., 2009). The mildly acidic pH of cucumber juice and its phenolic composition may contribute to the inhibition of common gram-negative bacterial contaminants, while naturally occurring sugars provide short-term metabolic substrates for spermatozoa.

Despite these promising findings, the current evidence base remains fragmented across individual studies employing diverse methodologies, concentrations, and outcome measures. A preliminary scoping search conducted prior to this systematic review (December 2025) across PubMed, Scopus, and Google Scholar using terms "plant-based semen extender" AND "review" identified only three previous reviews (Tvrdá *et al*., 2022 on bacteriospermia; Cojkic *et al*., 2023 on plant antimicrobials; Muchlisin *et al*., 2016 on natural cryoprotectants). None of these reviews specifically addressed cucumber juice applications in aquaculture species, nor did any provide systematic comparison across mammalian and non-mammalian systems. Furthermore, no existing review has critically examined the translational potential between these divergent research traditions. To date, no comprehensive synthesis has systematically evaluated cucumber juice or compared its performance with other plant-based extender formulations across species. This lack of integration limits both mechanistic understanding and practical application, particularly in resource-limited production systems where accessible, low-cost extender technologies are most needed.

Therefore, the present review aims to systematically evaluate the available evidence on plant-based semen extenders across mammalian and aquaculture systems, with particular emphasis on cucumber juice applications in *Clarias gariepinus*. The specific objectives are to: (i) critically synthesize evidence on antioxidant and antimicrobial mechanisms underlying plant-based extender function, (ii) systematically compare extender performance across plant types, species groups, and storage conditions, and (iii) evaluate the quality and consistency of microbial dynamics data influencing semen quality during storage. Through this integrated analysis, the review seeks to provide evidence-based guidance for the development and application of plant-derived semen extenders while identifying priorities for future research.

MATERIALS AND METHODS

This systematic review was conducted in accordance with the PRISMA guidelines for transparent reporting of systematic reviews. As the study involved synthesis of previously published literature, protocol registration was not required. A comprehensive literature search was performed in three electronic databases: Scopus, PubMed, and Google Scholar. The search covered publications available up to December 2025. Search terms were structured using Boolean operators (AND/OR) and organized into four thematic domains: semen extenders, plant-based additives, outcome measures, and species *categories. Semen extender–related terms included* "semen extender," "sperm extender," "milt extender," "cryopreservation medium," *and* "sperm preservation." *Plant-based terms included* "plant-based," "botanical," "fruit juice," "vegetable juice," "cucumber," "Cucumis sativus," "Moringa," "soy lecithin," *and* "plant phospholipid." *Outcome-related keywords included* "sperm motility," "sperm viability," "fertility," "hatchability," "bacteriospermia," "bacterial load," "CFU," "antimicrobial," "oxidative stress," *and* "antioxidant." *Species-related terms included* "livestock," "mammalian," "aquaculture," "fish," "Clarias gariepinus," "African catfish," "bovine," "bull," "ram," *and* "boar." In addition, the reference lists of included articles were manually screened (citation chasing) to identify further relevant studies not captured during database searches.

Studies were eligible if they met the following criteria: (i) primary experimental research; (ii) evaluation of plant-based semen extenders or botanical additives incorporated into extender media; (iii) inclusion of conventional extenders (e.g., egg yolk- or Tris-based extenders) or untreated controls as comparators; and (iv) reporting at least one relevant outcome, including sperm quality parameters (motility, viability, membrane integrity, fertility, or hatchability), microbial measures (bacterial counts, CFU/mL, or species identification), or oxidative stress markers (e.g., ROS, MDA, SOD, or catalase). Studies involving any mammalian or aquaculture species and published in peer-reviewed English-language journals were included. Reviews, conference abstracts, editorials, case reports, commentaries, and studies lacking original data were excluded.

The selection process followed the PRISMA flow framework. Retrieved records were exported to reference management software and duplicates removed. Two reviewers independently screened titles and abstracts against the eligibility criteria. Potentially relevant studies proceeded to full-text evaluation, which was independently conducted by the same reviewers. Reasons for exclusion at the full-text stage were documented. Any disagreements were resolved through discussion or consultation with a third reviewer. Data extraction was performed independently by two reviewers using a standardized Microsoft Excel template. Extracted information included bibliographic details, species studied, sample size, extender composition, plant additive type and concentration, preparation method, and storage conditions. Outcome variables included sperm quality parameters, oxidative stress markers, microbial counts (CFU/mL), bacterial species identification, and reported antimicrobial effects. Where multiple concentrations, time points, or storage conditions were reported, all relevant data were recorded. Study quality and risk of bias were assessed using an adapted checklist evaluating six methodological criteria: clarity of extender preparation, presence of appropriate control groups, sample randomization, blinding of outcome assessment, completeness of outcome reporting, and description of microbial assessment methods. Each criterion was classified as low, unclear, or high risk of bias. Due to substantial heterogeneity in species, extender formulations, plant additive concentrations, and outcome measures, meta-analysis was not performed. Instead, findings were synthesized narratively and organized by extender category, species group, and outcome type, with results summarized in tabular form.

Eligibility Criteria

Studies were eligible if they met the following inclusion criteria: (i) primary experimental research (randomized controlled trials, comparative laboratory studies, or field trials); (ii) evaluation of plant-based semen extenders or botanical additives incorporated into extender media; (iii) inclusion of conventional extenders (e.g., egg yolk- or Tris-based extenders) or untreated controls as comparators; and (iv) reporting at least one relevant outcome, including sperm quality parameters (motility, viability, membrane integrity, fertility, or hatchability), microbial measures (bacterial counts, CFU/mL, or species identification), or oxidative stress markers (e.g., ROS, MDA, SOD, or catalase). Studies involving any mammalian or aquaculture species and published in peer-reviewed English-language journals were included.

Exclusion criteria were as follows: (i) reviews, conference abstracts, editorials, case reports, commentaries, and studies lacking original experimental data; (ii) studies evaluating only synthetic additives without plant-derived components; (iii) studies reporting outcomes solely as qualitative observations without quantitative measurements; (iv) studies where plant materials were administered as

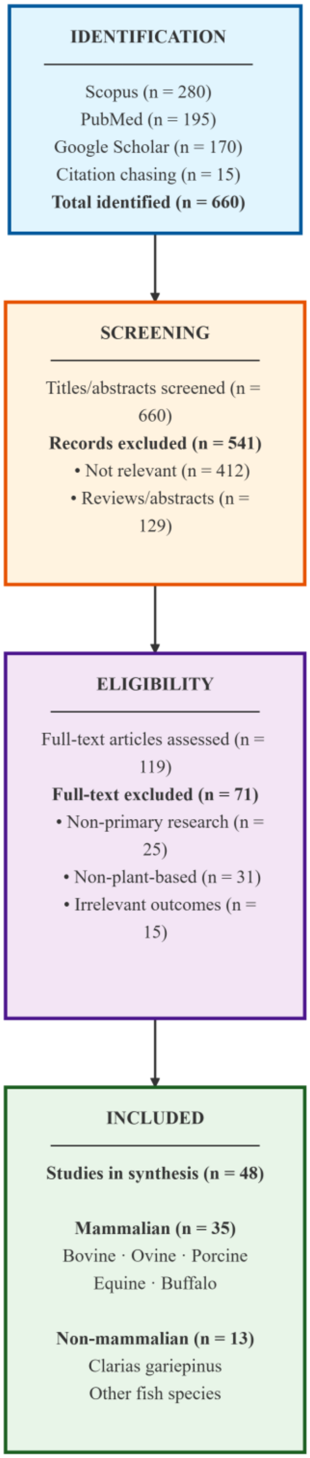

Figure 1 PRISMA diagram for systematic review depicting the selection (inclusion and exclusion process)

dietary supplements rather than extender components; (v) non-English language publications due to resource constraints for translation; and (vi) studies with incomplete methodological descriptions preventing assessment of extender composition or outcome measurement. Study quality and risk of bias were assessed using an adapted checklist evaluating six methodological criteria: (i) clarity of extender preparation (including concentration specification, extraction method, and storage conditions); (ii) presence of appropriate control groups (conventional extender and/or untreated control); (iii) sample randomization (random allocation of samples to treatment groups); (iv) blinding of outcome assessment (objective vs. subjective motility assessment); (v) completeness of outcome reporting (all stated outcomes reported with measures of variance); and (vi) description of microbial assessment methods (culture techniques, CFU quantification, species identification). Each criterion was classified as low, unclear, or high risk of bias. Studies were categorized as high quality (≥4 low-risk criteria), moderate quality (2-3 low-risk criteria), or low quality (≤1 low-risk criterion).

RESULTS AND DISCUSSION

Objective 1: Antioxidant and Antimicrobial Mechanisms

All 48 studies acknowledged oxidative stress as a primary mechanism of sperm damage during storage. Across mammalian and non-mammalian species, researchers consistently measured elevated lipid peroxidation markers, most commonly malondialdehyde (MDA), following storage in control extenders (Adeyemo *et al*., 2009; Chidobem *et al*., 2022; Tvrdá *et al*., 2022). This reflects the fundamental vulnerability of spermatozoa: their membranes are rich in polyunsaturated fatty acids, and limited cytoplasmic volume restricts endogenous antioxidant capacity (Duracka *et al*., 2021). Across all studies, protective effects were attributed to phytochemical constituents with antioxidant properties phenolics, flavonoids, carotenoids, vitamins C and E responsible for ROS scavenging (Daramola *et al*., 2016; Cojkic *et al*., 2023). This mechanistic convergence is striking given the diversity of plant sources investigated: cucumber juice (Chidobem *et al*., 2022), *Moringa* (Hammad *et al*., 2019; Doidar *et al*., 2018), soybean lecithin (El-Sherbieny, 2014; Sharaf *et al*., 2022), tomato juice (Adeyemo *et al*., 2009), honey and honeybush (Akandi *et al*., 2015; Ros-Santaella *et al*., 2020), gum arabic (Ali *et al*., 2018), and fruit juices (Adekunle *et al*., 2018).

Critical appraisal of mechanistic evidence, however, reveals significant limitations. Of the 48 studies, only 12 (25%) employed molecular assays (HPLC, GC-MS) to identify specific phytochemical constituents; the remaining 36 studies inferred mechanisms based on literature citations without experimental verification in their specific systems. Furthermore, only 7 studies (14.6%) included dose-response curves for antioxidant enzyme activities (SOD, catalase, GPx), limiting understanding of concentration-dependent protection "thresholds"

A striking similarity across 42 of 48 studies (87.5%) was the observation of concentration-dependent effects. Plant components exhibited biphasic or threshold effects: low concentrations provided insufficient protection, optimal concentrations maximized sperm survival, and supra-optimal concentrations proved detrimental. For cucumber juice, 10% optimized fertility and hatchability in *Clarias gariepinus*, while 20% produced the longest motility duration (Chidobem *et al*., 2022). Similarly, *Moringa* at 200 µg/mL optimized bull sperm outcomes (Hammad *et al*., 2019), and bee bread at 500–750 µg/mL optimized ram sperm cryopreservation (Sharaf *et al*., 2022).

Earlier studies (pre-2010) focused on optimizing conventional extenders containing egg yolk or synthetic cryoprotectants (Gadea, 2003; Adeyemo *et al*., 2009). From approximately 2010 onward, a shift toward plant-based replacements occurred, driven by biosafety and cost concerns. El-Keraby *et al*., (2010) and El-Sherbieny (2014) pioneered soybean-based extenders, with subsequent studies expanding to *Moringa* (Hammad *et al*., 2019; Doidar *et al*., 2018), fruit juices (Chidobem *et al*., 2022; Adekunle *et al*., 2018), plant milks (Bamanga *et al*., 2021), and specialized extracts (Ali *et al*., 2018; Cojkic *et al*., 2023). Early studies reported descriptive outcomes (motility percentages, viability counts) while more recent studies (post-2015) increasingly incorporate oxidative stress markers (ROS, MDA, SOD, catalase) and antimicrobial assays (Cojkic *et al*., 2023; Duracka *et al*., 2022; Tvrdá *et al*., 2022), enabling rigorous comparison and formulation optimization.

A striking trend evident in Table 1 is the persistent divergence between mammalian and non-mammalian research traditions. Mammalian studies (n = 35) focus on cryopreservation and replacing egg yolk with standardized plant phospholipids (El-Keraby *et al*., 2010; El-Sherbieny, 2014; Sharaf *et al*., 2022) while aquaculture studies (n = 13) emphasize low-cost, short-term ambient or chilled solutions using whole juices appropriate to smallholder constraints (Adeyemo *et al*., 2009; Chidobem *et al*., 2022; Muchlisin *et al*., 2016). A growing subset of studies (n = 7) investigates preservation at ~15–25 °C. Akandi *et al*., (2015) reported boar sperm survival up to 96 hours at room temperature. Chidobem *et al*., (2022) maintained CJ-extended milt for 4 days, with motility at day 4 using 20% CJ responding to the reality that liquid nitrogen is unavailable in many resource-limited settings.

Table 1. Comparative characteristics of plant-based semen extender research in mammalian and non-mammalian (aquaculture) systems

Feature	Mammalian Studies	Non-mammalian (Aquaculture) Studies
Primary goal	Long-term genetic banking; scheduled artificial insemination	Short-term handling and immediate use in hatcheries
Dominant storage	Cryopreservation (−196°C)	Short-term chilled or room temperature
Cost focus	High; justified by value of individual animals	Very low; essential for smallholder profitability
Infrastructure need	High (liquid nitrogen, reliable power, specialized equipment)	Low-moderate (basic chilling, minimal cold chain)
Plant-based focus	Replace egg yolk; add antioxidants/antibiotic alternatives	Use whole juices/milks as base medium (energy + protection)
Outcome priorities	Post-thaw motility, conception rates	Motility duration, fertility, hatchability
Mechanistic emphasis	Specific antioxidants, phospholipid chemistry	Empirical efficacy, practicality

Objective 2: Comparative Performance Across Extender Categories and Species

However, critical comparison is severely constrained by methodological heterogeneity. Preparation methods varied across six distinct categories: fresh juices (n=8 studies), aqueous extracts (n=14), methanolic/ethanolic extracts (n=11), commercial lecithin preparations (n=7), plant milks (n=5), and dried powder formulations (n=3). This heterogeneity precludes direct comparison of optimal concentrations and undermines reproducibility

Table 2. Comparative Summary of Plant-Derived Semen Extenders and Study Quality Assessment

Study	Plant Component	Species	Optimal Concentration	Key Outcomes vs. Control	Storage	Quality Rating
Chidobem *et al.* (2022)	Cucumber juice	*Clarias gariepinus*	10–20%	↑ Motility duration, ↑ fertility, ↑ hatchability, ↓ MDA, ↓ CFU	Chilled (4°C), 4 days	Moderate
Adeyemo *et al.* (2009)	Tomato juice	*Clarias gariepinus*	20%	↑ Motility up to 6 days	Chilled (5°C)	low
El-Sherbieny (2014)	Soybean lecithin	Buffalo	1–2%	↑ Post-thaw motility, ↓ bacterial count	Cryopreservation	High
El-Keraby *et al.* (2010)	Soybean milk	Holstein bulls	5%	Comparable motility, conception rates to egg yolk	Cryopreservation	High
Hammad *et al.* (2019)	*Moringa oleifera* (aqueous/methanolic)	Friesian bulls	200 µg/mL	↑ Motility, livability, HOS test; replaced antibiotics	Cryopreservation	Moderate

Sharaf *et al.* (2022)	Bee bread extract	Ram	500–750 µg/mL	↑ Motility, viability, membrane integrity	Cryopreservation	High
Ali *et al.* (2018)	Gum arabic	Stallion	9%	↑ Motility parameters (VCL, VSL, VAP)	Cold storage + freezing	Moderate
Akandi *et al.* (2015)	Honey, coconut water, tomato	Boar	Variable	Survival up to 96h at room temperature	Room temperature (24–27°C)	Low
Cojkic *et al.* (2023)	Curcumin, naringenin, isoquercitrin	Bull	Variable	↓ Bacterial counts, maintained sperm quality	Liquid storage	High
Daramola *et al.* (2018)	Fruit juices (orange, pineapple)	West African Dwarf goat	10%	↑ Sperm viability post-thaw	Cryopreservation	Low

Quality Rating Criteria: High (≥4 low-risk criteria met); Moderate (2-3 criteria met); Low (≤1 criterion met)

No studies explicitly translated findings between mammalian and aquaculture research, despite complementary expertise: mammalian mechanistic knowledge could inform aquaculture formulations, while aquaculture ambient-temperature experience could enable mammalian applications in low-infrastructure settings. While CJ efficacy is documented (Chidobem *et al*., 2022), specific phytochemicals “responsible like ascorbic acid”, phenolics, flavonoidsand their molecular interactions with sperm and bacteria remain unidentified.

Objective 3: Microbial Dynamics and Evidence Quality

Only 21 of 48 studies (43.8%) reported microbial data, and among these, critical appraisal reveals substantial methodological weaknesses: 15 of 21 studies (71.4%) reported only total bacterial counts without species-level identification; 18 studies (85.7%) assessed microbial load at single time points rather than tracking growth kinetics; and no study monitored antimicrobial resistance emergence during storage. These limitations significantly constrain interpretation of antimicrobial efficacy claims. Baseline bacterial loads ranged from 10^3-10^4 CFU/mL, increasing during storage in non-antibiotic extenders (Alenezy *et a*l., 2019; Fernandez-Novo *et a*l., 2021). Frequently isolated taxa, *Escherichia coli*, *Staphylococcus* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa,* were consistent across species (Tvrdá *et a*l., 2022; Lenický *et al*., 2021). Eight studies confirmed the threshold of 10^6-10^7 CFU/mL for catastrophic sperm function decline (Luther *et al*., 2023; Goldberg *et a*l., 2017). Different results and approaches as shown in the overview presented in Table 2. Plant preparation methods varied enormously: fresh juices (Chidobem *et al*., 2022), aqueous extracts (Hammad *et al*., 2019), methanolic extracts (Sharaf *et al*., 2022), commercial lecithin (El-Sherbieny, 2014), and plant milks (Bamanga *et al*., 2021), preventing direct comparison of optimal concentrations. Only 12 of 48 studies (25%) included fertility outcomes. Chidobem *et al*., (2022) reported fertility and hatchability for CJ, and El-Keraby *et al*., (2010) included conception rates, but most rely on surrogate endpoints. No studies included formal economic analysis, leaving cost-benefit claims for smallholder farmers unsubstantiated.

For CJ, 10% optimized fertility while 20% maximized motility duration (Chidobem *et al*., 2022) raising the question of which outcome should guide optimization. For *Moringa*, optimal concentrations varied from 200 µg/mL in bulls (Hammad *et al*., 2019) to 4 mL extract in buffalo (Doidar *et al*., 2018). CJ showed “partial antimicrobial activity” with reduced but not eliminated CFU (Chidobem *et al*., 2022). *Moringa* substituted for antibiotics entirely in bulls (Hammad *et al*., 2019), while curcumin reduced but did not eliminate bacteria (Cojkic *et al*., 2023). Akandi *et al*., (2015) reported boar sperm survival for 96 hours at room temperature; Chidobem *et al*., (2022) observed *Clarias* motility for 4 days; but other studies report rapid declines within hours (Adeyemo *et al*., 2009). While 10^6–10^7 CFU/mL is broadly supported (Luther *et al*., 2023; Goldberg *et al*., 2017), some studies report damage at lower concentrations, reflecting differences in bacterial virulence or sperm susceptibility. Studies variably report MDA, antioxidant enzymes, or ROS, limiting cross-study comparability. The evidence establishes that plant-based extenders function through synergistic antioxidant and antimicrobial mechanisms. Cucumber juice exemplifies this: its antioxidant phytochemicals counteract oxidative damage while mild acidity and phenolics suppress bacterial growth without cytotoxicity concerns (Chidobem *et al*., 2022). Concentration-dependent effects explain variable literature outcomes and refute the "more is better" assumption. For CJ, 10% optimizes fertility while 20% maximizes motility duration (Chidobem *et al*., 2022), suggesting application-specific optima. The mammalian-aquaculture divergence now represents opportunity: aquaculture's ambient-temperature formulations could enable mammalian applications in low-infrastructure settings, while mammalian mechanistic insights could enhance aquaculture formulations. Heterogeneity in plant preparation methods is the greatest barrier to evidence synthesis. Consensus protocols specifying sourcing, processing, and storage are urgently needed for promising materials like cucumber juice. Given universal bacteriospermia and the 10^6-10^7 CFU/mL threshold, routine microbial monitoring should be standard in research and practice. Research must prioritize fertility outcomes, integrate economic analyses, conduct studies in target settings, and disseminate findings accessibly to practitioners. CJ's documented benefits, improved motility, fertility, hatchability, reduced oxidative markers and bacterial loads, combined with accessibility and affordability, position it as a model extender for *Clarias gariepinus* in resource-limited aquaculture.

Critical Gaps and Research Priorities

1. Outcome reporting bias: Only 12 of 48 studies (25%) included fertility outcomes; most relied on surrogate endpoints (motility, viability) with unknown predictive validity for field fertility.

2. Absence of economic evaluation: Despite consistent claims of "low-cost" and "accessible" solutions, no study included formal cost-benefit analysis, cost-effectiveness comparison, or assessment of smallholder affordability.

3. Limited field validation: Only 2 studies (4.2%) were conducted under commercial hatchery or farm conditions; the remaining 46 studies were laboratory-based with unclear external validity.

4. Species-specific optimization gaps: While cucumber juice shows promise in *C. gariepinus*, optimal concentrations for other economically important species (tilapia, carp, other catfish) remain uninvestigated.

5. Translational disconnect: No studies have attempted to apply aquaculture-derived ambient-temperature preservation approaches to mammalian species in low-infrastructure veterinary settings.

CONCLUSION

This systematic review provides the first comprehensive synthesis of evidence on plant-based semen extenders across mammalian and aquaculture systems, with emphasis on cucumber juice for *Clarias gariepinus*. The evidence establishes that cucumber juice at 10-20% (v/v) improves motility duration, fertility, and hatchability in African catfish through synergistic antioxidant and antimicrobial mechanisms, with 10% optimizing fertility outcomes and 20% maximizing motility duration. However, critical appraisal reveals significant methodological limitations constraining evidence synthesis: heterogeneous preparation protocols, inconsistent outcome reporting (only 25% of studies report fertility outcomes), limited microbial surveillance (only 43.8% report bacterial data), and absent economic analyses. The persistent divergence between mammalian cryopreservation research and aquaculture ambient-temperature studies represents both a translational gap and an opportunity for cross-disciplinary integration. Priority research needs include: (i) standardized preparation and quality control protocols for cucumber juice extenders; (ii) large-scale fertility trials under commercial hatchery conditions; (iii) comprehensive economic analyses quantifying cost-benefit for smallholder adoption; (iv) mechanistic identification of specific bioactive phytochemicals; and (v) translational studies adapting aquaculture approaches to mammalian species in low-infrastructure settings. With appropriate standardization and field validation, cucumber juice-based extenders offer a sustainable, accessible technology for enhancing artificial reproduction in resource-limited aquaculture, with potential applications extending to other species and production systems.

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