Harnessing the Growth-Promoting Potential of Vermiwash for Sustainable Agriculture: A Meta-Analysis on Seed Germination Rate, Early Growth Parameters, and Optimal Dilution Ratios

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Abstract: Meeting the rising food demands of a growing global population requires innovative, eco-friendly agricultural solutions. Biofertilizers like vermiwash provide a sustainable alternative to chemical fertilizers, promoting plant growth while preserving soil health and minimizing environmental impact. This meta-analysis assesses the effectiveness of vermiwash in enhancing seed germination and early plant development across various crop types. Our findings demonstrate that vermiwash significantly increases germination rate with an effect size of 7.03 and germination percentage with an effect size of 8.89. In early plant development, it demonstrated an effect size of 6.08 for root length and 10.53 for shoot length. Notably, the seedling vigor index also showed marked improvement. The NPK composition of vermiwash varies by source, so farmers aiming to boost germination should select vermiwash made from a mix of leaf litter and cow dung. This blend, especially when prepared using *Eisenia foetida* and *Perionyx excavatus* earthworms, typically offers an ideal 1:2:1 NPK ratio that enriches the nutrient profile, supporting both germination and early seedling growth. Moderator analysis indicated that a 100% concentration of vermiwash enhanced germination properties, while dilutions below 25% promoted early growth parameters, supporting balanced nutrient absorption without the inhibitory effects associated with higher concentrations. This analysis also identifies potential publication biases, emphasizing a trend towards studies reporting larger effect sizes. We recommend that future primary studies report comprehensive statistical parameters and experimental details to support more robust meta-analyses. These findings highlight vermiwash's value as a sustainable bio-stimulant across various plant families, supporting global objectives for enhanced food production and environmental sustainability.

Key Words: Biofertilizers, Vermiwash, Seed Germination, Sustainable agriculture

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1. Introduction:

The Green Revolution dramatically increased global food production, yet the reliance on chemical fertilizers and pesticides has led to several ecological challenges. These include soil degradation, contamination of water sources, development of pest resistance, and disruptions to biodiversity. With these impacts accumulating, it has become essential to shift toward sustainable agricultural methods that not only support productivity but also safeguard soil and environmental health for future generations. Vermiwash, a liquid bio-fertilizer produced from vermiculture, shows great potential as an eco-friendly tool in sustainable agriculture. This nutrient-dense solution, obtained as a byproduct of vermicomposting, contains critical plant nutrients, growth regulators (such as auxins such as IAA, gibberellins, and cytokinins), beneficial enzymes, and a variety of microorganisms (1). Humic compounds in vermicompost play a dual role, they bind and slowly release essential nutrients (2) and act as non-nutritional growth-promoters by activating plant signaling pathways and enzymes (3).In addition to enhancing crop productivity as a fertilizer, vermiwash also aids in suppressing diseases and controlling pests due to its vital antimicrobial and pest-repelling compounds (4). Compared to solid vermicompost and conventional fertilizers, vermiwash is highly effective due to its readily absorbable nutrients, allowing rapid uptake by plants. This liquid biofertilizer can quickly reach targeted root zones, enhancing disease suppression and overall plant health. Moreover, vermiwash's versatility allows it to be applied both as a foliar spray and a soil amendment, making it an adaptable solution for various agricultural needs. Its diverse nutrient profile not only supports plant vigor but also encourages root and shoot development, along with enhancing soil microbial diversity and activity (5).Studies show that combining vermiwash with plant-based pest control methods enhances pest resistance, reducing infestations of pests like thrips and mites while improving crop yields (6,7).

Research has shown that vermiwash, an organic by-product derived from vermiculture, can significantly enhance seed germination and early seedling growth (8). Its potential to stimulate uniform crop establishment makes it especially valuable for sustainable and low-input farming systems. Recent studies suggest that treating seeds with 10% vermicompost tea for 8 hours can significantly improve Seed sprouting and seedling establishment (9). Applications of vermiwash at varied concentrations have demonstrated positive effects on critical growth parameters in early development stages, including germination speed, proportion of seeds that germinate, root extension, and shoot development. Recognizing these benefits, this study conducts a meta-analysis to systematically evaluate vermiwash's efficacy across different plant including legumes, non-legumes, monocots, and dicots, evaluating its impact on critical growth metrics like germination rate, germination percentage, and shoot and root lengths.

Vermiwash derived from various earthworm species most notably *Perionyx excavatus, Eudrilus eugeniae,* and *Eisenia fetida* has demonstrated significant effects on early plant growth parameters, including germination rate, root length, and shoot length. By synthesizing data through standardized mean differences (SMDs), this analysis aims to quantify vermiwash's impact with greater precision and to identify optimal dilution ratios for maximizing its effectiveness. These findings contribute to sustainable agriculture by promoting vermiwash as an eco-friendly seed treatment that enhances germination, reduces dormancy, and accelerates early development.

2. Methods:

2.1 Literature search:

Relevant literature was explored in Google Scholar search engine with a publication date range from 2000 to 2024. The keywords used included: "vermiwash" AND "seed germination" OR "germination rate" AND "early growth" OR "effect of vermiwash" AND "germination percentage." Initial screening involved evaluating, screening of titles and abstracts helped in identifying studies of potential interest. Full-text reviews were subsequently carried out on these selected articles, following predefined inclusion and exclusion criteria, to ensure relevance and quality for the study.

2.2 Inclusion and exclusion criteria:

Our inclusion criteria were (i) the presence of mean value along with the standard deviation for both vermiwash treatment and a control (mostly with water) for germination rate, proportion of seeds germinated, and shoot and root growth (ii) source of vermiwash and its effects particularly on seeds (iii) Data including plant growth and seedling vigor index (iv) no limitations of type of studies. Exclusion criteria were (i) study with incomplete data (ii) studies including combined effects of vermiwash and vermicompost.

2.3 Data collection:

A systematic search on Google Scholar yielded 58 research papers, from which 47 were excluded after abstract screening due to a lack of quantitative data. Eleven studies met the eligibility criteria, as they contained relevant quantitative data suitable for analysis. These 11 papers were included in the final dataset for the meta-analysis, following the PRISMA guidelines to ensure a transparent selection process. From the 11 selected papers, we identified four primary output variables to assess the effects of vermiwash on early plant growth: (i) germination rate, reported 120 times across 4 publications; (ii) germination percentage, with 160 occurrences in 7 publications; (iii) shoot length, recorded 200 times across all 11 publications; and (iv) root length, also reported 200 times across all 11 studies. However, study designs and plant species varied across the papers, adding diversity to the analysis. Due to limited data on the impact of vermiwash on seed vigor index, this variable was excluded from our analysis. Data were collected across several key aspects, including different sources of vermiwash, such as cattle dung, vermicompost, organic wastes, and leaf litter, as well as varied vermiwash concentrations. Furthermore, we documented the specific earthworm species used to produce vermiwash, and the concentrations of macronutrients nitrogen, potassium, and phosphorus in the vermiwash. Our analysis also considered the impact of vermiwash on various plant families, including legumes, *Malvaceae, Solanaceae*, and *Amaranthaceae*. Given the limited availability of quantitative data in Scopus-indexed journals on these topics, relevant non-Scopus articles were included in the data collection to ensure comprehensive coverage.

2.4. Statistical analysis:

To assess the effect size, the Standardized Mean Difference (SMD) was calculated for each variable of interest. A meta-analysis was conducted using JASP software to determine the cumulative effect size, enabling the synthesis of effect sizes across studies. JASP's meta-analysis module was utilized to compute and visually represent the cumulative effect size, facilitating a comprehensive analysis of the dataset.

2.5 Meta-analysis: Calculation of effect sizes:

In our meta-analysis, we evaluated the impact of vermiwash on seed germination and early plant development, focusing on four key variables: germination rate, germination percentage, shoot length, and root length. To standardize comparisons across studies with varying measurement scales, we calculated effect sizes using the Standardized Mean Difference (SMD), a method recommended by Borenstein et al., for studies where outcomes are measured on a physical scale (10). The SMD provides a consistent interpretation of treatment effects, enabling direct comparison between experimental and control groups across datasets that use diverse units (11). This metric is particularly useful when examining continuous outcomes, as it highlights the relative magnitude of effects, offering meaningful insights into the efficacy of vermiwash on seed germination and early development.

We also explored the impact of co-variables outlined in the experimental protocols, such as vermiwash dilution levels, to identify distinct groupings with varying effect sizes. Given the variability across studies, we chose a random-effects model to estimate a more generalized effect size. Unlike the fixed-effects model, which assumes homogeneity in study conditions and variances, the random-effects model accommodates greater conditions (10). By employing the SMD within a random-effects framework, we achieved a nuanced, comprehensive view of vermiwash's impact, accounting for variability across different study contexts and experimental designs. We computed the effect size for each study using Cohen's d, a standardized mean difference metric that allows for direct comparison across experiments with varying measurement scales. Cohen's d was calculated as follows:

Standardized mean difference

Cohen's d: $$d = \frac{\overline{X_1} - \overline{X_0}}{S_p}$$

$$S_p = \sqrt{\frac{(n_0-1)S_0^2+(n_1-1)S_1^2}{n_0+n_1-2}}$$

$$SE_d = \sqrt{\frac{1}{n_0} + \frac{1}{n_1} + \frac{d^2}{2(n_0+n_1-2)}}$$

X_1 and X_2 are sample means of two groups.

S_p=Pooled Standard Deviation

All figures in this study are based on the Standardized Mean Difference (SMD), which represents the effect of the vermiwash treatment compared to the vermiwash-free control. We indicate the magnitude of increase or decrease in seed germination and early plant development variables (such as rate and percentage of germination, as well as root and shoot lengths for the vermiwash treatment relative to the control. Each figure also includes the number of observations used in the calculations, providing clarity on sample size and the robustness of each effect size estimate.

3. Result and Discussion:

3.1 Effect of vermiwash on seed germination and early growth.

The addition of vermiwash in our meta-analysis significantly enhanced key and early development stages. Specifically, vermiwash increased germination rate with an effect size of 7.03 and germination percentage with an effect size of 8.89. In early plant development, it demonstrated an effect size of 6.08 for root length and 10.53 for shoot length, underscoring its strong influence on seed germination and seedling growth.

These results align with findings from individual studies across a variety of crops, including *Vigna radiata* (mung bean), *Oryza sativa* (rice), and *Solanum lycopersicum* (tomato), which show how vermiwash enhances germination rates, seedling vigor, and growth metrics such as shoot and root elongation (12,13,14). Consistent with our pooled effect sizes, these studies attribute the growth-promoting effects of vermiwash to bioactive compounds like auxins and cytokinins, which stimulate cell division and elongation for robust initial development (15,16,17).Similarly, research on cereals like *Zea mays* (maize) and *Triticum aestivum* (wheat) observed that applying vermiwash at a 50% concentration boosted germination rates by 7-9%, supporting our findings that moderate dilution levels are optimal for promoting early growth parameters. Previous studies further support the role of vermiwash in enhancing seedling growth. For instance, *Vigna radiata* seedlings treated with vermiwash exhibited abundant root hair development, an effect attributed to the indole-3-acetic acid (IAA) content in vermiwash, which stimulates root hair growth (18, 19, 20) also reported improved germination rates and enhanced seedling vigor in crops such as cowpea and paddy, with vermiwash proteases aiding germination and amylases providing readily accessible carbon sources to support plant growth and productivity (21). Additionally, Mujeera Fathima and Malathy Sekar (2014) showed that even low concentrations of vermiwash (10%) promoted growth in *Phaseolus vulgaris*, demonstrating effects that surpassed those of gibberellic acid (12). These studies collectively reinforce our meta-analysis results, highlighting vermiwash as a powerful bio-stimulant that enhances seed germination and early growth metrics across diverse crop types. In this study, we systematically evaluated the efficacy of vermiwash on key growth metrics across various plant species, dividing them into legumes and non-legumes to assess its impact on germination rate, germination percentage, shoot, and root lengths. Our meta-analysis revealed a positive effect size for both groups, indicating that vermiwash consistently promotes early growth across diverse plant types.

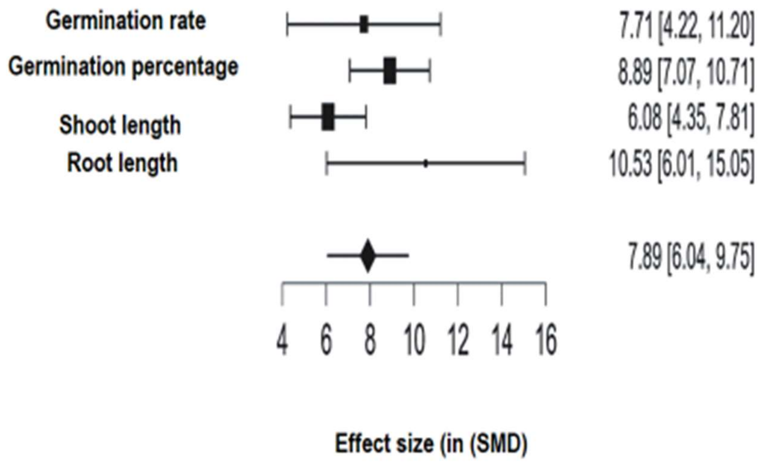

Figure 1: **Effect of Vermiwash Addition on Seedling Development Metrics with 95% Confidence Intervals:** The effect sizes (Standardized Mean Difference, SMD) for frequency and percentage of germination, as well as root and shoot lengths in seedlings treated with vermiwash. Each horizontal bar represents the 95% confidence interval for the respective effect size, indicating the span within which the true effect size is expected to lie. A confidence interval separate from the dashed line at zero suggests a statistically significant effect of vermiwash on that specific variable. The effect sizes quantify the increase in growth parameters following vermiwash addition. Analysis conducted using IASP software (0.19.1).

3.2 Cofactor analysis: Effect of Vermiwash Dilution on Seed Germination and Seedling Growth

In the present study, vermiwash demonstrated a positive influence on both germination and early seedling development across all tested dilutions. The optimal dilution range for enhancing germination was found to be between 50% and 100% of the growing medium volume, aligning with recommendations from various vermiwash producers who suggest this range to promote seed germination properties. Additionally, early seed development showed significant improvement at lower dilutions, specifically within the 1% to 25% range, indicating that less concentrated vermiwash applications can effectively enhance initial seedling growth. These findings underscore the versatility of vermiwash in supporting various stages of early plant development,with tailored dilution ranges to maximize specific growth parameters (22). At high Concentration (50-100%) the enhanced germination is due to the seeds likely to receive an optimal supply of nutrients and growth-enhancing hormones, such as cytokinins and gibberellins. Vermiwash contains various hormones, including cytokinins and auxins, as well as a mix of amino acids, vitamins, and a diverse range of enzymes such as proteolytic enzymes, amylolytic enzymes, urease, and phosphatase. Additionally, it includes other beneficial secretions and a rich community of useful microorganisms, including heterotrophic bacterial, fungal, and actinomycete populations, along with nitrogen-fixing bacteria such as azotobacter, agrobacterium, and rhizobium species.as well as phosphate-solubilizing microbes, also contribute to its bioactive profile (23) .Cytokinins and gibberellins are known to break seed dormancy and facilitate germination by promoting cell division and elongation in the embryonic tissues (24). Additionally, vermiwash is rich in enzymes like amylase, which aids in breaking down stored starches in the seed into simpler sugars, supplying the energy required for germination. This nutrient and hormone-rich environment accelerates the biochemical processes needed for quick and effective germination. Low Concentration (1-25%) of vermiwash may provide a milder nutrient environment that stimulates root and shoot growth without overwhelming the plant with high nutrient levels. Lower dilutions encourage the plant to actively develop its root system to access nutrients in the surrounding soil, enhancing root length and density. Auxins, present in vermiwash, are crucial for root development and elongation, especially at lower concentrations where they work more effectively without being countered by higher cytokinin levels. In addition, lower concentrations might reduce the risk of osmotic stress, allowing roots to absorb water efficiently, which further promotes root and shoot growth in young seedlings.

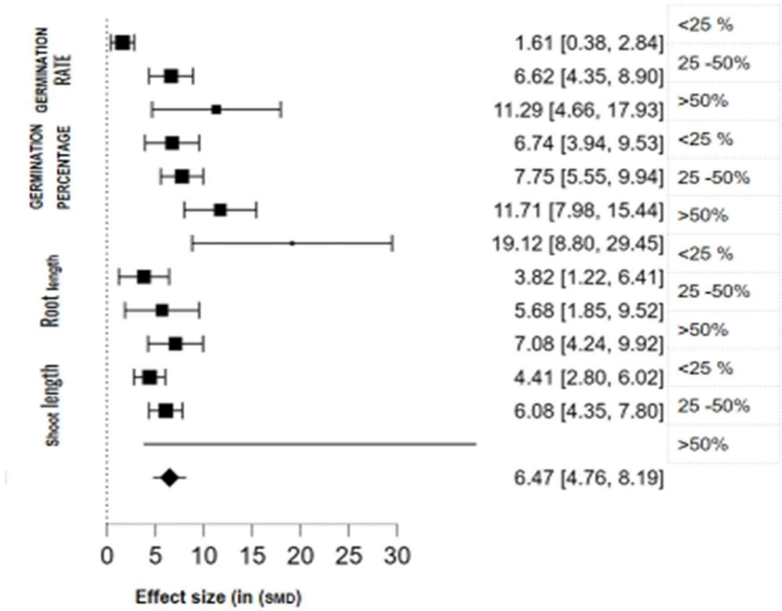

Figure 2: The influence of vermiwash dilution levels on four growth parameters: Germination rate, germination percentage, root length, and shoot length: Diamonds represent the standardized mean difference (SMD) effect sizes, with error bars indicating 95% confidence intervals. Confidence intervals that do not cross the dashed line at zero reflect statistically significant effects ($p < 0.05$).Higher dilutions of vermiwash (>50%) exhibit greater effects on germination rate and germination percentage, enhancing these parameters. Conversely, lower dilutions (<25%) show a more pronounced impact on shoot and root length, indicating that less concentrated vermiwash benefits early growth of these structural traits. Analysis conducted using IASP software (0.19.1)

3.3 Source-Based Nutrient Profile Variability in Vermiwash:

Our meta-analysis of NPK concentrations from various sources of vermiwash and organic fertilizers revealed substantial variability in nutrient profiles, highlighting the unique potential of vermiwash as a sustainable biofertilizer. Among the various vermiwash sources tested, the combination of leaf litter and cow dung emerged as the most nutrient-dense, offering 63 mg/dL of nitrogen, 67 mg/dL of phosphorus, and 72 mg/dL of potassium. This mixture closely aligns with the ideal 1:2:2 NPK ratio, which is particularly beneficial for seed germination, as the higher phosphorus content promotes enhanced root development and early plant growth. In comparison, other combinations such as cow dung mixed with gram bran, wheat bran, and rice bran also exhibited elevated nutrient levels but did not achieve the precise nutrient balance found in the leaf litter and cow dung mixture. For instance, cow dung alone showed moderate NPK concentrations (15.2 mg/dL nitrogen, 7 mg/dL phosphorus, and 4.8 mg/dL potassium), while mixtures with wheat bran and rice bran offered higher nitrogen and phosphorus but lacked the optimal balance. Additionally, cow dung mixed with vegetable waste and barley bran provided a balanced nutrient profile, though none matched the high phosphorus concentration of the leaf litter and cow dung mixture. Overall, leaf litter combined with cow dung stands out as the most effective vermiwash source, delivering superior nutrient profile that supports healthy seedling establishment and optimal early plant growth.

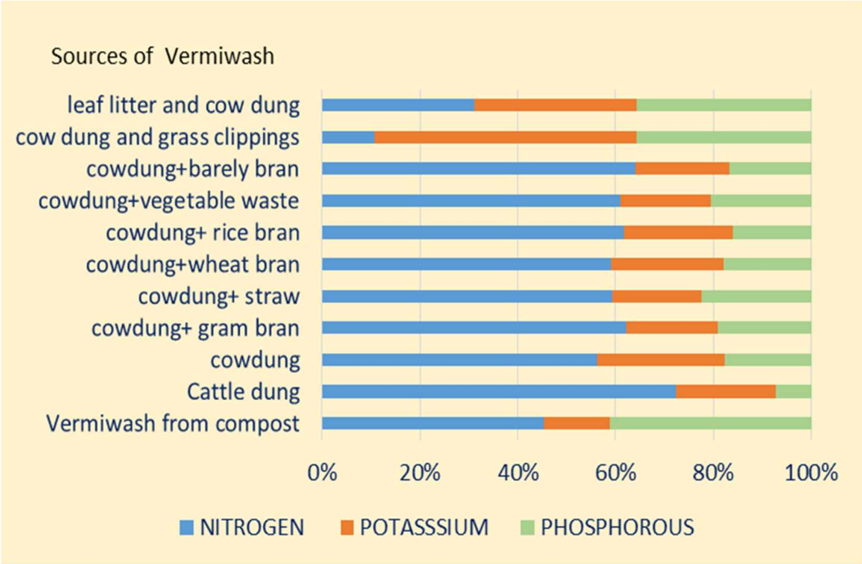

Figure 3: NPK Concentration across various Vermiwash Raw Material

3.3 Publication bias:

A meta-analysis may miss some research due to limitations in publication systems, database coverage, search engine algorithms, or keyword selection. If the missing studies have randomly distributed effect sizes, they should not influence the average effect size calculation. However, if missing studies have higher or lower effect sizes than the average, this would lead to bias (10). In this analysis, references were primarily retrieved from Google Scholar, potentially resulting in a sample that is not fully representative of all existing studies. Moreover, the available quantitative data generally involved small sample sizes, and research with larger effect sizes were more prone to yield significant results. This suggests a reporting bias favoring more effect sizes, increasing the risk of exaggerating the overall effect size. The high heterogeneity (T^2 and I^2) observed in shoot and root length effect sizes indicates substantial variation across studies, likely due to differences in experimental conditions or methodologies. Both the Rank Correlation and Egger's test revealed significant results, indicating funnel plot asymmetry and suggesting possible publication bias. This bias may stem from an overrepresentation of studies with large, significant effect sizes or selective reporting practices.

4. Conclusion:

This meta-analysis underscores the substantial benefits of vermiwash as a biofertilizer, demonstrating its significant positive effects on seed germination and early seedling growth. High effect sizes across germination rate, germination percentage, root length, and shoot length confirm vermiwash's ability to enhance early plant development. The findings suggest that optimal germination is achieved with vermiwash concentrations between 50% and 100%, while dilutions of 1% to 25% support robust seedling growth. Notably, vermiwash's NPK composition varies by source, making it essential for farmers aiming to enhance germination to select vermiwash derived from the combination of leaf litter and cow dung emerged as the most nutrient-dense, this blend typically offers a 1:2:1 NPK ratio ideal for promoting both germination and early seedling development.

Declaration. The authors declare no competing interest.

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