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Effect of Salinity (NaCl) Levels on Seed Germination and Seedling Vigor Traits of Mung bean (*Vigna radiata* L.)

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Abstract: This study evaluated the effects of four salinity treatments (T1–T4) on mung bean (*Vigna radiata* L.) seed germination and seedling vigor under controlled laboratory conditions. A completely randomized design (CRD) with three replicates per treatment was used; 50 seeds were placed in each Petri dish and incubated on filter paper moistened with: T1 distilled water (control), T2 5 g L^{-1} NaCl, T3 10 g L^{-1} NaCl, and T4 15 g L^{-1} NaCl. Germination percentage, germination energy (72 h), germination ability (168 h), shoot and root lengths, total seedling length, and Seedling Vigor Index (SVI = seedling length × germination %) were assessed, with mean separation by LSD at 5% and 1%. Results showed a consistent decline with increasing salinity (T1 > T2 > T3 > T4). Final germination remained very high in T1–T3 (100–98%) with no significant differences, but dropped sharply in T4 (63.33%), indicating a threshold effect at high salinity. Seedling growth traits were the most sensitive indicators, showing drastic reductions even when germination stayed high (notably in T3). SVI provided the clearest overall discrimination among treatments, highlighting that germination percentage alone may overestimate establishment potential under salinity stress.
Keywords: Mung bean, seed germination, seedling vigor, SVI, salinity, sodium chloride, seedling growth.

تأثير مستويات الملوحة (NaCl) على إنبات البذور وصفات قوة البادرات في فول الماش(.*Vigna radiata* L)

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المستخلص: هدفت هذه الدراسة إلى تقييم تأثير أربع معاملات ملوحة (T1–T4) باستخدام كلوريد الصوديوم NaClعلى إنبات بذورفول الماش (.*Vigna radiata* L) وقوة البادرات تحت ظروف معملية. نُفذت التجربة بتصميم عشوائي كامل (CRD)بثلاث مكررات لكل معاملة، حيث وُضعت 50 بذرة في أطباق بتري ورُطّبت أوراق الترشيح بمحاليل T1: ماء مقطر (شاهد)، T2 5جم /لتر، T3 10 جم /لتر، 15T4 جم /لتر. قيست نسبة الإنبات، طاقة الإنبات بعد 72 ساعة، قابلية الإنبات بعد 168 ساعة، أطوال الجذير والرويشة والطول الكلي، ثم حساب دليل قوة البادرة (SVI) .
أظهرت النتائج نمطًا واضحًا تنازليًا مع زيادة الملوحة (T1 > T2 > T3 > T4) حافظت T1–T3 على إنبات مرتفع (100–98%) دون فروق معنوية كبيرة، بينما انخفض الإنبات بشدة في (63.33%) T4 بفروق معنوية عالية. كانت صفات نمو البادرات الأكثر حساسية للملوحة إذ تراجع الطول الكلي ودليل القوة بشكل كبير حتى عندما بقي الإنبات مرتفعًا خصوصًا في T3 تؤكد النتائج أن الاعتماد على نسبة الإنبات وحدها قد يبالغ في تقدير القدرة على التأسيس تحت الإجهاد الملحي.
كلمات مفتاحية : فول الماش، إنبات البذور ، قوة البادرة، دليل القوة SVI ، الملوحة، كلوريد الصوديوم، نمو البادرات.

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek), also known as Mung bean / Green gram / Golden gram, is a short-duration grain legume of increasing nutritional and economic importance, widely cultivated in tropical and subtropical regions. Legumes in general represent a key component of sustainable agricultural production systems because of their high nutritional value and their role in improving soil fertility through symbiotic biological nitrogen fixation with rhizobia, which contributes to reducing the relative dependence on nitrogen fertilizers and improving the physical and chemical properties of the soil. In this context, Mung bean has further agronomic importance because its short field duration makes it suitable for inclusion in crop rotations and crop intensification programs, allowing farmers to exploit "seasonal windows" between seasons or between two main crops, thereby achieving more efficient use of land, water, and inputs (Nair *et al* **.,** 2019).

Despite these agronomic advantages, the successful cultivation and stable integration of mung bean within farming systems depend greatly on the efficiency of germination and field establishment. Germination and seedling emergence constitute a critical stage that determines plant population and uniformity of growth in the field, which directly affects crop competitiveness and later yield potential. Accordingly, any disturbance in germination speed, uniformity, or early seedling vigor may lead to variability in plant age and physiological status, reduced stand density, and potential yield losses. Mung bean, like many legume crops, faces challenges related to its sensitivity to environmental stresses during early growth stages. These include unfavorable sowing conditions such as moisture deficit, temperature extremes, poor seedbed preparation, and particularly soil salinity, which is among the most widespread and influential abiotic stresses in arid and semi-arid environments. Salinity disrupts water uptake and ionic balance in plant tissues, which may rapidly result in reduced germination percentage, delayed germination, and greater variation in germination time, in addition to weakened root and shoot growth at the seedling stage. In severe cases, these effects can cause substantial yield losses. Recent literature emphasizes that sustainable mung bean production requires prioritizing improved adaptation to stresses especially salinity within research and development agendas to support food security and provide high-value plant protein sources (Rahat *et al.*, 2023). The importance of this approach is reinforced by studies indicating that a rapid and practical screening for salinity response can be conducted during germination and early seedling growth stages to distinguish among genotypes/treatments in terms of tolerance or sensitivity (Podder *et al*., 2020). Accordingly, the research problem centers on the need to evaluate the effects of treatments (T1–T4) on mung bean establishment using an integrated set of germination, early growth, and vigor indicators, in order to identify the most effective treatment physiologically and statistically.Accordingly, the research aims to evaluate the effects of (T1-T4) treatments on mung bean germination using an integrated set of germination, early growth and viability indicators, in order to identify the most physiologically effective treatment, in line with calls to develop more salt-tolerant strategies and practices in arid and semi-arid regions.

MATERIALS AND METHODS

Seed material and laboratory supplies: Mungbean (*Vigna radiata* L.) seeds were used for all tests. The experiment was carried out under controlled laboratory conditions using standard germination supplies including Petri dishes, filter paper, a ruler (or measuring tape) for seedling-length measurements, a sensitive balance for dry-weight determination, distilled water, sodium chloride (NaCl) for preparing salinity treatments, and a 5% sodium hypochlorite (NaOCl) solution for surface sterilization.

Seed pre-treatment and surface sterilization: Prior to sowing, seeds were soaked in distilled water for 24 h to ensure uniform hydration. After soaking, seeds were surface-sterilized by immersion in 5% sodium hypochlorite for 2 min, then thoroughly rinsed several times with distilled water to remove residual disinfectant. Sterilized seeds were then transferred aseptically to Petri dishes lined with filter paper for germination assessment.

Preparation of salinity treatments: Four irrigation (moistening) treatments representing different salinity levels were prepared using NaCl solutions:T1: distilled water (control) / T2: 5 g L^{-1} / T3: 10 g L^{-1} /T4: 15 g L^{-1} Filter papers in each Petri dish were moistened with the corresponding solution for each treatment. During incubation, moisture was maintained by adding the assigned solution whenever necessary to prevent desiccation and to ensure consistent exposure of seeds to the intended salinity level.

Experimental setup and design: For each replicate, 50 seeds were placed evenly in a Petri dish (100mm x15mm). The experiment was arranged in a completely randomized design (CRD) with three replicates per treatment. Petri dishes were monitored throughout the test period, and germination and seedling growth were evaluated according to the defined observation times and traits described below.

Measured traits and calculations: Germination percentage (%) was determined using the formula: **Germination (%) =** (number of germinated seeds / total number of seeds) × 100 (Copeland& McDonald 2001). Germination energy was assessed as the percentage of seeds germinated after 72 h, while germination capacity (ability) was determined as the percentage of seeds germinated after 168 h (Bam *et al.*, 2006). Seedling growth parameters were measured at the final observation (14 days), including Shoot length/cm , Root length /cm, and total seedling length/cm, where total seedling length was calculated as Shoot length + Root length. For dry-weight determination, after recording shoot and root lengths at 14 days, seedlings from each Petri dish were oven-dried at 60°C until a constant weight was achieved, and then weighed using a sensitive balance. Seedling Vigor Index (SVI) was calculated according to:
SVI = (mean shoot length + mean root length) × germination (%) (Abdul-Baki & Anderson, 1973; Sagar *et al.*, 2019).

Statistical analysis: Data were subjected to analysis of variance (ANOVA) appropriate for a completely randomized design. Treatment means were compared using the least significant difference (LSD) test at 5% and 1% probability levels, based on the same error degrees of freedom and residual mean square obtained from the ANOVA.

Results and Discussion

The treatment means (Table 1) demonstrate a strong and coherent treatment effect on germination, early seedling growth, and integrated vigor. Across almost all traits, a consistent ranking is observed (T1 > T2 > T3 > T4), indicating a progressive reduction in the physiological capacity of seeds to germinate efficiently and establish vigorous seedlings as treatment severity increased. This general interpretation is consistent with the seed-science view that seed vigor is a complex trait governing rapid, uniform, and robust seedling establishment, particularly under suboptimal environments (Finch-Savage, & Bassel, 2016). A key feature of the dataset is that treatment effects were expressed more strongly in post-germination growth traits (shoot/root elongation and vigor index) than in final germination percentage under mild to moderate conditions. Such a pattern is frequently emphasized in the literature: seeds may retain high “maximum” germination under near-ideal conditions, while vigor-related outcomes (rate, uniformity, and seedling robustness) decline substantially, thereby lowering establishment potential (Finch-Savage, & Bassel, 2016).

Final germination percentage: stability under T1–T3 and a significant collapse under T4 Final germination remained (Table 1) very high in the first three treatments (T1 = 100%, T2 = 100%, T3 = 98%). Statistically, T1/T2 and T3 do not differ significantly because the largest difference (2%) is below LSD (5%) = 6.611. In contrast, T4 (63.33%) was sharply reduced, and the difference between T4 and the other treatments (e.g., 36.67 percentage points relative to 100%) far exceeds LSD (1%) = 9.62, confirming a highly significant reduction in germination success. Biologically, this indicates that while most seeds could still complete germination in T1–T3, T4 impaired completion of germination for a substantial fraction of seeds. This type of response is commonly reported for mungbean exposed to increasing NaCl levels: moderate salinity may reduce performance slightly or inconsistently, whereas higher levels typically cause a marked decline in germination percentage (Gothwal & Kumawat, 2020; Podder *et al.*, 2020). Mechanistically, severe salinity can reduce germination through (i) osmotic effects that limit water uptake and delay imbibition and (ii) ionic toxicity that disrupts metabolism and membrane integrity, often accompanied by oxidative stress. These processes are widely discussed in salt-stress studies on mung bean during germination and early seedling stages (Islam *et al.*, 2025).

Early germination dynamics: Germination Energy (GE) and Germination Ability (GA): Although final germination did not significantly separate T1–T3, the early germination indices help clarify whether treatments altered the speed/early completion of germination.GE values were clustered among T1 (50.00), T2 (48.67), and T3 (48.00). The largest difference among these (2.00) is below LSD (5%) = 2.431, indicating no significant differences in GE among T1–T3. However, T4 (21.33) was dramatically reduced; differences between T4 and T1–T3 (e.g., 28.67 compared with T1) exceed LSD (1%) = 3.537, confirming a highly significant decline in early germination vigor.
GA showed a similar structure: T1 (50.00), T2 (50.00), and T3 (49.00) are statistically comparable (differences far below LSD (5%) = 3.306), while T4 (31.67) is significantly lower than the others (e.g., 18.33 compared with T1 > LSD (1%) = 4.81). Together, GE and GA show that mild–moderate conditions did not measurably reduce early germination performance relative to LSD thresholds, but the most severe condition strongly reduced both early vigor and final capacity. Using fixed-time measures for early germination (e.g., 72 h and 168 h) is consistent with published approaches for describing early germination dynamics in seed quality/vigor studies (Podder *et al.*, 2020). From an applied perspective, this separation is important: even when final germination remains high (as in T3), early germination dynamics can still be compromised under stress, affecting uniformity and establishment. The literature on seed vigor and establishment emphasizes that vigor-related traits better reflect performance across a wider range of environments than germination percentage alone (Finch-Savage, & Bassel, 2016).

Seedling growth traits: the strongest and most sensitive treatment discriminato seedling growth traits showed very strong treatment sensitivity, strongly differentiating treatments even when germination remained high. Total seedling length (Table 1) fell sharply from 27.650 cm (T1) to 6.917 cm (T2) to 1.248 cm (T3) to 0.350 cm (T4). Differences between T1 and the remaining treatments are far larger than LSD (1%) = 1.882, confirming highly significant reductions in elongation as treatment severity increased. Differences between T2 and T3/T4 also exceed LSD (1%), demonstrating that growth impairment is substantial even when germination is still high. Notably, T3 vs T4 for total seedling length (difference = 0.898) was not significant because it is below LSD (5%) = 1.294, meaning that both treatments produced similarly restricted total elongation despite different germination percentages. This is consistent with the broader conclusion that post-germination growth can be more strongly constrained than germination completion, particularly under salt stress. Mung bean salinity studies commonly report reductions in seedling length and vigor index with increasing NaCl concentration (Gothwal & Kumawat, 2020; Podder *et al.*, 2020). Root length decreased from 6.450 cm (T1) to 4.300 cm (T2) and then collapsed to 0.317 cm (T3) and 0.283 cm (T4). T1 significantly exceeded T2 (difference = 2.150 > LSD(1%) = 1.441) and greatly exceeded T3/T4. Meanwhile, T3 and T4 were statistically indistinguishable (difference = 0.034 < LSD(5%) = 0.99), implying near-complete inhibition of root elongation in both treatments. Roots are often reported to be highly sensitive to salinity because they directly contact the saline medium, and reductions in root length under increasing NaCl are repeatedly documented in mungbean screening studies (Kamrul *et al.*, 2018;Benlioğlu & Özkan 2020;Gothwal & Kumawat, 2020). Shoot length (Table 1) showed extremely large contrasts: 20.100 cm (T1), 2.617 cm (T2), 0.955 cm (T3), and 0.067 cm (T4). T1 exceeded all other treatments by differences far beyond LSD (1%) = 1.775, indicating a highly significant treatment effect on shoot growth. The T2 vs T3 contrast (difference = 1.662) exceeded LSD(5%) = 1.22 but not LSD(1%), indicating significance at 5% but not at 1%.This pattern aligns with published evidence that salinity suppresses shoot elongation and biomass accumulation in mungbean seedlings, often in a dose-dependent manner (Gothwal & Kumawat, 2020; Podder *et al.*, 2020).

Seedling vigor index (SVI / seedling index): the most integrative indicator: The seedling index (vigor index) provided the clearest overall separation among treatments because it integrates germination with seedling growth into a single measure. The use of vigor indices has a strong basis in seed science, with vigor evaluation and the use of multiple criteria highlighted in classical work **(Bam *et al.*, 2006).** Moreover, seed-testing guidance explicitly states that SVI is calculated by multiplying germination (%) by seedling length and cites Abdul-Baki & Anderson (1973) as a foundational reference (Government of India 2008)Mungbean salinity studies also commonly compute SVI as (mean shoot length + mean root length) × germination percentage, as cited in published work (Podder *et al.*, 2020). In the present results, seedling index declined from 2655.00 (T1) to 691.67 (T2) to 124.59 (T3) to 22.27 (T4). Most contrasts exceeded LSD(1%) = 108.554, confirming highly significant treatment effects on integrated vigor. The T3 vs T4 contrast (102.32) was significant at 5% (> 74.604) but not at 1% (< 108.554). The key applied interpretation is that T3 maintained very high germination (98%) yet had a very low vigor index, because seedling growth was severely inhibited. This supports a central seed biology principle: final germination percentage can overestimate establishment potential, whereas vigor metrics better capture the ability to produce robust seedlings across stressful or variable environments (Finch-Savage, & Bassel, 2016).

Linking the statistical pattern to salt-stress biology: The combined statistical outcomes indicate two distinct “impact layers” of treatment severity: Mild–moderate conditions (T1–T3): final germination remained high, and GE/GA did not separate strongly, but seedling growth declined drastically, especially in T3. This suggests that post-germination processes (cell expansion, tissue differentiation, and sustained growth) were more sensitive than the initial completion of germination under those conditions. Severe condition (T4): both germination completion and seedling growth collapsed, indicating that salinity reached a threshold where seeds could neither germinate efficiently nor sustain early growth.Mungbean salt-stress papers describe similar trends and discuss the role of osmotic restriction, ion toxicity, and associated physiological disturbances during germination and early seedling development (Promila & Kumar 2000; Islam *et al.*, 2025 ;Viswanathan & Narayanan 2025).

Table:(1). Treatment Means and LSD-Based Interpretation of Germination and Seedling Vigor Traits

Treatment	Germination (%)	Germination Energy	Germination Ability	Seedling Length (cm)	Root Length (cm)	Shoot Length (cm)	Seedling Index

T1	100.00	50.00	50.00	27.650	6.450	20.100	2655.00
T2	100.00	48.67	50.00	6.917	4.300	2.617	691.67
T3	98.00	48.00	49.00	1.248	0.317	0.955	124.59
T4	63.33	21.33	31.67	0.350	0.283	0.067	22.27
LSD (5%)	6.611	2.431	3.306	1.294	0.99	1.22	74.604
LSD (1%)	9.62	3.537	4.81	1.882	1.441	1.775	108.554

Note: LSD (1%) was calculated using the same error d. f. (= 8), number of replicates (= 3), and residual mean square from the ANOVA output.

Conclusions: From a germination perspective, the results indicate that treatment 1 (T1) was the most suitable, achieving the highest growth rates and viability index. Treatment 2 (T2) maintained complete germination but exhibited a significant decrease in growth and viability compared to treatment 1 (T1), suggesting that even mild stress can impair germination potential. Treatment 3 (T3) maintained near-complete germination but produced very weak seedlings, indicating a high risk of poor germination despite a high germination rate. Treatment 4 (T4) caused the most damage, significantly reducing germination and leading to a collapse in seedling viability.This arrangement is consistent with studies examining the effects salinity of mung beans, which indicate progressive decreases in germination, particularly in seedling growth and viability index, with increasing sodium chloride levels (Gothwal & Kumawat, 2020; Podder *et al*., 2020).

Recommendations for future work: Because this study was conducted under controlled laboratory conditions, extrapolation to field performance should be made cautiously; field environments introduce additional factors (soil structure, fluctuating temperature, microbial activity, and heterogeneous salinity). Several mungbean salinity studies explicitly recommend field validation to confirm laboratory findings (Podder *et al.*, 2020). Future work could (i) quantify emergence and early biomass in soil-based assays, (ii) include physiological/biochemical markers (e.g., ion balance, membrane stability, oxidative stress markers), and (iii) test genotypic variation in response to the same salinity treatments, as done in recent mungbean salt-tolerance studies(Islam *et al.,* 2025).

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