



Extreme Acid Stress in a Red Mung Bean (*Vigna radiata*) Microecosystem: An Ecotoxicological Perspective on Plant Failure and Fungal Emergence at pH 2

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Abstract

Environmental pH is a fundamental physico-chemical factor influencing organismal performance, nutrient availability, chemical behaviour and biological interactions. Although extreme pH conditions are expected to impair seed germination and plant development, different biological components may respond differently to the same environmental stress. This study evaluated the responses of red mung bean (*Vigna radiata*) across a broad aqueous pH gradient, with particular attention to the unexpected emergence of visible fungal growth under extreme acidity. Seeds were exposed for seven days in Petri dish microcosms at pH 2, 3, 4, 5.5, 7, 8.5, 9.5, 10.5 and 12, with three replicates per treatment. Germination, seedling height, leaf development, general biological condition and cumulative biomass were assessed. Responses across the pH gradient were nonmonotonic, with mean germination ranging from 13.33% at pH 12 to 50.00% at pH 5.5. Mean germination at pH 2 was 16.67%, while cumulative plant biomass declined to zero. In contrast, pH 12 produced the lowest mean germination but retained measurable biomass, indicating contrasting biological responses at the two pH extremes. Most notably, conspicuous fungal colonization repeatedly developed in the pH 2 microcosms after approximately six days and became more extensive by Day 7, whereas comparable fungal growth was not recorded under the other pH treatments. The findings do not demonstrate that extreme acidity directly stimulated fungal growth. Instead, they support a testable ecotoxicological hypothesis whereby severe acid stress impaired seed viability and plant establishment, potentially generating deteriorating organic substrates that could subsequently be exploited by acid tolerant or opportunistic fungi. The study illustrates how an extreme environmental stressor may simultaneously suppress one biological component while permitting another to become conspicuous, emphasizing the importance of considering indirect biological responses and potential microecosystem shifts alongside conventional phytotoxicity endpoints.

Keywords: Acid Stress; Ecotoxicology; Fungal Colonization; Microecosystem; pH Stress; Red Mung Bean; *Vigna radiata*

Introduction

Environmental stress does not necessarily affect all organisms uniformly or in the same biological direction. This principle is central to ecotoxicology because chemical and physico-chemical stressors may suppress sensitive organisms while allowing more tolerant biological groups to persist or become relatively more successful, potentially altering ecological interactions, resource availability and ecosystem processes. Environmental pH is particularly important in this context because it influences cellular physiology, enzyme activity, membrane function, nutrient availability and the chemical speciation and bioavailability of environmental contaminants. Plants are especially vulnerable during germination and early seedling establishment, when substantial departures from favourable pH conditions can interfere with normal physiological development. Experimental evidence in tomato has shown that both acidic and alkaline pH stress can impair germination and seedling growth through disruption of reactive oxygen species homeostasis, enhanced membrane lipid peroxidation and altered hormonal regulation [1]. Thus, extreme pH represents more than a constraint on visible plant growth; it can initiate cellular and biochemical disturbances with consequences for subsequent establishment, biomass production and survival.

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Seed germination provides a sensitive biological system for examining such physico-chemical stress because it represents the transition from a relatively quiescent seed to an actively developing organism. Successful germination requires coordinated water uptake, mobilization of stored reserves, enzyme activation, membrane reorganization and cellular expansion. Disruption of these processes can manifest as reduced germination, abnormal root or shoot development and decreased biomass. Zhao *et al.*, [2] demonstrated that increasing acid stress reduced germination and seedling performance in *Neyraudia reynaudiana* while progressively damaging plasma membranes and subcellular structures. Membrane deterioration is particularly important from an ecotoxicological perspective because loss of cellular integrity can impair normal metabolism while allowing soluble cellular constituents to enter the surrounding environment [2]. Moreover, biological responses cannot necessarily be attributed to nominal pH alone. Vallabhaneni *et al.*, [3] demonstrated that different acids adjusted to comparable pH values produced contrasting effects on germination, biomass, leaf development and root and shoot growth in *Brassica juncea* and *Raphanus sativus*. Environmental acidity should therefore be regarded as a physico-chemical exposure whose biological effects can reflect both hydrogen ion concentration and the chemical characteristics of the acidifying environment.

The consequences of extreme pH may extend beyond the focal plant because different biological groups possess markedly different tolerance ranges. Fungi are especially relevant because numerous fungal taxa can tolerate conditions that severely constrain higher plants. Wheeler *et al.*, [4] examined 61 isolates of *Aspergillus*, *Penicillium* and *Fusarium* and found that nearly all tested species were capable of growth across a broad experimental range extending approximately from pH 2 to 11, although their growth optima and responses differed considerably. Physiological adaptation to strongly acidic environments has also been demonstrated experimentally in *Aspergillus niger* [5]. Such differences in tolerance have important ecological implications because severe plant injury may simultaneously generate deteriorating organic material that can be exploited by microorganisms. Consequently, extreme environmental stress may cause not simply a reduction in biological activity but a shift in its nature, from plant germination and growth towards tissue deterioration, microbial colonization and decomposition when conditions exceed plant tolerance but remain compatible with particular microorganisms.

Red mung bean (*Vigna radiata* (L.) R. Wilczek) provides a useful experimental model for investigating these responses because of its rapid germination and readily observable early seedling development. Previous work using *V. radiata* microecosystems demonstrated substantial developmental impairment under strongly alkaline conditions, particularly towards pH 10 to 12, and interpreted these responses within an ecotoxicological framework [6]. The present study extended this approach across a broader gradient encompassing extreme acidity, moderate acidity, neutral conditions and extreme alkalinity. Seeds were exposed in Petri dish microcosms to nominal pH values of 2, 3, 4, 5.5, 7, 8.5, 9.5, 10.5 and 12, and their germination, seedling height, leaf development, general biological condition and cumulative biomass were evaluated during a seven-day exposure period. Although variation among the conventional growth endpoints prevented a simple pH dependent interpretation, an unexpected and repeatedly observed phenomenon emerged under extreme acidity: conspicuous fungal colonization developed

specifically in the pH 2 microcosms after approximately six days and became more extensive by Day 7. This was particularly notable because pH 2 was also associated with severe plant impairment and zero cumulative plant biomass, whereas pH 12 strongly inhibited germination without comparable visible fungal proliferation.

Accordingly, the present study aimed to evaluate germination, early seedling development and biomass responses of *V. radiata* across a broad experimental pH gradient, with particular emphasis on interpreting the unexpected fungal emergence at pH 2 from an ecotoxicological perspective. Rather than assuming that extreme acidity directly stimulated fungal growth, the study considers the working hypothesis that severe acid stress damaged seed tissues, impaired normal plant establishment and potentially increased the availability of deteriorating organic substrates for colonization by acid tolerant or opportunistic fungi. The Petri dish is therefore considered not merely as a plant toxicity test system but as a simple experimental microecosystem in which different biological components may respond differently to the same physico-chemical stressor. This perspective broadens conventional phytotoxicity assessment by asking not only how extreme pH suppresses a sensitive organism, but also whether such suppression can create conditions under which another biological component becomes increasingly conspicuous.

Materials and Methods

Experimental organism and seed preparation

Red mung bean (*V. radiata*) seeds were used as the test organism for evaluating biological responses to a broad range of acidic, neutral and alkaline aqueous conditions. Seeds were selected from the same batch to minimize variation associated with seed origin and initial physiological condition. At the beginning of the experiment, the seeds were recorded as uniform and apparently healthy across all treatment groups. The use of seeds from a common batch was intended to reduce biological variability so that differences observed during the exposure period could be related primarily to the imposed pH treatments rather than initial differences among seed lots.

Seeds and early seedlings are particularly suitable biological materials for assessing environmental stress because germination and early establishment are sensitive developmental stages. Acid stress can alter germination performance, seedling growth, biomass production and cellular integrity, making these endpoints useful indicators of phytotoxicity [2]. Zhao *et al.*, [2], for example, evaluated germination, biomass and membrane integrity across an experimental acidity gradient and demonstrated pronounced impairment at sufficiently low pH.

The seeds were arranged in Petri dish microcosms containing a moist supporting substrate. Each Petri dish represented an independent experimental unit. Nine pH treatments were established, comprising pH 2, 3, 4, 5.5, 7, 8.5, 9.5, 10.5 and 12. Three replicate Petri dishes were prepared for each pH treatment, giving a total of 27 experimental units. The original experimental record documented that the initial seeds in all treatments were uniform and originated from the same batch.

Experimental pH gradient

The experimental treatments were selected to encompass a broad physico-chemical gradient extending from extreme acidity through neutral conditions to extreme alkalinity. The nine nominal treatments comprised pH 2, 3, 4, 5.5, 7, 8.5, 9.5, 10.5 and 12. This design enabled comparison of severe acid stress, moderate acidity, approximately

neutral conditions and progressively stronger alkaline stress within a common experimental system.

The wide pH gradient was considered ecotoxicologically relevant because hydrogen ion concentration can strongly affect germination and early plant development. Experimental studies have demonstrated that increasingly acidic conditions can reduce germination, root and shoot elongation and biomass while damaging plasma membrane and subcellular integrity [2]. Plant response is also not determined solely by nominal pH. Vallabhaneni *et al.*, [3] demonstrated that different acids adjusted to similar pH values produced contrasting effects on seed germination, biomass, leaf area and root and shoot development, indicating that acid identity can modify biological responses independently of hydrogen ion concentration alone.

Aqueous treatment solutions corresponding to the respective nominal pH values were used to maintain moisture within the Petri dish microcosms and expose the seeds during germination and early seedling development. Moisture condition was included among the routine observations because water availability influences seed imbibition, germination and subsequent development. The original laboratory records initially described moisture conditions as high across the treatments.

The chemical identities and concentrations of the acidifying and alkalinizing reagents were not documented in the available experimental record and therefore cannot be specified retrospectively. Likewise, continuous verification of treatment pH throughout the seven-day experiment was not documented. The stated pH values should therefore be regarded as nominal treatment values rather than continuously verified rhizosphere or seed surface pH. This distinction is relevant because plant responses to acidic exposure may depend upon both pH and the chemical nature of the acid used to achieve it [3].

Exposure period and biological observations

The Petri dish microcosms were maintained under laboratory conditions for seven days. During the exposure period, each experimental unit was inspected repeatedly for seed germination, seedling development and visible changes in biological condition. The principal quantitative endpoints were the number of germinated seeds, germination percentage, seedling height and number of leaves. Qualitative observations included general seed and seedling condition, moisture condition and visible abnormalities.

Germination percentage was determined from the proportion of seeds that successfully germinated within each replicate. Seedling height was recorded in centimetres, and leaf number was used as an additional indicator of early vegetative development. The use of multiple endpoints was intended to capture different stages of biological response because germination success does not necessarily correspond directly with subsequent seedling growth or biomass accumulation. Experimental evidence shows that acid stress may affect germination, root development, shoot development and total biomass to different degrees, demonstrating the importance of considering several developmental endpoints simultaneously [3].

The final replicate level dataset contained germination percentage, seedling height, leaf number and qualitative plant condition for all treatments. The pH 2 replicates displayed only limited germination and were recorded as showing fungal infection, whereas seedlings developed to varying degrees in the remaining treatments. These observations were subsequently summarized descriptively for

comparison across the complete pH gradient.

Photographic documentation and qualitative assessment

Photographic documentation was undertaken during the experiment to provide a visual record of germination, seedling morphology, deterioration and other biological changes within the Petri dish microcosms. The photographs complemented the quantitative endpoints by documenting treatment specific responses that were difficult to represent through germination percentage or seedling height alone.

The experimental record described fungal growth and seed deterioration at pH 2, whereas the pH 3 treatment showed moderate germination with limited seedling development. Better development was observed under less acidic conditions, and the report described pH 5.5 as supporting healthier germination and pH 7 as producing the healthiest and tallest seedlings. At progressively stronger alkaline conditions, reductions in seedling vigour were recorded, with pH 12 characterized by very poor germination and stunted development.

Photographic observations were treated as qualitative evidence. No automated image analysis was undertaken to quantify leaf area, lesion development or fungal surface coverage. Consequently, visual descriptors were retained as observational endpoints rather than converted retrospectively into quantitative measurements.

Observation of fungal colonization

Visible fungal growth emerged as an unexpected secondary biological endpoint during the experiment. Fungal colonization was recorded in the pH 2 treatment, where all three replicate dishes were described as showing fungal infection. In the final replicate observations, the three pH 2 dishes recorded germination percentages of 10%, 20% and 20%, respectively, and fungal infection was noted in each replicate.

The fungal growth became increasingly conspicuous following several days of exposure and was especially evident during the later stages of the experiment. Close up photographic documentation was undertaken on Day 7, and the laboratory record specifically identified visible fungal material growing within the Petri dish containing red mung bean exposed to pH 2.

Fungal occurrence was recorded because fungi can exhibit substantially different pH tolerances from higher plants. Wheeler *et al.* (1991) evaluated 61 isolates belonging to *Aspergillus*, *Penicillium* and *Fusarium* and found that nearly all of the examined species were capable of growth across a laboratory pH gradient of 2 to 11, although growth responses differed among taxa. Similarly, Park *et al.* (1996) demonstrated physiological adaptation of *Aspergillus niger* to strongly acidic environments, although direct exposure to pH 2 could inhibit unadapted hyphae. These studies provided the rationale for treating visible fungal development as an ecologically relevant secondary response rather than assuming that extreme acidity would necessarily inhibit all microbial growth.

No fungal isolation, culture-based identification, microscopy based taxonomic identification or molecular characterization was conducted. The observed organism is therefore referred to conservatively as visible fungal growth or fungal colonization. No claim is made that the fungus belonged to *Aspergillus*, *Penicillium*, *Fusarium* or any other particular taxon. Likewise, fungal biomass and colony surface coverage were not quantified. The observation was therefore treated as a qualitative and hypothesis generating endpoint.

Determination of cumulative plant biomass

Cumulative plant biomass was measured on Day 7 as an integrative endpoint representing the overall outcome of germination and subsequent seedling growth. Biomass is frequently used in seedling stress experiments because it integrates several underlying processes, including seed establishment, shoot and root development and cumulative resource allocation. Acid stress has previously been demonstrated to reduce seedling biomass alongside changes in germination and cellular integrity [2], while biomass responses may also vary according to both treatment pH and acid identity [3].

At the completion of the experiment, cumulative biomass was recorded for each pH treatment. The original dataset reported values of 0 at pH 2, 0.1853 at pH 3, 0.225 at pH 4, 0.274 at pH 5.5, 0.210 at pH 7, 0.240 at pH 8.5, 0.266 at pH 9.5, 0.250 at pH 10.5 and 0.170 at pH 12.

The available laboratory record does not specify whether biomass represented fresh mass or dry mass, and no measurement unit was provided. Accordingly, neither a unit nor a fresh or dry biomass designation was assigned retrospectively. Because treatment level cumulative values rather than replicate specific biomass measurements were available, the biomass data were evaluated descriptively and were not subjected to inferential statistical testing.

Experimental variability and quality considerations

Several uncontrolled environmental and operational factors occurred during the experiment and were considered in interpreting the resulting dataset. Rapid evaporation was observed in the Petri dish system, causing treatment water to become depleted faster than anticipated. This could have produced differences in water availability among experimental units and may also have altered the effective concentration of dissolved constituents as exposure progressed. Since germination and early seedling development depend strongly upon water availability, such variability may have contributed to differences among replicates.

Interference by ants was also observed during the experimental period. This biological disturbance could potentially have displaced seeds, disturbed the supporting substrate or otherwise altered conditions within individual experimental units. Ant interference was not part of the original experimental design and could not therefore be quantified as an independent factor.

Variation in the actual pH experienced by seeds represents an additional consideration. Seed tissues undergoing germination or deterioration can release biological compounds into their immediate surroundings, while evaporation can modify solution chemistry. Moreover, acid identity can significantly influence germination and seedling growth even where nominal pH is held approximately constant [3]. For these reasons, the experiment is interpreted as an exploratory Petri dish microcosm assessment rather than a definitive concentration response assay.

Results

The biological responses of *V. radiata* varied substantially across the experimental pH gradient (Table 1). At pH 2, germination was strongly inhibited, with only 1, 2 and 2 seeds germinating in replicates 1, 2 and 3, corresponding to germination percentages of 10%, 20% and 20%, respectively. Seedling development was also limited, with recorded heights of 0.90, 1.40 and 0.55 cm. Notably, all three pH 2 replicates were recorded as being infected by fungus. At pH 3,

germination increased to 40-60%, accompanied by greater seedling development. Responses remained variable among the intermediate treatments, although conspicuous shoot elongation occurred at pH 7, where seedling heights reached 29.80, 20.05 and 13.35 cm in the three replicates. Under increasingly alkaline conditions, seedling development generally became less vigorous. At pH 12, germination ranged from 0 to 30%, including complete failure of germination in replicate 2. These replicate-level observations demonstrate considerable biological variability across the pH treatments rather than a simple linear response to increasing or decreasing pH.

The treatment-level summary further demonstrated inhibition of germination toward both extremes of the pH gradient (Table 2). Mean germination was only 16.67% at pH 2 and reached its lowest value of 13.33% at pH 12. In contrast, the highest mean germination was recorded at pH 5.5 (50.00%), followed by pH 3 (46.67%) and pH 4 (40.00%). Mean germination at pH 7 and 8.5 was 30.00%, whereas values of 36.67% and 33.33% occurred at pH 9.5 and 10.5, respectively. Thus, germination did not follow a simple progressive response across the pH gradient. Instead, the two extreme treatments were characterized by particularly low germination. Importantly, although mean germination at pH 12 was slightly lower than at pH 2, fungal infection was specifically recorded in all three pH 2 replicates and not in the pH 12 treatment.

Cumulative biomass measured on Day 7 also varied among the treatments and did not display a simple monotonic relationship with pH (Table 3). No measurable plant biomass was recorded at pH 2. Biomass increased to 0.1853 at pH 3 and 0.225 at pH 4, reaching the highest recorded value of 0.274 at pH 5.5. Biomass values at pH 7, 8.5, 9.5 and 10.5 were 0.210, 0.240, 0.266 and 0.250, respectively, before declining to 0.170 at pH 12. The biomass data therefore did not demonstrate a straightforward dose dependent response across the entire pH gradient. Nevertheless, pH 2 was clearly distinctive because it was the only treatment for which the recorded cumulative plant biomass was zero.

The photographic and qualitative observations provided additional evidence of contrasting biological responses across the pH gradient (Table 4). At pH 2, conspicuous fungal growth occurred together with poor germination and deterioration of the seeds (Figures 1 and 2). At pH 3, moderate germination occurred but seedling development remained limited. Development progressively improved through the moderately acidic treatments, with pH 5.5 characterized by healthier germination and better seedling development. The pH 7 treatment produced the visually healthiest seedlings and particularly pronounced shoot elongation. At increasingly alkaline pH, seedling vigour declined. Reduced growth was observed at pH 9.5, only a few seedlings developed normally at pH 10.5, and pH 12 was characterized by very poor germination and stunted development.

The most distinctive response across the experiment was therefore not simply low germination at pH 2, because the mean germination percentage at pH 12 was slightly lower. Rather, pH 2 was characterized by the unusual combination of low mean germination (16.67%), zero cumulative plant biomass, fungal infection recorded in all three replicates, and conspicuous fungal colonization of the Petri dish microcosms. The fungal growth became visually extensive around the seeds and supporting substrate and was specifically documented during the final observations. In contrast, despite severe germination inhibition at pH 12, comparable fungal proliferation was not recorded. This contrasting response between the two extreme

Table 1: Germination and seedling responses of red mung bean (*Vigna radiata*) exposed to different pH treatments.

pH	Replicate	Germinated seeds (n)	Germination (%)	Seedling height (cm)	No. of leaves	Recorded general condition
2	1	1	10	0.9	0	Seeds infected by fungus
	2	2	20	1.4	2	Seeds infected by fungus
	3	2	20	0.55	0	Seeds infected by fungus
3	1	4	40	5.125	8	Not many seeds germinated
	2	4	40	7.775	4	Not many seeds germinated
	3	6	60	2.17	6	Not many seeds germinated
4	1	6	60	2.67	6	Not many seeds germinated
	2	3	30	4.4	0	Not many seeds germinated
	3	3	30	2.9	4	Not many seeds germinated
5.5	1	5	50	5.5	4	Not many seeds germinated
	2	6	60	3.73	2	Not many seeds germinated
	3	4	40	4.38	4	Not many seeds germinated
7	1	5	50	29.8	7	Seeds healthy but not all germinated
	2	2	20	20.05	4	Seeds healthy but not all germinated
	3	2	20	13.35	3	Seeds healthy but not all germinated
8.5	1	3	30	1.433	2	Only 1 or 2 seeds grew healthily
	2	4	40	5.025	4	Only 1 or 2 seeds grew healthily
	3	2	20	3.2	4	Only 1 or 2 seeds grew healthily
9.5	1	5	50	5.12	4	Only 1 or 2 seeds grew healthily
	2	2	20	12.7	4	Only 1 or 2 seeds grew healthily
	3	4	40	4.4	3	Only 1 or 2 seeds grew healthily
10.5	1	4	40	5.5	4	Only 1 or 2 seeds grew healthily
	2	4	40	3.1	2	Only 1 or 2 seeds grew healthily
	3	2	20	1.25	2	Only 1 or 2 seeds grew healthily
12	1	1	10	5.1	2	Only 1 or 2 seeds grew healthily
	2	0	0	0	0	Only 1 or 2 seeds grew healthily
	3	3	30	3.27	3	Only 1 or 2 seeds grew healthily

Table 2: Summary of germination responses of red mung bean (*Vigna radiata*) across different pH treatments.

pH	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	Mean germination (%)	Range (%)
2	10	20	20	16.67	10-20
3	40	40	60	46.67	40-60
4	60	30	30	40	30-60
5.5	50	60	40	50	40-60
7	50	20	20	30	20-50
8.5	30	40	20	30	20-40
9.5	50	20	40	36.67	20-50
10.5	40	40	20	33.33	20-40
12	10	0	30	13.33	0-30

Note: Mean germination percentages were calculated from the three replicate values.

pH treatments constitutes the principal qualitative finding of the experiment and provides the basis for subsequent ecotoxicological interpretation of severe acid stress, seed deterioration and fungal emergence.

Figure 1 shows extensive whitish fungal growth across the Petri dish substrate and surrounding the mung bean seeds, coinciding with severe seed deterioration, limited germination and poor seedling establishment, while Figure 2 presents close up

microscopic observations of material collected from the visibly colonized pH 2 microcosms, revealing filamentous material and rounded spore bearing or spore-like structures consistent with the macroscopic fungal colonization. Together, the observations provide complementary visual evidence of fungal presence under the extreme acidic treatment. However, because the fungal material was not isolated, cultured or subjected to taxonomic or molecular identification, the observed structures are conservatively described

Table 3: Cumulative biomass (g) of red mung bean (*Vigna radiata*) measured on Day 7 following exposure to different pH treatments.

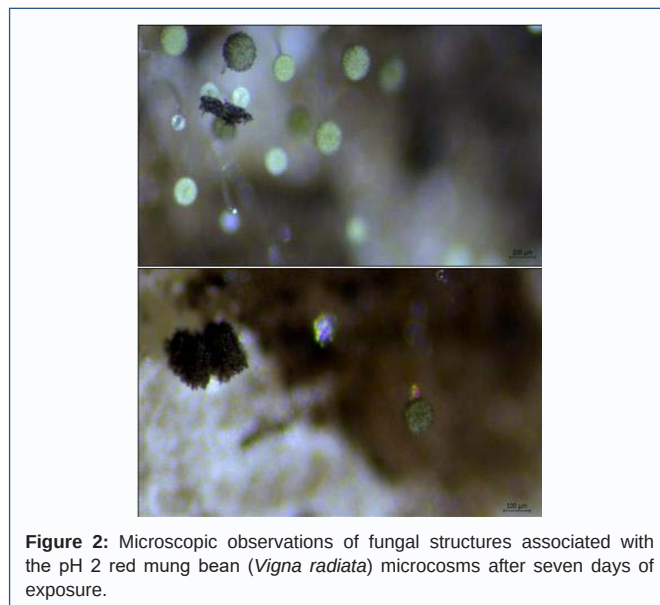
pH	Cumulative biomass
2	0
3	0.1853
4	0.225
5.5	0.274
7	0.21
8.5	0.24
9.5	0.266
10.5	0.25
12	0.17

as fungal structures without assignment to a particular genus or species, and the images alone do not demonstrate that pH 2 directly stimulated fungal growth.

Discussion

Contrasting phytotoxic responses across the pH gradient

The present findings demonstrate that red mung bean (*Vigna radiata*) responded substantially to both acidic and alkaline stress, although the responses did not follow a simple monotonic pattern across the complete pH gradient. Mean germination was highest at pH 5.5 (50.00%), followed by pH 3 (46.67%) and pH 4 (40.00%), whereas the lowest mean germination occurred at pH 12 (13.33%) and pH 2 (16.67%). These observations indicate that both extremes of the experimental pH range imposed severe constraints on seed germination. Nevertheless, individual biological endpoints responded differently. Particularly pronounced shoot elongation occurred at pH 7, where seedling heights reached 29.80, 20.05 and 13.35 cm among the three replicates, whereas the highest cumulative biomass was recorded at pH 5.5 rather than pH 7. Such differences illustrate an

**Figure 2:** Microscopic observations of fungal structures associated with the pH 2 red mung bean (*Vigna radiata*) microcosms after seven days of exposure.

important ecotoxicological principle: germination, shoot elongation and biomass represent different biological processes and need not respond identically to the same environmental stressor.

The strong suppression of mung bean development under highly acidic conditions is biologically plausible. A solution of pH 2 represents an extreme proton environment that is generally unsuitable for normal germination and early plant development. Acid stress can interfere with membrane integrity, metabolism and cellular organization. Zhao *et al.*, [2] demonstrated that acid stress caused substantial alterations to plasma membranes and subcellular structures during seed germination, while more recent evidence indicates that extreme acidic and alkaline conditions can disturb reactive oxygen species homeostasis, enhance lipid peroxidation

Table 4: Qualitative biological responses of red mung bean (*Vigna radiata*) across the experimental pH gradient.

pH	Observed germination and growth response	Fungal observation	Overall biological response
2	Very poor germination and severe seed deterioration	Conspicuous fungal growth	Severe phytotoxic response associated with fungal colonization
3	Moderate germination with limited seedling development	Not recorded	Strong acidic stress
4	Several seeds germinated with moderate seedling growth	Not recorded	Improved development relative to lower pH
5.5	Healthy germination and better seedling development	Not recorded	Relatively favourable growth
7	Tallest and visually healthiest seedlings	Not recorded	Favourable seedling development
8.5	Germination with moderate growth and reduced vigour	Not recorded	Mild alkaline stress
9.5	Reduced growth and lower seedling vigour	Not recorded	Increasing alkaline stress
10.5	Only a few seedlings developed normally	Not recorded	Strong alkaline stress
12	Very poor germination and stunted seedling growth	Not recorded	Severe alkaline stress

**Figure 1:** Visible fungal colonization of red mung bean (*Vigna radiata*) seeds following seven days of exposure to extreme acidic conditions at pH 2.

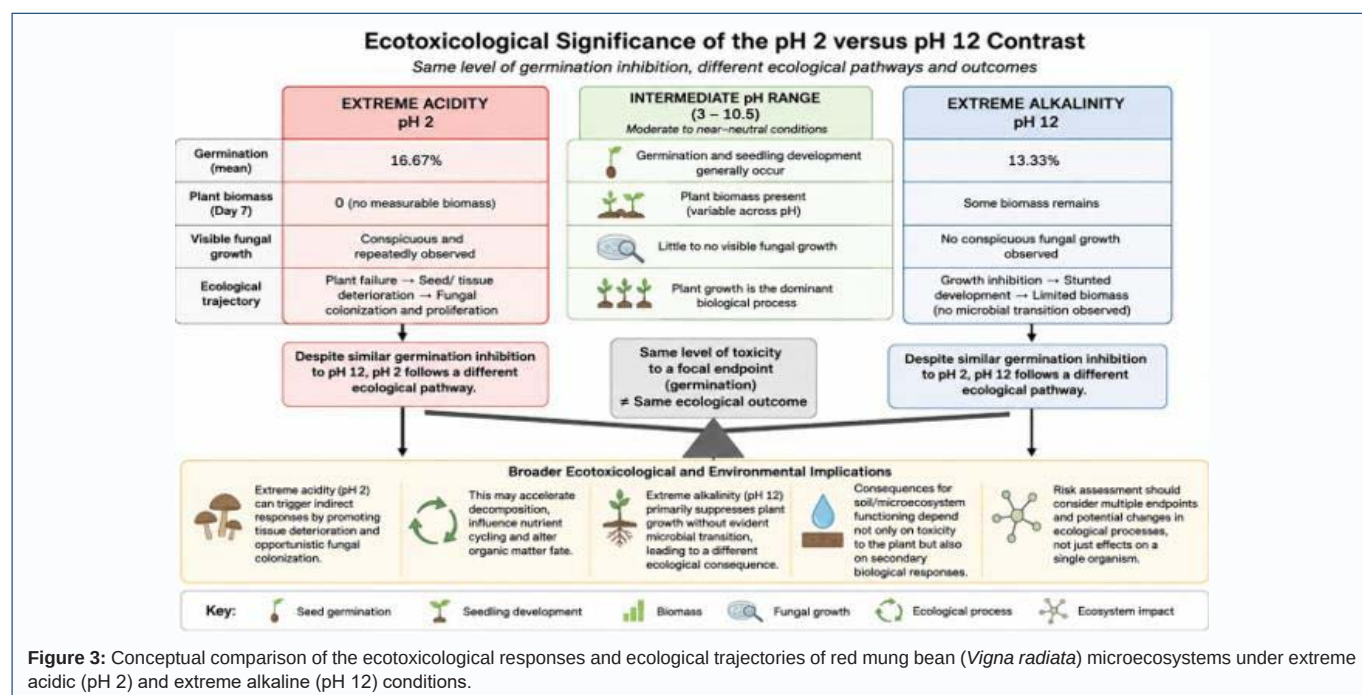


Figure 3: Conceptual comparison of the ecotoxicological responses and ecological trajectories of red mung bean (*Vigna radiata*) microecosystems under extreme acidic (pH 2) and extreme alkaline (pH 12) conditions.

and contribute to cellular damage during early plant development [1]. These mechanisms provide a physiological basis for the limited germination, short seedlings and eventual absence of measurable plant biomass observed at pH 2.

Importantly, however, the present data do not support describing pH 2 simply as the treatment producing the greatest germination inhibition. The mean germination percentage at pH 12 was slightly lower than that at pH 2. The biological significance of pH 2 instead arose from the combination of responses recorded at this treatment. Germination remained low, cumulative plant biomass declined to zero, and all three replicates were recorded as showing fungal infection. In contrast, pH 12 showed comparably severe germination inhibition and reduced biomass but no comparable fungal proliferation was recorded. This distinction between the two extreme pH treatments is central to interpreting the experiment from an ecotoxicological perspective.

Non-monotonic biomass responses and biological variability

Cumulative biomass did not decline progressively with increasing departure from neutral pH. Biomass ranged from 0.170 to 0.274 among treatments from pH 3 to pH 12, with the highest value occurring at pH 5.5, followed by pH 9.5 and pH 10.5. Consequently, the dataset does not demonstrate a conventional linear or monotonic pH response for biomass. Such variability is not unexpected in short-term biological experiments, particularly when several biological and microenvironmental processes operate simultaneously. Germination success, timing of emergence, elongation rate, seed reserve utilization and survival can each contribute differently to final biomass.

The absence of monotonicity should therefore not be interpreted as evidence that pH stress was biologically unimportant. Ecotoxicological responses commonly vary according to the endpoint examined, exposure duration and physiological state of the organism. In the present experiment, germination was severely inhibited at both pH extremes, while shoot elongation showed a different pattern

and biomass showed yet another. This endpoint-specific behaviour reinforces the need to avoid relying upon a single measurement when assessing environmental stress. The combined consideration of germination, seedling height, leaf development, biomass and visible biological condition provides a more informative representation of organismal response than any single endpoint alone.

Experimental factors probably contributed additional variability. Rapid evaporation, unequal depletion of treatment water and interference by ants were observed during the experimental period. These factors may have altered moisture availability and possibly the effective pH surrounding individual seeds. Because the Petri dish system contained relatively small volumes of treatment solution, evaporation and interactions between the solution, cotton substrate, seed coat and released biological compounds could also have altered the chemical microenvironment during exposure. Therefore, the intermediate biomass values should be interpreted cautiously. Nevertheless, the repeated occurrence of zero plant biomass and conspicuous fungal colonization at pH 2 remained biologically distinctive despite these sources of variation.

Why did conspicuous fungal growth occur at pH 2?

The most unexpected observation was the development of extensive fungal growth specifically in the pH 2 microcosms. At first sight, this appears paradoxical because the same highly acidic condition that severely inhibited mung bean development apparently permitted substantial fungal proliferation. However, environmental stress tolerance differs markedly among biological groups. Fungi are particularly important in this context because many species possess broad pH tolerance. Wheeler *et al.*, [4], examining isolates of *Aspergillus*, *Penicillium* and *Fusarium*, demonstrated that numerous fungal taxa could grow across remarkably wide pH ranges, including strongly acidic conditions. Acid adaptation has also been demonstrated experimentally in *Aspergillus niger*, where physiological adjustment enabled continued growth under low-pH environments [5]. Thus, severe acidity for a germinating seed does not necessarily

represent an equally severe environment for all fungi.

Nevertheless, the present observations should not be interpreted as demonstrating that pH 2 directly stimulated fungal growth. No fungal growth rate was quantified independently across treatments, and the fungal taxon or taxa were not isolated or identified. Furthermore, the nominal pH of the treatment solution does not necessarily represent the exact pH experienced by fungal hyphae around deteriorating seeds. Biological compounds released from injured tissues could have modified the immediate chemical environment. The most defensible interpretation is therefore that fungal organisms capable of tolerating or surviving the acidic microenvironment became conspicuous following extensive deterioration of the mung bean seeds.

The contrast with pH 12 strengthens this interpretation. Mean germination at pH 12 was actually lower than at pH 2, yet extensive fungal growth was not recorded under the strongly alkaline treatment. Thus, fungal emergence cannot be explained merely by poor germination or the presence of nonviable seeds. Rather, the acidic environment may have created a distinctive combination of physiological injury, substrate availability and microbial selection that differed from the conditions produced by extreme alkalinity. The present experiment cannot determine which of these mechanisms was dominant, but the repeated association between pH 2 and fungal colonization identifies a biologically meaningful phenomenon deserving further investigation.

Seed deterioration as a potential resource pathway

A plausible mechanism linking extreme acidity and fungal emergence involves acid-induced deterioration of seed tissues. Germinating seeds contain substantial stores of carbohydrates, proteins, lipids, amino acids and mineral nutrients required to sustain early seedling development. Under normal conditions, these reserves are mobilized internally and directed towards emerging roots and shoots. Severe environmental stress, however, can damage membranes and disrupt cellular compartmentalization. Zhao *et al.*, [2] demonstrated that acid stress could compromise membrane integrity and cellular structures during seed germination. Once membrane integrity deteriorates, soluble organic and inorganic constituents may become increasingly available outside the damaged cells.

From an ecological perspective, this represents a transfer of resources. Compounds no longer successfully incorporated into developing plant biomass may become available to heterotrophic microorganisms. Consequently, the same physiological damage that represents toxicity to the plant can simultaneously increase substrate availability for decomposers. In the pH 2 treatments, severe injury and failure of seedling establishment may therefore have transformed the seeds from actively developing organisms into increasingly available organic substrates. Acid-tolerant or opportunistic fungi present in the microcosm could subsequently exploit these resources.

Mung bean seeds themselves may also serve as carriers of fungal propagules. Seed-borne fungi reported from *V. radiata* include members of *Aspergillus*, *Penicillium*, *Alternaria*, *Rhizopus* and *Fusarium* [7]. Therefore, the fungal inoculum need not necessarily have originated from external laboratory contamination. Spores could have been associated with the seed surface or tissues before exposure. Alternatively, propagules could have originated from laboratory air, the substrate, Petri dishes, treatment water or handling. Since seeds were not compared systematically before and after surface

sterilization, the present experiment cannot distinguish among these possibilities.

A working mechanistic sequence can therefore be proposed:

Extreme acidity → physiological injury and membrane disruption → deterioration of seed tissues → suppression of normal germination and plant establishment → release or exposure of organic substrates → colonization by acid-tolerant or opportunistic fungi.

This pathway should be regarded as a testable hypothesis rather than a demonstrated causal mechanism. Nevertheless, it explains the simultaneous occurrence of severe plant impairment and extensive fungal colonization without requiring the assumption that acidity itself directly enhanced fungal growth.

From individual toxicity to microecosystem restructuring

The fungal observation expands the ecological interpretation of the experiment beyond conventional phytotoxicity. Plant toxicity tests frequently emphasize endpoints such as germination percentage, root length, shoot length and biomass. These remain essential indicators of stress, but they primarily describe the performance of the selected test organism. Ecological systems contain interacting organisms with different physiological tolerances and ecological roles. A stressor that suppresses one group may therefore alter competitive relationships and resource availability sufficiently to favour another group.

The pH 2 Petri dish can be interpreted as a simple experimental microecosystem in which extreme acidity altered the relative biological success of different organisms. Plant development was severely constrained, culminating in zero measurable plant biomass, while visually conspicuous fungal growth emerged around deteriorating seeds. Biological activity was therefore not simply eliminated. Instead, the dominant visible biological process apparently shifted from plant establishment towards fungal colonization and decomposition. This distinction is ecotoxicologically important because environmental stress can change biological organization even when total biological activity does not disappear.

The finding illustrates the concept of environmental filtering. Extreme physico-chemical conditions selectively exclude sensitive organisms while permitting organisms possessing appropriate physiological tolerances to persist or exploit newly available niches. In natural ecosystems, acidification associated with acid mine drainage, industrial discharges, acid sulfate soils and other environmental disturbances can similarly modify community composition, decomposition, nutrient transformations and species interactions. The present Petri dish system is clearly much simpler than a natural ecosystem, but it illustrates the underlying principle: environmental toxicity concerns not only how much biological performance is lost, but also which organisms decline, which persist and which subsequently become dominant.

Ecotoxicological significance of the pH 2 versus pH 12 contrast

As shown in Figure 3, despite similarly severe inhibition of germination, with mean germination of 16.67% at pH 2 and 13.33% at pH 12, the two pH extremes produced contrasting biological outcomes. At pH 2, no measurable plant biomass remained by Day 7 and conspicuous fungal colonization was repeatedly observed, suggesting a potential transition from plant failure and seed deterioration towards fungal colonization and decomposition. In contrast, some plant biomass remained at pH 12 despite severe

germination and growth inhibition, with no comparable visible fungal proliferation recorded. The conceptual comparison illustrates that similar toxicity at a single biological endpoint does not necessarily result in similar ecological outcomes and highlights the importance of considering secondary biological responses and their potential implications for decomposition, organic matter turnover, nutrient cycling and microecosystem functioning.

The comparison between pH 2 and pH 12 is particularly informative. Both conditions represented extreme departures from the intermediate pH treatments, and both strongly inhibited germination. Mean germination was 16.67% at pH 2 and 13.33% at pH 12. Thus, based solely on germination percentage, pH 12 could be regarded as at least as inhibitory as pH 2. However, the ecological trajectories of the two treatments differed. At pH 12, some plant biomass remained at Day 7, whereas biomass was zero at pH 2. More importantly, conspicuous fungal growth was repeatedly associated with pH 2 but not with pH 12.

These observations demonstrate why ecotoxicological interpretation should not depend exclusively upon ranking treatments according to a single endpoint. The same apparent level of toxicity to one component may lead to very different secondary biological outcomes. Strong alkalinity impaired germination and seedling development but did not produce the same visible microbial transition. Extreme acidity, by contrast, was associated with plant failure followed by fungal colonization. The two pH extremes therefore appear to represent different biological pathways rather than merely opposite ends of the same toxicity gradient.

This distinction may have broader implications for environmental risk assessment. Physico-chemical stressors can produce indirect ecological responses that are not predictable solely from effects on a focal test organism. If one stress condition promotes deterioration and decomposition while another principally suppresses growth, consequences for organic matter turnover, microbial activity and nutrient cycling could differ substantially even when plant toxicity appears superficially similar. The present observations therefore support a broader ecotoxicological perspective in which responses at the organism level are connected to potential changes in ecological processes.

Limitations and Future Experimental Direction

Several limitations prevent definitive causal interpretation of the present findings. Evaporation, unequal depletion of treatment water and interference by ants introduced uncontrolled variation during exposure. The actual pH experienced by individual seeds may also have changed over time as water evaporated and as biological compounds were released from germinating or deteriorating tissues. Maintaining a nominal starting pH therefore does not guarantee that identical exposure conditions persisted throughout the seven-day period. In addition, the biological effects of an acidic solution depend not only on hydrogen ion concentration but also on the chemical identity of the acid used to establish the treatment. Equal nominal pH values generated using different acids can result in different germination and seedling responses [3]. These methodological limitations should be considered when interpreting the present data.

Another major limitation concerns fungal identification and quantification. The visible fungal material was not cultured, isolated or taxonomically identified. Consequently, references to fungi in this

study describe the observed fungal-like colonization rather than a confirmed species or fungal community. Likewise, fungal biomass and percentage surface coverage were not quantified. The present evidence therefore supports a reproducible qualitative association between the pH 2 treatment and fungal emergence but does not establish a quantitative fungal dose response or prove that low pH directly promoted fungal proliferation.

A targeted follow-up experiment should distinguish between direct pH selection and indirect colonization following seed deterioration. Surface-sterilized and non-sterilized mung bean seeds should be tested simultaneously, together with sterile substrate controls containing treatment solution but no seeds. Treatments could include pH 2, 3, 5.5 and 7, with pH measured repeatedly throughout exposure and moisture maintained at standardized levels. Daily standardized photographs could be analysed quantitatively to determine percentage fungal surface coverage. Fungal isolates should subsequently be cultured and identified using morphological characteristics and, where possible, molecular methods such as internal transcribed spacer sequencing. Measurements of electrolyte leakage or conductivity could additionally provide evidence of acid-induced membrane damage and help test whether increasing seed deterioration precedes fungal colonization.

Conclusion

The present study demonstrates that extreme pH stress generated complex and endpoint-specific biological responses in red mung bean. Germination was strongly inhibited at both ends of the pH gradient, with the lowest mean germination occurring at pH 12 and the second lowest at pH 2. Biomass responses were non-monotonic across the intermediate treatments, emphasizing the biological and methodological complexity of short-term pH exposure. Nevertheless, the pH 2 treatment was biologically unique because limited germination coincided with zero cumulative plant biomass and conspicuous fungal colonization.

The fungal observation changes the interpretation of the experiment from a simple assessment of plant growth inhibition to an exploratory ecotoxicological study of biological restructuring. The evidence does not demonstrate that pH 2 directly stimulated fungal growth. A more plausible working hypothesis is that extreme acidity severely damaged the seeds, suppressed normal plant establishment and generated conditions in which acid-tolerant or opportunistic fungi could colonize deteriorating biological material. This interpretation remains to be tested experimentally, but the repeated occurrence of the phenomenon provides a strong basis for further investigation.

More broadly, the experiment illustrates that environmental stress cannot always be understood simply as an increase or decrease in biological performance. Different organisms respond differently to the same physico-chemical condition, and the suppression of one biological component may create opportunities for another. In this Petri dish microecosystem, extreme acidity apparently did more than inhibit mung bean development. It altered the direction of biological activity from plant establishment towards fungal colonization. From an ecotoxicological perspective, this shift is important because environmental disturbance concerns not only how much life is impaired, but also how stress changes which organisms succeed and which ecological processes become dominant.

Declaration on the Use of Artificial Intelligence

The authors declare that generative artificial intelligence tools were used in a supportive capacity during the preparation of this manuscript. These tools assisted with language refinement, structural organisation, and stylistic clarity of the text. All ideas, interpretations, reflections, and conclusions presented in this paper are the author's own and are grounded in personal experience and scholarly judgment. The authors retain full responsibility for the content, accuracy, originality, and integrity of the manuscript.

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