TOLERANCE OF MUNG BEAN (*VIGNA RADIATA* (L.) WILCZEK) TO LACTIC ACID, A POTENTIAL HERBICIDE: GROWTH AND MORPHOLOGY

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ABSTRACT

Lactic acid is used as an environmentally safe herbicide against a variety of grass and broadleaf weed species. However, it was found to be toxic to certain leguminous weeds and may also be toxic to important legume crops including mung bean. The effect of varying concentrations of lactic acid on the growth and morphological characteristics of two types of mung bean seedlings was determined to test the possibility of its safe use on the crop. Our findings show that although an 8% lactic acid concentration was toxic to both types of mung bean, they differed in their tolerance to lower concentrations in terms of root and shoot length and fresh and dry weight of roots and shoots. Minimum tolerance was observed at 2% lactic acid for both mung bean types. These tolerable concentrations are already much higher than those reported to be toxic to certain weed species, indicating that high concentrations of lactic acid may be safe to use on mung bean. The findings of this research can provide relevant information on the potential use of lactic acid as an organic herbicide and its possible effects on the growth of mung beans as well as other important legume crops.

Keywords: Lactic acid; Plant growth regulation; *Vigna radiata*; Mung bean; Plant morphology; Biopesticide; Herbicide; Weed management.

INTRODUCTION

Inorganic pesticides are commonly used to increase crop production by eliminating competition among plants and insects. However, these synthetically derived compounds have detrimental effects on the environment. They contaminate nearby bodies of water, alter soil structure and microbiota, destroy sensitive habitats, and eliminate non-target organisms (reviewed in Freemark and Boutin 1995; Aktar *et al*. 2009). Thus, interest in the use of naturally produced compounds as organic pesticides or biopesticides in crop production has been increasing recently (Chandler *et al*. 2011). One such compound is lactic acid. Lactic acid is widely used in the food processing industry as an acidulant, flavoring, pH buffering agent, or inhibitor of bacterial spoilage (Datta and Henry 2006). It is also used to increase the shelf life of poultry and fish packages, and its esters are used in baking as an emulsifying agent (Janaki and Manoharan 2012). Lactic acid has also found several applications in the manufacturing and pharmaceutical industries (Datta *et al*. 1995).

Lactic acid is registered as a biochemical pesticide with the United States Environmental Protection Agency for the control of mosquitoes (US EPA 2009). It is reported to have no known major negative health effects on humans. It is also non-toxic to pollinators, birds, aquatic organisms, and animal habitats and is highly biodegradable (Bowmer *et al*. 1998; US EPA 2009). Exogenous lactic acid and its polymers have been shown to have a growth-promoting effect on plants. Kinnersley *et al*. (1990) reported that the dimer L-lactoyllactic acid and polylactic acid both increased the biomass of duckweed and corn at low concentrations. A mixture of lactic acid and succinic acid (10 ppm) released by *Pseudomonas putida* into the rhizosphere of asparagus was also found to improve the plant's growth (Yoshikawa *et al*. 1993). Young (1989) reported on and patented the use of L-lactic acid as a growth promoter of several crops including tomatoes, grapes, and oranges at recommended concentrations of 10^{-9} M to 10^{-2} M, with the lower concentrations being more beneficial to plant growth.On the other hand, higher concentrations of lactic acid (from 10^{-5} to 2 M) were found to have inhibitory effects on the growth of several plant species. Hence, the use of lactic acid and its polymers as organic herbicides or as encapsulating materials for inorganic herbicides is gaining interest (Sinclair 1973; Stloukal *et al*. 2012). Charbonneau (2010) used a 25% mixture of lactic acid and citric acid to successfully control several leguminous weeds such as black medick and clover, although this herbicide also had some phytotoxic effects on the non-target turfgrass. Even though it has been shown to be a good herbicide for leguminous weeds, there is little evidence in the literature about the effect of lactic acid on legume crops. Chang *et al*. (1996) reported that low levels of polylactic acid and lactide (15 to 30 ppm) promoted growth and increased yield in soybeans, whereas higher concentrations (up to 900 ppm) were inhibitory. Furthermore, among the list of crops identified by Young (1989) as benefitting from low concentrations of lactic acid were peanuts and beans, whereas leguminous weeds identified that could be controlled with higher concentrations were several kinds of clover.

The mung bean (*Vigna radiata* (L.) Wilczek) is an important legume crop widely cultivated in many parts of South and Southeast Asia, as well as in Canada, China, and Australia, because of its high nutritional content (Oplinger *et al*. 1990; Sangiri *et al*. 2007; Chadha 2010). It is also utilized as a feed, green manure, and material for various industrial applications (Rosales *et al*. 1998). In the Philippines, mung bean is mainly utilized as a food crop: it is incorporated in soups, porridge, bread, noodles, and ice cream (Delfin *et al*. 2008). It also serves as a raw material for noodle and pastry production (Naryanan *et al*. 2004).

One of the major challenges facing mung bean production is weed management. Because of its short growth habit and slow growth during the early stages of establishment, the mung bean is easily overwhelmed by more competitive grass and broadleaf weeds. As with other dry beans, reduction in seed yield due to weed competition can reach up to 80% (cited in Yadav *et al*. 2008; Soltani *et al*. 2013). Control of weeds in mung bean is particularly difficult because the herbicides being commonly used also damage the crop (Oplinger *et al*. 1990; Kaushik 2006). Several studies on the effect of pre- and post-emergent inorganic herbicides on mung bean have reported reductions in shoot growth and dry weight, and necrosis of leaves, among other effects (Kaushik 2006; Yadav *et al*. 2008; Soltani *et al*. 2013). On the other hand, there are as yet no published study that we are aware of on the use and effect of organic herbicides, such as those containing lactic acid, on mung bean growth and development.

Therefore, an investigation was conducted to determine the effects of lactic acid on the growth and morphological characteristics of mung bean. In particular, the effect of varying concentrations of lactic acid on the morphological characteristics of newly sprouted mung bean seeds was investigated. The findings of this research can provide relevant information on the potential use of lactic acid as an organic herbicide and its possible effects on the growth of mung beans as well as other important legume crops.

MATERIALS AND METHODS

Mung Bean Seeds: Two locally cultivated varieties of mung bean seeds, yellow and green, were purchased from a local supermarket. All seeds used in the experiment were sourced from a single pack. The seeds were kept in a dry dark place at room temperature for two days and were subsequently checked for their viability by suspending them in double distilled water. The seeds that settled at the bottom were randomly sampled for the study. The seeds were first surface sterilized in a 0.5% hypochlorite solution for 20 minutes and were subsequently rinsed twice in double distilled water.

Lactic Acid Treatment: Lactic acid is an organic acid and among the most widely occurring carboxylic acids in nature. With an IUPAC name of 2-hydroxypropanoic acid, it is a three carbon organic acid: one terminal carbon atom is part of an acid or carboxyl group; the other terminal carbon atom is part of a methyl or hydrocarbon group; and a central carbon atom having an alcohol carbon group (Janaki and Manoharan 2012). Lactic acid is characterized as non-volatile, odorless, colorless, and has a mildly acidic taste. In this experiment, 80% lactic acid manufactured by Alyson's Chemical Enterprises, Inc. (Quezon City, Philippines) was used.

Experimental Procedures: Each experimental set up contained sixteen seeds (8 green and 8 yellow) germinated in Petri dishes (diameter = 150 mm) with a double layer of filter paper soaked in distilled water (control). A total of five identical experimental set ups were prepared. The eighty seeds were allowed to germinate for four days under natural daylight and room ventilation, which were similar in all the treatment setups. Germination of the seeds were set under a photoperiod of 12 hours with day and night temperature of 30±2ºC and 28±1ºC, respectively. After four days, baseline shoot and root lengths were measured.

Four different 25 mL solutions of lactic acid concentrations (2%, 4%, 6%, and 8%) were prepared with distilled water. Subsequently, 5 mL of each treatment solution was poured into the Petri dishes containing the four-day-old seedlings of each experimental set up. After 24 hours, the seedlings were harvested, the length, fresh and dry weight of the shoot and root were measured.

Root and Shoot Length: The root length was taken from the point below the hypocotyls to the end of the tip of the root, while the shoot length was measured from the base of the root-hypocotyl transition zone up to the base of the cotyledons. The root and shoot length was measured with the help of a thread and scale.

Fresh and Dry Weight: The fresh weight of root and shoot of seedlings was determined by weighing the root and shoot separately using an electronic weighing scale. After the fresh weight taken, the seedlings were kept in a hot-air oven at 60ºC for 48 hours, then the weight of the dry matter was recorded.

Statistical Analysis: The study employed a randomized completely block design with five treatment concentration levels of lactic acid and two varieties of mung bean as the blocking variable. The assignment of the seeds in the treatment group was done using simple random sampling with equal replication. One-way analysis of variance was used to identify significant differences in the means of root length, shoot length, shoot-to-root ratio, root and shoot fresh weight, and root and shoot dry weight of mung bean seedlings under varying concentration levels of lactic acid. Post-hoc multiple comparison tests were further generated via Tukey's studentized range test to determine which pairs of treatments were statistically different. Differences in the shoot-to-root ratio of the samples before and after the administration of the treatments were assessed using *t*-test for paired samples. Simple linear regression analysis was employed to determine the functional relationship between the outcome measures and the concentration levels of lactic acid. All statistical analyses were carried out at 5% level of significance using Statistical Analysis System (SAS) software with values reported as means± standard deviations.

RESULTS AND DISCUSSION

Baseline Morphological Characteristics: Using the control set up with distilled water, the growth and morphology of the germinated mung beans were assessed. The root and shoot length of the eighty germinated mung beans were measured, and these baseline values were compared to assess homogeneity of the samples prior to the introduction of the treatments. The means and standard deviations of the five experimental set ups were compared, using analysis of variance, the baseline means of root and shoot length in the five experimental set ups did not significantly differ (p=0.94 and p=0.74), suggesting homogeneity of the baseline morphological characteristics of the experimental units prior to treatment administration (Table 1). Moreover, the effect of the blocking variable, color of the mung beans, did not contribute any significant difference in the baseline morphological characteristics of the seedlings (Table 1).

Effects of Lactic Acid on Root and Shoot Length: The highest mean root length of 30.56±1.87 mm was attained in the control group, while the smallest mean root length of 24.16±1.85 was found in the 8% treatment group, and the mean root length was significantly different among the lactic acid concentration levels (p<0.0001) (Table 1). A comparison of the mean root length under different treatments revealed decreasing root length with increasing concentrations of lactic acid. However, post-hoc comparisons revealed no statistically significant differences among the 2%–4%, 4%–6%, and 6%–8% treatment pairs (Table 1).

When mean shoot length is compared across the different treatment groups, the control group (70.49±6.48) had the highest, while the smallest mean of 54.18±6.09 was in the 8% treatment group, which had a mean shoot length significantly different among the lactic acid concentration levels (p<0.0001). The mean shoot length under different treatments revealed a decreasing trend with increasing concentrations of lactic acid (Table 1). Further, the mean shoot length significantly differed among the control, 4%, and 8% treatment groups, whereas no significant statistical difference was noted in 0%–2%, 2%–4%, 2%–6%, 4%–6%, and 6%–8% treatment pairs (Table 1).

When the effect of the blocking variable, color of mung bean, was assessed in the different treatment groups, results showed that the highest mean root length was observed in the yellow mung beans under the control group, with no significant differences noted in the 4%, 6%, and 8% treatment groups under the two varieties of mung beans (Table 1). Moreover, the highest mean shoot length was identified in the green mung beans under the control group, but this did not significantly differ compared to the other variety under the control, 2%, and 4% treatment groups.

Using *t*-test for paired samples, an assessment of the shoot-to-root ratio of the germinated mung beans revealed significant increase in ratios across all the treatment groups (p<0.05), which suggests that exposure of mung beans to lactic acid resulted in delayed root elongation (Table 2). The color of the beans had no significant effect on the shoot-to-root ratio of the germinated mungs in both the pre- and post-treatment administration of lactic acid (Table 1).

Root Growth Characteristics: The length, fresh weight, and dry weight of roots of germinated mung beanssignificantly decreased with increasing concentrations of lactic acid exposure. The root length was noted to be longest in the yellow mung bean under the control group (Table 1). The mean root length in the yellow and control groups did not significantly vary from the green variety in the 0–2% treatment group. However, the lowest mean root length was observed in the green variety under the 8% treatment group. Consequently, the root fresh and dry weight means did not significantly differ in the 0–2% and in 4%–6%–8% treatment groups, but yellow mung beans in the control group had the highest mean root fresh and dry weight values (Table 1). Across the 2% to 8% treatment levels, the mean root fresh weight did not significantly differ between the two types of mung beans. However, there was a significant negative correlation between the mean root characteristics of mung beans and lactic acid concentration. Results showed that every percent increase in the concentration of lactic acid in the treatment corresponds to, on the average, a reduction of 0.742 mm, 0.565 mg, and 0.094 mg in the root length, fresh weight, and dry weight, respectively (Table 3).

Shoot Growth Characteristics: Results showed that the shoot measurements defined as shoot length, shoot fresh weight and dry weight of mung beans were affected when exposed to the different concentrations of lactic acid. The longest shoot length was observed in the control group, but it did not significantly differ from the 2% treatment group. Exposure to the highest concentration of the treatment resulted in the shortest shoot measurement (Table 1). However, exposure to 2%, 4%, and 6% concentrations did not render any significant differences in the mean shoot length. The green mung bean in the control group had the highest mean fresh weight, whereas the yellow type had the highest mean dry weight, and these values did not significantly differ among the 2% to 4% treatment groups (Table 1). A significantly negative correlation was found between the mean shoot characteristics and lactic acid concentration: for every percent increase in the concentration of lactic acid in the treatment, an average of 1.951 mm, 2.447 mg, and 0.168 mg reduction in the shoot length, fresh weight, and dry weight, respectively, occurred (Table 3).

Lactic acid (2-hydroxypropionic acid) is a naturally occurring by-product of fermentation that can also be created synthetically (Datta and Henry 2006). The acid, specifically its isomer L-lactic acid, has been shown to either promote or inhibit plant growth (Young 1989). As a growth promoter, it has the potential to increase yield of important crops; as an inhibitor, it has the potential to be used as an environmentally safe, organic herbicide. In this study, the inhibitory effects of different levels of lactic acid on the growth and morphological characteristics of mung bean were noted, but there appeared to be a tolerant concentration threshold at which no significant differences were observed with the control.

Although a 2% concentration of lactic acid inhibited root length of mung bean seedlings when compared to the control, it did not significantly inhibit shoot length and the fresh and dry weight of roots and shoots (Table 1). Higher levels (4–8%) of lactic acid had an inhibitory effect on all characteristics measured. No significant differences were found among the treatment levels, but the 8% concentration had the most inhibitory effect on seedling growth. When the mung bean variety was included as a variable, a slightly different response was observed between the green and yellow mung beans (Table 1). Yellow mung beans had a higher tolerance for lactic acid in terms of fresh and dry weight of shoots, with no significant differences found up to the 6% concentration level when compared with the control. For green mung beans, 4% was the maximum tolerable level. On the other hand, the root length of green mung beans tolerated lactic acid up to the 2% level only, whereas those of the yellow mung beans were already inhibited at the same concentration. The shoot length of both green and yellow mung beans tolerated lactic acid up to the 4% level. Root fresh and dry weights were not negatively affected up to the 6% level in both types of mung beans. These differences were confirmed with regression analysis (Table 3).

There is little available information in the published literature on plant tolerance to high lactic acid concentrations. Lactic acid is produced by plants during periods of hypoxia or anoxia particularly in roots exposed to flooding. High levels of the acid interfere with normal cellular metabolism and can cause irreversible cell damage due to cytoplasmic acidosis, resulting in the premature death of plant tissues (Roberts *et al*. 1985). But the production of lactic acid at the onset of anoxia is not prolonged, as the enzyme involved in its synthesis (lactate dehydrogenase) is inactivated once cytoplasmic pH dips below 7.0 (Drew 1997) and alcohol dehydrogenase is activated leading to ethanol production (Rivoal and Hanson 1994). Many plants do have mechanisms to efflux the acid into the rhizosphere (Xia and Saglio 1992) or transport it to the vacuole (Kennedy *et al*. 1992), thereby minimizing cellular damage (Ryan *et al*. 2001). Under normal, aerobic conditions, plant roots are exposed to varying levels of lactic acid in the soil through the action of lactic acid bacteria that live symbiotically in the rhizosphere (Jones 1998). In the soil, lactic acid may promote plant growth by the elimination of pathogens that would otherwise infect the plant. It is also involved in the solubilization of certain minerals, such as phosphates, making these essential nutrients more available for absorption by plant roots (Rodríguez and Fraga 1999). Hamed *et al*. (2011) also showed that tomato seeds treated with a culture of lactic acid bacteria showed improved growth even in the presence of the pathogenic fungus *Fusarium oxysporum*. More importantly, Takijima (1964) found that the ability of the type of soil to absorb and assimilate organic acids, such as butyric acid and acetic acid, was inversely related to the acids' ability to inhibit root growth of rice seedlings in test tube experiments. Organic acids that were more readily absorbed by the soil had a lower inhibitory effect on root growth. Takijima (1964) also showed that the amount of lactic, oxalic, and citric acids absorbed by different soil types was much higher than the amount of butyric, formic, and acetic acids. This could mean that lactic acid hardly reaches inhibitory levels in the soil even under apoxic conditions, and only upon exogenous additions can lactic acid influence plant growth, either positively or negatively.

The specific use of lactic acid as a growth inhibitor (i.e., herbicide) is not well studied, although published reports on its growth-promoting effects usually also report its inhibitory effect (see for example, Hildebrandt *et al*. 1954; Kinnersley *et al*. 1990). Chang *et al*. (1996) found that 150 ppm of polylactic acid (PLA) stimulated plant growth in soybeans similarly to 30 ppm. But when several concentrations of PLA were used to find the optimum growth-promoting range for soybeans, only the 7.5 ppm and 15 ppm were stimulatory, whereas concentrations higher than 30 ppm proved inhibitory. The potential to use higher concentrations of PLA to slow down the growth of other plants was noted by the author. According to Young (1989), a lactic acid concentration of as low as 10^{-2} M can already inhibit plant growth.

The effect of high concentrations of lactic acid on plant growth shows similarities with more well-recognized plant growth regulators. Several auxins were found to cause necrosis and death of hypocotyls of treated mung bean seedlings at a concentration of 10^{-3} M (Kollarova *et al*. 2005). Furthermore, exposure of micropropagated, cotyledonary nodal explants of mung beans to varying concentrations of benzylaminopurine, a cytokinin, resulted in decreased shoot proliferation at the highest concentration (5 ppm). When the shoot explants were transferred to growing media containing indole-3-butyric acid, an auxin, the number of developed roots decreased as the auxin concentration increased (Janaki and Manoharan 2012; Vats *et al*.2014). In our study, the lowest lactic acid concentration (2%) was at 0.22 M, which already caused the inhibition of the growth and morphological characteristics of the germinated mung bean seeds. Growth inhibition was most severe at 8% (0.87 M).

When used specifically as a herbicide, Coleman and Penner (2008) reported that a 1.5% (v/v) pelargonic acid formulation mixed with 1% L-lactic acid (v/v) was effective in the control of velvetleaf and lambsquarters. But in their study, L-lactic acid was used only as an adjuvant, with pelargonic acid being the main active ingredient. Furthermore, a 2.5% (v/v in water) formulation of pelargonic acid with sorbitan monolaurate as emulsifier and 8% or 15% lactic acid as adjuvant (0.2% and 0.38% v/v lactic acid final concentration), significantly controlled several dicot weed species, including grasses and the legumes red clover and white clover. Used alone, 1% (v/v) L-lactic acid was not effective in controlling these weeds. Stloukal *et al*. (2012) found that when used as an encapsulating material for an inorganic herbicide, metazachlor, PLA was effective in controlling the release of the pre-emergent herbicide. In the United States, Matratec® (Brandt Consolidated Inc., Illinois, USA) is a natural product, broad-spectrum foliar herbicide that contains lactic acid, but only as a minor active ingredient; its major active ingredient is clove oil. The most direct use of lactic acid as a herbicide was reported by Charbonneau (2010) who tested Organo-sol® by Lacto-Pro Tech, Canada (2% citric acid; 1.8% lactic acid) on several broadleaf weeds, especially the legumes black medick and white clover. At the recommended level of 25% herbicide (v/v) in water (0.45% v/v lactic acid final concentration), the product was effective against the weeds, but it was also phytotoxic to the non-target turfgrass. However, Siva (2014) later reported that the herbicide was not effective in controlling the same weeds. In our study, mung bean seedlings could tolerate up to 6% lactic acid depending on the variety, indicating that levels phytotoxic to common weeds may not be phytotoxic to certain mung bean varieties.

It should be noted, however, that this study was done *in vitro*. The effects of lactic acid on the growth of mung beans *in vitro* may vary physiologically and morphologically from those growing in soil with different microbial and nutritional conditions. Kinnersley *et al* (1990) found that the biomass of duckweed plants peaked at a 1000 ppm concentration of aqueous D-L-lactic acid in *in vitro*, sterile experiments. On the other hand, when corn seedlings were grown in soil or vermiculite, it was found that growth response to the dimer L-lactoyllactic acid was optimal at 1 ppm when vermiculite was the growing medium, whereas 10 ppm of L-lactoyllactic acid was the optimum when soil was used. It should also be noted that in this study, lactic acid was taken up by the mung bean seedlings through the roots. Many herbicides containing lactic acid, such as Organo-sol® and Matratec®, are applied as foliar sprays, and Coleman and Penner (2008) also applied their biopesticide containing lactic acid as a foliar spray. However, Kinnersley *et al*. (1990) and Chang *et al*. (1996) incorporated their lactic acid into the growing medium, and they also observed its inhibitory effects at higher concentrations. In fact, soil-incorporated lactic acid as an organic herbicide may be ideal and more efficient for mung bean because as a slow-growing field crop that loses out to faster growing weeds, it could have a starting advantage over lactic-acid-sensitive weeds.

Table 1. Effects of varying concentration levels of lactic acid on the root and shoot length, shoot-to-root ratio, fresh and dry weight of the two varieties of mung beans.

		Treatment (Concentration Levels of Lactic Acid)				
Characteristics	**Variety**	**0%**	**2%**	**4%**	**6%**	**8%**
Baseline root length (mm)	Green	20.44 ± 1.09^{a}	20.93 ± 1.13^{a}	20.65 ± 1.10^{a}	20.75 ± 1.12^{a}	20.71 ± 1.03^{a}
	Yellow	20.60 ± 1.05^{a}	20.41 ± 1.03^{a}	20.48 ± 1.08^{a}	20.33 ± 1.09^{a}	20.33 ± 2.06^{a}
	Combined	20.52 ± 1.03^{a}	20.67 ± 1.07^{a}	20.56 ± 1.06^{a}	20.54 ± 1.09^{a}	20.52 ± 1.03^{a}
Baseline shoot length (mm)	Green	47.56 ± 4.34^{a}	47.71 ± 5.38^{a}	46.93 ± 4.94^{a}	45.50 ± 4.41^{a}	45.41 ± 4.51^{a}
	Yellow	45.08 ± 4.61^{a}	44.14 ± 3.88^{a}	44.41 ± 4.19^{a}	44.58 ± 2.80^{a}	43.43 ± 3.80^{a}
	Combined	46.32 ± 4.51^{a}	45.93 ± 4.89^{a}	45.67 ± 4.61^{a}	45.04 ± 3.60^{a}	44.42 ± 4.16^{a}
Post-treatment root length (mm)	Green	29.91 ± 2.15^{ab}	28.31 ± 2.33^{abc}	25.95 ± 1.91^{cde}	25.91 ± 2.06^{cde}	23.64 ± 1.50^{e}
	Yellow	31.20 ± 1.38^{a}	27.73 ± 2.16^{bcd}	26.96 ± 2.02^{bcde}	26.00 ± 2.52^{cde}	24.69 ± 2.11^{de}
	Combined	30.56 ± 1.87^{a}	28.02 ± 2.19^{b}	26.46 ± 1.97^{bc}	25.96 ± 2.22^{cd}	24.16 ± 1.85^{d}
Post-treatment shoot length (mm)	Green	72.20 ± 6.28^{a}	65.85 ± 8.29^{abc}	61.71 ± 8.66^{abcd}	58.09 ± 7.56^{bcd}	54.91 ± 5.99^{cd}
	Yellow	68.79 ± 6.63^{ab}	63.20 ± 7.09^{abcd}	60.59 ± 6.28^{bcd}	58.16 ± 5.17^{bcd}	53.45 ± 6.50^{d}
	Combined	70.49 ± 6.48^{a}	64.53 ± 7.58^{ab}	61.15 ± 7.33^{b}	58.13 ± 6.26^{bc}	54.18 ± 6.09^{c}
Post-treatment shoot-to-root ratio	Green	2.42 ± 0.19^{a}	2.32 ± 0.18^{a}	2.37 ± 0.18^{a}	2.24 ± 0.14^{a}	2.32 ± 0.19^{a}
	Yellow	2.20 ± 0.17^{a}	2.28 ± 0.20^{a}	2.24 ± 0.11^{a}	2.24 ± 0.12^{a}	2.16 ± 0.11^{a}
	Combined	2.31 ± 0.21^{a}	2.31 ± 0.18^{a}	2.30 ± 0.16^{a}	2.24 ± 0.12^{a}	2.24 ± 0.17^{a}
Post-treatment root fresh weight (mg)	Green	21.43 ± 2.57^{ab}	20.20 ± 3.15^{abc}	18.36 ± 2.03^{abc}	18.35 ± 2.82^{abc}	16.61 ± 1.52^{c}
	Yellow	22.23 ± 2.69^{a}	19.93 ± 2.74^{abc}	19.06 ± 2.15^{abc}	18.26 ± 2.19^{abc}	17.50 ± 2.75^{bc}
	Combined	21.83 ± 2.58^{a}	20.06 ± 2.85^{ab}	18.71 ± 2.05^{bc}	18.31 ± 2.44^{bc}	17.06 ± 2.20^{c}
Post-treatment shoot fresh weight (mg)	Green	91.93 ± 9.05^{a}	83.88 ± 12.92^{abc}	79.73 ± 12.27^{abc}	74.11 ± 11.71^{bc}	69.89 ± 8.29^{c}
	Yellow	87.49 ± 10.01^{ab}	80.69 ± 11.68^{abc}	77.10 ± 8.46^{abc}	75.21 ± 8.11^{abc}	68.21 ± 10.48^{c}
	Combined	89.71 ± 9.50^{a}	82.28 ± 12.01^{ab}	78.41 ± 10.27^{bc}	74.66 ± 9.75^{bc}	69.05 ± 9.17^{c}
Post-treatment root dry weight (mg)	Green	3.64 ± 0.46^{ab}	3.44 ± 0.54^{abc}	3.14 ± 0.36^{abc}	3.13 ± 0.49^{abc}	2.85 ± 0.26^{c}
	Yellow	3.81 ± 0.48^{a}	3.44 ± 0.50^{abc}	3.28 ± 0.39^{abc}	3.16 ± 0.41^{abc}	3.01 ± 0.51^{bc}
	Combined	3.73 ± 0.46^{a}	3.44 ± 0.50^{ab}	3.21 ± 0.37^{bc}	3.14 ± 0.44^{bc}	2.93 ± 0.40^{c}
Post-treatment shoot dry weight (mg)	Green	6.04 ± 0.60^{ab}	5.48 ± 0.84^{abc}	5.20 ± 0.80^{abc}	4.86 ± 0.80^{bc}	4.91 ± 0.79^{c}
	Yellow	6.30 ± 0.85^{a}	5.80 ± 0.86^{abc}	5.56 ± 0.84^{abc}	5.41 ± 0.71^{abc}	4.91 ± 0.79^{bc}
	Combined	6.17 ± 0.72^{a}	5.64 ± 0.84^{ab}	5.38 ± 0.82^{bc}	5.14 ± 0.79^{bc}	4.76 ± 0.82^{c}

Means with the same superscript letters for a specific characteristic are not statistically different at 5% level of significance using Tukey's studentized range test.

Table 2. Pairwise comparison on the mean shoot-to-root ratio before and after administration of lactic acid across different concentration levels.

	Treatment (Concentration Levels of Lactic Acid)				
Shoot-to-Root Ratio	**0%**	**2%**	**4%**	**6%**	**8%**
Baseline	2.26±0.03	2.22±0.02	2.22±0.02	2.19±0.01	2.16±0.02
Post-treatment	2.31±0.21	2.31±0.18	2.30±0.16	2.24±0.12	2.24±0.17
p-value	0.0190	0.0046	0.0002	0.0095	0.0043

Table 3. Results of the regression analysis on the relationship between the varying concentration levels of lactic acid on the post-treatment morphological characteristics of *vigna radiata* seedlings.

Characteristics	Slope	Intercept*	F	*r*	*p*-value
Root length (mm)	-0.742	30.000	84.63	-0.72	<0.0001
Shoot length (mm)	-1.951	69.500	54.58	-0.64	<0.0001
Shoot-to-root ratio	-0.010	2.320	2.23	-0.17	0.1391
Root fresh weight (mg)	-0.565	21.45	35.02	-0.56	<0.0001
Shoot fresh weight (mg)	-2.447	88.609	38.06	-0.57	<0.0001
Root dry weight (mg)	-0.094	3.665	30.38	-0.53	<0.0001
Shoot dry weight (mg)	-0.168	6.084	30.76	-0.53	<0.0001

*all regression model intercepts are statistically significant at 5% level of significance.

Conclusion: In this study, it was found that mung bean seedlings, in general, can tolerate up to 2% (v/v) lactic acid as this did not affect shoot length and fresh and dry weight of roots and shoots. When the type of mung bean is considered, yellow mung beans can tolerate concentrations of up to 6%, whereas for green mung beans, it is 4%. This means that current herbicide formulations using lactic acid, which contain as much as 0.45% v/v lactic acid, may be safe to use in field production of mung beans to control weeds. The most common weeds of mung beans vary by location but normally include a variety of grasses and broadleaves. Although lactic acid was shown to have a damaging effect on leguminous weeds, we have shown that these levels may not be toxic to mung bean after all. Thus, lactic acid could be safe to use on weed control for this crop. It might also be safe to use higher levels of lactic acid for a more effective weed control. It is recommended to have field or greenhouse testing to verify this effect under normal growing conditions, particularly in relation to the soil environment, needs to be further studied.

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