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Dibutyl Dithiophosphate Increases Radish and Bush Beans Growth

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ABSTRACT. Hydrogen sulfide (H_2S) is a gasotransmitter that can promote growth and protect plants from environmental stressors. In this study, we tested the impact of dibutyl dithiophosphate (DBDTP), which releases H_2S as it is metabolized by plants, upon radish and bush bean growth. Experiments examined applications of DBDTP as a seed treatment in combination with soil drenches, and as a foliar spray under greenhouse and field conditions. In greenhouse trials, one application of 12.5 mM DBDTP as a seed treatment plus three applications of 0.5 mM DBDTP as a soil drench increased radish fresh and dry weight by 4%. Under field conditions, seed treatment using a solution of 12.5 or 25 mM DBDTP increased radish plant biomass by 5% to 7%, while radish root fresh weight was increased by 7.3% and 12.4%, respectively. We also examined the phytotoxicity of DBDTP foliar sprays and found that 0.5 mM DBDTP led to foliar damage at 8 and 15 d after germination. Bush bean seeds were treated with DBDTP at 12.5 mM, 25 mM, and 50 mM concentrations leading to trends of increased fresh weight and pod fresh weight. Our results suggest that DBDTP shows potential as an H_2S donor to enhance plant growth as a seed treatment. However, the mixed responses in seed treatments and the observed foliar phytotoxicity indicate that further research is required before broad use in agricultural settings can be recommended.

In 2002, Wang classified hydrogen sulfide (H_2S) as a plant gasotransmitter, along with nitric oxide (NO) and carbon monoxide (CO) (Wang 2002). Before 2002, H_2S was believed to have only negative effects on plants, but after its discovery as a gasotransmitter, it was shown to have strong, positive effects on plants at selected application rates. During the past few years, research has shown the effects of exogenous applications of H_2S on plants including abiotic and biotic stress resistance, delayed senescence, and improved seed germination (Bloem et al. 2004; Hancock and Whiteman 2014; Li and Zhu 2014).

H_2S as a gas is challenging to apply so the development of chemicals that slowly release H_2S is key to applying H_2S to plants economically. Dithiophosphates (DTPs) are stable chemicals that exist as solids at room temperature, are readily soluble in water, and slowly release H_2S by hydrolysis in the soil or within plants. They were recently formulated for their use in agriculture to slowly release H_2S to aid plant survival under abiotic stress and increase plant growth (Carter et al. 2019). Compared with other H_2S donors, DTPs have varying hydrolysis rates, degrade to release safe compounds such as fatty alcohols and monopotassium phosphate, and are inexpensive to produce (Carter et al. 2019). This study focuses solely on the DTP DBDTP.

The identification of low-cost seed or soil treatments that increase plant growth under normal or stressful conditions is

crucial to addressing decisive challenges in agriculture. Therefore, testing such compounds using different application methods and in different environments and crops is essential to ensure their proper use in agricultural settings.

Radishes (*Raphanus sativus*) are a widely grown vegetable worldwide and are cultivated for their roots, leaves, pods, and seeds, with distinct varieties in Eastern and Western cultures (Ahuja et al. 1987; Nishio 2017). Radishes are consumed as fresh and cooked vegetables and are important for their low-calorie content and for being a good source of antioxidants, electrolytes, minerals, dietary fiber, and vitamin C (Nishio 2017; Quais et al. 2013).

Bush beans (*Phaseolus vulgaris* L.) are a very important legume worldwide. They are consumed as dry seeds or unripe pods, and occasionally the leaves are eaten as a vegetable. In addition, the straw is used to feed livestock. The main forms of consumption of *P. vulgaris* are dry grain or green pods, highlighting their significance in human nutrition due to their high protein and fiber content (Broughton et al. 2003).

To elucidate the potential effects of DBDTP on radishes and bush beans, DBDTP was administered as a seed treatment, and plants were grown under field conditions. Additional greenhouse studies were carried out with radish. In the greenhouse, radishes were grown under two fertilizer treatments: 0 ppm nitrogen–phosphorus–potassium (NPK) and 100 ppm NPK (Mora-Leaf Plus 20–20–20 plus micronutrients; Wilbur Ellis, Denver, CO, USA). This setup was planned to assess the impact of H_2S on nutrient uptake, ensuring that any effects of H_2S would not be masked by fertility levels. In

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addition, five DBDTP treatments were tested as foliar applications in radishes to examine phytotoxicity.

Materials and Methods

The effect of DBDTP was evaluated on Rover F₁ radishes under greenhouse and field conditions in 2023 and 2024. In addition, it was assessed on Provider green bush beans under field conditions in 2023 and 2024. Rover and Provider seeds were obtained from Johnny Seeds (Albion, ME, USA).

DBDTP TREATMENT PREPARATION. DBDTP was applied first as a seed treatment and subsequently as a weekly drench application for radishes. To prepare the seed treatment, 7% of the weight of seeds treated within each treatment group was calculated. This percentage was used as the volume (dd water + surfactant) needed to dissolve the DBDTP. A surfactant (SPRET, Helena Holding Company, Collierville, TN, USA) was used to improve DBDTP surface contact and adhesion, at 0.125% (vol/vol).

The doses used in this experiment for seed treatment were 0, 12.5, 50, 100, and 200 mM DBDTP. Weekly drench application doses included 0, 0.5, 1, 2, and 10 mM DBDTP. A consistent water volume of 150 mL was used for drench applications, as the soil was saturated at this volume.

GREENHOUSE EXPERIMENTAL DESIGN. The DBDTP treatment experiment was divided into two groups: one group of plants was treated using DBDTP as a seed treatment plus three subsequent weekly drench applications beginning after germination resulting in a total of four DBDTP applications (DBDTP as seed treatment plus three DBDTP drench applications). The second group received only the seed treatment.

DBDTP as a seed treatment was applied at 0, 12.5, 50, 100, and 200 mM DBDTP. For the subsequent DBDTP drench applications, the concentrations were adjusted to 0, 0.5, 1, 2, and 10 mM DBDTP. In the first group, the seeds treated with 0 mM in the seed treatment corresponded to 0 mM in the drench application; 12.5 mM aligned with 0.5 mM, 50 mM with 1 mM, 100 mM with 2 mM, and 200 mM with 10 mM. For the drench application at 0 mM, only water was used.

Radishes were sown in 15 cm-diameter pots, with 10 seeds per pot using inorganic ceramic particles (Profile Greens Grade, Profile, Buffalo Grove, IL, USA). Ceramic particles resist compaction, eliminate localized dry spots, promote drainage, and facilitate healthy root systems (Reinders 2022). Seven days after planting, germination was recorded, and pots were thinned to five seedlings.

Plants were grown under two different fertilizer conditions: 100 ppm using 20N–20P–20K soluble fertilizer, and 0 ppm NPK. Pots were watered to saturation two to three times per week, depending on plant needs. The control pots (0 ppm) were watered in the same fashion but without fertilizer. According to Sparks (2023), when radishes are cultivated using soilless media in a flood-and-drain hydroponic system, the best fertilizer concentration ranges from 200 to 300 ppm N. Radishes grown under 100 ppm N tend to be very small, which is why we used 100 ppm NPK as a low fertilizer treatment.

The trial was planted in May 2024 and in Sep 2024. Overall fresh weight and root fresh weight were collected at harvest, 1 month after planting. A completely randomized design (CRD) with three replicates per treatment combination was used for this study, in which each replicate was composed of five plants per pot.

GREENHOUSE PHYTOTOXICITY TRIAL. In the greenhouse drench trial, the concentrations used in the drench application caused damage to the leaves on contact. To investigate this further, a new experiment was carried out using foliar application concentrations of 0, 0.5, 1, 2, and 10 mM DBDTP, as used previously. Five replications per treatment combination in a CRD with 10 radish seeds were sown per 15 cm pot filled with a 3:1 peatmoss perlite substrate. After germination, pots were thinned to five plants. Radishes were subjected to two different application times: 8 and 15 d after germination, using a different set of plants for each application time. The level of phytotoxicity was assessed using a visual scale of one to five, where one indicated no burns on the leaves; two represented small individual burns on some leaves; three indicated at least one leaf with spots that merged into a bigger spot but covering no more than one-fourth of the leaf; four implied larger spots in at least one leaf covering more than one-third of the leaves but without dead leaves; and five represented at least one true leaf dead and more leaves with scores of three and four or dead plant (Fig. 1). The trial was repeated twice. Radishes were harvested 8 d after the corresponding application time, and the overall fresh weight was measured.

RADISH AND BUSH BEAN FIELD EXPERIMENTAL DESIGN. In the field in 2023 and 2024, five DBDTP concentrations (0, 12.5, 25, 50, and 100 mM) were tested as a seed treatment on radishes and bush beans. Before the experiment, a composite soil sample (30 cm depth) was sent for soil analysis to Agvise Laboratories (Northwood, ND, USA) for both years of the study. In 2023, no pre-plant fertilization was needed. However, in 2024, a pre-plant

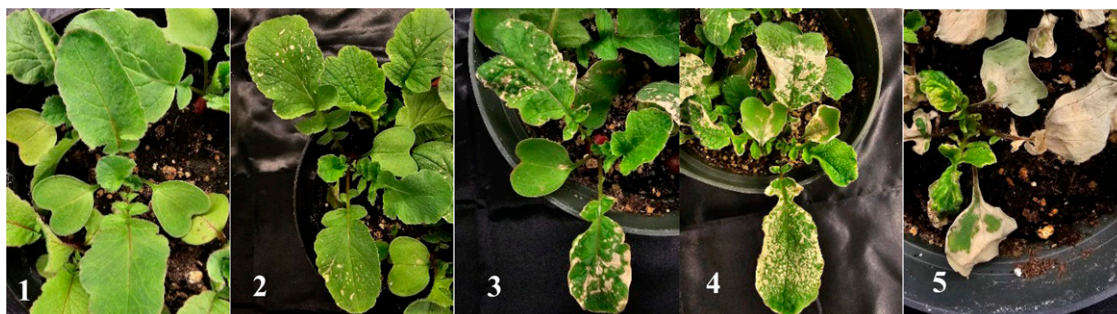


Fig. 1. Phytotoxicity scale used in testing the impact of foliar application of dibutyl dithiophosphate (DBDTP). A rating of one indicates no burns on the leaves; two represents small individual burns on some leaves; three indicates at least one leaf with spots that merged into a bigger spot but covering no more than one-fourth of the leaf; four represents bigger spots in at least one leaf covering more than one-third of the leaves but without dead leaves; and five indicates at least one true leaf dead and more leaves with scores of three and four or a dead plant.

broadcast fertilization of 64 kg·ha⁻¹ of urea was applied. No extra fertilization was applied during the growing season, and irrigation was performed twice a week for 2 h using a drip irrigation system. Different plots were used for each year's trial. In 2023, soil pH was 7.7, electrical conductivity (EC) 0.39 mmho·cm⁻¹, and organic matter 3.7%. In contrast, in 2024, soil pH was 7.5, EC 0.29 mmho·cm⁻¹, organic matter 4.0%, and cation-exchange capacity 22.7 meq/100 g soil.

For Provider green bush beans, 3% of the weight of the seeds was used for the seed treatment volume solvent, and for radishes, 7%. The change in the percentage used was due to the size of the seeds. The trials were conducted in a CRD with 15 replicates in 0.91 m-long rows in 2023 and with 20 replicates in 2024. The increase in replicates was due to greater field area availability in 2024, allowing us to increase replicates. For radishes, in 2023, seeds were sown 2.5 cm apart, with 30 cm between rows, and 30 seeds per replicate. For bush beans, 34 seeds were planted in a two-row plot with each seed spaced 5 cm apart. In 2024, 30 seeds were used for both cases, but the distance between rows increased to 76.2 cm because of more available space. Seedling emergence was measured 10 d after planting. Radishes were harvested 1 month after planting, and total plant fresh weight and root fresh weight were recorded. Bush beans were harvested 2 months after planting, and aboveground fresh weight, pod fresh weight, and number of pods per plant were recorded.

STATISTICAL ANALYSIS. Response variables from the greenhouse radish experiment were analyzed via analysis of variance (ANOVA) using a linear model with DBDTP concentration and application timing and their interaction using the *lm* function in R (R Foundation for Statistical Computing, Version 4.0.5, Vienna, Austria). Data were combined across trial dates (May and Sept) for germination percentage but analyzed separately for total plant fresh weight and root weight, the reason being interactions between DBDTP concentration and trial date were detected. Total plant fresh weight and root weight data were log-transformed to meet normality and constant variance assumptions. Estimated marginal means were back-transformed to the original scale for presentation. The 0 and 100 ppm fertilizer rates were analyzed separately as each level represented a distinct experiment, where each fertilizer level was analyzed as described previously.

For the radish phytotoxicity trials, phytotoxicity ratings from five plants were averaged for each treatment combination. Phytotoxicity data were analyzed via ANOVA using a linear model for a CRD combined across the two trial dates with application time, DBDTP concentration, and the interaction in the model. Total plant fresh weight for the 8 and 15 d applications was analyzed separately because they were harvested at different ages (8 d after the corresponding application). Therefore, direct comparisons between application times were not conducted. Data for fresh weight were log-transformed, and subsequently estimated marginal means were back-transformed to the original scale.

The data from the radish and bush bean field experiment were analyzed by combining the 2 years (2023 and 2024), using a linear model that included year, DBDTP doses, and their interaction using the *lm* function in R. Log transformation was not necessary, and the assumptions of normality and constant variance were met.

The focus in all experiments was to compare the control (0 mM DBDTP) vs. the tested DBDTP concentrations.

Differences with a *P* value < 0.05 were considered significant. All figures were created using Microsoft Excel (Redmond, WA, USA).

Results and Discussion

The effects of DBDTP were evaluated in 2023 and 2024 on radishes and bush beans under different experimental conditions. Greenhouse radish trials were carried out using 100 ppm NPK fertilizer and 0 ppm NPK, whereas field trials were carried out under normal fertilization conditions. For bush beans, DBDTP effects were tested only under normal field conditions.

Our research aimed to evaluate how H₂S influences germination percentage, overall fresh weight, and root fresh weight under 100 ppm NPK and 0 ppm NPK fertility.

THE EFFECT OF DBDTP ON RADISHES UNDER GREENHOUSE CONDITIONS AT 0 PPM NPK FERTILIZATION. Germination was recorded 7 d after planting. The combined data analysis from two independent trials indicated that DBDTP seed treatments did not impact germination (Fig. 2), although a 3% improvement was seen at 12.5 mM compared with the control, at 0 ppm NPK while germination rates trended down at 200 mM DBDTP for both fertilizer treatments.

Figures 3 and 4 display the doses corresponding to seed treatments on the x-axis, and these doses also correspond to the subsequent drench applications. Radishes were treated first using DBDTP as a seed treatment, and three drench treatments were applied after emergence, resulting in a total of four applications before harvesting.

Overall plant fresh weight was recorded 4 weeks after planting. Data were analyzed for each experiment separately. The results from the first trial revealed an interaction between DBDTP treatments and the number of times that DBDTPs were applied, showing that the 200 mM DBDTP and three applications of 10 mM

Radish Emergence Percentage with DBDTP Seed Treatment at two NPK levels in the Greenhouse

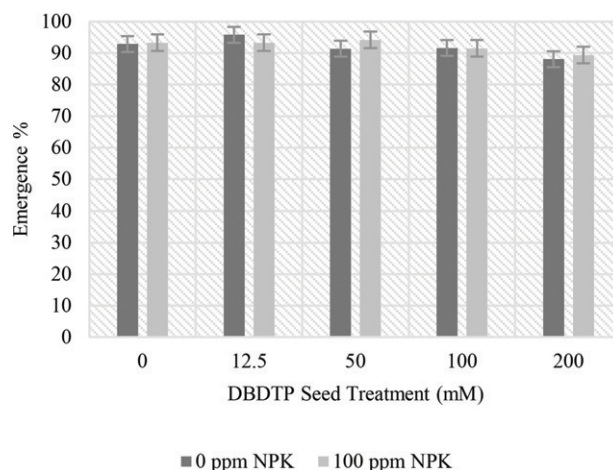
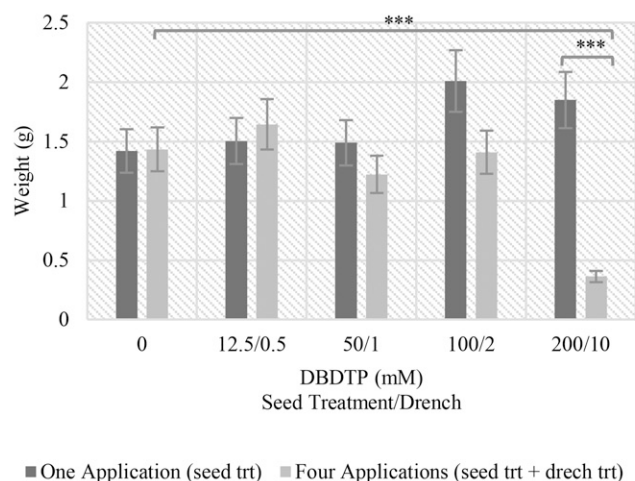
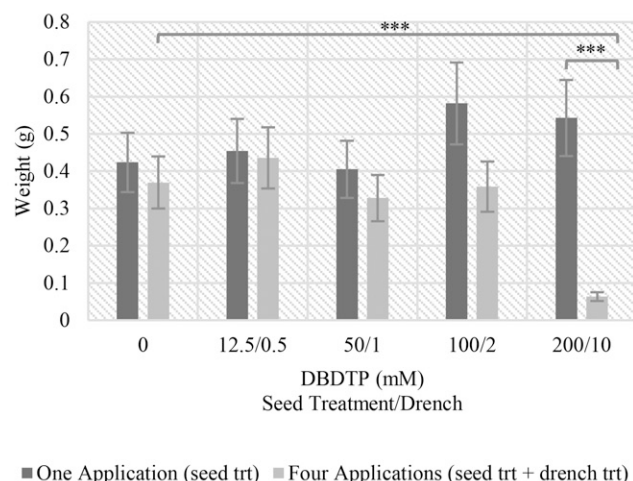


Fig. 2. Effect of dibutyl dithiophosphate (DBDTP) on radish germination. Seedling emergence percentage was measured 7 d after planting. Five DBDTP seed treatment levels were tested using 0 and 100 ppm NPK. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials.

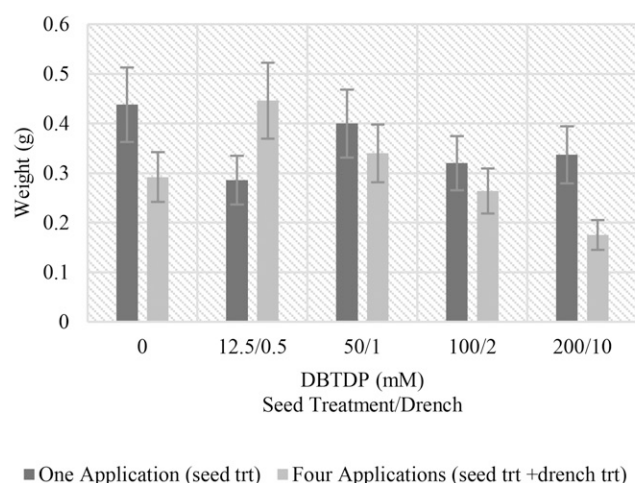
A Radish Plant Fresh Weight with DBDTP Treatments at 0 ppm NPK in the Greenhouse, May 2024 Trial



B Radish Root Fresh Weight with DBDTP Treatments at 0 ppm NPK in the Greenhouse, May 2024 Trial



C Radish Plant Fresh Weight with DBDTP Treatments at 0 ppm NPK in the Greenhouse, Sept 2024 Trial



D Radish Root Fresh Weight with DBDTP Treatments at 0 ppm NPK in the Greenhouse, Sept 2024 Trial

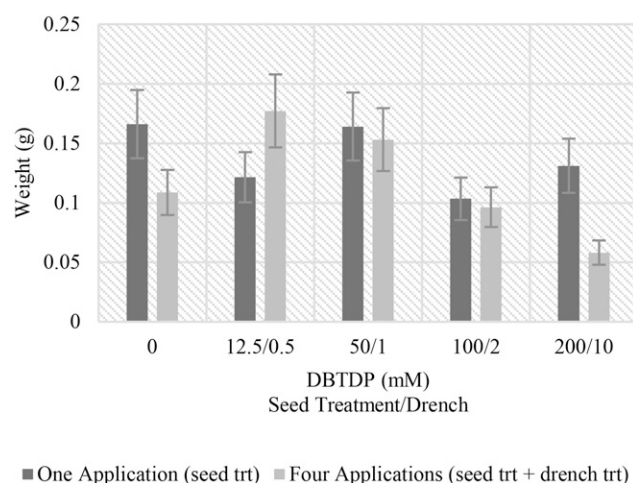


Fig. 3. Impact of dibutyl dithiophosphate (DBDTP) treatment on radish whole plant and root fresh weight. Radish plant and root fresh weight under greenhouse conditions for five DBDTP concentrations at 0 ppm NPK fertilization for the first (May 2024) and the second replication of the experiment (Sep 2024). The x-axis shows the doses used in seed treatments and the doses for the drench applications. (A) Overall fresh weight in May 2024 by number of applications and DBDTP treatments. (B) Root fresh weight by number of applications and DBDTP treatments in May 2024. (C) Overall fresh weight by DBDTP treatments and number of applications in Sep 2024. (D) Root fresh weight in Sep 2024 by DBDTP treatments and the number of applications. The term “one” refers to the treatment group that received only the seed treatment and “four” refers to the group that went through both seed treatment and drench treatments. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials analyzed individually. Statistical comparisons were made against the control in each group (0 mM DBDTP) and each dose by comparing one application vs. four applications. Comparisons were not made between different concentrations. Triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.

DBDTP significantly reduced total plant fresh weight and root fresh weight (Fig. 3A and 3B). This reduction in weight in the 200 mM/10 mM treatment group indicates that repeated high concentrations of H_2S are detrimental to radish growth (Fig. 3A and 3B).

In the second trial, we observed no significant effect for DBDTP concentrations and the number of applications on

overall plant fresh weight, but for root fresh weight, a significant effect was observed. This effect was not due to the difference between the control (0 mM) and the DBDTP concentrations (12.5/0.5, 50/1, 100/2, 200/10 mM) (Fig. 3C and 3D), but rather between DBDTP concentrations themselves.

Although no significant positive effects were detected in May and Sep 2024 with 0 ppm NPK combined with DBDTP treatments,

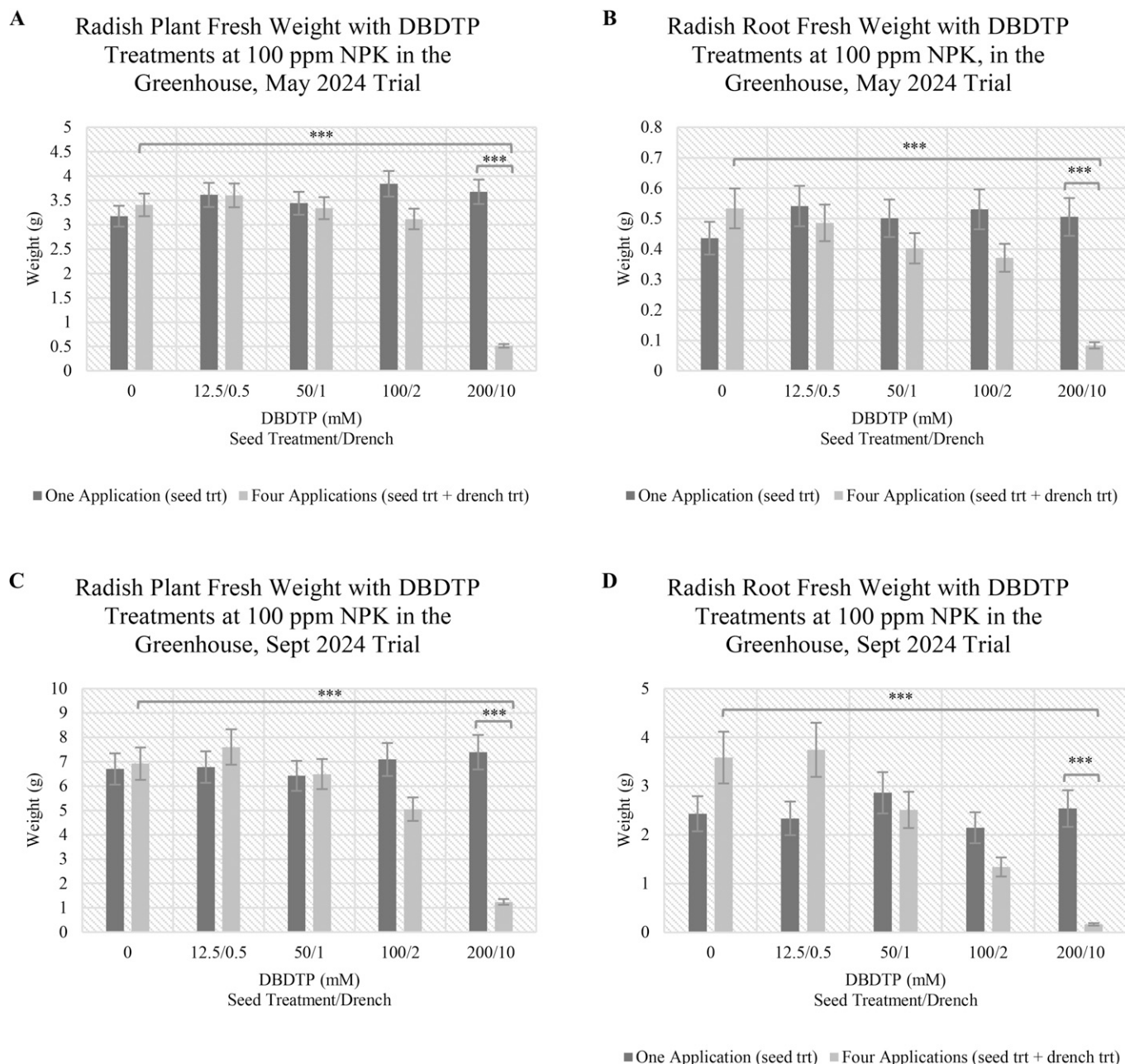


Fig. 4. Radish plant and root fresh weight under greenhouse conditions using 100 ppm NPK and varying levels of dibutyl dithiophosphate (DBDTP). The x-axis shows the doses used in seed treatments and the doses used in the drench applications. (A) Plant fresh weight in May 2024 by number of applications and DBDTP treatments. (B) Root fresh weight in May 2024 by the number of applications and DBDTP treatments. (C) Plant fresh weight in Sep 2024 by number of applications and DBDTP treatments. (D) Root fresh weight in Sep 2024 by the number of applications and DBDTP treatments. The term “one” refers to the treatment group that received only the seed treatment and “four” refers to the group that went through both seed treatment and drench treatment. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials analyzed individually. Statistical comparisons were made against the control in each group (0 mM DBDTP) and within each dose by comparing one application vs. four applications. Comparisons were not made between different concentrations. Triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.

interesting patterns emerged. In May, the 12.5 seed/0.5 mM drench treatment led to an increase of 14% overall fresh weight when DBDTP was applied in total of four times compared with the control, and an increase of 52% in Sep 2024 (Fig. 3A and 3C). However, when comparing a single 12.5 mM application with four applications, the difference between the two was only ~9% in May 2024 and 55% in Sep 2024. In addition, it was noted that a single application of 100 mM and 200 mM increased overall fresh

weight by 41% and 30%, respectively, compared with the control in May 2024, but such an effect was not seen in Sep 2024. In general, when these treatments were applied a total of four times, they resulted in a decrease in fresh weight in both trials (Fig. 3A and 3C). When sulfide is present in wetlands at high concentrations, it leads to toxicity in aquatic organisms (Fort et al. 2017). Lamers et al. (2013) reviewed historical studies of sulfide toxicity, in which a decrease in aboveground productivity has been observed at sulfide

levels ranging from 30 to 8000 $\mu\text{mol}\cdot\text{L}^{-1}$, depending on the species. Sulfide concentrations that are too high can lead to poor nutrient uptake and utilization. This results in the potential inhibition of various oxidases, compromising metabolic capacity, inducing oxidative stress, and reducing gas exchange in the root systems (Allam 1972; Armstrong and Armstrong 2005). Previous studies have shown that higher and continuous exposure to H_2S can lead to negative effects such as necrotic lesions and limitation in plant growth, and affect plant functioning depending on the conditions of the environment and species.

In May, we noticed that root fresh weight at 200 mM and 100 mM, one application, showed similar patterns to overall fresh weight. There was a nonsignificant increase in root weight of 37% and 28% for 100 mM and 200 mM, respectively, compared with the control, but in September, this effect was not present (Fig. 3B and 3D). In addition, the 12.5/0.5 mM treatment combination (total of four applications) led to a nonsignificant 17.8% increase in root weight in May and 63% in September compared with the control (Fig. 3B and 3D).

EFFECT OF DBDTP ON RADISHES UNDER GREENHOUSE CONDITIONS AT 100 PPM FERTILIZATION. Under 100 ppm soluble 20N–20P–20K conditions, DBDTP treatments did not have a significant effect on germination (Fig. 2); all treatments showed a similar response, with positive effects less than 1% compared with the control, suggesting that under suboptimal NPK for radish, DBDTP does not improve germination. Nevertheless, in May and September, a significant interaction was detected between DBDTP treatments and the number of times DBDTP was applied for overall plant and root fresh weight. Our results showed that the highest concentration reduced plant and root fresh weight compared with 0 mM when DBDTPs were applied in total four times (Fig. 4). The patterns seen between each trial varied slightly. In the May 2024 trial, the 12.5 mM treatment increased plant fresh weight by 13.7% after one application and 5.7% after four applications compared with the control (Fig. 4A) (nonsignificant). In the Sep 2024 trial, a nonsignificant 9.8% increase in plant weight was detected when DBDTP was applied in total four times, but no increase was observed with only one DBDTP application (Fig. 4C). In the May trial, the 12.5 mM seed/0.5 mM drench increased overall fresh weight similarly for both application frequencies (one vs. four applications); however, in the September trial, when it was applied four times, it resulted in a nonsignificant 12% weight increase (Fig. 4C) (one vs. four applications). Furthermore, we noted that one application of 100 and 200 mM DBDTP as a seed treatment resulted in a positive trend in overall plant weight by 21% and 15%, respectively, in May, and 5.8% and 10% in September, compared with controls (Fig. 4A and 4C). In the case of root fresh weight, when seed treatments of 12.5, 50, 100, and 200 mM were applied only once in May, they yielded nonsignificant increases of 24%, 15%, 21%, and 16% compared with the control (Fig. 4B). However, applying them four times led to a detrimental effect. In September, on the other hand, the 50 mM DBDTP seed treatment showed a nonsignificant increase of 17% and 200 mM showed 4.4% when they were applied once. The 12.5/0.5 mM treatment increased root weight by 4.4% when it was applied in total four times (nonsignificant), but no improvement was observed when it was applied only once (Fig. 4D).

Our results showed that the deficiency of fertilizer, combined with repeated applications of high DBDTP concentrations, negatively impacted radish growth. Optimal levels of N, P, and K are

vital for proper plant growth, and any deficiency negatively affects plants (Khalofah et al. 2022; Kumar et al. 2021). Previous studies in cabbage have reported that, under normal nitrogen levels, low concentrations of exogenous H_2S can increase biomass and crop quality. However, H_2S concentrations above 2.5 mM led to reductions in biomass and chlorophyll content (Montesinos-Pereira et al. 2016). Eleiwa et al. (2012) reported in potatoes that the application of varying NPK levels in combination with foliarly applied nutrients significantly affects yield, showing that medium or high NPK levels combined with a foliar treatments can lead to greater yields compared with low NPK levels alone. Similarly, Ofue et al. (2024) found that when full rates of NPK are applied alongside different concentrations of pyroligneous acid, tomato plants responded better when full NPK rates were used. These findings reinforce the idea of testing DBDTP under normal fertilizer conditions, especially because our results exhibited positive trends at low DBDTP concentrations under 100 ppm NPK levels.

The contrasting patterns between May and September trials at both 0 and 100 ppm NPK levels suggest that the interaction between NPK level and DBDTP treatments is not consistent across environments. Although 12.5, 50, 100, and 200 mM DBDTP seed treatments enhanced plant and root fresh weight under both 0 and 100 ppm in May, those effects were neutral to negative in the September trial. A likely explanation is that environmental fluctuations (e.g., greenhouse conditions, seasonal temperature and light, or irrigation frequency) affected the plant's response to DBDTP. Because irrigation was applied based on visual drying of the soil surface rather than at fixed intervals, differences in water availability between experimental runs may have influenced nutrient uptake and treatment efficacy. Infrequent watering has been shown to reduce yield in yams, affected flower quality, and decreased flowering in petunias (Santosa et al. 2004; Flohr and Conover 1994); therefore, further research is needed to elucidate clearly the effects of DBDTP under no fertilizer and varying fertilizer conditions in radishes. Nevertheless, 12.5 mM under both fertilizer conditions and in the two DBDTP application regimens showed a more stable behavior, suggesting the potential of these treatments for future experiments.

PHYTOTOXICITY EFFECT OF DBDTP IN FOLIAR APPLICATIONS. Foliar applications of DBDTP were applied 8 and 15 d after germination using concentrations we tested in our previous experiment (0, 0.5, 1, 2, and 10 mM). There was no difference between spraying times on phytotoxicity indicating 1 and 2 week-old plants behaved similarly in response to DBDTP. On the other hand, all DBDTP treatments showed a significant difference with each causing phytotoxicity (Fig. 5A). Even the smallest concentration (0.5 mM) caused a 23.5% increase in phytotoxicity compared with the control (Fig. 5A). Our results showed that on radishes, none of these concentrations should be used in foliar applications. There is a large variation in H_2S susceptibility between species as well as between cultivars of the same species due to plant morphology (Ausma and De Kok 2019), making it important to test different DBDTP concentrations in individual species.

In previous studies, very small concentrations of H_2S (50 μM NaHS) in foliar application improved fresh weight, shoot length, and physiology of soybean plants under cadmium (Cd) stress via sulfur metabolism pathways and expression of genes involved in glutathione synthesis (Oloumi et al. 2024). This indicates that

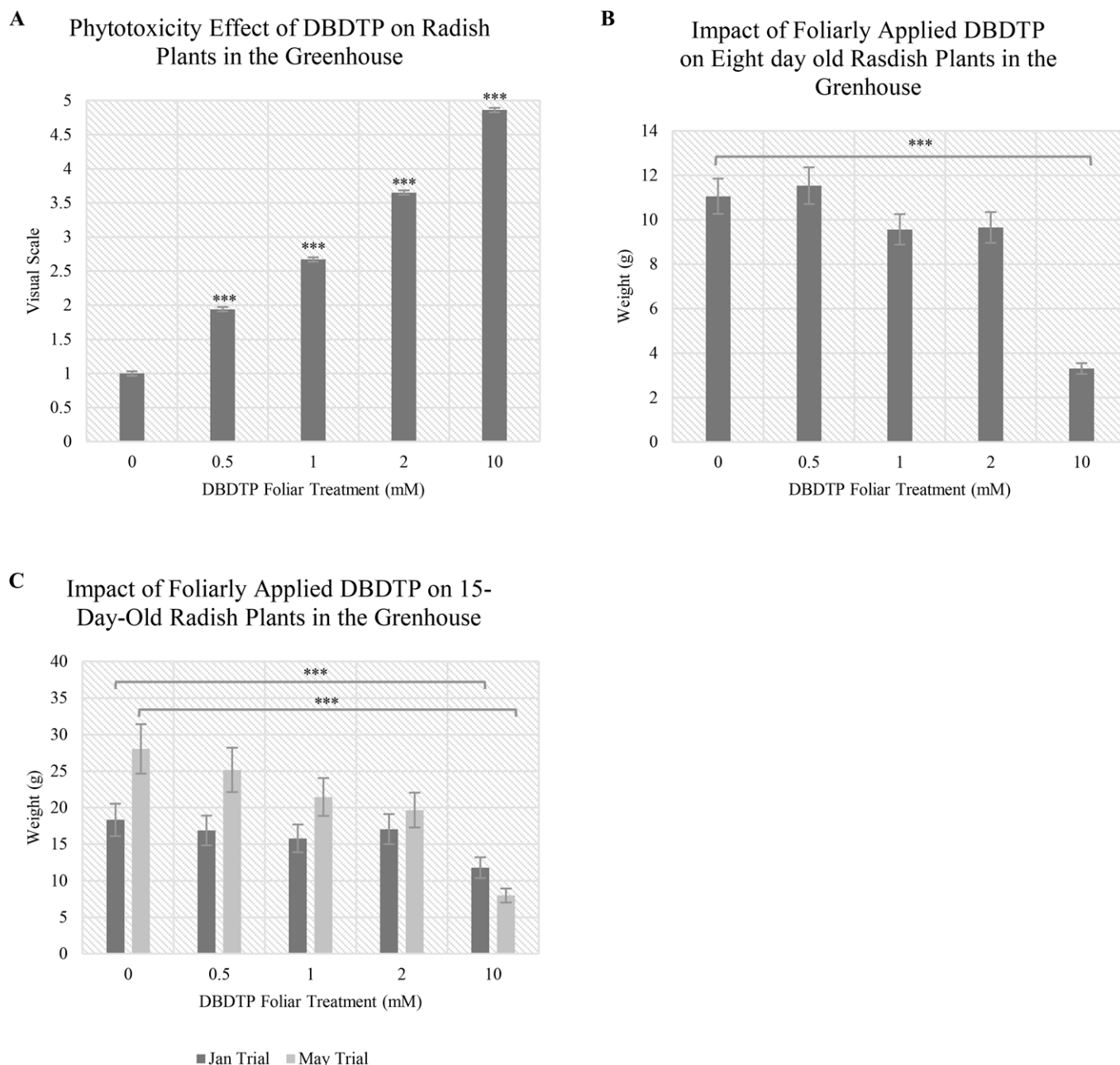


Fig. 5. Phytotoxicity effect and fresh weight of radish plants after dibutyl dithiophosphate (DBDTP) foliar applications. (A) Phytotoxicity effect of DBDTP foliar application on radishes applied at 8 and 15 d after germination (DAG). (B) Plant fresh weight by DBDTP treatments applied to 8 DAG radish plants. (C) Plant fresh weight by DBDTP treatments applied to 15 DAG radish plants. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials analyzed together. Statistical comparisons were made against the control in each group (0 mM DBDTP). Triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.

future experiments should test DDBTP under various scenarios, such as abiotic stress and at lower concentrations.

For plant fresh weight, data were analyzed for each application time individually by combining the data from both trials. This approach was necessary because plants for each application time were harvested 1 week following their respective application, making direct comparisons of weights between different application times impractical. Our analysis revealed that at 8 d after germination (DAG), the effects of DBDTP concentrations were statistically significant, whereas at 15 DAG, there was a significant interaction between DBDTP concentration and application

time. Fresh weight was significantly reduced in both application times when 10 mM was applied (Fig. 5B and 5C). In nearly all treatments after the application of DBDTP, we observed a reduction in fresh weight. These results once again emphasize the necessity of testing doses below 0.5 mM in foliar applications on radishes to improve fresh weight.

Our data illustrate that foliar application of high concentrations (10 mM) of DBDTP in radishes at 8 DAG and 15 DAG decreases plant weight by more than 35%. Prolonged exposures to H_2S in spinach reduces photosynthesis and growth (Maas et al. 1988). Photosynthesis is the cornerstone of plant growth and

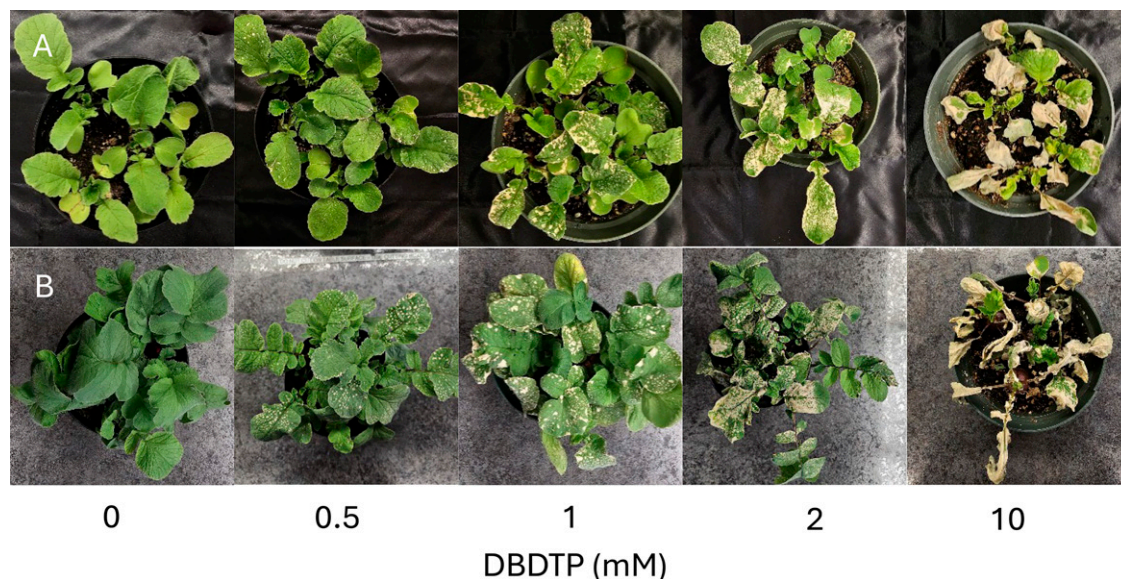


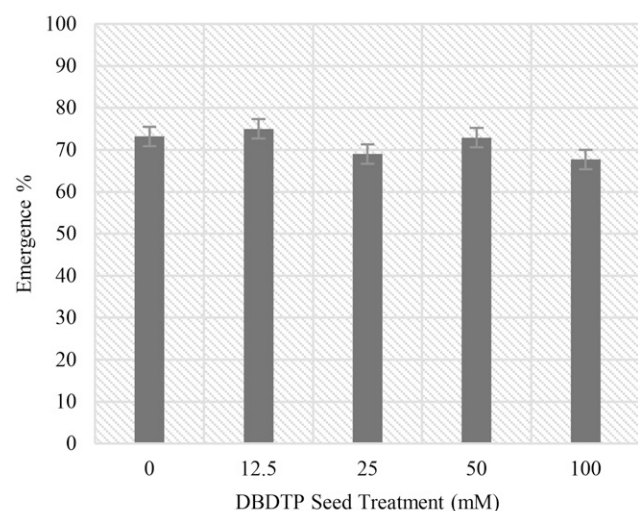
Fig. 6. Phytotoxicity effect of dibutyl dithiophosphate (DBDTP) foliar applications on radish. (A) Representative 8 DAG radish plants 1 week after a DBDTP foliar application. (B) Representative 15 DAG radish plants 1 week after a DBDTP foliar application.

crop productivity, as it provides the primary source of energy for plants (Anbarasan and Ramesh 2022; Gondim and Nolasco-Rondon 2020), therefore affecting the photosynthetic area in plants will affect their growth. Our results confirm that when too much damage is done to radish leaves due to H_2S exposure, a reduction in overall fresh weight results, suggesting that this effect may be due to decreased photosynthesis rates (Fig. 6).

EFFECT OF DBDTP ON RADISHES UNDER FIELD CONDITIONS. DBDTP was administered as a seed treatment and results were combined over 2023 and 2024. Comparisons were made between control plants and those grown from DBDTP-treated seeds. Germination was scored 10 d after planting and DBDTP

did not impact emergence (Fig. 7A). Our results align with those reported by Liu and Lal (2015), who found that H_2S did not significantly increase the emergence of lettuce under laboratory conditions; however, they found that a treatment of 0.01 mM H_2S significantly increased shoot length by 40% and 0.1 mM by 24%. Regarding plant and root fresh weight in this study, DBDTP seed treatments did not have a significant effect (Fig. 7B); however, low concentrations of DBDTP showed a positive trend for both plant and root fresh weight with a 5.2% and 7.2% increase in overall fresh weight, and 7.3% and 12.35% root fresh weight, for 12.5 mM and 25 mM treatments, respectively. These results suggest that DBDTP may improve fresh weight

A Emergence Percentage of Radishes in the Field with DBDTP Seed Treatment



B Plant and Root Fresh Weight of Radish in the Field with DBDTP Seed Treatment

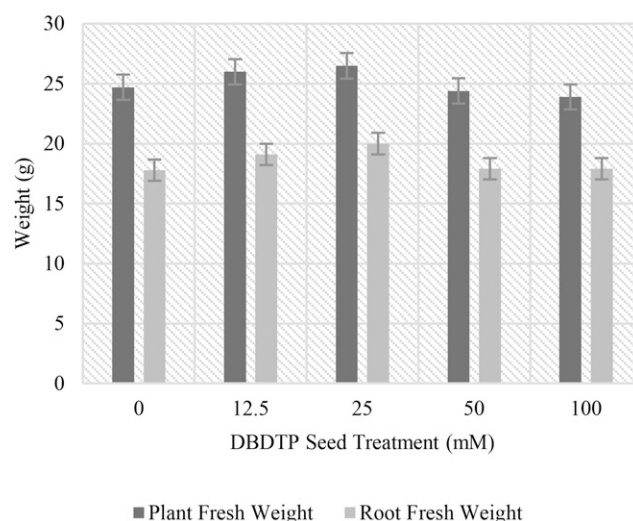
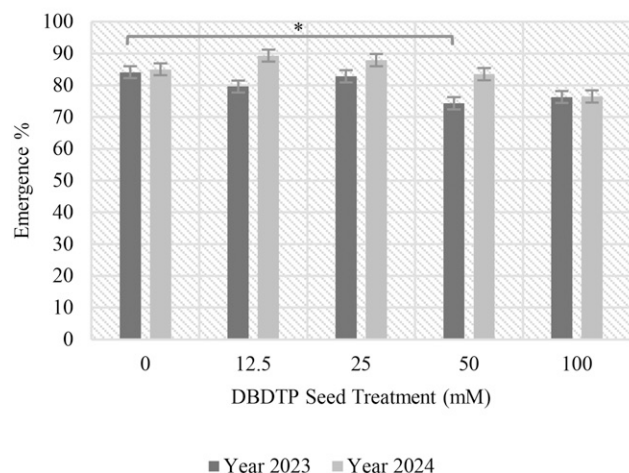
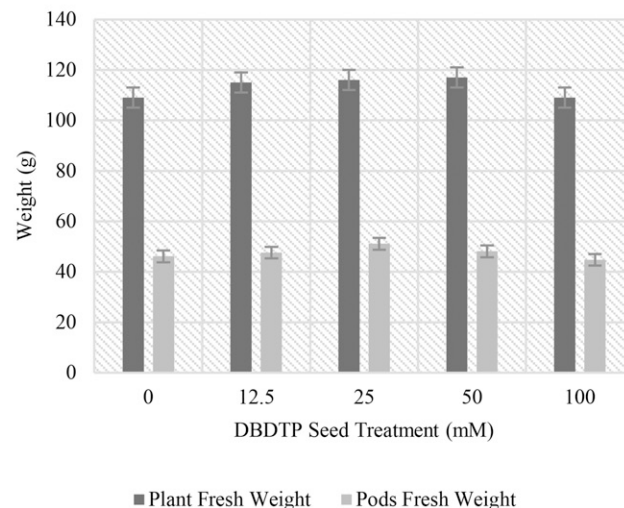


Fig. 7. The effect of dibutyl dithiophosphate (DBDTP) seed treatments on radish seedling emergence, plant, and root fresh weight under field conditions (data from 2023 and 2024). (A) Impact of DBDTP seed treatments on radish emergence. (B) Impact of DBDTP seed treatments on radish plant and root fresh weight. Error bars represent the standard error (*SE*) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm SE$) from two trials. Statistical comparisons were made against the control in each group (0 mM DBDTP). No significant differences were observed.

A Emergence Percentage of Bush Bean in the Field with DBDTP Seed Treatment per Year



B Plant and Pods Fresh Weight of Bush Beans in the Field with DBDTP Seed Treatment



C Number of Pods of Bush Bean in the Field with DBDTP Seed Treatment

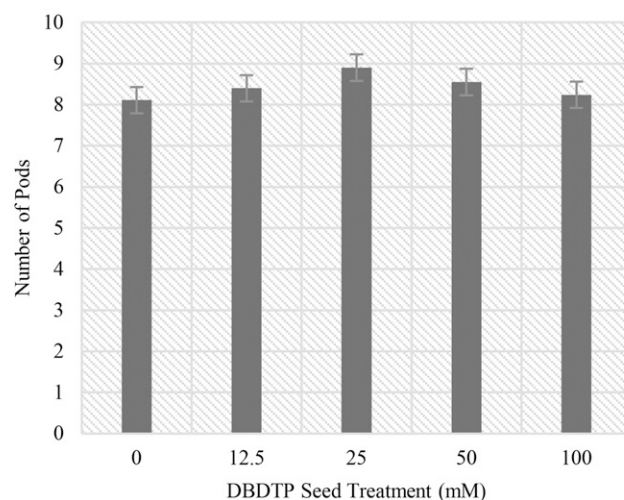


Fig. 8. Emergence percentage, fresh weight, and number of pods in bush beans per plant using dibutyl dithiophosphate (DBDTP) seed treatment in the field. (A) Germination percent of bush beans in the field in 2023 and 2024 using DBDTP seed treatments. (B) Overall fresh weight and pods fresh weight per plant by DBDTP seed treatments in bush beans. (C) Number of pods in bush beans in the field using DBDTP seed treatments. Error bars represent the standard error (*SE*) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ *SE*) from two trials. Statistical comparisons were made against the control (0 mM DBDTP). No significant differences were observed.

for doses within this range. In previous experiments, H_2S was used to boost early wheat growth; the overall mass of the plant, seeds, and roots was significantly higher than the controls for plants exposed to 1, 10, 100, and 500 μM H_2S (Dooley et al. 2013).

EFFECT OF DBDTP ON BUSH BEANS UNDER FIELD CONDITIONS.

The results of exposure of bush beans to DBDTP are presented as mean values averaged over trials in 2023 and 2024. Bean seedling emergence was negatively impacted by DBDTP seed treatment concentration in 2023 in which all DBDTP-treated seed had lower emergence, with a significant decrease in

the 50 mM DBDTP treatment group compared with 0 mM (Fig. 8A). However, in 2024, this effect was not present (Fig. 8A). In fact, in 2024, we observed a slight increase in emergence of 4.3% for 12.5 mM and 2.9% for the 25 mM DBDTP-treated seed.

No significant treatment effects were observed for bean plant or pod fresh weight or number of pods per plant (Fig. 8B and 8C). Nonetheless, treatments of 12.5 mM, 25 mM, and 50 mM exhibited a positive impact on overall fresh weight by 5.50%, 6.42%, and 7.33%, respectively. In addition, pod fresh weight trended 3.25%, 10.84%, and 4.33% higher for the same treatments.

The use of H₂S to mitigate stress has been tested on beans (*Phaseolus vulgaris* L., cv. Gina) grown in a greenhouse under salt stress, where foliar applications of 25 µM H₂S significantly increased shoot fresh mass by 11% (Ekinci et al. 2023). In another study under salinity stress and no salinity stress conditions in pots, using common bean (*P. vulgaris* L. cv. Nebraska), Dawood et al. (2022) reported that under no salinity stress, H₂S significantly enhanced shoot fresh weight by 8.57% at 50 µM and 15.17% at 100 µM compared with the control. These concentrations also significantly increased shoot fresh weight under salt stress conditions. Finally, Dooley et al. (2013) found a significant effect on fresh weight for bean (*P. vulgaris*) seedlings treated with 5 µM and 10 µM H₂S. These results aligned with our findings, indicating that H₂S positively affects fresh weight. These studies reinforce the importance of proceeding with further analysis of DBDTP under stress conditions.

Testing DBDTP as both a seed treatment and a foliar spray treatment provides valuable insights into the various ways in which the H₂S donor DBDTP can be used in agricultural settings. The practice of applying the same products through soil, spray, or seed treatments is well established in the agricultural industry, highlighting the importance of testing DBDTP across different application methods.

The application of agrochemicals in the form of seed treatments is an attractive and easy alternative that allows the reduction of the amount of a.i. per unit area compared with other types of application, the reduction of the release of a.i. into the environment, and the reduction of the use of machinery in the field (Matyjaszczyk 2017; Vojvodić and Bažok 2021). Seed treatment has the potential to fulfill crop micronutrient requirements, improve seedling emergence, stand establishment, yield, root development, early antioxidant metabolism, increase shoot dry weight, and combat diseases and insect pests associated with seed (Amirkhani et al. 2019; Farooq et al. 2012; Ma et al. 2019; Sharma et al. 2015).

Foliar nutrient spray in agriculture is also widespread, because they are potentially more target oriented as compared with other application methods, such as root treatments (Fernández and Eichert 2009). When plant amendments are applied foliarly, they are rapidly absorbed through leaf pores and dispersed across the surface of the leaf by foliar sprays; they can be ingested at rates that are typically higher than those through the roots (Gupta et al. 2023). When working with foliar sprays, it is important to consider various factors, including the environmental conditions present during treatment, volumes, and the physical-chemical properties of the solutions, such as pH, molecular weight, and the concentration of a.i. and adjuvants. These elements are responsible for the possible effects on plants (Fernández and Eichert 2009). The penetration of substances into leaves is a passive process driven by concentration gradients (Fernández and Eichert 2009). Foliar spray applications can be used successfully to supplement micronutrients, address specific nutrient deficiencies during specific growth stages, combat biotic and abiotic stress, and improve growth and yield (Gupta et al. 2023; Lovatt 2013; Matyjaszczyk 2017; Obreza et al. 2008).

In our research, DBDTP has been tested in different contexts: seed treatment, seed treatment in combination with drench application, and foliar treatment. Different application methods may require different amounts (grams, micrograms) of DBDTP depending on the concentration and volume required for each

solution's preparation. For seed treatments, small volumes are used, because seeds are small and merely imbibed to form a more or less continuous layer over the natural seedcoat (Scott 1989). On the other hand, for foliar treatments, higher volumes of water are required to ensure full coverage of the entire leaf area of the plant. Given that the amount of DBDTP (measured in mg) was calculated from volume and the tested concentrations, it is expected that a 100 mM concentration used for seed treatment (at 7% wt/vol) will yield a smaller quantity of DBDTP compared with a 0.5 mM concentration applied as a foliar treatment, in which volume calculations were based on the calibration time of the sprayer for full-sized plants.

The use of different application methods can impact the effect of the explanatory variable on the response variable. Ciccillo et al. (2002) demonstrated that *Burkholderia ambifaria* MCI 7 on maize differs from growth inhibition to growth promotion depending on the method used, showing how different modes of delivery affect the total bacterial community structure of the rhizosphere. In young potted apple trees, it was observed that the method of N application during the growing season significantly influences the growth and biomass partitioning of young apple trees, whereas soil N application promoted more shoot and root growth than foliar application, foliar application enhanced feeder root development and influenced subsequent autumn N uptake patterns (Dong et al. 2005). Similarly, Nadim et al. (2013) found that the application methods of micronutrients significantly impact the growth and yield of wheat. Their results indicated that among foliar treatment, sidedressing, and soil application, the soil application of boron at planting produced a higher number of tillers, number of grains per spike, and the grain yield of wheat.

Although our research did not concentrate on directly comparing all of our application methods, it is important to mention that the foliar spray treatments exhibited phytotoxicity owing to the higher amounts of DBDTP used in each treatment and the direct delivery of DBDTP on photosynthetically active tissue stomata. In contrast, seed treatments tolerated concentrations up to 100 mM without apparent negative effects, because of the smaller volumes used and the protective structures of the seed. Our results underscore that the same compound can produce different outcomes depending on how it is applied. This observation has pointed out the necessity of investigating lower concentrations for foliar applications, as well as the direct comparison of different application methods to determine the most effective way to use DBDTP in agricultural settings.

Conclusions

This work reported how radishes and bush beans responded to low applications of DBDTP that release H₂S under 0 or 100 ppm NPK fertilizer levels in a greenhouse. These results demonstrated that radish seed treatments followed by drench applications have a potential positive effect and that the proper dosing will be in the range of 12.5/0.5 mM for seed treatment/drench levels at 0 and 100 ppm NPK fertilizer levels. A DBDTP H₂S donor seed treatment could be beneficial for improving the growth of radishes under field conditions. This study shows that increased trends of 5.2% and 7.2% in overall fresh weight and 7.3% and 12.35% root fresh weight were observed for 12.5 mM and 25 mM DBDTP seed treatment groups compared with 0-mM DBDTP. Additional experiments are needed to fully

investigate the best application rates, including with less frequent drench applications. Investigating the effect of effect of DBDTP under recommended fertilization doses in soilless culture in the greenhouse is a future goal due to the important interaction between macronutrients in uptake, transport, or assimilation of each other (Khalofah et al. 2022; Kumar et al. 2021), which will likely yield strong, positive results with the application of DBDTP. A study in chickpeas showed that the use of proper fertilization or even an increase of 25% in combination with sulfur can improve yield, height, number of leaves, and number of branches (Mishra et al. 2023).

The phytotoxicity results showed a negative effect at all concentrations investigated. The foliar application of the treatments 0.5 mM, 1 mM, 2 mM, and 10 mM produced phytotoxicity when they were applied 8 or 15 DAG. These results suggest that lower concentrations of foliar treatments need to be tested.

The effect of DBDTP seed treatments on bush beans under field conditions showed that 12.5 mM, 25 mM, and 50 mM treatment levels showed positive trends in overall fresh weight, increasing 5.50%, 6.42%, and 7.33%, respectively, and pod fresh weight by 3.25%, 10.84%, and 4.33% compared with the control. These results suggest the potential of DBDTP for future testing under diverse conditions, such as when abiotic stress is present.

The importance of DBDTPs lies not only in their positive effect on improving plant growth but also in their ability to provide dual benefits to plants under stress. Although much literature emphasizes the positive effects of H₂S under abiotic stresses, only a few reports mention its benefits under normal growth conditions. Therefore, having a compound such as DBDTP that can improve plant growth and provide resistance to abiotic stress would be a valuable tool for agriculture. Although it is difficult to obtain statistically significant differences for small changes in yield, these small improvements in yield may become economically important for large-scale production.

References Cited

- Ahuja KL, Singh H, Raheja RK, Labana KS. 1987. The oil content and fatty acid composition of various genotypes of cauliflower, turnip and radish. *Plant Food Human Nutr.* 37(1):33–40. <https://doi.org/10.1007/bf01092298>.
- Allam AI. 1972. Sulfide inhibition of oxidases in rice roots. *Phytopathology.* 62(6):634–634. <https://doi.org/10.1094/Phyto-62-634>.
- Amirkhani M, Mayton HS, Netravali AN, Taylor AG. 2019. A seed coating delivery system for bio-based biostimulants to enhance plant growth. *Sustainability.* 11(19):5304. <https://doi.org/10.3390/su11195304>.
- Anbarasan S, Ramesh S. 2022. Photosynthesis efficiency: Advances and challenges in improving crop yield. *PSA.* 7(3):19–21. <https://doi.org/10.51470/PSA.2022.7.3.19>.
- Armstrong J, Armstrong W. 2005. Rice: Sulfide-induced barriers to root radial oxygen loss, Fe²⁺ and water uptake, and lateral root emergence. *Ann Bot.* 96(4):625–638. <https://doi.org/10.1093/aob/mci215>.
- Ausma T, De Kok LJ. 2019. Atmospheric H₂S: Impact on plant functioning. *Front Plant Sci.* 10:743. <https://doi.org/10.3389/fpls.2019.00743>.
- Bloem E, Riemenschneider A, Volker J, Papenbrock J, Schmidt A, Salac I, Haneklaus S, Schnug E. 2004. Sulphur supply and infection with *Pyrenopeziza brassicae* influence L-cysteine desulphydrase activity in *Brassica napus* L. *J Exp Bot.* 55(406):2305–2312. <https://doi.org/10.1093/jxb/erh236>.
- Broughton WJ, Hernández G, Blair M, Beebe S, Gepts P, Vanderleyden J. 2003. Beans (*Phaseolus* spp.) – model food legumes. *J Agric Plant Soil.* 252(1):55–128. <https://doi.org/10.1023/a:1024146710611>.
- Carter JM, Brown EM, Irish EE, Bowden NB. 2019. Characterization of Dialkylthiophosphates as Slow Hydrogen Sulfide Releasing Chemicals and Their Effect on the Growth of Maize. *J Agric Food Chem.* 67(43):11883–11892. <https://doi.org/10.1021/acs.jafc.9b04398>.
- Ciccillo F, Fiore A, Bevivino A, Dalmastri C, Tabacchioni S, Chiarini L. 2002. Effects of two different application methods of *Burkholderia ambifaria* MCI 7 on plant growth and rhizospheric bacterial diversity. *Environ Microbiol.* 4(4):238–245. <https://doi.org/10.1046/j.1462-2920.2002.00291.x>.
- Dawood MFA, Sofy MR, Mohamed HI, Sofy AR, Abdel-Kader HAA. 2022. Hydrogen sulfide modulates salinity stress in common bean plants by maintaining osmolytes and regulating nitric oxide levels and antioxidant enzyme expression. *J Soil Sci Plant Nutr.* 22(3):3708–3726. <https://doi.org/10.1007/s42729-022-00921-w>.
- Dong S, Cheng L, Scagel CF, Fuchigami LH. 2005. Method of nitrogen application in summer affects plant growth and nitrogen uptake in autumn in young Fuji/M.26 apple trees. *Commun Soil Sci Plant Anal.* 36(11–12):1465–1477. <https://doi.org/10.1081/CSS-200058491>.
- Dooley FD, Nair SP, Ward PD. 2013. Increased growth and germination success in plants following hydrogen sulfide administration. *PLoS One.* 8(4):e62048. <https://doi.org/10.1371/journal.pone.0062048>.
- Ekinci M, Turan M, Ors S, Dursun A, Yildirim E. 2023. Improving salt tolerance of bean (*Phaseolus vulgaris* L.) with hydrogen sulfide. *Photosynthetica.* 61(1):25–36. <https://doi.org/10.32615/ps.2023.005>.
- Eleiwa M, Ibrahim SA, Mohamed MF. 2012. Combined effect of NPK levels and foliar nutritional compounds on growth and yield parameters of potato plants (*Solanum tuberosum* L.). *Afr J Microbiol Res.* 6(24):5100–5109. <https://doi.org/10.5897/AJMR12.085>.
- Farooq M, Wahid A, Siddique KHM. 2012. Micronutrient application through seed treatments: A review. *J Soil Sci Plant Nutr.* 12(1):125–142. <https://doi.org/10.4067/S0718-95162012000100011>.
- Fernández V, Eichert T. 2009. Uptake of hydrophilic solutes through plant leaves: Current state of knowledge and perspectives of foliar fertilization. *CRC Crit Rev Plant Sci.* 28(1–2):36–68. <https://doi.org/10.1080/07352680902743069>.
- Flohr RC, Conover CA. 1994. Production and postproduction watering schedule effects on plant growth, quality and blooming of pansies and petunias. *Proc Fla State Hortic Soc.* 107:414–416. <https://journals.flvc.org/fshs/article/download/92449/88641>.
- Fort DJ, Todhunter K, Fort TD, Mathis MB, Walker R, Hansel M, Hall S, Richards R, Anderson K. 2017. Toxicity of sulfide to early life stages of wild rice (*Zizania palustris*). *Environ Toxicol Chem.* 36(8):2217–2226. <https://doi.org/10.1002/etc.3759>.
- Gondim E, Nolasco-Rondon J. 2020. The crucial role of photosynthesis in sustaining plant life and its impact on the global ecosystem, climate, and human survival. *Australian Herbal Insight.* 3(1):1–5. <https://doi.org/10.25163/ahi.319925>.
- Gupta S, Kaur N, Kant K, Jindal P, Ali A, Naeem M. 2023. Calcium: A master regulator of stress tolerance in plants. *S Afr J Bot.* 163:580–594. <https://doi.org/10.1016/j.sajb.2023.10.047>.
- Hancock JT, Whiteman M. 2014. Hydrogen sulfide and cell signaling: Team player or referee? *Plant Physiol Biochem.* 78:37–42. <https://doi.org/10.1016/j.plaphy.2014.02.012>.
- Khalofah A, Ghramh HA, Al-Qthanin RN, L'taief B. 2022. The impact of NPK fertilizer on growth and nutrient accumulation in juniper (*Juniperus procera*) trees grown on fire-damaged and intact soils. *PLoS One.* 17(1):e0262685. <https://doi.org/10.1371/journal.pone.0262685>.
- Kumar S, Kumar S, Mohapatra T. 2021. Interaction between macro- and micro-nutrients in plants. *Front Plant Sci.* 12:665583. <https://doi.org/10.3389/fpls.2021.665583>.
- Lamers LPM, Govers LL, Janssen ICJM, Geurts JJM, Van der Welle MEW, Van Katwijk MM, Van der Heide T, Roelofs JGM, Smolders AJP. 2013. Sulfide as a soil phytotoxin—a review. *Front Plant Sci.* 4:268. <https://doi.org/10.3389/fpls.2013.00268>.

- Li ZB, Zhu L. 2014. Hydrogen sulfide donor sodium hydrosulfide-induced accumulation of betaine is involved in the acquisition of heat tolerance in maize seedlings. *Braz J Bot.* 38(1):31–38. <https://doi.org/10.1007/s40415-014-0106-x>.
- Liu R, Lal R. 2015. Effects of low-level aqueous hydrogen sulfide and other sulfur species on lettuce (*Lactuca sativa*) seed germination. *Commun Soil Sci Plant Anal.* 46(5):576–587. <https://doi.org/10.1080/00103624.2014.998341>.
- Lovatt CJ. 2013. Properly timing foliar-applied fertilizers increases efficacy: A review and update on timing foliar nutrient applications to citrus and avocado. *HortTechnology.* 23(5):536–541. <https://doi.org/10.21273/HORTTECH.23.5.536>.
- Ma Y, Látr A, Rocha I, Freitas H, Vosátka M, Oliveira RS. 2019. Delivery of inoculum of *Rhizophagus irregularis* via seed coating in combination with *Pseudomonas libanensis* for cowpea production. *Agronomy.* 9(1):33–33. <https://doi.org/10.3390/agronomy9010033>.
- Maas FM, Van Loo EN, Van Hasselt PR. 1988. Effect of long-term H₂S fumigation on photosynthesis in spinach. Correlation between CO₂ fixation and chlorophyll *a* fluorescence. *Physiol Plant.* 72(1):77–83. <https://doi.org/10.1111/j.1399-3054.1988.tb06625.x>.
- Matyjaszczyk E. 2017. Comparison between seed and foliar treatment as a tool in integrated pest management. *J Agric Food Chem.* 65(30):6081–6086. <https://doi.org/10.1021/acs.jafc.7b01095>.
- Mishra A, Gupta H, Mishra US, Sirothia P, Darwai P. 2023. Interaction effect of sulphur and NPK on growth parameters and yield of mustard. *IJPSS.* 35(15):138–144. <https://doi.org/10.9734/ijpss/2023/v35i153089>.
- Montesinos-Pereira D, Barrameda-Medina Y, Baenas N, Moreno DA, Sánchez-Rodríguez E, Ruiz JM. 2016. Evaluation of hydrogen sulfide supply to biostimulate the nutritive and phytochemical quality and the antioxidant capacity of cabbage (*Brassica oleracea* L. “Bronco”). *J Appl Bot Food Qual.* 89:290–298. <https://doi.org/10.5073/jabfq.2016.089.038>.
- Nadim MA, Awan IU, Baloch MS, Khan N, Naveed K. 2013. Micronutrient use efficiency in wheat as affected by different application methods. *ResearchGate.* https://www.researchgate.net/publication/287063848_Micronutrient_use_efficiency_in_wheat_as_affected_by_different_application_methods.
- Nishio T. 2017. Economic and academic importance of radish, p 1–10. In: Nishio T, Kitashiba H (eds). *The Radish Genome. Compendium of plant genomes.* Springer International Publishing AG, Cham, Switzerland. https://doi.org/10.1007/978-3-319-59253-4_1.
- Obreza TA, Boman BJ, Zekri M, Futch SH. 2008. Methods of Fertilizer Application, p 46–47. In: *Nutrition of Florida Citrus Trees* (2nd Edition). Cooperative Extension Service, University of Florida, Institute of Food and Agricultural Sciences, FL, USA. <https://journals.flvc.org/edis/article/view/117225/115342>.
- Ofoe R, Nasir M, Thomas RH, Abbey L. 2024. Foliar application of pyroligneous acid acts synergistically with fertilizer to improve the productivity and phytochemical properties of greenhouse-grown tomato. *Sci Rep.* 14(1):1934. <https://doi.org/10.1038/s41598-024-52026-2>.
- Oloumi H, Maleki M, Habibipour L, Lotfi S. 2024. Foliar application of NaHS alleviates Cd toxicity in soybean plants through regulation of Glutathione metabolism. *Plant Stress.* 11:100363. <https://doi.org/10.1016/j.stress.2024.100363>.
- Quais M, Jahan S, Haque M, Khan M. 2013. Variation In Seed Quality Of Radish Preserved In Different Storage Containers. *Bangladesh J Agric Res.* 38(3):545–552. <https://doi.org/10.3329/bjar.v38i3.16981>.
- Reinders. 2022. Profile Products - Profile Greens Grade - 50 LB BAG. *Reinders.com.* <https://www.reinders.com/profile-products-profile-greens-grade-50-lbs-bag/>.
- Santosa E, Sugiyama N, Sulistyono E, Sopandie D. 2004. Effects of Watering Frequency on the Growth of Elephant Foot Yams. *Jpn J Trop Agric.* 48(4):235–239. <https://doi.org/10.11248/jsta1957.48.235>.
- Scott JM. 1989. Seed coatings and treatments and their effects on plant establishment. *Advances in Agronomy.* 42:43–83. [https://doi.org/10.1016/S0065-2113\(08\)60523-4](https://doi.org/10.1016/S0065-2113(08)60523-4).
- Sharma KK, Singh US, Sharma P, Kumar A, Sharma L. 2015. Seed treatments for sustainable agriculture-A review. *JANS.* 7(1):521–539. <https://doi.org/10.31018/jans.v7i1.641>.
- Sparks B. (2023, September 19). How mineral nutrition impacts indoor-grown radishes. *Greenhouse Grower.* <https://www.greenhousegrower.com/crops/how-mineral-nutrition-impacts-indoor-grown-radishes/>.
- Vojvodić M, Bažok R. 2021. Future of insecticide seed treatment. *Sustainability.* 13(16):8792. <https://doi.org/10.3390/su13168792>.
- Wang R. 2002. Two's company, three's a crowd: Can H₂S be the third endogenous gaseous transmitter? *FASEB J.* 16(13):1792–1798. <https://doi.org/10.1096/fj.02-0211hyp>.