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Article

Microbes Cultured from Garden Soil Positively Impact Seed Germination and Plant Growth

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SUMMARY

Soil microbes may act as pathogens — deteriorating the health of plants — or support the survival of plants. In the present study, we investigated the effect of microorganisms cultured from native soil on seed germination and plant growth. We hypothesized that the soil contained microorganisms supporting seed germination and plant growth. Our results showed that the addition of cultured microbes from domestic soil significantly enhanced the germination frequency of mung bean seeds compared to their controls, which received uncultured broth. Furthermore, the heights of these plants after seven days of intervention were significantly different — the microbe-supplied plants were taller than the controls. When we investigated the addition of similar cultured microbes into the soil of growing pumpkin and pea flower plants, we found that plants provided with microbial culture were significantly taller than their corresponding controls. Taken together, these findings show that domestic soil has beneficial microorganisms that assist in seed germination and plant growth. We can, therefore, better appreciate the beneficial plant-microorganism interactions and exploit them to enhance plant health and productivity.

INTRODUCTION

Plants are an important part of our ecosystem. They are the producers of two life essentials: oxygen and food. Thus, it is wise to ensure the health of these organisms. By understanding the process of plant propagation and maintenance, we can improve the dissemination and survival of plants on this planet, which in turn contribute to the overall health of the ecosystem.

Soil is vital for land plants. It essentially consists of a complex mixture of dirt, nutrients, microorganisms, insects, and worms. Thus, the soil is a dynamic habitat in which different organisms cooperate or compete to survive. In this regard, organisms in the soil affect plant health in two contradictory ways — they can enhance plant growth or cause plant diseases (1). Nonetheless, there are also documented soil microorganisms that have neutral interactions with plants, such as saprophytic microbes (2).

Beneficial soil microorganisms primarily affect plant health by increasing nutrient availability, producing favorable molecules, or suppressing plant diseases (3). In return, these organisms obtain energy from their host. Soil microbes often interact with the roots of plants; together they are known as rhizospheres (4). They convert certain nutrients in the soil, such as phosphorus, into forms that are readily available for plant root uptake (3). Additionally, soil microorganisms release some biological molecules, known as phytohormones, which promote plant growth (5). In terms of disease suppression, beneficial soil microorganisms feed on pathogenic ones or improve the plant's natural defense system (6). Furthermore, they compete with pathogenic microbes for nutrients or space to reduce their growth, produce metabolites that kill them, or block their access to the plant root (6).

Due to their assistance in maintaining plant health, beneficial soil bacteria and fungus are now commercially available (7). This includes isolates from the genera *Azospirillum*, *Rhizobium,* *Bacillus*, *Streptomyces*, *Pseudomonas*, *Trichoderma*, and *Glomus* (7, 8). The beneficial soil bacteria are often collectively referred to as plant growth-promoting rhizobacteria (PGPR) (9). They predominantly improve nutrient availability to the plant, though they can also control pathogen growth and prompt systemic resistance in some plant species (9). On the other hand, the mycoparasite *Trichoderma harzianum* helps prevent plant diseases (10), while the mycorrhizal fungi of the genus *Glomus* provide nutrients to host plants (11). Thus, different soil microorganisms can be advantageously utilized to increase agricultural crop yields and quality, as well as manage the health and appearance of domestic plants.

In this study, we investigated the role of the natural soil microbe in two different aspects of the plant life cycle — seed germination and plant growth. Germination is the process by which a seed emerges from the seed coat. It is a crucial step in a plant's life as it strongly influences the plant's subsequent growth. For germination to occur, the environmental conditions must trigger and allow the seed to go through the imbibition phase (12). Imbibition occurs when water absorbed by the seed tissues induces swelling until the seed splits open, allowing it to activate root growth (12). Among other environmental factors, soil conditions play a crucial role in this process (13). Similarly, soil factors also affect the growth of stem and roots (14). Thus, by testing the different soil variables, we can better understand and control the propagation and growth of plants.

Based on this collective knowledge, we hypothesized that the increased presence of natural microbes in the soil would positively impact seed germination and plant growth. To study this, we first cultured microbes from domestic soil and then added the culture onto a paper towel containing the seeds or into the soil of growing plants. Our findings revealed that the addition of microbes onto the paper towel containing mung bean seeds improved seed germination. Further, a similar addition of soil microbes into the soil of the growing plant

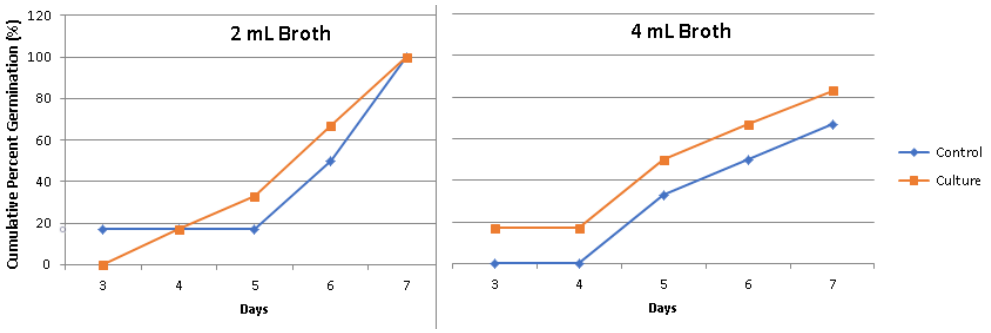

Figure 1: Addition of microbial culture to paper towel increased the percentage of germinated mung bean seeds. On Day 3-7, the cumulative percentage of germinated mung bean seeds (n = 6 seeds for each group) was recorded for seeds given 2 mL (left) or 4 mL (right) of either LB broth (control; blue) or microbial culture (orange).

DAY	Cumulative Percentage of Germinated Seeds (%)			
	2 mL Broth		4 mL Broth	
	CONTROL	CULTURE	CONTROL	CULTURE
3	16.6	0	0	16.6
4	16.6	16.6	0	16.6
5	16.6	33.3	33.3	50.0
6	50.0	66.6	50.0	66.6
7	100.0	100.0	66.6	83.3

Table 1: Cumulative percentage of germinated mung bean seeds grown in control broth or microbial culture

accelerated the growth of pumpkin and pea flower plants. Taken together, this study illustrates the beneficial role that soil microorganisms play in the life cycle of plants.

RESULTS

We first tested the normal sprouting and growth of a few plants, including pumpkin, pea flower, tomato, green bean, and mung bean plants. From these studies, we decided to test mung bean seeds for the germination study as it was easy to sprout the seeds on a moist paper towel. We selected pumpkin and pea flower plants for the growth study as they were relatively fast-growing, thereby having a measurable height change each day.

To test the effect of natural soil microbes on the germination of seeds, we first generated microbial cultures by inoculating a sample of garden soil in Luria-Bertani (LB) broth. We did not isolate any single type of soil microorganism for the culture as we aimed to observe the collective effect of all the microorganisms present in the soil.

On Day 0, we randomly segregated mung bean seeds into four groups, with each group containing six seeds. We then placed each group of seeds on separate paper towels. We added 2 mL or 4 mL of the microbial culture or the uncultured LB broth (experimental control) to the paper towels.

Germination of the seeds started on Day 3 onwards. Compared to their corresponding controls, seeds supplied with microbial culture had a faster germination rate (**Table 1 & Figure 1**). This was obvious on Day 6 — only 3 out of 6 seeds (50%) germinated for the two control groups compared to the 4 seeds that germinated (66.7%) in the microbial test groups (**Table 1**). Further, in the groups supplied with 4 mL of broth, only 4 seeds (66.7%) germinated in the control group, but 5 seeds (83.3%) germinated in the microbial test group at the end of the study (Day 7) (**Table 1**).

On Day 7, we measured the height of each germinated plant. In both experimental sets of plants, seeds supplied with the microbial culture had increased growth compared to their controls (**Figure 2**). However, only the 4 mL group (p-value = 0.04) showed a significant difference. This shows that the microbe in the culture did not only help germination, but it also helped the initial growth of the shoot and root.

We next investigated the effect of soil microbes on the growth of pumpkin and pea flower plants. After a week of growing in soil, plants were randomly segregated into three groups and supplied with microbial culture or uncultured LB broth (control). All plants had similar average heights before the addition of the broth to the soil (Day 0). Pumpkin plants supplied with microbial culture had increased growth compared to the LB broth control (**Table 2 & Figure 3**). This

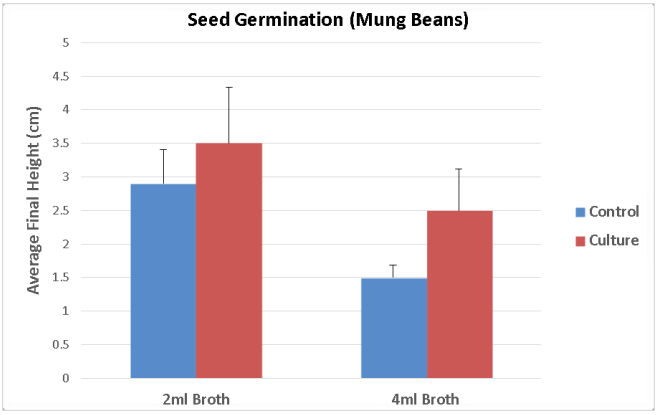

Figure 2: Addition of microbial culture to paper towel significantly increased average germinated mung bean seed height. The average final (Day 7) height (cm) of germinated mung bean seeds was measured and recorded for seeds given 2 mL (left) or 4 mL (right) of either LB broth (control; blue) or microbial culture (red). Error bars represent standard deviation. There was no significant difference between control and microbial culture groups with 2 mL (p-value = 0.08), but there was a significant difference in final height with 4 mL of microbial culture (p-value = 0.04).

Day	Plant Height (cm)					
	0	1	2	3	4	5
Control	1.5	3.5	3.8	5.6	6.4	6.8
	1.6	3.5	4.2	6.0	6.2	7.1
	1.7	3.5	4.0	5.8	6.6	6.7
Culture	1.6	3.8	6.0	6.8	7.5	7.8
	1.4	4.2	5.9	6.7	7.6	8.3
	1.8	4.0	6.1	6.9	7.4	7.5

Table 2: Heights of individual pumpkin plants measured in centimeters on different days.

increase was consistently noted from Day 1 until Day 5. The final average height of the plants (Day 5) was significantly higher (p-value = 0.009) in the microbial test group compared to the control group. This shows that the microorganisms in the culture aided in the growth of pumpkin plants.

Similar to the results of pumpkin plants, pea flower plants supplied with the microbial culture also showed an increase in growth rate compared to the control group (**Table 3 & Figure 4**). The difference was apparent from Day 3 onwards. At the end of the experiment, the height of the microbial test group was significantly higher than that of the control group (p-value = 0.005). Collectively, these results show that the microorganisms in the culture aided in the growth of both pumpkin and pea flower plants.

DISCUSSION

In this study, we showed that microorganisms cultured from soil taken from a domestic garden supported the germination and growth of plants. Our results showed that the addition of cultured soil microbes significantly increased the germination frequency of mung bean seeds and the height of the plants measured after seven days of intervention. Likewise, we found that growing pumpkin and pea flower plants provided with microbial culture were significantly taller than their corresponding controls that received uncultured broth. The results of this experiment may or may not be replicated in other plants or soil sources as different species of plants react with beneficial or pathogenic microbes differently.

In the seed germination study, mung bean seeds supplied with 4 mL of microbial culture, but not those supplied with 2 mL of culture, showed statistically significant increases in final plant heights. The observed difference between the two sets suggests that the 2 mL culture contained an insufficient number of microbes to induce a significant increase in plant heights on Day 7. The difference could also be due to the small sample size, e.g. six seeds per group; the 2 mL experimental group may have shown higher significance if the sample size was increased.

Interestingly, we observed a plastic-like layer, possibly the thickened cuticle, on the leaves of the pumpkin plants supplied with microbial culture, though the same was not observed in the control plants. As the waxy cuticle is essential to prevent water loss and external invasion (15, 16), we speculate that the cultured bacteria advantageously induced the thickening of the cuticle. However, whether this adaptation aided in the boosted growth warrants further investigation.

In our experiments, we used LB broth to culture the soil microorganisms. LB broth is ideal for the culture of bacteria; however, it may also support the growth of other types of microorganisms (17). We did not select or characterize the microorganisms present in our microbial culture, as it was beyond the scope of this study. Thus, the effect observed may be contributed by several types of soil microbes, including those from the well-documented bacterial genera *Azospirillum* and *Rhizobium* (7). Future studies can specifically isolate and study the detailed mechanisms of

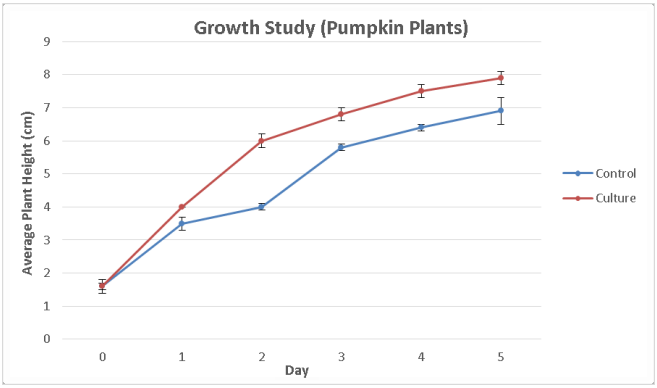

Figure 3: Addition of microbial culture to soil significantly increased average height of pumpkin plants. On Day 0-5, the heights of pumpkin plants (n = 3 plants for each group) was measured and recorded for plants given either LB broth (control; blue) or microbial culture (red). Error bars show the standard deviation of the replicates. There was a significant difference between the heights of control and microbial culture groups on Day 5 (p-value = 0.009).

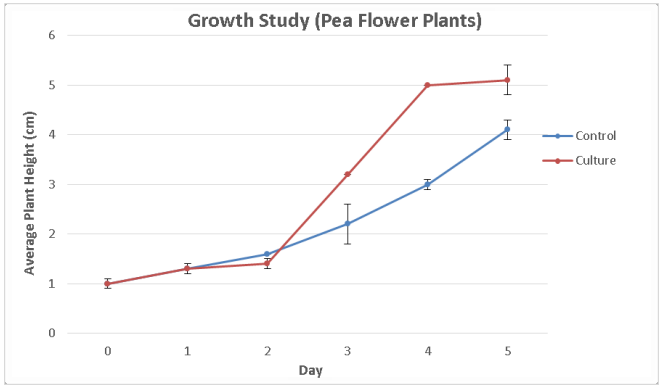

Figure 4: Addition of microbial culture to soil significantly increased average height of pea flower plants. On Day 0-5, the heights of pea flower plants (n = 3 plants for each group) was measured and recorded for plants given either LB broth (control; blue) or microbial culture (red). Error bars show the standard deviation of the replicates. There was a significant difference between the heights of control and microbial culture groups on Day 5 (p-value = 0.005).

Day	Plant Height (cm)					
	0	1	2	3	4	5
Control	0.9	1.3	1.6	2.2	3.1	4.0
	1.0	1.3	1.6	2.6	3.0	4.3
	1.1	1.3	1.6	1.9	2.9	3.9
Culture	1.0	1.3	1.4	3.2	5.0	4.7
	1.0	1.4	1.5	3.2	5.0	5.3
	1.0	1.2	1.3	3.2	5.0	5.2

Table 3: Heights of individual pea flower plants measured in centimeters on different days.

these microorganisms. It will also be interesting to note if these microorganisms are similar to those used commercially in agriculture (8).

Similarly, future studies could be improved by baking the soil in an oven before adding the microbial culture. This step can kill and offset the existing microorganism in the soil so that they do not influence the outcome of the study. Also, a separate experiment using baked (sterilized) and unbaked soil (control) could be conducted to investigate how removing microorganisms from the soil affects the germination and growth of the plants (18). These experiments would complement the results derived from the present study.

As we conducted the germination and growth study in the plant's natural environment, many other factors could have contributed to the outcome of this study. This includes the amount of sunlight, humidity, and the presence of wind. We did not grow the plants in a controlled environment (i.e. plant incubator or growth chamber) due to cost limitation. Therefore, it is important to consider these factors when generalizing the conclusions of this study.

Currently, there is a big knowledge gap concerning plant-microorganism interaction. This study provides useful information for us to appreciate the role of beneficial microbes in native soil. As these microorganisms are naturally present in the soil, we can identify soil rich in beneficial microbes to potentially preserve and cultivate them for agriculture. Further, if we can easily culture these microbes by inoculating the microbe-rich soil into less fertile land, we can optimize more land for crop production.

With more future studies investigating the plant-microorganism interaction, we can better utilize the natural soil biodiversity to enhance plant health in a more ecosystem-friendly manner.

MATERIALS AND METHODS

Microbial Culture Preparation

Soil from a domestic garden free from pesticides or other artificial chemicals was used as a source of microbes.

LB broth (BD Difco™) was prepared by following the manufacturer's recommendation and preserved at 4°C until further use. Before microbial culture preparation, all working surfaces were decontaminated using 70% ethanol. By using a flame sterilized inoculating loop, a very small sample of the soil was transferred to a test tube containing 8–12 mL of LB broth. The tube was then loosely covered with aluminum foil and placed in an orbital shaker at room temperature. The culture was shaken at 200 rpm. After 40 hours, the culture was removed and either immediately used for experiments or stored at 4°C for its use within the next 48 hours. The culture was thawed to room temperature before using in germination or growth studies.

Seed Germination Study

Twenty four healthy mung bean seeds were separated into four sets and were placed on different paper towels. Using Pasteur pipettes, 2 mL or 4 mL of LB broth containing the cultured microbe was then gently poured onto two of the paper towels (Day 0). The corresponding volumes of LB broth without any microbial culture were used as controls. All four sets of seeds were watered daily. The germination of seeds was monitored three times daily for a week. Using a ruler, the final heights of the germinated seed (including the shoot and roots) were measured on Day 7. As the height of mung bean plants was on average 3.4 cm (with a standard deviation of 3.1 cm) on Day 7 in our pilot study, plant heights of more than 9.6 cm (mean + 2 standard deviations) were considered as outliers and were omitted for statistical calculations in the final experiment.

Plant Growth Study

The pumpkin and pea flower seeds were planted on soil placed in egg trays (**Figure 5**). The egg trays were placed at a suitable location with good sunlight. Plants were grown for seven days, after which they were randomly separated into two groups — each containing three seeds. On the 7th day (Day 0), the initial measurement of the heights of the plant was taken using a ruler. Carefully measured 1 mL of LB broth

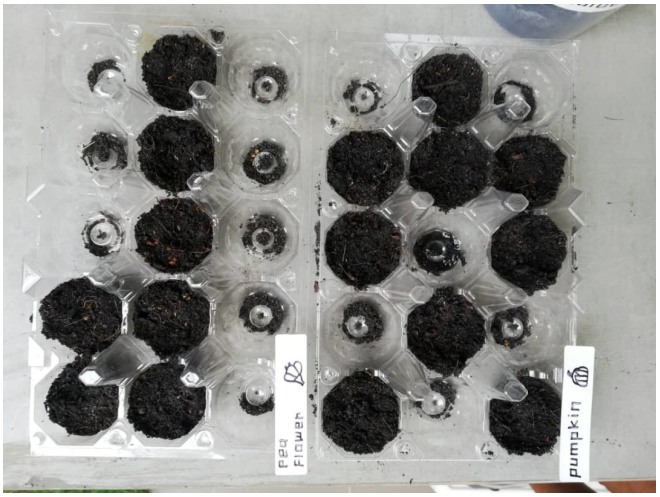

Figure 5: Experimental setting before planting the seed for growth study. Equal amount of soil was added to the compartments in egg trays before planting the pumpkin and pea flower seeds.

with or without cultured microbe was then added to each plant. For the subsequent days (Days 1–5), the heights of the plants were measured using a ruler.

Statistical Analysis

We used *t*-tests to compare two independent means in this study. A *p*-value of less than 0.05 was considered to be significant. The *p*-value was computed using online software (https://www.socscistatistics.com/tests/studentttest/default.aspx)

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