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Research Paper

Evaluating the Potential of Honey as Seed Germination Enhancer

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ABSTRACT

Seed germination is the process by which an organism grows from a seed, initiated by specific enzymes activated upon exposure to water. During this process, roots grow downward and shoots grow upward towards the soil surface. Various factors, including chemicals, influence seed germination. In this study, we examined the effects of honey and its proteins on seed germination. Acacia honey from north west Pakistan, was used as the specimen. We used honey to aid the germination of pea plants. Honey proteins were extracted using the acetone and salt precipitation method. Protein estimation was performed using the Lowry assay, and SDS-PAGE was conducted to analyze the extracted honey proteins and their molecular weight. The extracted honey proteins were then used for seed germination. Our study found that extracted honey proteins enhanced the rate of seed germination.

KEYWORDS: Acacia honey; Seed Germination; Honey Proteins; Seedling Development

INTRODUCTION

The germination rate in plant science refers to how many seeds of a specific plant species, or variety are expected to germinate over a specific time frame. The genetic makeup of the seed, the seed's physical characteristics, and environmental conditions all affect how quickly seeds germinate. To germinate, seeds require the right combination of temperature, oxygen, and water. Germination until the conditions are ideal for germination, seeds are dormant or inactive. To germinate, seeds require the right combination of temperature, oxygen, and water. Some seeds also need the right kind of light. Some seeds grow more effectively in direct sunlight, while others need shade to grow. A seed coat allows water and oxygen to enter when it is exposed to the right circumstances. The cells of the embryo begin to expand. The seed coat then splits open, allowing the shoot or plumule that contains the leaves and stem to emerge first, followed by the root or radicle. Poor germination is a common occurrence. Others need darkness to germinate, while some do well in broad sunlight. Water and oxygen are absorbed by seed through its seed coat when it is exposed to the right circumstances. Cells within the embryo start to grow. The seed coat then splits open, allowing a root or radicle to emerge first, then a shoot or plumule that is home to the leaves and stem. Poor germination is a complex issue that has several potential causes. Apple seeds need to be kept at very low temperatures for a while in order to germinate. The main categories of growth-stimulating and growth-inhibiting drugs, as well as a variety of pesticidal substances (nematicides, insecticides, fungicides, and herbicides), are reviewed. There have been studies on the rate of seed germination in the presence of various chemicals, but none have examined the rate of seed germination in the presence of honey.

Honey is a sweet, viscous natural product produced by honey bees and several other types of bees. Bees create honey via regurgitating, enzymatic activity, and water evaporation from the sugary secretions of plants or from the secretions of other insects. Honey is a delicious, thick liquid meal with

a dark golden hue that is made by different bee species from the nectar of flowers. By inverting a large percentage of the nectar's sucrose sugar into the sugars levulose (fructose) and dextrose (glucose) and by removing extra moisture, nectar is matured into honey. Natural honey's biochemical composition can be summarized as follows: it contains primarily 80–85 percent carbohydrates (primarily glucose and fructose), 15–17 percent water, 0.1–0.4 percent protein, 0.2 percent ash, and trace amounts of amino acids, enzymes, and vitamins, as well as other compounds like phenolic antioxidants. Honey's known activities, also referred to as its mechanisms of action, primarily involve interfering with a variety of molecular targets and cell signaling pathways, including apoptotic, anti-proliferative or cell cycle arrest, anti-inflammatory, estrogenic modulatory, anti-mutagenic, insulin modulatory, angiogenesis modulatory, and immunomodulatory pathways.

Bees are responsible for sucking up nectar to produce a sweet substance called honey. The main component of nectar is water, which contains different concentrations of dissolved sugars depending on the nectar source and environmental conditions [1]. The main component of nectar is sucrose[2]. Sucrose is broken down into fructose and glucose by invertases produced by honey bees [3]. The bee collects the nectar from the succulent flower by inserting its beak, then passing through the digestive system into the intestines, the final destination of the collected material is the honeycomb cells that make the wax. The soup thickens as the water evaporates until the honey is about 83 percent sugar. A layer of wax covers the ripened honey present in the honeycomb cells.

Honey contains carbohydrates and other components including proteins, enzymes, water, vitamins, organic acids and phytochemicals [3]. Fructose and glucose are the major sugars in honey at 38% and 31% [4], and this ratio varies with plant sources and the level of invertase produced by the honey. Triglycerides such as sucrose, maltose, isomaltose, tauranose, maltulose-containing sugars [5], isomaltose, albanose, isobanose, ketoacidose, 3-alpha-isomaltosyl, xanthose, 1-ketose-containing glucose are present in natural honey [6,7].

Here we described the effect of honey and honey proteins on seed germination.

MATERIALS AND METHODS

In our study, we used honey as potential promoter of seed germination. For this, we used three different concentrations of honey that are 1%, 3%, and 6%. The distilled water used for making honey concentrations and petri plates used in this study were autoclaved at 121°C for 30 minutes.

EXTRACTION OF HONEY PROTEINS

We used two different techniques to extract the honey proteins: precipitation by acetone and salting out.

Protein Precipitation by Acetone

In this method, the honey sample was diluted in water 50/50% (v/v) and 400 ml chilled acetone was added in 100 ml of the prepared honey sample. After that, The solution was vortex and incubated for 24 hours at -4 C. Afterwards, the mixture was centrifuged at 13000xg for 10 minutes the supernatant was discarded and a pellet of protein was dissolved in 2 ml of Tris-HCL (20mM) buffer and stored at -4 C.

Protein Precipitation by Salting Out

In this method, the 1gm of the honey sample was weighed and dissolved in filtered water to make it 10ml. The mixture was centrifuged at 4,000rpm for 10 min. The supernatant was collected and dissolved in 80% Ammonium Sulfate (5.67gm) and placed in shaker for an hour. Then samples were again centrifuged at 4,000 rpm for 10

min. The supernatant was discarded and the pellet was dissolved in 0.5 ml Tris buffer.

Protein Estimation

In the Modified Lowry Assay [2], 100 µl each sample and a reference blank of PBS were dissolved in 300µl PBS, then (400µl) x2 Lowry concentration mix was added and incubated at room temperature for 10 min. 0.2N Folin reagent (200µl) was added to each sample, quickly vortexing them to avoid reagent decomposition before it reacts with the protein and incubates at room temperature for 30 min. The absorbance of the sample was observed at 750 ŋm.

Seed germination assays

We used 1%, 3%, and 6% honey dissolved in distilled water for seed germination assays. Further, acacia honey proteins (100 µg) were also used in seed germination assays. All assays were carried out in the autoclaved glass petri plates and a 0.4-micron filter was used to pour diluted honey samples and honey proteins into the petri plates. The corn seeds were added into the plates incubated at 25 ℃ for five days.

Likewise, 1%, 3% and 6% glucose solutions and 1%, 3% and 6% sucrose solutions were also used in seed germination assays to check the effect of these sugars on seed germination.

RESULTS AND DISCUSSION

The concentration of honey proteins was estimated using the Lowry method. Bovine serum albumin (BSA) was used for the standard curve at different concentrations (1-100µg/mL). Regression analysis was used for the determination of protein concentration. The concentration of extracted honey proteins was found to be 8 µg/uL.

Honey protein mixture was subjected to SDSPAGE along with bovine serum albumin (66.5 kDa) (Figure 1).

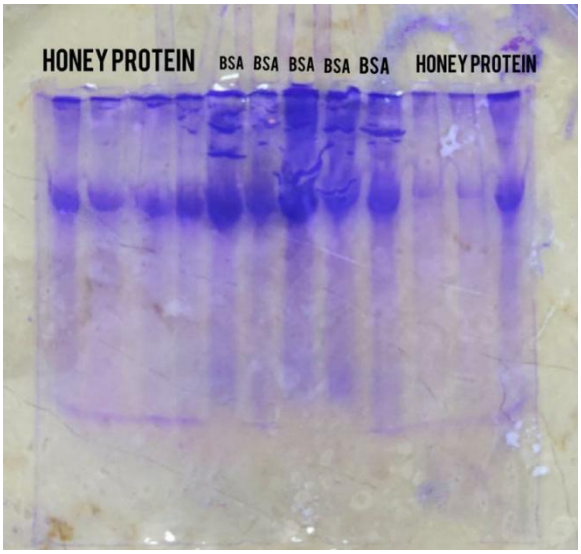

Figure 1: SDS PAGE of extracted honey protein and Bovine serum albumin (BSA).

The petri dishes containing 1% honey showed the growth after the 24 hours of incubation as compared to control in which roots and shoots were emerged after two days. However, the petri dish containing 3% and 6% honey did not show any seed germination for five days as shown in Figure 2.

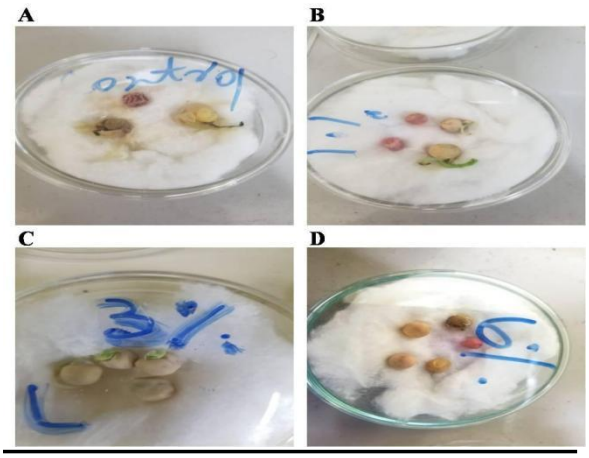

Figure 2: Effect of honey on pea seeds germination. The petri plates containing pea seeds and seedlings (A) pea seeds incubated with distilled water (B) 1% honey (C) 3% honey (D) and 6% honey.

The effects of glucose on seed germination was also evaluated. The petri dishes containing 1% glucose showed same rate of germination as compared to the control. The roots and shoots were observed in both,

however, the 3% glucose and 6% glucose did not show any growth for five days as shown in Figure 3.

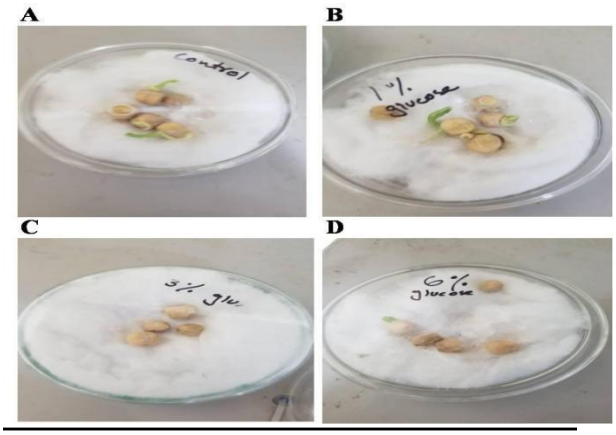

Figure 3: Effect of glucose on pea seeds germination. The petri plates containing pea seeds and seedlings (A) pea seeds incubated with distilled water (B) 1% glucose (C) 3% glucose (D) and 6% glucose.

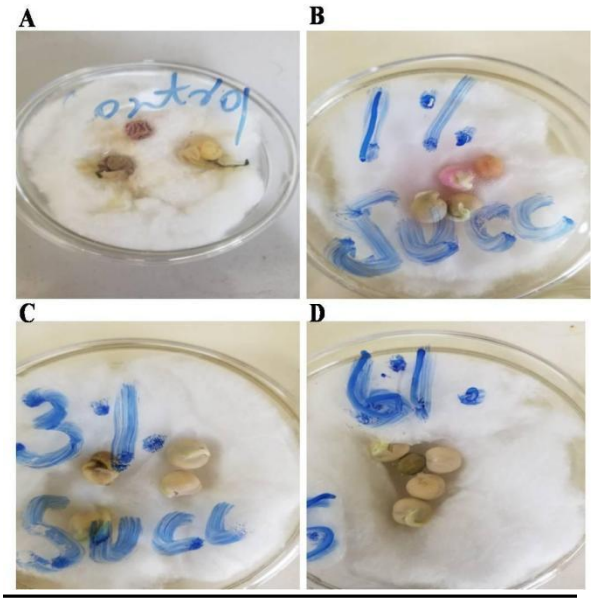

Figure 4: Effect of sucrose on pea seeds germination. The petri plates containing pea seeds and seedlings (A) pea seeds incubated with distilled water (B) 1% sucrose (C) 3% sucrose (D) and 6% sucrose.

The effects of sucrose on the germination of seeds were also evaluated. The petri dishes containing sucrose showed same rate of germination as compared to the control. The rate of germination remained same and sucrose did not affect rate of germination as shown in Figure 4.

Following that effect of honey protein on seed germination was also carried out. The findings showed that honey proteins (100 µg) are potent for increasing the rate of germination of seeds (Figure 5). These proteins should further be studied in order to understand the mechanism in which these proteins are playing important roles. The identification of these proteins would also help to enhance the rate of germination and break seed dormancy.

Figure 5: Effect of honey proteins on pea seeds germination. The petri plates containing pea seeds and seedlings (C) pea seeds incubated with distilled water (HP) Acacia honey protens (100 µg).

The seed dormancy is a process in which seeds are ready to sprout when the conditions are favorable. The process depends on many factors such as environmental and internal factors [10]. We investigated the role of extracted honey proteins on the effects of the rate of germination of seeds. Our study revealed that the honey proteins possess potential in stimulating the growth of plants by breaking the seed dormancy. Furthermore, our data showed that lower concentrations were found to be efficiently involved in elevating the rate of germination. As the concentration increases from 1% to 6% the seeds did not

show any growth. On the other hand, the glucose and sucrose did not show any significant change in the rate of germination. There are several studies that suggest that glucose is a potent inhibitor of seed germination [8,9] which is also aligned with our study as the seeds did not sprout in the presence of higher concentration of glucose. Another study conducted by Wang et al. concluded that sucrose did not have a similar role to glucose, his study showed that 167 mM sucrose delayed the rate of seed germination. The sucrose has multiple roles in seedling development. The higher concentration of sucrose resists the seed germination while also inhibiting the seedling development [10].

CONCLUSION

Our study showed that honey proteins has potential increase the rate of germination of seeds. These proteins should further be studied in order to understand the mechanism in which these proteins are playing important roles. The identification of these proteins would also help in the future in the field of agriculture to enhance the rate of germination and break seed dormancy

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