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Effect of Different Concentrations of Chitosan on Germination and Growth of Sweet Thai Basil

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ABSTRACT

Sweet Thai basil, *Ocimum basilicum var. thyrsiflora* is a medicinal plant that is widely used in many industries due to its richness in phytochemical contents. The present study was carried out to improve the germination growth and of sweet Thai basil. In the current research sweet Thai basil seeds were treated with different concentrations of chitosan (0, 2, 4 and 6 ml/L). The result showed that treatment at 4 ml/L chitosan produced seed germination percentage 100, seed germination speed (1.51), vigor index (6.93) taller seedlings (7.69 cm in height), with bigger stem diameter (0.90 mm), leaf area (1.59 cm²/leaf), and root length (3.19 cm). However, 4 ml/L chitosan concentration was best compare to 0, 2 and 4 ml/L concentration of chitosan. On the other hand, it is recommended for the seeds of sweet Thai basil to be treated with 4 ml/L of chitosan with different plant density.

Keywords: Sweet Thai basil, Germination, Growth, Phytochemical, Medicinal plants, and Chitosan.

INTRODUCTION:

Medicinal plants have been used for the centuries in the complementary medicine because of their health benefits. Some of the metabolites in plants have been successfully used directly in the prevention and treatment of infectious diseases by stimulating the immune system. Large numbers of drugs are derived from the plants, such as, Atropine from *Atropa*, Aswagandha from *Withania somnifera belladonna*, morphine from *Papaver somniferous*, Ephedrine from *Ephedra vulgaris*, (Prasongdee, 2015). Basil is one of the herbs that have been used widely as medicine, and also in food and beverages industries. It is belonging to the Lamiaceae family, under *Ocimum* genus. The name

'basil' was taken from the Greek word, which is the *Basileus*, meaning "King" due to its strong fragrance. Basil is primarily cultivated for its aromatic leaves (Teixeira *et al.*, 2002) and it is the extracted for its essential oil and also used for flavoring in either fresh or dried form (Nazir *et al.*, 2021). Sweet Thai basil (*Ocimum. basilicum var. thyrsiflora*) grown for the first time in Southeast Asia. The global demand for medicinal herbs is increasing yearly. This might be due to the consumer being exposed to the adverse effect of synthetic drugs and have realized the advantages of medicinal herbs. The production of basil species is in the range of 1,300-2,400 kg/ha (Pijic *et al.*, 2017). The global trade of basil as total export and import in 2013

is to be 820.16 - 8201.77 MT around the world, respectively as the major exporters were India, China, India, Madagascar, Egypt, and Mexico. The annual world production of basil oil was estimated at 14 tons in 1984, 43 tons in 1991 and 100 tons in the 1995 (Lachowicz et al., 1997). About 7 tons from Bulgaria, 5 tons from Egypt, 15 tons come from India, 2 tons from Israel, 4.5 tons from Pakistan, 1 ton from USA, 1 ton from Yugoslavia, 1 ton from the Madagascar, and smaller amounts from the other countries (Grayer et al., 1996). The most importance parts in this herb is its seeds, thus germination imbibed mature seed must quickly shift from a maturation- to a germination-driven program of the development and prepare for seedling growth (Rajjou et al., 2012). In basil, production through sexual (seeds) is more popular due to its availability. Basil needs soils with good drainage, a pH range of 6.0 to 7.5, and an optimum temperature of 20°C. Within 7 days of seed sowing, the temperature must be between 15 and 30 °C for seeds to germinate. Basil is a diploid and dicotyledonous plant with $2n = 48$ chromosomes (Prakash, 1990). Basil can be direct seeded or trans-planted into the field when the seedlings are about 15 cm tall. When direct seeding, germination percentage should be greater than the 80 to 90% (Leskovar & Cantliffe, 1993). Seed germination starts with the uptake of water by mature seed, followed by the protrusion of the radicle through the seed envelopes. One of the most important phases of the plant's life cycle is seed germination, which results in the elongation of the hypocotyl and radicle. The length of the germination (from the time of passive water intake to the point at which the seed coat

decomposes and the radicle appears) is the genetically determined, but the differentiation of embryos and zygotes during this phase is extremely susceptible to the environmental factors (Jakovljevic et al., 2020). Nevertheless, chitosan enhances seeds germination and the seedling growth of various plants such as soybean sprouts and sweet basil (Kim et al., 2005). However, chitosan has been studied in the many crop species, including cereal crops, ornamental, medicinal crops and fruits (Chmielewski et al., 2007; Chandrkrachang, 2003). Sweet Thai basil is considered as fast-growing & an easy herb, yet, the production is still insufficient to cater to the rising demand. An alternative plant growth substance, named chitosan which is widely used in agriculture. Chitosan has been used for decades in increasing crop productivity (Sharif et al., 2019; Malerba & Cerana, 2016).

It is safe, inexpensive (Khan et al., 2018) and widely available (Emami-Bistgani et al., 2017). Chitosan have positive effects on growth (Abdel-Aziz, 2019; Zayed et al., 2017) and phytochemicals in plants (Sae-lee et al., 2017; Vosoughi et al., 2018). Although the use of the chitosan sounds promising in improving plant germination & performance. Chitosan & their derivatives (**Fig. 1**) are environmental friendly, bio renewable, biocompatible, non-toxic, biodegradable & bio functional (Zargar et al., 2015). It has a great potential in agriculture, particularly in the production of crops enhancing. Chitosan also contains antibacterial and antifungal properties (El Hadrami et al., 2010; Shahen et al., 2019; Yin and Du, 2010).

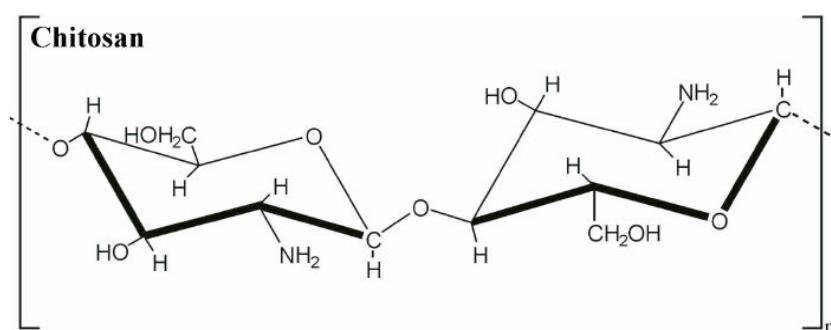


Fig. 1: Chemical structure of chitosan (López-García et al., 2014).

According to reports, chitosan stimulates the immune system that contributes to plants' resistance to pathogen invasion. Furthermore, the prior research points to

that, chitosan use of stimulate the vegetative growth, crop yield, seed germination, plant defense and quality of vegetable crops, such as in faba bean (El-sawy et

al., 2010), orchids (Nge *et al.*, 2006), cucumber and corn (Lizarraga-Paulin *et al.*, 2011). Nevertheless, Teixeira, (2002) reported that, chitosan showed a 100% germination rate after 10 days of germination, having all the seeds planted, grown and germinated. Chitosan are applied as spray and soaking. However, in most research's it is applied as soaking (Byczyńska, 2018; Mahdavi and Rahimi, 2013). Besides, most of the researchers applied chitosan with the concentration of 2ml, 4 ml, 5ml, and 10ml (Mahdavi and Rahimi, 2013; Byczyńska, 2018; Rashidi *et al.*, 2020). Application of (2ml and 4 ml) concentration of chitosan affected the seed vigour index, seed germination and germination rates in ajowan and basil plants seeds. Germination percentage, the germination rates, seed vigour index & radicle were increased with increasing chitosan concentration (Mahdavi and Rahimi, 2013; Rashidi *et al.*, 2020). Chitosan has strong film-forming abilities, making it simple to create a semipermeable film on the surface of seeds that may retain moisture in the seed and absorb moisture from the soil, so promoting seed germination. Despite numerous investigations, it is still unclear how chitosan affects seed germination. Cote and Hahn, (1994) reported, the oligosaccharides regulate plant growth, development, & morphogenesis as the phytohormone-like substances. Chitosan is thought to increase root growth, activate plant defense mechanisms, and produce specific enzymes including pectinases, chitinases & glucanases (Hien, 2004). Through altering cell osmotic pressure, chitosan increases the availability and uptake of vital nutrients and water, hence promoting germination percentage, germination rates, and plant growth (Guan *et al.*, 2009; Uddin *et al.*, 2023).

MATERIALS AND METHODS:

$$\text{Germination percentage} = \frac{\text{Number of seeds that germinated}}{\text{Number of seeds planted}} \times 100$$

Germination speed

Germination speed was evaluated as the proposed by Maguire, (1992). Germination speed was measured after 10 days of seeds germinated.

Seed vigor index

Seed vigor index was obtained by multiplying the total germination percentage by the total height of the seed-

The experiment was conducted at Field 10, Faculty of Agriculture, Universitiy Putra Malaysia. The seeds of sweet Thai basil were sown in 100% peat moss media. Seeds were sowed in germination tray with 4 replications with each replicate consists of 25 seeds. The seeds were watered with chitosan at different concentrations (0, 2, 4 and 6 ml/L) daily for 3 days. In order to determine the germination performance, the data on germination percentage, germination speed and seed vigor index were recorded. The seedling performance was then evaluated at 10 days after germination (DAG) which were plant height, stem diameter, leaf area and root length. The best treatment was chosen and used as the pretreatment for the seed germination in the next experiment. The seed testing experiment was designed in Complete Block Design (CBD) with 25 seeds in each replication for the 4 replications. Statistical Analysis System (SAS) was used to conduct an Analysis of Variance (ANOVA) on the results, and the means were compared (LSD) at 5%.

Data collection

Data on the performance of the seed germination as influenced by different concentrations of chitosan were collected.

Germination percentage

The germination percentage was calculated in order to obtain a greater perception of the number of plants that could be obtained with and without chitosan. The technique used was according to (ISTA rules, 2002). Germination was recorded daily, and the germination percentage was calculated after 10 days as there was no further germination occurred beyond 10 days. The calculation used was as below:

ling (10 days after sowing) divided by 100 (Amirkhani *et al.*, 2020).

Seedling height

The seedling height was measured from the soil surface until the shoot tip using a ruler. The data was collected 10 days after germination (DAG).

Stem diameter

Stem diameter was measured to determine the girth development as it is assumed that plants with bigger stem diameter are stronger and would be able to support a higher number of leaves and branches. Stem diameter was measured by using Vernier caliper. Data were collected from three different locations of the main stem randomly and the average of the stem diameter was expressed in mm/plant. The data was collected at 10 days after germination (DAG) and expressed by average of stem diameter (mm) per seedling.

Leaf area

Each leaf was placed flat and straight on Leaf Area Meter (Model: LI 3100 Area Meter, USA) one at a time. The total leaf area was calculated by adding all the leaves area and data was expressed as total leaf area per plant.

Total root length

Total of root length per plant was collected at 10 DAG during final data collection in order to evaluate the growth and development of root as affected by chitosan. As the seedlings were still small, the total of root length was measured manually by using a ruler with the accuracy 0.1 cm. The roots were washed with tap water before it was measured in order to clean it from growing media. The root length was measured by using a ruler.

RESULTS AND DISCUSSION:

Results indicated that germination percentage was significantly affected by the different concentrations of chitosan at $p<0.05$ (**Table 1**). Application of 2, 4 and 6 ml/L of chitosan were significantly different compared to the control treatment. Application of 2 and 4 ml/L chitosan was resulted in higher germination percentage compare to 0 and 6 ml/L chitosan which was 100%.

Table 1: Effect of different concentration of chitosan on Germination percentage of sweet Thai basil.

	Germination percentage
Chitosan ml/L	10 (DAG)
0	85c
2	100a
4	100a
6	95b

Besides, application 6 ml/L chitosan was the higher compare to control which was 95%.

Means with same letters within the column are not significantly different at $p<0.05$. Batool and Asghar, (2013) reported that in comparison to controls, *Carum copticum* seeds primed with various doses of chitosan demonstrated increased the germination percentage. Earlier studied also agreed with the finding where Shao et al. (2005) reported that, 70 to 95 % of maize germination percentage was increased by the chitosan. Chitosan may activate a signaling pathway associated with auxin production, which would then stimulate the synthesis of plant hormones like gibberellins and promote growth and the development (Uthairatanakij et al., 2007). Chitosan significantly affect the germination speed of sweet Thai basil (**Table 2**). 4 ml/L chitosan recorded the highest germination speed which was 1.51. As for other treated seeds, it showed non significantly different with the control where the germination speed was in between 1.07 until 1.24 only.

Table 2: Effect of different concentration of chitosan on germination speed of sweet Thai basil.

	Germination speed
Chitosan ml/L	10 (DAG)
0	1.07b
2	1.24b
4	1.51a
6	1.15b

Means with same letters within column are not the significantly different at $p<0.05$. Guan et al. (2009) reported that, Chitosan promotes plant germination through increasing the availability of the essential nutrients and uptake of water and through adjusting pressure of cell osmotic. Therefore, our result indicates that, Chitosan affected and enhanced the germination speed of Sweet Thai basil. Seed treated with chitosan increased germination percentage, germination speed and energy of germination in peanut. Also, Katchadat, (2005) recorded soybean germination increasing with application 4 ml/L chitosan concentration as compared to control. Zeng et al. (2012) suggested that, chitosan has strong film-forming abilities, making it simple to create a semipermeable film on the surface of the seed that can keep the seed moist and absorb soil moisture, thereby accelerating seed germination and seed germination.

nation speed. However, the results indicated that seed vigor index was significantly affected by the chitosan concentrations at $p<0.05$ (**Table 3**). Among treatments, the highest seed vigor index was obtained from the application of the 4 ml/L chitosan which was 6.93. Besides, there were no significant difference between 2ml/L and 6ml/L chitosan concentrations respectively. Nevertheless, the lowest seed vigor index was recorded under control treatment which was 5.74.

Table 3: Effect of different concentration of chitosan on seed vigor index of sweet Thai basil.

	Seed vigor index
Chitosan ml/L	10 (DAG)
0	5.74b
2	6.51ab
4	6.93a
6	6.09ab

Means with same letters within column are not the significantly different at $p<0.05$. Our result indicates that, application of chitosan enhanced the seed vigor index of Sweet Thai basil. Similar result was reported by Cho et al. (2008) increase in concentration of chitosan significant effect observed on morphological and vigor index of sunflower seedling. Nevertheless, significant different was found between different concentrations of chitosan. Result in **Table 4** indicated that, 4 ml/L of chitosan produced the tallest seedling which was 7.69 cm, compared to other concentrations. The untreated seedlings showed only 6.15 cm in height, which were not significantly different with 2 and 6 ml/L of chitosan.

Table 4: Effect of different concentration of chitosan on seedling height of sweet Thai basil.

	Seedling height (cm)
Chitosan ml/L	10 (DAG)
0	6.15b
2	6.51b
4	7.69a
6	6.51b

Means with same letters within column are not the significantly different at $p<0.05$. Chitosan promoted wheat growth in terms of the germination capacity, seedling height, the root length and increase in root activity. A study on the effect of chitosan application on vegetative growth of the Sridathip 3 tomato plants

indicated that 60 ppm chitosan enhanced the height of Sridathip 3 tomato plants. Furthermore, chitosan seed coating promoted the vegetative growth in sweet basil Kim et al. (2005), pearl millet (Sharathchandra et al., 2004).

According to Lee et al. (2005) and Kim et al. (2005), the use of chitosan in a variety of crops, including soy bean sprouts and sweet basil, was also found to promote the growth till now the mechanism of an action of chitosan on growth is not clear. Uthairatanakij and colleagues, (2007) research, auxin biosynthesis-related signaling pathways may be used by chitosan to stimulate the synthesis of gibberellins and other plant hormones that promote growth and development. Besides, the average of stem diameter was significantly affected by the chitosan concentrations at $p<0.05$ (**Table 5**). Among the treatments, the highest stem diameter was obtained from the application of 4 ml/L chitosan at 10 DAG which was 0.90 mm/plant. The control seedlings having 0.62 mm of the seedling diameter, which was non-significantly with those treated with 2 ml/L of chitosan. This showed that the lowest concentration tested in this study was not sufficient to enhance bigger diameter in seedlings. As the concentration was increased from 4 ml/L to 6 ml/L, there was a reduction in stem diameter, whereby the seedlings shown to have only 0.76 mm of the stem diameter at 6 ml/L.

Table 5: Effect of different concentration of chitosan on stem diameter of sweet Thai basil.

	Stem diameter (mm)
Chitosan ml/L	10 (DAG)
0	0.62c
2	0.71bc
4	0.90a
6	0.76b

Means with same letters within column are not the significantly different at $p<0.05$. Chitosan significantly enhanced the stem diameter of the Sweet Thai basil. Chitosan enhanced growth factors in terms of the average stem length, stem diameter, the number of growing leaves, including leaf width and length as well as the number of flowers per bush (Wanichpongpan et al., 2001). In addition Salehi and Rezayatmand, (2017) stated that chitosan improved stem dry weight and

stem diameter of savory plants. Although the important economic parts of sweet Thai basil are its leaves and seeds, but in some cases the whole shoot of sweet Thai basil is also used as herbal product (Bucktowar *et al.*, 2016). Strong stems can become a great connector between leaves and roots. Data in **Table 6** shows a significant different between the concentrations of chitosan. Those treated with 4 ml/L of the chitosan produced 1.59 cm²/leaf which was the highest among the other treatments. Besides, the lowest leaf area was recorded to be 0.98 cm² with application of for the 2ml/ L. There were differences in other treatments with different concentrations of chitosan. Furthermore, there was not significant with 4 M/L and control.

Table 6: Effect of different concentration of chitosan on average leaf area of sweet Thai basil.

	Average leaf area (cm ²)
Chitosan ml/L	10 (DAG)
0	1.30ab
2	0.98b
4	1.59a
6	1.18b

Means with same letters within column are not the significantly different at p<0.05. The increment of leaf area (**Table 6**) is assumed to increase the plant growth performance. Based on the previous research, chitosan had improved growth parameter leaf area in lily species and number of leaf of *Triticum aestivum L.* plants (Masjedi *et al.* 2017), which contributed to the increase in fresh weight. This was similar with) Khan *et al.* (2019) and Ahmad *et al.* (2017who reported the increased of fresh weight in *Mentha piperita L.* plants and *Fagonia indica*, respectively under application of chitosan. Besides, based on the previous report, chitosan at early growth stage is beneficial as the rate of auxin activity is high and chitosan enhance more auxin as it regulates its accumulation (Lopez-Moya *et al.*, 2019). Nevertheless, Total root length per seedling of sweet Thai basil was affected by the concentrations of chitosan at p<0.05 (**Table 7**). Result indicates that, the root length was higher in those treated with 4ml/ L chitosan application compared to 2 ml/L, 6ml/L and control, which was 3.19 cm. The lowest root length was recorded under control treatment which was 2.54 cm and not significantly different with 2 ml/L and 6 ml/L of chitosan.

Table 7: Effect of different concentration of chitosan on total root length of sweet Thai basil.

	Root total length (cm)
Chitosan ml/L	10 (DAG)
0	2.54b
2	2.66b
4	3.19a
6	2.53b

Means with same letters within column are not the significantly different at p<0.05. Plants with established root system are more efficient in nutrient uptake from the soil and can contribute to increasing the yield. Besides, chitosan provides nutritive elements for the plants & improve plant's defense mechanism (Becker *et al.*, 2000). In the plants, auxin is well-known as a growth hormone, which could stimulate root system (Tanimoto, 2005), while chitosan was the previously reported to stimulate the biosynthesis of auxin in plant cells (Iglesias *et al.*, 2019. Therefore, the enhancement of root length accumulation could be related to the increase in level of auxin in the root system under the application of chitosan (Lopez-Moya *et al.*, 2019).

CONCLUSION:

The germination and growth of sweet Thai basil were affected by the different concentrations of chitosan. Application of 4ml/ L chitosan concertation was better compare to 2, 6ml/L and control. However, application of the 4 ml chitosan concentration improved the seedling height, stem diameter, average leaf area, germination speed, seed vigor index and total root length. The performance of sweet Thai basil investigated under drench application of chitosan concentrations led to the increment of sweet Thai basil leaves and germination. Plants treated with 4ml/L chitosan showed greater performance compared to 2, 4 ml/L and control. Therefore, it is recommended for the sweet Thai basil to be treated with chitosan at the concentration of 4 ml/L and applied at different time. As a limitation of this research, a commercial mixed soil was used, thus the result may have the minor difference if the farmers use different types of soil.

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CONFLICTS OF INTEREST:

Authors do not have any conflict of interest.

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