Knowex Food and Agriculture Journal
Vol.2, No.1, 10-19, 2021, ISSN 2682-728X (online)
https://doi.org/10.17501/2682728X.2021.2102

IMPACTS OF DIFFERENT CONCENTRATIONS AND TIMES OF APPLICATION OF CHITOSAN ON SWEET BASIL (Ocimum basilicum L.)

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Abstract: Sweet basil is one of the popular herbs that contains various types of antioxidants and is widely used in pharmaceutical, food and cosmetic industries. Although sweet basil is considered as an easy and fast-growing herb, yet, the production is still insufficient to cater to the rising demand. Thus, the aim of this study is to increase biomass of sweet basil by the application of chitosan at different timing. The experiment was arranged in Randomized Complete Block Design with four replications. Plants were treated with different concentrations of chitosan (0, 2, 4 and 6 ml/L) at three different times of application (20, 40 and 20+40 days after transplanting), and kept for 60 days under open field. Data was analysed by using Statistical Analysis Software (version 9.4), Analysis of Variance was used and means were separated using Least Significant Differences. Based on the findings, all treated plants showed greater value compared to the control treatment. Among the treatments, it was found that plants treated with 4 ml/L at 20 days after transplanting performed the best where the plants reached highest stem fresh weight (44.59 g/plant), root dry weight (2.83 g/plant), leaf fresh weight (54.28 g/plant) and leaf dry weight (8.80 g/plant). The yield was higher than control treatment at 43.45% and 59.71% based on its leaf fresh weight and leaf dry weight, respectively. Therefore, it is recommended for sweet basil to be treated with 4 ml/L at 20 days after transplanting. Besides, more details study on compound profiling and its fraction from sweet basil leaf extract can be conducted in the future.

Keywords: dry weight, fresh weight, physiology, sweet basil, yield

Introduction

Sweet basil (*Ocimum basilicum* L.) belonging to the Lamiaceae family is an annual aromatic herb native to Afghanistan, Iran, Pakistan and India (Kőszeghi *et al*. 2014). Due to its strong fragrance and valuable pharmaceutical potentials, sweet basil is synonymous with the title of “Aromatic King” among the pharmaceutical and aromatic herbs (Filip, 2017) and is widely used in pharmaceutical, food (Slougui *et al*. 2018) and cosmetic industries (Mith *et al*. 2016). Sweet basil poses over 200 phytochemicals (Ghasemzadeh *et al*. 2016) with phenolics and flavonoids being the main phytochemical compounds (Filip, 2017). Due to its richness in phytochemicals, sweet basil is considered as one of the most important herbs (Costa *et al*. 2015) and acts as natural source of antioxidant compounds (Murali and Prabakaran, 2018). Previously, it had been reported that the consumption of natural antioxidants reduce the risk of critical illnesses such as cancers, cardiac disease, respiratory diseases and skin disorders (Ch *et al*. 2015; Filip, 2017; Murali and Prabakaran, 2018). Sweet basil is said to have beneficial properties such as anticancer (Pereira *et al*. 2009), antiviral (Koca and Karaman, 2015), antidiabetic (Ch *et al*. 2015) and anti-inflammatory (Ghasemzadeh *et al*. 2016). In nature, the content of phytochemical compounds in plants is low (less than 1% of dry weight) and highly dependent on its physiology and development (Akula and Ravishankar, 2011). It will be beneficial for the industries if the amount of the phytochemical is increased.

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Sweet basil is considered as an easy and fast-growing herb, yet, the production is still insufficient to cater to the rising demand. An alternative plant growth substance, named chitosan is a biopolymer-based which is widely used in agriculture. Chitosan has been used for decades in increasing crop productivity (Malerba and Cerana, 2018). It is safe, inexpensive (Khan *et al*. 2018) and widely available (Emami-Bistgani *et al*. 2017). Chitosan has been reported to have positive effects on growth, yield (Zayed *et al*. 2017; Abdel-Aziz, 2019) and phytochemicals in plants (Sae-lee *et al*. 2017; Vosoughi *et al*. 2018). Although the use of chitosan sounds promising in improving plant performance, it highly depends on its concentration and time of application. Previous studies showed that lower concentrations of chitosan increase the productivity of potato and vice versa (Falcón-Rodríguez *et al*. 2017). The same trend was reported by (Abdel-Mawgoud, 2010) in strawberries. Besides, Mondal *et al*. (2012) stated hat the application of chitosan is more effective when applied at early growing stage compared to later stage in okra.

Thus, the aim of this study is to increase the biomass of sweet basil as influence by different concentrations of chitosan and applied at different timings

Material and Methods

Experimental field activity

The experiment was carried out in 4X3 factorial RCBD with four replications in an open field condition in field 15th of University Putra Malaysia in Malaysia country. Seedlings were then transplanted when reaching 3 to 4 leaf-stage into 14 inches sized polybags containing mixed soil (Brand: Bio-soil, Melayu ImpraTM) in an open area. Plants were then treated with four different concentrations of chitosan which were 0, 2, 4 and 6 ml/L at rate of 250 ml/plant through a drenching method. Each concentration was applied at three different times of application specifically at 20, 40 and 20+40 days after transplanting (DAT). Data were collected at 60 DAT such as fresh weight of stem, root and leaf. As for the dry weight, leaf and root dry weight were also measured in this study.

Data collection and analysis

Plants at age of 60 DAT were harvested at the field and brought into the laboratory immediately. The plant's parts were separated into leaves, stems and roots and were weighted using digital weighing scale (Mettler Toledo, Model: B303-S, Switzerland) in order to measure the fresh weight. As the organ of economic important of sweet basil is leaves, thus, leaves dry weight was measured by drying the leaves at 55°C until it reached the constant weight. As the treatments were applied through drenching method, dry weight of root was measured by drying the roots at same temperature with leaves. After this, data was analysed by using Statistical Analysis Software version 9.4 (SAS 9.4). And, Analysis of Variance was used and means were separated using Least Significant Differences (LSD) at 95% significant different.

Results and Discussion

Fresh weight of stem

The results indicated that stem fresh weight was significantly affected by the interaction of chitosan concentrations and times of application at $p<0.01$ (Table 1). Among treatments, the highest stem fresh weight was obtained from the application of 4 ml/L chitosan at 20 DAT which was 44.59 g/plant. This treatment was 9.96 % higher compared to the control treatment. Concentration of 6 ml/L which was applied at 20 DAT resulted in significantly higher value of 43.27 g/plant compared to the control treatment. Concentration of 2 ml/L applied for two times on 20 and 40 DAT was not significantly different with single application at 20 DAT. The results of other two concentrations applied at 20+40 DAT were similar to the control treatment. From the result, it can be stated that sweet basil plants which were treated with 4 ml/L chitosan at 20 DAT have better growth and stem development which can lead to the yield of more number of leaves.

Table 1: effects of different concentrations of chitosan and times of application on stem fresh weight

	Stem fresh weight (g/plant)			
	Time of Application (DAT)			
Chitosan (ml/L)	20	40	20+40	Means
0	40.55de	40.04e	40.46de	40.35B
2	42.38bcd	41.20cde	42.85abc	42.14A
4	44.59a	40.66de	41.63bcde	42.29A
6	43.27ab	41.59bcde	39.93e	41.60A
Means	42.70A	40.87B	41.22B	

Means with different letters in a same column and row are significantly different at $p < 0.01$ by LSD. Lower case and upper case indicate analysis by single factor and factorial (4 X 3), respectively.

Based on the data of fresh weight of stem (Table 1), it can be stated that both factors are important in enhancing these variables. Early reports have confirmed that the application of chitosan increased fresh and dry weight of shoots in Phaseolus vulgaris (Sheikha and Al-Malki, 2011; Zayed *et al*. 2017). Similar result was observed by Khan *et al.* (2019) on Fagonia indica plants.

In addition, Salehi and Rezayatmand (2017) stated that chitosan reversed negative influence of salt stress and improved stem dry weight of savory (Satureja isophylla L.) plants. Although the important economic parts of sweet basil are its leaves and seeds, but in some cases the whole shoot of sweet basil is also used as herbal product (Bucktowar *et al*., 2016). On the other hand, strong stems can become a great connector between leaves and roots. Therefore, it would be important to improve stem in sweet basil plants.

Fresh and dry weight of root

Results indicated that root fresh weight was not significantly affected by the concentrations of chitosan and times of application at $p<0.05$ (Table 2). Application of 2, 4 and 6 ml/L of chitosan at 20, 40 and

20+40 DAT were not significantly different compared to the control treatment. The range of root fresh weight obtained in this study was in the range of 10.61 to 9.83 g/plant.

Table 2: ANOVA results for root fresh weight of Ocimum basilicum L. affected by the chitosan concentrations and times of application

Source	df	Pr > F
Block	3	0.7143ns
Time of application	2	0.2722ns
Chitosan concentration	3	0.2414ns
Time of application*Chitosan concentration	6	0.8913ns
Error	33	

Although fresh weight of root is not affected by the treatments, interestingly, the root dry weight is significantly affected by the interaction of chitosan concentrations and times of application at $p<0.01$ (Table 3). Plants treated with chitosan concentrations showed significantly higher root dry weight compared to untreated plants. The best time of application of chitosan for sweet basil plants is 20 DAT with 4 ml/L which resulted in 2.83 g/plant of root dry weight. At 20 DAT, 2 ml/L and 6 ml/L gave similar root dry weight which was around 2 g/plant. When the application of chitosan was delayed to 40 DAT, different concentrations of chitosan were not significantly different with each other. The plants did not respond to the concentrations (1.7 – 1.8 g/plant of root dry weight), but the plants still performed better than the untreated plants (1.16 g/plant). The same pattern was also observed when chitosan was applied for two times, which were at 20 and 40 DAT.

Table 3: effects of different concentrations of chitosan and times of application on root dry weight

	Root dry weight (g/plant)			
	Time of Application (DAP)			
Chitosan (ml/L)	20	40	20+40	Means
0	1.16d	1.16d	1.12d	1.14C
2	2.05b	1.76bc	1.88bc	1.89B
4	2.83a	1.75bc	1.95bc	2.17A
6	1.94bc	1.66c	1.98bc	1.86B
Means	1.99A	1.58B	1.73B	

Means with different letters in a same column and row are significantly different by LSD. Lower case and upper case indicate analysis by single factor and factorial (4 X 3), respectively.

Chitosan can be directly absorbed in the root system and then utilized by the plants (Ohta *et al*. 2004). 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 (Becker *et al*. 2000) and improve plant's defence mechanism (Malerba & Cerana, 2018).

Root fresh weight is influenced by the amount of internal water inside the roots as well as during the harvesting of the roots. In this study, there was no significant difference among the treatments on the fresh weight of roots (Table 2). Thus, it might be caused by the method in handling the root during harvesting. As the roots were washed to remove the growing medium, it was therefore contributing to its fresh weight. However, Sheikha and Al-Malki (2011) and Zayed *et al*. (2017) stated that root fresh weight of Phaseolus vulgaris significantly improved under the application of chitosan. In the case of root dry weight (Table 3), it is solely contributed by the solid mass of the roots. Sheikha and Al-Malki (2011) and Zayed *et al*. (2017) reported higher root dry weight due to chitosan application. In the plants, auxin is well-known as a growth hormone, which could stimulate root system (Tanimoto, 2005), while chitosan was previously reported to stimulate the biosynthesis of auxin in plant cells (Iglesias *et al*. 2019. Therefore, the enhancement of root dry weight 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).

Fresh and dry weight of leaf

Results from statistical analysis showed that the leaf fresh weight was significantly affected by the interaction of chitosan and time of application (Table 4) at p<0.01. Chitosan at 4 ml/L and applied at 20 DAT produced the highest leaf fresh weight which was 54.28 g/plant. This was followed by 2 ml/L of chitosan applied at 20 DAT. Without the application of chitosan, sweet basil recorded to have around 37.3 g/plant to 38.9 g/plant of leaf fresh weight only which was 28.3% lower. Application of chitosan at 40 DAT and 20+40 DAT showed non-significant difference among the concentrations of chitosan. The leaf fresh weight in those treatments was in the range of 39.1 g/plant and 43.6 g/plant which is better than those without the application of chitosan.

Table 4: Effects of different concentrations of chitosan and times of application on leaf fresh weight

	Leaf fresh weight (g/plant) Time of Application (DAT)			
Chitosan (ml/L)	20	40	20+40	Means
0	37.84ef	38.88def	37.31f	38.01C
2	50.49b	39.93def	39.61def	43.34AB
4	54.28a	38.78def	39.84def	44.30A
6	41.16cde	41.73cd	43.64cd	42.18B
Means	45.94A	39.83B	40.10B	

Means with different letters in a same column and row are significantly different at p < 0.01 by LSD. Lower case and upper case indicate analysis by single factor and factorial (4 X 3), respectively.

The increment of leaf fresh weight (Table 4) is assumed to lead to the increase in plant growth performance. Based on the previous research, chitosan had improved growth parameters such as leaf length and leaf area in lily species (Khalafi, 2019) and number of leaf of Triticum aestivum L. plants (Masjedi *et al*. 2017), which contributed to the increase in fresh weight. This is in agreement with Ahmad *et al*. (2017) and Khan *et al*. (2019) who reported the increase of fresh weight in Mentha piperita L. plants and Fagonia indica, respectively under application of chitosan. It could be further explained that chitosan has the property of improving the defence system of plants, which helps plants against unfavourable biotic and abiotic factors leading to optimum growth (Katiyar *et al*. 2015). In addition, chitosan provides essential and nutritive elements for plants, which stimulate plant growth and development (Becker *et al*. 2000). On the other hand, results show that application of chitosan at early growing stage is more efficient compared to other times of application. This could be due to the high rate of auxin activity in the early growth stage of the plants (Shang *et al*. 2004), since chitosan regulates its accumulation (Lopez-Moya *et al*. 2019).

Results indicate that leaf dry weight was significantly affected by the interaction of chitosan concentrations and times of application at p<0.01 (Table 5). Concentrations of 2 ml/L and 4 ml/L chitosan applied on 20 DAT significantly increased leaf dry weight by 7.19 g/plant and 8.80 g/plant, respectively compared to the control treatment (5.51 g/plant) which was 59.71 % higher. Plants treated with chitosan on 40 DAT were not significantly different compared to the control treatment. All chitosan concentrations applied for two times (20 DAT and 40 DAT) produced around 7.11 until 7.53 g/plant of leaf dry weight. It is important to measure leaf dry weight in order to measure the solid biomass of the leaves as affected by the treatments. The solid biomass of leaves is contributed by the amount of primary metabolites existed in the leaves, number of cells and its constituents. Other than that, it will give the knowledge and platform for dry herb producers.

Table 5: effects of different concentrations of chitosan and times of application on leaf dry weight

	Leaf dry weight (g/plant) Time of Application (DAT)			
Chitosan (ml/L)	20	40	20+40	Means
0	5.51f	5.78ef	5.75ef	5.68C
2	7.19bc	6.19ef	7.11b	6.83B
4	8.80a	6.11ef	7.53b	7.48A
6	6.54cde	6.30def	7.38bcd	6.74B
Means	7.01A	6.09B	6.94A	

Means with different letters in a same column and row are significantly different at p < 0.01 by LSD. Lower case and upper case indicate analysis by single factor and factorial (4 X 3), respectively.

Increment of leaf dry weight (Table 5) by increasing the concentration of chitosan under 20 DAT is in agreement with Emami-Bistgania *et al*. (2017) who reported on the increment of dry plant materials in Thymus daenensis under chitosan application. Higher concentration of chitosan tends to reduce leaf dry weight, which is in agreement with the result of Mondal *et al*. (2011) in Basella alba L. plants. Besides, data on leaf dry weight may be linked to data on fresh weight. The increment in fresh and dry biomass could be associated with the increment in plant growth and development. On the other hand, previous researchers had confirmed the growth enhancement of chitosan on several crops (Anusuya and Sathiyabama, 2016; Yan *et al*. 2017; Nuengjamnong and Angkanaporn, 2018). Hence, increment in fresh and dry leaf yield could be a great contribution to the farmers.

Conclusion

The performance of sweet basil investigated under drench application of chitosan concentrations and times of application led to the increment of biomass of sweet basil leaves. Plants treated with chitosan at one time in early growing stage showed greater performance compared to late and frequent applications. Therefore, it is recommended for the sweet basil to be treated with chitosan at concentration of 4 ml/L and applied at 20 DAT. As a limitation of this research, a commercial mixed soil was used, thus the result may have minor difference if the farmers use different types of soil. Future study is needed to focus on chemical profile, and development and standardizing of new medicines from sweet basil leaf extract.

Conflict of interest

The authors declare no conflict of interest related to this paper.

Acknowledgement

We acknowledge Universiti Putra Malaysia for providing opportunity and financial support to complete this work. We would also like to acknowledge Dasatim Sdn Bhd for providing chitosan to be used in this study.

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