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# EFFECTS OF DIFFERENT CONCENTRATIONS OF CHITOSAN AND APPLICATION METHODS ON GROWTH AND YIELD CHARACTERISTICS OF CHILLI PEPPER (*Capsicum annum*)

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## ABSTRACT

***Chitosan is a natural polysaccharide that is commonly made by chitin deacetylation, which is the structural component in sea crustaceans. It is beneficial to plant growth and development. Its use to improve chilli pepper production had been shown to elevate environmental and health issues. Hence, different concentrations of Chitosan and application methods on growth and yield characteristics of chilli pepper were investigated. Three replications were used in the split plot design experiment. Chitosan application types (foliar and media) were assigned to the main plots while Chitosan concentration levels were assigned to the sub plots. Apart from number of functional leaves and chlorophyll content that had significant one-way interaction of Chitosan application method ($p < 0.05$), all other growth and yield parameters were not significant ($p > 0.05$) for both sole and interactions. Highest number of functional leaves and chlorophyll content occurred under foliar application of Chitosan. Greater plant height and stem diameter was achieved under the media application of Chitosan with a rate of 50 ppm and 100 ppm respectively at 6 weeks after planting. Foliar application of Chitosan as a sole factor improved all the yield attributes of chilli pepper.***

**Key words:** Chitosan, foliar, media, chilli pepper, yield

## INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is one of the common vegetables cultivated by smallholder farmers because they are comparatively easy to grow, and tolerant to a wide range of climatic conditions (Saavedra *et al.,* 2014), with about 31 million tons produced on roughly 1.9 million hectares of land. The production of chili peppers in Ghana is relatively profitable (Mohammed *et al.,* 2016). Due to the significant increase in demand for vegetables on the domestic and global markets, farmers now have a great opportunity to increase national production, increase their income, and ultimately better their financial status.

Chili pepper contains protein, lipids, carbs, calcium, vitamins A, $B_1$, and C (Amaechi *et al.,* 2012) as well as bioflavonoids, capsaicin, and capsicum (Dludla *et al.,* 2003). Its potential health benefits have recently received more attention in order to increase production, yield, nutrients value and income, whiles reducing field pests and diseases without compromising on its effects on the environment. Chili pepper has seen a sharp increase in demand in Ghana as a fresh vegetable crop (Edusei *et al.*, 2012; Assefa *et al.*, 2020). Consequently, it is essential that pepper growing methods adopt good agricultural practices. One of the requirements is to ensure food security by minimizing the use of chemical fertilizers and/or pesticides and switching to the usage of biomaterials like Chitosan.

The recent increase in the world's food demand, the consequences of climate change, the risky use of farmland, and consumers' increasing attention to high quality, safe, and ecologically friendly fruits and vegetables have all contributed to the need for alternative biological ways to meet this demand. One of the alternatives presently being studied to avoid using chemical products to manage plant diseases and enhance the growth of fruits and vegetables like pepper is the usage of Chitosan.

Chitosan is a biopolymer as well as a natural polymer derived from chitin and found in the shells of crabs and shrimps. It is known for its growth promotion potentials such as improving nutrient uptake efficiency in plants. (Chakraborty *et al.*, 2020). The chemical structure of chitosan is a linear polysaccharide composed of randomly distributed β-(1→4)-linked D- glucosamine (deacetylated unit) and N- acetyl-D-glucosamine (acetylated unit) (Figure 1). Chitosan can chelate with metal ions in the soil, making them more available to plants. Chitosan promotes root development leading to an increase in nutrient and water uptake enhancing plant growth (Makhlouf *et al.*, 2022). Becker *et al.* (2000) reported that Chitosan contains nitrogen in the basic unit of its formula. When the nitrogen contained in the Chitosan is dissolved, it penetrates gradually and remains in the soil for a long period of time. It was reported that Chitosan improved the transportation

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of nitrogen in the functional leaves which enhanced plant growth and development (Chibu and Shibayama, 2003; Gornik *et al.*, 2008). In addition, Chitosan led to a strong increase in extracellular peroxidase activity (Ortmann and Moerschbacher, 2006). Many investigators reported that using Chitosan as foliar spray increased vegetative growth, yield and quality of vegetable crops including cucumber (Farouk *et al.*, 2008), strawberry (Abdel-Mawgoud *et al.*, 2010), sweet pepper (Ghoname *et al.*, 2010), cowpea (El-Tanahy *et al.*, 2012), garlic (Fawzy *et al.*, 2012), okra (Mondal *et al.*, 2012), cucumber (Shehata *et al.*, 2012) and mung bean (Mondal *et al.*, 2013).

It is a good option for many uses because of its non-toxicity, biodegradability, and biocompatibility (Hawrylak-Nowak *et al.*, 2021). With minimum toxicity to mammals and no environmental impact, these compounds have shown to be effective against infections in a respectable manner while improving growth and yield characteristics (Mao *et al.*, 2001). Additionally, these biomaterials can increase the productivity of variety of crop plants, such as tomato, pepper and eggplant (Kumar *et al.*, 2021; Shahrajabian *et al.*, 2021; Sun et al., 2022), all while lessening the requirement for extensive use of chemical fertilizers and risky farming practices. Besides harnessing secondary messengers to activate the stress absorption pathway, it also improves physiological response and minimizes the negative consequences of abiotic stress (Hidangmayum *et al.*, 2019). Additionally, it can react with heavy metals to produce chemicals that can be employed in soil phytoremediation and bioremediation (Girma, 2015). Additionally, this is applied directly to a lot of plants as an anti-transpirant agent, which saves water and ensures protection from additional side effects (Hidangmayum *et al.*, 2019). Due to changing climates, Chitosan is employed in sustainable farming methods based on its advantageous qualities (Kashyap *et al.,* 2015). Based on foliar and medium treatment, some authors have reported favourable significant impacts on fruits and vegetable production (Karakurt *et al.*, 2009; Davarpanah *et al.*, 2016; Rouphael *et al.*, 2018). The medium and foliar application of Chitosan on chili peppers, however, has received little attention. The current study looked at how the growth and yield of chilli pepper were affected by different concentrations of Chitosan and its application methods.

## METHODOLOGY

### Experimental Site

The research study was carried out in the Upper East Region of Ghana at the ornamental garden of the Department of Ecological Agriculture, Bolgatanga Technical University, Sumburungu campus.

### Plant Materials and Treatments

Pepper seedlings were planted immediately in buckets filled with growth garden soil. Garden soil was collected from a farm near the university. It consists mainly of topsoil collected from 0-15 cm depth. The newly planted seedlings were incubated for three days by covering them with transparent plastic cups to maintain favorable relative humidity for immediate recovery of the seedlings. However, these cups are slightly tilted periodically for ventilation. The buckets were placed in the open field for the entire duration of the experiment. Garden soil was tested for pH, nitrogen, phosphorus, potassium and organic matter.

### Experimental Design and Procedure

In the experiment, the growth response of pepper seedlings as affected by different treatment levels of Chitosan concentrations was conducted. Seedlings were treated with the following Chitosan concentration levels: 0, 50, and 100 mg $l^{-1}$. The experimentation was laid out in a split plot in completely randomized design (CRD) with three treatments, three replications and six plants per treatment. Chitosan application type (foliar and media) represented the main plots while Chitosan concentration levels represented the sub plots.

### Foliar and Media Application of Chitosan

Different concentrations of Chitosan prepared were applied two weeks after transplanting using a hand sprayer and biweekly subsequently. The plants were sprayed uniformly until the leaves were completely wet. At the time of spray, growth media were covered with plastic sheet to prevent spray drift on media. In the media, 20 ml was applied per plant for the entire duration of the experiment (Figure 2).

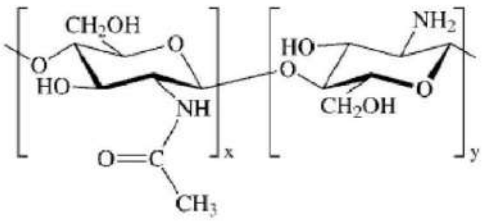


**Figure 1**: Structure of Chitosan

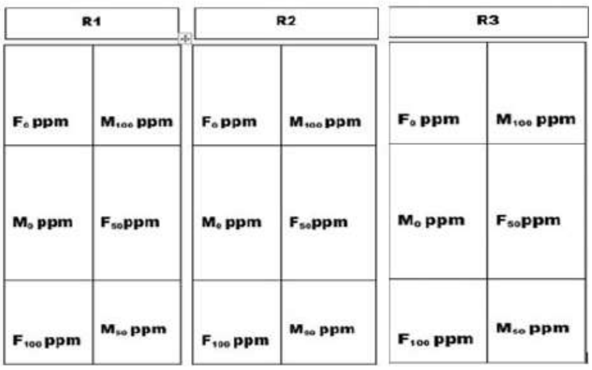


**Figure 2:** Experimental layout of *F*-folia application, *M*- media application

### Vegetative Growth Parameters

Height, stem diameter, the number of functioning leaves, and chlorophyll content of the plant were measured as growth characteristics. For the purpose of collecting vegetative data, three plants were chosen at random from each treatment and replication, tagged, and used.

### Yield Parameters Gathered

The average fruit weight, fruit diameter, and fruit length of samples of the chilli pepper fruits in each sub-plot were measured after harvest. Besides this, the overall yield as tonne/fed was calculated by weighing the fruits in each treatment after they were harvested twice weekly.

### Statistical Data Analysis

The data from the experiment were analyzed using the analysis of variance of split-plot in completely randomized design. Comparison of means was done using Tukey's LSD test at $p \leq 0.05$.

## RESULTS

### Soil Physico-Chemical Properties

The physico-chemical characteristics of the soils at the experiment site (Table 1) demonstrated that the soil medium was appropriate for pepper cultivation.

### Growth Attributes of Pepper

There was no 2-way interactive effect or 1-way interactive effect on plant height of chilli pepper at all weeks ($p > 0.05$). Among the treatments, greater plant height (50.30 cm) was achieved under the media application of Chitosan with a rate of 50 ppm at 6 weeks after planting (Figure 3). Also, plant height recorded under foliar and media application of Chitosan at the rate of 100 ppm was at par (Figure 3). The least plant height (8.85 cm) was however observed under media application of Chitosan at a rate of 0 ppm at 2 weeks after planting (Figure 3).

Similarly, stem diameter was not statistically significant for the 2-way or 1-way interaction effect ($p > 0.05$) for all the weeks observed. Among the interactions however, the media application of Chitosan at a rate of 100 ppm at 6 weeks after planting gave the highest stem diameter (6.17 g) of chilli pepper (Figure 4). This was followed by media application of Chitosan at a rate of 50 ppm at 6 weeks after planting and the least stem diameter (1.51 g) recorded under media application of Chitosan at a rate of 0 ppm at 2 weeks after planting (Figure 4). While there was no 2-way interaction effect on number of functional leaves of chilli pepper. Number of functional leaves of chilli pepper was however significantly ($p < 0.05$) affected by Chitosan application method (Figure 5). Among the treatments, foliar application of Chitosan recorded more number of functional leaves than media (Figure 5).

**Table 1:** Physico-chemical properties of soils at the experimental site

| Soil parameter | Initial soil analysis |
|---|---|
| pH | 6.82 |
| Organic carbon (%) | 3.90 |
| Available nitrogen (ppm) | 17.00 |
| Available phosphorus (ppm) | 16.50 |
| Potassium (ppm) | 41.50 |
| *Particle size distribution (%)* | |
| Sand | 52.05 |
| Silt | 47.63 |
| Clay | 0.32 |
| Texture | Sandy loam |

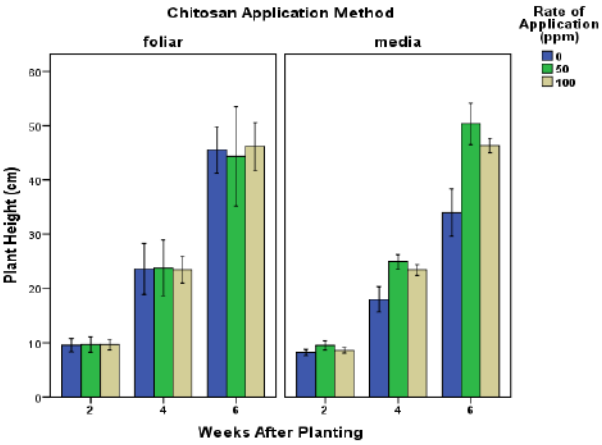


**Figure 3:** Effect of Chitosan and its application rate on plant height of chilli pepper grown in the Sudan savanna zone of Ghana. Data taken during the 2021 and 2022 cropping seasons. Bars represent standard error of mean.

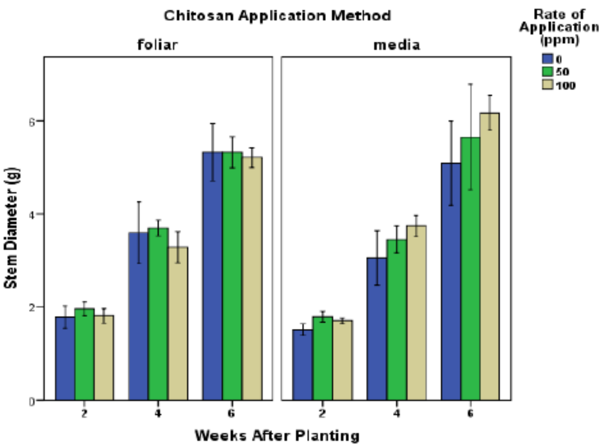


**Figure 4:** Effect of Chitosan and its application rate on stem diameter of chilli pepper grown in the Sudan savanna zone of Ghana. Data taken during the 2021 and 2022 cropping seasons. Bars represent standard error of mean.

Although the interaction effect of Chitosan application method × rate of its application did not affect significantly ($p > 0.05$) on chlorophyll content of chilli pepper, Chitosan application method as a sole factor significantly ($p < 0.05$) affected chlorophyll content (Figure 6). Foliar as a method of Chitosan application recorded the highest chlorophyll content (26.10) of chilli pepper (Figure 6).

LSD $_{(0.05)}$ = 14.97

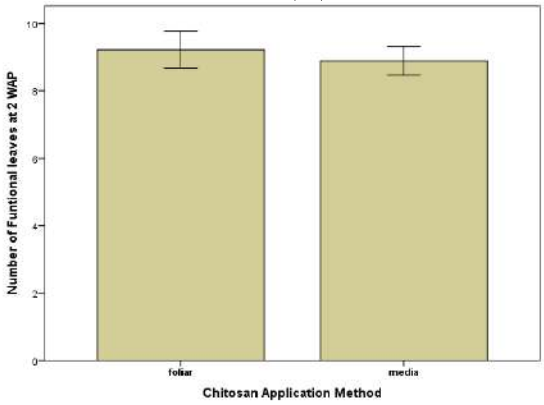


**Figure 5:** Effect of Chitosan application method on number of functional leaves of chilli pepper grown in the Sudan savanna zone of Ghana. Data taken during the 2021 and 2022 cropping seasons. Bars represent standard error of mean.

LSD $_{(0.05)}$ = 2.61

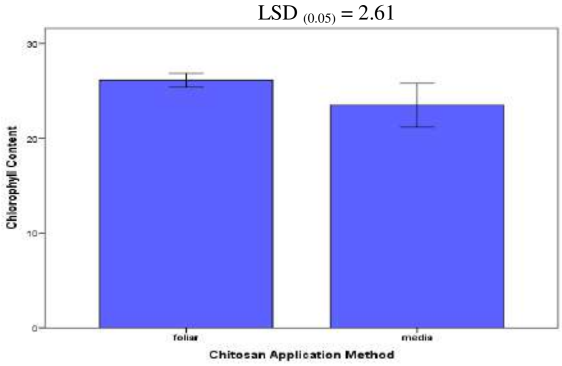


**Figure 6***:* Effect of Chitosan application method on chlorophyll content of chilli pepper grown in the Sudan savanna zone of Ghana. Data taken during the 2021 and 2022 cropping seasons. Bars represent standard error of mean.

### Yield Attributes of Chilli Pepper

There was no significant two-way or one-way interaction effect ($p > 0.05$) of the factors on flowering, fruit diameter, fruit length and number of fruits of chilli pepper (Table 2). Among the treatments, foliar application of Chitosan as a sole factor was found to improve all the yield attributes of chilli pepper (Table 2). Also, Chitosan application rate of 100 ppm improved the number of fruits (16) and fruit diameter (7.23 mm) of chilli pepper although the difference in the results observed under the application of 50 ppm of Chitosan was not significant (Table 2). For the two-way interactions, media × 100 ppm application of Chitosan gave the highest mean number (21.30) of chilli pepper fruits, whereas the least mean number (3.70) of chilli pepper was recorded under the interaction of media × 0 ppm (Table 2). Also, fruit diameter of 8.38 mm and fruit length of 6.65 cm were favored by the application of foliar × 50 ppm of Chitosan (Table 2).

## DISCUSSION

The soil of the experiments is sandy loam textured, slightly acidic, low in total nitrogen and low in available phosphorus (Table 1). With such low inherent crop available nutrient, one will expect to have a good response of crops to soil amendment. According to Fageria (2001) and Havlin (2020), there is a positive response of crops to the supply of limited nutrient elements in the soil. The increase in plant height associated with increased Chitosan rate of 50 and 100 ppm is a reflection of the effective role of the nutrient element N, which was increased. Fageria and Baligar (2005) similarly observed that increasing of nitrogen level generally improved plant vegetative development. Additionally, an upsurge in the main enzyme activities of nitrogen metabolism (nitrate reductase, glutamine synthetase, and protease) as well as improved photosynthesis may be contributing factors to Chitosan's significant influence on growth/development of plants (Gornik *et al.*, 2008; Mondal *et al.*, 2012). Better growth and performance of seedlings occurs with application of nutrients through the roots or leaves (Fageria *et al.*, 2009).

It is worth mentioning that increasing the level of Chitosan in the media caused an enhancement in the stem diameter of chilli pepper. This may be attributed to an increase in the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure, and reducing the

**Table 2:** Effect of Chitosan and its application on yield attributes and stem diameter of chilli pepper grown in Sudan Savanna zone of Ghana

| Treatment | Flowering | Fruit diameter (mm) | Fruit length (cm) | Number of fruits |
|---|---|---|---|---|
| *Application method* | | | | |
| Foliar | 5.89 | 8.04 | 6.02 | 13.80 |
| Media | 4.44 | 5.40 | 4.08 | 13.00 |
| LSD ($p$ = 0.05) | NS | NS | NS | NS |
| *Rate of application* | | | | |
| 0 ppm | 5.17 | 6.40 | 4.06 | 9.30 |
| 50 ppm | 6.17 | 6.53 | 5.56 | 14.80 |
| 100 ppm | 4.17 | 7.23 | 5.53 | 16.00 |
| LSD ($p$ = 0.05) | NS | NS | NS | NS |
| *Interactions* | | | | |
| Foliar × 0 ppm | 7.33 | 7.81 | 5.36 | 15.00 |
| Foliar × 50 ppm | 7.00 | 8.38 | 6.56 | 15.70 |
| Foliar × 100 ppm | 3.33 | 7.92 | 6.13 | 10.70 |
| Media × 0 ppm | 3.00 | 4.99 | 2.76 | 3.70 |
| Media × 50 ppm | 5.33 | 4.68 | 4.56 | 14.00 |
| Media × 100 ppm | 5.00 | 6.54 | 4.92 | 21.30 |
| LSD ($p$ = 0.05) | NS | NS | NS | NS |

Data taken during the 2021 and 2022 cropping seasons. NS - not significant accumulation of harmful free radicals by increasing antioxidants and enzyme activities (Singh *et al.*, 2015). Also, the observed increased in number of functional leaves of chilli pepper under foliar application of Chitosan might be attributed to increased photosynthetic activity and increased production and accumulation of carbohydrates in the leaves. Foliar application of fertilizers is a substitute for soil application of fertilizers (Alam *et al.*, 2010), while it is still crucial to select the appropriate source and concentration of the material used (Fageria *et al.*, 2009; Patil and Chetan, 2018). In turn, less harm is done to the leaves while effectiveness is increased. Also, foliar application of Chitosan might have improved leaf concentrations of N, K, Mg, Ca and Zn, leading to growth promoting effect of these nutrient elements and their possible semi-hormonal effects (Farid *et al.*, 2022). Furthermore, Chitosan may simulate physiological processes by enhancing nitrogen transfer in functioning leaves and, hence, vegetative growth and development (Chibu and Shibayama, 2003; Gornik *et al.* (2008).

Leaf chlorophyll and soluble carbohydrate content are significant determinants of seedling quality. Leaf chlorophyll index and soluble sugars depend on the time of seedling development, the amount of nutrients, and the intensity of the light (Li *et al*., 2017; Kumar *et al.,* 2021). In order to increase leaf greenness and sugar status, seedlings may need to be provided with critical nutrients (Wingler *et al.,* 2006; Engels *et al.,* 2012). N-carboxymethyl Chitosan applied to maize increased the amount of N and N protein as well as the activity of vital N metabolic enzymes (Sajid *et al*., 2020; Boamah *et al.,* 2023). The nutrient nitrogen is well-known for being necessary for the synthesis of numerous enzymes, cytokinin, and chlorophyll.

Foliar application of chitosan as a sole factor improved all the yield attributes of chilli pepper. These results are similar to those of Hasani *et al.* (2012), Shafeek *et al.* (2014), Haleema *et al.* (2018) who showed that the application of some minerals as foliar spray caused an enhancement in plant growth and fruit yield. A number of studies found that using Chitosan as a foliar spray improved the vegetative growth, production, and quality of vegetable crops such cucumber (Farouk *et al.*, 2008), strawberry (Abdel-Mawgoud *et al.*, 2010), and sweet pepper (Ghoname *et al.*, 2010). Chitosan application as fertilizer has been shown to improve okra (Mondal *et al.*, 2012), cowpea (El-Tanahy *et al.*, 2012), and mungbean (Hidangmayum *et al.,* 2019) growth and yield characteristics. According to Chibu and Shibayama (2003) and Gornik *et al.* (2008), Chitosan dissolved, penetrated the soil progressively, and remained there for a while, improving the transportation of nitrogen in the functional leaves and enhancing plant growth characteristics. This is evidenced by the observed increase in the number of chilli peppers under media × 100 ppm application of Chitosan.

## CONCLUSION

From this study, Chitosan promoted growth and yield of chilli pepper plants to different application methods of Chitosan. Foliar application of Chitosan increased the photosynthetic rate of the leaves of chilli plant which enhanced their growth and development. In addition, foliar feeding of pepper seedlings using different concentrations of Chitosan demonstrated promising effects on seedling growth, quality and yield. More so, the addition of Chitosan to the soil at different rates can improve the single fruit quality and yield of chilli pepper. Pepper production increased when Chitosan was added to the soil at the right rate. Along with increasing photosynthetic rate, it also markedly improved the leaf chlorophyll index and photochemical efficiency. This study showed that Chitosan, employed as a soil supplement, promoted the growth of chilli peppers and may have application in the sustainable production of chilli peppers. High levels of Chitosan, however, could have a deleterious effect on the development and growth of chilli peppers. For chilli pepper field production, more research is required to optimize the application method, timing, and rate.

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