E-ISSN-2580-7129
Print- ISSN-1978-6107

TECHNO: JURNAL PENELITIAN
Journal homepage: http://ejournal.unkhair.ac.id/index.php/Techno
Volume 12 Number 02 October 2023 DOI: https://doi.org/10.33387/tjp.v12i2.6639

Effect of Chitosan on Chlorophyll Content and Phytotoxicity in *Brassica Juncea L.*

Sulistia Ningsih[1*], Dahlia Wulan Sari[2]

[1] *Faculty of Agriculture, Nahdlatul Ulama University West Kalimantan, Indonesia, ningsihsulistia92@gmail.com*
[2] *Faculty of Agriculture, Nahdlatul Ulama University West Kalimantan, Indonesia, dahliawulansari@gmail.com*

Received : 08-09-2023
Accepted : 28-10-2023
Available online : 30-10-2023

ABSTRACT

The positive impact of chitosan has been widespread and has attracted interest from farmers with the pretext that it is safe for the environment. However, information on the adverse effects of using chitosan on plants still needs to be available. This study aims to discover the side effects of applying chitosan on the *Brassica juncea*. This test is done using Complete Random Design (CRD). The test is carried out by applying a solution of chitosan with five series of concentrations (0.5%, 1.5%, 2.5%, 3.5%, and 4.5%) plus control of acetic acid 1% (AA) to the upper and lower surfaces of the *B. juncea* leaves (old and young leaves) using a brush smooth to drip wet. The trial was repeated four times. Data was analyzed using ANOVA (Analisis of Variance). When there is a significant difference between the treatments, further tests are conducted using DMRT at a substantial level of 5%. In two week and three week *B. juncea* plant, the highest chlorophyll content is observed in the chitosan with a concentration of 3.5% and 0.5% with the same value of 0.74 mg/g, while the lowest concentration is at 1.5% 0.61 mg/g at the age of two weeks and 0.50 mg/g at three weeks. Using chitosan at high concentrations (2.5-4.5%) indicates phytotoxic occurrence in the leaves of two week or three week old *B. juncea* plants. Phytoxic indications are seen in rolling leaves, thickening, and structurally more hardinge when compared with low concentration (0.5% and 1.5%) and control treatments. It is best to pay attention to the use of concentrations of chitosan so that plants get optimal positive effects without causing adverse side effects.

Keywords: Acetic acid, Leaf structure, Malformation, Photosynthesis, Stomata

ABSTRAK

Dampak positif kitosan telah banyak dilaporkan dan menarik minat petani dengan dalih berbahan alami yang aman terhadap lingkungan. Akan tetapi, informasi efek negatif penggunaan kitosan pada tanaman masih jarang dilaporkan. Penelitian ini bertujuan mengetahui efek samping pengaplikasian kitosan pada tanaman *Brassica juncea*. Pengujian ini dilakukan dengan menggunakan Rancangan Acak Lengkap (RAL). Pengujian dilakukan dengan cara mengoleskan larutan kitosan dengan lima seri konsentrasi (0,5%, 1,5%, 2,5%, 3,5%, dan 4,5%) ditambah kontrol asam asetat 1% (AA) ke permukaan atas dan bawah daun tanaman *B. juncea* (daun tua dan muda) menggunakan kuas halus hingga basah menetes. Percobaan diulang sebanyak empat kali. Data dianalisis menggunakan ANOVA (*Analisis of Variance*). Apabila terdapat beda nyata antar perlakuan, maka dilakukan uji lanjut menggunakan DMRT pada taraf nyata 5%. Pada tanaman *B. juncea* umur dua minggu dan tiga minggu, rerata kandungan klorofil tertinggi secara berurutan terdapat pada kitosan dengan konsentrasi 3,5% dan 0,5% dengan nilai yang sama yaitu 0,74 mg/g, sedangkan terendah pada konsentrasi 1,5% yaitu 0,61 mg/g pada umur dua minggu dan 0,50 mg/g pada umur tiga minggu. Penggunaan kitosan dengan konsentrasi tinggi (2,5-4,5%) menunjukkan terjadinya fitotoksik pada daun tanaman *B. juncea* umur dua minggu maupun tiga minggu. Indikasi fitotoksik terlihat pada bentuk daun yang menggulung, menebal dan struktur yang cenderung lebih keras jika dibandingkan dengan perlakuan kitosan konsentrasi rendah (0,5%

dan 1,5%) serta kontrol. Sebaiknya perlu memperhatikan penggunaan konsentrasi kitosan agar tanaman mendapatkan efek positif yang optimal tanpa menimbulkan efek samping yang merugikan.

Kata kunci: Asam asetat, Fotosintesis, Malformasi, Stomata, Struktur daun

INTRODUCTION

The world's high demand for food has triggered the increased use of chemicals in agriculture. Agricultural chemicals are used in plant production processes to prevent and control plant pests and diseases, improve the quality and productivity of crops, and reduce agricultural loss (Popp et al., 2013). In addition to the benefits that can benefit farmers, agricultural chemicals can also have a negative impact. These negative impacts include reduced environmental quality, reduced biodiversity, water and soil pollution, and can even negatively impact human health (Eddleston et al., 2002; Jeyaratnam, 1990). For food demand to remain satisfied without degrading the quality of the environment and harming other living creatures, it is necessary to implement a sustainable agricultural system in agricultural cultivation. By implementing this sustainable farming system, it is expected that the sustainability of agricultural production can continue to beined in the long term by minimizing environmental damage.

One of the efforts that can be made to increase agricultural production by minimizing environmental damage is to use materials that are environmentally safe, easily degradable, and quickly found and abundant in nature. One of the potential compounds to be effective in increasing agricultural production is chitosan (Maluin & Hussein, 2020). Chitosan results from deacetylation of chitin, the second most abundant natural polysaccharide biopolymers after cellulose (Meyers & Bligh, 1981). Chitosan has extensive applications in the industrial world due to its natural, biodegradable, biocompatible, and non-toxic properties. Chitosan has been developed extensively in almost every area of life, especially agriculture.

In agriculture, chitosan has been widely used as a natural insecticide that reduces the number of eggs and the image of females *Aphis gossypii, Callosobruchus chinensis,* and *C. maculatus* (Sahab et al., 2015). Ningsih et al. (2020) also state that chitosan can inhibit *Spodoptera litura* eating activity both in the method of choice and without choice. Not only does it act as an insecticide, but it is also effective as a fungicide that can inhibit the growth and reproduction of mycelium in *Colletotrichum Gloeosporioides*, *Alternaria* spp, *Fusarium oxysporum*, and *F. graminearum* (Barrera-Necha et al., 2018; Brunel et al., 2013; Kaur et al., 2018; Kheiri et al., 2016, 2017). In addition to being used in plant protection, chitosan can promote plant growth; for example, it can increase the total fresh mass of roots and leaves and increase leaf catalase and plant peroxidase activity *Capsicum annuum* (Asgari-Targhi et al., 2018). Another study also carried out by (Khati et al., 2017; Zayed et al., 2017) showed that chitosan was able to increase the flowering of *Phaseolus vulgaris* seeds after 72 hours under salinity, increase seed flowering, plant height, and leaf width of *Zea mays*. In addition, chitosan was also able to improve the percentage of fruit set, relative leaf water content, and chlorophyll content of the *Salacca zalacca* plant (Sunarka et al., 2015) increase chlorophyll, leaf breadth, dry leaf weight, improve the maximum photochemical efficiency of leaves and photochemical results, improve average electron transport on leaves, as well as improve the rate of leaf photosynthesis and stomata conductivity of *Lactuca sativa* plant (Xu & Mou, 2018).

In previous research, the application of chitosan to plants used the method of pouring it on the soil and spraying it on the leaves, but this study used the method of rubbing it on the leaves. In addition, previous research has examined the impact of chitosan on plant pests and diseases, plant growth and development, chlorophyll content, and seed germination. However, until now research on the impact of chitosan on plant phytotoxicity is still very limited. Therefore, this research aims to determine the side effects of chitosan application on *Brassica juncea*. It is hoped that the results of this research can be a reference for farmers in using chitosan at the right dose so that it does not cause bad side effects on plants.

METHODOLOGY

This test is done using the Complete Random Design (CRD). The chitosan is a pure chitosan derived from the skin of the Black Tiger shrimp, with a purity of 90% obtained from CV. Bio Chitosan Indonesia (Cirebon, Indonesia). The test was carried out by applying the solution with five series of concentrations (0.5%, 1.5%, 2.5%, 3.5%, and 4.5%) plus acetic acid (AA) controls to the upper and lower surfaces of two weeks and three weeks old *Brassica juncea* leaves (old and young leaves) using a brush smooth to dripping wet. The trial was repeated four times.

Five days after treatment, plants are harvested, and chlorophyll levels are analyzed. The sample is analyzed by crushing and smoothing with a mortar using an ethanol solvent. Then, the sample solution is inserted into a measuring glass and filtered using Whatman paper. Then, the sample solution is injected into the cuvet until the boundary mark, and then its absorption is measured at wavelengths of 649 nm and 665 nm.

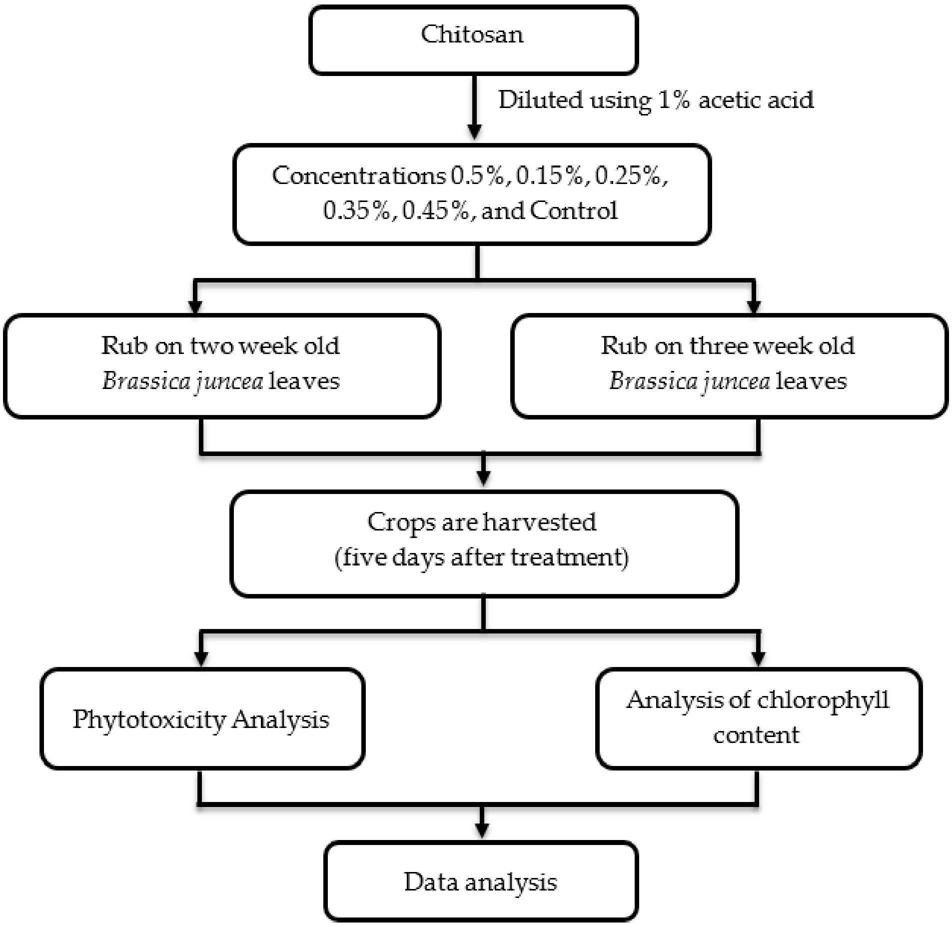


Figure 1. Flow chart of research process

Chlorophyll levels can be calculated with the following equation.

Chlorophyll a = 13.95A665 − 6.88A649 …………………………… (1)

Chlorofyll b = 24.96A646 − 7.32A665 …………………………… (2)

Total chlorophyll = Chlorophyll a + Chlorophyll b ……………….… (3)

A665 and A649 are absorption values at 665 nm and 659 nm (Lichtenthaler & Buschmann, 2001).

Chlorophyll content data was analyzed using ANOVA (Analisis of Variance). When there's a real difference in treatment. The data was then tested further using DMRT (Duncan's Multiple Range Test) at a 5% accurate level using the SPSS 16.0 program. The flow chart of research process can be seen in Figure 1.

RESULTS AND DISCUSSION

Chlorophyll Content in *Brassica juncea*

Based on the results of the trials, the effects of chlorophyll concentrations on chlorophyll content in two week and three week *Brassica juncea* plants vary significantly between 0.5%, 1.5%, 2.5%, 3.5%, and 4.5% treatments (Table 1). In two weeks and three weeks old *B. juncea* plants, the highest chlorophyll concentration ratio is observed in chloroplasts with 3.5% and 0.5%, with the same concentration of 0.74 mg/g, while the lowest concentration is at 1.5% of 0.61 mg/g at two weeks old and 0.50 mg/g at three weeks of age.

This study's results align with a study conducted by Sunarka et al. (2015) in which antitranspiration of chitosan in the 15-45% concentration range significantly affects the chlorophyll content of *Salacca zalacca* leaves in the second half of the season. Furthermore, Xu & Mou (2018) found that chitosans in the 0.05-0.30% concentrations range can significantly increase chlorophyll content in *Lactuca sativa*. Another study by Moolphuerk & Pattanagul (2020) showed that chitosan can increase the chlorophyll content of *Oryza sativa* in drought conditions. It is known that chitosan can release nitrogen, one of the photosynthesis components (Shangguan et al., 2000). Besides, it can also increase the opening of the stomata as an entrance of CO_2 to accelerate the rate of photosynthesis (El Amerany et al., 2022).

At the highest concentration of 4.5%, chlorophyll content tends to be lower than the control and chlorophyll concentrations below. This is due to the thickness of the chitosan layer that covers the surface of the *B. juncea* leaf, thus supposedly inhibiting the absorption of sunlight, resulting in a decrease in the chlorophyll content. The thickness of the cyanide layer on the leaf surface can also cover the stomata, thus blocking the entry of CO_2. The lack of CO_2 in the plant will affect the activity of photosynthesis.

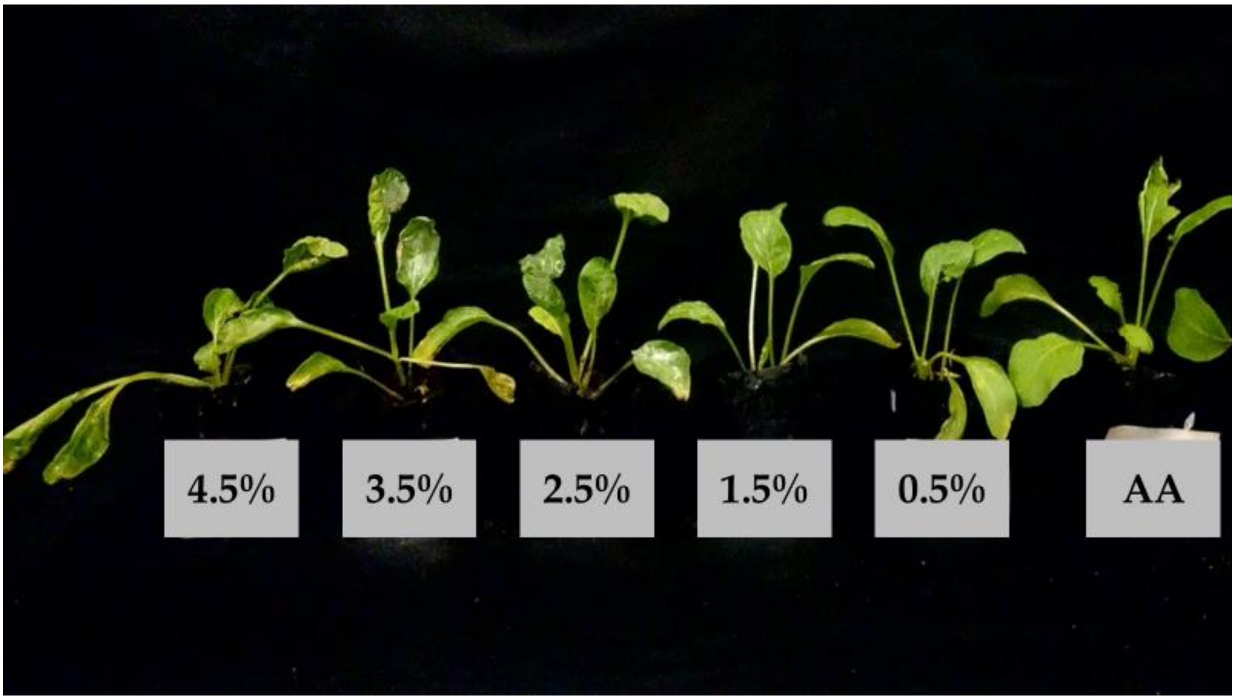
Table 1. Effects of chitosan on chlorophyll content in *B. juncea* plants at different ages

Concentration	Chlorophyll content rate	
	Two weeks old (mg/g) ± sd	**Three weeks old (mg/g) ± sd**
AA	0.69 ± 0.05 ab	0.62 ± 0.08 ab
0.5%	0.70 ± 0.06 ab	0.74 ± 0.16 b
1.5%	0.61 ± 0.06 a	0.50 ± 0.03 a
2.5%	0.70 ± 0.04 ab	0.71 ± 0.13 b
3.5%	0.74 ± 0.09 b	0.69 ± 0.09 b
4.5%	0.64 ± 0.06 ab	0.69 ± 0.06 b

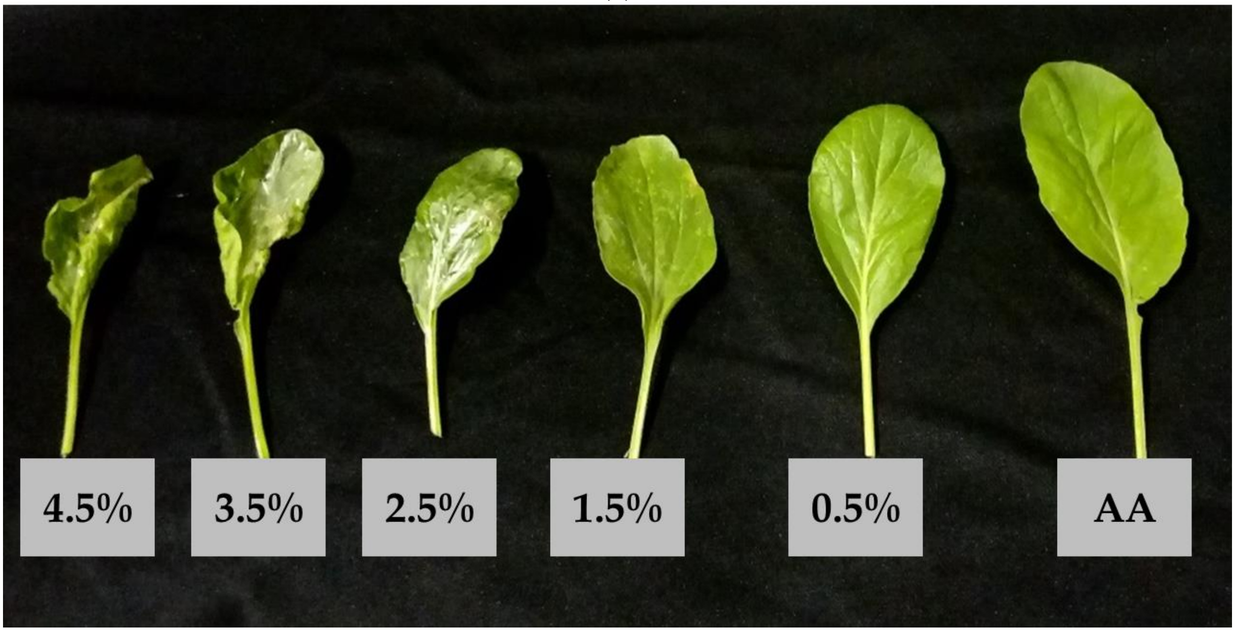
Description:
The ratio at each concentration followed by the same letter in the same column does not differ significantly at the DMRT test level (α=5%)

Phytotoxicity of Chitosan in *Brassica juncea*

Using chitosan in high concentrations (2.5-4.5%) indicates phytotoxic changes in shape and structure in the leaves of two week old *Brassica juncea* plants (Figure 2) or three weeks old. (Figure 3).

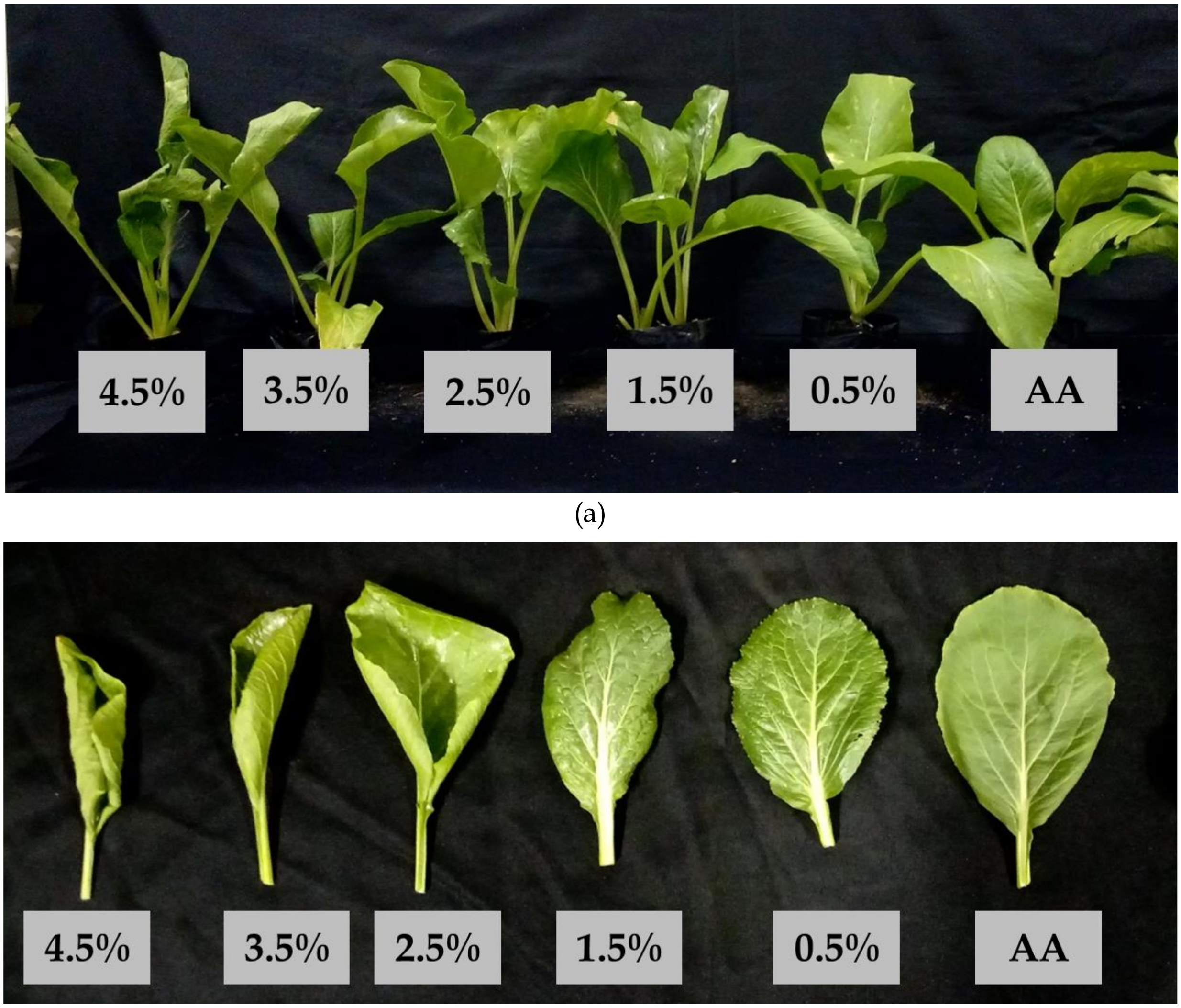


(a)



(b)

Figure 2. The shape of the leaf *B. juncea* due to the treatment of the chitosan (two weeks old)



(a)

(b)

Figure 3. The shape of the leaf *B. juncea* due to the treatment of the chitosan (three weeks old)

The changes in shape and structure are the edges of the rolling leaves. In addition, the plant leaves also showed thickening and structure that tended to be more hardinge when compared to low concentration (0.5% and 1.5%) and control treatment. This means that applying chitosan at high concentrations can cause the leaves of *B. juncea* plants to suffer malformations. This study aligns with a study by Xu & Mou (2018) that reports that using chitosan in high concentrations can harm the growth and development of *Lactuca sativa* plants. (Asgari-Targhi et al., 2018) also showed that chitosan in nano-sized and bulk at higher concentrations (5, 10, 20, and 100 mg/L) is phytotoxic to the growth and physiology of *Capsicum annuum*. Chitosan mixed with a nutrient solution of hoagland and cadmium applied through the leaves showed symptoms of increasing phytotoxicity on the middle-aged leaves and the stem broccoli plants (Pérez-Millán et al., 2022).

Applying chitosan to plants alters the character of plants by inducing a variety of plant defense responses. The plant's defense responses include the accumulation of phytoalexin, PR (pathogenesis-related), proteinase inhibitors, and various enzymes, including the peroxidase enzyme (El Hadrami et al., 2010). This triggers *B. juncea* leaves to thicken and tend to be harder if applied with high concentrations of chitosan. Therefore, it is necessary to pay attention to the use of chitosan concentrations in its application to plants so that plants get a positive effect and can reduce adverse side effects.

CONCLUSION

The highest average chlorophyll content (0.74 mg/g) was found in two and three week old *B. juncea* at chitosan concentrations of 3.5% and 0.5%, while the lowest chlorophyll content was obtained at a chitosan concentration of 1.5% in two and three week old *B. juncea* respectively with a value of 0.61 mg/g and 0.50 mg/g. The use of chitosan with high concentrations (2.5-4.5%) shows phytotoxicity at all ages of plants. So its application, chitosan with a concentration of 0.5% is the best concentration in increasing the chlorophyll content and does not have a toxic effect on *B. juncea*. Further research is needed regarding the mechanism of action of chitosan on plants, especially at the molecular level.

REFERENCES

Asgari-Targhi, G., Iranbakhsh, A., & Ardebili, Z. O. (2018). Potential Benefits and Phytotoxicity Of Bulk And Nano-Chitosan On The Growth, Morphogenesis, Physiology, And Micropropagation Of *Capsicum Annuum*. *Plant Physiology and Biochemistry*, *127*, 393–402. https://doi.org/10.1016/j.plaphy.2018.04.013

Barrera-Necha, L. L., Correa-Pacheco, Z. N., Bautista-Baños, S., Hernández-López, M., Jiménez, J. E. M., & Mejía, A. F. M. (2018). Synthesis and Characterization of Chitosan Nanoparticles Loaded Botanical Extracts with Antifungal Activity on Colletotrichum Gloeosporioides and Alternaria Species. *Advances in Microbiology*, *8*(04), 286–296. https://doi.org/10.4236/AIM.2018.84019

Brunel, F., El Gueddari, N. E., & Moerschbacher, B. M. (2013). Complexation of Copper(II) with Chitosan Nanogels: Toward Control of Microbial Growth. *Carbohydrate Polymers*, *92*(2), 1348–1356. https://doi.org/10.1016/J.CARBPOL.2012.10.025

Eddleston, M., Karalliedde, L., Buckley, N., Fernando, R., Hutchinson, G., Isbister, G., Konradsen, F., Murray, D., Piola, J. C., Senanayake, N., Sheriff, R., Singh, S., Siwach, S. B., & Smit, L. (2002). Pesticide Poisoning in The Developing World: A Minimum Pesticide List. *The Lancet*, *360*, 1163-1167. https://cgspace.cgiar.org/handle/10568/41246

El Amerany, F., Rhazi, M., Balcke, G., Wahbi, S., Meddich, A., Taourirte, M., & Hause, B. (2022). The Effect of Chitosan on Plant Physiology, Wound Response, and Fruit Quality of Tomato. *Polymers*, *14*(22). https://doi.org/10.3390/polym14225006

El Hadrami, A., Adam, L. R., El Hadrami, I., & Daayf, F. (2010). Chitosan in Plant Protection. *Marine Drugs 2010, Vol. 8, Pages 968-987*, *8*(4), 968–987. https://doi.org/10.3390/MD8040968

Jeyaratnam. (1990). Acute Pesticide Poisoning: A Major Global Health Problem. *World Health Statistics Organization: Geneva, Switzerland*, *43*, 139–144.

Kaur, P., Duhan, J. S., & Thakur, R. (2018). Comparative Pot Studies of Chitosan and Chitosan-Metal Nanocomposites as Nano-Agrochemicals Against Fusarium Wilt of Chickpea (*Cicer arietinum* L.). *Biocatalysis and Agricultural Biotechnology*, *14*, 466–471. https://doi.org/10.1016/J.BCAB.2018.04.014

Khati, P., Chaudhary, P., Gangola, S., Bhatt, P., & Sharma, A. (2017). Nanochitosan Supports Growth of *Zea Mays* and Also Maintains Soil Health Following Growth. *3 Biotech*, *7*(1). https://doi.org/10.1007/S13205-017-0668-Y

Kheiri, A., Moosawi Jorf, S. A., Malihipour, A., Saremi, H., & Nikkhah, M. (2017). Synthesis and Characterization of Chitosan Nanoparticles and Their Effect on Fusarium Head Blight and Oxidative Activity in Wheat. *International Journal of Biological Macromolecules*, *102*, 526–538. https://doi.org/10.1016/J.IJBIOMAC.2017.04.034

Kheiri, A., Moosawi Jorf, S. A., Mallihipour, A., Saremi, H., & Nikkhah, M. (2016). Application of Chitosan and Chitosan Nanoparticles for The Control of Fusarium Head Blight of Wheat (*Fusarium Graminearum*) in Vitro and Greenhouse. *International Journal of Biological Macromolecules*, *93*(Pt A), 1261–1272. https://doi.org/10.1016/J.IJBIOMAC.2016.09.072

Lichtenthaler, Y. H. K., & Buschmann, C. (2001). *Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy*. Current Protocols in Food Analytical Chemistry by John Wiley & Sons, Inc. https://sci-hub.se/https://doi.org/10.1002/0471142913.faf0403s01

Maluin, F. N., & Hussein, M. Z. (2020). Chitosan-based Agronanochemicals as a Sustainable Alternative in Crop Protection. In *Molecules* (Vol. 25, Issue 7). MDPI AG. https://doi.org/10.3390/molecules25071611

Meyers, S. P., & Bligh, D. (1981). Characterization of Astaxanthin Pigments from Heat-Processed Crawfish Waste. *Journal of Agricultural and Food Chemistry*, *29*(3), 505–508. https://doi.org/10.1021/JF00105A017

Moolphuerk, N., & Pattanagul, W. (2020). Pretreatment with Different Molecular Weight Chitosans Encourages Drought Tolerance in Rice (*Oryza Sativa* L.) Seedling. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *48*(4), 2072–2084. https://www.notulaebotanicae.ro/index.php/nbha/article/view/12018/9045

Ningsih, S., Putra, N. S., & Trisyono, Y. A. (2020). Feeding Inhibition by Chitosan on Larvae of *Spodoptera litura* (Lepidoptera: Noctuidae). *Jurnal Perlindungan Tanaman Indonesia*, *24*(2), 182. https://doi.org/10.22146/jpti.44032

Pérez-Millán, R., Cámara-Zapata, J. M., Fernández-Zapata, J. C., Simón-Grao, S., Alfosea-Simón, M., Zavala-González, E. A., Shahid, M. A., & García-Sánchez, F. (2022). Application of Biocat G, Selenium, and Chitosan to Counteract the Negative Effects of Cd in Broccoli Plants Grown in Soilless Culture. *Agronomy*, *12*(6). https://doi.org/10.3390/agronomy12061327

Popp, J., Pető, K., & Nagy, J. (2013). Pesticide Productivity and Food Security. A Review. *Agron. Sustain. Dev*, *33*, 243–255. https://doi.org/10.1007/s13593-012-0105-x

Sahab, A., Waly, A., Sabbour, M., & Nawar, L. S. (2015). Synthesis, Antifungal and Insecticidal Potential of Chitosan (CS)-G-Poly (Acrylic Acid) (PAA) Nanoparticles Against Some Seed Borne Fungi and Insects of Soybean. *Int.J. ChemTech Res*, *8*(2), 589–598. https://www.researchgate.net/publication/281738061_Synthesis_antifungal_and_insecticidal_potential_of_chitosan_CS-g-poly_acrylic_acid_PAA_nanoparticles_against_some_seed_borne_fungi_and_insects_of_soybean

Shangguan, Z. P., Shao, M. A., & Dyckmans, J. (2000). Nitrogen Nutrition and Water Stress Effects on Leaf Photosynthetic Gas Exchange and Water Use Efficiency in Winter Wheat. *Environmental and Experimental Botany*, *44*(2), 141–149. https://doi.org/10.1016/S0098-8472(00)00064-2

Sunarka, I. K., Rai, I. N., & Kartini, D. N. L. (2015). Pengaruh Konsentrasi Antitranspiran Chitosan Terhadap Pembuahan dan Produksi Salak Gula Pasir di Luar Musim. *Agrotrop*, *5*(1), 30–36.

Xu, C., & Mou, B. (2018). Chitosan as Soil Amendment Affects Lettuce Growth, Photochemical Efficiency, and Gas Exchange. *Hort Technology*, *28*(4), 476–480. https://doi.org/10.21273/HORTTECH04032-18

Zayed, M. M., Elkafafi, S. H., Zedan, A. M. G., & Dawoud, S. F. M. (2017). Effect of Nano Chitosan on Growth, Physiological and Biochemical Parameters of *Phaseolus vulgaris* under Salt Stress. *Journal of Plant Production*, *8*(5), 577–585. https://doi.org/10.21608/JPP.2017.40468