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Investigation of the Use of *Arthrospira (Spirulina) platensis* and *Cladophora glomerata* Algae in *Agaricus bisporus* (white button mushroom) Cultivation to Increase Growth and Yield

Göksal Sezen^a *^{ib}, Mustafa Turunçoğlu^a ^{ib}

^aHarran University, Faculty of Arts and Sciences, Department of Biology, Şanlıurfa, Türkiye

*Corresponding Author: sezen@harran.edu.tr

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Abstract: This study was conducted with three replicates using three different doses of *Cladophora glomerata* and *Arthrospira (Spirulina) platensis* algae (*C. glomerata* dry weight dose 10 g, 25 g, and 50 g; *A. platensis* dry weight dose 5 g, 12.5 g, and 25 g) in order to develop and determine the most suitable growing media for *Agaricus bisporus* species. Between 28.04.2021 and 10.07.2021, research trials were conducted in a private mushroom production enterprise in Korkuteli District of Antalya Province. The composts in which the seed mushroom mycelia of "Amycel Company" were planted and inoculated were obtained from "SMS Ersanlar Compost Company" in this district. During the research, some distinguishing characteristics of mushroom quality such as mushroom yield, average mushroom weight, mushroom cap and stem weights, mushroom cap diameter, and height, mushroom stem diameter and length, total mushroom length, dry weight, ash weight, and pH were analyzed. It was observed that Algae treatments to composts generally gave better results than the control group. Cld 250 and Spr 250 application doses increased the total mushroom yield by 7% and 15%, respectively.

Keywords: *Agaricus bisporus*, *Arthrospira (Spirulina) platensis*, *Cladophora glomerata*, Culture mushroom cultivation, Edible mushroom

Öz: Bu çalışma, *Agaricus bisporus* türünün en uygun yetiştirme ortamlarının geliştirilmesi ve belirlenmesi amacıyla, *Cladophora glomerata* ve *Arthrospira (Spirulina) platensis* alglerinin üç farklı dozu (*C. glomerata* kuru ağırlık dozu 10 gr, 25 gr ve 50 gr; *A. platensis* kuru ağırlık dozu 5 gr, 12,5 gr ve 25 gr) kullanılarak üç tekerrürlü olarak yapılmıştır. 28.04.2021 ile 10.07.2021 tarihleri arasında Antalya İli'nin Korkuteli İlçesi'nde özel bir mantar üretim işletmesinde araştırma denemeleri yapılmıştır. "Amycel Firması"'nın tohumluk mantar miselleri içinde ekilmiş ve aşıllı olan kompostlar, "SMS Ersanlar Kompost Firması"'ndan yine bu ilçe içinden temin edilmiştir. Araştırmada sırasında mantar kalitesinin bazı ayırt edici özelliklerinden olan; mantar verimi, ortalama mantar ağırlığı, mantar şapka ve sap ağırlıkları, mantar şapka çapı ve yüksekliği, mantar sap çapı ve uzunluğu, toplam mantar uzunluğu, kuru ağırlık, kül ağırlığı ve pH gibi kıstaslar incelenmiştir. Mantar kompostlarına alg uygulamalarının genelde kontrol grubuna göre daha iyi sonuçlar verdiği görülmüştür. Cld 250 ve Spr 250 uygulama dozlarının toplam mantar verimini sırasıyla % 7 ve % 15 oranda arttırdıkları tespit edilmiştir.

Anahtar Kelimeler: *Agaricus bisporus*, *Arthrospira (Spirulina) platensis*, *Cladophora glomerata*, Kültür mantarı yetiştiriciliği, Yenilebilir mantar

1. Introduction

Since ancient times and due to their intriguing nature, fungi have attracted great interest from scientists. These researchers are discovering new species of fungi, with a discovery rate reaching 1200 species per year in the last decade. Today, there are more than 3000 species considered edible mushrooms, including economically and commercially cultivated species and species produced on an industrial scale. However, the white button mushroom (*Agaricus bisporus*) is still one of the world's most produced mushroom species and today is the fourth most cultivated mushroom species worldwide, accounting for 15% of global mushroom production. By 2025, it is expected to dominate the global market [1]. World mushroom production is 50 million tons and 80% of this production is met by Asian countries, 10% by European countries and 5% by the USA. Turkey has a share of 0.0012% with a production value of 61460 tons [2]. According to the data of Turkish Statistical Institute (TurkStat), mushroom cultivation area reached 895 decares and mushroom production reached 61460 tons in 2021 and Korkuteli district of Antalya ranked first with 280 decares of cultivated area and 28000 tons of product and 45.55% on a national basis [3, 4].

It is especially preferred by consumers interested in vegan and 'clean' diets due to its high nutritional value and many health benefits. In addition to identifying the main growth directions and requirements, this fungus has gained a large

share of scientific studies investigating methods to optimize each of the key steps of the mushroom production cycle, such as composting, spawning, sheathing, pinning, trimming and harvesting, and to develop new species with higher yield capacity and resistance to specific diseases [1].

Bacteria and algae in particular are the main component of culture beds, some of which have the ability to increase mushroom yield and quality [5]. Not only in *Agaricus bisporus*, but also in other mushrooms such as *Pleurotus ostreatus*, the addition of bacterial cultures to the mushroom growing medium has led to faster growth of mushroom mycelia [6]. These effective bacteria produce specific metabolites that can initiate sporophore formation. Phytohormones such as indole 3-acetic acid are the most important factors proposed for the growth stimulating effect of these microorganisms. Cyanobacteria and some other algae are a group of microorganisms that can influence the mycelial growth of fungi. Cyanobacteria and some eukaryotic algae produce a wide range of secondary metabolites, including antibiotics, algicides, toxins, pharmaceuticals and plant growth regulators. Among the growth regulators, gibberellin, auxin, cytokinin, ethylene, abscisic acid and jasmonic acid have been identified in cyanobacteria and some eukaryotic algae [7-11].

In this study, it was aimed to investigate the effects of the addition of *Spirulina plantesis* and *Cladophora glomerata*, which are among the micro and macro algae with high nutritional values and content used in plant nutrition, animal feeds, human food supplements, on the yield and quality of *Agaricus bisporus* mushroom cultivation in the mycelial development stage.

2. Material and Method

Arthrospora (Spirulina) platensis

Arthrospora (Spirulina) platensis was obtained from Çukurova University, Faculty of Fisheries. The culture was produced in 20 l plastic bottles in Harran University, Faculty of Arts and Sciences, Department of Biology, Department of Hydrobiology - Algology Laboratory, at a constant temperature of 30 ± 1 °C, pH 9.8-10.3, 24 hours of white LED lamp illumination adjusted to 4000 ± 100 lux light intensity and continuously mixed with fresh air by aquarium pump. The culture reached maximum density every 8-9 days and was harvested by filtering through a 20 µ plankton mesh. The harvested *Arthrospora platensis* was dried in the shade, pulverized with a 46 thousand speed blender (Tefal Ultra High Speed Blender - shredder) and stored in a -20 °C deep freezer. *A. platensis* was cultured with "Spirulina medium" [12].

Cladophora glomerata

Cladophora glomerata required for the experiment was collected from Karkamış Dam in Birecik, Şanlıurfa. The collected algae were cleaned from stones, sand and invertebrates (mussels and snails), dried in the shade, pulverized with a 46 thousand speed blender (Tefal Ultra High Speed Blender - shredder) and stored in -20 °C deep freezer.

Table 1. The systematics of *Agaricus bisporus*, *A. platensis* ve *C. glomerata* are given in Table 1.

Empire	Prokaryota	Eukaryota	Eukaryota
Kingdom	Eubacteria	Plantae	Fungi
Subkingdom	Negibacteria	Viridiplantae	
Phylum	Cyanobacteria	Chlorophyta	Basidiomycota
Class	Cyanophyceae	Chlorophytina	Homobasidiomycetes
Subclass	Oscillatoriothycidae	Ulvophyceae	Homobasidiomycetidae
Order	Oscillatoriales	Cladophorales	Agaricales
Family	Microcoleaceae	Cladophoraceae	Agaricaceae
Genus	<i>Arthrospira platensis</i> Gomont 1892	<i>Cladophora glomerata</i> (Linnaeus) Kützing 1843	<i>Agaricus bisporus</i> (J.E.Lange) <i>Imbach</i>

Mushroom compost

The experiment was carried out in a private mushroom production enterprise in Korkuteli District of Antalya Province. The compost used in the experiment was obtained from "SMS Ersanlar Kompost" company operating in Antalya / Korkuteli district. Seed mycelia were obtained from "Amycel Company" as planted in the compost. The peat used as cover material of mushroom compost was obtained from "Keskin Torf Company" from Çebiçli District of Burdur.

Establishment of the Experiment and Application Method

The compost to be used in the experiment was placed in the mushroom house warehouse where the experiment would be carried out. After the internal temperature of the bag was determined, the necessary air conditioning conditions were provided. The composts placed in the warehouse in the form of presses were placed in bags of 10 kilograms each, weighed and placed on the bunks. The study setup was prepared according to factorial experimental design with 4 replications. In

the experiment, 21 bags of 10 kg each were used. The total amount of compost used was 210 kg. The daily procedures performed in the experiment are given in Table 2 and the changes in the temperature and humidity of the growing room and compost are given in Table 2.

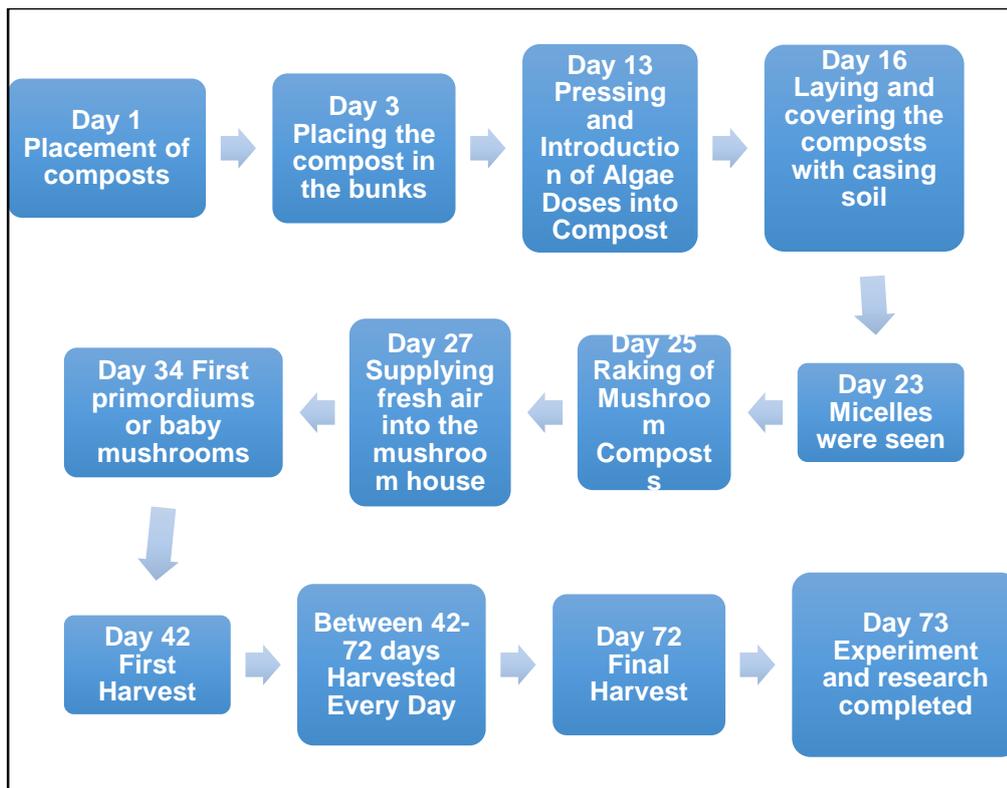


Figure 1. Daily treatments in the experiment

Table 2. Variation of temperature and humidity of the growing room and compost

Process Periods Room	Temperature Bag	Internal Temperature	In Room Humidity
Mycelial pre-growth period	18-22 °C	20-25 °C	75-80
Application of cover soil	20-22 °C	22-28 °C	80-85
Aeration after raking	15-17 °C	17-19 °C	75-80
Harvest time	15-17 °C	15-17 °C	70-75

Powdered *Cladophora glomerata* was diluted to 10%. The powdered *Arthrospira (Spirulina) platensis* used in the study was diluted with water to 5%. After disinfection of the prepared mixtures, applications were made. After the completion of the mycelial pre-development period, the printing process was carried out. After the printing process was completed, labeling process representing the application doses and groups was performed in the test bags. Three groups were formed as Spirulina, Cladophora and control group and each group was applied 100 ml, 250 ml and 500 ml mixture homogeneously with 3 repeated injections. The depth of application was 3-4 cm in the compost. In the control group, 100 ml, 250 ml and 500 ml of water were applied. Table 3. shows the dry weight of algae applied to the experimental groups in grams.

Table 3. Dry weights of algae applied to the experimental groups

Application Groups	Cld 100	Cld 250	Cld 500	Spr 100	Spr 250	Spr 500	Cont
In dry weight amounts of algae (gr)	10	25	50	5	12,5	25	0

Analysis and Measurements in the Experiment

The mushrooms were harvested daily from each trial bag and the necessary measurements were made to determine the total yield during the one-month harvest period. In order to determine the characteristics of each group, 10 samples with the characteristics that reflect the general characteristics of the group were separated. If there were not enough samples, the necessary measurements were made on the samples obtained. At the same time, the samples required for analysis in the groups were taken for freezing and drying.

Physical measurements and analyses to determine mushroom quality were performed according to total carpophore weight, average carpophore weight, total number of carpophores, cap diameter (mm), cap height (mm), cap weight (g), stem length (mm), stem diameter (mm), stem weight (g), average carpophore height (mushroom full height), ash content, dry matter percentage and pH [13, 14].

3. Conclusion and Discussion

Total number of carpophores (mushrooms)

The lowest and highest number of carpophores was 182 in the Spr 500 group and 246.33 in the Cld 250 group, which is about 12% higher than the control groups. The data obtained are shown in Figure 4.1. It was found that the increased activity of nitrifying bacteria was related to the high level of extractable nitrate in *Agaricus* compost, which increased slowly before the completion of the cropping process, and that the total number of carpophores (fungi) could be increased by adding cyanobacteria such as *Nostoc sp.* to the compost [15]. In a similar study conducted by various researchers, it was observed that various bacteria, algae and organic matter added to the compost increased mushroom yield, quality and total carpophore (mushroom) count compared to the control group [16-20].

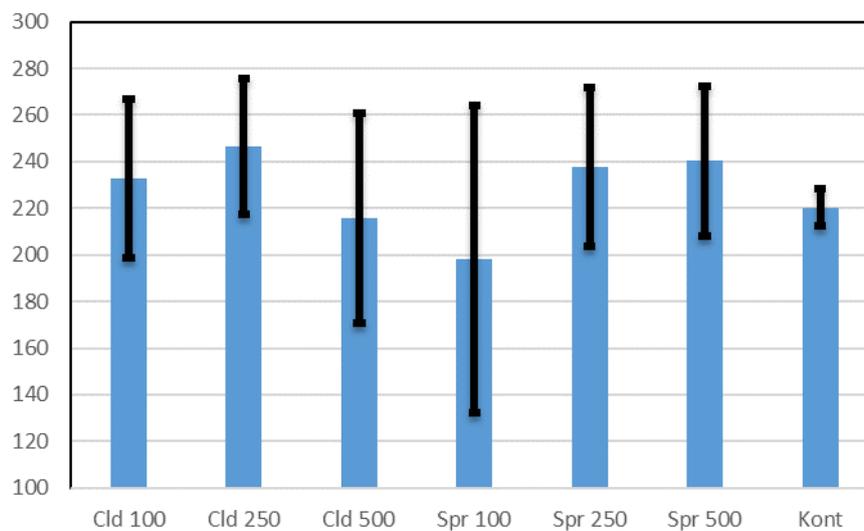


Figure 2. Effect of different dose applications on total carpophore number

Mushroom full length (mm)

The lowest and highest mushroom full length was 42.21 mm in Spr 100 group and 45.72 mm in Spr 500 group, 7% more than the control group. The data obtained are shown in Figure 4.2. In similar studies, it was observed that various bacteria and organic substances added to the mushroom compost increased the mushroom full length compared to the control group [17, 19, 21].

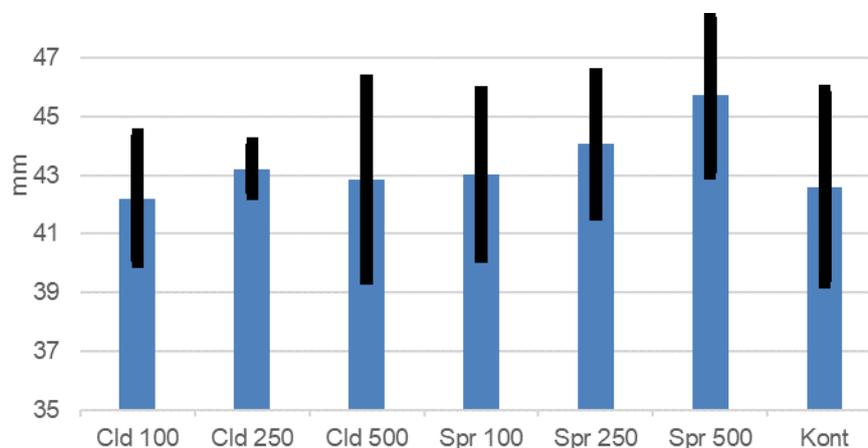


Figure 3. Effect of different dose applications on mushroom full length

Mushroom cap diameter (mm)

The lowest and highest mean hat diameter was 38.11 mm in the Cld 250 group and 43.16 mm in the Spr 500 group, which were 13% higher than the control group. The data obtained are shown in Figure 4.3. In the experiments carried out by various researchers, it was observed that various bacteria and organic substances added to the compost increased the mushroom cap diameter [17, 21-25].

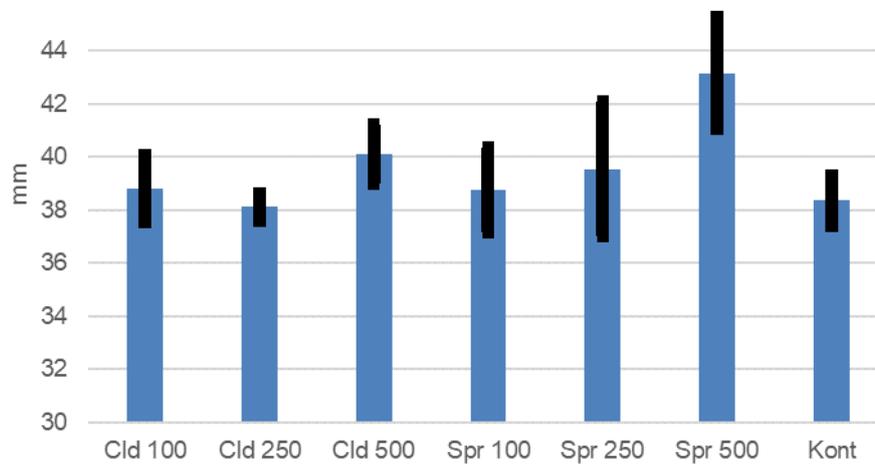


Figure 4. The effect of different dose applications on hat diameter

Height of mushroom cap (mm)

The lowest and highest average hat height was 18.53 mm in Spr 100 group and 32.14 mm in Spr 500 group, which were 70% higher than the control group. The data obtained are shown in Figure 4.4. In some studies, it was determined that various bacteria and organic substances added to mushroom compost increased the height of mushroom cap compared to the control group [17, 21].

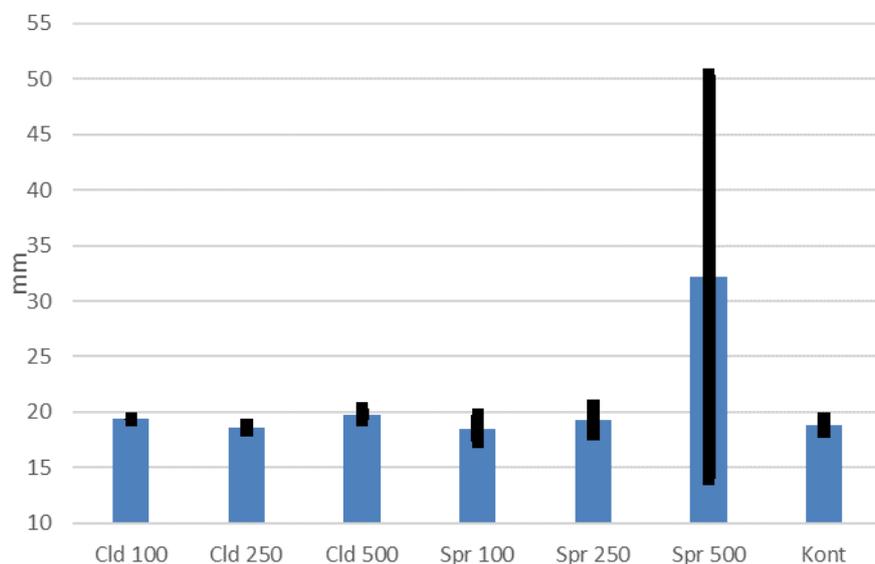


Figure 5. Effect of different dose applications on average hat height

Mushroom stem diameter (mm)

The lowest and highest mean stem diameter was 16.76 mm in the Cld 250 group and 18.72 mm in the Spr 500 group, 4% more than the control group. The data obtained are shown in Figure 4.5. In similar studies, it was found that various bacteria and organic substances added to the mushroom compost increased the mushroom stem diameter compared to the control group [17, 21].

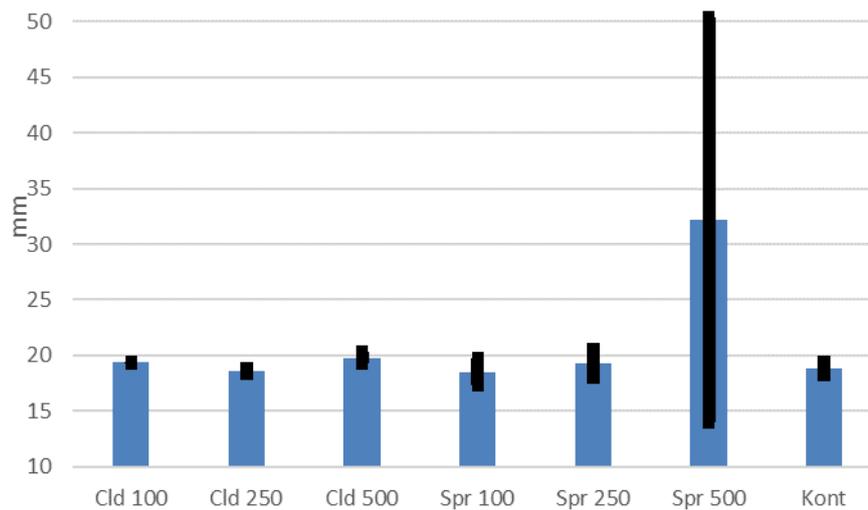


Figure 6. Effect of different dosage treatments on stem diameter

Mushroom stem height (mm)

The lowest and highest mean stem height was 30.91 mm in Cld 100 group and 34.92 mm in Spr 500 group, which were 10% higher than the control group. The data obtained are shown in Figure 4.6. In a similar study, stem length values were observed in the range of 14.45-17.21 cm, the highest value was observed in BM2 group with 17.21 cm and the lowest value was observed in SR8 group with 14.45 cm [21]. In similar studies conducted by various researchers, it was observed that various bacteria, algae and organic substances added to mushroom compost increased the stem length values compared to the control group [17, 18, 22-27].

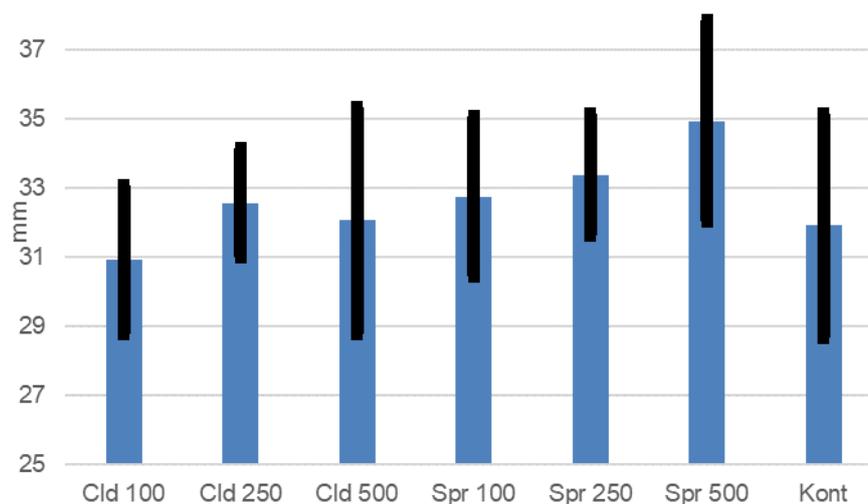


Figure 7. Effect of different doses on stem height

Total carpophore (mushroom) weight

According to the application dose, the lowest total carpophore weight was 2744.93 g and the highest was 3572.89 g, which was 15% more than the control group. The data obtained are given in Figure 4.7. In a study investigating the effect of *Scytalidium thermophilum* inoculation of mushroom composts on yield, it was determined that mushroom yield was twice higher in inoculated composts compared to pasteurised controls [28]. A large increase in the yield of *A. bisporus* mushroom was observed by spraying the photosynthetic bacteria on the cover soil [29]. They observed that the application of nitrogenobacters mixed into mushroom composts did not change the yield of *A. bitorquis* much, but *Bacillus thuringiensis*, *Bacillus circulans* -II and *Alcaligenes feacalis* significantly increased mushroom yield compared to non-inoculated groups [30]. *Streptomyces violaceorubidus*, *Microbacterium humi*, *Gordonia hydrophobica*, *Curtobacterium citreum*, *Agaricola taiwanensis*, *Advenella incenata* and *Actinomycetales bacterium* isolates isolated from the cover soil of *Agaricus blazei* increased fresh mushroom yield from 70% to 115% [31]. In another study, it was reported that isolates containing *Pseudomonas fluorescens*, *P. putida* and *Bacillus mycoides* bacteria, which were isolated in *A. bisporus* cultivation and had good interaction in the cover soil, provided yield increase in the range of 8-40% compared to the

control, and *P. putida* bacteria provided the best result [32]. In a recent study, the highest mushroom yield values of two different commercial microbial fertilisers named BM-MegaFlu and SS-Super Root with different bacterial contents were found in the range of 1063.3 - 2768.5 g, the highest value was found in BM8 with 2768.5 g and the lowest value was found in BM8 application with 1063.3 [21]. In similar studies conducted by various researchers, it was determined that various bacteria, algae and organic substances added to mushroom composts increased the total carpophore (mushroom) weight values compared to the control group [15-17].

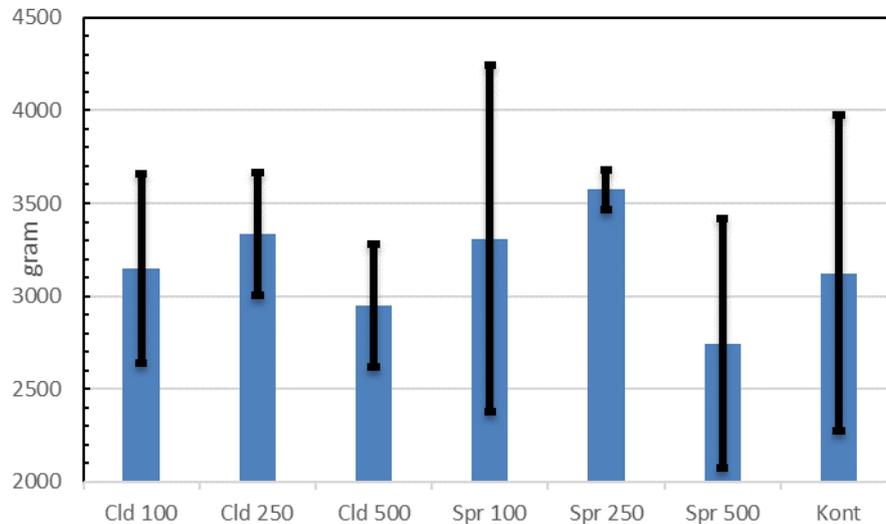


Figure 8. Effect of different dose applications on total carpophore weight

Average carpophore (mushroom) weight

The lowest and highest average carpophore weight was 16.35 g in Cld 250 group and 19.89 g and 22.57 g in Spr 500 and Cld 500 groups, respectively, which were 18% and 34% higher than the control group. The data obtained are shown in Figure 4.8. In the study of application of photosynthetic bacteria to mushroom composts, it was observed that the average mushroom weight varied between 10.71 - 11.20 g [29]. In similar studies conducted by various researchers, it was determined that various bacteria, algae and organic substances added to the composts increased the average carpophore (mushroom) weight values compared to the control group [17, 21]. It is thought that the differences observed in these studies in terms of mushroom weights may be due to the genetic structure of fungi, differences in treatments and environments.

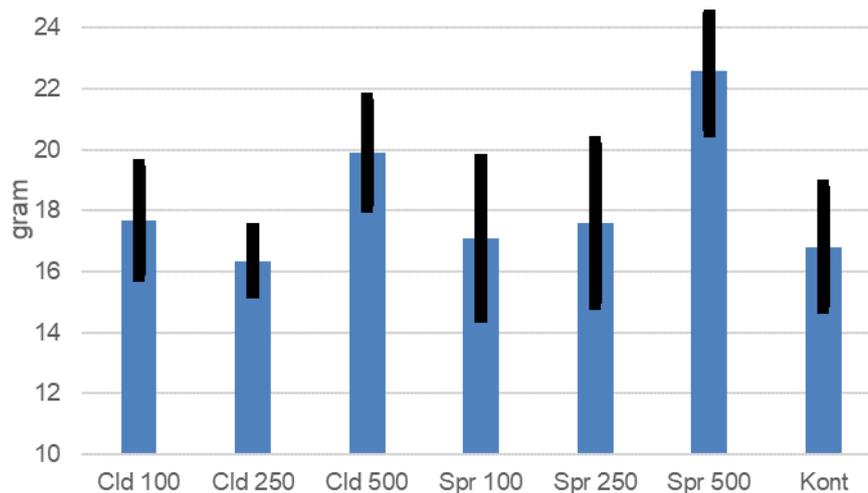


Figure 9. Effect of different dose applications on average carpophore weight

Mushroom hat weight

The lowest and highest average hat weight was found to be 10.63 g in Cld 250 group and 15.75 g in Spr 500 group, 43% more than the control group. The data obtained are shown in Figure 4.9. In similar researches and studies carried out by various researchers, it was determined that various bacteria, algae and organic substances added to mushroom composts increased mushroom cap weight values compared to the control group [17, 21].

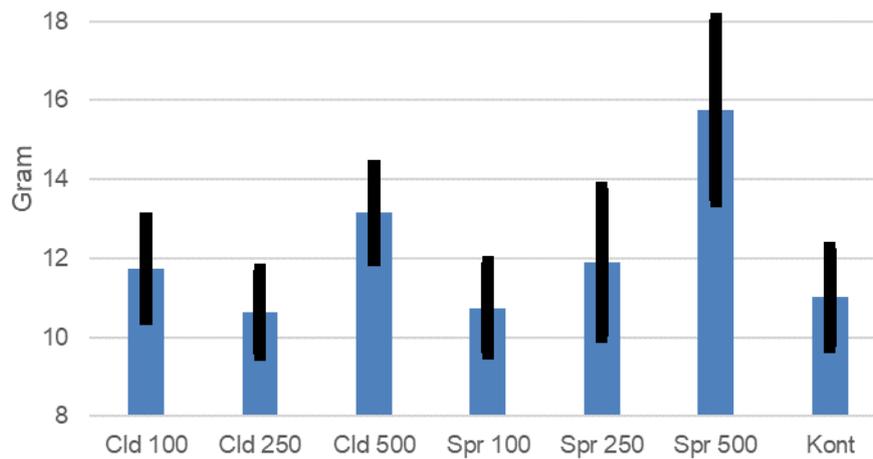


Figure 10. The effect of different dose applications on hat weight

Mushroom stem weight

The lowest and highest average stem weight was 5.74 g in Cld 250 group and 7.14 g in Spr 500 group, which were 27% higher than the control group. The data found are shown in Figure 4.10. In similar studies carried out by various researchers, it was determined that bacteria, algae and organic substances added to mushroom composts increased mushroom stem weight values compared to the control group [17, 21].

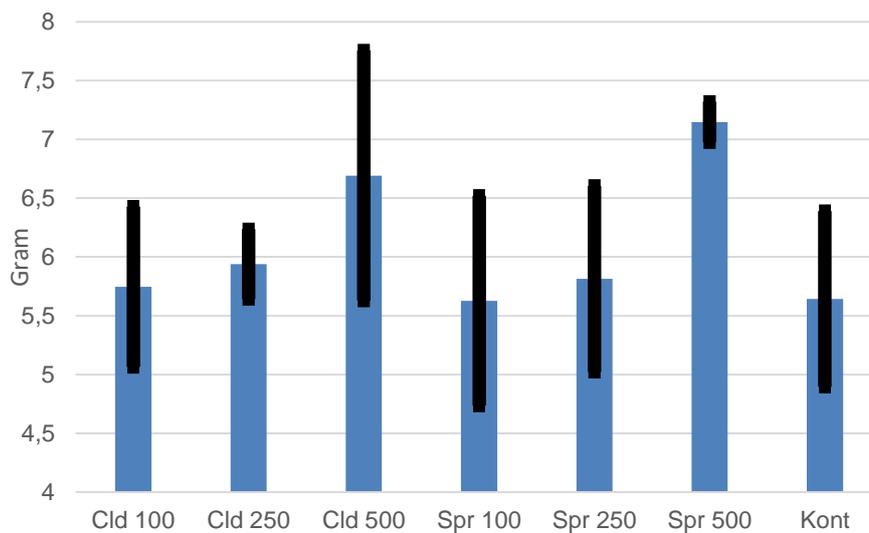


Figure 11. Effect of different dosage treatments on mushroom stem weight

Mushroom wet weight

The lowest and highest mushroom wet weight values were 24.87 g in Cld 100 group and 36.00 g in Cld 500 group, which were 77 % higher than the control group. The wet weights of the mushrooms in the experiments are given in Figure 4. 11. In similar studies carried out by various researchers, it was determined that bacteria, algae and organic materials added to mushroom composts increased mushroom wet weight values compared to the control group [16, 17, 21].

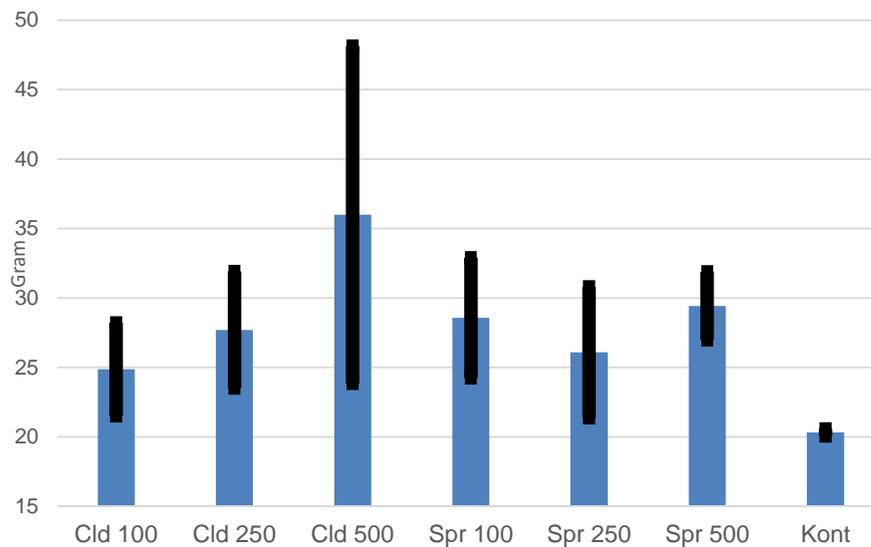


Figure 12. Effect of different dose applications on mushroom wet weight

Mushroom dry weight

Depending on the application dose, the values obtained from the experimental groups varied between 1.79 g. The lowest and highest mushroom dry weight values were 0.91 g in Cld 100 group and 1.79 g in Cld 500 group, which were 112 % higher than the control group. The data obtained are given in Figure 4.12. In a study, it was determined that 9 - 12 % of 100 g fresh mushroom was dry matter and the amount of dry matter of mushroom was influenced by the species and varieties of mushrooms, cultural processes, nutrient content of the mushroom compost medium [33]. In another study, the amount of dry matter was found to be 6.38% [27], and in another study, it was found to be in the range of 7.67 - 9.10%, however, it was also stated that the amount of dry matter decreased in the second and third harvest period compared to the first harvest period [34]. In similar studies carried out by various researchers, it was found that bacteria, algae and organic materials added to mushroom composts increased mushroom dry weight values compared to the control group [15-17, 20, 21].

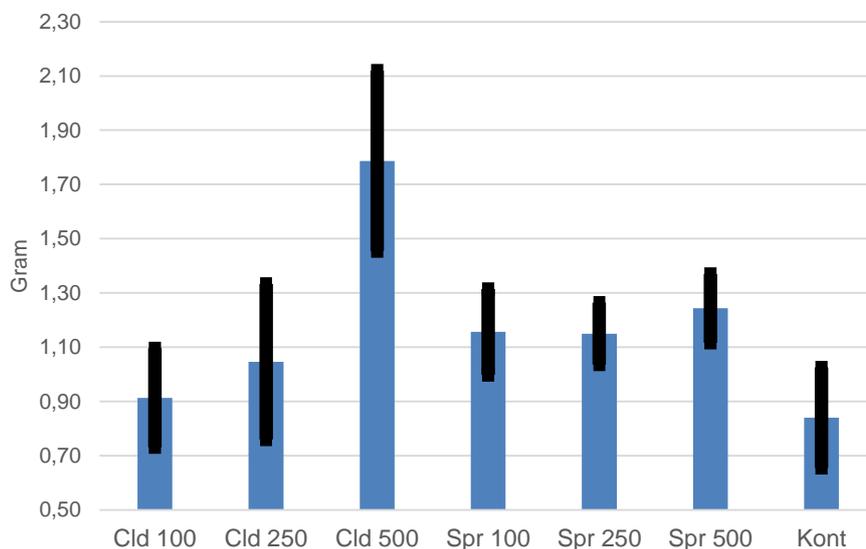


Figure 13. Effect of different dose applications on mushroom dry weight

Mushroom ash weight

The lowest and highest values were 0.33 g in Cld 100 group and 0.70 g in Cld 500 group, which were 109 % higher than the control group. The data obtained are shown in Figure 4.13. In a study, it was found that the amount of mushroom ash was higher in compost formulas and recipes with bacteria and organic fertiliser, the highest was 12.33% ST+B and the lowest was 11.26% ST [35]. In similar studies, it was found that bacteria, algae and organic materials added to mushroom composts increased mushroom ash weight compared to the control group [15, 17, 20, 21].

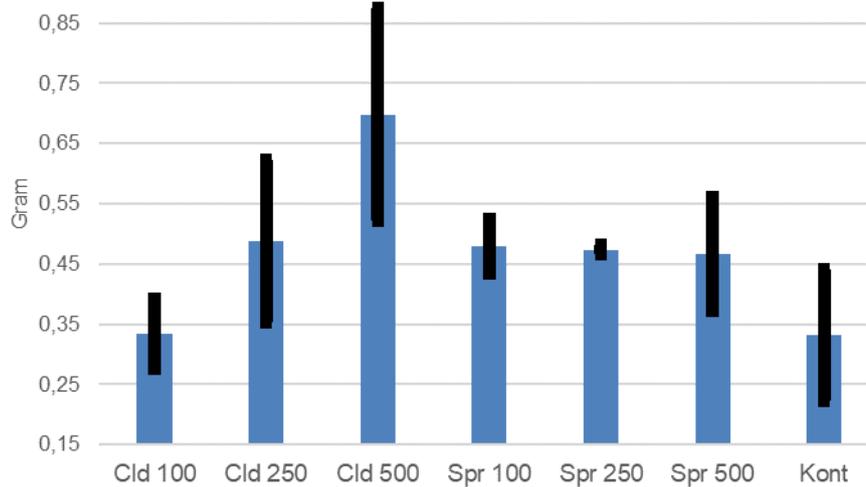


Figure 14. Effect of different doses on mushroom ash weight

Mushroom (%) moisture content

The lowest value was 94.82 % in Cld 500 group and the highest value was 96.35 % in Cld 100 group. The data obtained are shown in Figure 4. 14.

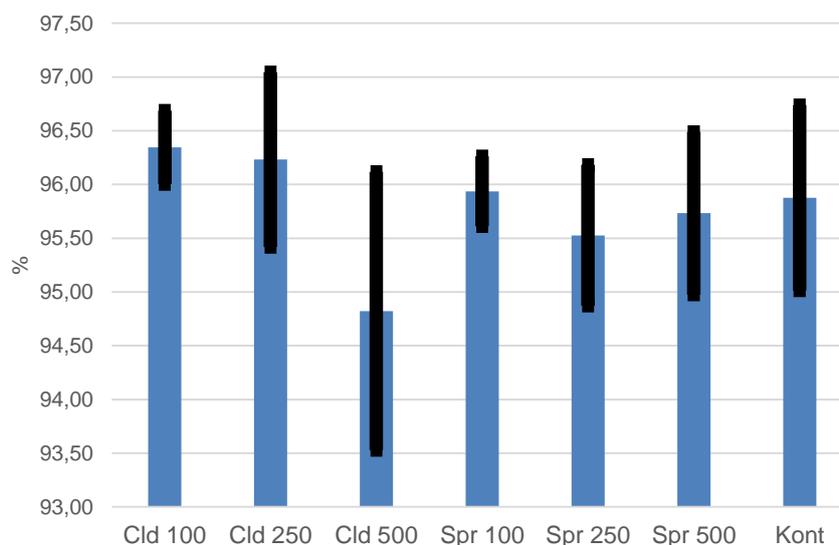


Figure 15. Effect of different dose applications on the moisture content of mushroom (%)

Mushroom pH values

The lowest value was found in Cld 500 group with 6.62 and the highest value was found in Spr 500 group with 7.31, which was 5% higher than the control group. All of the Spirulina sp. treatments were slightly higher than the control groups. It is thought that this may be due to the fact that Spirulina sp. grows in alkaline conditions with pH >10 and pH values are high. The pH result data obtained are given in Figure 4. 15. In a study [21], the pH measurements of the mushroom were measured between 6.76 (BM6) - 7.53 (BM3). These results are in parallel with the studies carried out by different researchers and they stated that different environments and applications may be effective on the pH of mushroom [17, 22, 26, 36].

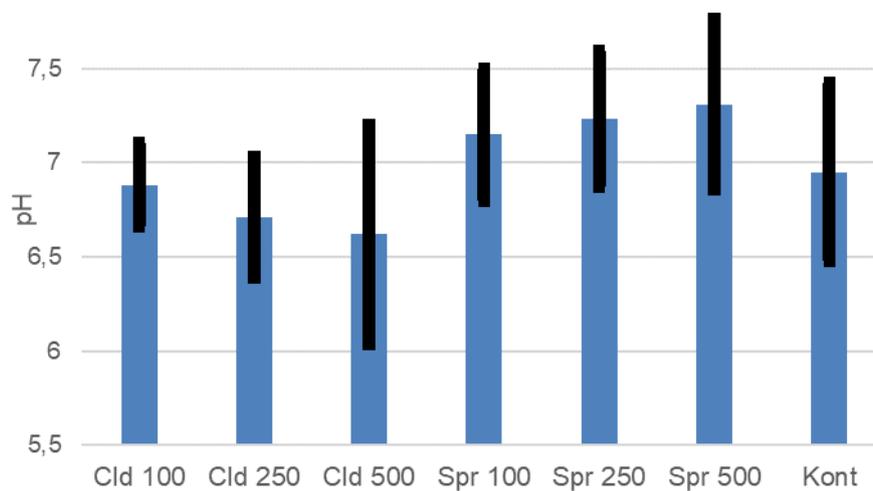


Figure 16. Effect of different dose applications on mushroom pH values

4. Conclusions and Recommendations

This study was conducted with three replicates using three different doses of *Cladophora glomerata* and *Arthrospira (Spirulina) platensis* algae (*C. glomerata* dry weight dose 10 g, 25 g and 50 g; *A. platensis* dry weight dose 5 g, 12.5 g and 25 g) in order to develop and determine the most suitable growing media for *Agaricus bisporus* species. Between 28.04.2021 and 10.07.2021, research trials were conducted in a private mushroom production enterprise in Korkuteli District of Antalya Province. The composts planted and inoculated in the seed mushroom mycelia of "Amycel Company" were obtained from "SMS Ersanlar Compost Company" in this district. During the research, some distinguishing characteristics of mushroom quality such as mushroom yield, average mushroom weight, mushroom cap and stem weights, mushroom cap diameter and height, mushroom stem diameter and length, total mushroom length, dry weight, ash weight and pH were analysed. It was observed that algae treatments to mushroom composts generally gave better results than the control group. Cld 250 and Spr 250 application doses increased the total mushroom yield by 7% and 15%, respectively.

In recent years, the demand for organic agricultural products has increased due to the negative effects of conventional agricultural techniques (pesticides, chemical fertilisers, antibiotics, hormones, etc.). These are biofertilisers of plant and animal origin. In addition, in recent years, various bacteria and algae, which have the effects of increasing plant growth, earliness and long harvest period, have started to be used. Many researchers are using macroalgae, which are abundant and cheap in nature, and a large number of bacteria and algae species and varieties isolated from different sources, inexpensively reproduced or cultivated with advanced techniques and used as biofertilisers at appropriate doses.

Soil microorganisms, especially bacteria, are the main component of mushroom compost, some of which have the ability to increase mushroom yield and quality [1]. Not only in *Agaricus bisporus*, but also in other mushrooms such as *Pleurotus sp.*, the addition of bacterial culture to the mushroom growing medium made the mushroom mycelia work faster [1]. These effective bacteria produce specific metabolites that can initiate sporophore formation. Phytohormones such as indole 3-acetic acid are the most important factors proposed for the growth-stimulating effect of these microorganisms. Cyanobacteria or blue-green algae (BGA) are another group of soil microorganisms that can influence the mycelial growth of fungi. Cyanobacteria produce a wide range of secondary metabolites, including antibiotics, algicides, toxins, pharmaceuticals and plant growth regulators. Among the growth regulators, gibberellin, auxin, cytokinin, ethylene, abscisic acid and jasmonic acid have been identified in cyanobacteria [7- 11, 17].

In this study, the effects of macroalgae and microalgae which can be used as organic and biofertilisers on mushroom yield and quality were investigated. It was found that mycelial growth in composts treated with organic fertiliser, bacteria and algae occurred in a shorter time, covered the compost more quickly, harvesting could be done earlier and positive effects in terms of yield increase were determined. Organic and biofertiliser added compost formulas and recipes can be easily used by producers for mushroom cultivation due to these features.

In terms of the main characteristics and criteria used in the quality of mushrooms, very important effects of these applications were observed. Positive effects of biofertilisers on compost formulas and recipes were determined in terms of quality parameters such as hat, stem and whole weight of mushroom, whole length of mushroom, hat length, hat diameter, and stem length weight.

In nature or in our environment, many algae and some bacterial groups (Plant Growth Promoting Rhizobacteria or PGPRs, Cyanobacteria -are photosynthetic bacterias-, etc.) can be used as biofertilizers. In this study, only two algae species were used. Considering that there may be algae and bacterial species that will have much more effects, we think that more

research and studies should be carried out in this field and subject. Especially in the case that these biofertilisers to be obtained may be expensive, and the properties of other cheap materials that can be found are not very good, the use of these biofertilisers even at a very small rate and amount may give much better results. We think that more research, trials and studies should be carried out on these issues and we propose them to all researchers.

Conflict of Interest

The authors have no conflicts of interest to declare.

Ethics Committee Approval

Not applicable

Author Contribution

All authors have read and agreed to the published version of manuscript.

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