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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2024 21(3): 644-657. **OPEN ACCESS**

Efficacy of exogenous application of Seaweed (Ascophyllum Nodosum) extract on production of Oyster and king oyster mushrooms

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Oyster and king oyster mushrooms are kinds of mushrooms that are cultivated in different parts of the world. A research trial was conducted in the Medicinal and Mushroom Lab, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, to check the growth and behavior of oyster mushrooms with the exogenous application of seaweed extract (*Ascophyllum Nodosum*). There were 4 treatments (To = distilled water, T_1 = 2 mM, T26 mM, and T3 10 mM), each replicated thrice. Application of seaweed was done after completion of the mycelia stage and an 8-hour interval was followed. The purpose of this research work was to study the possibility of Ascophyllum Nodosum utilization in combination with cotton waste to produce oyster and king oyster mushrooms and to find the optimum dose and assess the impact of foliar application of Ascophyllum Nodosum for a better physical, and chemical quality and yield of mushrooms. A decreasing trend was recorded with the increase in the concentration of seaweed extract. The overall result showed that T_1 and T2, represent the better result among oyster mushrooms Whereas, Ti performed best in physiological parameters while T3 and To showed minimum performance respectively. The maximum fresh weight of the mushroom and highest yield was obtained from T_1 (2mM seaweed extract). Different chemical analysis shows that total soluble solids in brix, titratable acidity percentage, ascorbic acid level, total sugars level, reducing sugar level and non-reducing sugars level are influenced positively by all seaweed treatments. It is suggested that cotton waste supplemented with various concentrations of seaweed gave significant results as compared to the control. Overall results showed that maximum significant results on cotton waste with foliar application supplemented (T1) 2mM seaweed extract for oyster and king oyster mushroom. As compared to king oyster mushrooms seaweed extract's effect on oyster mushrooms showed higher results.

Keywords: Mushrooms, seaweed extract, non-reducing sugars, ascorbic acid level and Oyster and King Oyster Mushrooms

INTRODUCTION

Mushrooms are classified into several genera. There are approximately 12,000 species that are members of the mushroom family. Despite many species, only 2000 are known to be edible; only 35 of which are grown commercially, but 25 have been accepted for consumption as a food source (Sur et al. 2019). Most of the Pleurotus species belong to the genus Agaricales and the family Pleurotaceae generally produces white spores. Pleurotus species are commonly cultivated, ranked third in the world, and share 25% of the world's

edible mushroom production (Sur et al. 2019). About 40 species of oyster mushrooms are mostly found in the tropics and temperate regions. Most of these species are also well-known and cultivated in Asia, America and Europe on a commercial scale (Mortada et al. 2020). Pleurotus ostreatus (Oyster Mushroom), Pleurotus eryngii (King Oyster), Pleurotus citrinopileatus (Golden Oyster Mushroom), Pleurotus flabellatus, Pleurotus djamor var. roseus (Pink Oyster Mushroom) and Pleurotus florida (White Oyster mushroom) these are the species of mushroom which are reported to be cultivated on different agricultural waste rich in lignin (Golak-Siwulska et al. 2018).

Mushroom cultivation on locally available agriculture waste and by-products of industry can increase the valorization of agro-industrial waste (Kumar et al. 2020). Many lignin-rich agricultural wastes have been shown to have 100 percent biological efficiency in oyster mushroom cultivation (Mandeel et al. 2005). Agriculture waste, industrial by-products and household waste containing high levels of cellulose and hemicellulose, such as wheat (straw), rice (straw), paddy (straw), cotton (hulls), maize (cobs), sugarcane (bagasse), wood (sawdust), office (paper) and trees (leaves), can all be used to make mushroom compost (Sharma et al. 2013). Mostly, this kind of waste is mishandled, dumped and burned, causing environmental pollution and posing health risks to humans. Mushroom farming can turn these waste products into valuable resources (Girmay et al. 2016). In Pakistan, cotton waste is used for the commercial cultivation of oyster mushrooms due to its availability in abundance. Cotton waste is very effective because mushrooms grow well on it. Cotton waste pasteurization is easier than other substrates and takes less time too (Ali et al. 2007).

Oyster mushrooms are easily available because of the large-scale production around the world in different climatic zones (Li et al. 2021). Pleurotus species were required to be grown at a medium temperature range of 18–35 °C, with a wide range of moisture content in the substrate (50–80%), less than 2000 ppm CO2, and light levels ranging from 500 to 1000 lux. The incubation period and temperature are determined by the species. During its six-week incubation period, 25°C or 30°C temperatures are recorded around the world (Iqbal et al. 2018). Oyster mushrooms are becoming popular in the world due to health reasons. They are a well-known source of nutritional, medicinal and tonic content. It has a high protein content (a meat substitute), low lipid, calorie, and sugar content (better than eggs, meat, and pulses), low levels of cholesterol, sodium, and fat, and is high in fiber, carbohydrates, vitamins, and essential amino acids, making it an ideal food for health-conscious people (Reddy, 2015; Thakur, 2020; Li et al. 2021).

Pleurotus mushrooms are used as functional foods because of their appealing flavor and odor, as well as their nutritional and therapeutic benefits (Knop et al. 2014). In recent years, researchers have discovered that these edible mushrooms have high nutritional contents in terms of essential nutrients as well as a mixture of physiologically bioactive chemicals that are good for human health. The morphological characteristics of Pleurotus species are unstable due to varying agro-climatic conditions and different substrates used for cultivation (Kong, 2004). Therefore, some morphological differences were found between Pleurotus oyster and Pleurotus eryngii. Oysters have a white to grey or tan to dark broad cap that ranges from 5 to 25 cm, while king oysters have a medium-sized cap that is initially round but becomes tube-like after some days. Oyster stipe is white, tight, and varies in hardness (Zadrazil et al. 2004; Kalmıs et al. 2008).

Ascophyllum nodosum L. brown seaweed with a perennial life cycle can be found growing along the temperate coasts of the United Kingdom (Bozorgi, 2012). It contains a large amount of organic carbon (especially carbohydrates, alginic acid, and laminaren). Seaweed extract also contains a lot of polysaccharides but relatively little N.P.K. Due to its growth-promoting and stress-tolerance properties, A. nodosum use has increased in horticultural crops (Battacharyya et al. 2015). Seaweed acts as a soil conditioner, mineral sink, and biostimulant in the production of plants, fruits, and vegetables.

Extracts of Ascophyllum nodosum (AN) have been shown to boost crop growth and yield while also improving crop quality. It activates specialized metabolic pathways from which different organic compounds are produced within treated plants, which have diverse effects on them (Battacharyya et al. 2015). It stimulates plant growth and root development which is thought to be the effect of the chemicals and hormones of seaweed on plant gene regulation of treated plant (Arioli et al. 2015).

These bioactivities of complex organic components enhance growth and product quality (Ali et al. 2021). Recently, lots of studies have been done on this seaweed due to its ability to boost plant growth (Chai et al. 2021). The purpose of this research work was to study the possibility of Ascophyllum Nodosum utilization in combination with cotton waste to produce oysters and king oyster mushrooms. The aim was to find the optimum dose and assess the impact of foliar application of Ascophyllum Nodosum for a better physical, and chemical quality and yield of mushrooms.

MATERIALS AND METHODS

The experiment was conducted at the Mushroom and Medicinal Lab, Institute of Horticultural Sciences (IHS), Faculty of Agricultural Sciences, University of Agriculture Faisalabad (UAF) during 2021-2022. Different ratios of seaweed extract were used to evaluate their effect on the growth and quality of oyster mushrooms.

Experimental Layout

The impact of foliar spray of Ascophyllum Nodosum (C7H14O7S) on oyster and king oyster mushroom growth, yield, and quality were studied by conducting a trial in Complete Randomized Design (CRD) with a two-factor factorial arrangement.

Experimental detail

There were a total of three treatments (2 mM, 6 mM, and 10 mM) that were applied as foliar feeding along with control (distilled water) on the two different varieties of mushrooms, respectively. Each treatment was replicated thrice. The first spraying was done on the complete mycelium

Treatments	Foliar spray
T0	Control
T1	2mM (A. Nodosum) (C7H14O7S)
T2	6mM (A. Nodosum) (C7H14O7S)
T3	10mM (A. Nodosum) (C7H14O7S)

Compost Preparation:

Compost was prepared in the greenhouse area of the Medicinal and Mushroom Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Cotton (20kg), and Ca (OH)2 (3kg) with adequate amount of water were used for the preparation of the compost.

Substrate Preparation:

In this experiment, cotton was the main ingredient as it is a very valuable substrate to produce oyster mushrooms. Cotton was collected from a textile industry and was then soaked in water containing 4% lime. The substrate was covered with a plastic sheet for seven days until it observed 70% moisture. After that 4kg of maize waste was added in the cotton substrate and mixed well.

Pasteurization technique:

Pasteurization is an important step in mushroom production. For this purpose, polythene bags were used. They were filled with substrates and then loosely tied with rubber bands. They were placed on the iron stand in pasteurization drum on the iron stand. Water was added below the stand to produce steam for pasteurization. The temperature was kept at 58-60 °C for three hours. After three hours, to lower the temperature of the pasteurization drum gas supply were stopped. To prevent the burning because of the high temperature in the drum, bags were taken out and let them cool. Fungi and actinomycetes favor the temperature zones in compost which was lowered over 24 hours gradually when the pasteurization was completed.

After that, the temperature of the compost was kept 52 °C until all the traces of ammonia were left which were formed during composting process.

Spawning:

Mushroom spawns were placed on top of the compost and bags were loosely tied by thread and small holes were made in these bags. After spawning polythene bags were kept in a growth chamber.

Temperature and Humidity Control:

The temperature of the growth room amid spawn running was maintained at 25 °C while the relative humidity was (RH) 80 to 90 %.

Application of Seaweed extracts:

Foliar application of seaweed extracts, applied at three different concentrations according to the treatments mentioned above.

Cropping and watering:

The temperature of the growth room was kept between 16-19 °C after finishing spawn running. After successful spawn running formation of fruiting body was started and substrate moisture contents were visually checked daily. Provision of adequate moisture content to the substrate is crucial for mushroom mycelium growth. Too dry or too wet substrate is not suitable for mushroom mycelium growth. A drought condition on substrate can produce a fine delicate mycelium growth which was not suitable for fruiting. Over-irrigated substrate controls mycelium development and delivers extremely intense mycelia and dry compost at spawn addition must be lightly watered or misted with a sprinkler to improve moisture in compost. So, water was sprinkled on substrate twice a day to maintain 70 % relative humidity.

Parameters Studied:

Time taken for initiation of pinhead formation (in days), Number of pinheads/bags,, Number of days for completion of 1st flush, Number of days for completion of 2nd flush, Number of days for completion of 3rd flush, Total flushes/bags, Weight of 1st flush, Weight of 2nd flush, Weight of 3rd flush, Yield per bag (g), Mushroom Moisture (%), Non-reducing sugars (%), Reducing sugars (%), Titratable Acidity (%) (Hortwitz 1960), Total sugar (%), TSS (Brix) (Hasan *et al.* 2020), Vit. C contents (mg/100ml) (Ruck 1961)

Physiological Attribute:

Time taken for initiation of pinhead formation (in days):

Numbers of days were counted from spawning to first pinhead formation.

Weight of first flush (g):

Weight of first flush was recorded in grams until the initiation of second flush.

Weight of Second Flush (g):

Second flush weight was recorded in grams till the initiation of third flush.

Weight of Third Flush:
The third flush weight was recorded in grams till harvesting.

Total number of pinheads per Bag:
Pinheads harvested were counted to calculate the total yield.

Number of days for completion of 1st flush:
The total numbers of days were counted from the formation of the first flush to second flush.

Number of days for completion of 2nd flush:
A total number of days was counted from second flush to the formation of the third flush.

Number of days for completion of 3rd flush:
The total number of days was counted from the third flush to the final harvest.

Total flushes/bags:
Flushes were recorded in each bag.
Yield per bag (g):
The total yield of bags was noted.
Bio-Chemical Analysis:

Moisture percentage:
By measuring media before and after water is removed the moisture percentage is calculated.
Moisture percentage was calculated by the following:
Moisture percentage = initial – dried /initial × 100

Total soluble solids TSS (oBrix):
For the determination of TSS (oBrix) the digital refract meter (Atago Japan, Rx 500.) was used. On the prism of the refract meter, the mushroom juice drop was placed; the lid was closed and at room temperature range 25-28 °C the TSS (oBrix) was recorded directly from the digital scale of the refractro meter.

Titratable Acidity (%):
To calculate the TA first, 10 g mushroom pulp were taken from the sample then water was added to make the homogeneous mixture, and 100 ml volume was made which was titrated by following the titration method of Hortwit (1960). The results were expressed in percentage of citric acid.
T.A(%) = (N/10 NaOH used ×0.0064)/ (volume or wt of the sample used) × 100

Preparation for dye:
In a 200 ml volumetric flask, 42 mg NaHCO3 AND 52 mg 2, 6-dichlorophenol indophenol was combined to make a dye. Distilled water filtered and freshly prepared dye, was used to get the volume up to the required level.

Vitamin C (mg/100ml):
By using (Ruck, 1961) Vitamin C contents were computed. Ascorbic acid was measured fixed in keeping with 2, 6-dichloroindophenol-dye method. For 100 ml solution distilled water was added in 2.5 ml of 20 % metaphosphoric acid fruit sample of mushroom is 10 gm up to mark. 10 ml of the suspension was titrated with standard 2.6-dichloroindophenol freshly made for 15 sec to appear light pink color. In each sample, vitamin C was computed as mg/ 100 grams. Average was computed and examined statistically.
Ascorbic acid = (1×R1×V)/(R×W×V1) ×100
Were,
V= Volume of aliquot made up of 0.4% oxalic acid
V1= ml of juice taken for titration
R1= ml of dye used against V1 of aliquot (sample reading)
W= ml of juice taken
R= ml of dye used to titrate against 2.5 ml (1 ml standard ascorbic acid + 1.5 ml 0.4% oxalic acid) of reference solution (standard reading)

Preparation for dye:
In a 200 ml volumetric flask, 42 mg (NaHCO3) and 52 mg (2, 6-dichlorophenol indophenols) were used to make the dye. Distilled water filtered and freshly prepared dye, was used to get the volume up to the required level.

Total sugars:
In a 100 mL volumetric flask 25 mL of solution which is already prepared, and 5 mL of concentrated hydrochloric acid and 20 ml distilled water are added to the flask. Then we kept the solution for 24-48 hours for absolute hydrolysis in preparation for non-reducing sugar. Manufacture the volume of distilled water produced the next day and as an indicator use phenolphthalein and 0.1 NaOH for neutralization. For total sugars analysis, a solution present in a burette was titrate with 10mL Fehling solution. With this formula, we can determine total sugar.
Total sugars = 25 × (A/C)
A = 10 mL of Fehling's used to find solution standard sugar solution (mL)
C Sample aliquot by Volume (mL) titrated against 10 mL of Fehling's solution

Reducing sugar contents (%):
This solution was made with 10 mL Fehling solution adding 2-3 drops of methylene blue trial hydrate solution was grasped against a 2mL burette and with aliquot drops on boiling the solution to that time the brick color originates. The quality of aliquot use was written down and further action to observe reducing sugars is as
Reducing sugars (%) = 6.25× (A/B)
A = 10mL Fehling's solution was titrated against standard sugar solution
B = 10 mL Fehling's solution against sample of aliquot Volume (mL)
Non-reducing sugar contents (%):

By (Hortwitz, 1960) the formula for non-reducing sugar was calculated as:
N-R-S (%) = 0.95 × (Total inverted sugar – Reducing sugars)

Preparation for dye:

52 mg of 2,6- dichlorophenol indophenols added in a 200 ml volumetric flask with 42 mg NaHCO3 Dye was prepared. Filtered and freshly prepared dye was inevitably used. Volumes were completed by adding distilled water up to the mark.

Ascorbic acid (mg/100ml juice) = 1 x R1 x V x 100/ R x W x V1

Were

R1 = To titrate against 2.5ml Dye used in ml used (1.5 ml 0.4% oxalic acid + 1 ml standard ascorbic acid) solution (reading standard)

R = Against V1 of aliquot dye used to titrate in ml (Sample reading)

The dye used ml to titrate against an aliquot of V1 (Sample evaluation)

V = Oxalic acid 0.4% made by aliquot volume

V1 = for titration juice taken in ml

W = Juice ml

Statistical Analysis:

Statistix8.1® software (Analytical Software, Tallahassee, USA) will be used to run data in a completely randomized design under factorial arrangements. Total significance of the data will be employed using Analysis of Variance (ANOVA) whereas the significant differences among treatment means will be linked using Least Significant Difference (LSD) at 5% probability level (Steel et al. 1997).

RESULTS AND DISCUSSION

Fresh weight of 1st flush:

Data for the Weight for 1st flush in grams for fresh mushroom were collected and analyzed statistically and graphical representation shown in fig 4.1. The result depicted significant differences (P=0.00) among the Weight for 1st flush in grams for fresh mushroom of oyster mushroom. The oyster and king oyster mushroom grown with foliar application foliar application of seaweed (T_3 10mM seaweed extract) showed the minimum mean values of weight for 1st flush in grams that were 79 and 43 respectively. The weight of flushes could be affected due to different impurities temperature of mushroom culture and relative humidity present in the substrate.

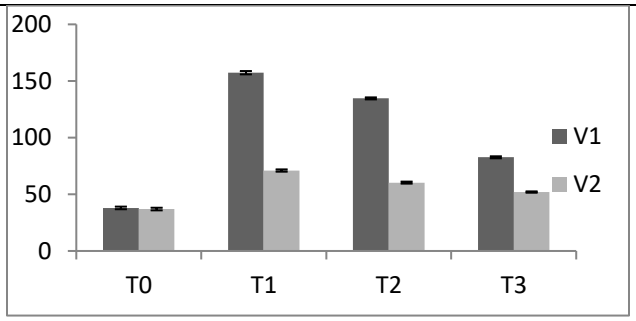

Figure 1: Effect of seaweed extract enriched cotton waste media on weight of 1st flush of oyster and king oyster mushrooms.

T_0= Distilled water
T_1= Seaweed extract at 2mM concentration
T_2=Seaweed extract at 6mM concentration
T_3=Seaweed extract at 10mM concentration
V_1= Pleurotus ostreatus
V_2= Pleurotus eryngii

Fresh weight of 2nd flush:

Data for the weight for 2nd flush in grams for fresh mushrooms were collected and analyzed statistically representation shown in fig 2. It indicated oyster and king oyster mushroom grown with foliar application of seaweed extract (T_1 2mM seaweed extract) and (T_2 6mM seaweed extract) showed maximum mean values (T_1 120 and 55) (T_2 115 and 45) respectively. While oyster and king oyster mushroom grown with foliar application of seaweed (T_3 10mM seaweed extract) showed the minimum mean values.

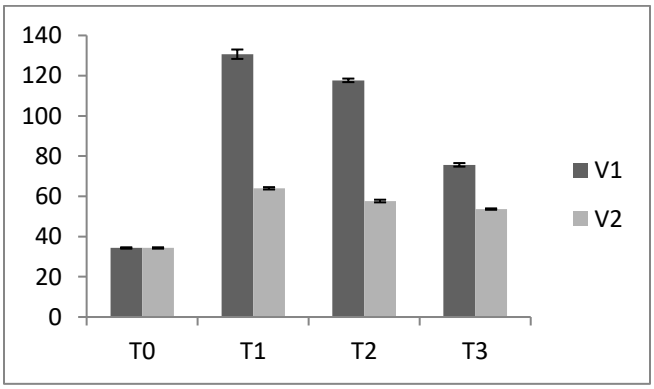

Figure 2: Effect of seaweed extract enriched cotton waste media on weight of 2nd flush of oyster and king oyster mushrooms

Fresh weight of 3rd flush:

Data for the weight for 3rd flush in grams for fresh mushrooms were collected and analyzed statistically and presented in fig 3. It indicated oyster and king oyster mushroom grown with foliar application of seaweed extract (T_1 2mM seaweed extract) and (T_2 6mM

seaweed extract) showed maximum mean values (T_1 105 and 55) (T_2 85 and 45) respectively. While oyster and king oyster mushrooms grown with foliar application of seaweed (T_3 10mM seaweed extract) showed the minimum mean values.

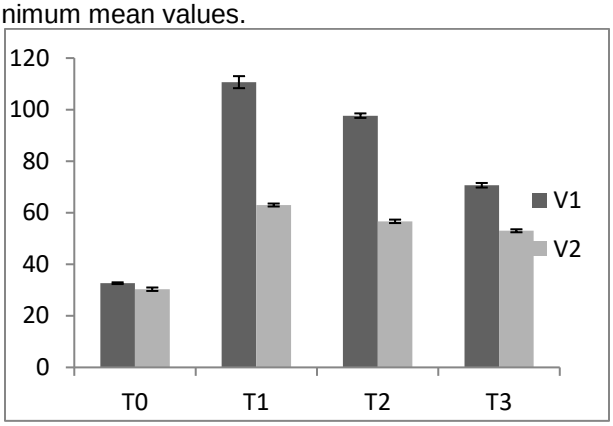

Figure 3: Effect of seaweed extract enriched cotton waste media on fresh weight of 3rd flush of oyster and king oyster mushrooms

Total number of Flushes:

Data for a total number of flushes obtained per bag were collected and analyzed statistically representation shown in fig 4, treatments as compared to control revealed significant results. The result depicted non-significant differences among the T×V of oyster and king oyster mushroom. It shows oyster mushroom grown with foliar application of seaweed extract influenced by all treatments. Maximum mean values at T_1 (2mM seaweed extract) 4.3333 and 3.6667 and minimum value at T_0 (control) 3.3000 and 2.3333 were shown.

Pinhead number could be affected due to mycelium spreading which depends on the humidity and other phenolic compound present in the substrate**.**

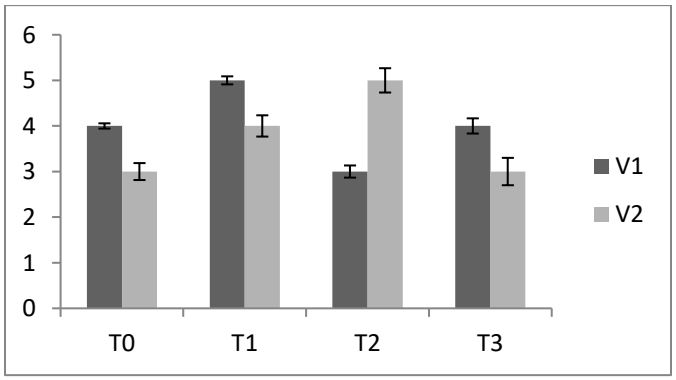

Figure 4: Effect of seaweed extract enriched cotton waste media on total number of flushes of oyster and king oyster mushrooms

Number of days taken to complete 1st flush:

Data for the number of days between first and second flush were collected and analyzed statistically shown in Fig 5. Oyster and king oyster mushroom grown with the foliar spray of 2mM seaweed took the least days (7 days), (5 days) respectively, between the development of first and second flush while oyster mushrooms grown without any foliar spray (control) took more days (17 days) between the development of first flush to second flush**.**

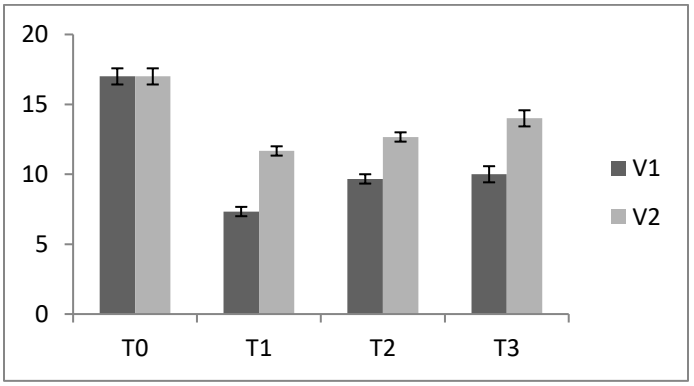

Figure 5: Effect of seaweed extract enriched cotton waste media on number of days taken to complete 1st flush of oyster and king oyster mushrooms

Number of days taken to complete 2nd

Oyster mushroom grown with the foliar spray of 2mM and 6mM of seaweed extract took less days (12 days), (16 days) respectively, between the oyster and king oyster mushroom grown without any foliar spray (control) took more days to complete second flush.

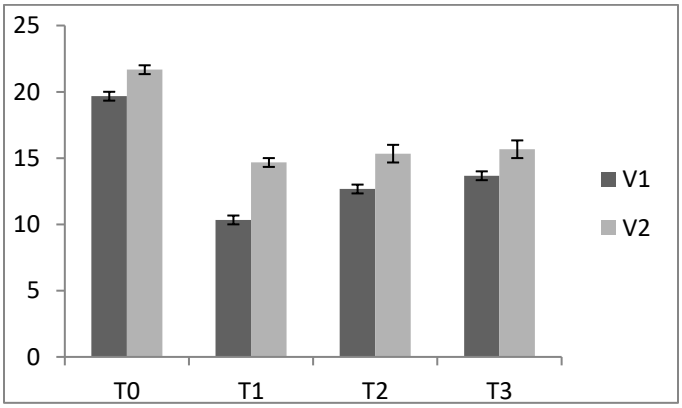

Figure 6: Effect of seaweed extract enriched cotton waste media on number of days taken to complete 2nd flush of oyster and king oyster mushrooms

Number of days taken to complete 3rd flush:

Oyster mushroom grown with the foliar spray of 2mM of seaweed extract took least days (15 days) respectively, between the oyster and king oyster mushroom grown without any foliar spray (control) took more days to complete second flush.

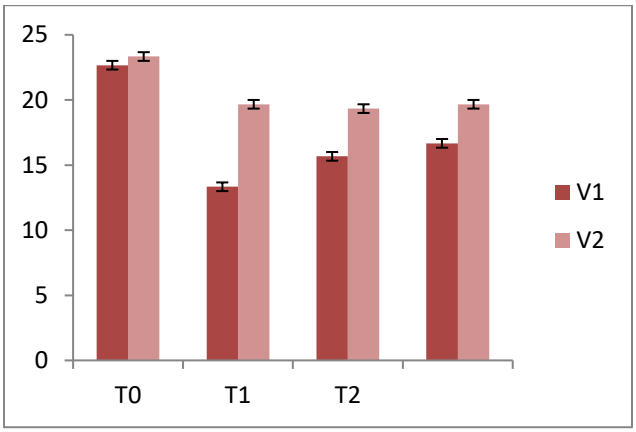

Figure 7: Effect of seaweed extract enriched cotton waste media on number of days taken to complete 3rd flush of oyster and king oyster mushrooms.

Number of pinheads in 1st flush:

Results depicted significant differences (P= 0.01) between 43 the number of pinheads at the first flush of oyster mushroom and king oyster mushroom. Oyster and king oyster mushrooms grown with the foliar application of 2mM seaweed extracts, 6mM seaweed extracts and 10mM of seaweed extracts showed a greater number of pinheads at first flush respectively.

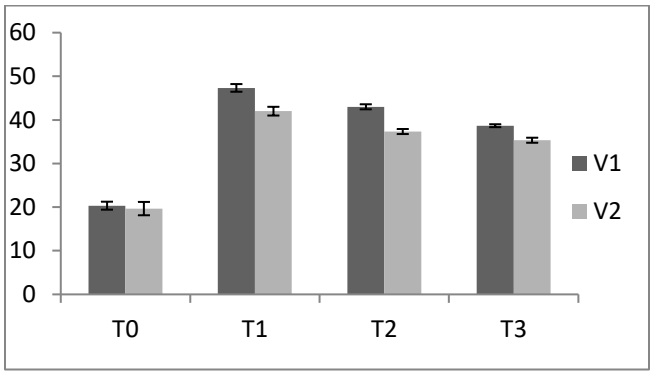

Figure 8: Effect of seaweed extract enriched cotton waste media on number of pinheads in 1st flush of oyster and king oyster mushrooms

Number of pinheads in 2nd flush:

Results are like the first flush. All treatment showed better results than control but several pinheads per bag decreased from the first flush. Our results are closely related to those of Kitamoto et al. (1975). Due to the lower humidity, the number of fungi is lower, and the number of pinheads decreases when the temperature is out of range (Singh et al. 2011).

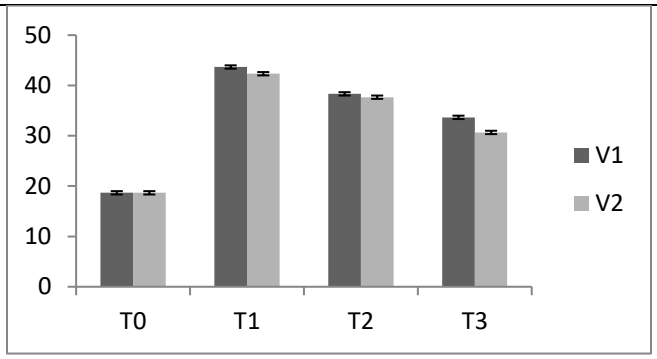

Figure 9: Effect of seaweed extract enriched cotton waste media on number of pinheads in 2nd flush of oyster and king oyster mushrooms

Number of pinheads in 3rd flush:

Results are similar to the first flush and second flush all treatments show better result than control but number of pinheads per bag decrease from first and second flush.

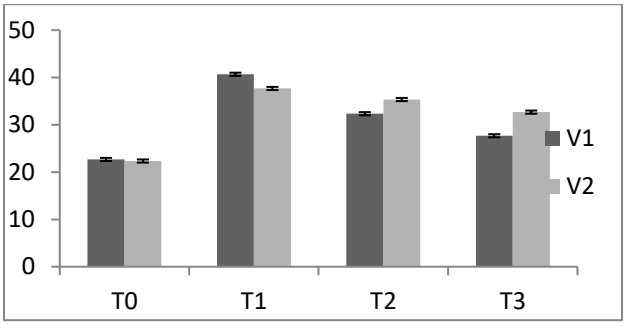

Figure 10: Effect of seaweed extract enriched cotton waste media on the number of pinheads in 3rd flush of oyster and king oyster mushrooms

Days taken for initiation of pinheads at 1st flush:

Pinhead initiation depends upon the mycelium spreading, It could be affected by moisture contents or other impurities present in the substrate. Data for the Time required for starting of pinhead formation in days were collected and analyzed statistically and presented as a comparison representation shown in fig 11, the result depicted non-significant differences among the starting of pinhead formation in days of oyster and king oyster mushroom.

Ahmed (1998) found oyster mushroom took 23-27 days to start pinhead. The number of pinhead initiatives is influenced by temperature, light, humidity, fructose, glucose, and the occurrence of other substances in the substrate (Kitamoto et al. 1995).

Kahn et al. (2001) reported oyster mushroom propagation on various growing substrates and pointed out that it acquired 7-8 days after spawning.

Shah et al. (2004) reported mushroom culturing in various media. He noticed that the structure formed 6-7

days after the leak. The degree of pinhead formation is affected by a variety of factors such as temperature, humidity, substrate properties, tensile quality or strength. If the temperature is outside the range of the number of wire heads, the number of wire heads will be adversely affected if the number of wire heads is lost due to insufficient yield of the mold and lack of water (Singh et al. 2011).

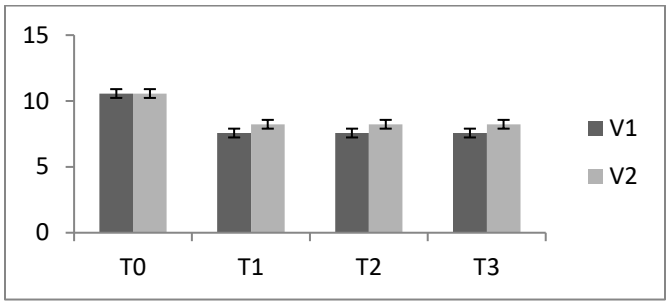

Figure 11: Effect of seaweed extract enriched cotton waste media on days taken for initiation of pinheads at the first flush of oyster and king oyster mushrooms

Days taken for initiation of pinheads at 2nd flush:

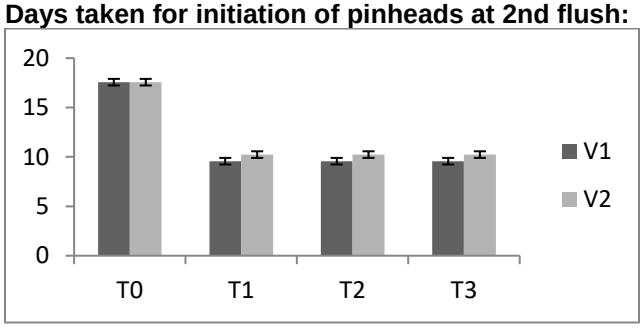

Figure 12: Effect of seaweed extract enriched cotton waste media on days taken for initiation of pinheads at 2nd flush of oyster and king oyster mushrooms

Days taken for initiation of pinheads at 3rd flush:

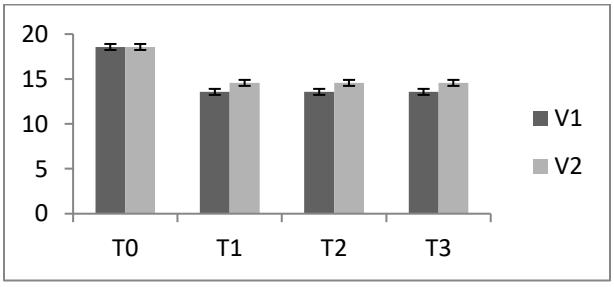

Figure 13: Effect of seaweed extract enriched cotton waste media on days taken for initiation of pinheads at 3rd flush of oyster and king oyster mushrooms

Yield per bag:

Data for the total yield per bag in grams was collected and analyzed statistically and presented as a comparison in fig 4.14 treatments and their interaction all revealed significant results. Oyster mushroom grown with foliar application of seaweed extract (T_2 2mM seaweed extract) showed maximum mean values of 350 for white oyster and 175 for king oyster mushrooms respectively. While oyster mushrooms grown with an application of seaweed (T_3 10mM seaweed extract) showed the minimum mean values of total yield per bag that were 200 for white oyster and 125 for king oyster mushroom.

The result of the ostreatus pleurotus on cotton waste was because of the occurrence of cellulose and hemicellulosic substances in the cotton waste. Present results are closely related to results (Khan et al. 2010)

Payapanon et al. (1994) stated that an adequate quantity of supplementation added to medium paddy straw exploited the yield of Pleurotus spp at best production cost. For the correlation study the connection is strong because the greater the fresh weight, the greater the amount of mushroom content for sale (economic yield) as these mushrooms are sold in kilograms as soon as they are harvested.

These results are also under the observation of Sunarpi et al. (2010) who reported that seaweed extracts increase rice plant growth and development. Amino acids proved to alleviate several negative effects of different stresses on plants hence, improving plant growth.

The yield of several plants increases by the application of seaweed extract as it works as a yield booster for many species of plants. It promotes the production of proteins, nucleic acid, and chlorophyll. Biostimulants are rich sources of several micro and macro molecules along with cytokinin and auxins thus promoting the vegetative growth of plant (Shehata et al. 2011).

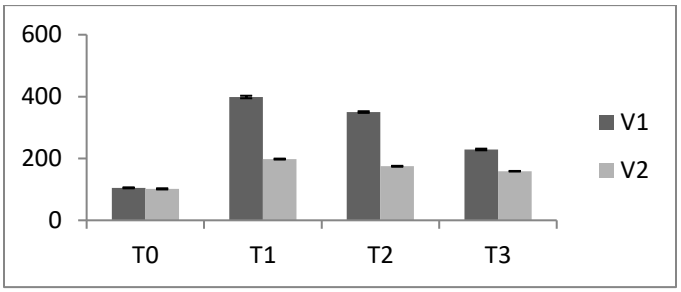

Figure 14: Effect of seaweed extract enriched cotton waste media on yield of oyster and king oyster mushrooms

Total Soluble Solid:

Comparison indicated that total soluble solids (brix^o) showed highest value in T_2 (6 mM seaweed extract) while mushroom with second most significant values of T_3 (10 mM seaweed extract). While oyster mushroom grown without any foliar application control showed the

lower mean values of total soluble solids that were (4.47). Total soluble solids are important for controlling mushroom value and extending its stability and shelf life. Total soluble solids are also needed to sustain the shape of the fungus and improve its excellence. Solids dissolve in substances called totally soluble solids. Sugar is a common example of TSS, vitamins and minerals and other solids. The soluble solids of whole apples and other fruits are important quality parameters related to texture and composition (Peng et al. 2000).

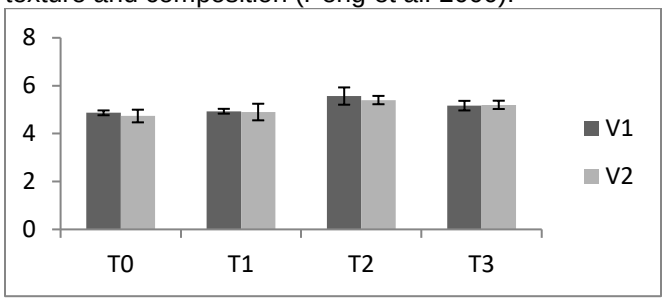

Figure 15: Effect of seaweed extract enriched cotton waste media on yield of oyster and king oyster mushrooms

Moisture %:

A comparison indicated that oyster mushrooms grown with foliar application of seaweed extract (T_1 2mM seaweed extract) and showed maximum mean values of 96.673 and 92.184 for oyster and king oyster mushroom respectively. While oyster and king oyster mushrooms grown without any foliar application (T_0 control) showed lower mean values of total moisture contents which were 83.826 and 83.284 respectively.

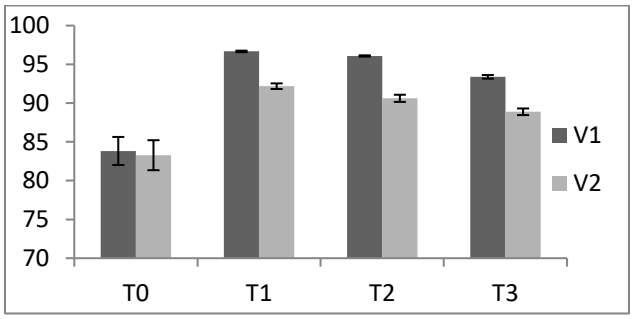

Figure 16: Effect of seaweed extract enriched cotton waste media on moisture % of oyster and king oyster mushrooms

Total Sugars:

Means comparison showed total sugar content of oyster and king oyster mushrooms was significantly affected by all the treatments of seaweed extract had maximum total sugar content (84.000) (8.7667) at T_1 2mM of seaweed extract and T_3 10 mM of seaweed extract and alike results are found at T_2 and T_3 for oyster mushroom 8200 total sugar content while and increasing trend were seen in king oyster mushroom from T_1 towards T_3. Minimum sugar content was recorded at T_0 control.

Abo Sedera et al. (2010) showed that spraying strawberry plants with amino acids (peptone) at concentrations of 0.5 and 1.0 g/L significantly increased the total nitrogen, phosphorus and potassium content of plants, as well as total yield, weight, TSS, vitamin C and total sugar. Compared to comparative therapy. It is clear from the results that the influence of seaweed extract and indole-3-butyric acid shows a considerable rise in the total sugar content of mushrooms. The control conditions yield an earlier rise in the sugar content of the of button and extends its storage treatment. The rise in the sugar content is just due to the transformation of certain carbohydrates into glucose, sucrose, and fructose. The starch is hydrolyzed by certain enzymatic reactions into simpler sugars (Nishizawa et al. 2008).

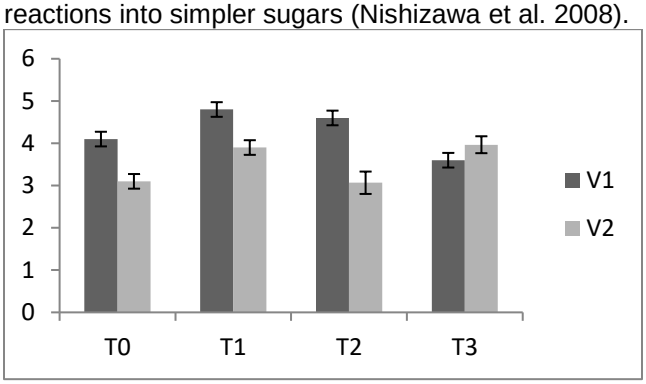

Table 17: Analysis of variance for the influence of treatment and varieties on total sugars of Pleurotus ostreatus and Pleurotus eryngii

Reducing sugar content:

Means comparison showed reducing sugar content of oyster and king oyster mushrooms was significantly affected by all the treatments of seaweed extract had maximum reducing sugar content (4.6000 and 4.8000) at T (2mM Seaweed extract) respectively and minimum reducing sugar content (2.1000 and 3.1000) at T_0(control) respectively were recorded Fig.18. Non-reducing sugar maximum in mushroom at maturity stages and decreased when mushroom stored. Due to different substrates present in waste material mushrooms may be affected (Bhatti et al. 2007).

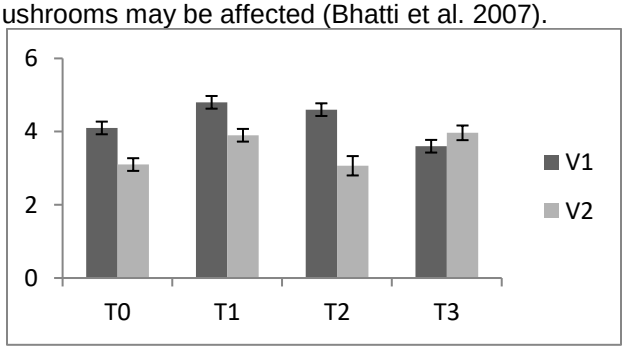

Table 18: Analysis of variance for the influence of treatment and varieties on reducing sugars of Pleurotus ostreatus and Pleurotus eryngii

Non-reducing sugar:

Means comparison showed non-reducing sugar content of button mushrooms had significantly affected by all the treatments of seaweed extract had maximum non-reducing sugar content T_1 and T_3 4.8000 and 3.9667 for oyster and king oyster mushrooms respectively and minimum non-reducing sugar content at T_2 (3.067) and (4.1000 and 3.1000) were recorded at T_0 control. Increasing order were recorded in the oyster mushroom from T_1 to T_3 (Fig.19.).

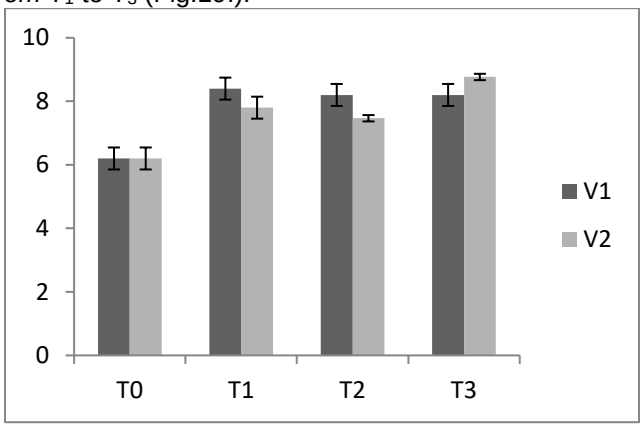

Table 19: Analysis of variance for the influence of treatment and varieties on non-reducing sugars of Pleurotus ostreatus and Pleurotus eryngii

Vitamin C:

Means comparison showed vitamin C content of oyster and king oyster mushrooms had maximum VC content 28.099 and 26.446 at T1(2mM of seaweed extract) and minimum VC content 13.223 and 11.570 at T_0 (control) and T_3 similar value of 21.488 were recorded for oyster and king oyster and decreasing trend are seen in both mushrooms from T_1 to T_3 in both mushrooms (Fig.20).

Ascorbic acid is the main source of vitamin C for humans and plants. It acts as an antioxidant and regulates plant growth and development during hormone signaling (Pastori et al. 2003). Abo Sedera et al. (2010) showed that spraying strawberry plants with amino acids (peptone) at concentrations of 0.5 and 1.0 g/L significantly increased the total nitrogen, phosphorus and potassium content of plants, as well as total yield, weight, TSS, vitamin C and total sugar. compared to comparative therapy.

The exogenous application of seaweed extract increases the level of ascorbic acid in grapevines and the rise in vitamin C content is observed in the harvested grapes (Khan et al. 2012). Similar results of the application of seaweed extract were observed in apricot (El Wahab, 2015).

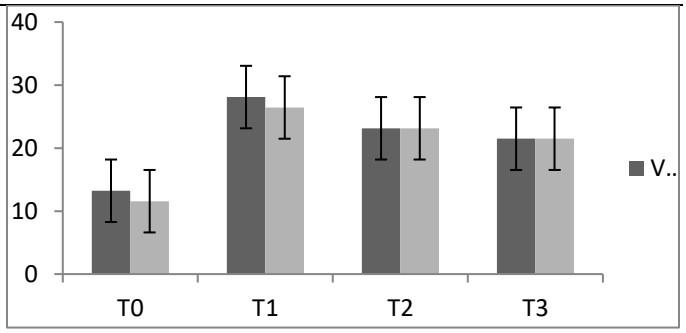

Figure 20: Analysis of variance for the influence of treatment and varieties on vitamin C of *Pleurotus ostreatus* and *Pleurotus eryngii*

Titratable Acidity:

Results depicted significant differences among treatments while there is a non-significant difference between varietal and treatment × variety acidity present in oyster and king oyster mushrooms. Oyster mushrooms grown with the foliar application of T_1 2mM of seaweed extracts showed high values of acidity 0.2667 and 0.2633 for oyster and king oyster mushrooms respectively, while oyster mushrooms grown with foliar application of $T_3$10mM of seaweed extract showed least acidity 0.2433 and 0.2367 and T_0 0.2167 and 0.2233 respectively.

The variation in total acidity is due to the presence of organic acids present in the oyster fruits. These results are in line with the study of Spann and Little, (2011) who reported that seaweed extracts are an important source of antioxidants and improve overall plant health. These results can also be proved with the study of Sadak et al. (2014) who reported a reduction in salinity in faba bean with the foliar application of amino acids leading to quality crop production.

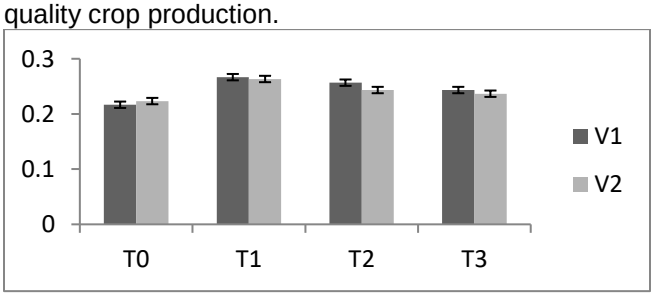

Table 21: Analysis of variance for the influence of treatment and varieties on titratable acidity of *Pleurotus ostreatus* and *Pleurotus eryngii*

CONCLUSIONS

The purpose of this research work was to study the possibility of Ascophyllum Nodosum utilization in combination with cotton waste to produce oyster and king oyster mushrooms and to find the optimum dose and assess the impact of foliar application of Ascophyllum Nodosum for a better physical, chemical quality and yield of mushrooms. Different chemical

analysis shows that total soluble solids in brix, titratable acidity percentage, ascorbic acid level, total sugars level, reducing sugar level, and non-reducing sugars level are influenced positively by all seaweed treatments. It is examined from our research work that cotton waste supplemented with various concentrations of seaweed gave significant results as compared to control. Overall results showed that maximum significant results on cotton waste with foliar application supplemented (T_1) 2mM seaweed extract and (T_2) 6mM seaweed extract for oyster and king oyster mushroom. When we compare the seaweed extract effect on oyster and king oyster mushrooms, oyster mushroom showed higher value than king oyster mushroom. A decreasing trend was recorded with the increase in the concentration of seaweed extract.

Supplementary materials

This article's supplementary material/support can be found online and downloaded from Google Scholar, Science Direct, and Publon.

Author contributions

Mr. Muneeb designed the Research Layout and conducted the research and Miss Maria helped with statistics and the remaining authors contributed to proofreading and the final review

Funding statement

This study was not funded.

Institutional Review Board Statement

The University of Agriculture, Department of Horticulture, Faisalabad Bioethical Committee approved the study.

Informed Consent Statement

Not applicable.

Data Availability Statement

All of the data is included in the article/Supplementary Material.

Acknowledgments

We thank all authors for their contributions.

Conflict of interest

The authors declare no conflict of interest.

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