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Effects of Humic and Folic acid on Quantity and Quality Related Traits of Button Mushroom (*Agaricus bisporus*)

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ABSTRACT: In order to study the effect of humic and folic acid on quality and quantity related traits of white button mushroom, an experiment was conducted in factorial based on completely randomized design with three replications in Zabol University under in vitro conditions. Experimental treatments consist of humic acid (A factor) and folic acid (B factor) that including three levels such as 0, 1.5 and 3cc per liter. Results showed that effects of the folic and humic acid on the mycelium emergence percentage, cap diameter, cap color, yield, biological output and protein percentage were significant at 1%, and on the cap to base proportion was significant at the 5% probability level. Humic and folic acid interaction for the cap diameter was significant at 5%, and for other traits at 1% probability level. Application of humic and folic acid, resulting an increase of quantity and quality yield of white button mushroom. Humic and folic acid interactions showed that to get the maximum quantitative and qualitative yield performance, 3 cc per liter folic acid and 1.5cc per liter humic acid can be used.

Keyword: Diameter of the Cap, Protein percent, Cap Color

INTRODUCTION

Protein is one of the most important components of foodstuff used by human beings. Natural human body need for them shows the necessity of using them in family food baskets. From a long time ago meat especially red meat was the main source of providing the needed protein but now due to the change from traditional way of life to the industrial lifestyle and reduced physical activity of people in everyday life, the use of meat has decreased due to the large amounts of fatty substances. Instead the use of foodstuff that while having adequate amounts of protein is not detrimental to health has got a lot of attention. Cereals, soybeans and mushrooms are among the most important non animal protein sources used widely nowadays. Meantime mushroom markets experiencing a growing trend due to the increased awareness of the society about its benefits and its methods of use and by the use of modern technology and the optimal use of agricultural byproducts and processing industries mushroom's unit per area production has increased (Giri and Prasad, 2007).Button mushroom is the most widely cultivated mushroom throughout the world and it contributes about 40% of the total world production of mushroom (Giri and Prasad, 2007). Recently due to the environmental considerations the use of a variety of organic acids has found great popularity for improving the yield and quality of agricultural and garden crops. Very small amount of organic acids has significant impacts on improving the physical, chemical and biological soil characteristics and due to hormonal compounds are beneficial in increasing the production and improving the quality of agricultural products (Samavat and Malakooti, 2005). Humus compounds of organic materials has two types of organic acids called humic acid and folic and humin component which are extracted from different sources such as soil, humus, peat, oxidized lignite and coal and they differ in molecular size and chemical structure (Sebahattin and Necdet, 2005). Humic acid with molecular weight of 30-300 kg Dalton and Folic acid with a molecular weight of less than 30 kg Dalton respectively result in forming stable complexes and insoluble with micro elements. Humic acid has a higher carbon percentage compared to folic acid but there is more oxygen in folic acid the amount of carboxyl groups of Folic acid is higher than that of humic acid (Michael, 2001). Humic acid and folic acid increase the element's uptake and increase soil's fertility with chelating essential elements while not harming the environment (Michael, 2001). Sladky (1959) observed in a study that the use of 50 mg of humic acid or folic acid increased respiration of leaves and root and chlorophyll content of leaves and the same result was taken from spraying a solution of humic acid on Begonia. Sladky and Tichy (1959) considered the increased dry weight of the Begonia sprayed with humic acid due to chlorophyll and photosynthesis increase. Vaughan and Malcolm (1979) in studying the effects of several humic acids on the activity of the root phosphatase enzyme found that humic acid acts as an inhibitor of phosphatase enzyme activity in wheat root by combining and forming a complex with the enzyme.

Lee and Bartlett (1976) stated that the addition of 8 mg of sodium humate in liter to the soil with low organic matter in which corn was planted significantly increased the amount of root. Liu *et al.* (1996) in studying the effect of humic acid on Bent grass plant found that the humic acid has significantly increased photosynthetic rate, development of root biomass and plant nutrient content. This increase was especially observed at a concentration of 400 mg L of humic acid. Vaughan and Malcolm (1976) investigated the rate of phosphor 32 uptake in winter wheat root cells In the presence of humic acid and found that concentrations of 5 to 50 mg in liter of humic acid significantly increased phosphor uptake. Wang *et al.* (1995) in a field study added humic acid with phosphorus fertilizer to the soil and found that phosphorus uptake increased by 25% compared to the absence of humic acid. Pinton *et al.* (1999) studied the effect of humic substances on nitrate uptake by maize roots. The result of the research showed that nitrate uptake of humic acid in the plasma membrane of root cells was significantly increased. It appears that the activation of membrane proton pump is the initial response to humic acid in nutrient uptake. Sabzevari *et al.* (2009) stated that the most effective concentration of humic acid on the growth of roots and shoots of wheat is 300 mg l and the best response was related to Sabalan type. Therefore, the use of this organic acid can be very useful in root development and wheat establishment which is considered a problem at the beginning of the growing season. Xudan (1986) found that spraying the leaves of wheat with humic and folic acid in greenhouse and field caused an increase in the chlorophyll amount of the leaves. Chen and Aviad (1990) reported that by increasing concentrations of humic substances an increase in plant growth was observed. The researchers noted that one of the causes of plant growth by humic substances was its impact on recruitment and increased permeability of the metal ions. Tan and Tantiwiramanond (1983) stated that increasing humic acid organic matter as an organic material to the cultivation medium increased the number of nodes in soybeans. Sabzevari *et al.* (2010) stated that humic acid through increasing the speed and reducing germination time improves wheat seed's germination particularly in rain fed types and by increasing seedling vigor index and the ratio of root to shoot increased seedling establishment and ultimately improves farm's green level. Low levels of organic acids due to hormonal compounds have very useful effects on increasing production and improving the quality of agricultural products (Samavat and Malakuti, 2005). Use of a variety of natural fertilizers including humic acid and folic acid without the environmental damaging effect can be useful to enhance the performance of mushroom, especially in changing environments because humic acid and folic acid are sited as environmental friendly organic fertilizers (Samavat and Malakuti, 2005). This study was done to investigate the effects of humic and folic acid on the characteristics associated with the quantity and quality of white button mushroom.

MATERIAL AND METHODS

A. Location of experiment

The experiment was done at Zabol University Laboratory under controlled conditions. Compost blocks with spawns were purchased from the University of Mashhad and were placed under laboratory conditions to accelerate the spawn's production. The blocks were placed in a plastic container with dimensions of (10 × 50 × 70 cm) and the standard form used in the country. Test method was planting in separate plots and environmental conditions of breeding saloons were in accordance with mushroom breeding terms namely 25°C in the growth stage and a temperature of 17°C in reproductive growth stage in moisture content of 85-90 percent and total darkness during the entire period and using cooler for cooling and providing relative humidity and the use of fans to reduce carbon dioxide.

B. Field experiment

To study the effect of humic acid and folic acid on quantity and quality of button mushroom, a factorial experiment in a randomized way with three replications was conducted in 2012.Humic acid as "A" factor and folic acid as "B" factor were tested each in three levels of 0, 1.5 or 3 ml per liter of water. 20 days after soiling and the start of reproductive growth sampling from platforms was done. Sampling was done 3times during 3 pickings due to appropriate growth of mushrooms.

C. Quantitative traits

Quantitative traits were such as cap diameter, the emergence of mycelium in top soil during mycelium injection phase in the soil, cap ratio to the stem, performance, biological yield and quality traits were the color of mushrooms' cap and fungal protein. Biological yield was obtained by dividing the average performance (g) by the total weight of the seed bed (g). Cap color change from white to cream or brown will reduce the quality so the cap color was classified in three colors, white, cream and brown and each was given a different value. White was given the "1" value, cream color the "2" value and brown the value of "3".

D. Protein rate

The protein rate of mushrooms was determined by using Kjeldahl method and semi-automatic Kjeldahl Gerhardt machine Model 45 VAP. Excel program was used to draw the charts.

E. Data collect

Data analysis was done using version 9.1of SAS software and comparison of averages with least significant difference test (LSD) done at the 5% probability level test.

RESULTS AND DISCUSSION

Quantitative and qualitative results of variance analysis are shown in Table 1, the results of the comparison of separate averages of humic acid and folic acid in Table 2 and results of the comparison of averages of humic acid and folic acid interactions are shown in Table 3.

Table 1: Analysis of variance for quantitative parameters of Mushroom.

		Means of square						
SOV	**df**	**E.M**	**CD**	**Cap/base**	**CC**	**Yield**	**BE**	**PP**
Folic acid(F)	2	133.3**	1.06**	0.33*	73.5**	3733940.3**	128.97**	131.78**
Humic acid(H)	2	115.9**	2.84**	0.44*	42.0**	1361611.4**	46.84**	152.47**
F*H	4	146.4**	3.72*	0.79**	48.0**	829067.2**	29.00**	113.78**
error	18	21.29	0.10	0.07	0.01	70190.3	2.36	17.69
C.V (%)	-	8.47	5.82	10.5	6.9	6.57	6.4	16.85

*, **, ^{ns} significant at p<0.05, p<0.01 and Non significant, respectively
EM: Emergence of Mycelium; CD: Cap Diameter; CC: Color Cap; BE: Biological Efficiency; PP: Protein percent

Table 2: Means of quantities characteristics of Mushroom as influenced by Folic and Humic acid.

PP	**BE**	**Yield(gm^{-2})**	**CC**	**Cap/base**	**CD**	**E.M**	**Level**	**Treatment**
23.1c	22.3b	3797.2b	1.77b	2.57ab	5.44a	58.88a	0 cc/lit	
24.9b	20.7c	3528.9c	2.38a	2.81a	5.53a	52.22b	1.5 cc/lit	Folic acid
26.9a	27.9a	4754.2a	1.66b	2.43b	5.36a	52.22b	3 cc/lit	
22.5c	22.9b	3912.0b	2.27a	2.78a	5.63a	40.55c	0 cc/lit	
25.0b	26.2a	4460.0a	1.83b	2.68a	5.43ab	50.00b	1.5 cc/lit	Humic acid
27.4a	21.7b	3707.0b	1.72b	2.36a	5.27b	72.77a	3 cc/lit	

Any two means not sharing a common letter differ significantly from each other at 5% probability
EM: Emergence of Mycelium; CD: Cap Diameter; CC: Color Cap; BE: Biological Efficiency; PP: Protein percent

Table 3: Interaction Folic and Humic acid on quantities characteristics of *Mushroom*.

							Treatment	
PP	**BE**	**Yield(gm^{-2})**	**CC**	**Cap/base**	**CD**	**E.M**	**Humic acid**	**Folic acid**
20.8c	21.25cd	3620.0d	2.6bc	2.47bc	5.40ab	15.00f	0 cc/lit	0 cc/lit
21.9c	23.80c	4053.3cd	2.4a	2.55bc	5.70a	70.00c	1.5 cc/lit	0 cc/lit
26.7b	21.86cd	3718.3d	2.0b	2.70bc	5.23ab	91.66a	3 cc/lit	0 cc/lit
20.8c	19.93d	4390.0bc	2.5c	3.60a	5.63a	26.66e	0 cc/lit	1.5 cc/lit
25.8b	21.06cd	3590.0d	2.5c	2.82b	5.70a	53.33d	1.5 cc/lit	1.5 cc/lit
28.1a	21.20cd	3606.6d	2.1bc	2.02d	5.26ab	76.66bc	3 cc/lit	1.5 cc/lit
25.0b	27.76cd	4728.3b	2.0b	2.28cd	5.86a	80.00b	0 cc/lit	3 cc/lit
27.4ab	33.73a	5736.6a	2.0b	2.67bc	4.90b	26.66e	1.5 cc/lit	3 cc/lit
28.3a	22.30cd	3797.6d	1.0a	2.35bcd	5.33ab	50.00d	3 cc/lit	3 cc/lit

Any two means not sharing a common letter differ significantly from each other at 5% probability
EM: Emergence of Mycelium; CD: Cap Diameter; CC: Color Cap; BE: Biological Efficiency; PP: Protein percent

A. Emergence of Mycelium

Folic acid and humic acid at 1% probability level showed a significant effect on the percentage of emergence of mycelium in topsoil (Table 1). Most of the emergence of mycelium in top soil was obtained at the first level of folic acid (58.88 %) and the third level of humic acid (72.77%) (Table 2).

The interaction between humic and folic acid showed that the highest percentage in mycelium emergence was obtained at the first level of folic acid with the third level of humic acid (91.66) which compared to the controls (15.00) showed a significant increase (Table 3). This showed the positive effect of humic acid on the percentage of the emergence of mycelium in top soil after 12 days.

B. Diameter of the Cap

Humic and folic acid at 1% probability level had a significant effect on the diameter of the cap. Humic and folic acid interaction at 5% probability level showed a significant effect on cap diameter (Table 1). The maximum diameter of the button mushroom cap was obtained at the second level of folic acid (5.53 cm). Non-application of humic acid (the control) obtained the highest cap diameter (5.63 cm) (Table 2). The interaction between humic and folic acid showed that the maximum cap diameter was observed at the third level of folic acid and the first level of humic acid (5.86cm) (Table 3). Therefore, the use of folic acid rather than humic acid had a greater impact on cap diameter. Liu and Cooper (2000) stated that folic acid with chelating essential elements cause the nutrient uptake rise and increase the plant's performance.

C. Proportion of mushroom Cap to the Stem

The proportion of mushroom cap to the stem is an indicator of mushroom's quality. The greater the ratio the better is the quality. Folic acid and humic acid at 5% probability level had a significant effect on the ratio of the stem to the cap. While the interaction between folic acid and Humic acid at the probability level of 1% showed a significant effect on the trait (Table 1) the greatest proportion of cap to the stem in the button mushrooms was obtained at the second level of folic acid (2.81) and the first level of humic acid (2.78) (Table 2). The interaction between folic acid and humic acid showed that the highest proportion of cap to the stem was at second level of folic acid and first level of humic acid (3.6) which compared to the control (2.47) had caused an increase in the proportion of cap to the stem. Therefore, the application of humic acid and folic acid had increased the proportion of cap to the stem and consequently increased the quality of the button mushroom (Table 3).

D. Cap Color

Folic acid, humic acid and the interaction of folic and humic acid at the probability level of 1% (Table 1) had a significant effect on the cap color of button mushroom (Table 1). The best cap color was obtained from the third level of folic acid (1.66) and the third level of humic acid (1.72) (Table 2). The interaction between folic acid and humic acid showed that the best color was obtained at the third level of folic acid and the third level of humic acid which was equal to 1 and compared to the control (2.6) showed an increase in the quality. Use of folic and humic acid caused the production of white mushrooms and the increase of quality. With increasing concentrations of folic and humic acid the quality of mushrooms were enhanced.

E. Yield of button mushroom

Folic acid, humic acid and the interaction of folic acid and humic acid at the probability level of 1% (Table 1) had a significant effect on the yield of button mushroom (Table 1). The most button mushroom yield was obtained at the third level of folic acid (47545.2 $g.m^{-2}$) and the second level of humic acid (4460 gm^{-2}) (Table 2). The interaction between folic and humic acid showed that the highest yield of white button mushroom was obtained at the third level of folic acid and the second level of humic acid (5736.6 $g.m^{-2}$) which showed 58.47 % of increase in the yield over the control group. The results show the positive effect of humic acid and folic acid on the increase of button mushroom yield (Table 3). Karkut *et al.* (2009) investigated the effect of humic acid on yield and quality of pepper at 5 concentrations under soil or foliar care and found that humic acid significantly increased fruit weight and total yield compared to the control. In an experiment the effect of spraying humic acid and nitrogen on durum wheat was studied. The results showed that humic acid caused a significant increase in shoot and root dry weight of wheat. The results also showed that grain yield and spike fertility was increased in both cases. This increase was much higher in spraying nitrogen concurrently with humic acid (Delfine *et al.*, 2005).

F. Biological Efficiency

The effect of folic and humic acid and folic and humic acid interaction on biological efficiency was significant at 1% level (Table 1). Most of the biological efficiency was obtained at the third level of folic acid (27.93%) and the second level of humic acid (26.2%), respectively (Table 2). Interaction of folic acid and humic acid showed that the highest biological yield of button mushroom was obtained at the third level of folic acid and the second level of humic acid (33.73%) which showed 58.73 percent efficiency increase over the control. These results reflect the positive impact of humic and folic acid on biological efficiency of button mushroom (Table 3).

G. Protein Percentage

Folic acid, humic acid and the interaction of folic acid and humic acid at the probability level of 1% (Table 1) had a significant effect on protein percentage (Table 1). The most protein percentage was obtained at the third level of folic acid (26.9%) and the third level of humic acid (27.4%) (Table 2). The interaction between folic acid and humic acid showed that the highest protein percentage of button mushroom was obtained at the third level of folic acid and the third level of humic acid (28.3%) which showed 36.06 % increase over the control group.

Protein rate of button mushroom has been under different levels of humic and folic acid and with the increase of the amount of humic and folic acid the protein content and consequently the quality of button mushroom was enhanced. Karkut *et al.* (2009) investigated the effect of humic acid on yield and quality of pepper at 5 concentrations under soil or foliar care and found that reduction of sugar content of fruit with the use of humic acid increased in either way. In an experiment the effect of spraying humic acid and nitrogen on durum wheat was studied. The results showed that humic acid caused a significant increase in the amount of protein the seeds in both cases. This increase was much higher in spraying nitrogen concurrently with humic acid (Delfine *et al.*, 2005).

DISCUSSION

Nutritive supply is essential for growth of mycelium in compost. As long as the nutritive is supplied, the production continues. Therefore we can enrich the compost by using dietary supplements resulting in increased production and performance. Humic and folic acid can be convenient and affordable as a new organic fertilizer and can be usedin order to improve plant's yield and strength and preserve of nutrients. Humic and folic acid cause an increase in root volume and consequently result in improved absorption of all elements. These materials also play the role of a good chelating one and are not harmful for the environment. Humic and folic acid due to being acids can directly release different elements from minerals attract them and at the proper time place at roots' disposal. Besides nutritive and growth stimulants are useful soil micro organisms and they also help in different ways to release nutrients in the soil. Therefore, humic and folic acid directly or indirectly help to better release and uptake of elements. The use of high levels of humic acid (3 cc) had the greatest impact on the emergence of mycelium which can be considered as an administrative approach in adverse conditions. Folic acid had the greatest impact on the performance of white button mushroom in 3 cc per liter. The interaction of humic and folic acid on the function showed that to obtain the best performance it is better to use 3cc per liter of folic acid and 1.5cc per liter of humic acid. What was obtained from studying the results of variance analysis and comparison of average of data indicated the positive effect of organic fertilizers on the yield and quality of white button mushroom.

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