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Int. Agrophys., 2012, 26, 375-380
doi: 10.2478/v10247-012-0052-4

Enhancement in growth and yield of mushroom using magnetic field treatment

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Received June 2, 2011; accepted November 4, 2011

A b s t r a c t. The magnetic treatment effects on mushroom spawn growth and yield were studied. The spawn of mushroom were exposed to full-wave rectified sinusoidal magnetic field. The spawn were grown after magnetic field treatment under controlled laboratory conditions. The magnetic field treatment resulted in significant increase ($P<0.05$) in the growth and yield of mushroom. The number of pin heads formed, number of pin heads developed into mature mushrooms, fresh (wet) and dry masses increased up to 38.18, 34.83, 76.43, and 38.26%, respectively, while reduction in number of days for spawn complete running and number of days for appearance of pin heads was found to be -3.14 and -26.86%, respectively.

K e y w o r d s: magnetic field treatment, mushroom, spawn running, yield

INTRODUCTION

Over the last few decades, there has been a growing concern of higher food production due to stress of increased population; on the other hand, there are various natural factors opposing the germination, growth and ultimately yield of crops (Fujimaki and Kikuchi, 2010; Li *et al*., 2011). In this regard, the agricultural scientists and food technologists are trying to search for methods which must be proficient, eco-friendly and inexpensive. Biological, chemical and physical treatments are being used to obtain higher growth and yield (Ćwintal *et al*., 2010; Hernandez-Aguilar *et al.,* 2009; Perveen *et al.,* 2010). Chemical treatments were found to be effective for enhancing the growth and yield, but might be detrimental at later stages of development (Dao-liang *et al.,* 2009). The effect of magnetic field treatment on biological systems has been studied by various researchers for enhancing germination, vigour as well as growth at later stages of development (Florez *et al.,* 2007; Marks and Szecówka, 2010). It is believed that magnetic field treatment changes the free radicals, concentration of ions and electrical charges without any degradation/alteration in the chemical profile of seed and makes the membranes more permeable (Iqbal *et al.,* 2012), and this free movement of ions activates the metabolic pathways by enhancing the biochemical and physiological feedback (Pietruszewski, 2007).

Various researchers have studied and reported that magnetically treated maize, wheat, sunflower, barley, corn, beans, tomato, fruits and mushrooms *etc*. showed high performance in terms of plant growth, height, yield, mass per spike as well as shoot and root length and assimilation of fresh and dry matter (Anggoro *et al*., 1999; Fischer *et al.,* 2004; Pietruszewski and Kania, 2010; Zepeda-Bautista *et al.,* 2010). Furthermore, the MF strength, exposure duration and modulation are also important in this regard (Tkalec *et al.,* 2009). Iqbal *et al.* (2012) reported that the magnetic field treatment at 10 mT for 40 h boosted up the height, mass and crop yield. Similarly, Vashisth and Nagarajan (2010) reported positive results in the growth of maize, chickpea and sunflower seeds exposed to static magnetic field.

Mushroom is one of man earliest foods which have come to be recognized as highly nutritive, low in calories, rich in proteins and certain vitamins. A distinctive feature of mushroom protein is that it covers all of the essential amino acids and has a high digestible value. The world production of mushroom was estimated to be 3 577 632 t in 2009, China producing 87% of the world supply. Mushroom is the easiest to produce and the least expensive to grow for small scale cultivation as well as at commercial level with limited budget. Various *Pleurotus* species grow on a wide array of forest, agricultural and industrial materials. The cultivated species of *Pleurotus* are *ostreatus, pulmonarius, systetiosus,*

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eryngii, djmor, citrinopileatus and *Pulmonarius*. The cultivation of mushroom as a source of fungi growing has been suggested as one of the ways to meet the demand of growing population. Further, mushroom can be grown on almost all ligno-cellulosic agricultural and industrial wastes. Being a protected crop, it does not compete for area with any other crop because it is usually produced indoor and thus is safe from the vagaries of natural effects (Ali *et al.,* 2007).

Mushrooms are consumed as a delicious food item. Both fresh and preserved fruiting bodies of tens of species can be culinary-processed in different manners. All mushrooms continue to grow, mature, respire and senesce after harvest, resulting in mass loss along with other undesirable changes and spoilage, which leads to poor value and even total loss of the production (Arumuganathan *et al*., 2010). So far, despite their importance, little attention has been given for the improvement of mushrooms native to Pakistan.

The aim of this paper was to investigate the effect of magnetic field (MF) produced by a home-made electromagnet for the treatment of spawn on its growth and yield.

MATERIALS AND METHODS

The spawn of mushroom were obtained from the Culture Bank, Department of Horticulture, University of Agriculture, Faisalabad. The selected spawn were exposed to controlled MF in the Department of Physics and sown in the Vegetable Seed Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. The presowing MF treatments were administered using an electromagnet consisting of two pairs of cylindrical coils, each formed of 4 000 turns of 0.42 mm enamelled copper wire. Each pair of coils was wound 10 cm apart on an iron bar (dimensions 40×3.5 cm). The two bars were placed one above the other, their ends held by metallic supports. The coils were connected in series and fed through a power source using a variable transformer. A 50 Hz full wave rectified sinusoidal voltage was fed to the coils. When electric current passed through the coils MF was generated in the air space between the two bars. The MF treatment was applied according to the method of Iqbal *et al.* (2012). Briefly, the spawn were kept in Petri dishes and held between the poles of the electromagnet one by one. The strength of electromagnet was changed using variable voltage through coils. The MF strength in the region between the poles was measured using a magnetic flux meter (ELWE, Germany). The distribution of magnetic field between pole pieces of the electromagnet for an applied voltage of 220 V is shown in Fig. 1.

The mushroom spawn were multiplied on malt extract agar (MEA) medium at 25°C. The medium was sterilized in an autoclave at 15 psi, at 121°C for 30 min, and was then poured into 90 mm Petri dishes. The bacteria *streptopenicilline* were poured into the sterilized medium at the rate of 1 g l^{-1}. Four strains were inoculated into MEA medium in 90 mm Petri dishes in triplicate at 25°C for mycelia growth.

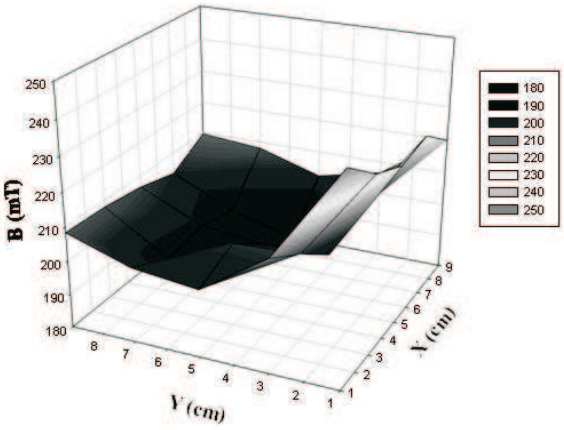

Fig. 1. Distribution of magnetic field between pole pieces of the electromagnet taken at 220 V.

T a b l e 1. MF treatment parameters used for mushroom spawn treatment

MF (mT)	2 min	5 min	15 min
5	T_1	T_2	T_3
15	T_4	T_5	T_6
25	T_7	T_8	T_9
100	T_{10}	T_{11}	T_{12}

Spawn was prepared on wheat grains (Ali *et al.,* 2007). Whole wheat grain was boiled for 30 min and mixed with 2% calcium carbonate ash and 4% calcium sulphate to avoid culming of grains. The grains were sterilized at 121°C for 1 h and inoculated with three strains of *Pleurotus* spp. The spawn were considered ready when mycelia covered the grains and then were subjected to the MF doses (strength and exposure duration) (Table 1).

Non-exposed spawn were used as control (T_0). A rectangular glass dish with mushroom spawn was placed between the poles of the electromagnet for the required duration of exposure and MF strength. The strength of the MF was controlled by regulating the voltage in the coil of the electromagnet. After respective treatment for specific period and MF strength the spawn were kept in controlled lab conditions. All treatments in the experiment were run simultaneously along with controls under same conditions. The temperature was kept at 23-24°C and high humidity was maintained to minimise drying of the substrate surface.

Waste cotton (textile industry by-product) was used as the substrate. The substrate was soaked in water for 72 h and spread on inclined cement floor to remove excessive water from the substrate until 70% water content. This material was put into polypropylene bags of 8"×12" size. Total mass of substrate in each bag was 1kg and the mouth of the bags was plugged with cotton wool. Substrate filled bags were subjected to the following pasteurization techniques:

– hot-water treatment with boiling water for 30 min,
– pasteurization with steam at 80°C for 1 h,
– chemical sterilization with formalin, in which 0.5 l of formalin was diluted 10 time with water and used for 1 m^3 of substrate.

Now the bags were ready and spawning was done at the rate of 5% of the net mass of the substrate. The inoculated bags were kept at 25°C in complete darkness for spawn running until the substrate became white due to impregnation by fungal mycelium (Fig. 2). After the completion of spawn running the mouths of the bags were cut with the help of scissors and the bags were placed on a shelf of growing room for cropping under controlled lab condition. Data for days taken to spawn running (DTSR) and days taken to formation of pin head (DTPPH) were recorded on the completion of mycelia growth and appearance of pin heads, respectively. For NPH formation and pin heads transformed into mature mushrooms (PHMM), the total number of promordia greater than 2 mm and total number of pin heads developed into mature mushrooms were counted. For wet mass, the masses of mushroom were determined on each flush and total yield was calculated at the end of the cropping period. For measurement of dry mass, the mushrooms were dried by heating at 37-40°C.

The data were analyzed using SPSS-16 software. For the laboratory experiment, two factor analysis of variance (ANOVA) was performed on a factorial experiment adopting the MF strength as the first factor and exposure duration as the second one. The significance levels ($P<0.05$) of differences for all measured traits: MF strength, exposure time and interactions were estimated.

RESULTS AND DISCUSSION

After magnetic field treatment, more uniform and faster mycelia growth as well as higher yield of mushroom were observed as compared to control. Prespawning MF treatment significantly enhanced the growth characteristics such as the number of pin heads formed, NPH, while the effect of MF treatment on the number of days to complete spawn running (DTSR) and on the time taken till the appearance of pin heads (DTPPH) was found to be marginal. Table 2 depicts the mean values of DTSR and DTPPH. A marginal difference between magnetically treated and untreated spawn was observed for the doses of T_6 followed by T_9 and T_{10} for DTSR, while DTPPH value was found to be higher for T_6 and T_9 doses as compared to control. The reduction in the number of DTSR was found to be 1.34 days, while the pin head formation took place 6 days faster versus control. The number of pin heads (NPH) formed was enhanced significantly ($P<0.05$). The treatment T_6 and T_9 showed the highest positive effect, followed by T_{10}, while T_{12} showed a negative effect and treatments T_2 and T_{11} demonstrated no response as compared to control. Similarly, the effect of MF

Fig. 2. Images of mature stage of: a – oyster mushroom, b – oyster mushroom (*Pleurotus* spp.), c – pin heads, d – general view in mushroom growth laboratory.

Table 2. Effect of different MF strength and exposure duration on DTSR, DTPPH, PHMM and wet and dry masses of mushroom

MF dose	DTSR	DTPPH	NPH	PHMM	Wet mass (g)	Dry mass (g)
T_0	42.67 ± 2.31	22.33 ± 1.76	18.33 ± 1.04	15.33 ± 2.52	45.02 ± 4.4	12.31 ± 3.59
T_1	42.85 ± 1.76	18.00 ± 1.32	17.00 ± 1	14.33 ±1.04	46.60 ± 3.03	11.07 ± 0.31
T_2	42.33 ± 1.61	17.67 ± 2.75	18.33 ± 2.31	14.67 ±2.53	52.60 ± 6	14.98 ± 4.64
T_3	42.03 ± 1.04	16.0 ± 1.32	18.60 ± 1.53	15.50 ± 1.00	77.73 ± 3	16.27 ± 0.87
T_4	42.67 ± 1.89	22.67 ± 1.26	14.33 ± 3.12	10.00 ± 0.87	40.86 ± 4.10	9.28 ±0.90
T_5	41.99 ± 0.76	20.00 ± 1	18.00 ± 1.76	11.00 ± 0.5	47.43 ± 4.5	10.63 ± 1.66
T_6	41.33 ± 1.76	18.67 ± 0.76	25.00 ± 3.33	20.67 ± 3.07	79.43 ± 7.0	17.02 ± 5.22
T_7	43.33 ± 1.89	21.00 ± 1.8	14.67 ± 2.36	11.00 ± 1.73	46.37 ± 3.4	10.99 ±1
T_8	43.67 ± 1.61	20.00 ± 1.8	15.33 ± 3.25	12.33 ± 3.25	47.43 ± 4.6	12.99 ± 1.61
T_9	41.5 ± 1.5	16.33 ± 2.02	25.33 ± 1.76	17.67 ± 1.73	60.10 ± 2.22	13.00 ± 0.77
T_{10}	41.5 ± 1.5	21.00 ± 2.02	22.33 ± 3.82	16.00 ± 2.29	47.43 ± 3.03	12.40 ± 1.56
T_{11}	43.67 ± 2.02	22.00 ± 3.06	18.00 ± 3.3	14.67 ± 2.52	29.43 ± 4.5	8.68 ± 2.02
T_{12}	42.67 ± 1.53	26.33 ± 3	11.76 ± 1	12.00 ± 0.88	24.01 ± 4.05	6.91 ± 0.72

DTSR – days taken to spawn running, DTPPH – days taken till the appearance of pin heads, NPH – number of pin heads, PHMM – pin head transformed into mature mushrooms.

treatment on the transformation of pin heads into mature mushrooms was found to be same as in the case of NPH. The increment in fresh/wet and dry masses was found to be remarkably significant ($P<0.05$), MF pretreatment of spawn enhanced the fresh and dry mass as compared to untreated samples. The maximum values of wet and dry masses were observed for treatment T_6 and T_3, while treatment T_4, T_{11} and T_{12} showed a negative response. Overall, the MF treatment had a marginal effect on mushroom growth in the early stages, and a profoundly significant one at later stages of development; it was also found that the MF treatment parameters (exposure time and strength) are interrelated. For example, the treatment of low MF strength for longer time and high MF strength for shorter time of exposure were found to be effective throughout the study.

Table 3 shows the percentage effect (positive/negative) of MF treatment over untreated samples. The percent change was found to be -3.14 and -26.86% (negative sign indicates the reduction in the number of days) for DTSR and DTPPH, respectively, and that of wet and dry masses percent gain was found to be 76.43 and 38.26%, respectively. The percentage increase in NPH and the number of pin head transformed into mature mushrooms was 38.18 and 34.83%, respectively, *vs.* control.

The effect of MF pretreatment strength, duration of exposure and their interactions are given in Table 4. Of the MF strength and exposure time, 5, 15, and 25 mT for 15 min and 100 mT for 2 min exposure were found to be better as compared to all other treatments as well as control. In the case of DTPPH and the number of pin heads transformed into mature mushrooms, the doses of 15 and 25 mT for 15 min were found to be better, while DTSR and NPH showed higher response for 15, 25 and 100 mT for 15, 15 and 2 min, respectively. The MF strength of 5, 15 and 25 mT for 15 min for wet mass and 5, 5 and 15 mT for 5, 10 and 15 min for dry mass were found to be superior *vs*. control.

In summary, the growth and yield parameters of mushroom samples treated with MF were significantly higher than and superior to the control. It was found that the MF treatments had acceleratory effects on NPH and the number of pin heads that converted into mature mushrooms, as well as on wet and dry masses; DTSR and DTPPH increased marginally. Furthermore, the low MF strength for longer exposure or high MF strength for shorter exposure duration were more effective as compared to other combinations.

After MF treatment, faster and more uniform growth, assimilation of masses as well as greater NPH formation in mushroom were observed as compared to control. Our results are in accordance with the work of other researchers, which have been reported for a verity of crops. Florez *et al.* (2007) and Vashista and Nagarajan (2010) noted a considerable enhancement in germination characteristics such as seedling vigour, shoot and root growth in maize, chickpea and sunflower seeds when treated magnetically. Similarly, Fischer *et al.* (2004) observed higher germination and growth of sunflower as compared to untreated seed samples. Marks and Szecówka (2010) reported an impact of variable MF stimulation on growth of aboveground parts of potato which increased as a result of presowing magnetic treatment. Florez *et al*. (2007) reported enhancement in germination in rice when exposed to 125/250 mT MF for specific time intervals, which indicates that the better results are dependent to

T a b l e 3. Percentage change (%) (positive/negative) of various parameters of mushroom after MF irradiation

MF dose	DTSR	DTPPH	NPH	PHMM	Wet mass	Dry mass
T_1	0.42	-19.39	-7.25	-6.62	3.50	-10.07
T_2	0	-20.89	0	-4.30	16.83	21.68
T_3	-1.49	-28.34	1.47	1.1	72.65	32.16
T_4	0	1.52	-21.82	-34.76	-9.24	-29.61
T_5	-1.59	-10.43	-1.8	-28.24	5.35	-13.64
T_6	-3.14	-16.39	36.38	34.83	76.43	38.26
T_7	1.54	-5.95	-19.96	-28.24	2.99	-10.72
T_8	2.34	-10.43	16.36	-19.56	5.35	5.52
T_9	-2.74	-26.86	38.18	15.26	33.49	5.60
T_{10}	-2.74	-5.95	21.82	4.37	5.35	0.73
T_{11}	2.34	-1.47	0	-4.2	-34.62	-29.48
T_{12}	0	17.91	-36.33	-21.72	-46.66	-43.86

Explanations as in Table 2.

T a b l e 4. Effect magnetic field strength, duration of exposure and their interaction by analysis of variance (ANOVA) for variables

Parameter	MF (mT)		Time (min)		MF× time	
	F	*P*	*F*	*P*	*F*	*P*
DTRS	0.378	0.48	0.07	0.98	0.21	0.76
DTPPH	0.91	0.45	0.324	0.512	0.575	0.62
NPH	4.806*	0.031	1.428	0.15	4.8*	0.04
No. of mature PH	1.056	0.212	0.818	0.49	5.503*	0.009
Wet mass (g)	5.959*	0.01	19.471*	<0.001	0.499	0.563
Dry mass (g)	1.934	0.103	7.383*	<0.001	6.548*	0.001

Explanations as in Table 2. Statistical significance level (P<0.05).

appropriate MF strength and exposure duration combination. It is well understood from literature that the best outcome of growth and development at later stages are possible when optimal exposure doses are applied, and these results are in accordance with Iqbal *et al.* (2012) who noted considerable positive changes in pea plant after MF treatment, when the MF strength was low for longer time of irradiation or high MF strength for shorter exposure time. Similarly, Aguilar *et al*. (2009) observed that the positive effects are dependent on the irradiation parameters *ie* the intensity and exposure time used in the presowing seed treatment.

The best results were obtained with CL-12 X CL-11 maize genotype when irradiated with magnetic flux density of 100 mT for 7.5 min. Dominguez *et al.* (2010) found an increment in emergence rate and seedling emergence and on seedling growth at magnetic induction levels of 160 and 560 mT as compared to control and other MF treatments. The results of growth, spawn running, NPH formation as well as wet and dry masses showed that specific combinations of MF doses such as T_6 and T_9 (15 and 25 mT, 15 min) for DTSR, DTPPH, NPH formation and number of pin heads transformed into mature mushrooms, and T_3 and T_6 (5 and 15 mT, 15 min) for wet and dry masses and also T_9 (100 mT for 2 min) for DTSR and NPH formation, were found superior to control (Tables 2 and 3). This observation indicates that growth and development take place at appropriate combinations of MF exposure duration and strength. We found that MF pretreatment could enhance growth in mushrooms, which is consistent with various previous studies (Anggoro *et al*., 1999). It is obvious from this study that appropriate MF treatment for specific time duration could significantly accelerate the growth and enhance wet and dry masses in mushrooms. Our results of mushroom growth and yield in the present study are in agreement with Iqbal *et al.* (2012). The combined effects of MF and exposure duration accounted in this study are in agreement with those reported by various other workers (Fischer *et al.,* 2004; Pietruszewski and Kania, 2010).

The MF mechanisms of action on growth is not well known yet, however several theories have been proposed, including biochemical changes or altered enzyme activities. The higher growth response might be attributed to a combined effect of biochemical, physiological, metabolic as well as enhanced enzymatic action. It is assumed that the MF treatment influences the structure of cell membrane and in this way increases its permeability and ion transport in the ion channels, which as a result affects the metabolic pathways. The enzymes which are necessary for germination at particular stages were found higher in magnetically treated objects during germination and growth. It is well known that the MF affects the biological objects by non-conventional spins, free radicals, liquids crystals or mobile electron charges. Chemically these free radicals are very active species which take part in fast reactions and cause changes in the biochemical and physiological processes during germination and growth. An increase in water uptake rate as a result of MF treatment was also reported, which might be responsible for increased growth and development (Iqbal *et al.,* 2012).

CONCLUSIONS

1. The prespawning magnetic field treatment significantly enhanced the number of pin heads, pin heads transformed into mature mushroom as well as wet and dry masses, while the increment in days taken to spawn running and days taken till the appearance of pin heads was found to be marginal *vs*. control.

2. Among the various combinations of magnetic field strength and duration; 5, 15, 25 and 100 mT for 15, 15, 15 and 2 min exposure, respectively, yielded superior results.

3. Low magnetic field strength for longer time of exposure or high magnetic field strength for shorter duration treatments showed superior and better response as compared to other treatments as well as control.

4. Highly significant assimilation of masses and the formation of a greater number of pin heads suggests that magnetically treated mushroom spawn can be used practically to obtain higher yields.

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