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STUDIES ON THE EFFECT OF DIFFERENT AMOUNTS AND METHODS OF APPLYING SPAWN ON THE YIELD OF *PLEUROTUS SAJOR-CAJU* (Fr.) SINGER

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Wheat grain spawn of *Pleurotus sajor-caju* (Fr.) Singer was used in various experimental inoculations of paddy straw beds in earthen trays. Maximum yield of sporophores was obtained with 250g of spawn per tray. although good yields were possible with as little as 30g per tray. Best results were obtained when spawn was spread only on the middle layer of the straw bed. Growth of spawn inoculum and subsequent development of sporophores were enhanced by combined addition of $CaCO_3$, $CaSO_4$ and mixed trace elements.

Key words : Amount, Method, Applying spawn, Yield, *Pleurotus sajor-caju*

INTRODUCTION

With a view of popularizing cultivation of *Pleurotus sajor-caju* and its processing as a rural based industry, extensive researches have been conducted on the cultivation of *Pleurotus sajor-caju* using different agricultural wastes as mushroom bed substrates and on factors influencing the yield of this crop (Jandaik and Kapoor, 1974 ; Sivaprakasam and Kandaswami, 1980 ; Garcha, 1981 ; Eswaramurthy *et al.*, 1986 ; Goswami *et al.*, 1986). In mushroom production technology the spawn is compared to the seed in crop plants (Pal, 1980). Production of spawn and spawning of substrate are two important steps in the cultivation of *Pleurotus sajor-caju* using earthen trays. In the present investigation attempts have been made to find out : (a) the optimum amount of spawn required for the maximum production of crop ; and (b) the best method for spawning for the optimum yield of crop.

MATERIALS AND METHODS

Test Organism

The tissue culture of *Pleurotus sajor-caju* was prepared from a fresh sporocarp of the mushroom obtained from Santoshpur, West Bengal during the rainy season.

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The cultures were made on 3% maltagar medium and maintained in the same medium by:snbculturing regularly at 30 days intervals The cultures were maintained at 25°C in complete darkness (Jandaik and Kapoor, 1976).

Preparation of wheat grain spawn in bottles

Clean and healthy wheat grains were boiled in water for about 30 min. until the grains became soft, after which they were spread on blotting paper or wire netting to remove the excess water. The boiled grains were then mixed with gypsum and calcium carbonate in the ratio of 100 : 6 . 2 to form a spawn growth medium (Pal, 1980). The spawn growth medium was then placed in 500 ml milk bottles (250 g bottle) and plugged with non-absorbent cotton wool. The bottles were sterilized by autoclaving at 15 lbs. p.s.i. for 1 h for two consecutive days. After autoclaving, the bottles were cooled, shaken and then inoculated aseptically with a 1 cm disc of pure mycelial culture of *Pleurotus sajor-caju* raised on 3% maltagar medium. Each bottle was inoculated at the surface only and incubated at 25°C for 15-20 days. During the incubation period the bottles were shaken 3 to 4 times at regular intervals to ensure uniform and rapid spread of mycelium.

Cultivation Technique

Soaking of straw

Paddy straws were chopped into small pieces (about 10 mm length) and soaked in water for 24 h. The straws were then dipped into hot water (65°C ± 5°C) for half an hour. After cooling down to 20-25 C (Kurtzman, 1975a, 1979b ; Bano *et al.*, 1979), the excess water was drained off and discarded and the straws were placed in earthen trays.

Inoculation of straw bed

Earthen trays each of 60 cm diameter and 9 cm depth with perforated base, were used for the purpose of cultivation. A layer (3 cm thick) of water-soaked chopped paddy straws was placed on each tray. 10 g of NPK fertilizer (15 : 15 : 15) was placed over the straw layer of each tray and was again covered with 1 cm layer of wet straw. A mixture of $CaSO_4$ and $CaCO_3$ (1 : 3) was sprinkled over this layer at the rate of 10 g/tray and then grain spawn was uniformly sprinkled on it. The spawn was finally covered with another 1 cm thick layer of wet straw.

Casing and aftercare

After spawning the earthen trays were covered with casing soil. The casing soil used was a mixture of sand and clay (3 : 1) in which the pH had been adjusted to pH 7 using $CaCO_3$ and $CaSO_4$ (Samajpati *et al.*, 1986). After casing each tray was covered with a black polythene sheet to cut off sunlight entirely during the

spawn run period. Watering was done once a day in such a way so as to keep the substrate moist without the accumulation of free water. Polythene sheets were removed as soon as the buds started appearing in the trays. Yield was recorded periodically by picking fully grown fruit bodies and recording their number, weight in each replication for different treatments (Grewal and Sohi, 1987).

Different Methods of Spawning

For different methods of spawning the prepration of the mushroom beds in earthen trays was slightly altered from those described earlier. In this case, 25 cm of straw was placed on each tray. 10 g NPK fertilizer (15 : 15 : 15) was sprinkled over this layer and was then covered with another 0.5 cm thickness of wet straw. This formed the bottom layer for spawning. On this bottom layer, another 2 cm thick layer of straw was placed which formed the middle layer for spawning. On this middle layer was placed a final 1 cm thick layer of straw which formed the surface layer for spawning Here 10 g of $CaCO_3$ and $CaSO_4$ (6 : 2) mixture was used in each tray, but the amount was equally divided and sprinkled over each of the spawning layers. Four different methods of spawning were used.

(i) Spawning in three layers : In this case spawn was applied on all the three layers-surface, middle and bottom.

(ii) Spawning in two layers : In this case spawn was applied on just two layers-surface and middle layers, middle and bottom layers, surface and bottom layers.

(iii) Spawning in one layer : Spawn was applied only on one layer-surface layer, middle layer, or bottom layer.

(iv) Spawning by thorough mixing : The spawn was thoroughly mixed with all layers.

In all these methods 250 g of spawn was used per tray. In the case of spawn applied to 2 or 3 layers, the above amount of spawn was equally divided into 2 and 3 parts respectively for application to each layer.

RESULTS AND DISCUSSION

A. *Effect of different amount ot spawn on the yield*

To evaluate the quantity of spawn needed to give the maximum yield the straw beds in earthen trays were inoculated at different rates. Ten different amounts of spawn were used and their effects on the period of spawn run, number of buds, mature sporophores and fresh weight of sporophores per tray were recorded. The results presented in Table 1 show that with gradual decrease in the amount of spawn used per tray there is a gradual increase in the spawn run period.

Table 1. *Effect of different amount of spawn on the yield of* P. sajor-caju

Amount of spawn used (g) per tray	Period of spawn run (days)	No. of buds per tray	No. of mature sporophores per tray	Fresh weight of sporophores (g) per tray
500	17(±2), 10(±2)	50(±3)+30(±3) =80(±6)	15(±3)+5(±1) =20(±4)	140(±5)+80(±7) =220(±12)
250	18(±2), 11(±2)	60(±4)+34(±5) =94(±9)	14(±2)+5(±2) =19(±4)	150(±8)+80(±6) =230(±14)
125	19(±2), 10(±1)	46(±3)+26(±3) =72(±6)	18(±3)+6(±2) =24(±5)	116(±6)+110(±3) =226(± 9)
60	20(±1), 11(±2)	56(±3)+34(±2) =90(±5)	21(±4)+15(±3) =36(±7)	113(±8)+104(±6) =217(±14)
40	21(±2), 12(±1)	50(±3)+40(±3) =90(±6)	24(±2)+16(±2) =40(±4)	140(±6)+ 70(±6) =210(±12)
30	20(±2), 11(±2)	57(±3)+25(±2) =82(±5)	20(±2)+10(±2) =30(±4)	120(±10)+88(±6) =208(±16)
25	21(±2), 11(±2)	50(±3)+30(±3) =80(±6)	40(±3)+15(±2) =55(±5)	90(±6)+50(±5) =140(±11)
20	22(±2), 12(±2)	44(±3)+24(±2) =68(±5)	35(±3)+13(±3) =48(±6)	85(±5)+26(±4) =111(± 9)
15	23(±2), 14(±2)	30(±3)+18(±3) =48(±6)	24(±2)+15(±2) = 9(±4)	50(±4)+25(±4) = 75(± 8)
10	24(±2), 14(±2)	22(±3)+14(±3) =36(±6)	15(±2)+ 8(±2) =23(±4)	35(±3)+18(±2) = 53(± 5)

*Data are average of 20 trays. The first and second figures indicate the data for the first and second flushes respectively.

Maximum yield, in terms of fresh weight of sporophores, was produced from trays inoculated with 250 g of spawn. These same trays also gave the highest number of buds, although less than a quarter of those buds developed into mature sporophores. The actual number of sporophores collected from these trays (mean of 19 per tray) was the least of any treatment, but they were the largest of the sporophores obtained in the experiment, averaging over 12 g each. Nearly three times as many sporophores (mean of 55 per tray) were collected from trays inoculated with only 25 g of spawn, but these averaged less than 3 g each.

Graphical comparison between the different measures of sporophore production(Fig.1) show that their responses to changing amounts of spawn fell into two categories. At lower levels of inoculum in the range 10-30 g spawn per tray, small changes in the inoculum level produced substantial changes in all measures of the yield. At higher levels of inoculum within the range 30-500 g per tray, large changes in inoculum levels produced only small changes in the yield. For example, in the lower inoculum range a threefold increase in spawn resulted in a fourfold increase in fresh weight yield (increased from 50 to 200 g per tray), while in the higher inoculum range an eightfold increase in spawn very little additional yield

Table 2. *Effect of different methods of spawning on the yield of* P. sajor-caju*

Method of spawning	Period of spawn run (days)	No. of buds per tray	No. of mature sporophores per tray	Fresh weight of sporophores (g) per tray
Thorough mixing	20(±2), 11(±2)	60(±3)+13(±2) =73(±5)	25(±3)+10(±3) =35(±6)	110(±5)+65(±3) =175(±8)
One layer spawning				
Surface	21(±2), 13(±2)	54(±3)+20(±2) =74(±5)	38(±4)+16(±2) =54(±6)	107(±5)+80(±2) =187(±7)
Middle	20(±3), 12(±2)	70(±4)+38(±2) =108(±6)	40(±3)+13(±2) =53(±5)	160(±6)+64(±3) =224(±9)
Bottom	25(±2), 14(±3)	24(±2)+16(±2) =40(±4)	16(±2)+ 8(±2) =24(±4)	60(±5)+36(±3) =96(±8)
Two layers spawning				
Surface and Bottom	24(±2), 12(±2)	28(±2)+23(±3) =51(±5)	25(±3)+12(±2) =37(±5)	82(±5)+55(±3) =137(±8)
Surface and Middle	22(±2), 12(±2)	60(±3)+44(±2) =104(±5)	44(±3)+40(±2) =84(±5)	106(±5)+94(±5) =200(±10)
Middle and Bottom	21(±3), 12(±2)	70(±3)+34(±2) =104(±5)	44(±4)+20(±2) =64(±6)	104(±6)+78(±5) =218(±11)
Three layers spawning				
Surface, Middle and Bottom	24(±2), 12(±3)	50(±4)+40(±4) = 90(±8)	40(±3)+24(±2) =64(±5)	100(±6)+80(±6) =180(±12)

*Data are average of 20 trays. The first and second figures indicate the data for the first and second flushes respectively.

Table 3. *Effect of pretreated wheat grain spawn on the yield of* P. sajor-caju*

Spawn	Period of spawn run (days)	No. of buds per tray	No. of mature sporophores per tray	Fresh weight of sporophores (g) per tray
Set I	21(±2), 13 (±2)	14 (±2) +8 (±2) =22 (±4)	12 (±2) +5 (±2) =17 (±4)	75 (±4) +30 (±3) =105 (±7)
Set II	20(±2), 12 (±2)	20 (±2) +10 (±2) =30 (±4)	14 (±2) +7 (±2) =21 (±4)	90 (±5) +35(±5) =125 (±10)
Set III	25(±2), 12 (±2)	16 (±2) +13 (±2) =29 (±4)	11 (±2) +8 (±2) =19 (±4)	60 (±4) +40(±4) =100 (±8)

* Data and average of 20 trays. The first and second figures indicate the data for the first and second flushes respectively.

(increased from 210 to 230 g per tray) was obtained. The consequence of the sharp inflection in the yield/inoculum curve is that, while 250 g of spawn per tray is technically the optimum treatment because it gave the maximum fresh weight yield of sporophores, 30 g of spawn per tray might be considered the most economical treatment because it gave close to the same yield for the expenditure of very much less inoculum.

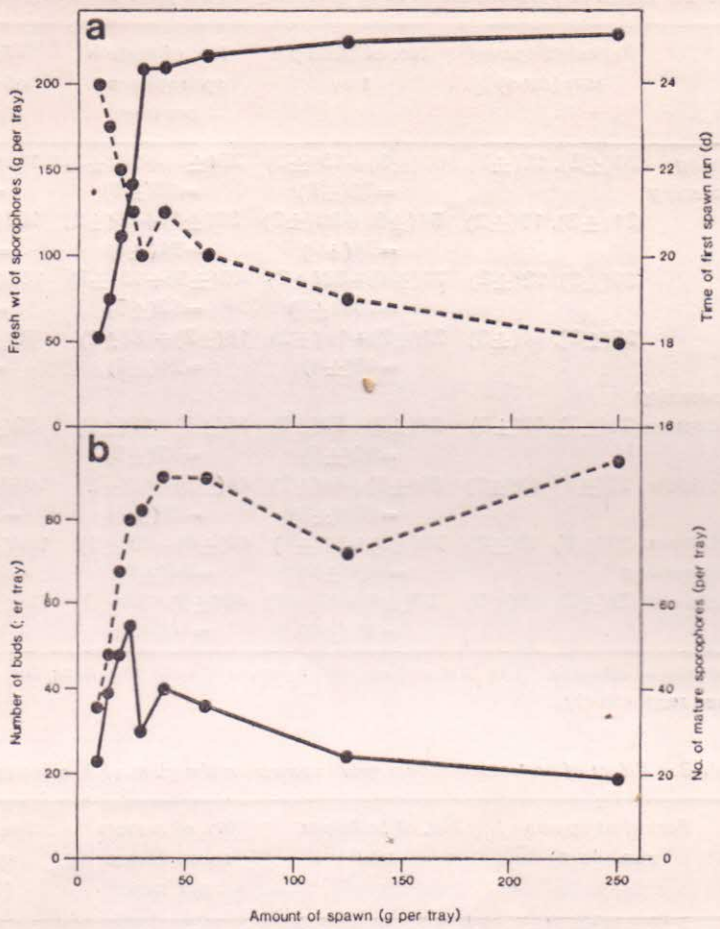

Figure 1. Relationship between amount of spawn and production of sporophores.

a) Time taken for first spawn run (broken line) and total fresh weight of sporophores over two harvests (continuous line), in relation to amount of spawn applied.

b) Total number of buds (broken line) and total number of sporophores (continuous line) produced over two harvests, in relation to amount of spawn applied.

At low inoculum levels the graphs (Fig. 1) indicate a positive correlation between the amount of spawn applied and each of the following : (i) number of buds ; (ii) number of sporophores ; and (iii) fresh weight yield. There was also a negative correlation between amount of spawn and (iv) the time taken for the first spawn run. These correlations have been confirmed by statistical analysis.

Linear regression analysis, using a Hewlett Packard programmed calculator, gave coefficients of determination (r^2) as follows : (i) 0.89, (ii) 0 96, (iii) 0.9, and (iv) 1.00. These values show that the mean data fit very well with a straight line. The various sporulation parameters also all correlate very strongly with each other over that same range (r^2 values ranged between 0.83 for no. of buds versus fresh weight yield, to 0.96 for time for first spawn run versus no. of sporophores). It is evident then, that below 30 g spawn per tray, the amount of inoculum applied is a limiting factor which controls all quantitative aspects of yield.

Above 30 g per tray (25 g per tray in the case of no. of mature sporophores) the correlations between inoculum input and sporophore production break down. Fresh weight yield seems to approach a limiting maximum, presumably controlled at this stage by the amount of nutrients available to the fungus from the trays of paddy straw. The number of buds which appeared, also seemed to plateau at around 40 g spawn per tray, so presumably this was also limited by the size or nutrient capacity of the trays. However, time for the first spawn run continued to decrease with increasing inoculum, although less steeply than at lower inoculum levels. It is interesting to note that the number of buds which succeeded in developing into mature sporosphores, also decreased with increasing levels of inoculum above 25 g per tray. But inspite of this the fresh weight yield continued to rise slightly because there was a compensatory increase in the mean size of mature sporophores. This is interpreted as meaning that there was competition between developing sporophores for the resources acquired by the mycelium.

Zadrazil and Kurtman (1978) said that "all key factors for primordia formation and development of fruiting bodies in *Pleurotus* production are concentrated on the following ecological factors : temperature, light, air humidity and air composition'. However, the present results obtained under the same environmental conditions, emphasise that the intrinsic fungus factors are also important. Amount of spawn applied as inoculum at lower concentrations had a profound effect on yield and sporophore production. This is supported by similar observations reported by Sur *et al.* (1980) for *Volvariella diplasia*. And at higher levels of inoculum there were significant effects on the number and size of mature sporophores which requires further investigation.

B. Effect of different methods of spawning on the yield.

To evaluate the best placement of spawn in the mushroom bed substrate for maximum yield, spawning was done by four different methods—spawning by thorough mixing, one layer of spawning, two layers of spawning and three layers of spawning. The results presented in Table 2 show that spawning by thorough mixing and in three layers gave moderate yield. In the case of two layers of spawning comparatively high yields were obtained when spawning were done on

middle + bottom layers and surface + middle layers. However, poor yield was obtained by spawning the surface + bottom layers. This confirms the earlier reports of Chakravarty *et al.* (1978). In the case of spawning in one layer, poor yield was obtained by spawning the bottom layer while moderate yield was obtained by spawning the surface layer. However, among all the methods, one layer of spawning within the middle layer gave the best yield. Probably the middle layer is most suitable in terms of humidity, aeration and temperature for the growth of the fungus.

C. Effect of pretreated wheat grain spawn on the yield.

Three different sets of wheat grain spawns were used. In the first set (I) of the spawn, the grains were mixed with calcium carbonate and gypsum (100 : 6 : 2). In the second set (II) of spawn the wheat grains were with calcium carbonate, gypsum (100 : 6 : 2) and 1.25 ppm of seven trace elements—Zn, Fe, Mg, Mo, Mn, B and Cu. In the third set (III) of spawn the wheat grains were not treated with any nutrients and it served as control.

Results presented in Table 3 show that highest yield with lowest spawn run period was obtained when spawning was done with set (II) spawn. The trace elements were found to have a better stimulatory role on the yield of sporophores. This confirms the findings of Bhattacharjee *et al.* (1988) who reported the stimulatory effect ofelements on the submerged mycelial growth of *P.sajor-caju* in liquid culture. Highest spawn run period with lowest yield was obtained with control set (III) of spawn while moderate yield was obtained with set (I) of spawn. While conducting experiments it was observed that mixing $CaCO_3$ and gypsum (6 : 2) with grains appreciably reduced the contamination of spawn by other fungi and improved the visibility of the fungal growth inside the glass bottles. Observation at this stage also indicated that the presence of trace elements in the set II spawn enhanced the mycelial growth, leading to the subsequent rapid colonization of the mushroom bed substrate and thereby reducing the spawn run period.

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