AN INNOVATIVE METHOD OF PREPARATION OF HEALTHY GRAIN SPAWN

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ABSTRACT

Mushroom spawn is prepared on cereal grain media by inoculating them with pure cultures of selected mushroom species under sterile conditions, yet the problem of contamination of spawn is a major bottle-neck in the growth and spread of mushroom farming in developing countries. Species of *Trichoderma, Aspergillus, Penicillium, Rhizopus,* etc. besides wet spot causing bacteria are known to be major contaminants of mushroom spawn in eastern India, including Jharkhand affecting commonly grown mushrooms viz., *Pleurotus* spp, *Hypsizygus ulmarius* and *Volvariella volvacea*, which need urgent action for their management. In view of reports of appearance of fungicide-resistant strains of *Trichoderma,* therefore, a Neem- based herbal formulation, viz., Mahaneem containing 0.15% Azadirachtin was tried as a prophylactic pre-treatment of the wheat grains used for the spawn medium together with some empirical changes in the current method of preparation of the grain spawn medium. The modified method tried successfully for raising healthy, contamination-free and productive master and planting spawns of oyster and paddy straw mushrooms has been discussed.

Keywords: spawn, oyster-mushroom, azadirachtin

INTRODUCTION

Grain spawn is a medium that is impregnated with mycelium made from a pure culture of the chosen mushroom strain, and spawn production is a fermentation process in which the mushroom mycelium increases by growing through a solid organic matrix. Although, grain spawn is currently prepared by inoculating sterilized grains with complete sterile precaution, yet small and medium size spawn- laboratories still face the problems of contamination i.e. unwanted intrusion of harmful and competing organisms in the spawn, making them useless and dangerous for mushroom production due to their potential for infecting the beds and the crop as a whole. Mushrooms have since long been there in the diet of various tribes of Jharkhand, mostly drawn from wild collections during monsoon season, and hence several mushroom farming units have come up during last few years both in the urban and rural areas of the state. Gradually spawn laboratories both in public and private sectors are also coming up in major towns to serve the growers. However, due to humid and wet weather conditions particularly during rainy season, 20-40% losses are reported to be caused by bacterial and fungal contaminants of commercial spawn, restricting the availability of healthy and quality spawn in time and in required quantities, which is seriously hampering the growth and spread of mushroom farming in the area.

It has been roughly estimated that about 1 million microbes are killed while preparing only 1 kg of pure spawn. Moreover, during the process of spawn making, contamination is likely to come from various sources like aerospora of the lab, impure inoculum, incomplete sterilization of grain medium and contaminated spawn bags/tools/hands of the workers, etc, which obviously make pure spawn making a highly risky activity. Moreover, cereal grains carry lacs of microbes in them, particularly those escaping full sterilization, either due to their thermo-tolerance, insect damage, mechanical injury or those ruptured by over-boiling. Yet, in the prevalent methodology of spawn-preparation, the grains are not given any sort of pre-treatment to reduce their inoculum-load, although while preparing the beds for oyster and milky mushrooms, their substrates (wheat or paddy straw etc) are widely being treated with chemicals/fungicide [1] or with Neem-oil based bio-formulations [2] for the same purpose. In the year 1985, the button mushroom Farms of Ireland and again in 1990s of Europe, America, Canada, Mexico, etc faced devastating on slaughts of Green Mould disease which led to the use of systemic fungicides to control the malady. Grogan *et al.* [3] reported that grain-spawn treated with benomyl or carbendazim or thiabendazole could effectively control the causal fungus *Trichoderma harzianum* biotype Th2. However, large scale use of Benzimidazole fungicides soon led to the emergence of a fungicide-resistant biotype Th4 of *T. harzianum* (syn. *T. aggressivum, f. aggressivum),* against which the same treatments proved ineffective [4], which led to the banning of

those fungicides in several affected countries. Obviously, such an action has left the commercial growers of those areas under the possible threat of another epiphytotic.

The small growers of eastern states of India are mostly raising seasonal crops of oyster, milky and paddy straw mushrooms with low cost and low input technology and are more prone to diseases and pests.Yet, only some preventive measures to manage them are likely to be adopted both by the growers and spawn producers of the area. Hence, in the present investigation experiments have been carried out to get rid of as much of the contaminating microbial load on the grains as possible by their pre-treatment with potent bio-formulations together with suitable modification in the spawn making methodology so as to ensure effective sterilization of the spawn medium.

MATERIALS AND METHODS

Modified method of spawn preparation

Spawn is a potent source of contamination to cultivated mushrooms [5,6] . Hence, with a view to remove the contaminating microbes from the spawn, an attempt to modify the standard method of spawn preparation now in practice was made in this investigation. Required quantities of good quality wheat grains were procured from the local market and their initial moisture content was determined. Subsequently, the grains were washed 2-3 times to remove any dirt and artifacts and soaked in water for 30 minutes to get rid of any pesticide-residues. Neem oil (Mahaneem) dilutions containing 1.5 and 0.15 ppm Azadirachtin were prepared in quantities just sufficient to submerge the grains and to absorb most of the solution so as to make their final moisture-content to not less than 55% as calculated on the basis of their initial moisture-content. The initial pH of the 2 sets of dilutions were kept at 8.5 and 9.0, in which the grains were soaked for more than 18 hours or overnight and all the treatments were replicated 4 times with two controls, viz., grains soaked i) in plain water, and ii) in 0.05% carbendazim solution. During soaking, the containers were covered with plastic sheets to prevent any loss of the liquid. The soaked grains were later spread thinly on a clean surface in shade to dry the free water sticking on the grains, to which calcium carbonate was added @ 2% and mixed thoroughly to make the grain spawn medium ready. The medium was filled in 1 litre glass bottles up to 2/3rd capacity and plugged with non-absorbent cotton, which were autoclaved at a pressure of 20 lb psi for not less than 45 minutes. The bottles were then shaken to avoid clumps and kept over-night in the inoculation-room before inoculating them with 1x1mm inocula taken from the growing edges of freshly raised pure cultures of *Pleurotus florida* and *Hypsizygus ulmarius*. For *Volvariella volvacea* also the grain medium was similarly prepared except that wheat grains were replaced by Paddy grains. The inoculated media bottles were incubated at 28±2 °C/30±2 °C for oyster and paddy straw mushrooms respectively.

Growth performance of modified grain spawn

The inoculated bottles during incubation were shaken at regular intervals to enhance and ensure uniform growth of the mushroom mycelium. After 2 weeks of incubation their final growth-performance, vigor, abnormality /contamination were noted by visual observation. Further, the spawn bottles were monitored during storage in refrigerator for 3 weeks at regular intervals for any late contaminants.

Yield-performance of spawn prepared by modified method

Spawn prepared by modified method were tested for their productivity by raising crops of *P. florida*, and *H. ulmarius* on paddy-straw in polythene bags as per Vijay and Sohi [1]. Experiment on *P. florida* was laid with modified spawn treated with dilutions of Neem-oil @ 1.5 and 0.15 ppm Azadirachtin adjusted to pH 8.5, and followed by straw treatment with 500 ppm formalin- solution. Two controls viz., i) Modified spawn treated with 1.5 and 0.15 ppm of Azadirachtin at their natural pH 5.6 -5.9 followed by straw treatment with 500 ppm formalin; and ii) Modified spawn without treatment but straw treated with solution of 75 ppm Bavistin and 500 ppm formalin. Experiment on *H. ulmarius* was however designed to assess the performance of the Modified spawn with grains treated with 0.1% Bavistin instead of Neem oil, and straw treated with 37.5 ppm carbendazim and 500 ppm of formalin and the 2 controls kept were: 1) untreated spawn + treated straw with carbendazim and formalin; and 2) both spawn and straw untreated. The crops were grown in an

improvised seasonal growing room in the RKMV university campus, with temperature ranging between 26-29 °C during the day.

RESULTS AND DICUSSION

Modified grain spawn medium

Soaking of wheat grains in measured quantity and known strength of Azadirachtin dilutions for a long duration of 18 hrs or so, ensured maximum possible absorption and retention of the bio-pesticide in the internal tissues of the seeds making them least prone to attacks by the contaminants. Moreover, prolonged soaking of the grains could avoid the process of 30-35 minutes boiling, now in practice, saving thereby leaching of nutrients of the grains and their cracking, as well as the costly fuel and valuable time spent over boiling. Besides, the possibility of killing of the heat- resistant bacterial endospores during autoclaving was also very much enhanced due to their germination to vegetative phase during prolonged soaking. This was amply revealed by the absence of bacterial soft-rot infection in any of the spawn-bottles prepared by the modified method.

Growth performance of modified grain spawn

Grain spawns of 3 mushroom species, viz. *P. florida*, *H. ulmarius* and *V. volvacea* were prepared by the modified method using two dilutions of Azadirachtin adjusted at 2 different pH in case of former 2 species. However, for all the 3 species 2 controls were kept, one soaked in 0.05% carbendazim and the other in sterile water only. In Tables 1, 2 & 3, observations on their mycelial growth attained 2 weeks after incubation have been given. It is noted that *H. ulmarius* attained faster growth at pH 8.5 as compared to pH 9.0, irrespective of dilutions of the Neem-oil. carbendazim soaking at 0.05% concentration also supported faster growth-rate of the spawn. Data in Table 2 exhibit the same trend of growth response by *P. florida* as noted for *H. ulmarius* both in the case of Azadirachtin as well as carbendazim. The spawn of *P. florida* also grew faster at pH 8.5 than at pH 9. However, the growth-rate of *V. volvacea* was slightly retarded due to soaking in 1.5 ppm Azadirachtin as compared to its lower conc. (0.15 ppm) and also 0.05% carbendazim (Table 3). It is notable that none of the Modified spawn bottles under different treatments/replications exhibited any contamination what so ever (Figs.1 & 2).

Table 1. Performance of *Hypsizygus ulmarius* spawn prepared by modified method and treated with Neem oil at different pH

Treatments	Azadirachtin (ppm)				Carbendazim	Water
	pH 8.5		pH 9			
	1.5	0.15	1.5	0.15	Control	Control
Growth on 13th day	++++	++++	+++	+++	++++	+++
Remarks	No Contamination					

++++ = Complete, +++ = Incomplete

Table 2. Performance of *Pleurotus florida* spawn prepared by modified method and treated with Neem oil at different pH

Treatments	Azadirachtin (ppm)				Carbendazim	Water
	pH 8.5		pH 9			
	1.5	0.15	1.5	0.15	Control	Control
Growth on 13th day	++++	++++	+++	+++	++++	+++
Remarks	No Contamination					

++++= Complete, +++= Incomplete

Table 3. Performance of *Volvariella volvacea* spawn prepared by modified method and treated with Neem oil at different pH

Treatments	Azadirachtin (ppm)		Carbendazim*	Water
	1.5	0.15*		
Growth on 14th day	+++	++++	++++	+++
Contamination	No Contamination			
Remarks	*Profuse formation of chlamydospores by 18th day			

++++= Complete, +++= Incomplete

Figure 1. *Hypsizygus ulmarius* spawn with Neem Oil 10^{-3} dilution at pH 9

Figure 2. *Volvariella volvacea* spawn treated with Neem Oil dilution 10^{-4} and carbendazim 0.05%

Further it was also noted that 0.15 ppm Azadirachtin and 0.05% carbendazim treated spawn profusely developed brick-red colored chlamydospores, which are known to be indicators of healthy and vigorous spawn [7].

Yield-testing of spawn prepared by modified method

Spawn sprepared by Modified method were used to raise crops of *Hypsizygus ulmarius, Pleurotus florida* and *Volvariella volvacea* to test their productivity under *in vivo* conditions on paddy straw as substrate (Figs.3, 4 & 5). Data obtained from replicated Experiments on the former two species in Tables 4 & 5 indicated that the modified spawn treated either with Azadirachtin (1.5 & 0.15 ppm) or carbendazim (0.05%) could give good yield of *P. florida* (71-78.33% BE) and *H. ulmarius* (56.73-79.20% BE), respectively. However, in case of both the mushroom species, even untreated modified spawn gave equally good yield either on formalin+carbendazim treated straw or untreated straw, which might possibly be due to the vigorous and healthy nature of the spawn grown on a more nutritious medium. The health and vigor of the spawn was also indicated by the high yield obtained from *P. florida* bags even though most of the bags with treated spawn(but only formalin-treated straw), got locally infected with green mould below the points where holes were made for fruiting. Another important observation noted in the performance of *H. ulmarius* was the negative effect of carbendazim on the productivity of that mushroom giving the lowest yield, when spawn and straw both were treated with the fungicide (Table 5).

A crop of *Volvariella volvacea* was also raised with Azadirachtin treated modified spawn by the Cage-culture method , but it could not be replicated and its yield performance could not be assessed. However, good fruiting was recorded with attractive and healthy sporocarps (Fig. 5). Use of cereal grains for mushroom spawn was introduced by Sinden [8] as early as in1931 the superiority of which was established by Szudyga and Mallinowske [9] even in the year 1974, although, various modifications continued to be proposed therein. Lemke [10] suggested that the grains should contain 50% moisture and the pH of the spawn medium be adjusted between 6.5-6.7, while Kumar *et al.* [11] recommended the use of

Figure 3. *H. ulmarius* in fruiting

Figure 4. *P. florida* in fruiting

Figure 5. *V. volvacea* in fruiting

Table 4. Yield Performance of *P. florida* spawn prepared by modified method and treated with Neem oil on chemically pasteurized straw

Treatments			Room Temp.	Yield	B.E	Remarks
Spawn	Straw		(°C)	(g)	%	
	Carbendazim (ppm)	Formalin (ppm)				
Azadirachtin (1.5ppm) @ 8.5 pH	-	500	27-29	1175	78.33	Contamination of *Trichoderma* sp.
Azadirachtin(0.15ppm) @ 8.5 pH	-	500	27-29	1140	76.00	
Azadirachtin(1.5ppm) at natural pH (5.6-5.9)	-	500	27-29	1085	72.33	
Azadirachtin (0.15ppm)	-	500	27-29	1065	71.00	
Untreated control natural pH	37.5	500	27-29	1115	74.33	No contamination
CD 5%		46.76				

calcium carbonate and gypsum in the ratio of 1:3 for better spawn growth. Mantel [12] earlier in 1973 had perfected the method of grain spawn preparation in India, which is still being practiced all over the country and its vicinity with minor changes only, with little attention to quality and health.

Besides high productivity and consumer-friendly traits of the mushroom strain, maintenance of health and vigor of spawn are very important aspects, which are inter-related. A healthy spawn must be free from contaminants to be vigorous.

Table 5. Yield Performance of *H. ulmarius* spawn prepared by modified method and treated with carbendazim on chemically pasteurized straw

Treatments		Room Temp.	Yield	B.E	Remarks
Spawn	Straw	(^{0}C)	(g)	%	
Carbendazim 0.1%	Carbendazim + Formalin	26- 29	851	56.73	No contamination observed
– Do -	Untreated	26- 29	967	64.47	
Untreated	Carbendazim + Formalin	26- 29	1075	71.67	
Untreated	Untreated	26- 29	1188	79.20	
CD 5%		101.15			

Similarly, only a vigorous spawn can remain contamination-free [13]. Obviously, both these aspects depend heavily on nutrients available in the spawn-medium. Avoidance of boiling of the grains and absorption of almost all the water used for soaking in the modified method being discussed here very well prevent the loss of valuable nutrients by leaching and hence the grain medium so prepared is more nutritious and well set to support a healthy and vigorous spawn. Further, prolonged soaking in aqueous solution is most likely to induce the heat-resistant endospores of *Bacillus* sp, causing wet-spot disease, to germinate into heat-sensitive vegetative phase leading to their elimination, as also of some thermo-tolerant species of *Aspergillus*, during the standard process of autoclaving. Thus, the modified grain spawn medium is more likely to be contamination-free.

The current scenario of spawn health and quality in India and abroad is, however, worrying. Soriano [6] surveying the Central Luzon recorded the problem of spawn contamination among 91% mushroom growers, 67% of whom referred it as occasional, and 16% each as frequent and seldom. In India, 5-years data from a public sector Mushroom Research and Development Laboratory in Haryana, reported 5-25% spawn contamination by various moulds as well as wet-spot causing *Bacillus* sp. [14]. Earlier, Ahlawat *et al*. [15] also reported appreciable damage to *A. bisporus* spawn due to *Bacillus subtilis* infection at NRCM, Solan. From the North Eastern State of Assam also, 8 fungal and 1 bacterial species were reported as common contaminants of oyster mushroom spawn, being more frequent during the monsoon months [16].

Thus, in the present challenging scenario [17] of disease management, every effort needs to be made right at the starting point, i.e. preparation of spawn by keeping them fully free from the contaminants as well as maintaining their quality and health. Also, mushrooms themselves being fungi as also most of the contaminants, they cannot be managed with fungicides and other hazardous chemicals alone for obvious reasons [18]. Therefore, preventive measures like preparation of contamination- free grain spawn medium like the one described here, together with observance of full sterile precautions, cleanliness and hygiene are essential to be adopted in all spawn laboratories, which might ensure the availability of healthy and vigorous spawn to the growers at large.

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