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USE OF MEDICINAL PLANTS IN DIFFERENT COMPOSTS FOR YIELD IMPROVEMENT OF VARIOUS STRAINS OF OYSTER MUSHROOM

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Abstract

Different of concentration of four medicinal plants viz., *Eucalyptus camaldulensis, Azadirachta indica, Citrus lemon, Cymbopogon marginatus* were investigated for the effect of certain active components in their parts, capable of increasing mushroom yield and controlling mushrooms pathogenic microbes which cause great loss in mushroom yield. Four strains of Oyster mushroom were selected on the basis of their well mycelial growth on MEA. For selection of best compost simple composts were also prepared without any medicinal plant products i.e., cotton, wheat, paddy straw. Corn stover composts and cotton compost gave the maximum yield. The dried leaves of the Citrus lemons, lemon grass and Neem cake (dried) were crushed, and the sawdust of the logs of *Eucalyptus* were incorporated with different doses of 2%, 3%, 4%, 5% w/w of substrates with cotton substrate before compost fermentation. Each of the compost bag having specific medicinal plant product with specific concentration were spawned with selected four strains of Oyster mushroom i.e., two local strain *Pleurotus florida* (P-17), *Pleurotus ostreatus* (P-19) and two exotic strains *Pleurotus (florida) ostreatus* (WC536), *Pleurotus ostreatus* (WC-522). Spawn running and mushroom fruitification were allowed to develop under optimum environmental condition. The mushroom yield data of compost bags with different concentration of medicinal plant products plants were calculated. The results showed that presence of Neem cake and Citrus lemon in the substrate increased the yield of Oyster mushroom strains i.e. *Pleurotus (florida) ostreatus* (WC-536) followed by *P. ostreatus* (WC-522) strain. Neem cake and Citrus lemon were more promising in improving yield of mushroom. These results led to the conclusion that addition of specific medicinal plants concentration to compost increases the yield of Oyster mushroom by reducing the incidence of microbes and is more preferable than chemicals due to their lethal effects during human consumption of mushroom.

Introduction

The mushroom (*Pleurotus ostreatus*) is an edible basidiomycete having excellent flavour and nutritional value. Oyster mushroom has high protein content and has many other constituents such as vitamin B1 and B2, and low calorie levels. This makes it very popular for consumption among people who are dieting (Bhandari & Mukerji, 1959). *P. ostreatus* is in class Basidiomycetes, subclass Hollobasidiomycetidae, order Agricales. Edible mushrooms are attacked by various diseases of fungal, bacterial, viral origin and cause great loss in yield of mushroom. In Oyster mushroom cultivation there is problem with contamination of pure mushroom culture from foreign micro-organisms that cause effects on the growth of myelia, which grow at sub-optimum levels and give low yields. Up to 50% of *P. ostreatus* were contaminated by green fungi in the farmer's mushroom house at Mahasarakham province in the northeast of Thailand (Sopit Vetayasuporn, 2006). So these diseases must be controlled to save crop production. Pathogenic bacteria and fungi can be controlled by chromium and copper elements (Dawoud *et al.*, 2005). Hundred percent increase in the number of pests/diseases has resulted from the

indiscriminate use of synthetic pesticides in agriculture (Ray *et al*., 2004). Hot water extract of *Curcuma aromatica* inhibited the mycelial growth of the causative agent of dry root rot of cotton and wilt of tomatoes (Raja & Kurcheve, 1999). The main objective of this study was to incorporate some effective medicinal plants products with different levels of concentration in the compost of mushroom for phytochemical control of pathogenic microbes. The plants used were Neem cake, Citrus lemon (Dawoud & Eweis, 2006), Eucalyptus and Lemon grass (Abd-El-Khair & Omima 2006). These medicinal plants contain many substances as antibiotics, antiseptics, phenolic compounds, alkaloids (Bhonde *et al*., 1999), tannins, volatile oils, triterpenoids (Johnson *et al*., 1999), antibacterial, antifungal (Steinhauer, 1996; Murthy & Sirsi, 1957) and antiviral compounds (Rao *et al*., 1969). The fine powder of these medicinal plants substances were mixed with mushroom substrate, its effect on growth of mushroom and its yield was studied. This investigation was carried out for the selection of the medicinal plants and their appropriate concentration that increases the yield of Oyster mushroom by any mechanism of action and probably by inhibiting the growth of pathogenic microbes.

Materials and Methods

Two local *Pleurotus florida* (P-17) and *Pleurotus ostreatus* (P-19) were obtained from the Department of Plant Pathology,UAF Pakistan and other five exotic strains viz., *Pleurotus (florida) ostreatus* (wc536), *Pleurotus ostreatus* (wc-522) and *Pleurotus (sajor-caju) pulmonarius* (wc-537), *Pleurotus cystidiosus* (wc-609), *Pleurotus (flabellatus) djamor* (R-22), were taken from mushroom laboratory obtained from Penn State University,USA. The selected strains were multiplied on Malt Extract Agar (MEA) with malt extract 20g/L, dextrose 20g/L, agar 20g/L and peptone 1g/L at 25°C.

The medicinal plants used were: **1**. Eucalyptus (*Eucalyptus camaldulensis*) an evergreen growing to 100m, **2**. Neem cake (*Azadirachta indica*) fast growing tree that can reach a height of 15-20 m, **3**. Citrus lemon (*Citrus lemon*). True lemon tree reaches (3-6 m) in height, edible fruits. **4**. Lemon grass (*Cymbopogon marginatus*) perennial grass growing to 6 feet, forms dense clumps of typical grass leaves, tough and sharp.

The substrates used for mushroom were cotton waste, paddy waste, wheat straw, corn stover and other ingredients were calcium carbonate, urea and wheat bran.

Selection of *Pleurotus* strains: Pure culture of 7 strains of oyster mushrooms were inoculated in Malt Extract Agar medium (Ibekwe *et al*., 2008), in 90 mm Petri plates. These Petri plates were kept at 25°C. Data regarding radial growth were recorded 4, 6 and 8 days after inoculation. Two local strain of *Pleurotus florida* (P-17*), Pleurotus ostreatus* (P-19) and two exotic strains *Pleurotus (florida) ostreatus* (wc536), *Pleurotus ostreatus* (wc-522) were selected.

Selection of compost: The experiment included preparation of various composts and selection of only one that gave the high yield of Oyster mushroom selected strains.

Mushroom yield experiment: The experiment included preparation of mushroom spawn (seed), preparation of various composts, after selecting the preparation of cotton compost having different medicinal plant products with different levels of concentration, yield parameters. Incubation was performed under certain conditions for vegetative growth (mycelial production), incubation conditions were changed for fruiting bodies production (yield) and three flushes were harvested.

Spawn preparation: Unbroken sorghum grains after washing 2-3 times were boiled in tap water and mixed with 2% Calcium carbonate. The grains were transferred in wide mouth bottles (500 ml capacity), plugged and sterilized at 121°C for one hour, cooled to room temperature, inoculated separately with different strains of mushroom mycelia and incubated at 25°C for 21 days. This was then ready for use (El-Kattan, 1998).

Compost preparation: Cotton waste, Wheat straw, Paddy straw, Corn stover were soaked in water and nitrogen sources like urea, diammonium phosphate, ammonium nitrate and wheat bran were added in water @ 10 g/L of water to absorb in substrate. Later on these substrates were covered with polythene for five days for fermentation. Paddy straw and wheat straw were soaked only for 3-4 hours (Oei, 1991). Then these materials (paddy straw, wheat straw, cotton waste and Corn stover) were filled in polythene bags @ 1kg / bag. These bags were tied with rubber bands. The bags were sterilized in an autoclave at 121°C 15 psi for 1/2 hours. The spawning was done on the next day. Three replications were used of each substrate having different mushroom strains.

Compost preparation with medicinal plants: Cotton compost was prepared according to above mentioned composition along with different concentrations of (fine powder) medicinal plant products viz., Eucalyptus, Neem cake, Citrus lemon, Lemon grass. The Neem cake powder was prepared, as the Neem cake (NC), a by- product left after the extraction of oil from Neem seed, was dried, crushed and converted into fine powder using a Glen Creston grinder fitted with a 2 mm pore size sieve. The moisture content of dry cake powder was 6.34%. The powder was stored in metal containers at 4°C for experimental use. Similarly the sawdust of the logs of Eucalyptus was prepared and the dried leaves of the Citrus lemons and lemon grass were crushed and made into fine powder, medicinal plants products were applied to substrate at 2% w/w before keeping the bags for fermentation**.** After the fermentation period the composts were inoculated with spawn.

Experimental design: The compost at the rate of 1kg/bag was filled according to completely randomized design.

Incubation: The spawned compost with different concentrations of medicinal plants products were filled into polythene bags @ 1kg/bag. Each treatment having compost + specific Oyster mushroom strain + specific concentration of medicinal plants products were repeated three times. For spawn running of Oyster Mushroom the temperature was maintained at 25 ± 2°C.In growing room, the relative humidity was maintained 85-90% by humidifier (Disc humidifier) and data was recorded twice a day from 8.00 am to 2.00 p.m. Room having spawn running bags was kept in dark for 10-15 days till complete colonization of the compost with fungal mycelium (El-Kattan & El-Hadded, 1998).

Fruiting and harvest: After the completion of spawn running temperature was dropped to 15-17°C, relative humidity was raised to 90-95% and light of 50-250 lux (White fluorescent) was provided daily to spawned compost bags for eight hours for the fruitification of mushroom. Furthermore, fresh air wash was given to growing room by exhaust fan to lower the carbon dioxide below 1000ppm after every 3-4 hours. Pin head appeared after 5-9 days from aeration. Mushroom harvesting was done at maturity, the three mushroom flushes were picked with the interval of 17 days when the cap was still tight over a short stem.

Yield measurement: Yield of selected four strains Oyster mushroom was expressed as gram fresh fruiting body/bag of compost (1kg) in three flushes of mushroom.

Statistical analysis: The experiment followed complete randomized design. The obtained data was subjected to DMR test. The least significant difference was used to compare means of treatment at 5% probability.

Results and discussion

Mycelial growth: The results showed that the most effective strains among seven different local and exotic strains were *Pleurotus ostreatus* (P-19) and *Pleurotus (sajor-caju) pulmonarius* (WC-537), which showed mycelial growth 8.70 mm and 8.50 mm after 4 days, the least significantly effective strain was *Pleurotus (flabellatus) djamor* (R-22) showed mycelial growth of 5.00mm (Table 1).

After 6 days the significantly effective strain was *Pleurotus (florida) ostreatus* (WC-536) which showed mycelial growth of 37.60 mm, followed by *Pleurotus ostreatus* (WC-522) which showed mycelial growth of 36.40 mm and again the least significantly effective strain was *Pleurotus* (*flabellatus*) *djamor* (R-22) wich showed mycelial growth of 23.40 mm. After 8 days (table 1) the significantly effective strain was *Pleurotus (florida) ostreatus* (WC-536) with mycelial growth of 50.10 mm, followed by *Pleurotus ostreatus* (WC-522) having mycelial growth of 47.80 mm. The next significantly effective strains were *Pleurotus ostreatus* (P-19) and *Pleurotus florida* (P-17) with mycelial growth of 45.20 mm and 43.20 mm respectively. The least significantly effective strain here also was *Pleurotus (flabellatus) djamor* (R-22) with mycelial growth of 34.00mm. So these top four strains (two local and two exotic) on the basis of mycelial growth were selected for spawn preparation and further yield experiment.

Compost selection: The results (Table 2) showed yield performance of various strains of *Pleurotus ostreatus* on different composts, the yield of all four strains of Oyster mushroom were maximum in case of cotton compost i.e., 1003.75 g (Leong, *et al*., 1978; Tan, 1981), followed by corn stover 787.25 g and least yield was shown by paddy straw 648.50 g. Cotton compost was selected for further experiment on the basis of yield performance of all the four strains of Oyster mushroom. The results also showed that the yield of *Pleurotus (florida) ostreatus* (wc536) gave maximum yield (887.75 g) of these four strains followed by *Pleurotus ostreatus* (wc-522) which gave yield of 842.50 g, while the *P.ostreatus* (P-19) gave 719.50 g and *P. florida* (P-17) gave 717.75 g, however statistically the yields of last two strains were not significantly different. On the other hand, there was a significant increase in the yield of mushroom especially growing on compost with medicinal plants.

Yield on compost with different medicinal plants products*:* The results (Table 3) showed that the yield of mushroom was increased on addition of eucalyptus plants product 2% w/w of compost and this increase in yield was continued up to 4%. On addition of 5% eucalyptus plants product the mushroom yield started to decrease (1282.50 g). So at 4% maximum yield was obtained i.e., 1286.00 g and that was due to antimicrobial activity of the eucalyptus plants product (Khalil, 2001).

Table 1. Mycelial growth on malt-extract agar (MEA).

Strains	Mycelial growth (mm) in days			Means
	4	6	8	
P.cystidiosus (WC-609)	6.3 p	24.8 l	40.4 f	23.83 E
P. florida (P-17)	8.2 n	26.6 k	43.2 d	26.00 D
P.(florida) ostreatus(WC536)	7.4 o	37.6 h	50.1 a	31.70 A
P. ostreatus (P-19)	8.7 n	29.7 j	45.2 c	27.87 C
P.(flabellatus) djamor (R-22)	5 q	23.4 m	38.7 g	22.37 F
P. ostreatus (WC-522)	8.3 n	36.4 i	47.8 b	30.83 B
P. (sajor-caju) pulmonarius (WC-537)	8.5 n	26 k	42.5 e	25.67 D
Means	7.49 C	29.21 B	43.99 A	

*Data within the same letters and columns are not significantly different

Table 2. Yield performance in grams of various strains of *Pleurotus ostreatus* on different composts.

Strains of oyster mushroom	Cotton waste	Wheat straw	Paddy straw	Corn stover	Means
P. ostreatus (WC-522)	1060 b	753 g	693 hi	864 e	842.50 B
P. (florida) ostreatus (WC-536)	1105 a	823 f	733 gh	890 de	887.75 A
P. ostreatus (P-19)	935 c	672 i	598 j	673 i	719.50 C
P. florida (P-17)	915 cd	664 i	570 j	722 gh	717.75 C
Means	1003.75 A	728.00 C	648.50 D	787.25 B	

*Data within the same letters and columns are not significantly different

Table 3. Yield performance (in grams) of various strains of *Pleurotus ostreatus* on cotton substrates with *Eucalyptus* as a medicinal plant product added (2%, 3%, 4%, and 5% of compost w/w).

Strains of oyster mushroom	Different levels of medicinal plants products in addition with cotton substrate				Means
	2%	3%	4%	5%	
P.(florida) ostreatus (WC-536)	1385 d	1402 c	1408 a	1405 b	1400.00 A
P. florida (P-17)	1183 k	1198 j	1203 i	1197 j	1195.25 C
P. ostreatus (P-19)	1154 n	1170 m	1175 l	1170 m	1167.25 D
P. ostreatus (WC-522)	1338 h	1351 g	1358 e	1354 f	1350.25 B
Means	1265.00 D	1280.25 C	1286.00 A	1281.50 B	

*Data with the same letters and columns are not significantly different

Table 4. Yield performance (in grams) of various strains of *Pleurotus ostreatus* on cotton substrates with neem cake as a medicinal plants products added 2%, 3%, 4%, and 5% of compost w/w.

Strains of oyster mushroom	Different levels of medicinal plants products in addition with cotton substrate				Means
	2%	3%	4%	5%	
P.(florida) ostreatus (wc-536)	1488 f	1518 c	1540 b	1547 a	1523.25 A
P. florida (P-17)	1275 n	1293 m	1309 j	1312 i	1297.25 C
P. ostreatus (P-19)	1253 o	1274 n	1295 l	1301 k	1280.75 D
P. ostreatus (WC-522)	1439 h	1465 g	1492 e	1498 d	1473.50 B
Means	1363.75 D	1387.50 C	1409.00 B	1414.50 A	

*Data within the same letters and columns are not significantly different

Table 5. Yield performance (in grams) of various strains of *Pleurotus ostreatus* on cotton substrates with citrus lemon as a medicinal plants products added 2%, 3%, 4%, and 5% of compost w/w.

Strains of oyster mushroom	Different levels of medicinal plants products in addition with cotton substrate				Means
	2%	3%	4%	5%	
P.(florida) ostreatus (wc-536)	1425 d	1448 c	1460 b	1466 a	1449.75 A
P. florida (P-17)	1232 m	1249 j	1256 i	1259 h	1249.00 C
P. ostreatus (P-19)	1210 n	1231 m	1240 l	1244 k	1231.25 D
P. ostreatus (WC-522)	1384 g	1408 f	1419 e	1424 d	1408.75 B
Means	1312.75 D	1334.00 C	1343.75 B	1348.25 A	

*Data within the same letters and columns are not significantly different

The results (Table 4) showed that the yield of mushroom was increased on addition of Neem cake @ 2% w/w of compost and this increase in yield was continued up to 4% (1409.00 g), on addition of Neem cake 5% w/w of compost the mushroom yield was increased but at a decreasing rate (1414.50 g).

Similarly the results (Table 5) showed that the yield of mushroom was increased on addition of Citrus lemon at 2% w/w of compost and this increase in yield was continued up to 4% (1343.75 g), on addition of Neem cake 5% w/w of compost the mushroom yield was increased but to a smaller extent (1348.25 g).

The results (Table 6) showed that the yield of mushroom was increased on addition of Lemon grass plants product 2% w/w of compost and this increase in yield was continued up to 4% (1286.00 g) as in case of eucalyptus and on addition of 5% Lemon grass plants product the mushroom yield started to decrease (1282.50 g). So at 4% maximum yield was obtained i.e., 1286.00 g and that was due to antimicrobial activity of the Lemon grass plants product (Joarder & Khatun, 1982).

These results showed that mushroom yield increased significantly on increasing the concentration of eucalyptus and lemon grass plant products in cotton compost but after exceeding 4% there was decrease in mushroom yield which may be due to adverse effect of the biochemical substances present in them. In case of other two medicinal plants i.e., Citrus lemon and Neem cake the yield of Oyster mushroom strains increased rapidly up to addition of medicinal plants products 4% w/w of compost and on addition of 5% the

yield increased with a small difference and this may also be due to increase in concentration of substances present in Neem cake which contain a mixture of tetranortriterpenoids (Govindachari *et al*., 1998), flavonoid, nimbosterol, liminoids, quercentinm, tannic acid, and substances found in citrus lemon i.e., volatile oil (Zeringue & Bhatnagar, 1994), limonene, alpha-pinene, beta-pinene, citral, coumarins, biflavonoids (Sammbamurty & Subrahmanyan, 2000), In addition to these benefits Neem plant products have extremely low mammalian toxicity (Kleeberg, 1992) and are relatively safe to non-target organisms (Schmutterer, 1995). Moreover the results also show that Neem cake and citrus lemon are promising in improving the yield of Oyster mushroom. This may be due to the effect of the antimicrobial substances found in Neem cake which contain a mixture of tetranortriterpenoids (Govindachari *et al*., 1998), flavonoid, nimbosterol, liminoids, quercentinm, tannic acid, along with nutrients i.e., nitrogen, phosphorus, potassium (Hoitink & Boehm 1999; Jefferson, *et al*, 2000) and due to substances found in citrus lemon i.e., volatile oil (Zeringue & Bhatnagar, 1994), limonene, alpha-pinene, beta-pinene, citral, coumarins, biflavonoids (Sammbamurty & Subrahmanyan, 2000). The results in Table 7 showed that on increasing the concentration of medicinal plants products the yield of the Oyster mushroom strains also increased but on moving toward higher level the rate of output (mushroom yield) decreased per unit of input (concentration of medicinal plants products).

Table 6. Yield performance (in grams) of various strains of *Pleurotus ostreatus* on cotton substrates with lemon grass as a medicinal plants products added 2%, 3%, 4%, and 5% of compost w/w.

Strains of oyster mushroom	Different levels of medicinal plants products in addition with cotton substrate				Means
	2%	3%	4%	5%	
P.(florida) ostreatus (wc-536)	1379 d	1401 c	1411 a	1408 b	1399.75 A
P. florida (P-17)	1168 m	1184 k	1191 i	1186 j	1182.25 C
P. ostreatus (P-19)	1145 o	1164 n	1172 l	1168 m	1162.25 D
P. ostreatus (WC-522)	1340 h	1361 g	1370 e	1368 f	1359.75 B
Means	1258.00 D	1277.50 C	1286.00 A	1282.50 B	

*Data within the same letters and columns are not significantly different

Table 7. Effect of substrate with different levels of medicinal plants products on yield (in grams) of various strains of oyster mushroom.

Substrate with different levels of medicinal plants products	Mean yield of all strains of oyster mushroom
Cotton substrate + 2% medicinal plants products	1299.88 C
Cotton substrate + 3% medicinal plants products	1319.81 B
Cotton substrate + 4% medicinal plants products	1331.19 A
Cotton substrate + 5% medicinal plants products	1331.69 A

*Data within the same letters and columns are not significantly different

References

Abd-El-Khair, H. and O.M. Hafez. 2006. Effect of aqueous extracts of some medicinal plants in controlling the green mould disease and improvement of stored "Washington" Navel Orange Quality. *Journal of Applied Sciences Research*, 2(10): 664-674.

Bhandari, P.R. and B. Mukerji. 1959. The neem: Indian lilac. *East Pharmacist*, 1: 1-22.

Bhonde, S.B, S.G, Deshpande and R.N. Sharma. 1999. *In vitro* evaluation on inhibitory nature of some neem formulations against plant pathogenic fungi. *Hindustan Antibiotic Bulletin*, 41(1-4): 22-24.

Dawoud, M.E.A and M. Eweis.2006.Phytochemicals control of edible mushrooms phytogenic bacteria. *J. Food, Agri. Environment*, 4(1): 321-324.

Dawoud, M.E.A., M. Eweis and M.A. Rizk. 2005. Chemical control of some edible mushroom pathogenic bacteria. *N. Egypt. J. Microbiol*., 12: 24-37.

El-Kattan, M.H. 1998. Production of Oyster Mushroom.Regional Training course on mushroom production.FAO/UNSCO/ARC/TMF,Cairo.

El-Kattan, M.H. and S.A. El-Hadded. 1998. *Mushroom Biology and Spawn Production*. Regional Training course on mushroom production.FAO/UNSCO/ARC/TMF,Cairo.

Govindachari, T.R., G. Suresh, G. Gopalakrishnan, B. Banumathy and S. Masilamani. 1998. Identification of antifungal compounds from the seed oil of *Azadirachta indica*. *Phytoparasitica*, 26: 109-116.

Hoitink, H.A.J. and M.J. Boehm. 1999. Biocontrol within the context of soil microbial communities: A substrate-dependent phenomenon. *Annual Review of Phytopathology*, 37: 427-46.

Ibekwe, V.I., P.I. Azubuike, E.U. Ezeji and E.C .Chinakwe. 2008. Effects of Nutrient sources and Environmental Factors on the cultivation and yield of oyster mushroom (*Pleurotus ostreatus*). *Pak. J of Nutrition*, 7(2): 349-351.

Jefferson, L.D.A., J.A. Menge and W.L. Casale. 2000. Biological control of Phytophthora rot-rot of avocado with micro organisms grown of avocado with micro organisms grown in organic mulches. *Brazilian Journal of Microbiology*, 31: 239-46.

Joarder, G.J. and M. Khatun. 1982. Inhibitory effects of lemon grass oil on indigenous microflora. Part 1-Inhibition of *Aspergillus niger*. *Bangladish J. Sci. Ind. Res*., 17: 219-226.

Johnson, S., E.D. Morgan and C.N. Peiris. 1996. Development of the major triterpenoids and oil in the fruit and seeds of neem (*Azadirachta indica*). *Ann. Bot*., 78: 383-388.

Khalil, A.R.M. 2001. Phytofungitoxic properties in the aqueous extracts of some plants. *Assiut J. Agric. Sci*., 23: 135-143.

Kleeberg, H. 1992. The NeemAzal conception: test of systemic activity. *Proc. 1st Workshop on Practice Oriented Results on Use and Production of Neem Ingredients* (Wetzlar, Germany), pp. 5-16.

Leong, P.C., T.A. Young and S.E. Chua. 1978. The use of cotton waste for the production of straw mushroom in Singapore. *Singapore J. Primary Industry*, 6(2): 63-68.

Murthy, S.P. and M. Sirsi. 1957. Pharmacological studies on *Melia azadirachta*- Part I. Antibacterial, antifungal and antitubercular activity of neem oil and its fractions. *Symp. Utilization Indian Med. Pl., Lucknow*. 55 p.

Oei, P. 1991. Manual on mushroom production.*Food Science and Agricultural Chemistry*, 1(2): 1948.

Raja, J. and V. Kurcheve. 1999. Fungicidal activity of plant and animal products. *Ann. Agric. Res*., 20(1): 113-115.

Rao, A.R., S. Sukumar, T.B. Paramsivam, S. Kamalakshi, A.R. Parashuraman and M. Shantha. 1969. Study of antiviral activity of tender leaves of Margosa tree (*Melia azadirachta*) vaccinia and variola virus. A preliminary report. *Indian J. Med. Res*., 57: 495.

Ray, A.B., B.K. Sarma and U.P. Singh. 2004. *Medicinal properties of plants (Antifungal, Antibacterial, Antiviral Activities*). Army printing press, Sadar cantt, Lucknow, p.11.

Sammbamurty, A.V.S.S. and N.S. Subrahmanyan. 2000. *Medicinal Plants in Industry*. C.B.S. Publishers, New Delhi, pp. 22-64.

Schmutterer, H. 1995. Side effects of beneficials and other ecologically important non-target organisms. pp. 495-517. In: *The Neem Tree. Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes*. (Ed.): H. Schmutterer. [Ed.] VCH Verlagsgesellschaft, Weinheim, Germany.

Sopit, Vetayasuporn. 2006. Oyster mushroom cultivation on different cellulosic substrates. *Research Journal of Agriculture and Biological Sciences*, 2(6): 548-551.

Steinhauer, B. 1996. Fungicidal activity of commercial neem products. *Proc. 4th Workshop on Practice Oriented Results on Use and Production of Neem Ingredients and Pheromones* (Bordigherra, Italy, 1994), pp. 105-108.

Tan, K.K. 1981. Cotton waste is a fungus (*Pleurotus*) good substrate for cultivation of *Pleurotus ostreatus* the oyster mushroom. *Mush. Sci*., 11: 705-710.

Zeringue, H.J.Jr. and D. Bhatnagar. 1994. Effects of neem leaf volatiles on submerged cultures of aflatoxigenic Aspergillus parasiticus. *Appl. Environ. Microbiol*., 60: 3543-3547.

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