International Journal of Advance Research in Science and Engineering
Volume No.07, Special Issue No.04, March 2018
www.ijarse.com
IJARSE
ISSN: 2319-8354

Efficacy of Fungicides against Mycogone perniciosa causing Wet Bubble disease of White Button mushroom (Agaricus bisporus) in Kashmir

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ABSTRACT

Wet Bubble (Mycogone perniciosa) is a devastating disease in the crop production of mushrooms. In India, it has been reported to cause serious crop losses. The aim of the present study was to check the in vitro and in vivo efficacy of fungicides against Wet Bubble (M. perniciosa) associated with the cultivation of Agaricus bisporus. The in vitro efficacy of ten fungitoxicants against A. bisporus and M. perniciosa revealed that captan and prochloraz manganese with the mean inhibition of 95.71 and 99.52%, respectively, were highly inhibitory to the pathogen among non-systemics. Prochloraz manganese completely inhibited the mycelial growth of the pathogen at 50-500 μg ml^{-1} with least inhibition (10.83%) of A. bisporus mycelium, whereas captan did so at only 500 μg ml^{-1} concentration, with 15.41% inhibition of A. bisporus mycelium. Among systemic fungicides carbendazim proved highly inhibitory to the pathogen (97.35%), with the least inhibition (1.45%) of A. bisporus mycelium. Bitertanol and myclobutanil with the inhibition percentage of 93.05 and 93.70% were the next best with least (7.08 to 9.78%) inhibition of A. bisporus mycelium.

Keywords: Cultivation, Mushroom, Efficacy, Fungicides, Pathogen

I.INTRODUCTION

Mushrooms include a large group of heterogeneous fungi having various shapes, sizes and colours. Mushrooms are quite different in character, appearance and edibility. Mushroom production is an eco-friendly activity where agricultural or industrial wastes are utilized and recycled to produce protein rich fruiting bodies called mushrooms. Mushrooms are also good source of vitamins and minerals, especially those of B complex group, but are relatively poor in fat soluble vitamins, A, D, E and K. Among B complex vitamins, mushrooms are specially rich in thiamine (B_1), riboflavin (B_2), niacin and biotin (Manzi, *et al*, 2004). During last four decades, mushroom has attained the status of commercial crop. At present, it is being produced in about 100 countries. In countries like Europe and America, it is under hi-tech industry. Total world production is estimated to be 5 million tones, with increasing rate at 7% /annum (Tewari, 2003).

Presently mushrooms are being cultivated in about 100 countries with an annual production of 3,206,738 million tons (Anonymous, 2004). Out of the large groups of mushrooms with more than 2000 edible species, about 300

species belonging to 70 genera are reported from India (Chadha and Sharma, 1995). However, only a few have been brought under cultivation on commercial scale. Among the cultivated mushrooms, white button mushroom (*Agaricus bisporus*) is an important mushroom cultivated in India. The white button mushroom has the highest growth rate and potential for production.

So far, in modern mushroom production panorama, button mushroom (*Agaricus bisporus*) reigns supreme with 1,424,000 tonnes of production during 1989-90 (Munshi and Ghani, 2003).

Mushrooms are subject to attack by disease-causing organisms, including fungi, bacteria, viruses and nematodes. Some of them are capable of reducing the yield significantly or even result in complete crop failure depending upon their severity and stage of appearance. One of the mycoparasites, *M. perniciosa* (the cause of wet bubble disease) has been reported as one of the serious diseases from almost all major mushroom growing countries of the world. Wet bubble disease causes heaviest losses among all diseases in mushroom beds. The natural incidence of wet bubble disease of button mushrooms ranged from 1 to 100 per cent in northern India. Loss in yield in *A. bisporus* (S-11) due to this disease under artificial inoculation conditions has been reported to vary from 15.72 to 80.13%. Bhatt and Singh (2000) have reported the yield loss up to 100% as a result of artificial inoculation of *M. perniciosa*. Management of this fungal disease of *A. bisporus* poses problems because both the host and pathogen are fungi. Consequently, there is a need to select fungicides which have least effect on the growth of the mushroom. Hence, an attempt was made to evaluate different fungicides against the pathogen to manage this devastating disease.

II.MATERIALS AND METHODS

The present study was conducted during 2008 and 2009 at Mushroom Research and Training Centre, Division of Plant Pathology, SKUAST-Kashmir, Shalimar, Srinagar. Survey of the mushroom units located in three districts *viz*., Srinagar, Budgam and Pulwama, of Kashmir Division was conducted in both spring and autumn crop seasons of 2008 and 2009, to ascertain the status of wet bubble disease (*Mycogone perniciosa*) of white button mushroom, *Agaricus bisporus* (Lange) Imbach. Nine representative locations/mushroom farms were randomly selected in each district.

***In vitro* evaluation of fungitoxicants:**

In the present study, five non systemic and five systemic fungitoxicants (Table 1) were assayed *in vitro* against *M. perniciosa* and *A. bisporus* mycelium using poisoned food technique Nene and Thapliyal (2000). Each non systemic fungitoxicant was evaluated at concentrations of 25, 50, 100, 200 and 500 µg ml^{-1}, whereas the systemic fungitoxicants were evaluated at concentrations of 5, 10, 25, 50 and 100 µg ml^{-1}. 25 ml double strength sterilized PDA in 150 ml Erlenmeyer flask, was amended aseptically with 25 ml double strength test fungitoxicant concentration. The contents were thoroughly but gently shaked and aseptically poured in pre-sterilized petriplates. Mycelial discs of 7 and 14 day old culture, respectively, of *M. perniciosa* and *A. bisporus* were separately and aseptically placed at the centre of each petriplate and incubated at 23±2^{o}C. Each treatment was replicated three times. *M. perniciosa* and *A. bisporus* allowed to grow separately on PDA amended with sterilized water in petriplates served as checks. Data on radial mycelial growth were recorded when the test

pathogen nearly covered the petriplate in check. The mycelial growth inhibition as index of fungicidal efficacy was computed using the formula given by Vincent (1947).

$$PI = \frac{(C - T)}{C} \times 100$$

Where PI = Percent inhibition

C = Growth of pathogen in control (mm)

T = Growth of pathogen in treatment (mm)

Table 1: Systemic and non-systemic fungitoxicants used with their chemical names

Common name	Trade name	Chemical name
a) Non systemic		
1. Prochloraz manganese	Sportek WP	Manganese, dichlorotetrakis [N-propyl-N-[2-(2,4,6-trichlorophenoxy) ethyle]-1H-imidazole-1-carboximide]
2. Chlorothalanil	Kavach 75 WP	2, 4, 5, 6 tetrachloro isophthalonitrile
3. Mancozeb	Dithane M-45	Manganese ethylene bis dithio-carbamate plus zinc
4. Captan	Captaf 50 WP	N-(trichloromethylthio)-4-cyclo hexane-1, 2-dicarboximide
5. Propineb	Antracol 70 WP	3-(2-methylpirperidinno) propyl-3, 4-dichloro-benzoate
b) Non systemic		
1. Carbendazim	Bavistin 50 WP	[2 (methoxy-carbamoyl) benzimidazole]
2. Bitertanol	Baycor 25 WP	1-(1, 1-biphenyl)-1 H-I, 2, 4-triazole-1-1 ethanol
3. Myclobutanil	Systhane 10 WP	A-butyl-α (4-chlorophenyl)
4. Triademiphon	Bayleton 25 WP	1-(4-chlorophenoxy)-3,3-dimethyl-1 (1H-1, 2,4-trizol-1-yl)-2-butanone

5.	Diniconazole	Sumi 25 WP	4-dimethyl-2-(1, 2, 4-triazol-I-yl)-1)-1-pentene-3-l

Presence of pathogen in casing materials

Composite samples of different casing materials such as peat, garden soil, virgin soil, spent compost, farm yard manure (FYM) and sand were collected, sieved through a 200 mesh sieve and put in pre-sterilized plastic vials. One gram of the material was separately used for assessing the *M. perniciosa* propagules using serial dilution method.

Spent compost as inoculum source

500 g of composite samples of spent compost were collected in triplicate from the production trays, showing the signs of wet bubble disease soon after termination of spring and autumn cropping season of 2008 and placed separately in plastic jars. The jars were kept outdoors under natural conditions and 5 g of compost samples drawn at monthly intervals for assessing the presence of pathogen *M. perniciosa* using serial dilution method.

***In vivo* evaluation of fungitoxicants:**

Fungicides which have shown promising results during *in vitro* evaluation and were least inhibitory to mushroom mycelium, were evaluated *in vivo* during the present studies. The systemic fungicides viz., carbendazim 50 WP, myclobutanil 10 WP and bitertanol 25 WP were evaluated at 0.025, 0.05 and 0.1% concentrations, whereas the non-systemic fungicides viz., prochloraz manganese 50 WP, chlorothalonil 75 WP and captan 50 WP were evaluated at 0.05, 0.1 and 0.2% concentrations. Each fungicidal concentration was admixed with casing mixture at the rate of 100 ml/kg casing mixture before inoculation of the pathogen (*M. perniciosa*). Casing was done by spreading 2-3 cm thick layer of casing mixture on spawn-run compost in 10 kg polybags. Each bag received about 2 kg casing soil. Inoculation of the pathogen was done by spraying spore suspension (1×10^8 spores ml^{-1}) obtained from 10 day old culture, on casing soil at the rate of 20 ml/bag of 30 cm diameter. Each treatment represented by a single bag was replicated three times. The control was run without any fungicidal treatment with and without inoculation of pathogen. Per cent disease intensity and yield for a cropping period of one month and also other quality characters were recorded during first flush as per the procedure given earlier.

Statistical analysis

The data collected were subjected to statistical analysis wherever needed. The differences exhibited by the treatments in various experiments were tested for their significance as per the methods suggested by Gomez and Gomez (1984). The 'Minitab' computer software was used for data analysis.

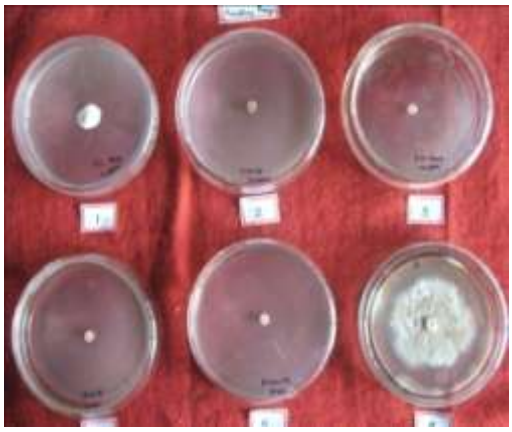
III.RESULTS

***In vitro* evaluation of non-systemics**

Five non-systemic fungicides *viz*., prochloraz manganese, chlorothalonil, mancozeb, captan and propineb were evaluated at 25, 50, 100, 200 and 500 µg ml^{-1} concentrations by poisoned food technique for their inhibitory effects on pathogen *Mycogone perniciosa* and the host *Agaricus bisporus* mycelium . (Plate-1).

Effect on mycelial growth of pathogen

Prochloraz manganese proved most effective and provided a growth inhibition of 99.52% followed by captan exhibiting inhibition percentage of 95.71%. Mancozeb was the next best fungicide showing an inhibition of 86.56%, whereas propineb was the least inhibitory (41.20%) fungicide against the test pathogen (Table-2). A significant interaction between fungicides and their concentrations also existed. The results further revealed that an increase in fungicide concentration resulted in a corresponding increase in per cent growth inhibition of test pathogen, such that a maximum overall inhibition of 91.98% was obtained at the highest concentration of 500 µg ml^{-1}, whereas 25 µg ml^{-1} fungicide concentration exhibited overall growth inhibition of 72.35%.



(a)

(b)

(c)

(d)

Plate-1 :*In vitro* evaluation of non-systemic fungicides

a, b, c = Against pathogen *M. perniciosa*

d = Against host *A. bisporus*

Prochloraz manganese at a concentration of 50 µg ml^{-1} and more and captan at a concentration of 500 µg ml^{-1} completely restricted the growth of the test pathogen. Captan at 200 µg ml^{-1} concentration was next best fungicide exhibiting a growth inhibition of 99.76 per cent followed by again prochloraz manganese at 25 µg ml^{-1}, captan at 100 µg ml^{-1} and mancozeb at 500 µg ml^{-1}. Propineb was the least effective fungicide in checking the growth of the test pathogen.

Effect on mycelial growth of host

The results (Table-3) revealed that the test fungicides varied significantly in their inhibitory effects on the host mycelium. In overall comparison, prochloraz manganese recorded minimum (10.83%) growth inhibition of *A. bisporus* mycelium followed by chlorothalonil and captan (13.22-15.41%), whereas propineb exhibited maximum growth inhibition (38.01%) of the test fungus. On an average, the per cent growth inhibition increased with increase in fungicide concentration such that minimum (1.87%) inhibition was obtained at 25 µg ml^{-1} and maximum (54.36%) at 500 µg ml^{-1}. A significant interaction between fungicides and their concentrations also existed. Prochloraz manganese, chlorothalonil, mancozeb and captan at upto 50 µg ml^{-1} and mancozeb at 25 µg ml^{-1} did not show any growth inhibition of host mycelium. Prochloraz manganese and captan at 100 µg ml^{-1} and mancozeb at 50 µg ml^{-1} were the next least inhibitory (3.15-3.64%) fungicide concentrations followed by chlorothalonil 100 µg ml^{-1} (7.29%) and propineb 25 µg ml^{-1} (9.37%). Mancozeb and propineb at 500 µg ml^{-1} exhibited maximum inhibition (62.49-68.22%) of the test fungal mycelium.

***In vivo* evaluation of non-systemics**

Effect on disease development

The data (Table-4) revealed that all the fungicidal treatments reduced the per cent disease intensity as compared to pathogen-infested and untreated check-I. Compared to a wet bubble intensity of 16.67 per cent obtained in pathogen infested and untreated check-I, the disease was reduced to 0.0-0.37 per cent by application of captan 50 WP at 0.1-0.2% or prochloraz manganese 50 WP at 0.2%, exhibiting the disease control of 97.78-100%, which was statistically at par with uninfested and untreated check-II. Captan 50 WP at 0.05%, prochloraz manganese 50 WP at 0.1% and chlorothalonil 75 WP at 0.2% were the next best treatments exhibiting wet bubble intensity of 1.11-1.85 per cent with a disease control of 88.90-93.34 per cent. Prochloraz manganese 50 WP and chlorothalonil 75 WP at 0.05 and 0.1% were the least effective fungicidal treatments exhibiting wet bubble intensity of 4.07-5.92 with a disease control of 64.48-75.58 per cent.

Effect on yield and yield components

It is evident from the (Table-5) that the application of captan 50 WP at 0.2% concentration exhibited minimum (90.16) number of fruit-bodies per kg mushroom followed by prochloraz manganese 50 WP and chlorothalonil 75 WP at 0.2% and captan 50 WP at 0.1% yielding 91.50-92.50 fruit-bodies per kg mushroom, lesser than 94.16 and 92.83 fruit-bodies per kg mushroom obtained in infested-untreated and un-infested, untreated checks, respectively.

The average fruit-body weight also varied significantly with different fungicidal treatments. Prochloraz manganese 50 WP and captan 50 WP each at 0.2% concentration exhibited maximum (10.86-10.89 g) average weight of single fruit-body similar to that obtained in uninfested-untreated check (10.93 g). Captan 50 WP (0.10%) and chlorothalonil 75 WP (0.20%) were the next best treatments showing average fruit-body weight of 10.69-10.77 g compared to 10.68 g obtained in infested-untreated check.

The button yield per quintal compost also improved significantly with the application of non-systemic fungitoxicants. Captan 50 WP (0.10-0.20%) and prochloraz manganese 50 WP (0.20%) exhibited maximum button yield of 14.23-14.83 kg / quintal compost as compared to that of 6.18 and 12.84 kg / quintal compost obtained in infested-untreated and uninfested-untreated checks, respectively. Prochloraz manganese 50 WP (0.10%) or captan 50 WP (0.05%) were the next best treatments with the average yield of (12.59-13.48) kg / quintal compost. Prochloraz manganese 50 WP and chlorothalonil 75 WP each at 0.05% concentration were the least effective fungicides.

Effect of quality parameters of sporophores

The non-systemic fungicidal applications on infested casing also significantly affected the quality parameters of sporophores such as pileus weight, pileus dia, stipe weight and stipe dia (Table-6).

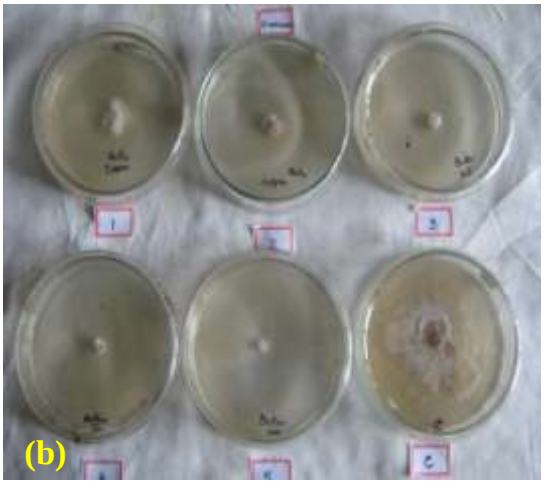
- **Pileus weight:-** The pileus weight of 5.32 g as obtained in infested- untreated check was found to significantly improve to 6.01-6.02 g in the treatments receiving captan 50 WP (0.20%) or prochloraz manganese 50 WP (0.20%). Chlorothalonil 75 WP (0.10%) or captan 50 WP (0.10%) or Prochloraz manganese 50 WP (0.10%) were the next best treatments providing the pileus weight of 5.70-5.79 g.
- **Pileus dia:-** The maximum pileus dia (3.54 cm) among different fungicidal treatments was exhibited by captan 50 WP (0.20%) which was statistically at par with uninfested-untreated check (3.56 cm). Prochloraz manganese 50 WP (0.20%), chlorothalonil 75 WP (0.20%) and captan 50 WP (0.05-0.10%) were the next best treatments providing pileus dia of 3.50-3.51 cm.
- **Stipe weight:-** The stipe weight was maximum (4.80 g) in treatment receiving captan 50 WP (0.20%) as compared to that (4.29 g) in infested-untreated check. Captan 50 WP (0.10%) or chlorothalonil 75 WP (0.20%) or prochloraz manganese 50 WP (0.20%) were the next best treatments providing stipe weight of 4.68-4.77g. The average weight of stipe in uninfested-untreated check was 3.56 cm.
- **Stipe dia:-** The maximum stipe dia of 1.31 cm was exhibited by the treatments receiving captan 50 WP (0.20%) and prochloraz manganese 50 WP (0.20%), as compared to that of 1.23 cm observed in infested-untreated check.

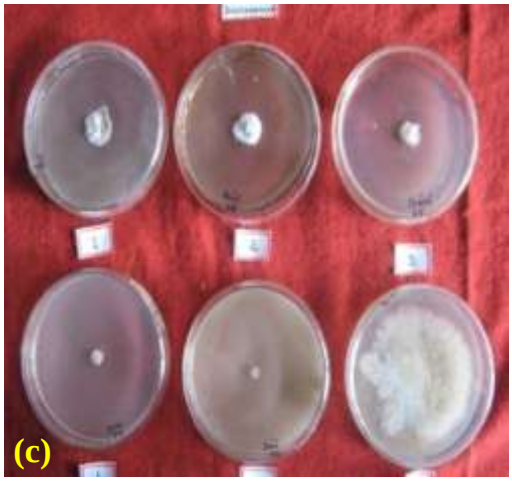
***In vitro* evaluation of systemics**

Five systemic fungicides *viz*., carbendazim, myclobutanil, bitertanol, triademephon and diniconazole were evaluated at 5, 10, 25, 50 and 100 µg ml^{-1} concentrations by poisoned food technique for their inhibitory effects on pathogen *M. perniciosa* and host *A. bisporus* mycelium (Plate-2).

Effects on pathogen

On an average, carbendazim proved most effective and provided growth inhibition of 97.35 per cent followed by myclobutanil and bitertanol exhibiting inhibition percentage of 93.05-93.70, respectively. It is also clear from Table-7, that with increase in concentration, the per cent inhibition over control also increased significantly, such that a maximum growth inhibition of 99.02 per cent was obtained at 100 µg ml^{-1} concentration and a minimum of 81.39 per cent at 5 µg ml^{-1} concentration. A significant interaction between fungicides and their concentrations also existed. Myclobutanil at 50-100 µg ml^{-1} and carbendazim and bitertanol at 100 µg ml^{-1} concentration exhibited maximum (100%) growth inhibition of the test pathogen. Carbendazim and bitertanol 50 µg ml^{-1} were the next best fungicide concentrations exhibiting the growth inhibition of 99.16-99.53 per cent followed by again carbendazim 25 µg ml^{-1} giving the inhibition percentage of 98.13 per cent.





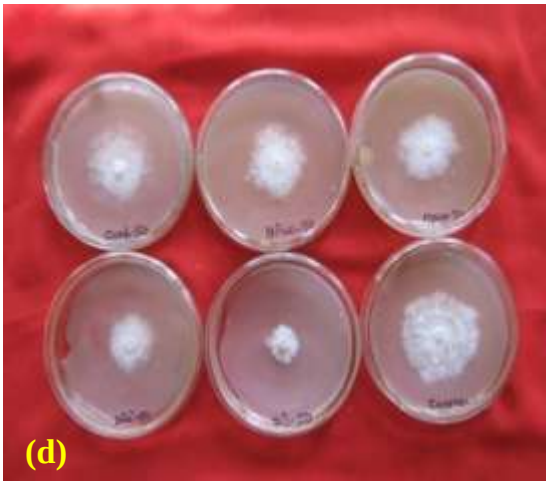


Plate-2: *In vitro* evaluation of systemic fungicides

a, b, c = Against pathogen *M. perniciosa*

d = Against host *A. bisporus*

The other fungicides in order of their increasing inhibitory effect were triademephon at 10 µg ml^{-1} (75.05%) < diniconazole at 5 µg ml^{-1} (75.98%) < triademephon at 25 µg ml^{-1} (81.81%) < diniconazole 10 µg ml^{-1} and bitertanol 5 µg ml^{-1} (82.27-83.94) < myclobutanil at 5 µg ml^{-1} and 10 µg ml^{-1} (84.61-85.31%) < triademephon at 50 µg ml^{-1} and diniconazole 25 µg ml^{-1} (87.41-89.74%) < carbendazim at 5 µg ml^{-1} and 10 µg ml^{-1} (93.70-95.80%) and bitertanol 25 µg ml^{-1} (94.40%) < carbendazim µg ml^{-1} (98.13%) and bitertanol 50 µg ml^{-1} (99.53%).

Effects on host mycelium

All the test fungicides varied significantly for their inhibitory effects against host mycelium (Table-8). In overall comparison, carbendazim recorded minimum (1.45%) growth inhibition of *A. bisporus* followed by myclobutanil (7.08%). Bitertanol was the next least inhibitory (9.78%) to the host fungus, whereas triademephon and diniconazole exhibited maximum inhibition (56.34-56.55%). The mycelial inhibition was found to show a continuous increase with increase in fungicide concentration. A significant interaction between fungicides and their concentrations also existed. Carbendazim at upto 25 µg ml^{-1} and bitertanol at 5 µg ml^{-1} did not show any inhibition of the host fungus. The same fungicides respectively at 50-100 and 10-25 µg ml^{-1} and bitertanol at 5-10 µg ml^{-1} were next least inhibitory (2.60-4.68%) to the test fungus. The other fungicides in order of their increasing inhibitory effects on *A. bisporus* were bitertanol 25 µg ml^{-1} < bitertanol and myclobutanil 50 µg ml^{-1} < myclobutanil 100 µg ml^{-1} < bitertanol 100 µg ml^{-1}, triademephon 5 µg ml^{-1} and diniconazole 10µg ml^{-1} < triademephon and diniconazole 25 µg ml^{-1} < diniconazole 50 µg ml^{-1} < triademephon 50 µg ml^{-1} < triademephon 100 µg ml^{-1} < diniconazole 100 µg ml^{-1}

***In vivo* evaluation of systemics**

Effect on disease development

The data on the effect of systemic fungicides incorporated in casing on wet bubble disease of white button mushroom (Table-9) revealed that all the fungicidal treatments reduced the per cent disease intensity as compared to pathogen infested-untreated check-I. Compared to a wet bubble intensity of 16.67 per cent obtained in pathogen uninfested-untreated check-I, the disease was reduced to 1.11-1.48 per cent by applications of carbendazim 50 WP or bitertanol 25 WP at 0.1%, exhibiting the disease control of 91.12-93.34 per cent. Carbendazim 50 WP or bitertanol 25 WP @ 0.05% or myclobutanil 10 WP @ 1% were the next best treatments exhibiting wet bubble intensity of 2.59-3.70 per cent with a disease control of 77.80-84.46 per cent. Myclobutanil 10 WP @ 0.025 - 0.05% and carbendazim 50 WP and bitertanol 25 WP @ 0.025% were the least effective fungicidal treatment exhibiting wet bubble intensity of 5.55-7.41 per cent with a disease control of

55.54-62.62 per cent. However, a wet bubble-free crop without any disease intensity was obtained in pathogen uninfested-untreated check-II.

Effect on yield and yield components

The fungicidal application on pathogen-infested casing was found to show significant effects on yield and yield components such as number and weight of fruit-bodes. It is evident from Table-10 that minimum (88.83-91.67) number of fruit-bodies per kg mushroom was recorded in treatments which received carbendazim 50 WP or bitertanol 25 WP as compared to infested-treated check (92.16). Myclobutanil 10 WP (0.05%) and carbendazim 50 WP (0.05%), were next best fungicides providing as many fruit-bodies per kg mushroom (93.17-93.50) as obtained in uninfested-untreated check. Carbendazim 50 WP and bitertanol 25 WP each at 0.025% did not significantly influence this yield component compared to infested-untreated check (96.33-97.50 fruit-bodies per kg mushroom).

The application of systemic fungicides in *M. perniciosa*-infested casing significantly affected the average weight of fruit-bodies. The average weight of fruit-body of 10.23 g as obtained in infested-untreated check was found to improve significantly to 10.46-11.12 g, with the application of myclobutanil 10 WP (0.05-0.1%), carbendazim 50 WP (0.1%) or bitertanol 25 WP (0.1%) similar to that (10.83 g) obtained in infested-untreated control. The application of carbendazim 50 WP (0.025-0.05%), myclobutanil 10 WP (0.025-0.05%) or bitertanol 25 WP (0.05%) were the next best fungicide treatment providing fruit body weight of 10.46-10.69 g.

The button yields (kgs per quintal compost) also improved significantly with the application of fungicides; carbendazim 50 WP and bitertanol 25 WP each at 0.1% concentration exhibited maximum yield 12.29-12.97 kg/qtl compost, as good as that obtained in uninfested-untreated check (12.84 kg / qtl. compost). Bitertanol 25 WP, carbendazim 50 WP each at 0.05% and myclobutanil 10 WP at 0.1% concentration were the next best fungicides showing the average yield of 10.23-10.49 kg q^{-1} compost compared at 6.18 kg q^{-1} compost obtained in infested-untreated check. Of all the evaluated fungicidal concentrations, myclobutanil 10 WP application at 0.025% did not significantly improve the button yield compared to infested-untreated check.

Effect on quality parameters of sporophores

The fungicidal application on infested casing significantly affected some of the sporophore quality parameters such as pileus dia and stipe weight, whereas the pileus weight and stipe dia did not experience any significant change (Table-11).

- **Pileus weight:**-The fungicidal application in pathogen-infested casing did not significantly change the pileus weight of the *A. bisporus* sporophores.
- **Pileus dia:**-The pileus dia of 3.36 cm as obtained in infested-untreated check was found to significantly improve to 3.58-3.63 cm in treatments receiving bitertanol 25 WP (0.025-0.1%) or myclobutanil 10 WP (0.05-0.1%) or carbendazim 50 WP (0.1%). Carbendazim 50 WP (0.025 - 0.05%) or myclobutanil 10 WP (0.025%) applications were the next best treatments providing the pileus dia of

3.36-3.39 cm, similar to that obtained in infested-untreated check. However, none of the fungicidal applications provided the pileus dia of 3.82 cm obtained in uninfested-untreated check.

- **Stipe weight:-**The stipe weight significantly improved to 4.72-4.83g in treatments which received carbendazim 50 WP or myclobutanil 10 WP or bitertanol 25 WP each at 0.1% as compared to that of 4.23 g obtained in infested-untreated check and 4.59 obtained in uninfested-untreated check. Carbendazim 50 WP (0.025-0.05%), myclobutanil 10 WP (0.05%) and bitertanol 25 WP (0.025-0.05%) were the next best treatments providing the average stipe weight of 4.61-4.00g.
- **Stipe dia:-**The fungicidal application did not significantly change the stipe diameter of *A. bisporus* sporophores

IV.DISCUSSION

The effective management of wet bubble disease in mushroom production houses requires preferably the use of components other than chemicals (fungicides) such as botanicals and bio-control agents. Some fungicides can also be used for economical management of the disease. However, all these applications and amendments aim at checking or inhibiting the growth and proliferation of *M. perniciosa* with no or least such effects on *A. bisporus*. These components were, therefore, evaluated both *in vitro* and *in vivo* against both these fungi to select the most suitable ones for containing the disease. During the present investigations, prochloraz manganese followed by captan showed maximum *in vitro* inhibition against *M. perniciosa* and least against *A. bisporus* amongst non-systemic fungicides; whereas among systemic carbendazim, bitertanol and myclobutanil were *in vitro* most inhibitory to *M. perniciosa* and least inhibitory to *A. bisporus in vitro*. These fungicides, therefore, show promise for controlling wet bubble disease of white button mushroom. Similarly, inhibitory effects of these fungicides have also been reported by several other workers (Dhar and Kapoor, 1990; Bhat and Singh, 2002 and Sharma and Jarial, 2000).

V.CONCLUSION

The *in vitro* efficacy of ten fungitoxicants against *A. bisporus* and *M. perniciosa* revealed that prochloraz manganese was highly fungitoxic among non-systemics completely inhibiting the mycelial growth of the pathogen at 50-500 µg ml^{-1} with least inhibition of *A. bisporus* mycelium. This finding is in agreement with F.J Gea, *et al*; 2010, who reported that *in vitro* experiments showed that prochloraz exhibit maximum efficacy against *M. perniciosa.* The other fungitoxicants inhibiting the mycelial growth of pathogen in order of their efficacy were captan, mancozeb, chlorothalonil and propineb.

Among systemic fungicides carbendazim proved highly fungitoxic, inhibiting the mycelial growth of the pathogen at different test concentrations. F.J Gea, *et al*; 2010 also reported that Carbendazim was the most effective fungicide for inhibiting the mycelial growth of *M. perniciosa.* The other systemic fungicides inhibiting the growth of the pathogen in order of their efficacy were myclobutanil, bitertanol, diniconazole and triademiphon. Carbendazim, myclobutanil and bitertanol at concentration 100 µg ml^{-1} completely inhibited the pathogen mycelium, while as myclobutanil did so at 50 µg ml^{-1}. Carbendazim also recorded no growth

inhibition of *A. bisporus* mycelium at 5-25 µg ml^{-1}. This findings is in partial agreement with the results obtained by Shaiesta Shah, *et al*; 2013, who reported the least inhibition of mushroom mycelium *in vitro*. The other fungitoxicants in order of their least inhibitory effects against host fungus are bitertanol, myclobutanil, triademiphon and diniconazole.

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International Journal of Advance Research in Science and Engineering
Volume No.07, Special Issue No.04, March 2018
www.ijarse.com
IJARSE
ISSN: 2319-8354

Table-2 : *In vitro* evaluation of different non-systemic fungitoxicants against *Mycogone perniciosa*, the causal agent of wet bubble disease of white button mushroom (*Agaricus bisporus*)

Conc. (µg ml^{-1})	Growth inhibition over control (%)					Mean
Fungicide	**25**	**50**	**100**	**200**	**500**	
Prochloraz manganese 50 WP	97.46 (80.63)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00	100.00 (90.00)	**99.52 (88.12)^{a}**
Chlorothalonil 75 WP	59.58 (50.18)	68.64 (55.75)	76.75 (60.89)	80.75 (63.67	95.51 (77.07)	**76.24 (61.51)^{d}**
Mancozeb 75 WP	78.07 (62.07)	82.31 (65.12)	85.85 (67.90)	88.68 (70.33	97.91 (81.68)	**86.56 (69.42)^{c}**
Captan 50 WP	86.79 (68.03)	94.10 (75.48)	97.91 (81.43)	99.76 (88.08	100.00 (90.00)	**95.71 (80.60)^{b}**
Propineb 75 WP	19.84 (25.82)	27.15 (31.08)	32.10 (34.02)	51.19 (45.38	75.71 (60.23)	**41.20 (39.30)^{e}**
Mean	**72.35 (56.08)^{E}**	**73.59 (62.82)^{D}**	**77.81 (66.25)^{C}**	**83.51 70.92)^{B}**	**91.98 (77.40)^{A}**	

	S.E±	**CD(p=0.05)**
Fungicide	(0.50)	(0.83)
Concentration	(0.50)	(0.83)
Fungicide x Concentration	(1.13)	(1.89)

Means of three replications; figures in parentheses are angular transformed values; means followed by similar letter(s) are statistically identical.

Table-3: *In vitro* evaluations of different non-systemic fungicides against *Agaricus bisporus* mycelium

Conc. (µg ml^{-1})	Growth inhibition over control (%)					Mean
Fungicide	**25**	**50**	**100**	**200**	**500**	
Prochloraz manganese 50 WP	0.0 (2.86)	0.0 (2.86)	3.64 (10.94)	12.49 (20.67)	38.02 (38.06)	**10.83 (15.07)^{a}**
Chlorothalonil 75 WP	0.0 (2.86)	0.0 (2.86)	7.29 (15.63)	15.10 (21.53)	43.74 (41.40)	**13.22 (16.85)^{b}**
Mancozeb 75 WP	0.0 (2.86	3.62 (10.55)	14.06 (21.99)	39.06 (38.68)	68.22 (54.07)	**24.99 (25.63)^{c}**
Captan 50 WP	0.0 (2.86)	0.0 (2.86)	3.15 (10.21)	14.57 (22.35)	59.37 (50.40)	**15.41 (17.73)^{b}**
Propineb 75 WP	9.37 (17.71)	22.39 (28.22)	43.22 (41.10)	52.60 (46.48)	62.49 (52.23)	**38.01 (37.14)^{d}**
Mean	**1.87 (5.83)^{A}**	**5.20 (9.47)^{D}**	**14.27 (19.97)^{C}**	**26.76 (29.94)^{B}**	**54.36 (47.23)^{A}**	
	S.E±			**CD(p=0.05)**		
Fungicide	(0.61)			(1.23)		
Concentration	(0.61)			(1.23)		

Fungicide x concentration	(1.22)	(2.46)	

Means of three replications; figures in parentheses are angular transformed values

Table-4: Effect of non-systemic fungitoxicants incorporated in casing on per cent intensity of wet bubble disease (*Mycogone perniciosa*) of white button mushroom (*Agaricus bisporus*) during spring 2009 and 2010

Treatments		Spring 2009	Spring 2010	Mean	Disease control (%)
P. manganese 50 WP	0.05%	5.18 (13.09)	6.66 (14.82)	5.92 (13.96)[d]	64.48
	0.1%	1.48 (6.66)	1.48 (6.66)	1.48 (6.66)[b]	91.12
	0.2%	0.0 (2.86)	0.74 (4.76)	0.37 (3.81)[a]	97.78
Chlorothalonil 75 WP	0.05%	4.44 (12.16)	7.41 (15.75)	5.92 (13.96)[d]	64.48
	0.1%	3.70 (10.96)	4.44 (12.16)	4.07 (11.56)[c]	75.58
	0.2%	2.22 (8.56)	1.48 (6.66)	1.85 (7.61)[b]	88.90
Captan 50 WP	0.05%	0.74 (4.76)	1.48 (6.66)	1.11 (5.71)[b]	93.34
	0.1%	0.00 (2.86)	0.0 (2.86)	0.0 (2.86)[a]	100
	0.2%	0.0 (2.86)	0.0 (2.86)	0.0 (2.86)[a]	100
Check I (infested - untreated)		16.29 (23.73)	17.04 (23.24)	16.67 (24.03)[a]	-
Check II (uninfested - untreated)		0.0 (2.86)	0.0 (2.86)	0.0 (2.86)[a]	-
S.E±		**(1.05)**	**(1.26)**	**(0.94)**	
CD(p=0.05)		**(2.14)**	**(2.57)**	**(1.89)**	

Means of three replications; figures in parentheses are angular transformed values;
means followed by similar letter(s) are statistically identical.

Table- 5: Effect of non-systemic fungicides incorporated in *M. perniciosa* infested casing on the number and weight of fruit bodies and button yield during spring 2009-2010 (pooled over years)

Fungicide		No. of fruit bodies per kg mushroom	Weight of fruit bodies (g)	Button yield (kgs/q compost)
P. Manganese 50 WP	0.05%	95.83^{f}	10.38^{e}	8.89^{d}
	0.1%	94.00^{de}	10.60^{d}	10.59^{c}
	0.2%	91.50^{b}	10.86^{a}	14.25^{a}
Chlorothalonil 75 WP	0.05%	96.00^{f}	10.30^{e}	8.51^{d}
	0.1%	94.50^{e}	10.59^{d}	10.90^{c}
	0.2%	92.66^{bc}	10.77^{b}	13.48^{b}
Captan 50 WP	0.05%	94.33^{e}	10.62^{cd}	13.47^{b}
	0.1%	92.50^{b}	10.69^{bc}	14.23^{a}
	0.2%	90.16^{a}	10.89^{a}	14.83^{a}
Control I (infested - untreated)		94.16^{e}	10.68^{c}	6.18^{e}
Control II (uninfested - untreated)		92.83^{cd}	10.93^{a}	12.84^{b}
SE±		**(0.62)**	**(0.04)**	**(0.50)**
CD (p=0.05)		**(1.25)**	**(0.08)**	**(1.01)**

Means of three replications; means followed by similar letter(s) are statistically identical.

Table: 6 Effect of non-systemic fungicides incorporated in *M. perniciosa* infested casing on quality parameters of white button mushroom *(Agaricus bisporus)* during spring 2009-2010 (pooled over years)

Fungicide		Weight of pileus (g)	Diameter of pileus (cm)	Stipe Weight (g)	Stipe Diameter (cm)
P. manganese 50 WP	0.05%	5.62^{c}	3.29^{e}	4.57^{e}	1.26^{a}
	0.1%	5.79^{b}	3.37^{d}	4.62^{d}	1.29^{a}
	0.2%	6.02^{ab}	3.50^{b}	4.70^{c}	1.31^{a}
Chlorothalonil 75 WP	0.05%	5.26^{e}	3.37^{d}	4.57^{e}	1.23^{a}
	0.1%	5.70^{cd}	3.44^{c}	4.64^{d}	1.25^{a}
	0.2%	5.58^{bc}	3.51^{b}	4.77^{b}	1.25^{a}
Captan 50 WP	0.05%	5.63^{c}	3.45^{bc}	4.61^{de}	1.19^{a}
	0.1%	5.71^{b}	3.50^{b}	4.68^{c}	1.21^{a}
	0.2%	6.01^{a}	3.54^{a}	4.80^{a}	1.24^{a}
Control I (infested - untreated)		5.32^{be}	3.29^{e}	4.29^{f}	1.23^{a}
Control II (uninfested - untreated)		6.26^{a}	3.56^{a}	4.26^{e}	1.25^{a}
SE±		**(0.16)**	**(0.02)**	**(0.02)**	**(0.03)**
CD (p=0.05)		**(0.31)**	**(0.05)**	**(0.05)**	**(0.07)**

Means of three replications; means followed by similar letter(s) are statistically identical

Table-7: ***In vitro*** **evaluation of different systemic fungicide against** ***Mycogone perniciosa*****, the causal agent of wet bubble disease of white button mushroom (*Agaricus bisporus*)**

Conc. (µg ml^{-1})	Growth inhibition over control (%)					Mean
Fungicide	**5**	**10**	**25**	**50**	**100**	
Carbendazim 50 WP	93.70 (75.09)	95.80 (77.53)	98.13 (80.63)	99.16 (84.76)	100.00 (90.00)	**97.35 (81.60)a**
Myclobutanil 10 WP	84.61 (66.42)	85.31 (67.39)	98.60 (82.040	100.00 (90.00)	100.00 (90.00)	**93.70 (79.17)b**
Bitertanol 25 WP	83.94 (60.90)	87.41 (68.87)	94.40 (75.84)	99.53 (86.17)	100.00 (90.00)	**93.05 (65.17)e**
Triademiphon	68.76 (55.54)	75.05 (59.56)	81.81 (64.39)	87.41 (68.87)	95.80 (77.53)	**81.76 (65.17)e**
Diniconazole	75.98 (60.22)	82.27 (64.89)	89.74 (70.76)	97.43 (99.50)	99.30 (85.37)	**88.94 (72.18)d**
Mean	**81.39 (63.63) ^{E}**	**85.16 (67.64) ^{D}**	**92.53 (74.77) ^{C}**	**96.70 (81.86) ^{B}**	**99.02 (86.58) ^{A}**	

	S.E±	**CD(p=0.05)**
Fungicide	(0.69)	(1.15)
Concentration	(0.69)	(1.15)
Fungicide x concentration	(1.55)	(2.59)

Means of three replications; figures in parentheses are angular transformed values;
means followed by similar letter(s) are statistically identical.

Table-8: ***In vitro*** **evaluation of systemic fungicide against** ***Agaricus bisporus*** **mycelium.**

Conc. (µg ml^{-1})	Growth inhibition over control (%)					
Fungicide	**5**	**10**	**25**	**50**	**100**	**Mean**
Carbendazim 50 WP	0.0 (2.86)	0.0 (2.86)	0.0 (2.86)	2.60 (9.27)	4.68 (12.38)	**1.45 (6.91)^{a}**
Myclobutanil 10 WP	2.60 (9.27)	3.64 (10.73)	7.29 (15.65)	12.49 (20.69)	22.91 (28.59)	**9.78 (16.98)^{c}**
Bitertanol 25 WP	0.0 (2.86)	2.60 (9.27)	4.68 (12.38)	11.45 (19.74)	16.67 (24.00)	**7.08 (13.63)^{b}**
Triademiphon	22.91 (28.55)	36.97 (37.40)	55.72 (48.30)	78.12 (62.08)	88.02 (69.75)	**56.34 (49.21)^{d}**
Diniconazole	22.91 (28.55)	41.14 (39.89)	54.16 (47.39)	72.39 (58.30)	92.18 (73.81)	**56.55 (49.58)^{d}**
Mean	**9.68 (14.41)^{E}**	**16.87 (20.03)^{D}**	**24.37 (25.31)^{C}**	**35.41 (34.01)^{B}**	**44.89 (41.70)^{A}**	
		S.E±			**CD(p=0.05)**	
Fungicide		(0.80)			(1.62)	
Concentration		(0.80)			(1.62)	
Fungicide x concentration		(1.61)			(3.24)	

Means of three replications; figures in parentheses are angular transformed values;
means followed by similar letter(s) are statistically identical.

International Journal of Advance Research in Science and Engineering
Volume No.07, Special Issue No.04, March 2018
www.ijarse.com
IJARSE
ISSN: 2319-8354

Table- 9 Effect of systemic fungitoxicants incorporated in casing on per cent intensity of wet bubble disease (*Mycogone perniciosa*) of white button mushroom (*Agaricus bisporus*) during spring 2009 and 2010

Treatments		Spring 2009	Spring 2010	Mean	Diseases control (%)
Carbendazim 50 WP	0.025%	6.67 (14.96)	5.92 (14.02)	6.92 (14.49)^{de}	62..26
	0.05%	5.18 (13.09)	2.22 (8.56)	3.70 (10.82)^{c}	77.80
	0.1%	1.48 (6.66)	0.74 (4.76)	1.11 (5.71)^{b}	93.34
Myclobutanil 10 WP	0.025%	7.41 (15.75)	7.41 (15.75)	7.41 (15.75)^{a}	55.54
	0.05%	5.92 (14.02)	5.18 (13.09)	5.55 (13.56)^{d}	66.70
	0.1%	2.96 (9.76)	2.22 (7.86)	2.59 (8.81)^{c}	84.46
Bitertanol 25 WP	0.025%	6.67 (14.96)	5.92 (14.02)	6.23 (14.49)^{de}	62.62
	0.05%	4.44 (11.89)	2.96 (9.06)	3.70 (10.48)^{c}	77.80
	0.1%	1.48 (6.66)	1.48 (5.96)	1.48 (6.31)^{b}	91.12
Check I (infested - untreated)		16.29 (23.73)	17.04 (24.34)	16.67 (24.03)^{f}	-
Check II (uninfested - untreated)		0.0 (2.86)	0.0 (2.86)	0.0 (2.86)^{a}	-
S.E±		**(1.17)**	**(1.51)**	**(1.08)**	
CD(p=0.05)		**(2.40)**	**(3.08)**	**(2.16)**	

Means of three replications; figures in parentheses are angular transformed values;
means followed by similar letter(s) are statistically identical.

Table-10: Effect of systemic fungicides incorporated in *M. perniciosa* infested casing on the number and weight of fruit bodies and button yield during spring 2009-2010 (pooled over years)

Fungicide	No. of fruit	Weight of	Button yield

		bodies per kg mushroom	fruit bodies (g)	kgs/q compost
Carbendazim 50 WP	0.025%	96.33^{cd}	10.33^{c}	9.18^{c}
	0.05%	93.50^{bc}	10.57^{bb}	10.25^{b}
	0.1%	88.83^{a}	11.09^{a}	12.29^{a}
Myclobutanil 10 WP	0.025%	95.67^{c}	10.57^{b}	6.43^{e}
	0.05%	93.17^{b}	10.78^{ab}	8.00^{d}
	0.1%	90.00^{a}	11.12^{a}	10.49^{b}
Bitertanol 25 WP	0.025%	97.50^{d}	10.23^{c}	8.25^{cd}
	0.05%	95.50^{c}	10.46^{bc}	10.23^{b}
	0.1%	91.67^{ab}	10.90^{a}	12.97^{a}
Control I (infested -untreated)		96.33^{cd}	10.23^{c}	6.18^{e}
Control II (uninfested -untreated)		92.16^{b}	10.83^{a}	12.84^{a}
SE±		**(1.46)**	**(0.20)**	**(0.47)**
CD (p=0.05)		**(2.93)**	**(0.40)**	**(0.95)**

Means of three replications; means followed by similar letter(s) are statistically identical.

Table-11: Effect of systemic fungicides incorporated in *M. perniciosa*-infested casing on quality parameters of white button mushroom *(Agaricus bisporus*) during spring 2009-2010 (pooled over years)

Fungicide	Weight	Diameter of	Stipe	Stipe

		of pileus (g)	pileus (cm)	Weight (g)	Diameter (cm)
	0.025%	5.69	3.36 [c]	4.66 [c]	1.27
Carbendazim 50 WP	0.05%	5.83	3.29 [c]	4.69 [b]	1.30
	0.1%	6.06	3.63 [b]	4.80 [a]	1.32
	0.025%	5.32	3.38 [c]	4.60 [c]	1.22
Myclobutanil 10 WP	0.05%	5.74	3.47 [b]	4.61 [bc]	1.25
	0.1%	5.97	3.53 [b]	4.72 [ab]	1.26
	0.025%	5.70	3.46 [bc]	4.63 [b]	1.20
Bitertanol 25 WP	0.05%	5.91	3.47 [b]	4.69 [b]	1.23
	0.1%	6.10	3.58 [b]	4.83 [a]	1.26
Control I (infested - untreated))		5.69	3.36 [c]	4.23 [d]	1.24
Control II (uninfested- untreated)		6.20	3.82 [a]	4.59 [c]	1.26
SE±		**(0.48)**	**(0.09)**	**(0.05)**	**(0.00)**
CD (p=0.05)		**(NS)**	**(0.18)**	**(0.11)**	**(NS)**

Means of three replications; means followed by similar letter(s) are statistically identical.