Eco. Env. & Cons. 29 (1) : 2023; pp. (402-411) *Copyright*@ EM International ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2023.v29i01.060

Organization of *Mycogone perniciosa* triggering Wet Bubble Disease (WBD) of White Button Mushroom

Kanika Mahajan¹, Anil Rao², Sunil Kumar^{3*}, Vandana Kumari⁴, Ambrish Kumar Mahajan¹ and Anil Kurmi⁵

 ¹School of Earth & Environmental Sciences, Central University of Himachal Pradesh, Distt.-Kangra 176 206, H.P., India
 ²ICAR-Directorate of Mushroom Research-DMR, Solan 173 212, H.P., India
 ³School of Life Sciences, Central University of Himachal Pradesh, Distt.- Kangra 176 206, H.P., India
 ⁴Department of Biosciences, Himachal Pradesh University, Summer Hill, Shimla 171 005, H.P., India
 ⁵Krishi Vigyan Kendra, Indira Gandhi National Tribal University, Anuppur 484 887, M.P., India

(Received 17 August, 2022; Accepted 30 October, 2022)

ABSTRACT

The most extensively farmed mushroom in the world is Agaricus bisporus (Lange) Imbach. WBD, which is predominantly instigated by *Mycogone perniciosa*, might pose a severe danger to A. bisporus output over the world. Because of the similarity between A. bisporus and M. perniciosa, it was predicted that the current study would choose reliable antimycotic agents that could favorably treat this fungal disease on mushrooms. The antimycotic susceptibility of host and pathogen was investigated *in vitro* using six different fungicides. The effects of chlorothalonil, carbendazim, thiophanate-methyl, azoxystrobin, difenoconazole, and kresoximmethyl on *M. perniciosa*, the mycoparasite that origins white button mushroom wet bubble disease, were evaluated in vitro and in vivo. Chlorothalonil and carbendazim were the potent antimycotic agents for reducing M. perniciosa mycelial growth in vitro, with inhibitions of 96.93 percent and 94.15 percent, respectively. Chlorothalonil inhibited the pathogen's mycelial growth at 25-500 ppm, whereas carbendazim did so at 5 to 100 ppm, with least (16.67 percent) inhibition of A. bisporus mycelium. Difenoconazole, kresoximmethyl and azoxystrobin among other fungicides, were shown to be very repressive to the pathogen (91.81 percent, 83.26 percent and 71.05 percent) with the largest percentage of inhibition (87.77 percent, 84.44 percent and 75.55 percent) of A. bisporus mycelium. Chlorothalonil and carbendazim and thiophanatemethyl continued to handle WBD in field experiments with a smaller impact on mushrooms than other fungicides.

Key words : Cultivation, Mushroom, Efficacy, Fungicides, Pathogen.

Introduction

The white button mushroom, *Agaricus bisporus* (Lange) Imbach, is the utmost widely farmed mushroom in the world, belonging to the Agaricaceae family. China is one of the largest producers of *A. bisporus* on the planet (Royse *et al.*, 2017). The white

button mushroom is becoming a more common part of people's diets because of its nutritional worth, medical benefit (antitumor activity and hypocholesterolemic effects), and ecologically acceptable approach (Zhang *et al.*, 2014; Du *et al.*, 2017). *Mycogone perniciosa* (Magnus) Eugene Delacroix, which causes wet bubble disease however, have a significant impact on *A. bisporus* assembly (Tu and Liao, 1989; Sharma and Singh, 2003; Fu *et al.*, 2016). *Mycogone perniciosa*, initially reported in 1888 from Paris and from time to time, the disease has been reported to spread to other important mushroom-growing countries such as the United States, United Kingdom, the Netherlands, the, China, Brazil, Australia, Africa, Hungary, Taiwan and Europe. This pathogen was first discovered in India in the year of 1978 in mushroom fields in Jammu and Kashmir (Kaul *et al.*, 1978). Himachal Pradesh and Haryana were later reported to have contracted the disease. Several workers exhibit the symptoms of wet bubble at various phases of mushroom cultivation.

Artificial vaccination of M. perniciosa has resulted in yield losses of up to 100%, according to Bhatt and Singh (2000). Because *M. perniciosa* causes a eclectic array of symptom severity during infection, the ailment is normally managed by cultural practices and the use of fungicides (Kaul et al., 1978). Because both the host and the infective agent are fungi, management of this fungus disease of A. bisporus is complicated. As a result, it's critical to choose fungicides that have the fewest effects on mushroom growth. Aimed at the control of many plant diseases, chemical management is an effective strategy (Shi *et al.*, 2020). The usage of various fungicides is a typical disease management approach used on farms all over the world. Several compounds have been rejected for use in Europe as a result of recent chemical evaluations. As a result, disease management with no or few pesticides in the smallest mode is a severe problem for mushroom farmers in the twenty-first century. Antifungal resistance amongst pathogen populations, which is handled by presently accessible pesticides, and the introduction of novel infections are two examples of problems (Grogan, 2008). The effectiveness of fungicides is determined by the frequency with which they are used (Bonnen and Hopkins, 1997), also their perseverance in higher concentrations in mushroom covering soil (Grogan and Jukes, 2003). When fungicides from the category of methyl benzimidazole carbamates (MBC) were developed in 1960s, they provided exceptional treatment of a variety of fungal infections of cultivated mushrooms (Delp, 1987; McKay et al., 1998; Grogan and Gaze, 2000; Potonik et al., 2007, 2008). Carbendazim and Thiophenate methyl fungicides belong to MBC (Methyle Benzimidazole Carbamate) group and their target site is β -tubulin assembly in mitosis. Carbendazim was likewise revealed to be the utmost efficient fungicide for reducing M. perniciosa mycelial evolution, according to Gea et al., (2010). However, resistant pathogen strains emerged after many years of extensive usage (Bollen and van Zaaven, 1975; Bonnen and Hopkins, 1997; Potonik et al., 2008b). Many fungal infections on mushrooms are effectively inhibited by imidazole demethylation inhibitors (DMIs), by preventing the demethylation phase in sterol biosythesis, a vital molecule accountable for the permanency and function of mushrooms (Hamamoto et al., 2000). Strobilurin are the fungicides isolated from the mushrooms (Basidiomycetes) (Anke *et al.*, 1977) are also known as QUI fungicidesinhibit the mitochondrial respiration by blocking the electrons transfer at cytochrome, they include kresoxim- methyl, Azoxystrobin, pyraclostrobin, trifloxystrobin and fluoxastrobin (Isamu and Makoto, 2005; Rodrigues et al., 2013; Khandelwal et al., 2014; Zubrod et al., 2019).

Pesticide biodegradation is a remarkable environmental feature because it prevents hazardous compounds from accumulating in the atmosphere, however there must be a balance between the chemical

Common name	Trade name	Chemical name
Chlorothalonil	Kavach	2,4,5,6-tetrachlorobenzene-1,3-dicarbonitrile
Carbendazim	Bavistin	methyl N-(1H-benzimidazol-2-yl)carbamate
Thiophanate Methyl	Ditto	Dimethyl N,N2-[1,2-phenylenebis
		(azanediylcarbonothioyl)] dicarbamate
Azoxystrobin	Amistar	Methyl (2E)-2-(2-{[6-(2-cyanophenoxy)pyrimidin-4-yl]oxy}phenyl)-3- methoxyprop-2-enoate
Difenoconazole	Score	1-[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-
		2-yl]methyl]-1,2,4-triazole
Kresoxim -methyl	Ergon	methyl (E)-methoxyimino[á-(o-tolyloxy)-o-tolyl]acetate

Table 1. Technical details of fungicides used under the study

treatment against its target microorganism and the time it takes for it to break down into harmless components at the end (Grogan, 2008). The concentration of thiabendazole in covering soil persisted high through the harvesting period, but carbendazim and prochloraz manganese concentrations decreased dramatically in the end of the subsequent flush (Grogan and Jukes, 2003; Papadopoulos, 2006). Carbendazim is identified to be vulnerable to microorganism squalor in soils (Fletcher et al., 1980; Yarden et al., 1990), which might lead to a reduction in pathogen control in zones wherever the antifungal is still effective. These of fungicides in efficacy tests on cultivated mushrooms is quite unusual. Highly specific fungicides are essential for the chemical management of fungal infections so as to prevent infection without hindering the growth of A. bisporus. Due to the few studies and stringent registration procedures, commercial fungicides certified for mushroom growing are not widely available (White head, 2002; Stoddart et al., 2004). The objective of this research was to find fungicides that could help with WBD control. Table 1 shows technical details of fungicides used in this study. As a result, we evaluated the sensitivity of six fungicides in vitro and in vivo against M. perniciosa and A. bisporus.

Material and Methods

The extant study was steered at the Central University of Himachal Pradesh in the department of environment science to check the standing of wet bubble disease in white button mushrooms.

In vitro testing of fungitoxicants

Using the Nene and Thapliyal poisoned food technique (2000), six fungitoxicants were inspected in vitro against to M. perniciosa and A. bisporus mycelium. The systemic fungitoxicant was tested at concentrations of 5, 10, 25, 50 and 100 ppm whereas the non-systemic fungitoxicant was tested at concentrations of 25, 50, 100, 200, and 500 ppm. A 25 ml double strength test fungitoxicant concentration was aseptically added to double strength sterilised PDA in a 150 ml Erlenmeyer flask. After being thoroughly but gently shaken, the contents were aseptically placed on pre-sterilized Petri plates. Mycogone perniciosa and A. bisporus culture mycelial discs were retained aseptically in the Petri plate and incubated at 23±2°C for 7 and 14 days, respectively. Three times each experiment was repeated. As controls, M. *perniciosa* and *A. bisporus* were permitted to breed disjointedly in Petri plates. After the test pathogen had nearly filled the Petri plate, data on radial mycelial development was collected. Vincent's approach (1947) was used to determine mycelium growth inhibition as a measure of fungicidal activity.

Percent mycelial growth inhibition =
$$\frac{(C-T)}{C} \times 100$$

Where C = Radial mycelial growth (mm) under control

T = Radial mycelial growth (mm) within the treatment

In vivo evaluation of fungitoxicants

During the current study, fungicides that had good results in vitro and showing minimum inhibition to host mushroom mycelium were applied in vivo as well. Chlorothalonil was evaluated at doses of 0.05, 0.1, and 0.2 percent. The systemic fungicides carbendazim and thiophanate-methyl were tested at 0.025, 0.05, and 0.1 percent. Before inoculating the pathogen, apiece fungicidal concentration was combined with exterior mixture at the rate of 100 ml/kg casing mixture. 1 x 108 spores ml⁻¹ solution obtained from a 10-days old culture, put to covering soil at the degree of 20 ml/bag of 30 cm diameter, apiece treatment was replicated three times. Experiment was run with inoculated and uninoculated control without any fungicidal treatment. The experiment was conducted without the use of any software. There were three mushroom flushes that were looked into. For each treatment, the number of healthy and sick mushrooms as well as the overall weightiness of strong fruiting bodies was noted. Mushroom fruiting times, hues, and shapes were examined and noted. For each treatment, the yield of healthy mushrooms was estimated. Founded on the proportion of the quantity of infected sporophores to the entire number of cropped mushrooms, disease prevalence was determined as a fraction. Each fungicide's efficacy to control disease, was evaluated according to the following formula (Gea et al., 2010).

Statistical analysis

Statistical investigation was accomplished on the

MAHAJAN ET AL

acquired data. Bestowing to the procedures recommended by Gomez and Gomez, the alterations shown by the behaviors in several studies were evaluated for their implication (1984).

Results

In vitro evaluation of Fungitoxicants

The inhibitory effects of fungicides, namely chlorothalonil, carbendazim and thiophanate-methyl, difenoconazole, kresoxim-methyl and azoxystrobin on the *Mycogone perniciosa* and *Agaricus bisporus* mycelium, were assessed using the poisoned food technique at 5, 10, 25, 50, 100, 200, and 500 ppm concentrations.

Effect on pathogen's mycelial development

Chlorothalonil was the most effective, inhibiting growth by 96.93 percent, followed by carbendazim, which inhibited growth by 94.15 percent and azoxystrobin was the least effective fungicide against the test pathogen (75.55 percent) (Table 2).

The results (Fig.1) also demonstrated that as the fungicide concentration was increased, the myce-

lium growth inhibition of the pathogen increased as well, chlorothalonil caused overall inhibition of 97.84 percent, attained at the highest dose of 500 ppm, whereas a fungicide dose of 25 ppm inhibited total growth by 73.72 percent. Carbendazim was the second-best fungicide, inhibiting growth by 94.15 percent followed by difenoconazole (93.81 percent), thiophanate methyl (91.66 percent), kresoxim-methyl (83.26 percent) and azoxystrobin (71.05 percent).

Effect on the host's mycelial development

The results (Table 3) demonstrated that the inhibitory possessions of the test fungicides on the host varied significantly. In comparison, carbendazim inhibited *A. bisporus's* development by the least amount (16.67 percent, followed by thiophanate methyl and chlorothalonil (23.26-32.36 percent), while difenoconazole was the least effective, greatest growth inhibition was 87.77 percent.

The percent growth inhibition increased with increasing fungicide concentration, with the lowest (2.88 percent) inhibition obtained at 25 ppm and the highest (32.36 percent inhibition obtained at 500



Fig. 1. *In vitro* evaluation of fungitoxicants against pathogen *Mycogone perniciosa:* (a) Chlorothalonil fungitoxicants against *Mycogone perniciosa* (b) Cabendazim fungitoxicants against *Mycogone perniciosa* (c) Thiophanate methyl fungitoxicants against *Mycogone perniciosa* (d,f) Azoxystrobin, Difenoconazole, Kresoxim-methyl.

Fungicide	% growth	% growth inhibition over control at diverse concentrations (μg ml ⁻¹)				
0	5 (25)*	10 (50) *	25 (100) *	50 (200) *	100 (500) *	
Chlorothalonil	73.72	79.14	86.12	91.74	96.93	85.53
	(59.19)**	(62.82)	(68.13)	(73.35)	(80.01)	(68.70)
Carbendazim	67.76	70.13	75.27	85.13	94.15	78.49
	(55.39)	(56.87)	(60.15)	(67.40)	(76.14)	(63.19)
Thiophanate methyl	57.37	63.96	71.37	81.65	91.66	73.20
	(49.22)	(53.08)	(57.79)	(64.61)	(73.20)	(59.55)
Difenoconazole	32.76	71.62	72.83	87.63	91.81	71.46
	(34.90)	(57.79)	(58.56)	(69.39)	(74.06)	(58.94)
Kresoxim-methyl	54.98	51.54	64.99	81.85	83.26	67.32
,	(47.84)	(45.86)	(53.70)	(64.76)	(65.83)	(55.60)
Azoxystrobin	52.41	61.76	67.25	59.28	71.05	62.35
	46.36	(51.81)	(55.07)	(50.33)	(57.43)	(52.20)
	Mean	56.50	66.36	72.97	81.21	88.25
		(48.82)	(54.70)	(58.87)	(64.97)	(71.11)
	CD	0.05	S.	S.E(d)		m)
Fungicide	1.0	1.07		0.53		7
Concentration	0.9	0.98		0.48		4
Fungicide x Concentration	n 2.4	2.40		1.19		4

 Table 2. In vitro assessment of diverse fungitoxicants w.r.t inhibitory effects on the evolution of Mycogone perniciosa, the cause of wet bubble disease

*Concentrations of chlorothalonil

** Figures are angular transformed values

Table 3.	In vitro assessment of d	lifferent fungtoxicants v	v.r.t inhibitory e	effects on the g	rowth of Agaricu	s bisporus myce-
	lium					

Fungicide	% gro	% growth inhibition over control at diverse concentrations (μg ml ⁻¹)					
0	5 (25)*	10 (50)*	25 (100)*	50(200)*	100(500)*		
Chlorothalonil	2.88	5.49	15.40	28.30	32.36	16.89	
	(9.73)**	(13.47)	(23.07)	(32.04)	(34.60)	(22.58)	
Carbendazim	3.13	7.63	10.98	14.07	16.67	10.49	
	(10.15)	(16.02)	(19.30)	(21.99)	(24.02)	(18.30)	
Thiophanate methyl	3.74	6.51	18.58	18.65	23.26	14.15	
1 2	(11.07)	(14.76)	(25.49)	(25.54)	(28.82)	(21.13)	
Difenoconazole	66.66	75.55	77.77	83.33	87.77	78.21	
	(54.78)	(60.39)	(61.94)	(65.94)	(69.81)	(62.57)	
Kresoxim- methyl	66.66	63.33	74.42	78.88	84.44	73.54	
, ,	(54.72)	(52.83)	(59.72)	(62.72)	(67.05)	(59.41)	
Azoxystrobin	47.77	53.99	63.33	68.88	75.55	61.90	
5	(43.65)	(47.32)	(52.85)	(56.17)	(60.50)	(52.10)	
Mean	31.81	35.42	43.41	48.68	53.34		
	(30.68)	(34.13)	(40.40)	(44.07)	(47.47)		
	CD _{0.05}		S.E(d)		S.E(m)		
Fungicide	2.8	2.84		1.41		1.00	
Concentration	2.5	59	1.	1.29		0.91	
Fungicide x Concentrat	ion N,	/S	3.	3.17		2.24	

*Concentrations of chlorothalonil

** Figures are angular transformed values

MAHAJAN ET AL

ppm in chlorothalonil. The least inhibiting fungicide concentrations were carbendazim and thiophanate methyl at 5 ppm followed by chlorothalonil at 25ppm. At 100 ppm, difenoconazole and kresoximmethyl inhibited the test fungal mycelium the most (87.77-84.44 percent) (Fig. 2).

In vivo assessment of fungitoxicants

Table 4 represents the outcome of fungitoxicants merged in exterior on % incidence of wet bubble disease of white button mushroom (*Agaricus bisporus*). The data discovered that entirely the fungicidal treatments abridged the % disease strength as equated to inoculated unprocessed check-I. Associated to a wet bubble strength of 31.55 % attained in inoculated crude check-I, the disease was abridged to 3.49 per cent by application of chlorothalonil at 0.2% unveiling the disease control of 88.93 %. Carbendazim and Thiophanate methyl at 0.1% were the subsequent best actions revealing wet bubble intensity of 7.83-8.04 percent with a disease control of 75.08-74.51 percent.

The application of thiophanate methyl at 0.1 percent concentration yielded the less fruit-bodies per kg mushroom (79.50), trailed by carbendazim and chlorothalonil yielding 80.13-82.66 fruit-bodies per kg mushroom (Table 5), With different fungicidal treatments, the average fruit-body weight also varied greatly. Chlorothalonil and carbendazim at 0.2 percent and 0.1 percent concentration each showed maximum (14.24-14.14 g) average, compare to that of an inoculated and uninoculated control (10.62-12.51 g). Chlorothalonil and carbendazim (0.2 percent and 0.1 percent) produced the highest button yields of 17.40-15.61 kg/quintal compost, compared to 16.6 6kg/quintal compost in the uninoculated control. The least efficient fungicides were thiophanate methyl (0.1 percent) with average yields of (14.78) kg / quintal compost.

Effect of quality parameters of sporophores

The characteristics of sporophores were also considerably impacted by fungicidal sprays on infested casing (Table 6). The pileus weight of 4.9 g in the inoculated control was observed to improve remarkably to 7.78 g-6.86g in the chlorothalonil and carbendazim treatments (0.2 percent and 0.1 percent). In the uninoculated control, the pileus weight improved remarkably to 6.9 g. The next best treatments were thiophanate-methyl, which provided a



Fig. 2. *In vitro* evaluation of fungitoxicants against host *Agaricus bisporus: (a)* Chlorothalonil fungitoxicants against *Agaricus bisporus* (b) Cabendazim fungitoxicants against *Agaricus bisporus* (c) Thiophanate methyl fungitoxicants against *Agaricus bisporus* (d,f) Azoxystrobin, Difenoconazole, Kresoxim-methyl.

pileus weight of 6.02 g. Among the different fungicidal treatments, chlorothalonil (0.20 percent) had the largest pileus diameter (3.60 cm), which was comparable to the uninoculated control (3.57cm). The next best treatments were carbendazim and thiophanate-methyle (0.1 percent), which provided pileus dia of 3.77-3.44cm. In the carbendazim fungicide (0.1 percent), the stipe weight was highest (7.15 g). The next best treatments were chlorothalonil and thiophanate-methyl (0.20 and 0.1 percent percent), which provided stipe weights of 6.59-6.07g. In the inoculated control, the average stipe weight was 5.2 g, compared to 6.23 g in uninoculated control. The treatments receiving carbendazim and chlorothalonil had the largest stipe diameter of 1.51-1.32cm, compared to 1.16 cm in the inoculated control, compared to 1.33 cm in uninoculated control.

Discussion

Chlorothalonil, carbendazim and thiophanate methyl exhibited the greatest *in vitro* inhibition against *M. perniciosa* and the tiniest against *A. bisporus*. At 100 ppm, carbendazim, thiophenate-methyl, and difenoconazole reduced the pathogen mycelium at large extent, but at 200 ppm, chlorothalonil did the

Table 4. Outcome of fungitoxicants unified in covering on % incidence of wet bubble disease of white button mushroom (*Agaricus bisporus*)

Fungicide	Concentration (µg ml ⁻¹)	Disease Incidence	Disease Control (%)
Chlorothalonil	0.05%	7.27 (2.86)	76.95
	0.1%	5.47 (2.53)	82.66
	0.2%	3.49 (2.10)	88.93
Carbendazim	0.025%	11.46 (3.38)	63.67
	0.05%	10.93 (3.45)	65.35
	0.1%	7.83 (2.97)	75.08
Thiophanate methyl	0.025%	12.93 (3.73)	59.01
1 2	0.05%	10.62 (3.40)	66.33
	0.1%	8.04 (3.00)	74.51
Check I (inoculated - untreated)		31.55	
Check II (unioculated - untreated)	0.00	
S.E±	0.122	0.122	
CD _{0.05}	0.28	0.28	

Table 5. Effect of three selected fungitoxicants on *Mycogone perniciosa* and agronomical traits of white button mushroom (*Agaricus bisporus*) under mushroom house conditions

Fungicide	Concentration	No. of fruit bodies per kg of mushroom	Weight of fruit bodies (g)	Button yield kgs/q compost
Chlorothalonil	0.05%	83 (9.16)	12.77 (3.71)	11.65 (3.55)
	0.1%	83.83(9.21)	12.99 (3.74)	14.41 (3.92)
	0.2 %	82.66 (9.14)	14.24 (3.90)	17.40 (4.28)
Carbendazim	0.025%	81.50 (9.08)	11.63 (3.55)	11.50 (3.53)
	0.05%	81.66 (9.09)	12.84 (3.72)	13.40 (3.79)
	0.1 %	80.13 (9.01)	14.14 (3.89)	15.61 (4.07)
Thiophanyte methyl	0.025%	81.83 (9.10)	11.23 (3.49)	9.76 (3.28)
1 2 2	0.05%	80.50 (9.02)	11.74 (3.56)	11.19 (3.50)
	0.1 %	79.50 (8.97)	12.74 (3.70)	14.78 (3.92)
Inoculated control		81.33	10.62	5.8
Un-inoculated control	82.66	12.51	16.66	
CD _{0.05}		0.28	0.08	0.07
S.E(d)		0.12	0.04	0.03
S.E(m)		0.08	0.02	0.02

same. At 25 ppm carbendazim had 16.67 percent maximum repressive outcome on the evolution of A. bisporus mycelium, however chlorothalonil and thiophanate methyl inhibited the 32.36-23.26 percent growth of host. Carbendazim, a systemic fungicide, was shown to be extremely fungitoxic, reducing the pathogen's mycelial growth at various test dosages. However, Chalaux et al., (1993) discovered that carbendazim was hazardous to A. bisporus in the past. Agaricus bisporus strain X22 was found by Chrysayi-Tokousbalides et al., (2007) to have a poor sensitivity to carbendazim. Potnik et al., 2010 and Gea et al., 2010 reported that fungicide group MBC was quite operative to control M. perniciosa in mushroom industry although they don't improve the any efficiencies of A. bisporus. It was observed that carbendazim has slightly reduced the efficiency of pathogen inhibition as compare to chlorothalonil and A. bisporus strain NBS5 was susceptible to the fungicide carbendazim and has shown minimum inhibition among other fungicides. Findings of Fletcher et al., (1980) indicated the same thing during the advance stage of crop cycle there is small decline in the efficacy of carbendazim and this finding is also supported by Grogan and Jukes (2003). Chlorothalonil was reported to cause toxicological issues in mushroom mycelial development at different concentrations (Challen and Elliott, 1985; Fletcher, 2002; Beyer and Kremser, 2004). However, at low doses Chalaux et al., (1993) found no evidence of toxicity of that fungicide to some selected strains

of A. bisporus which is consistent with our results. France, Poland, and Spain have also allowed the usage of chlorothalonil. American law authorizes the usage of methyl benzimidazole carbamate (MBC) fungicide and chlorothalonil formulations in mushroom harvests. Chlorothalonil and carbendazim were the efficient fungicides against the pathogen Mycogone perniciosa and gave maximum growth inhibition as comparison to other fungicides but chlorothalonil has shown much growth inhibition against host A. bisporus compare to the carbendazim, this finding is in favor with the finding of (Fletcher, 2002; Beyer and Kremser, 2004) who reported the carbendazim was the best and suitable fungicide in fungicide sensitivity from A. bisporus with the slightly reduction in the crop yield (Table 3).

A momentous interaction existed amongst fungicides and their concentrations. Difenoconazole, Kresoxim methyl and azoxystrobin were the other fungitoxicants that had shown good efficacy against the mycelium growth of pathogen. 91.81 percent efficacy was observed from difenoconazole fungicide but it has shown highest toxicity against the host mycelium (87.77) percent followed by the kresoximmethyl has shown 84.44 percent inhibition at 100 ppm. Triazole group of fungicides has shown significant inhibition against a variety of pathogens in mushroom crop described by several investigators (Chrysayi-Tokousbalides *et al.*, 2007; Shi *et al.*, 2020) which is conflicting with our results of

	ē				
Fungicide	Concentration	Weight of pileus (g)	Diameter of pileus (cm)	Stipe Weight (g)	Stipe Diameter (cm)
Chlorothalonil	0.05%	5.82 (2.61)	3.10 (2.02)	6.18 (2.67)	1.58 (1.60)
	0.1%	6.60 (2.75)	3.44 (2.10)	6.53 (2.74)	1.27 (1.50)
	0.2%	7.78 (2.77)	3.60 (2.14)	6.59 (2.75)	1.32 (1.52)
Carbendazim	0.025%	5.21 (2.49)	3.33 (2.08)	6.05 (2.65)	0.91 (1.38)
	0.05%	5.97 (2.64)	3.55 (2.13)	6.59 (2.75)	1.13 (1.46)
	0.1%	6.86 (2.79)	3.77 (2.18)	7.15 (2.85)	1.51 (1.58)
Thiophanyte methyl	0.025%	5.12 (2.47)	3.24 (2.06)	5.80 (2.60)	0.85 (1.36)
	0.05%	5.34 (2.51)	3.34 (2.08)	6.76 (2.78)	1.04 (1.42)
	0.1%	6.02 (2.65)	3.44 (2.10)	6.07 (2.65)	1.22 (1.49)
Inoculated Control	4.9	2.69	5.2	1.16	
Uninoculated Control	6.9	3.57	6.23	1.33	
CD _{0.05}		0.05	0.02	0.02	0.07
S.E (d)		0.02	0.01	0.01	0.03
S.E (m)		0.01	0.00	0.00	0.02

Table 6. The effect of fungitoxicants introduced in Mycogone perniciosa verminous casing on quality parameters of white button mushroom (Agaricus bisporus).

difenoconazole, showing good inhibition against pathogen but very toxic to host A. bisporus. Many researchers reported the unsatisfactory efficacy of Strobillurin group of fungicide in terms of their interference with the A. bisporus growth (Potocnik et al., 2009; Chrysayi-Tokousbalides et al., 2007). QUI fungicide Azoxystrobin and Kresoxim methyl have showed significant efficacy against M. perniciosa at low doses but also inhibited the growth of A. bisporus at high extent so they were not use in vivo studies since they failed to inhibit the development of the M. perniciosa isolates without affecting the host growth (Table 3). This finding supported bymany researchers reported the unsatisfactory efficacy of Strobillurin group of fungicide in terms of their interference with the A. bisporus growth (Potocnik et al., 2009; Chrysayi-Tokousbalides et al., 2007). Three out of six tested fungitoxicants carbendazim, chlorothalonil and thiophanate methyl may be recommended effective against *M*. perniciosa. Chlorothalonil has shown maximum inhibition and azoxystrobin has the least inhibition against pathogen M. perniciosa. The best fungicide was the carbendazim have given better disease control without impairing vegetative development and fructification of A. bisporus.

References

- Anke, T., Oberwinkler, F., Steglich, W. and Schramm, G. 1977. The strobilurins-new antifungal antibiotics from the basidiomycete *Strobilurus tenacellus* (Pers.exFr.) Sing. *J. Antibiot.* 30: 806–810.
- Bhatt Nand and Singh, R.P. 2000. Chemical and biological management of major fungal pathogens *Agaricus bisporus*Lange) Imbach. *Mush. Sci.* XV : 587-593.
- Bonnen, A.M. and Hopkins, C. 1997. Fungicide resistance and population variation in *Verticillium fungicola*, a pathogen of the button mushroom. *Agaricus bisporus*. *Mycological Research*. 101: 89-96.
- Bollen, G.J. and van Zaayen, A. 1975. Resistance to benzimidazole fungicides in pathogenic strains of *Verticillium fungicola*. Netherlands Journal of Plant Pathology. 81: 157-167.
- Beyer, D.M. and Kremser, J.J. 2004. Evaluation of fungicide tolerance and control for three fungal diseases of mushrooms. In: Romaine, C.P., Keil, C.B., Rinker, D.L., Royse, D.J. (Eds), Science and Cultivation Fedible and Medicinal Fungi. Mushroom Science XVI. (pp521-529). Pennsylvania, PA, USA : Penn State University, University Park.
- Chalaux, N., Savoie, J.M. and Olivier, M. 1993. Growth inhibition of *Agaricus bisporus* and associated ther-

mophilic species by fungicides used in wheat cultivation. *Agronomie*. 13: 407-412.

- Challen, M.P. and Elliott, T.J. 1985. The *in vitro* responses to a range of fungicides of two strains of the mushroom *Agaricus bisporus* and the pathogen *Verticillium fungicola*. *Mycopath*. 90: 161-164.
- Chrysayi Tokousbalides, M., Kastanias, M.A., Philippoussis, A. and Diamantopoulou, P. 2007. Selective fungi toxicity of famoxadone, tebuconazole and trifloxystrobin between *Verticillium fungicola* and *Agaricus bisporus*. *Crop Prot.* 26 : 469–475.
- Delp, C.J. 1987. Benzimidazole and releted fungicides. In: H. Lyr (Ed.), Modern Selective Fungicides: Properties, Applications, Mechanisms of Action (233-244). New York, USA: Longman Scientific and Technical; John Wiley and Sons.
- Du, P. Q., Wu, X., He, H., Zhang, Y., Xu, J., Dong, F. S., Zheng, Y. Q. and Liu, X. 2017. Evaluation of the safe use and dietary risk of beta-cypermethrin, pyriproxyfen, avermectin, diflubenzuron and chlorothalonil in button mushroom. *Scientific Reports.* 7: 8694–86100.
- Gea, F.J., Tello, J.C. and Navarro, M. J. 2010. Efficacy and effects of yield on of different fungicides for control of wet bubble disease of mushroom caused by mycoparasite *Mycogone periniciosa. Crop Protection.* 29: 1021-1025.
- Fletcher, J.T., Connolly, G., Mountfield, E.I. and Jacobs, L. 1980. The disappearance of benomyl from mushroom casing. *Annals of Applied Biology*. 95: 73-82.
- Fletcher, J.T., Hims, M.J. and Hall, R.J. 1983. The control of bubble diseases and cobweb disease of mushrooms with prochloraz. *Plant Pathol.* 32: 123–131.
- Fletcher, J.T. 2002. Cobweb diseas , a new challenge. *Mush-room News*. 50 : 20–23.
- Fu, Y.P., Wang, X.X., Li, D., Liu, Y., Song, B., Zhang, C. L., Wang, Q., Chen, M. Y., Zhang, Z.W. and Li, Y. 2016. Identification of resistance to wet bubble disease and genetic diversity in wild and cultivated strains of *Agaricus bisporus*. *International Journal of Molecular Sciences*. 17 : 1568–1579.
- Gomez, K.A. and Gomez, A.A. 1984. Statistical procedures for agricultural research. John Wiley and Sons. New York, p. 680.
- Grogan, H.M. and Gaze, R.H. 2000. Fungicide resistance among *Cladobotryum* spp.—causal agents of cobweb disease of the ediblemushroom *Agaricus bisporus*. *Mycol. Res.* 104 : 357–364.
- Grogan, H.M. and Jukes, A.A. 2003. Persistence of the fungicides thiabendazole, carbendazim and prochloraz- Mn in mushroom casing soil. *Pest Management Science*. 59 : 1225-1231.
- Grogan, H.M. 2008. Challenges facing mushroom disease control in the 21st century. In Lelley, J.I., Buswell, J.A. (Eds.), Proceeding of the Sixth International Conferenceon Mushroom Biology and Mushroom Prod-

ucts (pp 120- 127). Bonn, Germany: WSMBMP.

- Grube, B.J., Eng, E.T., Kao, Y.C., Kwon, A. and Chen, S. 2001. White button mushroom phytochemicals inhibit aromatase activity and breast cancer cell proliferation. *The Journal of Nutrition*. 131: 3288–3293.
- Hamamoto, H., Hasegawa, K., Lee, Y.J., Makizumi,Y., Akutsu, K. and Hibi, T. 2000. Tandem repeat of a transcriptional enhancerup stream of the sterol14ádemethylasegene (CYP51) in Penicilliumdigitatum. Applied and Environmental Microbiology. 66: 3421– 3426.
- Isamu, Y. and Makoto, F. 2005. Recent topic sonaction mechanisms of fungicides. J. Pestic. Sci. 30: 67–74.
- Jeong, S.C., Jeong, Y.T., Yang, B.K., Islam, R., Koyyalamudi, S.R., Pang, G., Cho, K.Y. and Song, C.H. 2010. White button mushroom (*Agaricus bisporus*) lowers blood glucoseand cholesterol levels in diabetic and hypercholesterolemic rats. Nutrition Research. 30: 49–56.
- Kaul, T.N. 1978. Diseases and competitors of mushroom farmsin Kashmir valley. Indian Mush. Sci. 1: 193-203.
- Khandelwal, A., Gupta, S., Gajbhiye, V.T. and Varqhese, E. 2016. Degradation of kresoxim-methyl in water: impact of varying pH, temperature, light and atmospheric CO₂ level. *Bull. Environ. Contam. Toxicol.* 96: 130–136.
- McKay, G. L., Egan, D., Morris, E. and Brown, A.E. 1998. Identification of benzimidazole resistance in *Cladobotryum dendroides* using a PCR-based method. *Mycological Research*. 102 : 671-676.
- Papadopoulos, G. 2006. *The fate of prochloraz in mushroom casin* - PhD thesis, University of Reading, UK.
- Potoènik, I., Milijaševic, S., Rekanovic, E., Todorovic, B. and Stepanoviæ, M. 2008. Sensitivity of Verticillium fungi colavar. fungicola, *Mycogone perniciosa* and *Cladobotryum* spp. to fungicides in Serbia. In: M. Van Greuning (Ed.), Science and cultivation of edible andmedicinal fungi: Mushroom Science XVII, *Proceeding of the17th Congress of the International Society for MushroomScience* (pp 615-627). Cape Town, South Africa: ISMS.
- Potoènik, I., Vukojeviæ, J., Stajiæ, M., Tanoviæ, B. and Todoroviæ, B. 2008b. Fungicide sensitivity of selected Verticillium fungi cola isolates from *Agaricusbis porus* farms. *Archives of Biological Sciences*. 60(1): 151-158.
- Potoènik I., Vukojeviæ, J., Stajiæ, M., Rekanoviæ, E., Stepanovic, M., Milijaševic, S. and Todorovic, B. 2010a. Toxicity of biofungicide Timorex 66 EC to

Cladobotryum dendroides and *Agaricusbisporus*. *Crop Protection*. 29 : 290-294.

- Rodrigues, E. T., Lopes, I. and Pardal, M.A. 2013. Occurrence, fate and effectsof azoxystrobin in aquatic ecosystems: a review. *Environ. Int.* 53: 18–28.
- Royse, D.J., Baars, J. and Tan, Q. 2017. Current Overview of Mushroom Production in the World. In: *Edible and Medicinal Mushrooms: Technology and Applications;* Diego, C.Z., Pardo-Giménez, A., Eds.; John Wiley & Sons Ltd.: Hoboken, NJ, USA, pp. 5–13.
- Sharma, V. P. and Singh, C. 2003. Biology and control of Mycogone Perniciosa Magn. causing wet bubble disease of white button mushroom. *Journal of Mycology* And Plant Pathology (India). 33: 257–264.
- Shi, N.N., Ruan, H.C., Gan, L., Dai, Y.L., Yang, X. J., Du, Y. X. and Chen, F. R. 2020. Evaluating the sensitivities and efficacies of fungicides with different modes of action against *Phomopsis asparagi*. *Plant Disease*. 104: 448–454.
- Stoddart, H., Garthwaite, D.G. and Thomas, M.R. 2004. Pesticide Usage Survey Report 197: Mushroom Crops in Great Britain 2003. Department of Environment, Food and Rural Affairs and Scottish Executive Environment and Rural Affairs Department.
- Szumigaj-Tarnowska, J., Uliňski, Z. and Œlusarski, C. 2012. Assessment of effectiveness of biological agents in the control of fungal diseases of the white button mushrooms. *Progress in Plant Protection/ Postêpy w Ochronie Roælin.* 52(4): 963-968.
- Tu, C. C. and Liao, Y.M. 1989. Major diseases of cultivated mushroom and their control in Taiwan. *Mushroom Science*. 12: 615–626.
- Vincent, J. M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*. 15 (9): 850.
- Whitehead, R. 2002. *The e-UK Pesticide Guide*. British Crop Protection Council CABI Publishing.
- Yarden, O., Salomon, R., Katan., J. and Aharonson, N. 1990. Involvement of fungi and bacteria in enhanced and nonenhanced biodegradation of carbendazim and other benzimidazole compounds in soil. *Canadian Journal of Microbiology*. 36: 15-23.
- Zubrod, J. P., Bundschuh, M., Arts, G., Brühl, C. A., Imfeld, G. and Knäbel, A. 2019. Fungicides: an overlooked pesticide class? *Environ. Sci. Technol.* 53: 3347–3365.
- Zhang, Y., Geng, W., Shen, Y., Wang, Y. and Dai, Y.C. 2014. Edible Mushroom Cultivation for Food Security and Rural Development in China: Bio-Innovation, Technological Dissemination and Marketing. Sustainability. 6: 2961–2973.