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Evaluation of non-conventional chemicals against *Verticillium fungicola* causing dry bubble disease in *Agaricus bisporus* button mushroom

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Abstract
Verticillium fungicola is an important pathogen causing dry bubble disease in button mushroom *Agaricus bisporus*. Present investigations were carried out on both host and pathogen by covering an aspect of management of dry bubble disease causing pathogen. Therefore, non-conventional chemicals such as salicylic acid and jasmonic acid with different concentrations (0.1, 0.2 and 0.4 mM) were evaluated and growth inhibition was 44.96% recorded at concentration 0.4 mM with salicylic acid, followed by jasmonic acid (23.42%) against *V. fungicola*. In *In vivo* both salicylic acid and jasmonic acid resulted in reduction of lesions size on *A. bisporus* to the extent of 69.69% and 39.93%, respectively at concentration of 0.4mM. Similarly, the number of lesions reduction was 81.96% (salicylic acid) and 54.64% (jasmonic acid) at same concentration. In host *A. bisporus* growth inhibition was 16% with salicylic acid and 11% with jasmonic acid at the same concentration.

Keywords: *Verticillium fungicola*, *Agaricus bisporus*, non-conventional chemicals, growth

Introduction
Mushroom is a macro-fungus that may grow above or below the ground with a distinctive fruit body that can easily be seen by naked eyes and easily picked up by hands. Mushroom imparts diversification in any farming system and helps in addressing the problems of quality food, health and environmental sustainability. Mushroom farming is today being practiced in more than one hundred countries. The most fascinating concept of mushroom science is "The cultivation of highly nutritious fruit bodies of excellent taste from waste". Therefore the cultivation of edible mushrooms converts different types of agricultural and household wastes into nutrition rich food and their commercial production is a great source of income in almost every part of the world (Wani *et al*., 2010) [16]. About 1.5 million species of fungus are known (Hawksworth, 1991) [6] and out of these it has been estimated that 14,000 species produce fruiting bodies that are desirable to be considered as mushrooms (Hawksworth, 2001) [7]. About 7,000 species of edible mushrooms are known out of which 200 are experimentally grown and 10 have been produced at the industrial scale (Chang and Miles, 2004) [3].
In India, mostly four species of edible mushrooms *viz*., *Agaricus bisporus* (white button mushroom), *Volvariella* spp. (paddy straw mushroom), *Pleurotus* spp. (oyster mushroom) and *Calocybe indica* (milky mushroom) are commercially cultivated. Mushroom cultivation is affected by a large number of biotic and abiotic factors. Fungi, bacteria, viruses, nematodes, insects and mites are different biotic factors that damage the mushroom crop directly or indirectly (Sharma *et al*., 2011) [12]. Among the various factors responsible for low production and productivity of mushroom in our country, fungal diseases play a major role. The fungal pathogens, *Verticillium fungicola*, *Mycogone perniciosa*, *Trichoderma* spp. and *Papulaspora byssina* are the predominant mycopathogens. Amongst these, *Verticillium fungicola* var. *fungicola* (Preuss) is the important pathogen of the *Agaricus bisporus* (Lange) Imbach and annual losses to the growers are estimated to be 2–4% of total revenue (Berendsen *et al*., 2010) [1]. The pathogen induces various symptoms like bubbles (undifferentiated spherical masses), bent and/or split stipes (blowout) and spotty caps. Inoculation of *A. bisporus* crop with isolates of *V. fungicola* var. *fungicola* of various degrees of aggressiveness showed that the more aggressive isolates induced higher numbers of bubbles (Largeteau and Savoie, 2008) [8]. The *Verticillium* dry bubble is the most prevalent disease and if left uncontrolled in the mushroom growing environment; the disease can wipe out an entire crop in 2–3 weeks (Sharma *et al*., 2002) [11]. Moreover, the disease may be devastating for years following the initial infection because spores are capable of resting in debris and re-infecting crop year after year (Berendsen *et al*., 2010) [1].

It has been noticed that, exogenously application of phytohormones like salicylic acid and jasmonic acid increased the level of host resistance Braaksma *et al.* (2001). Singh *et al.*, (2011) [12, 13] reported that the problems being faced in controlling the spread of disease is the fungal nature of both pathogen as well as the host. Thus the various control measures to be applied *i.e.* cultural practices, sanitation, bio-agents, botanical extracts and chemicals at various stages of crop cycle in order to effectively management of diseases. The chemicals measures significantly inhibit the mycelial growth of cultivated strains and leave harmful residue in fruit bodies. Therefore, it is essential to select safe and eco-friendly control measures of pathogen without affecting the growth of *A. bisporus*.

Materials and Methods

Evaluation of non-conventional chemicals against *V. fungicola* and *A. bisporus*

In vitro sensitivity of non-conventional chemicals against host and pathogen

In vitro sensitivity of non-conventional chemicals against the *V. fungicola* and *A. bisporus* were determined by poisoned food technique (Grover and Moore, 1962) [5]. The chemicals salicylic acid (SA) and jasmonic acid (JA) were used with different concentrations (mM) *i.e.* 0.1, 0.2 and 0.4. Stock solutions of these chemicals were prepared by dissolving required quantity in sterilized distilled water. Autoclaved potato dextrose agar (PDA) medium was amended with different stock solutions to obtain the desired concentration of extracts before being poured into Petri plates. The Petri plate with un-amended PDA served as check. Three Petri plates for each concentration of the chemicals were inoculated with *V. fungicola* and *A. bisporus* by placing five mm actively growing mycelial disc of 12 days old culture. The radial growth (mm) was recorded after four days interval of incubation in BOD incubator at 25±1ºC up to nine days. The percent growth inhibitions of the pathogen and host at various concentrations of extracts were calculated over the check by using Vincent's formula (1947). [PI =100 (C – T)/C].Where, PI - Per cent inhibition, C - Radial growth (mm) in control, T - Radial growth (mm) in treatment

In vivo efficacy of non-conventional chemicals against *V. fungicola* on cut fruit bodies

In vivo efficacy of the non-conventional chemicals against *V. fungicola* on cut mushrooms fruit bodies were also studied. Cut mushrooms fruit bodies were allowed to absorb stocks solution of salicylic acid and jasmonic acid with different concentrations and after 12 hrs three cut mushrooms fruit bodies for each concentration of the chemicals were inoculated with uniform spore suspension ($\leq 10^4$ spores/ml) of *V. fungicola* and uninoculated served as control. Incubate in an isolated room at a temperature 25±1°C with relative humidity of more than 85 per cent. The size (mm) and number of lesions were measured on fruiting bodies at 24 hrs regular interval up to three days.

Results and Discussion

Evaluation of non-conventional chemicals against *V. fungicola* and *A. bisporus*

In vitro sensitivity of non-conventional chemicals against the *V. fungicola* and *A. bisporus* were determined by poisoned food technique. The chemicals salicylic acid (SA) and jasmonic acid (JA) with different concentrations (mM) *i.e.* 0.1, 0.2 and 0.4 were used respectively. The radial growth (mm) was recorded after four days interval of incubation in BOD incubator at 25±1ºC up to 12 days. The percent growth inhibitions of the pathogen and host at various concentrations were calculated over the control.

From the data in Table-1 and Fig-1, it was depicted that percentage inhibition over control towards *V. fungicola* was 44.96% at concentration of 0.4 mM in case of salicylic acid followed by 23.42% in jasmonic acid and on the other hand, the percentage inhibition was very low 7.80% (jasmonic acid) and 17.08% at concentration of 0.1 mM after 12 days of incubation. The percentage inhibition in *A. bisporus* (Table-2) was 16.28% at concentration of 0.4 mM in salicylic acid followed by 11.11% in jasmonic acid. While, minimum percentage inhibition over control of host *A. bisporus* was 2.95% (jasmonic acid) and 6.66% (salicylic acid) at concentration of 0.1 mM after 12 days of incubation.

During these studies the evaluation of non-conventional chemicals *i.e.* salicylic acid (SA) and jasmonic acid (JA) with different concentrations (mM) *i.e.* 0.1, 0.2 and 0.4 against the *V. fungicola* in response to size and number of lesions development on cut fruiting bodies of *A. bisporus* (Fig-2). Perusal data in Table-3, showed that percentage size reduction over control of *V. fungicola* was 69.69% at concentration of 0.4 mM in case of salicylic acid followed by 39.93% in jasmonic acid and on the other hand, the percentage size reduction was very low 20.12% (jasmonic acid) and 30.03% (salicylic acid) at concentration of 0.1 mM after 72 hrs. Similarly, from Table-4, the number of lesions reduction over control of *V. fungicola* was 81.96% at concentration of 0.4 mM in case of salicylic acid followed by 54.64% in jasmonic acid, while the percentage number of lesions reduction over control was very low 9.01% (jasmonic acid) and 45.35% (salicylic acid) at concentration of 0.1 mM after 72 hrs.

Host Fruiting body

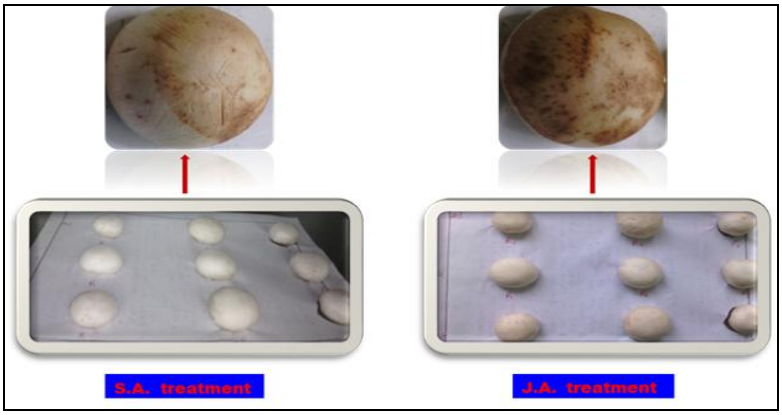

Fig 1: Effect of non-conventional chemicals on fruiting bodies of button mushroom against *verticillium fungicola*

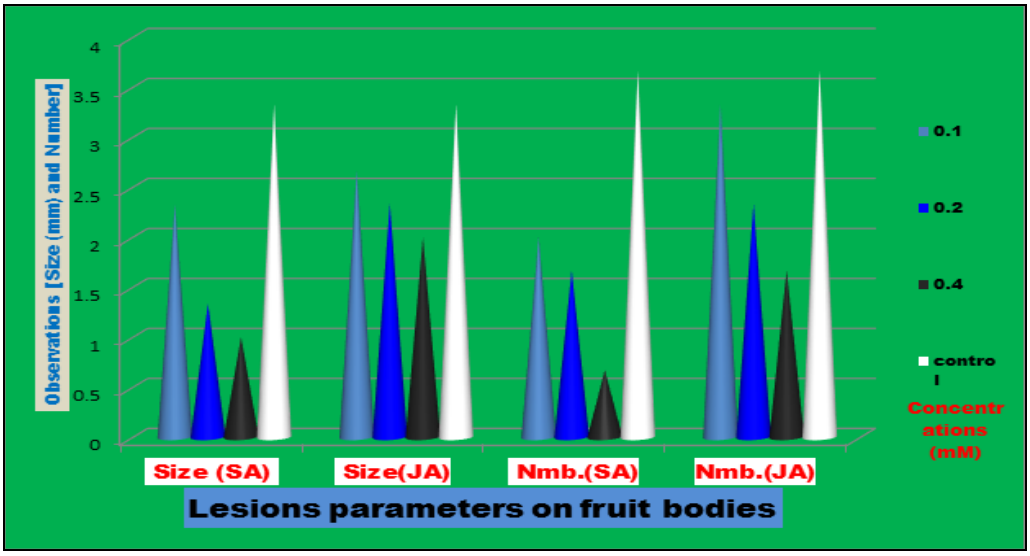

Fig 2: Evaluation of non-conventional chemicals against *verticillium fungicola*

Table 1: Evaluation of non-conventional chemicals against *Verticillium fungicola*

S. No	Conc. (mM)	Observation** on radial growth* of *Verticillium fungicola*									
		Salicylic acid					Jasmonic acid				
		4DAI	8DAI	12DAI	Mean	PIOC#	4DAI	8DAI	12DAI	Mean	PIOC#
1	0.1	16.00	29.50	37.17	27.56	17.08	17.00	30.67	41.33	29.67	7.80
2	0.2	12.33	22.00	31.00	21.78	30.84	14.67	24.67	37.33	25.56	16.72
3	0.4	9.50	16.83	24.67	17.00	44.96	12.67	19.00	34.33	22.00	23.42
4	Control	17.50	31.50	44.83	31.28		17.50	31.50	44.83	31.28	
5	Mean	13.83	24.96	34.42			15.46	26.46	39.46		
Factors		A	B^{1}	A× B^{1}			A	B^{II}	A× B^{II}		
CD at 5%		1.39	1.60	2.78			1.29	1.49	2.57		

*Average of three replications, ** observation (DAI) days after inoculation, # PIOC- percent inhibition over control (12DAI), A- concentration & B – observation

Table 2: Evaluation of non-conventional chemicals against *Agaricus bisporus*

S. No	Conc. (mM)	Observation** on radial growth* of *Agaricus bisporus*									
		Salicylic acid					Jasmonic acid				
		4DAI	8DAI	12DAI	Mean	PIOC#	4DAI	8DAI	12DAI	Mean	PIOC#
1	0.1	14.33	27.83	42.00	28.06	6.66	16.00	30.00	43.67	29.89	2.95
2	0.2	14.00	27.00	40.00	27.00	11.11	14.83	28.00	42.67	28.50	5.17
3	0.4	12.33	25.33	37.67	25.11	16.28	13.00	26.33	40.00	26.44	11.11
4	Control	15.33	30.83	45.00	30.39		15.33	30.83	45.00	30.39	
5	Mean	14.00	27.75	41.17			14.79	28.79	42.83		
Factors	A	B^{1}	A× B^{1}			A	B^{II}	A× B^{II}			
CD at 5%	1.98	2.29	N/A			1.89	2.18	N/A			

*Average of three replications, ** observation (DAI) days after inoculation, #PIOC- percent inhibition over control (12DAI), A- concentration & B – observation

Table 3: Evaluation of non-conventional chemicals against lesions size development in *Verticillium* dry bubble disease

S. No	Conc. (mM)	Size* (mm) of lesions on fruit bodies									
		Effect of Salicylic acid (SA)					Effect of Jasmonic acid (JA)				
		24hrs	48hrs	72hrs	Mean	PSROC#	24hrs	48hrs	72hrs	Mean	PSROC#
1	0.1	0.00	1.66	2.33	1.33	30.03	0.00	2.33	2.66	1.66	20.12
2	0.2	0.00	1.33	1.33	0.88	60.06	0.00	2.00	2.33	1.44	30.03
3	0.4	0.00	0.00	1.00	0.33	69.96	0.00	1.66	2.00	1.22	39.93
4	Control	0.00	3.00	3.33	2.11		0.00	3.00	3.33	2.11	
5	Mean	0.00	1.50	2.00			0.00	2.25	2.58		
Factors		A	B^{1}	A× B^{1}			A	B^{II}	A× B^{II}		
CD at 5%		0.46	0.54	0.93			0.46	0.54	N/A		

*Average of three replications, #- percent size reduction over control (PSROC), A- Concentration, B^{I} – Size of lesions in SA, B^{II} –Size of lesions in JA, Control- for pathogen *Verticillium fungicola* without treatment

Table 4: Evaluation of non-conventional chemicals against number of lesions development in *Verticillium* dry bubble disease

S. No	Conc. (mM)	Number* of lesions on fruit bodies									
		Effect of Salicylic acid					Effect of Jasmonic acid				
		24hrs	48hrs	72hrs	Mean	PLROC#	24hrs	48hrs	72hrs	Mean	PLROC#
1	0.1	0.00	1.33	2.00	1.11	45.35	0.00	2.33	3.33	1.88	9.01
2	0.2	0.00	0.66	1.66	0.77	54.64	0.00	1.66	2.33	1.33	36.33
3	0.4	0.00	0.00	0.66	0.22	81.96	0.00	1.33	1.66	1.00	54.64
4	Control	0.00	2.66	3.66	2.11		0.00	2.66	3.66	2.11	
5	Mean	0.00	1.16	2.00			0.00	2.00	2.83		
Factors	A	B^{I}	A× B^{I}			A	B^{II}	A× B^{II}			
CD at 5%	0.34	0.40	0.69			0.40	0.46	0.79			

*Average of three replications, #- percent lesion reduction over control (PSROC), A- Concentration, B^{I} – number of lesions in SA, B^{II} - Number of lesions in JA, Control- for pathogen *Verticillium fungicola* without treatment

Out of two non-conventional chemicals *i.e.* salicylic acid and jasmonic acid evaluated at with different concentrations (mM) *i.e.* 0.1, 0.2 and 0.4 significantly inhibited the radial growth of *V. fungicola*. The inhibition was measured 50% at 0.4 mM concentration by salicylic acid, while jasmonic acid inhibited the growth to the extent of 23%. In host *A. bisporus* growth inhibition was 16% with salicylic acid and 11% with jasmonic acid at the same concentration. *In vivo* the both of salicylic acid and jasmonic acid when evaluated at same concentration against *V. fungicola* in terms of size and number of lesions development on cut fruiting body of *A. bisporus*. The lesions size reduction over control of *V. fungicola* was 70% with salicylic acid followed by 40% with jasmonic acid. While, the maximum number of lesions reduction over control of *V. fungicola* was 82% at concentration of 0.4 mM with salicylic acid and 55% with jasmonic acid. It has been reported that, phytohormones like salicylic acid and jasmonic acid enhance the level of resistance against the pathogen in mushroom crop when applied exogenously and the effect of defense-associated phytohormones on *V. fungicola* has been investigated by Braaksma *et al.* (2001). Similarly, both salicylic acid and jasmonic acid enhance resistance in plants against pathogen when applied exogenously (Pieterse *et al*., 1998). The salicylic acid has also been shown to inhibition of the growth of *Fusarium oxysporum* (Di *et al*., 2017) [4]. Several studies indicate altered disease resistance of crops, when treated with salicylic acid, jasmonic acid and ethylene as defense elicitors. Our finding also support the work of Wang *et al*. (2012) [15] who reported that lesions development by *Sclerotinia sclerotiorum* infection on leaves was arrested by salicylic acid and jasmonic acid treatments in oilseed rape. Similarly, Lee and Hong (2014) [9] reported salicylic acid, jasmonic acid and ethylene mediated defenses all together to combat pathogens having different infection strategies and to achieve broad spectrum disease resistance.

Summary and Conclusion

The non-conventional chemicals such as salicylic acid and jasmonic acid when evaluated at different concentrations (0.1, 0.2 and 0.4 mM) both against pathogen and host revealed that salicylic acid gave maximum growth inhibition (44.96%) against *V. fungicola* at 0.4 mM concentration followed by 23.42% with jasmonic acid. Whereas, in host *A. bisporus* the percentage inhibition was 16.28% and 11.11% by application of salicylic acid and jasmonic acid, respectively at 0.4 mM concentration.

In *in vivo* studies evaluation of non-conventional chemicals against the *V. fungicola* in terms of size and number of lesions development on cut fruiting bodies of *A. bisporus*, revealed that lesion size reduction was maximum (69.69%) at concentration of 0.4 mM in salicylic acid application, while minimum (39.93%) with jasmonic acid. Similarly, reduction in number of lesions was also maximum (81.96%) at concentration of 0.4 mM in case of salicylic acid followed by jasmonic acid (54.64%). Hence, salicylic acid proved to be far better than jasmonic acid in the management of the disease.

References

1. Berendsen RL, Baars JPP, Kalkhove SIC, Lugones LG, Wosten HAB, Bakker PAHM. *Verticillium fungicola*: causal agent of dry bubble disease in white-button mushroom. Molecular Plant Pathology. 2010; 11:585-595.
2. Braaksma A, Schaap DJ. Kwaliteit van champignons: geremde hoedopening en meer smaak. Wageningen: Agrotechnology & Food Innovations BV, 2005.
3. Chang ST, Miles PG. Mushrooms: Cultivation, Nutritional Value, Medicinal effect and Environmental Impact (2nd edition). Boca Raton, CRC press. 2004, 6.
4. Di X, Gomila J, Frank LWT. Involvement of salicylic acid, ethylene and jasmonic acid signalling pathways in the susceptibility of tomato to Fusarium oxysporum. Molecular Plant Pathology. 2017; 18:1024-1035.
5. Grover RK, Moore JD. Taxonomic studies for fungicides against brown rot organism *Sclerotinia fructicola* and S. laxa. Phytopathology. 1962; 52:876-880.
6. Hawksworth DL. The fungal dimension of biodiversity: magnitude, significance and conservation, Mycological Research. 1991; 95:641-655.
7. Hawksworth DL. Mushrooms: the extent of the unexplored potential. Int. J Med. Mush. 2001; 3:333-337.
8. Largeteau ML, Savoie JM. Effect of the fungal pathogen *Verticillium fungicola* on fruiting initiation of its host, *Agaricus bisporus*. Mycological Research. 2008; 112:825-828.
9. Lee YH, Hong JK. Differential defence responses of susceptible and resistant kimchi cabbage cultivars to anthracnose, black spot and black rot diseases. Plant Pathology, 2014. DOI: 10.1111/ppa/12262.
10. Pieterse Z. *Mycogone perniciosa*, a pathogen of *Agaricus bisporus*. M.Sc. thesis, University of Pretoria, 2005, 1-38.
11. Sharma SR, Satish K, Sharma VP. Diseases and Competitor Moulds of Mushrooms and their Management. Journal of Early Republic. 2002; 22:509-516.
12. Sharma VP, Kumar S, Kamal S, Singh SK. Etiology and molecular characterization of wet bubble disease causing fungus (*Hypomysis perniciosus*) in *Agaricus bisporus*. Mushroom Research. 2011; 20(1):21-25.
13. Singh M, Vijay B, Kamal S, Wakchaure GC. Mushroom Cultivation, Marketing and Consumption. Directorate of Mushroom Research (ICAR), Solan HP. India, 2011, 1-22.
14. Vincent JM. Distortion of fungal hyphae in presence of

certain inhibitor. 1947; 150:1-850.
15. Wang Z, Tan X, Zhang Z, Gu S, Li G, Shi H *et al.* Defense to *Sclerotinia sclerotiorum* in oilseed rape is associated with the sequential activations of salicylic acid signaling and jasmonic acid signaling, Plant Science. 2012; 184:75-82.
16. Wani BA, Bodha RH, Wani AH. Nutritional and medicinal importance of mushrooms. Journal of Medicinal and Plant Research. 2010; 4:2598-2604.