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Tyrophagus putrescentiae as causative agent of dry bubble disease and green mould disease in *Agaricus bisporus*

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Abstract
A study was conducted to assess the role of *Tyrophagus putrescentiae* in transmission of disease. *Verticillium sp.* and *Trichoderma* sp. are causative agents of dry bubble and green mould disease, respectively in mushrooms. 10 pairs of *T. putrescentiae* were released on *V. fungicola* and *T. harzianum* culture plates under aseptic condition. *T. putrescentiae* started feeding as soon as they were released on culture plates. A peak population (47.40 Mites), (60.80 Mites) was recorded after 12 days and 14 days. After 14 days, 15 pairs of *T. putrescentiae* were released from *V. fungicola* and *T. harzianum* infected plates to *A. bisporus* culture plates under aseptic conditions. After few days brownish mouldy growth shown by *V. fungicola*, colour appearing white to pale brown and *Trichoderma* sp. led to complete green mouldish growth on *A. bisporus culture*. The mite number significantly increased with increase in observation period till 14th day. At this stage peak in the population on both fungus species were recorded 138 and 131.21 mites respectively.

Keywords: *Agaricus bisporus*, dry bubble disease, green mould disease, *Verticillium fungicola*, *Trichoderma harzianum*, *Tyrophagus putrescentiae*

1. Introduction
In India, the total production of mushroom is about 1.2 million tonnes [1], out of which major share (85%) goes to button mushroom. Haryana is the leading state in seasonal mushroom production contributing approximately 10,000 tonnes per year [2] and ranks third in the country. For commercial production, more than 60 Genera of mushrooms are cultivated throughout the world as food [3]. In addition to mite attack, the cultivation of the mushroom is susceptible to many competitive organisms and weed fungi [4, 5], causing substantial economic loss to the growers due to decrease yields. *T. putrescentiae* can spread viruses causing diseases in mushrooms [6] and are also responsible for the dispersal of weed and pathogenic fungal spores. Red pepper mites are also considered as vectors of *Trichoderma* and *Hypocrea nigricans*. The mites swarming on the casing and mushrooms bodies spread the spores of *Trichoderma* spp. [6]. Erban *et al.* [7] studied the presence of *Wolbachia*, *Cardinium*, *Bartonella*, *Blattabacterium* and *Solitalea* in the eggs of *T. putrescentiae* which indicated mother to offspring (vertical) transmission. Dry bubble disease is severe impairment in the profitable cultivation of white button mushroom (*A. bisporus*). *Verticillium sp.* and *Trichoderma* sp. are causative agents of dry bubble and green mould disease, respectively in mushrooms. Wet bubble disease, are the most common fungi causing severe losses in yield of *A. bisporus* throughout the world. In India, this disease was reported for the first time in 1978 from Jammu and Kashmir [8] and later from Himachal Pradesh, Haryana and Maharashtra [9, 10, 11].
Keeping in view of the above facts, the present study has been undertaken to identify the vector responsible dry bubble and green mould disease in *Agaricus bisporus* (Strain-U-3).

2. Materials and Methods
Stock culture of *T. putrescentiae* was maintained in laboratory at 27±1^{0}C and 80-85 percent relative humidity in Department of Zoology, CCS Haryana Agricultural University, Hisar. Pure strains of *Agaricus bisporus* (U-3), *Verticillium fungicola* and *Trichoderma harzianum* were taken from Mushroom Testing Laboratory Department of Plant Pathology, CCSHAU and maintained on Potato Dextrose Agar (PDA) in the Acarology Lab, Department of Zoology, CCSHAU, Hisar from January to March, 2017.

2.1 Experimental Set Up

To assess the role of *T. putrescentiae* in transmission of dry bubble disease and green mould disease, culture of pathogenic fungi *Verticillium fungicola* and *Trichoderma harzianum* was used. Under this experiment, Potato Dextrose Agar (PDA) medium was prepared. Petri plates were inoculated with a bit of *V. fungicola* and *T. harzianum* under Laminar flow. After five days of inoculation of *V. fungicola* and *T. harzianum* 10 pairs of *T. putrescentiae* were released in plates under aseptic and replicate conditions. With five replicates experiments were conducted for 14 days. Observations on mite feeding and multiplication were recorded after each alternative day. After 14 days, 15 pairs of *T. putrescentiae* were picked from *V. fungicola* infected plates and released in *A. bisporus* culture U-3 plates. Same processor followed by *Trichoderma sp*. Observations on the *T. putrescentiae* population build up and development of mouldy growth on strain of *A. bisporus* was recorded to observe the transmission of disease.

2.2 Statistical Analysis

The reported data are the mean of triplicates and was subjected to ANOVA to analyze the significant differences using software 'OPSTAT', developed at the Computer Center, College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar.

3. Results

To assess the role of *T. putrescentiae* in transmission of dry bubble disease, experiments was laid out in completely randomised block design. 10 pairs of *T. putrescentiae* were picked from culture and released on *V. fungicola* under aseptic condition. *Verticillum sp.* is causative agent of dry bubble disease. The plates were kept in BOD at 27± 1^0C and 80-85 percent and percent relative humidity and mites were allowed to feed and multiply on *V. fungicola*. Observations were recorded after every alternative day in each of the 5 replicate. Thereafter, fifteen mite pairs were picked from *Verticillum* culture plate after 14 days and shifted to *A. bisporus (U-3)* culture plates under aseptic conditions. Observations on the development of disease symptoms, brownish mouldy growth of mycelium on *Agaricus bisporus* and population build up of *T. putrescentiae* was recorded. Results are presented with the help of tables and plates.

3.1 Population of *Tyrophagus putrescentiae* on *Verticillium fungicola*

The data pertaining to *T. putrescentiae* population build up on *V. fungicola* is presented in Table1. *T. putrescentiae* started feeding as soon as they were released in culture plates. Continuous significant increase in number at each observation period (CD= 3.01; p= 0.05) (Table 1). It was 20.00, 26.80, 30.00, 34.00, 37.20, 41.80, 47.40 and 42.80 at 2, 4, 6, 8, 10, 12 and 14 day. A peak in population (47.40mites) was recorded after 12 days. Thereafter, a gradual but significant decline in population was recorded with day respectively.

Table 1: Population of *Tyrophagus putrescentiae* on *Verticillium fungicola*

Observation (Days after release of mites)	10 pairs of mite/ strain of *V. fungicola*
0	20.00 ± 0.00
2	26.80 ±0.93
4	30.00 ± 0.74
6	34.00 ±0.55
8	37.20 ± 0.45
10	41.80 ± 0.58
12	47.40 ±1.66
14	42.80 ± 1.54
CD(p = 0.05)	3.01
SE(m)	1.04

Population of *Tyrophagus putrescentiae* on *V. fungicola*

The data pertaining to *T. putrescentiae* population build up on *Trichoderma harzianum*. is presented in Table2. *T. putrescentiae* started feeding as soon as they were released in culture plates. Significant increase in number at each observation period (CD=4.52; p= 0.05) (Table 2). It was 20.00, 27.40, 30.00, 36.60, 41.00, 45.80, 52.60 and 60.80 at 2, 4, 6, 8, 10, 12 and 14 day. A peak in population (60.80 mites) was recorded at 14 days. Thereafter, a gradual but significant increased in population was recorded with day respectively.

Table 2: Population of *Tyrophagus putrescentiae* on *Trichoderma harzianum.*

Observation (Days after release of mites)	10 pairs of mite/ strain of *Trichoderma* sp.
0	20.00 ± 0.00
2	27.40 ± 0.62
4	30.00 ± 0.74
6	36.60 ±0.68
8	41.00 ± 1.57
10	45.80 ± 1.52
12	52.60 ±2.09
14	60.80 ± 1.99
CD(p = 0.05)	4.52
SE(m)	1.56

After 14 days, 15 *T. putrescentiae* pairs were released from *Verticilium* infected plates to *A. bisporus* culture plates under aseptic conditions. After 3rd day, brownish growth started appearing on *A. bisporus* culture. Within 14 days, it spread to whole culture plate. The colour of culture changed from white to pale brown. The statistical analysis showed significant effect of observation period on population build up of *T. putrescentiae* (CD= 3.05; p= 0.05) (Table 3). The mite number significantly increased with increase in observation period till 14th day. At this stage peak in the population was recorded (138.00 Mites).

Table 3: *Verticillium fungicola* reared population of *Tyrophagus putrescentiae* mites on *Agaricus bisporus* (Strain-U-3)

Observation (Days after release of mites)	15 pairs of *Tyrophagus putrescentiae* / strain of *A. bisporus U-3.*
0	30.0±0.00
2	32.0±0.63
4	36.60±0.81
6	46.40±0.74
8	67.60±1.07
10	91.40±1.50
12	117.00±1.44
14	138.00±1.34
CD(p = 0.05)	3.05
SE(m)	1.05

Under aseptic conditions 15 pairs *T. putrescentiae* from *T. harzianum* infected plates were relaesed to *A. bisporus* culture plates. Greenish mouldy growth was started appearing on *A. bisporus culture* after 4 day. The statistical analysis showed significant effect of observation period on population build up of *T. putrescentiae* (CD= 4.78; p= 0.05) (Table 4). At this stage peak in the population was recorded (131.80 mites). Afterwards, fungus *Trichoderma* sp. led to complete green mouldish growth on *A. bisporus* culture plates.\

Table 4: *Trichoderma harzianum* reared population of *Tyrophagus putrescentiae* mites on *Agaricus bisporus* (Strain-U-3)

Observation (Days after release of mites)	15 pairs of *Tyrophagus putrescentiae* / strain of *A. bisporus U-3*
0	30.00±0.00
2	30.80±0.66
4	38.60±1.20
6	52.80±1.93
8	65.40±1.16
10	84.60±1.69
12	105.40±2.97
14	131.80±1.77
CD(p = 0.05)	4.78
SE(m)	1.653

4. Discussion

The present discussion encompasses the work on mite *T. putrescentiae* which act as carrier of dry bubble disease and green mould disease. *T. putrescentiae* started feeding as soon as they were released in culture plates of both pathogen fungus *V. fungicola* and *T. harzianum*. They multiplied and peak population (47.40 mites), (60.80 mites) was recorded after 12 days and 14 days. *T. putrescentiae* pairs shifted from *V. fungicola* and *T. harzianum* infected plates to *A. bisporus* culture transmitted spores of fungus. After few days dry bubble disease and green mould disease appeared on *A. bisporus* culture and spread to whole culture plate within 14 days. The colour of culture changed from pale brown to dark brown in dry bubble disease and green in green mould disease.

In addition to *T. putrescentiae*, few more mite species like *Trochometridium*, [12], *Siteroptes cerealium* [13], *Tarsonemus ips* [14], *Imparipes haeseleri, I. apicola* and *I. breganti* [15,16] feeds on fungi and help in transportation of fungal spores. Itisha *et al*. [17] reported that *T. putrescentiae* act as carrier of wet bubble disease in white button mushroom, white bubble disease, caused by *Mycogone perniciosa* (Magnus) hampers the yield. In all cases, only females were responsible for fungal transfer. Although there is no data concerning the viability of the spores transported in the sporotheca, mites might act as fungi spreaders transporting spores inside or outside their bodies to other possible substrates, rewarded at the same time by being provided with mycelia for a new colony of mites [18, 19, 20].

5. Conclusion

In the present study we focused on the role of *T.putrescentiae* as vector of dry bubble and green mould disease caused by fungus *Verticillium fungicola* and *T. harzianum*, respectively was also ascertained during the present study. *V. fungicola* reared *T. putrescentiae* population on *Agaricus bisporus* spread the disease within 14 days whole culture plates. Similarly, *T. harzianum* reared *T. putrescentiae* population on *Agaricus bisporus* spread the green mould disease to culture plates. More studies are required in this aspect, need to know viability of the fungal spores transportation by mites through their bodies to other possible substrates.

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