

Original Research Article

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Tyrophagus putrescentiae as Causative Agent of Wet Bubble Disease in *Agaricus bisporus*

Itisha*, Rachna Gulati, Manoj, Anita and Surjeet Singh

Department of Zoology, Chaudhary Charan Singh Haryana Agriculture University,
Hisar – 125004, Haryana, India

*Corresponding author

ABSTRACT

Keywords

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A study was conducted to assess the role of *Tyrophagus putrescentiae* in dispersal of *Mycogone perniciosa*, a causative agent of wet bubble disease in *Agaricus bisporus*. *T. putrescentiae* started feeding as soon as they were released in *M. perniciosa* culture plates. A continuous significant increase in mite number was recorded at each observation period with peak (150.50 mites) after 20 days which was statistically comparable with 145.50 mites at 18 days. After 15 days, 10 *T. putrescentiae* pairs were released from *M. perniciosa* infected plates to *A. bisporus* culture plates under aseptic conditions. After 6 days, whitish mouldy growth was started appearing on *A. bisporus* culture which soon spread to whole culture plate within 18 days. Mites were feeding and multiplying at this stage. The mite number significantly increased with increase in observation period on *A. bisporus* culture plates till 22nd day. At this stage peak in the population was recorded (177.25 mites).

Introduction

Mushroom growing is one of the fastest growing and most technologically sophisticated horticultural industries in the world. Three species of edible mushroom, white button mushroom (*Agaricus bisporus*), paddy straw mushroom (*Volvariella* spp.) and oyster mushroom (*Pleurotus* sp.) are commercially grown in India. Button mushrooms are subject to a range of diseases and pests which have the capacity to cause serious crop losses. Sciarid fly, phorid fly, spring tails and mites are important arthropod pests of cultivated mushroom in India. Fifty four mite species have been found associated

with mushrooms (Tripathi, 2005), out of which *Tarsonemus myceliophagus* Hussey, *Tyrophagus lantneri* Osb, *Caloglyphus mycophagus* (Megnin) (Kumar *et al.*, 2004), *Tyrophagus putrescentiae* Schrank, *Uroobovella* sp. (Rana, 2008) are considered economically important pests.

Tyrophagus putrescentiae, commonly called as mould mite, copra mite or cheese mite, belong to the family Acaridae of the order Astigmata. Their small size makes early infestation by mites difficult to detect and enables them to enter packaging and exploit

food residues in very small cracks and crevices. The life cycle consists of the egg, larva, protonymph, tritonymph, and adult stages. Development from the egg to adult takes about 1 to 3 weeks, and could take 118 days depending on environmental conditions and the food type on which the mite is reared (Kheradmand *et al.*, 2007).

It is reported as an important vector of dispersing weed fungi throughout mushroom cultivation facilities (Okabe *et al.*, 2001; Czajkowska, 2002). These include *Fusarium* sp., *Alternaria* sp., *Geotrichum* sp., *Mucor* sp. and *Trichophyton* sp. 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. (Hussey *et al.*, 1969). Erban *et al.*, (2016) studied the presence of *Wolbachia*, *Cardinium*, *Bartonella*, *Blattabacterium* and *Solitalea* in the eggs of *T. putrescentiae* which indicated mother to offspring (vertical) transmission. In white button mushroom, the incidence of wet bubble disease caused by the fungus, *Mycogone perniciosa* is a serious problem (Gahukar, 2014) whose carriers are presumed to be pests but no detailed study is available. It produces symptoms like dense white growth on gills; swollen stems and caps from which reddish-brown liquid oozes, chlorine like smell and crinkled, walnut-like bodies on the surface of the casing. *T. putrescentiae* can spread viruses causing diseases in mushrooms (Hussey *et al.*, 1969) and are also responsible for the dispersal of weed and pathogenic fungal spores, so the present study has been undertaken to assess the role of *Tyrophagus putrescentiae* in wet bubble disease dispersal.

Materials and Methods

To assess the role of *T. putrescentiae* in transmission of wet bubble disease, culture of pathogenic fungi (*Mycogone perniciosa*) and

A. bisporus was reared on Potato Dextrose Agar (PDA) medium. Petriplates were inoculated with a bit of *Mycogone perniciosa* under Laminar flow. After five days of inoculation of *M. perniciosa*, 10 pairs of *T. putrescentiae* were released in plates under aseptic and penta-replicate conditions.

Experiments were conducted for 28 days at $27\pm 1^{\circ}\text{C}$, 95-99 percent relative humidity in the BOD incubator. Observations on mite feeding and multiplication were recorded after each alternative day. After 15 days, 10 pairs of *T. putrescentiae* were picked from *M. perniciosa* infected plates and released in *A. bisporus* culture ABL-2 plates. Observations on the *T. putrescentiae* population build up and development of whitish mouldy growth of mycelium on strain of *A. bisporus* was recorded to observe the transmission of disease. Counting of mites was done under the stereozoom microscope under 10X magnification.

Statistical analysis

Critical Difference (CD) was calculated for *Tyrophagus putrescentiae* population on fungi to see the effect of observation periods. The Software 'OPSTAT', developed at the Computer Centre, College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar, was used for the analysis.

Results and Discussion

The data pertaining to *T. putrescentiae* population build up on *M. perniciosa* is presented in Table 1. *T. putrescentiae* started feeding as soon as they were released in culture plates. Eggs were laid by *T. putrescentiae*, a continuous significant increase in number at each observation period (CD= 6.62; p= 0.05). It was 28.00, 35.00, 61.00, 71.75, 84.75, 101.75, 115.50, 129.25, 145.50 mites and 150.50 at 2, 4, 6, 8, 10, 12,

14, 16, 18 and 20 day. A peak in population (150.50 mites) was recorded after 20 days which was statistically comparable with 145.50 mites at 18 days. Thereafter, a gradual but significant decline in population was recorded with 137.25, 123.75 and 111.00 mites at 22, 24 and 26 day respectively.

After 15 days, 10 *T. putrescentiae* pairs were released from *M. pernicioso* infected plates to *A. bisporus* culture plates under aseptic conditions. After 6 days, whitish mouldy growth was started appearing on *A. bisporus* culture (Plate I). It soon spread to whole culture plate within 18 days. Mites were feeding and multiplying at this stage (Plate

II). The colour of culture changed from pale brown to white. The statistical analysis showed significant effect of observation period on population buildup of *T. putrescentiae* on *A. bisporus* culture (CD= 6.73; p= 0.05) (Table 2). The mite number significantly increased with increase in observation period till 22nd day. At this stage peak in the population was recorded (177.25 mites). Afterwards, it gradually declined to the lowest population count of 105.50 mites at 32 day. The mite count was statistically non-significant at zero and second day, 20th and 26th days, 18th and 28th days, 16th and 30th days. Mite number at 14th and 32nd days was also found to be at par with each other.

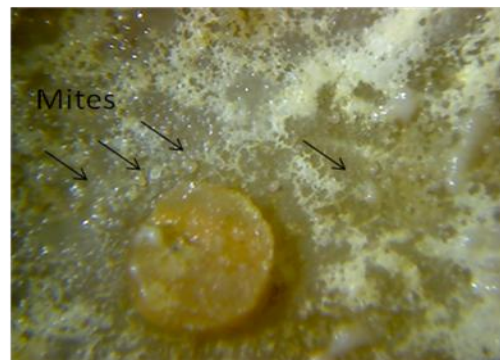
Table.1 Population of *Tyrophagus putrescentiae* on *Mycogone pernicioso*

Observation (days after 10 pairs of mite/ strain of release of mites)	<i>Mycogone pernicioso</i>
0	20.00 ± 0.00
2	28.25 ± 1.43
4	35.00 ± 1.78
6	61.00 ± 2.82
8	71.75 ± 4.55
10	84.75 ± 3.22
12	101.75 ± 1.93
14	115.50 ± 2.39
16	129.25 ± 1.93
18	145.50 ± 2.10
20	150.50 ± 1.44
22	137.25 ± 1.93
24	123.75 ± 1.88
26	111.00 ± 2.34
SE(m)	2.31
CD(p = 0.05)	6.62

Table.2 Population of *Tyrophagus putrescentiae* on *Agaricus bisporus*

Observation (days after release of mites)	10 pairs of mite/ strain of <i>Agaricus bisporus</i>
0	20.00 ±0.00 ^a
2	26.00 ±0.81 ^a
4	37.25 ±1.54
6	50.25 ±2.05
8	62.50 ±1.75
10	74.00 ±2.48
12	89.25 ±1.54
14	102.75 ±1.93 ^c
16	113.50 ±2.95 ^d
18	129.50 ±3.12 ^c
20	150.50 ±1.70 ^b
22	177.25 ±2.21
24	164.50 ±2.10
26	152.25 ±1.79 ^b
28	131.25±3.70 ^c
30	118.75 ±3.35 ^d
32	105.50 ±3.66 ^e
SE(m)	2.36
CD(p = 0.05)	6.73

Plate.1 Appearance of wet bubble disease on *A. bisporus*
 Plate II: Spreading of wet bubble disease



Most of the mites feed on wild fungus (contaminants in cultivated mushrooms) and help in dispersal of their spores (Price, 1976). Wild fungi act as competitive fungi in cultivated mushroom beds thus affecting the overall yield (Okabe, 1999). The life cycle of *C. cinerea* along with the mite *T. putrescentiae* starts with the mite feeding on the mushroom tissues including the spores (González, 2008).

In nature, the spores of coprophilous fungi survive their passage through the animal digestive system when consumed by herbivorous (Richardson, 2001). Basidiospores ingested by the mites will not be digested. Faecal pellets containing a number of fungal spores are formed and excreted. The spores in the faecal pellets easily germinate and form colonies. Mites can affect fungi by grazing, and

they may be integrally involved in the dominance of mycotoxigenic fungal species due to their role in dispersal of fungal spores and also influenced the increase of aflatoxin production from the fungus (Franzolin *et al.*, 1999; Hubert *et al.*, 2003).

In the present study, results showed that *T. putrescentiae* act as carrier of wet bubble disease. *T. putrescentiae* started feeding as soon as they were released in culture plates of *M. pernicioso*. The colour of culture changed from pale brown to white. In white button mushroom, white bubble disease, caused by *Mycogone pernicioso* (Magnus) hampers the yield (Gahukar, 2014). Krantz (1978) earlier reported that mites collected from infested cultures were *Tyrophagus putrescentiae*. The mites are found in environments suitable also for fungi, particularly environments with high humidity and elevated temperatures (Sánchez-Ramos *et al.*, 2007). Since *T. putrescentiae* transfers fungal spores to food, another common name was given as mold mite (Hubert *et al.*, 2004).

In addition to *T. putrescentiae*, few more mite species like *Trochometridium*, (Lindquist, 1985), *Siteroptes cerealium* (Suski, 1973), *Tarsonemus ips* (Moser, 1985), *Imparipes haeseleri*, *I. apicola* and *I. breganti* (Ebermann and Hall, 2003) feeds on fungi and help in transportation of fungal spores. 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 (Ramírez-Suárez *et al.*, 2002). Eggs were laid among the faecal pellets, indicating that fungi play an important role in the mites' cycle of development (Ebermann and Hall, 2003). Upon germination of fungal mycelium from the basidiospores, hatching larvae are supplied directly with food for their further development. Gazeta *et al.*, (2012) recorded an association between *T. putrescentiae* and pathogenic bacteria such as

Klebsiella sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. When agar plates were infested with mites, 100 percent bacterial colonies developed. Considering the role of *T. putrescentiae* as vector for *M. pernicioso* causing wet bubble disease in *A. bisporus* spawn, further research is needed to understand the ecology of this organisms.

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