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Bio-management of Nematodes in *Agaricus bisporus* by *Fictor composticola* and its compatibility with Neem Seed Kernel Water Extract

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ABSTRACT

Bio-management of *Aphelenchoides swarupi* in white button mushroom (*Agaricus bisporus*) was done by *Fictor composticola* in different inoculum levels and at different stages of mushroom cultivation, like, prey and predator at spawning, prey and predator at casing, prey at casing and predator at spawning, prey at spawning and predator at casing with uninoculated control. The treatments where *A. swarupi* was inoculated at spawning, the final population was higher than the treatments where it was inoculated at casing. The yield was significant when prey was inoculated at casing. Similarly, the spawn run was good when *A. swarupi* was inoculated at casing and poor when it was inoculated at spawning and *Fictor composticola* at casing. Reproduction factor of *A. swarupi* was maximum when it was inoculated at spawning and predator at casing but minimum when predator at spawning and prey at casing. Even at the lowest concentration (1 %) of neem seed kernel water extract (NSKWE), caused 99.5 % nematode mortality after 24 h. Hence, its compatibility cannot be recommended with *F. composticola* for the management of mushroom nematodes.

KEYWORDS

Fictor composticola, Neem Seed Kernel Water extract (NSKWE), mycophagous nematodes, Aphelenchoides swarupi, Biomanagement

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INTRODUCTION

Button mushroom, *Agaricus bisporus* (Lange) Singer contributes more than 80 % of total mushroom production in India (NRCM (2007). Mushroom growing is a young progressive industry all over the world today and is one of the fastest growing small-scale industries especially in rural areas. In India, its commercial cultivation is picking up in states like Himachal Pradesh, Haryana etc. It contains some bioactive constituents, such as phenolic compounds, terpenes, steroids, polysaccharides (Royse, 2014).

The successful growing of this delicacy needs attention and hygienic conditions which is nearly impossible in farmers' unit. This unhygienic condition invites many pests, like insects, nematodes, fungi, bacteria and other such biotic and abiotic stresses that reduce the quality and quantity of the mushroom (Bellettini *et al*, 2016) and (Sharma *et al*, 2019). One of the major limiting factors is, myceliophagous nematodes, reported from many parts of India (Bajaj and Kanwar (2011), Singh and Sharma (2016) and Kumar *et al* (2019)). The mycophagous nematodes are noxious pests which once introduced into the beds, are very difficult to manage. The mycophagous nematodes like *Aphelenchus avenae*, *Aphelenchoides spp*., *Ditylenchus myceliophagus* are common in mushroom beds (Bajaj and Kanwar, 2011). They feed on the mushroom mycelia by piercing the mycelial wall with their hollow stylet and suck the contents of the pierced cells. They may attack the mycelium at any time from spawning onwards. Due to their greater initial numbers, they may cause a steady decline in mushroom yield and production may suffer a loss. They may cause patchy to no growth of mycelium, sinking and foul smell of spawn run in mushroom bed leading to severe reduction in the mushroom yield (Kumar *et al*, 2008).

To get good productivity, this crop needs to be protected from nematodes. Mushroom cropping often continues for 6-8 weeks, and are harvested and consumed soon after they appear. Therefore, applying any nematicide during cropping, is not safe for health because of toxicity and residual problems. Also, mushroom is very sensitive to many chemicals and the sporophores on the surface, are sometimes more sensitive than the mycelia. Thus, the role of biocontrol agents become important in the management of these mycophagous nematodes. And the naturally occurring predators can be a good source for the management of these mycophagous nematodes.

The bio-control agents are the cheaper, non-toxic and provide pollution free control of pests. Amongst these biocontrol agents, predaceous nematodes have vital role in nematode management (Devi and George, 2018). The majority of predatory nematodes belong to four major taxonomic groups of nematodes - Mononchida, Dorylaimida, Diplogasterida and Aphelenchida of which Diplogasterids are the excellent example of predators. Their feeding apparatus is cutting and sucking type. They are generally found abundantly in decomposing organic manure and predates on different

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groups of nematodes (Keshari, 2016) and (Bajaj and Kanwar, 2015). *Fictor composticola*, a diplogasterid predatory nematode, was described from mushroom compost (Khan *et al*, 2008) and was commonly found, holds promise as bio-control of mushroom feeding nematodes (Kanwar *et al*, 2009). It also managed the root knot nematodes when applied along with organic amendments (Sidhu and Kanwar, 2019) . *F. composticola* predated on the preys by random movement. It fed on any part of the prey body and fed on the contents for 3-4 minutes at a time and became motionless before it shifted to another prey (Keshari and Kanwar, 2015). The males encountered more prey species but in most of the cases, the prey escaped after probing while females in 80 % chances, grasped the first prey, killed and completely consumed it (Kanwar and Keshari, 2019).

It was found prevalent in compost used for cultivating button mushroom (*Agaricus bisporus* (Lange) Singer) in Haryana and Bihar states of India (Khan *et al*, 2008). It is a voracious feeder of myceliophagous nematodes as well as other nematodes also. It has a very short life cycle and high fecundity. It also survives and multiplies on bacteria. These qualities make them a potential biological control agent (Keshari, 2016). Kanwar *et al* (2009) and Bajaj and Kanwar (2015) conducted preliminary studies on this nematode. The present investigation was planned to explore its potential as bio control agent of nematode pests of mushroom

The use of neem seed kernel extract (NSKWE) is proved to be a good control over the nematodes but if predatory nematodes are used, will it be compatible with these nematodes. So, the effect of NSKWE on predatory nematodes was also studied. Javed *et al* (2007) reported that the neem (*A. indica*) leaves, neem cake and a commercially refined product Aza (azadirachtin) extracted from neem seed significantly reduced the number of females and egg masses in roots. All the neem formulations significantly reduced the number of eggs per egg mass on the un-treated root portion. Even after 16 weeks all the treatments significantly reduced the galling index and number of egg masses but their effectiveness declined over time.

The aqueous extracts of neem (*A. indica*) crude formulations (leaves and cake) at 10 %, 5 %, and 2.5 % w/v caused immobility and mortality, in root-knot nematode (*Meloidogyne javanica*) whereas a refined product, Aza at 0.1% w/v caused neither immobility or mortality of juveniles. When egg masses were placed in extracts of these formulations, hatching did not occur in any of the concentrations (10 %, 5 %, 2.5 % and 1.25 % w/v) of the crude formulations (Javed *et al*, 2008).

Lynn *et al* (2010) reported that the isolated soil nematodes (*M. incognita*) when exposed to various concentrations of azadirachtin, Neema, and Neema-plus, the immobility of juvenile nematodes showed no change at 2 h after treatment, whereas a reduction of 36.3 % was observed at day 1 with 10 ppm of azadirachtin. Nevertheless, the effects of neem formulations were faster and much higher than those of azadirachtin. At a cucumber greenhouse, soil treatments with neem formulations significantly reduced the numbers of soil nematodes and plant root-knots; the reduction with Neema was 12.1 and 9.0 %, and with Neema-plus 26.4 and 24.6 % of the control, respectively. Furthermore, soil treatment with Neema-plus greatly improved the growth of cucumber plants in nematode-infested pots. So, the neem formulations may have effect on predatory nematodes also, which was studied here.

MATERIALS AND METHODS

The experiment was carried out in polythene bags of five kg capacity using pasteurized compost. U_3 strain of *A. bisporus* obtained from, Department of Plant Pathology, CCS HAU, Hisar was used for experimentation. Spawn raised on boiled wheat grains was mixed in compost @ 50 g/kg compost at the time of filling the bags. The treatments were laid out as follows in three replications-

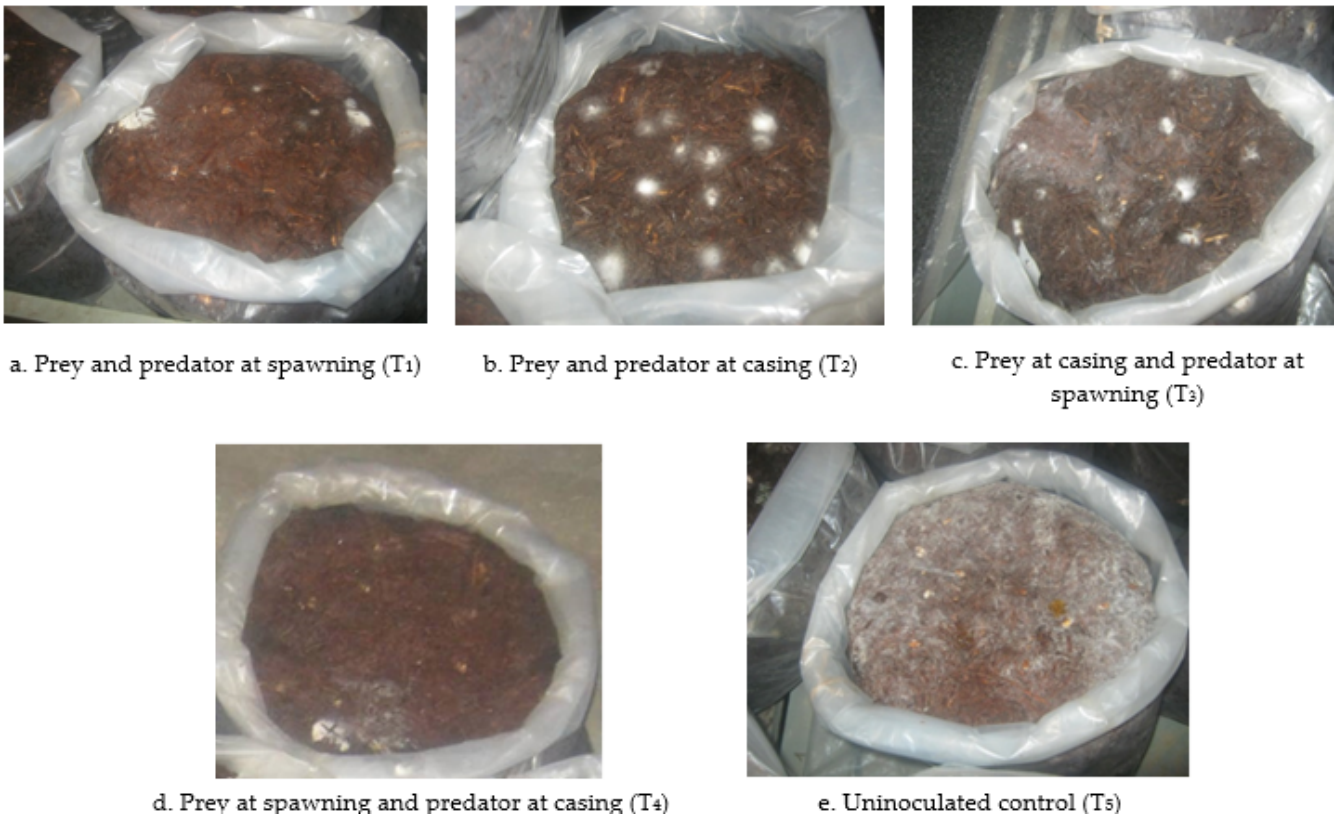
- Prey and predator at spawning (T_1)
- Prey and predator at casing (T_2)
- Prey at casing and predator at spawning (T_3)
- Prey at spawning and predator at casing (T_4)
- Uninoculated control (T_5)

Casing was done with ash of rice husk, 21 days after spawning. The inoculum levels for the predator (*F. composticola*) and prey (*Aphelenchoides swarupi*) were kept 10 and 100 nematodes per kg compost, respectively. The observations were taken for spawn run, population of prey and predator at harvesting and yield per bag (in 5 kg bags only), spawn run and nematode population. The data were analysed by CRD.

A concentration of 4 % neem seed kernel water extract (NSKWE) was prepared by dipping 4 g coarsely ground neem seed kernel in 100 ml water for 24 hours. This was strained through 4-ply muslin and used in experiment after further dilutions of 2 % and 1 %. Fifty handpicked *F. composticola* were transferred in 5 ml of each concentration in 5 cm dia. Petri plates with four replications. Sterile water was kept as control. Mortality of *F. composticola* was recorded after 24 and 48 h in NSKWE and compared with control.

RESULTS AND DISCUSSION

The population recovered after the harvest of the crop, was maximum (55.23) at T_4 when the prey was inoculated at spawning and predator at casing Figure 1 d, and minimum (29.76) when the time of inoculation was reversed for both the prey and predator at T_3 (Figure 1 c). The population of *A. swarupi* recovered after harvest was significantly higher in treatments, T_1 and T_4 where prey and predator were inoculated at spawning (44.39) (Figure 1 a) and when prey was inoculated at spawning and predator at casing (55.23) (Figure 1 d) than the treatments, T_1 and T_4 where prey and predator were inoculated at casing (35.7) (Figure 1 b) and when prey was inoculated at spawning and predator at casing (29.76) (Figure 1 d) (Table 1). The spawn run was best in T_5 when



a. Prey and predator at spawning (T_1) b. Prey and predator at casing (T_2) c. Prey at casing and predator at spawning (T_3)

d. Prey at spawning and predator at casing (T_4) e. Uninoculated control (T_5)

Fig. 1: Different management treatments

there was no prey and predator, i.e., uninoculated control (Figure 1 e).

The reproduction factor was maximum (152.77) among all the treatments, when prey was inoculated at spawning and predator at casing (Figure 1 d). In this treatment, the spawn run was also poor. It was minimum (44.51) when prey was inoculated at casing and predator at spawning (Figure 1 c). Spawn run was moderate when both prey and predator were inoculated at spawning (Figure 1 a). It was found good in the treatments where prey and predator were inoculated at spawning and prey at casing with predator at spawning. Significant reduction in the mean weight of buttons between treated and control bags also occurred (Table 1). The maximum yield reduction (60.7 %) was recorded when the prey was inoculated at spawning and predator at casing and the minimum (14.3 %) when the prey was inoculated at casing and predator at spawning. Population of *F. composticola* was not recovered at the time of termination of experiment.

Table 1: Population of *Aphelenchoides swarupi*, spawn run and yield in button mushroom compost

Treatments	Population of *A. swarupi* (200 cc compost)	Rf	Spawn run	Yield per bag (g)	Yield Reduction (%)
Prey and predator at spawning	2006.25 (44.39)	100.31	Moderate	540	50.0
Prey and predator at casing	1279.0 (35.70)	63.95	Good	625	25.6
Prey at casing and predator at spawning	890.25 (29.76)	44.51	Good	720	14.3
Prey at spawning and predator at casing	3055.5 (55.23)	152.77	Poor	327.5	60.7
Uninoculated control	-	-	Good	840	-
CD at 5%	(6.46)	-	-	14.56	-

Number of nematodes inoculated : Prey : 500 per bag Predator : 50 per bag
The figures in parentheses are square root transformedvalues

treatments. The mortality at different periods (24 and 48 h) was non-significant. The treatments where NSKWE was used as 4 %, 2 % and 1 % concentrations, were statistically at par. The mortality of *F. composticola* at 4 %, 2 % and 1 % NSKWE was 99.24 %, 99.24 % and 99 %, respectively. The interaction results between treatments and time periods were found non-significant.

Table 2: Number of *Fictor composticola* killed in different concentrations of neem seed kernel extract after 24 and 48 hours

Treatments	Number of *Fictor composticola* killed after		
	24h	48h	Mean
4% NSKWE	49.75 (7.19)	49.5 (7.16)	49.62 (7.17)
2% NSKWE	49.5 (7.17)	49.75 (7.19)	49.62 (7.18)
1% NSKWE	49.75 (7.19)	49.25 (7.16)	49.5 (7.17)
Sterile water	0.0 (1.41)	0.0 (1.41)	0.0 (1.41)
Mean	37.25 (5.74)	37.12 (5.73)	

CD @ 5%
Treatment (T) =0.04 Period (P) = NS Interaction (T × P) = NS
The data in parentheses representsquare root transformed values

The mortality of nematodes, *F. composticola* at 4 %, 2 % and 1 % concentrations of NSKWE for 24 and 48 hours, is presented in Table 2 which revealed that the treatment where only sterile water was used with *F. composticola*, was significantly very different (lower) with the other three

The spawn run was good in the treatments where both the prey and predator were inoculated at casing time or when prey was inoculated at casing time and predator at spawning time. It was so because by the time of casing, spawn run was complete and *A. swarupi* inoculated at this time, got less time to damage the spawn as compared to when it was inoculated at spawning. *F. composticola* managed the myceliophagous nematodes significantly when inoculated at spawning resulted in good spawn run and maximum yield (Keshari and Pathak, 2019).

F. composticola may have a role in reduction of the number of *A. swarupi*. Population of *A. swarupi* was recorded maximum when it was inoculated at spawning and predator at casing due to the fast multiplication of *A. swarupi* in absence of the predator up to casing, and availability of spawn. The reproduction factor was also maximum in this treatment. Maximum reduction of yield over control (60.7 %) was found when prey was inoculated at spawning and predator at casing. This may be due to the reasons that *A. swarupi* got longer time to multiply, affecting spawn run and, the predator was introduced in the bags at casing time when the temperature was low.

So, *F. composticola* was not able to suppress prey population as after casing the temperature dipped to less than 10 °C affecting the predation and life cycle of *F. composticola*. This also seems the reason for non-recovery of *F. composticola* in the compost. This is also supported by our observations where in spite of presence of high population of *F. composticola* (2700/200 cc compost), it was not recovered in the crop season from the compost in Mushroom Technology Laboratory, Department of Plant Pathology, CCS HAU, Hisar. Bajaj and Kanwar (2015) also suggested that F. composticola can not survive at temperature < 15 °C.

Significant differences in the mean weight of the mushroom buttons between treated and control beds, were recorded. *F. composticola* might have helped in increasing the yield of mushroom indirectly by reducing the populations of *A. swarupi* **.** It may be presumed that *F. composticola* has played role in declining the population of prey at initial stage when temperature was favourable.

In absence of recovery of *F. composticola* at the end of crop season, it can not be concluded with certainty that to what extent the predator controlled *A. swarupi* in compost bags. However, at higher densities under commercial mushroom farming where optimum temperature can be maintained, it may serve as an important bioagent for management of mushroom nematodes.

Majorities of small farmers prepare compost by long method and grow mushroom in traditionally low cost mushroom houses. In the North region of India like Himachal Pradesh, Haryana where mushroom cultivation is done, the temperature goes very low after the month of November (below 10 °C) and at such low temperature, the activity and survival of *F. composticola* are adversely affected. This poses a problem in use of this predator as a biocontrol agent of the mushroom nematodes.

Further studies are required with higher population and repeated release of predator at different timings. The large number of predators increase the probability of encounters between predators and prey, and chances of survival under unfavourable conditions. *F. composticola* also feeds on bacteria. Therefore, its food preference between nematodes and bacteria also needs to be investigated.

For the management of myceliophagous nematodes in mushroom, the chemical method of control is not adopted generally due to residual problem in sporophores. Use of plant parts or their products may provide an alternative as they have been found promising for managing plant parasitic nematodes . This study was planned with a view to integrate NSKWE with *F. composticola*, for the management of *A. swarupi* in button mushroom.

NSKWE (4 % @ 7.5 litre/q compost) has been found effective and recommended to control the myceliophagous nematodes in mushroom without affecting yield**.** But, when NSKWE was tested against *F. composticola* at 1, 2 and 4 % for 24 and 48 hours, even at 1 %, it killed the predator after 24 hours (Table 2), showing its incompatibility with predator. SO, it cannot be recommended with *F. composticola.*

CONCLUSIONS

The treatments where *A. swarupi* was inoculated at spawning, the final population of *A. swarupi* was higher than the treatments where it was inoculated at casing time. The yield was better in the treatments where prey was inoculated at casing. Similarly, the spawn run was good when *A. swarupi* was inoculated at casing and poor when it was inoculated at spawning and *F. composticola* at casing. The reproduction factor of *A. swarupi* was maximum when it was inoculated at spawning and predator at casing but minimum when predator at spawning and prey at casing. *F. composticola* was not recovered after the harvest in any of the treatments. The minimum population of *A. swarupi* was recorded when *F. composticola* was inoculated at spawning although no population of *F. composticola* was recovered from compost at the end of crop season. Under in vitro test, *F. composticola* could not survive even at 1 % neem seed kernel water extract (NSKWE) after 24 h exposure. So, it was not found compatible with NSKWE.

Further studies with its higher inoculum levels under controlled conditions in mushroom and for management of plant parasitic nematodes are required.

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