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Research Note

Biocontrol of *Haematobia irritans* horn flies with entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*)

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ABSTRACT: The bio-control of adult *Haematobia irritans* flies, its eggs and pupae with two fungal agents *viz*, *Beauveria bassiana* and *Metarhizium anisopliae* was studied. High concentration of ($1X10^8$ conidia /ml) *B. bassiana* and *M. anisopliae* showed mortality of 62.5% and 93.7% respectively against eggs of *Haematobia irritans*. The pupae of *H. irritans* treated with high concentration *B. bassiana* and *M. anisopliae* resulted in 50% and 52% mortality respectively. Adult *H. irritans* when treated with high concentration of *B. bassiana* and *M. anisopliae* showed mortality of 90% and 80% respectively. These two fungal agents led to the reduced emergence of adults form treated eggs and pupae. The growth of mycelia was observed on the body dead adult flies after 4 days.

KEY WORDS: *Haematobia irritans*, biological control, *Beauveria bassiana*, *Metarhizium anisopliae*, efficacy.

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Haematobia irritans (horn flies) belonging to the family Muscidae are one of the most important obligate ectoparasites of pastured cattle in many areas in the world and causes anemia, blood loss, reduced weight gain and general weakness.

Horn flies mechanically transmit *Anthrax*, *Trypanosomosis* and they act as biological vector for the filarial nematode *Stephanofilaria stilesi*. The horn flies also cause damage to cattle hide, increasing the occurrence of hyperaemia. *Haematobia irritans* flies may also cause a periorbital and ventral ulcerative dermatitis in cattle and horse the lesions of which may become infected by *Habronema* worms.

Biological control as an alternative is being investigated for control of the horn flies. One of the biological weapon used is entomopathogenic fungi such as *Metarhizium anisopliae, Beauveria bassiana* and *Paecilomyces fumosoroseus* species (Steenberg *et al.*, 2001). A study was therefore undertaken to evaluate the in-vitro efficacy of two fungal agents on *Haematobia irritans* and its developing stages.

Haematobia irritans flies were collected from naturally infested cattle in different farms *viz*., Indo Danish farm, KVAFSU farm and Nandini sperm station, Bangalore during 2010.

In the present study for the biological control of horn flies two entomopathogenic fungi (*Metarhizium anisopliae & Beauveria bassiana*) were used and these were evaluated against different stages of horn flies including eggs, pupae and adults. The seed cultures of these fungi were identified and obtained from the National Bureau of Agriculturally Important Insects of ICAR in Bangalore.

A medium was prepared from Sabouraud dextrose agar with 1% yeast extract (HiMedia Laboratories Pvt. Ltd. Mumbai, India). To 9 gms of Sabouraud Dextrose Agar in a conical flask, 200 ml of distilled water was added and dissolved. It was autoclaved at 121°C for 15 minutes and then 500 ppm of chloramphenicol was added. It was then incubated at 25– 27°C for 21 days to observe the fungal growth. The 21 day old fungi were used in the present study and the following steps were adopted to extract the fungal conidia and spores (Moorhouse, 1993).

The fungus was scraped by using a sterile loop. Then the scrapings were suspended in 9 ml distilled water containing 1% Tween 20. To a sterile dish this suspension was poured and mixed for about 3-4 min. By using a muslin cloth this suspension was filtered. The spores were then counted by using a haemocytometer.

Counting the spores of fungi

The count of conidia per ml of suspension (spores) was done in a haemocytometer.

1. Nine µl of the above cell suspension was carefully added in to the WBC counting chambers and then a cover slip was placed on it and the conidia were counted under 40X magnification.

2. The spores counted in each of the four 0.1 mm^3 corner squares labeled A to D were counted.

The total number of spores was counted in the four corner squares. The spore count was estimated as per the following equation.

Spores/ml = (n) x 10^4

n = the average cell count per square of the four corner squares counted.

Efficacy on eggs

Twenty eggs of *H. irritans* were taken on the cattle manure medium and inoculated with 4 ml of conidial suspension at different concentrations of two fungal agents viz. 1x 10^6, 1x 10^7 and 1x 10^8 conidia /ml.

Control group was sprayed with sterile distilled water with 0.1% Tween 20. These plates were kept in room temperature of 25° ± 2°C. The mortality of eggs was recorded until adult emergence was complete.

Efficacy on pupae

Twenty pupae of less than 48 hr old were taken in petridishes containing two moistened filter paper layers. 4 ml of different concentrations of conidial suspension inoculated on pupae in petridishes. Control group was sprayed with sterile distilled water containing 0.1% of Tween 20. These petridishes were kept at room temperature of 25-27°C & 70% RH. The mortality of pupae was recorded.

Efficacy on adult horn flies

Ten adult flies of less than 48 hr old were immobilized by exposing them to cold temperature of (4 ± 1°C) for 2-3 minutes (Watson *et al*., 1995. Crosby *et al.*, 1991). Then these adult flies were placed on moistened double lined filter paper in a plastic box of 10 cm height X 10 cm diameter and each group was sprayed with different concentrations of two fungal agents.

Control group was sprayed with sterile distilled water with 0.1% Tween 20 (Poprawsky *et al*., 1985). These boxes were kept at temperature 27° ± 1°C. After 48 hrs of inoculation dead adults were counted at 24 hrs interval.

In this study application of the high concentration of 10^8 conidia /ml of *Beauveria bassiana* and *Metarhizium anisopliae* against the eggs of *Haematobia irritans* caused high mortality in larvae and pupal stage up to 62.5% and 93.7%, at the dose of 10^7 concentration 50% and 68.7% mortality was observed and up to 37.5% and 31.5% was found on the application of low dose of concentration of 10^6 conidia/ml of suspension respectively (Table 1).

Angel *et al.* (2005) had reported 33% and 27% reduction in emergence of adult flies after treatment with *Metarhizium anisopliae* and *Beauveria bassiana* on eggs respectively.

In the present study up to 50% and 52% mortality was observed with 10^8conidia/ml. and 38.8% and 36.8% mortality of pupae was observed at dose of 10^7conidia/ml. and low concentration of 10^6 conidia /ml resulted in 22% and 21% mortality. Dead pupae showed growth of mycelia on the body after 4 days of treatment

Laboratory bioassay was conducted by Dinalva *et al*. (2010) who observed that groups of 20 pupae of *Haematobia irritans* flies respectively bathed and sprayed with fungal isolate suspension containing 10^6, 10^7 and 10^8 conidia / ml.

Mortality of pupae was observed at a concentration of 1X10^7 & 1X10^8 conidia / ml of *M. anisopliae* and high pupal mortality was observed at 1X10^8 conidia / ml concentration of *B. bassiana*. In this study different concentration of fungi resulted in variable mortality against pupae of horn flies.

In the present study application of high dose of conidia of 10^8 showed 90% and 80% mortality of adult flies after 3 days of application, 70% mortality occurred with 10^7conidia/ml of both the fungi and at low concentration of 10^6conidia/ml of suspension 50% and 40% mortality was observed respectively.

In vitro trial of B. bassiana on adult horn flies was evaluated by Steenberg *et al.,* 2001 indicating that 100% mortality of adult *H. irritans* was caused by *B. bassiana* caused after seven days of inoculation. They reported higher mortality than that in the present study; it could have been because of the length of the treatment or differences in the concentration used.

Steenberg *et al*. 2001 had reported susceptibility of adult horn fly to another fungus *viz., Paecilomyces farinosus* with mortality rate of 90% after seven days of inoculation. Laboratory bioassay was conducted by Dinalva *et al*. (2010) on groups of 30 adult flies each which were respectively bathed and sprayed with fungal isolate suspensions containing 10^6, 10^7 and 10^8 conidia/ml. *M. anisopliae* and *B. bassiana* induced death in 100% of the flies at the 10^8 conidia / ml suspension.

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Table 1. Comparison of *in vitro* trials with fungi against different biological stages of *Haematobia irritans*

Metarhizium anisopliae				*Beauveria bassiana*			
% mortality of adults	% mortality of pupae	% efficacy on eggs	Concentration	% mortality of adults	% mortality of pupae	% efficacy on eggs	Concentration
90	50	62.5	1×10^8conidia /ml	80	52	93.7	1×10^8conidia /ml
70	38.8	50	1×10^7conidia /ml	70	36.8	68.7	1×10^7conidia /ml
50	22	37.5	1×10^6conidia /ml	40	21	31.5	1×10^6conidia /ml
0	10	20	Control group	0	5	20	Control group

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