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Median Lethal Concentrations and Times of *Beauveria bassiana* (Bals.) Vuill. and M*etarhizium Anisopliae* (Metsch.) Sorokin Against the Third Instar Larvae of *Spodoptera litura* (Fab.)

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ABSTRACT

The median lethal concentrations (LC_{50}) and times (LC_{50}) of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin were determined against the third instar larvae of *Spodoptera litura* (Fab.) by dipping the larvae in fungal spore suspension concentrations varying from 25 to 125x10^4 spores/ml and 100 to 300x10^4 spores/ml, respectively. The LC_{50} recorded with *B.bassiana* and *M. anisopliae* against the third instar larvae of *S.litura* were 20.4x10^4 and 9.4x10^4 spores/ml, and 265.2 x 10^4 and 98.9 x 10^4 spores/ml at nine and eleven days after treatment, respectively. Whereas, the LT_{50} values were ranged from 180.0 to 302.4 hours in case of *B. bassiana* and 196.8 to 256.8 hours in case of *M. anisopliae*.

Key words : Beauveria bassiana, Metarhizium anisopliae, Spodoptera litura

Concerns about the negative effects of chemical insecticides have led to emphasis on biological agents for pest control that are eco-friendly, bio-safe, economically viable and socially acceptable. One such effective group of bioagents is the entomopathogenic fungi, which have been evaluated as pest control products. The most successful by so far has been the fungi belonging to fungi imperfecti. They are *Beauveria bassiana* (Bals.) Vulli. and *Metarhizium anisopliae* (Metsch.) Sorokin that causes fatal white and green muscardine diseases in insects by the production of a cyclodepsipeptide, beauvericin and insecticidal metabolities, destruxins, respectively. Over 200 species of insects in nine orders, mainly Lepidoptera and Coleoptera have been recorded as hosts of *B. bassiana* (Li, 1988). Like wise *M. anisopliae* is known to attack insects covering seven orders including coleopterans as the most common hosts (Yendol and Robert, 1971). Keeping this in view, the present investigations were undertaken with selected strains of *B. bassiana* and *M. anisopliae* to study the efficacy using bioassays that quantified the relationship between conidial dosage and infection, and time required to kill 50 per cent of the test larvae, *Spodoptera litura* (Fab.)

MATERIAL AND METHODS

Pure cultures of *B. bassiana* and *M. anisopliae* were obtained from the biological control laboratory, Acharya N G Ranga Agricultural University, Rajendranagar, Hyderabad and were inoculated aseptically with the respective conidia into Saboraud dextrose agar medium (Delarosa *et al*. 1997) in the Department of Entomology, Agricultural College, Bapatla. After 10 days, the conidia were harvested by gently scraping the surface of the culture with a sterile bacteriological loop and maintained at 4^0C for further use.

Bioefficacy of the test pathogens, *B. bassiana* and *M. anisopliae* against the tobacco cutworm, *Spodoptera litura* (Fab.) was evaluated from the point of median lethal concentrations (LC_{50}) and lethal times (LT_{50}). The third instar larvae of *S. litura* were dipped for few seconds (Desh Mukh and Mathai, 1991) in different concentrations of fungal spore suspensions of *B. bassiana* and *M. anisopliae* prepared by serial dilution. After air-drying, the treated larvae were fed with fresh and clean castor leaves in the laboratory (28±2^0C and 60 to 70% RH).

Twenty larvae per treatment with four replications were maintained for each experiment. The data on larval mortalities were recorded daily till pupation. The dead larvae were maintained in a

Table 1. Median lethal concentrations (LC_{50}) of *B. bassiana* and *M. anisopliae* as pure cultures against the third instar larvae of *S. litura.*

Sl.No	Entomopathogenic Fungi	DAT	χ^2 value	Slope (b)	$LC_{50}x10^4$ spores/ml	Fiducial Limits (95%)
1	*B. bassiana*	9	5.388	1.9942	20.4	17.6 - 25.1
2	*B. Bassiana*	11	0.188	2.3200	9.4	8.6 - 15.7
3	*M. anisopliae*	9	6.102	0.1462	265.2	219.5 - 386.1
4	*M. anisopliae*	11	1.162	0.2097	98.9	63.3 - 122.9

DAT: Days after treatment

Table 2. Median lethal times (LT_{50}) for different concentrations of *B. bassiana* as pure culture against the third instar larvae of *S. litura.*

Sl.No	*B. bassiana* Concentrations spores/ml	χ^2 value	Slope (b)	LT_{50} (Hours)	Fiducial Limits (95%)
1	$5x10^4$	0.011	1.3927	*	*
2	$10x10^4$	1.353	2.1745	302.4	217.2 - 384.0
3	$15x10^4$	0.481	3.2181	261.6	244.8 - 292.8
4	$20x10^4$	3.068	4.7500	220.8	206.4 - 242.4
5	$25x10^4$	0.176	5.3960	180.0	163.2 - 196.8

*: LT_{50} could not be calculated as 50 per cent larval mortalities were not resulted.

controlled humidity chamber to stimulate the development of fungal mycelia to confirm that the death of the larvae was due to fungal infection. The larval mortality data was subjected to probit analysis (Finney, 1981) using multiple linear programme on computer.

RESULTS AND DISCUSSION

The LC_{50} values of *B. bassiana* at nine and eleven days after treatment (DAT) were $20.4x10^4$ and $9.4x10^4$ spores/ml. Whereas, the LC_{50} values of *M. anisopliae* at nine and eleven DAT were $265.2x10^4$ and $98.9x10^4$ spores/ml, respectively. The values of x^2, regression equations and fiducial limits corresponding to different LC_{50} values are also presented in the table1.

The present LC_{50} of $9.4x10^4$ spores/ml for *B. bassiana* as pure culture after eleven DAT is in agreement with the earlier findings of Sivasankaran *et al*. (1990) against the second and third instar larvae of *Chilo infuscatellus* Snellen (10^5 spores/ml) and Manjula and Padmavathamma (1999) against the third instar larvae of *Helicoverpa armigera* (Hub.) (10^5 spores/ml). Similarly for *M. anisopliae* the present LC_{50} of $265.2x10^4$ spores/ml obtained was almost nearer to the value reported by Ajay Kumar Pandey and Kanaujia (2003) against the fourth instar larvae of *S. litura* ($2.65x10^6$ conidia/ml).

Similarly, the present LC_{50} of *M. anisopliae* as pure culture after eleven DAT *i.e.,* $98.9x10^4$ spores/ml obtained in the present investigation is in close agreement with the earlier findings of Daykar and Kanaujia, 2001 ($12.53x10^5$ conidia/ml) against the second instar larvae of *S. litura*, Dayakar and Kanaujia, 2003a ($14.85x10^5$ conidia/ml) against the third instar larvae of *S. litura* and Dayakar and Kanaujia, 2003b ($9.8x10^5$ conidia/ml) against the second instar larvae of *Ergolis merion* L.

Table 3. Median lethal times (LT_{50}) for different concentrations of *M. annisopliae* as pure culture against the third instar larvae of *S. litura.*

Sl.No	*M. annisopliae* Concentrations spores/ml	χ^2 value	Slope (b)	LT_{50} (Hours)	Fiducial Limits (95%)
1	$100x10^4$	1.532	3.1563	256.8	244.8 - 273.6
2	$150x10^4$	3.074	3.9563	240.0	230.4 - 252.0
3	$200x10^4$	0.353	4.1600	237.6	228.0 - 247.2
4	$250x10^4$	4.261	5.5084	199.2	187.2 - 211.2
5	$300x10^4$	4.014	5.9606	196.8	184.8 - 211.2

The LT_{50} values of *B. bassiana* against *S. litura* were 180.0, 220.8, 261.6 and 302.4 hours at $25x10^4$, $20x10^4$, $15x10^4$ and $10x10^4$ spores/ml concentrations, respectively. At $5x10^4$ spores/ml concentration *B. bassiana* did not result in 50 per cent larval mortality (Table 2). Lack of sufficient number of spores that adhere to the body of *S. litura* larvae to result in 50 per cent mortality might be the reason for not attaining LT_{50} values in the lower concentration $5 x10^4$ spores/ml. Whereas, the LT_{50} values of *M. anisopliae* against *S. litura* larvae were 196.8, 199.2, 237.6, 240.0 and 256.8 hours at $300x10^4$, $250x10^4$, $200x10^4$,$150x10^4$ and $100x10^4$ spores/ml, respectively (Table 3). The values of x^2, regression equations and fiducial limits corresponding to the LT_{50} values of *B. bassiana* and *M. anisopliae* are presented in Tables 2 and 3, respectively.

The present LT_{50} valves 180.0 to 302.4 hours for *B. bassiana* as pure culture were in close proximity with the earlier reports of Sivasankaran *et al.,* 1990 (110.4 to 148.8 hours) and Dayakar and Kanaujia, 2003a (123.12 to 154.8 hours) against the second and third instar larvae of *C. infuscatellus* (10^5 spores/ml) and third instar larvae of *S. litura* ($14.85x10^5$ spores/ml), respectively.

Similarly, the present LT_{50} values of *M. anisopliae* against *S. litura* larvae, 196.8 to 256.8 hours were in close proximity with the LT_{50} values of 123.12 to 154.8 and 168.24 hours reported earlier by Dayakar and kanaujia (2003a) and Ajay Kumar Pandey and Kanaujia (2003) against the third (1.73 to $6.42x10^6$ conidia/ml) and fourth (14.85 to $71.69x10^5$ conidia/ml) instar larvae of *S. litura*, respectively.

The differences in the LC_{50} and LT_{50} values of the present study compared to the earlier reports of both the pathogens might be due to variations in the test insects, concentrations tested and strains of entomopathogenic fungi used.

Further, the LC_{50} and LT_{50} values of both the entomopathogenic fungi tested decreased with increase in the period of exposure of the larvae to the pathogens and increase in the spore concentrations tested. This might be due to nutritional stress because of reduced food consumption in the infected larvae that enhances the insect susceptibility to fungal diseases (Ramoska and Todd, 1985). The fungal metabolites produced during the early stages of infection process act initially to stimulate and then inhibit food consumption (Fargues *et al.,* 1994).

Thus from the results it is concluded that between the two entomopathogenic fungi, either from the pathogenicity or the susceptibility of *S. litura* point of view, *B. bassiana* is more efficacious.

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