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INTERACTION OF SOME GREEN MUSCARDINE FUNGI WITH LABORATORY CULTURED BENEFICIAL INSECTS

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Abstract

The entomopathogenic fungi *Metarhizium flavoviride* and *Nomuraea rileyi* used for the management of fruit borer were tested for their safety to some of the laboratory reared beneficial insects *viz., Trichogramma chilonis* (Ishii.), egg-larval parasitoid, *Chelonus blackburni (Cam.)* and pupal parasitoid, *Tetrastichus israeli* (Fer.). In the laboratory experiment there was no significant harmful effect on *T. chilonis.* The variations observed in parasitoids were also not significant for many parameters observed. Thus, infection by *N. rileyi* did not affect the development of parasitoid in the host eggs. Emergence of egg-larval parasitoid *C. blackburni* from *M. flavoviride* infected *Helicoverpa* larvae was affected by fungi in laboratory conditions. The emergence of parasitoid from 100 host eggs was 12 to 46 per cent in comparison with control (65%). The fungi *M. flavoviride* and *N. rileyi* were not harmful to the adults of *T. israeli* and the variation recorded from control was not significant although adult emergence was affected in the laboratory conditions. As far as *M. flavoviride* was concerned, there was no variations from control.

Key Words: Entomopathogenic fungi; *Metarhizium flavoviride; Nomuraea rileyi;* beneficial insects; *Trichogramma chilonis*.

Introduction

Trichgramma is an egg parasitoid belonging to family Trichogrammatidae. It is a naturally occurring biocontrol agent. The parasitoid is well exploited for large scale field use for the management of lepidopteran pests. Fungi are not highly host specific due to their unique mode of action *i.e.,* cuticular penetration. Hence, they need to be seen in relation to their pathogenicity to benefical insects of crop pests. The fungus *Metarhizium* was reported to be safe to predators like and *Lycosa pseudoannulata* (Bosenberg et Strand) and parasitoids like *Trichogramma japonicum* (Ishii) (Rao, 1989; Urs and Govindu,1971). Predatory insectsincluding coccinellids were reported to be susceptible to *Metarhizium* (Megalhaes *et al*., 1988). *Microplitis croceipes* (Cresson), a parasitoid attacking *Helicoverpa* larvae was susceptible to *N. rileyi* infection (Powers *et al.,* 1986). Parasitised larvae were much more susceptible to *N. rileyi* infection (King and Bell, 1978). *Metarhizium* is a potential biopesticied worldwide (Kirshmeir *et al*., 2004). In the present study, safety of *Metarhizium* and *Nomuraea* were studied against laboratory certain laboratory reared beneficial insects.

Materials and methods

Experiments were conducted to test the safety of the fungi to the biocontrol agents used for the management of tomato fruit borers viz., egg-parsitoid, *Trichogramma chilonis* (Ishii.), the egg-larval parasitioid, *Chelonus blackburni* (Cameron.) and the pupal parasitoid, *Tetrastichus israeli* (Fer.). Test insects were obtained from Center for Plant Protection Studies, Coimbatore for the study.

Trichogramma

The safety of fungus to the egg parasitoid was studied by treating the eggs of host insect, *Corcyra*

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cephalonica (Stainton). Freshly laid eggs of *C. cephalonica* were pasted on strips of cards (2 x 5 cm) at the rate of 200/card. Each treatment was replicated three times. Conidial suspension containing 10^6, 10^8, 10^{10}, 10^{12} conidia per ml was applied on egg card with the help of an atomizer, shade dried for 5 to 10 min and kept in polythene bags and secured by a rubber band at room temperature.

Egg cards sprayed with distilled water served as control. The experiment was conducted in Completely Randomised Design. Newly emerged parasitoids of *T. chilonis* at the rate of 50 parasitoids per treatment were released on the treated egg cards. The parasitoids were allowed to parasitise *C. cephalonica* eggs for two days and then removed. Upon death, the parasitoids were observed for mycosis. *Corcyra* larvae emerging from unparasitised eggs were removed daily. Observations on the per cent parasitism, per cent adult parasitoid emergence, total life cycle and adult longevity were recorded.

C. blackburni

Newly emerged *C. blackburni* adults were allowe d to parasitise the fungus treated *Corcyra* egg cards for 4¸h at the rate of 10 parasitoids per card. The parasitoid egg cards were transferred to plastic containers (3 x 8 cm) containing broken pearl millet grains (100 g) and covered with muslin cloth. Parasitoids were observed for mycosis after death. Data on parasitoid emergence was recorded daily from the fourth week onwards. The experiment was conducted with *M. anisopliae, M. flavoviride* and *N. rileyi* respectively. The treatments were replicated thrice with twenty pupae per application.

T. israeli

Newly emerged parasitoids were allowed to parasitise fungus infected pupae of *Spodoptera* and observed for the number of adults emerged from pupa and adult longevity. The treatments were replicated three times with 20 pupae per replication.

Statistical analysis

The data expressed in terms of percentage in different experiments were transformed to corresponding angles (arc sine percentage). Data on larval population from field experiments were transformed to as per the method developed by Poisson for statistical analysis (Panse and Sukhatme, 1957) and analysis of variance was done.

Results

The entomopathogenic fungi used for the management of fruit borer were tested for their safety to egg parasitoid, *Trichogramma chilonis* (Ishii0), egg- larval parasitoid, *Chelonus blackburni* (Cam.) pupal parasitoid, *Tetrastrichus israeli* (Fer.).

T. chilonis

In the laboratory experiment, there was no signif icantharmful effect of *Nomuraea rileyi* (Sam.) on the parasitoid on various parameters observed in the study (Table 1). The variations observed in percentage parasitism, adult emergence, adult life span and total life-span were also not significant. Parasitoid emergence from host eggs was not affected (Fig1.). Thus, infection by *N. rileyi* did not affect parasitoid development in the host eggs. However, percentage parasitism of host eggs by *T. chilonis* was affected by *M. flavoviride* at all concentrations tested. It was 70.36 to 73.30 per cent in *M. flavoviride* treated trichocards. There was no significant variations among the concentrations of the fungus.

Table 1 . Effect of topical application of entomopathogenic fungi on egg parasitoid *Trichogramma chilonis*

Sl. No.	Treatments (spores /ml)	Parasitism (%)	Adult emergence (%)	Adult longevity (days)	Total life – span (days)
1	*M. flavoviride*(10^6)	73.46	92.3 ± 0.1	3 ± 0.2	9.8 ± 0.1
2	*M. flavoviride*(10^8)	71.00	90.3 ± 1.2	3 ± 0.1	10.1 ± 0.1
3	*M. flavoviride* (10^{10})	70.70	91.5 ± 0.6	3 ± 0.2	10.1 ± 0.1
4	*M. flavoviride* (10^{12})	73.30	90.6 ± 1.1	3 ± 0.2	10.2 ± 0.1
5	*N. rileyi* (10^6)	75.10	89.5 ± 0.2	3 ± 1.6	10.0 ± 0.1
6	*N. rileyi* (10^8)	80.00	93.8 ± 0.7	3 ± 1.2	9.8 ± 0.1
7	*N. rileyi* (10^{10})	76.50	90.2 ± 0.2	3 ± 0.2	10.1 ± 0.2
8	*N. rileyi* (10^{12})	78.20	88.5 ± 0.1	3 ± 0.8	10.1 ± 0.4
9	Untreated check	82.60	92.8 ± 0.1	3 ± 0.2	10.1 ± 1.0

(replications: 3)
CD = 7.10
n.s.- (P=0.05) non significant

C. blackburni

Variations observed in parasitism, adult longevity and total life-span were not significant among the treatments. However, emergence of egg-larval parasitoid from fungus infected *Helicoverpa* larvae was affected at all the doses of fungus applied in laboratory conditions. The emergence of parasitoid from 100 host eggs contaminated by *N. rileyi* spores was only 13.60 per cent for conidial spray at a concentration of 10^{12}/ ml and 46.00 for *N. rileyi* sprayed at 10^{12} spores/ml (Table 2). Adult emergence from *M. flavoviride* infected larvae ranged from 12.00 to 32.60 per cent in various concentrations of the fungus. This harmful effect was relatively higher (32.60%) than *N. rileyi* (46.00%). It was highly significant compared to the untreated check (65.00%). This showed

that *C. blackburni* was affected by the two fungi in the laboratory conditions.

Table 2. Effect of topical application of entomopathogenic fungi on egg- larval Parasitoid, *Chelonus blackburni*

Sl. No.	Treatments (spores /ml)	Parasitism (%)	Adult emergence (%)	Adult longevity (days)	Total life – span (days)
1	*M. flavoviride* (10^6)	72.3 ± 1.9	32.6 ± 1.6	8 ± 0.2	42.0 ± 1.0
2	*M. flavoviride* (10^8)	68.9 ± 1.5	24.1 ± 1.1	8 ± 0.1	46.7 ± 1.3
3	*M. flavoviride* (10^{10})	68.1 ± 2.2	14.2 ± 0.5	8 ± 0.2	36.1 ± 2.0
4	*M. flavoviride* (10^{12})	71.2 ± 0.1	12.0 ± 2.2	8 ± 0.2	36.0 ± 1.5
5	*N. rileyi* (10^6)	73.5 ± 0.1	46.0 ± 3.1	8 ± 1.6	34.9 ± 2.0
6	*N. rileyi* (10^8)	67.0 ± 1.6	28.3 ± 1.1	8 ± 1.2	40.4 ± 1.1
7	*N. rileyi* (10^{10})	70.0 ± 1.8	22.1 ± 2.5	8 ± 0.2	39.5 ± 0.6
8	*N. rileyi* (10^{12})	74.0 ± 2.2	13.6 ± 3.0	8 ± 0.8	41.0 ± 1.0
9	Untreated check	74.0 ± 0.5	65.0 ± 1.1	8 ± 0.2	41.3 ± 2.1

(replications: 3)
CD = 2.66 n.s.*
n.s. (P=0.05) * non significant

T. israeli

M. flavoviride was relatively safe to the development of the pupal parasitoid in comparison to *N. rileyi.* In *N. rileyi* infected larvae, the number of pupal parasitoids emerged were mere 15 to 28 adults per pupa. In untreated check, it was 220 adults per pupa. Other parameters of parasitoid development were not affected. The fungi *M. flavoviride* and *N. rileyi* were not harmful to the adults of *T. israeli* and the variations recorded from control was not significant (Table 3). Green muscardine fungi were safe to several species of spiders cultured in the captivity. Yet there was a case of mycosis caused by *Beauveria bassiana* (fig 1C). This showed differential pathogenicity of green muscardines and white muscardines. This is taken up for further investigation.

Table 3. Effect of topical application of entomopathogenic fungi on pupal parasitoid *Tetrastichus* spp.

Sl. No.	Treatments (spores /ml)	Parasitism (%)	Adult emergence (%)	Adult longevity (days)	Total life – span (days)
1	*M. flavoviride*(10^6)	86.4±2.3	192 ±12	13 ± 0.5	42.0 ±1.0
2	*M. flavoviride*(10^8)	84.3±1.0	186 ± 9	13 ± 0.1	46.7 ±1.3
3	*M. flavoviride*(10^{10})	82.3±1.8	189 ± 8	13 ± 0.6	36.1±2.0
4	*M. flavoviride*(10^{12})	92.8±1.3	161 ± 5	13 ± 0.2	36.0 ±1.5
5	*N. rileyi* (10^6)	89.0±0.5	28 ± 3	13 ± 0.6	34.9±2.0
6	*N. rileyi* (10^8)	86.5±0.2	22 ± 2	13 ± 1.2	40.4 ±1.1
7	*N. rileyi* (10^{10})	92.6±0.8	20 ± 1	13 ± 0.1	39.5±0.6
8	*N. rileyi* (10^{12})	96.0±0.2	15 ± 3	13 ± 0.4	41.0 ±1.0
9	Untreated check	91.5±2.0	220 ± 1	13 ± 0.2	41.3 ±2.1

(replications: 3)
CD =8.5 n.s. ; n.s. = (P=0.05) *non significant

C. carnea: Third instar grub of the green lace wing was sprayed with fungal spore suspension and provided with *Corcyra* eggs as prey and observed for mortality due to mycosis. Each treatment was replicated three times with 20 third instar grubs per replication. The experiment was conducted with *M. anisopliae, M. flavoviride* and *N. rileyi.* The results showed no pathogenicity. All insects were survived without significant moratlity and were similar to control group.

Fig. 1. Left: storage of trichocards
Fig. 1 Right : Top: parasitisation of host eggs by parasitoid; Middle: Healthy trichocard; Bottom: Trichocard infected by green muscardine fungus under forced paper strip exposure and storage; early mycosis and complete mycosis

Discussion

Entomopathogenic fungi are polypathogenic in nature provided the wounds in the cuticle and haemocoelic entry of propagules are ensured. There are several earlier reports of fungal infection in natural enemies like, hibernating cocinellids, carabids, syrphids, notonectids, salticid spiders, pteromalids, hymenopterans (Fargues, 1981). However, safety record of fungi to the natural enemies of *Helicoverpa* is not available. Adults of egg parasitoid, *Trichogramma chilonis*, was found not infected by the fungi in the laboratory conditions in the present studies. Adult life-span, total life-span and adult emergence remained unaffected. However, it was possible to infect the host egg with fungi. Percentage parasitism of the parasitoid emerging from the *M. flavoviride* treated host egg was affected. The number of hosts that adults of *Trichogramma* can parasitise when the hosts are available depends on variations in fecundity. This fecundity is influenced by microbial associations (Girin and Bouletreau,1995). From the study, it can be presumed that fungus *M. flavoviride* might have had

negative association with the parasitoid while *N. rilleyi* did not. The trichocards stored under refrigerated condition could get fungal infection. This imply that trichocards should be stored without fungal contamination. Otherwise, this contamination could be a limiting factor in the storage and shelf-life of the parasitoid. Reports on egg infection by pathogenic fungi are scarce. Wester (1910) observed that in freshly laid eggs, egg shells of lepidopterans had no chitin and hence fungi did not attack. In this study, eggs were also affected. This could be due to the fact that as the development of embryo occurred, blastoderm cells lay down a membrane upon chorion which contained chitin. At this stage, *Corcyra cephalonica* (Stainton) eggs became susceptible. Moreover, in trichocards, the egge are in dense array (15000 to 18000 eggs/card) and Steinhaus (1949) reported crowding as a factor in fungal disease in the field conditions as regard to natural enemies. Rao (1989) reported that conidial spray of *M. anisopliae* and *M. flavoviride*, at a concentration of 10^8/ml and reported no adverse effect on *Trichogramma japonicum*, (Ashmed), similar to the present conformation. Also, he confirmed the safety to *Tetrastichus shoenobii* (Ferriere).

In the case of larval and pupal parasitoids, infection of the host and the sequence of host attack play an important role. Emergence of *Chelonus blackburni* (Cam.) was only 22% in fungus infected *Helicoverpa*, larvae. Similarly this emergence of pupal parasitoid, *T. israeli*. In the studies on interactions between larval parasitoid, *Microplitis croceipes*, *N. rileyi*, and laboratory reared fruit borer larva, *H. zea* parasitised larvae, infected hosts were found to be more susceptible to fungus (King and Bell, 1978). This is in confirmity with the observations made in respect of entomopathogenic fungus on *C. blackburni*. Fungal infection of the host stage was detrimental to the development of the parasitoid in *Corcyra* larva. The fungi under study did not harm the parasitoids directly by virtue of mycosis in parasitoid insect, but the depletion of contents of the host due to mycelial ramification probably could be the reason. The competition for the nutrients among the developing parasitoid and host stage could result in harmful effect on parasitoid emergence.

In the field conditions, pest - defender ratio is highly skewed in favour of the pest only. Hence, the possibility of these parasitoids being infected by fungi is less. Field tests conducted by Jones and Poprawski (1996) also conformto this. They demonstrated that mycoinsecticides are less lethal to the foraging parasitoids and fungus was unable to colonise hymenopteran parasitoids. With this exhaustive information it is necessary to deal the safety aspects of fungi in a case by case approach. The knowledge of the specificity of fungi, already used or promising candidates for use against pests and of the mechanism playing role in determining the host specificity, is an essential factor for the choice of fungal agent. The observations recorded in this study emphasize the need to keep the commercial insectaries meant for parasitoid production, free from contamination by fungi. The selected agent should be tested by oral ingestion and infection through wounds before large scale field application especially in crop ecosystems rich in diversity of natural enemies. Spore applications were made to host egg cards. The results generally demonstrated that fungus was unable to colonise the eggs. No infection of parasitoid was successful. Rates of successful parasitism were abobe 90 per cent. Adult egg parasitoids emerged without any difficulty. Field tests in vegetables especially tomato demonstrated that application of the the fungus *Nomuraea rileyi* against fruit borer was less lethal to foraging parasitoids than insecticides.

References

Girin, C. and M. Bouletreau : Experientia, 51, 398-400, (1995).

Fargues, J: Comprehensive Insect Physiology, Biochemistry and Pharmacology., p.313-346, 12. eds., Kerkut, G.A. and L.I. Gilbert, Pergamon Press, New York, (1981).

Jones, W.A. and T. J. Poprawski: Proceedings of XX International Congress of Entomology, August 25-31, held in Firenze, Italy, (1996).

King, E.C. and J.V. Bell: J. Invertebr. pathol., 31: 337-340, (1978).

Kirchmair, M, L. Huber, M. Porten and J. Rainer and H. Strasser, 2004. BioControl 49: 295–303.

Magalhaes, B.P., J.C. Lord., S.P. Wraight., R.A. Daoust and D.W. Roberts: J. Invertebr. Pathol., 52: 471-473, (1988).

Panse, V.G. and P.V. Sukhatme: Statistical methods for agricultural workers. Second edition. 328pp. ICAR, New Delhi, (1957).

Powers, W., N. Wilding., P.J. Brobyn, and S.J. Clark : Entomophaga, 31, 293 - 302, (1986).

Rao, R.M: Ph.D thesis submitted to Tamil Nadu Agricultural University, Coimbatore, pp 138, (1989).

Steinhaus, E.A: Principles of Insect Pathology, McGraw Hill Pub., New York, (1949).

Urs, N.V.R.R. and H.C. Govindu: Mycopathol. Mycol. Appl., 44 : 317-320, (1971).

Wester, D.H: Biol. Tiere, 28, 531-558, (1910).