LABORATORY EXPERIMENTS ON MICROBIAL CONTROL OF *PITYOGENES CHALCOGRAPHUS* L. (COL., SCOLYTIDAE) WITH ENTOMOPATHOGENIC FUNGI

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ABSTRACT

The entomogenous fungi *Beauveria bassiana* (Bals.) Vuill. (Bba 3), *Metarhizium anisopliae* (Metch.) Sorok. (Ma 3, Ma 6 & Ma 43), *Paecilomyces fumosoroseus* (Wize) Brown et Smith (Pfr 11) and *P. cicadae* (Miquel) Samson (Pc 3) were tested in the laboratory for virulence on *Pityogenes chalcographus*. 20 adults of *P. chalcographus* contaminated with conidia by releasing on agar plates for two minutes were reared on bark of *Picea abies* (4 x 4 cm). *B. bassiana*, *M. anisopliae* (Ma 3, Ma 6, Ma 43) and *P. fumosoroseus* caused 100 percent mortality within 10 days, while in control and *P. cicadae* that was 20 and 30 percent, respectively. Conidial suspensions of *B. bassiana* (Bba 3), *M. anisopliae* (Ma 6) and *P. fumosoroseus* (Pfr 11) in Tween 80 (0.1%) were sprayed at the concentration of 2 x 10^7 conidia/ml on logs (40 cm x 15-20 cm dia.) of *P. abies*. 410 *P. chalcographus* adults were released in each treatment and mortality was estimated from the emergence of adults in next generation. Population in next generation was reduced in the order, *B. bassiana* (46.9%), *M. anisopliae* (15.1%) and *P. fumosoroseus* (1.4%).

Key words: *Pityogenes chalcographus, Beauveria bassiana, Metarhizium anisopliae, Paecilomyces fumosoroseus* and *P. cicadae*.

INTRODUCTION

Spruce bark beetle, *Pityogenes chalcographus* L. (Col., Scolytidae) is a notorious pest of *Picea abies* L. in Western Europe. It becomes particularly serious after extensive storm felling and in warm summers. The beetles bore and feed beneath bark, because of that withering starts from top and trees ultimately die. In case of felled wood, quality is impaired which results in reduced market value. In addition, logs also serve as breeding sites for the pest. So far, integrated control practices carried out, include debarking, use of pheromone traps, complete wetting of logs, planting trap trees and insecticide application. Except insecticides, all other practices are mainly of precautionary nature, so main emphasis is laid on pesticides. But the pesticides are hazardous to the ecosystem, thence attempts are being done to minimize their uses. Therefore, it is the need of time to reorientate integrated control programmes.

Entomopathogenic fungi are well known to attack many agricultural and forest insect pests. Among these, *Metarhizium anisopliae* (Metch.) Sorok. and *Beauveria bassiana* (Bals.) Vuill. are distributed world wide.They have more than several hundred insect hosts, mainly coleopterous and lepidopterous (Zimmermann, 1993; Feng et al., 1994). *B. bassiana* has given 44.7 and 62.5 percent mortality in scolytid beetle (*Blastophagus piniperda* L.) when sprayed one week and just before they bored, respectively (Nuorteva and Salonen, 1968). Similarly, Doberski (1981 a) obtained different levels of pathogenicity in

Scolytus scolytus larvae and adults by contaminating with *B. bassiana*, *M. anisopliae* and *Paecilomyces farinosus* in laboratory. In addition, he has also found an influence of temperature and relative humidity on the development of infection by these three pathogens in *S. scolytus* (1981b). Wulf (1983) has described that *B. bassiana* penetrates through the cuticle of *P. chalcographus* and infests muscles and fat tissues. After death, mycelium emerges on body surface and under favourable conditions it starts sporulation. Humidity of bark plays a principal role in the development of both fungus and beetle rather than the relative humidity outside the galleries.

Pehl and Kehr(1993) have reported 95.3% mortality in *P. chalcographus* adults contaminated directly with *M. anisopliae* conidia. When the conidia were sprayed in aqueous suspension on bark of *P. abies*, 26.8% population reduction was recorded in the next generation.

Keeping these findings in view, the present study was conducted in the laboratory with various entomogenous fungi to test their pathogenicity on *P. chalcographus* adults to control the pest.

MATERIALS AND METHODS

Virulence Tests

Six fungus isolates, i. e., *B. bassiana* (Bals.) Vuill. (Bba3), *M. anisopliae* (Metch.) Sorok. (Ma3, Ma6 & Ma43), *P. fumosoroseus* (Wize)Brown et Smith (Pfr11) and *P. cicadae* (Miquel) Samson (Pc3) were tested against *P. chalcographus* L. For each test isolate, 20 beetles were released on sporulating cultures grown on malt extract (3%)-peptone (0.3%)-agar for 2 minutes (Müller-Kögler, 1966). Then, adults contaminated with fungal propagules were released on bark pieces (4x4cm) of *P. abies* in petri dishes (8.5cm dia.) and stored at 23°C and 65±5%RH. In the control, 20 beetles were set free on the same size of bark without any treatment. After 4 and 8 days of the treatment, excavated frass was weighed to know the extent of damage and activity of the bark beetles. On 10th day galleries were opened to record mortality.

Application on Bark

One strain of *B. bassiana* (Bba3), *M. anisopliae* (Ma3) and *P. fumosoroseus* (Pfr11) were used. Conidia from one month old cultures grown on malt extract-peptone agar (Merck) were washed in Tween 80(0.1%). The suspension was shaked for 10 minutes on reciprocal shaker and conidia were counted with the help of a haemocytometer from 1:100 dilution. Consequently, one concentration of $2x10^7$ conidia/ml for all test strains was prepared. Of the same concentration germination test was carried out. 95±3% conidia of the three test fungus species germinated in germination test.

Four trees of *P. abies* were felled and from each tree four stem pieces, 40cm long and 15-20cm dia., were selected. These 16 pieces were mixed together and then divided into four groups at random. Thus per variant(including check) four logs were sprayed. In the control only Tween 80(0.1%) was taken. Each log was sprayed with about 45ml using a common hand spray machine. After treatment, logs were dried at room temperature and transferred to cages (70x45x45cm). Later on 410 adults of *P. chalcographus* were released on 1st (210) and 2nd (200) day after spraying, respectively. After two months, photoeclectors were built (70cmx45cmx45cm) to collect newly emerging beetles. The experiment was carried out at 20°C and 65-70%RH.

RESULTS

Virulence Tests

The contaminated beetles had bored into the bark within 20-30 minutes and started to excavate frass. The results on the frass activity and the mortality are displayed in Table 1. The frass, excavated by the beetles contaminated directly with *B. bassiana* (Bba3) and *M. anisopliae* (Ma3, Ma6 & Ma43) was reduced more than 50 percent within

8 days. Feeding was stopped in the treatments of *B. bassiana* and *M. anisopliae* after 4 days, but it remained continuous in *P. fumosoroseus, P. cicadae* and in the control. In second 4 days 8.47%, 42.86% and 30.14% more frass was produced by the adults contaminated with *P. fumosoroseus, P. cicadae* and in the control, respectively. However, there was no significant difference in frass excavation in *P. cicadae* and the control treatments. Within 10 days, 100% mortality was found in all test isolates of *B. bassiana, M. anisopliae* and *P. fumosoroseus*; whereas 30 % were recorded by *P. cicadae* and 20% mortality in the control.

Table 1: Frass excavated (g) and mortality (%) of *P. chalcographus* contaminated with entomogenous fungi.

Entomogenous fungi	4 days (g)	8 days (g)	Mortality (%)
Control	0.73 -	0.95 -	20**
B. bassiana 3	0.41 (-43.8%)*	0.41 (-56.8%)*	100
M. anisopliae 3	0.43 (-41.1%)	0.43 (-54.7%)	100
M. anisopliae 6	0.42 (-42.4%)	0.42 (-55.8%)	100
M. anisopliae 43	0.39 (-46.6%)	0.39 (-58.9%)	100
P.fumosoroseus 11	0.59 (-19.2%)	0.64 (-32.6%)	100
P. cicadae 3	0.70 (-04.1%)	1.00 (+5.3%)	30

* reduction over control calculated with the formula **(Fc-Ft)÷Fcx100**, where Fc frass in control and Ft frass in respective entomogenous treatment.

** mortality after 10 days

Application on bark

Two months after spraying logs and releasing the bark beetles, newly emerged *P. chalcographus* adults were collected from photoeclectors to observe the extent of control in terms of percent reduction in the population within the next generation (Table 2). The results have indicated that overall pattern of emergence of adults was same in all treatments. Population was significantly reduced by *B. bassiana* throughout the emergence period. During the first two weeks, emergence of adults was very slow in *B. bassiana* treatment and similarly, at the end of the 9th week (26.8.-09.9.94) emergence was reduced considerably. Maximum adults were emerged in the 5-9th week in all variants. Maximum reduction in population over control was incurred by *B. bassiana* (46.9%) followed by *M. anisopliae* (15.1%) and *P. fumosoroseus* (1.4%).

Table 2: **Number of *P. chalcographus* adults emerged from stem logs treated with different entomogenous fungi.**

Period	Control	*B. bassiana*	*M. anisopliae*	*P. fumosoroseus*
15.7-21.7.94	30(7.3%)	10(2.4%)	16(3.9%)	19(4.6%)
22.7-28.7.94	23(5.6%)	11(2.7%)	12(2.9%)	21(5.1%)
29.7-04.8.94	72(12.6%)	98(23.9%)	96(23.4%)	102(24.9%)
05.8-11.8.94	185(45.1%)	136(33.1%)	208(50.7%)	264(64.4%)
12.8-18.8.94	328(80.0%)	210(51.2%)	281(68.5%)	349(85.1%)
19.8-25.8.94	307(74.8%)	239(58.3%)	308(75.1%)	299(72.9%)
26.8-01.9.94	331(80.7%)	116(28.3%)	280(68.2%)	307(74.9%)
02.9-08.9.94	367(89.5%)	149(36.3%)	264(64.3%)	280(68.3%)
09.9-15.9.94	258(62.9%)	98(23.9%)	191(46.5%)	251(61.2%)
16.9-22.9.94	162(39.5%)	45(10.9%)	96(23.4%)	142(34.6%)
Total	2063	1112	1752	2034
% reduction		46.9%	15.1%	1.4%

% calculated on the basis of 410 beetles

% reduction = **(Pc-Pt)÷Pcx100**, where Pc population in control and Pt population in entomogenous treatment.

DISCUSSION

Results indicate that the amount of frass produced by beetles is directly related to mortality and can be taken as a factor to judge the efficacy of test pathogens. In pathogenicity tests, 100 percent mortality by *B. bassiana* and *M. anisopliae* displays the capability of these fungi as promising biocontrol agents against the spruce bark beetle. In the presence of a suitable concentration of the pathogen, control mortality can be secured by *B. bassiana* and *M. anisopliae* and more than 50% damage of logs can also be avoided. The mortality caused by *M. anisopliae* is comparable with that of Pehl and Kehr (1993) but reduction in population, by spraying in the next generation, is incomparable because of differences in the climatic conditions. These have profound impact on the development of infection and germination of fungal propagules (Walstad et al., 1970; Doberski, 1981b; Zimmermann, 1982; Wulf, 1983). Temperature and humidity during trials were 20°C and 65-70%, while optimum temperature and humidity determined for these fungi are about 23-25°C and 90%RH. These are the same climatic conditions which favour epidemics of the pest. The difference in these factors may have played a role in low reduction of population (46.9% & 15.1% by *B. bassiana* and *M. anisopliae*, respectively) of the beetle on sprayed logs.

Another decisive factor for efficient microbial control is the contamination and infection of the host which depends upon the formulation of fungal propagules. Formulation is selected according to the behaviour of the pest, e.g. when *B. bassiana* was used against *Pantorhytes plutus* beetle, the estimated value for the LD_{50} of oil formulation was 36 times lower than the water formulation, and the LD_{95} was 111 times lower than that of water (Prior and Jollands, 1988). Therefore, for control purposes different formulations namely, aqueous, oil and dust need to be tried against spruce bark beetle. According to the behaviour of *P. chalcographus,* oil and dust formulations in higher concentrations may be suggested more suitable than aqueous one.

CONCLUSION

From these studies it can be concluded that *B. bassiana* and *M. anisopliae* have potential as microbial control agents against *P. chalcographus*.

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