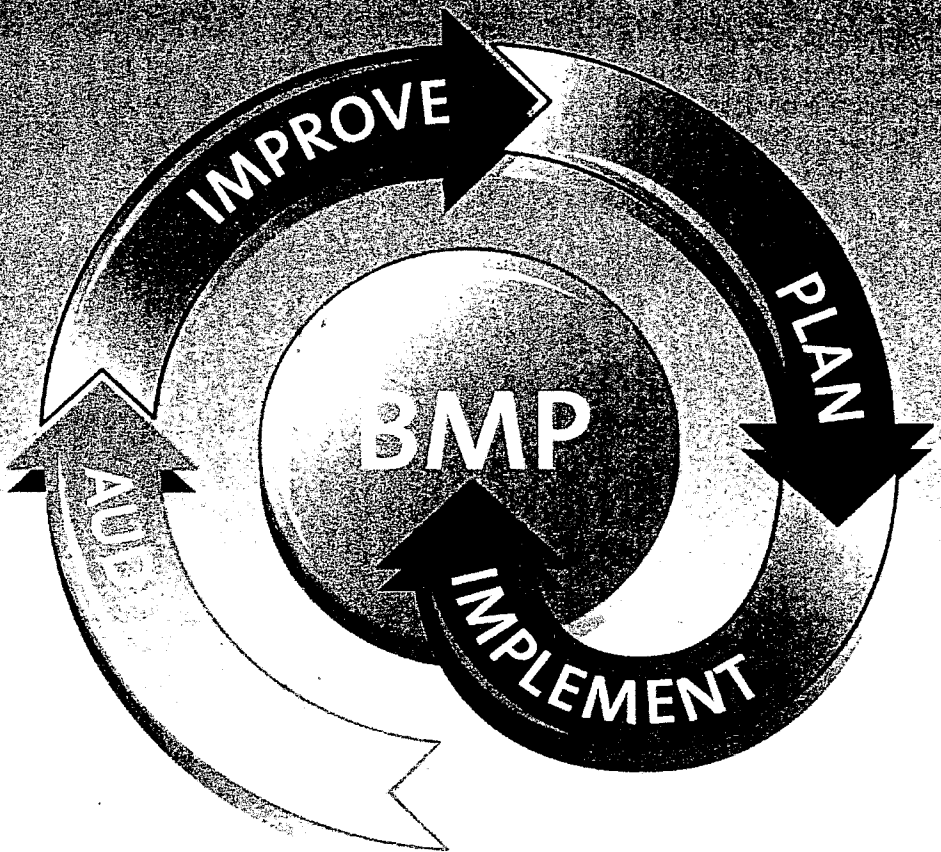


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Influence of entomopathogenic hyphomycetes and bacteria (*Pseudomonas* sp.) on locusts

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INTRODUCTION

The possibility of using entomopathogenic hyphomycetes (*Beauveria bassiana* and *Metarhizium anisopliae*) for management of locust populations has been shown by many researchers (Lomer *et al.*, 2001). However, mycoses are characterized by their long latency time. Index LT_{90} usually varies from 7 to 34 or more days. Some authors (Bajan, 1973; Логинов & Павлюшин, 1987) have indicated a shortening of latency time and increased mortality of various insects when using a mixture of microorganisms from close or distant taxons. In this work we studied different species and strains of entomopathogenic fungi and bacteria which were virulent to locusts (*Locusta migratoria*, *Calliptamus barbarus* and a complex of species in the tribe Dociostaurini).

METHODS

Entomopathogenic hyphomycetes were isolated from dead locusts collected in the steppe zone of Western Siberia. The bacteria (*Pseudomonas* sp.) were isolated from a laboratory population of crickets (*Gryllus bimaculatus*) at the Institute of Systematic and Ecology of Animals. The nymphs were infected by once washing in the aqueous suspension of the conidia and/or bacterial cells. The dilution of fungal and bacterial suspensions was varied from 5×10^5 to 5×10^7 conidia or cells per ml. The nymphs were placed in the 700 ml plastic hatcheries which were then covered with cloth. Each treatment was replicated four times. For each replication 5–10 nymphs were used. The mortality was measured daily for 13–17 days.

RESULTS AND DISCUSSION

Following infection of *L. migratoria* with *B. bassiana* and *M. anisopliae*, a 5-day latency time was observed. Subsequently, there was rapid nymphal mortality: thus, mortality of *L. migratoria* nymphs was 95–100% 12–15 days after inoculation; similarly, mortality of *L. migratoria* nymphs was c. 30–50% 3–6 days after infection with *Pseudomonas* sp. Subsequent mortality of the locusts was not observed. With synchronous inoculation of *L. migratoria* with fungi and bacteria, nymphal mortality was more rapid than with monoinfections (Table 1); LT_{50} was about 3 days. Very similar dynamics of mortality occurred for Dociostaurini and *C. barbarus* infected with fungi and bacteria.

Individuals which died in the first few days of the experiment had typical symptoms of bacteriosis. Those that died subsequently were mummified: typical of mycosis. Microbiological analysis of the dead insects shown that co-existence of both pathogens in the infected locusts is possible. To determinate the antagonism of the fungi and *Pseudomonas* on the synthetic nutrient medium the blocking method was used. The fungi did not influence the growth of the bacteria, and *Pseudomonas* had little (an insignificant) effect on that of the fungi. Zones of growth-inhibition (when using blocks of 12 mm diameter) were 4.5 mm for *B. bassiana* and 7.5 mm for *M. anisopliae*.

The most effective concentrations for the concurrent use of fungi and bacteria were 1×10^7 and 5×10^6 , respectively (for 2nd- and 3rd-instar nymphs of *Dociostaurini* and *C. barbarus*) and 1×10^7 for fungi and 5×10^7 for bacteria (for 5th-instar nymphs of *L. migratoria*). Increasing the concentration of one or both pathogens two- or five-fold, levelled all differences in the dynamics of nymphal death resulting from bacteriomycosis and monoinfections. After the dilution of the pathogens we observed a decrease in the additive effect.

Two main factors can have an additive effect, depending on the composition of the pathogens present. Firstly, bacterial gut infection can lead to poisoning and subsequent death of the insects. Secondly, bacterial infection can reduce of growth, limit eating and delay moulting. These conditions favour the germination of fungal hyphae into the cuticle and the haemolymph, and also stimulate mycosis in the insects. Our data demonstrate that a mixture of bacteria (*Pseudomonas* sp.) and hyphomycete fungi may be unique for producing a combined preparation for the regulation of locust populations.

Table 1. Dynamics of the mortality of fifth instar nymphs of *Locusta migratoria* infected with *B. bassiana* (1×10^7 conidia/ml) and *Pseudomonas* sp. (5×10^7 cells/ml)

Treatment	Mortality in days (%)					
	3	5	7	9	11	13
<i>B. bassiana</i>	5 ± 5	15 ± 5	50 ± 15	90 ± 1	100	100
<i>Pseudomonas</i> sp.	28 ± 5	33 ± 5	45 ± 3	50 ± 4	53 ± 5	55 ± 3
<i>B. bassiana</i> + <i>Pseudomonas</i> sp.	55 ± 4	70 ± 1	75 ± 5	85 ± 6	95 ± 5	100
Control (water)	0	2.5 ± 2	7.5 ± 2	12.5 ± 5	12.5 ± 5	20 ± 7

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