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A NOVEL CHEMICAL -MICROBIAL HYBRID PESTICIDE IN THE MANAGEMENT OF *S.ORYZAE* L.

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ABSTRACT

Mortality of rice weevil, *Sitophilus oryzae* (L.) caused by *Metarhizium anisopliae* (Metschnikoff) Sorokin alone and in combination with a neonicotinoid insecticide, acetamiprid was evaluated in laboratory bioassays. Spray application of *M. anisopliae* alone at a spore concentration of 6 x 10^7 conidia/ml required 6 days to cause 10 percent mortality whereas 4 x 10^7 conidia/ml required 8 days to cause 10 percent mortality. Acetamiprid alone at a concentration of 30.00 µg/dl required 96h to cause 10% mortality and at 20.00 µg/dl required 120 h to cause 20% mortality. In combination with acetamiprid, *M. anisopliae* killed *S.oryzae* significantly faster than without acetamiprid. *M. anisopliae* at a spore concentration of 3 x 10^7 conidia/ml with 7.50 µg/dl acetamiprid killed 100 percent of the *S. oryzae* in 96 h and at 2 x 10^7 conidia/ml with 5.00 µg/dl acetamiprid killed 100 percent in 120 h. *S. oryzae*, *M. anisopliae* - acetomiprid combination resulted in higher mortality. Statistical analysis demonstrated synergistic interaction between these two insecticides.

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KEY WORDS

Metarhizium anisopliae, acetamiprid, *Sitopilus oryzae*, synergism, hybrid pesticide

INTRODUCTION

Sitophilus oryzae L. (Coleoptera: Curculionidae) a serious pest of various food grains, is managed using organophosphates[1], methyl chloroform[2] fenitrothion and bromphos[3] methyl bromide and phosphine[4] and non-judicious use of chemicals leads to resistance development in stored grain pests.[5,6,7,8] Hence, there is a global need for new environmentally safe pesticides to manage stored grain pests. Bacteria, fungi, virus, protozoans and nematodes with insecticidal properties are being tried as alternatives to chemical pesticides. Entomopathogenic microbes can be used along with chemical pesticides to produce synergistic effect on insects resulting in reduced doses of the *two* components[9,10].

Among the different microbes, entomopathogenic fungi such as *Beauveria bassiana (*Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Nomuraea rileyi (*Farlow) Samson*, and Paecilomyces farimosus* (Dick) Brown and Smith have been successfully used against insect pests.[11,12,13,14]. *M.anisopliae* is more economical and efficicent by virtue of its ability to grow on cheap substrates, high virulence, transcuticular penetration, broad host range and target specific being safe to human beings, animals and plants.[15,16,17,18] Several studies reported increased virulence of *M.anisopliae* in combination with the organic pesticides imidacloprid, chloropysifos, propetanphos and cyfluthrin.[19,20,21,22.] In none of these studies the chemical pesticide was blended with the microbial component to form a homogeneous suspension carrying virulent conidiospores. In the present study, *M.anisopliae* conidiospores were suspended in a liquid preservative, in which acetamiprid was already present in a concentration non-toxic to the spores. This hybrid pesticide with dual insecticidal components was prepared following the method developed by Anitha[23.] The toxicity of the two-component hybrid pesticide were tested against *S. oryzae* adults and compared with the individual toxicity of the two components in the same test insect.

MATERIALS AND METHODS.

(i) Acetamiprid Spray Preparation

Commercial Acetamiprid (20 percent w/w) dispersible powder (DENOCIL), procured from the local retailer was used for preparing 5% acetamiprid stock solution in sterile distilled water based on the active ingredient of the pesticide. Further dilutions ranging from 10 to 120 µg/dl were made from the stock just before spraying.

(ii) M.anisopliae conidiospore harvesting and preparation of spray solution

Pelleted spores of *M.anisopliae* were obtained from The Institute of Microbial Technology (IMTECH), Chandigarh, India. The spores were subcultured in Potato Dextrose Agar (PDA) under sterile conditions. From 16-18 days old cultures, conidiospores were harvested in sterile preservative solution. The spore suspension taken in a conical flask was mixed well in a shaker, serially diluted and 1ml of the spore suspension from different dilutions was placed on a haemocytometer and the concentration of spores was determined by counting the conidiospores. Dilutions with spore concentrations ranging from 2 - 18 x 10^7 spores /ml were sprayed on *S.oryzae* adults.

(iii)Preparation of the hybrid pesticide

Mature conidiospores of *M.anisopliae* were transferred into a conical flask containing the spore preservative in which acetamiprid was already mixed at 5% concentration. This suspension was diluted using distilled water ranging from 2.5 to 30.00 µl /dl acetamiprid and 1to 12×10^7 spores /ml *M.anisopliae* were used for spraying on *S.oryzae*

(iv)Probit analysis

The mortality response of *S. oryzae* adults after 96 and 120 h of exposure was analyzed using Probit analysis[24] to obtain the corresponding LC^{50} values and their upper and lower confidence intervals.

(iv)Fate of spores in hybrid pesticide

The spores contained in the hybrid pesticide were periodically tested for their viability and virulence. The spores were transferred to PDA medium and allowed to germinate. The second generation spores produced by *M. anisopliae* raised from hybrid pesticide were checked for their virulence against *S. oryzae.* These tests ensured the compatibility of acetamiprid and *M. anisopliae* spores.

RESULTS

Response of *S. oryzae* adults after spray application of Acetamiprid, *M. aniso*pliae and Acetamiprid + *M. anisopliae* hybrid combination.

Treatments	Hours / days	Lower concentration	Mortality %	Higher concentration	Mortality	LCL	LC_{50}	UCL
Acetamiprid	96 h	30	10	90	100	46.38	54.18	63.28
(µg/dl)	120 h	20	20	80	100	32.07	37.98	44.97
M.anisopliae	6 day	6	10	14	100	8.35	9.50	10.79
spore	8 day	4	10	10	100	5.59	6.53	7.66
suspension								
($\times 10^7$								
conidia/ml)								
	96 h	7.5	10	22.5	100	11.59	13.55	15.63
Acetamiprid		+		+		9.0	5.42	6.33
(µg/dl)		3		9				
+	120 h		10			8.02	9.50	11.25
M.anisopliae					100	3.21	3.80	4.49
spore		5.00		20.00				
suspension		+		+				
($\times 10^7$		2.00		8.00				
conidia/ml)								

M. anisopliae **colony spores isolated from hybrid pesticide**

A concentration of 90 µg/dl acetamiprid caused 100 percent mortality of *S. oryzae* in 96 h. *M. anisopliae* conidial suspension in the spore concentration of 14.0 x 10^7 conidia/ml killed all the exposed *S. oryzae* in 6 days of exposure. When hybrid pesticide was applied, the concentration amounting to 22.50 µg/ dl acetamiprid and 9.00 x 10^7 conidia/ml *M. anisopliae* spores resulted in 100 percent mortality of *S. oryzae* in 96 h.

DISCUSSION

The results clearly established a synergistic interaction between acetamiprid and *M. anisopliae* conidiospores. Virulence of *M. anisopliae* was greatly enhanced and mortality of *S. oryzae* was significantly accelerated by the addition of acetamiprid. Fungus emergence from the hybrid pesticide was normal indicating that acetamiprid at 5 percent level was well tolerated by *M .anisopliae* spores. In addition, results of the virulence bioassay showed that conidia produced from emerged fungus on petriplates retained the same virulence as that of the original inoculum. This was confirmed by a study that recorded a high compatibility of imidacloprid and fipronil with *B. bassiana* and *M. anisopliae,* in a formulation of water dispersible granules[25.] In another study[26], a high concentration of acetamiprid significantly reduced germination, vegetative growth and conidia production in *M. anisopliae*.

Acetamiprid did not affect the entomopathogenic fungus, *M. anisopliae* as recorded in a report[27] exemplifying synergistic effect between wettable powder insecticides and *M. anisopliae*. In fungi, physiological mechanisms exist for metabolizing insecticides and liberating compounds that can be used by the fungus as secondary nutrients that enhance conidia production. Another possible explanation is that substances present in the insecticide formulations can be used directly as nutrients increasing the vegetative growth and conidia production of the pathogen. *M. anisopliae* enters in to the insect hemocoel after penetration of the exoskeleton which is then followed by the production of hyphae and toxins that kill the host[28]. Several studies have focused on the potential use of entomopathogenic fungi *M. anisopliae,* in combination with sub lethal doses of organic insecticides against various insect pests.[29]

This study very clearly demonstrates a synergistic interaction between an entomopathogenic fungus *M. anisopliae* and an organic pesticide, acetamiprid which significantly decreases environmental contamination. The main advantage of using *M. anisopliae* - acetamiprid hybrid combination is the fast killing action exhibited by it at a comparatively low concentration.

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