



Technical Information Brochure



MYCOINSECTICIDES

New Products in the fight against
Helicoverpa armigera Pest in Pulses

A joint project between the **National Chemical Laboratory** (NCL) in Pune, Atlas Agro GmbH and the **Research Station for Agroecology and Agriculture** (FAL) in Zurich, Switzerland under the Indo-Swiss Collaboration in Biotechnology (ISCB)

Involved investigators

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- ◆ Mounir Hassani, Atlas Agro GmbH, Zurich, Switzerland
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The problem

The gram pod borer, *Helicoverpa armigera* (Hub.) (Lepidoptera: Noctuidae) is considered as one of the most important insect species responsible for major crop losses in pulses and cotton. In India, the decline in yield due to *H. armigera* in pulses is approximately 30-40% of the total annual crop losses. This corresponds to Rs. 2,000 crores (US\$ 400 million) loss per year.

The developments of high levels of resistance to the conventional insecticides and the negative impact of pesticides on the environment have given impetus to evaluate alternative strategies for chemical control. The use of **entomopathogenic fungi** is one of the methods of biocontrol measures.



Figure 1:

Helicoverpa armigera larvae damage to tomato, pigeon pea and cotton (left side)

Helicoverpa armigera on chickpea and pigeon pea (below)





Our approach

The objective of the Biopesticide Project (BP1) is to develop a commercial microbial pesticide based on entomopathogenic fungi against *Helicoverpa armigera* in pulses following the process shown in Figure 2.

The project is part of the Indo-Swiss Collaboration in Biotechnology Programme (ISCB), which is a bilateral agreement between the Indian and Swiss Governments. The ISCB promotes research partnerships between Swiss and Indian institutions in various areas.

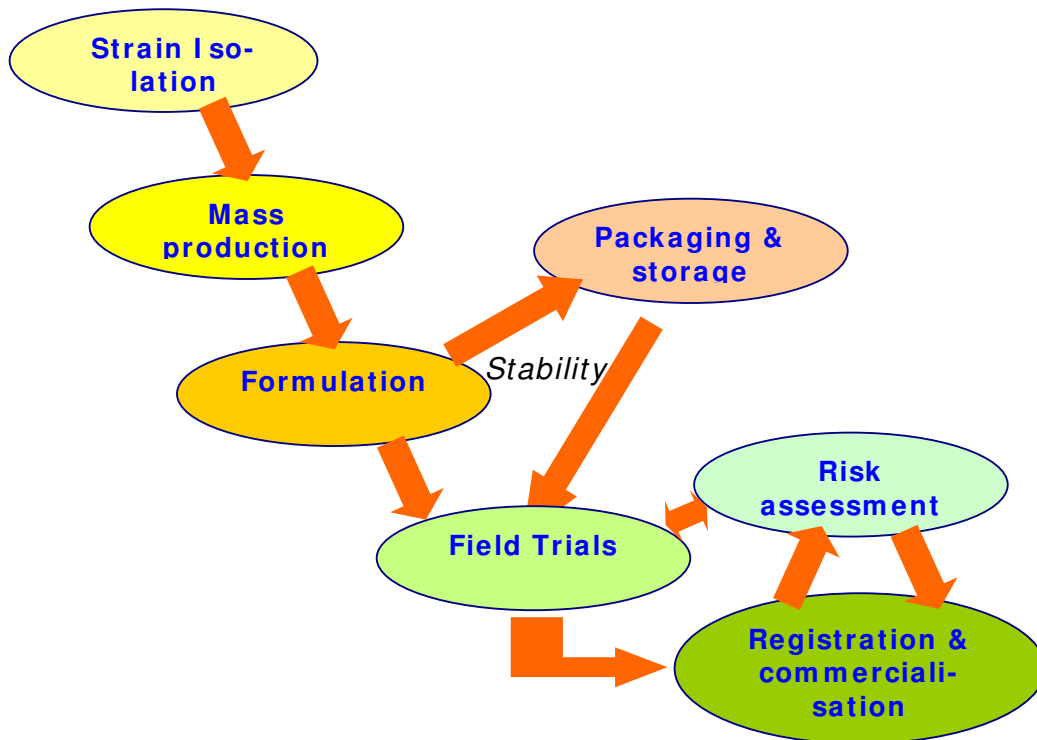


Figure 2: Approach and strategy of BP 1 for the development of mycoinsecticides against *H. armigera* in pulses



Set-up & results (1)



Strain isolation

The field campaigns in the Pune region (Maharashtra State, India) were undertaken in September 2000 and up date more than 50 fungal isolates (22 *Metarhizium*; 7 *Beauveria* and 15 *Nomuraea*) have been isolated from the infected caterpillars as well as by separate *Galleria* bait and soil plating method.

Figure 3: *M. anisopliae* culture, caterpillar of *Spodoptera litura* and *Galleria* sp. infected with *Nomuraea rileyi* and *M. anisopliae*



Mass production

The mycoinsecticides are produced using liquid and solid state fermentations (Figure 4). In the first stage, fungal biomass is produced in submerged fermentation. Further, solid substrates are inoculated with the biomass and incubated for 1-2 weeks. After drying (< 20% moisture content) the conidia are harvested from the substrate using the MycoHarvester (CABI Bioscience Biopesticides Programme, Ascot, UK).

Figure 4: From stock culture over liquid fermentation to solid state fermentation

Figure 5: MycoHarvester (CABI Bioscience Biopesticides Programme, Ascot, UK)



Barley, rice, maize, wheat, sorghum and pearl millet seeds were evaluated as solid substrate for conidia production of the 3 most promising isolates *M. anisopliae* (M34412), *B. bassiana* (B3301) and *N. rileyi* (N812). The highest spore yield for all three isolates was obtained on rice.



Figure 6: Spore yield of fungal isolates per kg substrate after 14 days incubation at 25°C in solid state fermentation studies



Set-up & results (2)



WP ES OF

Figure 7: Water powder (WP), Water Emulsion (ES) and Oil flowable (OF) formulation tested

Formulation

In our study we investigated the viability and the virulence of the *M. anisopliae* aerial conidia in different carriers and formulation (Figure 7). The *M. anisopliae* (M34412) spores suspended in Tween 80 (0.1%), Diesel/Sunflower (7:3) and the water emulsion oil Emoleo R2 (John L Seaton & Co Ltd, UK) showed the highest germination rate (Figure 8) and mortality against *H. armigera* larvae under laboratory condition compared to all other carriers (Figure 9).

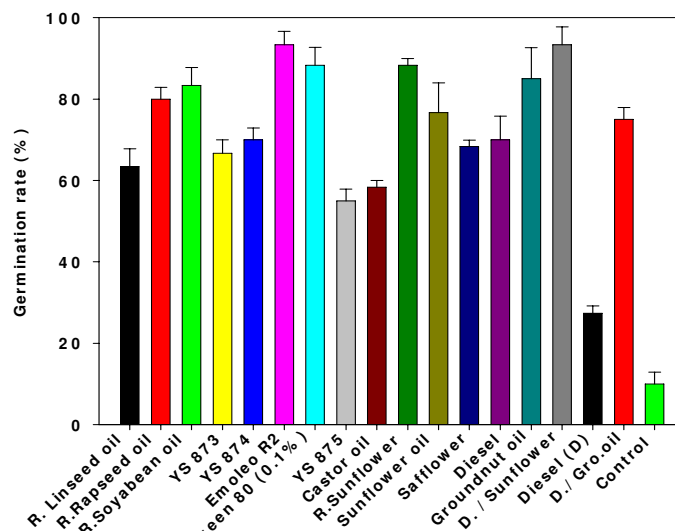


Figure 8: Germination rate of *M. anisopliae* (M34412) aerial conidia formulated in different carriers



Set-up & results (3)

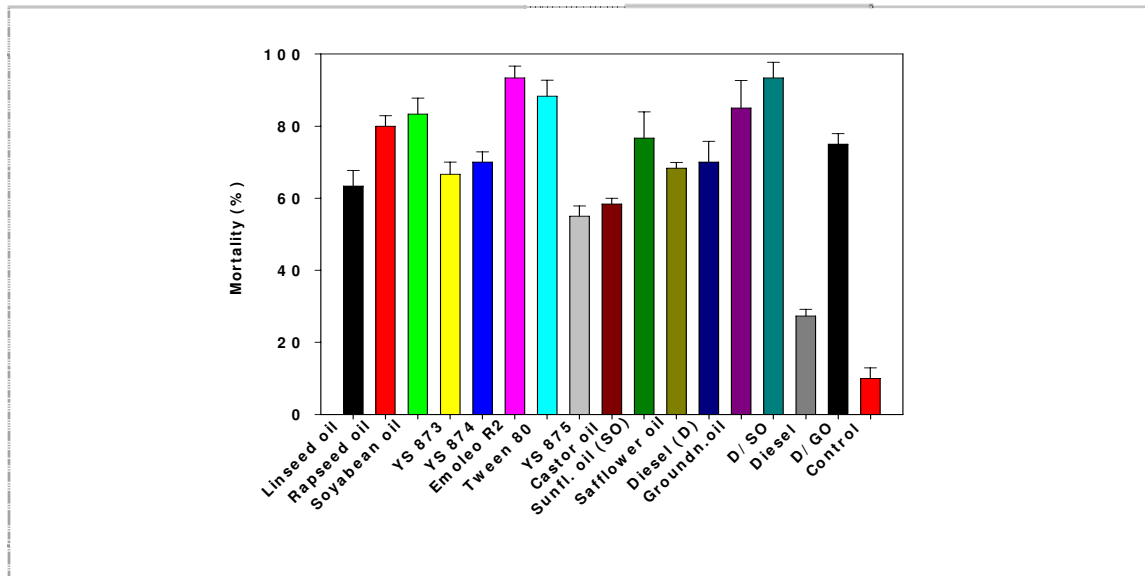


Figure 9: Virulence of *M. anisopliae* (M34412) aerial conidia formulate in different carriers against 3rd instars larvae of *H. armigera*



Fig. 10



Fig. 11



Fig.12

Field testing

The field efficacy trials were conducted at College of Agriculture in Pune, Maharashtra, India on pigeon pea (*var.* ICPL 87) during the *Kharif* season, 2001 (June to Sept.) (Figure 10 & 11), and on chickpea (*var.* Vikas) from December to March 2002. Three promising fungal isolates were compared with recommended insecticide Endosulfan (Endocel 35 EC, Excel Industries Limited, Mumbai, India) and the biological product HaNPV (Heliokill, MPKV, Rahuri, India).

These studies showed highest decline in the *H. armigera* population and pod damage which was reflected by increased yield in plots treated with the *M. anisopliae* strain (M34412). It is followed by two other fungal isolates, namely *B. bassiana* (B3301) and *N. rileyi* (N812), Endosulfan, HaNPV and Control (Figure 10 and 11).

Figure 10 & 11: Mycoinsecticides application in pigeon pea using Ultra Low Volume CDA sprayer

Figure 12: Chick pea field



Set-up & results (4)

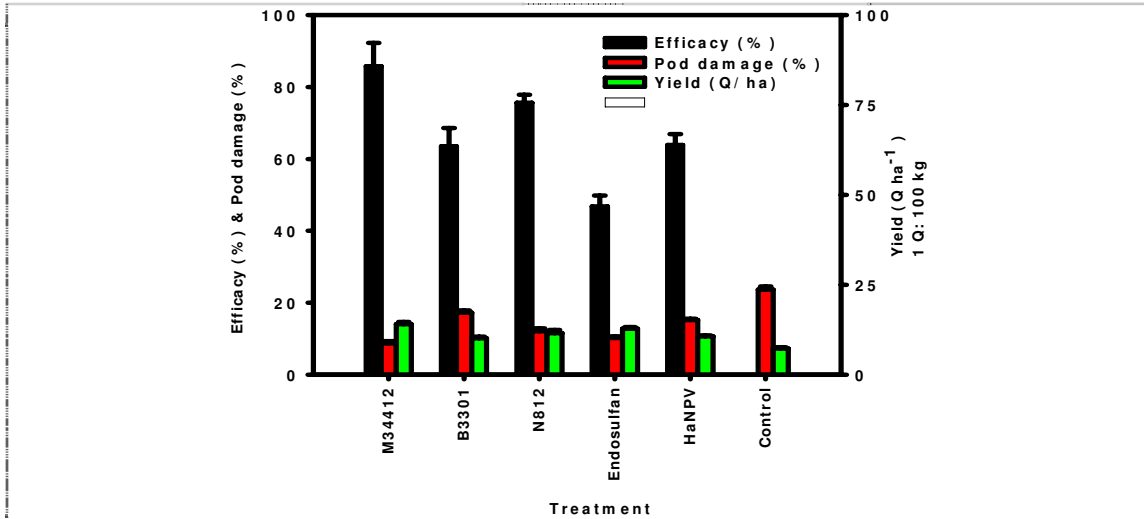


Figure 13: Efficacy of different entomopathogenic isolates against *H. armigera* on pigeon pea under field condition

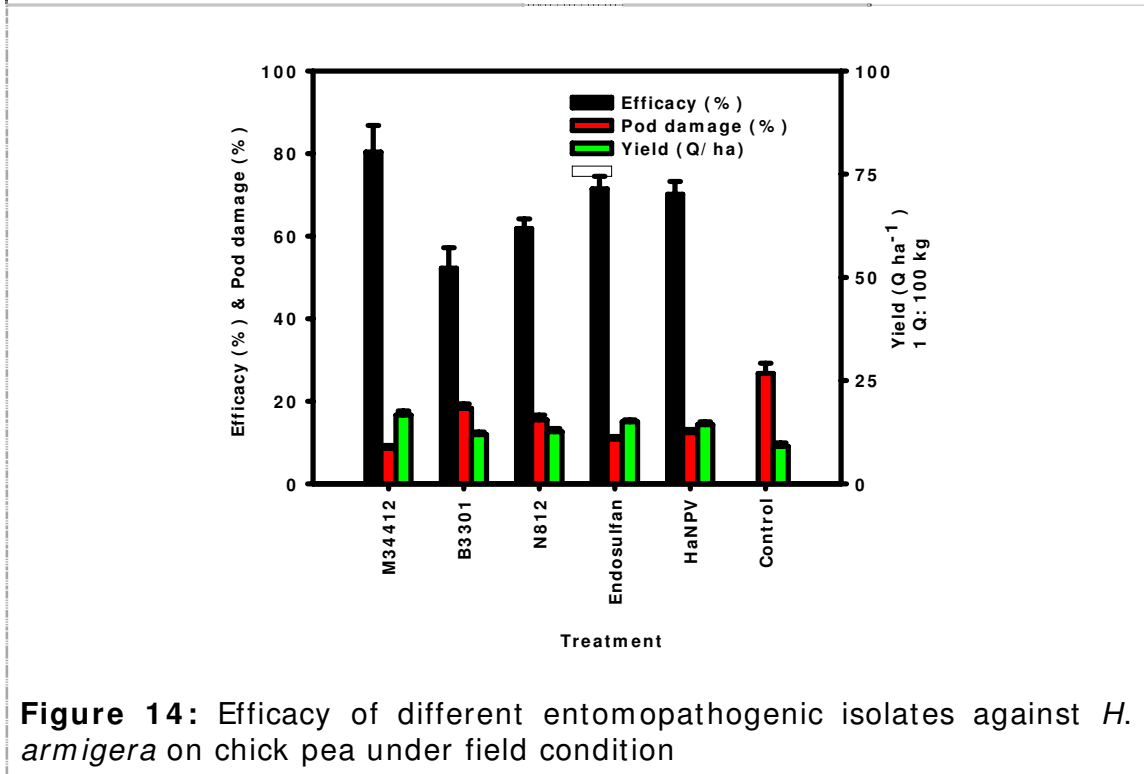


Figure 14: Efficacy of different entomopathogenic isolates against *H. armigera* on chick pea under field condition



Our capacities and the proof-of-concept (1)



Figure 15: Member of ISCB BP1 team with farmers

Team

- Molecular biologist
- Entomologist
- Agronomist
- Insect Pathologist
- Chemist
- Microbiologist
- Mycologist



Figure 16: Lab scale production

Infrastructure

- Molecular biology tools
- Molecular monitoring tools/equipment
- Lab Scaling up to Produce 25-50 kg dry conidia of *M. anisopliae* (M34412) per year to spray 200-400 ha



Figure 14: dry and formulated conidia spore of *M. anisopliae* (M34412)

Proof-of-Concept (prototype product)

- High virulence isolate against *H. armigera* larvae under lab and field conditions
- Production (extraction of 40 g dry conidia per kg rice. Only 1.5 kg rice are needed to produce the needed active ingredient for 1 ha spray)
- Application method (the oil flowable formulation (Diesel/Sunflower) showed good efficacy with application rate of 3 l ha⁻¹ using the Ultra Low Volume sprayer)
- Efficiency (reduction of population of *H. armigera* in the Pigeon pea and chick pea fields, increase of yield and decrease of pod damage rate in pigeon pea and chick pea)



Our capacities and the proof-of-concept (2)

Proof-of-Concept

- High sporulations of the infected caterpillars in the field, which allow the second, pick up of the spore from healthy larvae.

What's up in the pipeline

- Molecular tools for monitoring
- Risk assessment under laboratory and field conditions
- Quality control
- Storage

Publications/ presentations (1)

- Chandele, A.G., Hadapad, A., Bucher, T., Hassani, M., Nahar, P., Kulye, M., Yadav, P., Keller, S., Tuor, U. and Deshpande M. V. (2002). Comparative evaluation of indigenous fungal isolates, *Metarhizium anisopliae* M34412, *Beauveria bassiana* B3301 and *Nomurea rileyi* N812 for the control of *Helicoverpa armigera* (Hub.) on pigeon pea. "Proceedings on Biological control of Lepidoptera Pests". July 17-18, 2002, PDBC, Bangalore, India
- Deshpande, M. V., Chandele, A.G., Nahar, P., Hadapad, A., Patil, G., Ghormade, V., Keller, S., and Tuor, U. (2001). Entomopathogenic fungi: Mycoinsecticides useful against lepidopteran pest in pulses. IOBC/WPRS Bull. (In press)
- Deshpande, M.V., Chandele, A.G., Hadapad, A. B., Nahar, P., Keller, S and Tuor, U. (2001). ISCRISAT. Workshop "Helicoverpa Management: The Journey ahead" 21-22 December, 2001. ICRISAT Patancheru 502324, Hyderabad, Andhra Pradesh, India.
- Deshpande, M.V., Chandele, A.G., Nahar, P., Hadapad, A., Patil, G., Ghormade, V., Keller, S. and Tuor, U. (2001). Entomopathogenic fungi: Mycoinsecticides useful against lepidopteran pest in pulses, 8th European meeting of the IOBC/WPRS working group "insect pathogens and parasitic nematodes", 29 May – 2 June 2001, Athens, Greece



Our capacities and the proof-of-concept (3)

Publications/ presentations (2)

- Deshpande, M.V., Chandele, A.G., Nahar, P., Hadapad, A., Patil, G., Keller, S., Hassani, M. and Tuor, U. (2001). Entomopathogenic fungi as Myco-insecticides. National Symposium on "Plant Protection Strategies for Sustainable Agri-Horticulture, at Jammu, India, October 12-13, 2001.
- Deshpande, M. V. (2002) Fungal morphogenesis and development of antifungal agents. Technology Day Celebration, Hindustan Antibiotics Ltd., Pimpri, May 11th, 2002.
- Hadapad, A., Patil, G., Nahar, P., Chandele, A.G., Keller, S., Tuor, U. and Deshpande, M.V. (2001). Microbial control of pests: Entomopathogenic fungi as Mycoinsecticides, ISCB Inauguration Symposium, New Delhi, Feb 12-13, 2001
- Hadapad, A., Nahar, P., Patil, G., Chandele, A. G., Tuor, U. and Deshpande, M. V. 2001. Optimization studies for the large scale production of infective propagules of entomopathogenic fungi. Proceedings of symposium on biocontrol based pest management for crop protection in the current millennium organized by Indian Society for the advancement of insect science and Society for Biocontrol advancement at Punjab Agricultural University, July 18-19, 2001, Punjab, India, pp .94
- Hassani, M., Keller, S., Deshpande, M.V. and Tuor, U. (2001). Effect of various liquid culture media on growth, propagule production and morphology of the entomopathogenic mitosporic fungus *Nomurea rileyi*, 8th European meeting of the OBC/WPRS working group "Insect pathogens and parasitic nematodes", 29 May-2 June 2001, Athens, Greece
- Hassani, M., Bucher, T., Hadapad, A., Nahar, P., Chandele, A., Tuor, U., Keller, S., Deshpande, M.V. (2002). Lab-scale mass production of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in pulses, XXXV Annual Meeting of the Society For Invertebrate Pathology, August 18-23, 2002, Foz do Iguassu, Brazil



Future perspectives

What we are aiming at in a strategic partnership

- ❖ Development of pilot production unit
- ❖ Large scale field evaluation
- ❖ Risk assessment study
- ❖ Registration
- ❖ Commercialisation
- ❖ Farmer training for the use biocontrol methods
- ❖ Collaboration with farmers and extensions service for large use of the Mycoinsecticides

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