A novel, natural anti-microbial product for use as an agricultural bactericide and fungicide

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R&D Microbials

Koppert Biological Systems

Berkel & Rodenrijs, the Netherlands

1st Annual Biocontrol Industry Meeting, October 23-24, 2006, Lucerne, Switzerland
• **Introduction to the Lactoperoxidase-system**
  • Koppert’s LP-system
  • How does LP work?
  • LP as a fungicide
  • LP as a bactericide
  • LP against viruses
  • Compatibility
  • Conclusions
What is the Lactoperoxidase system?

- Occurs in animals and humans
- Important non-immune defence system
- Active system in milk, saliva, tears, etc.
- Used in food products and oral care products
- Koppert and DMV/Campina have developed it as a natural fungicide/bactericide
What is the LP system?

- Natural system based on:
  - 1 enzyme + 2 substrate components: lactoperoxidase, SCN⁻ and H₂O₂
- Based on the formation of reactive oxygen species that inhibit or kill bacteria
- FAO: milk preservation: addition of SCN⁻ and H₂O₂
MANUAL ON THE USE OF THE LP-SYSTEM IN MILK HANDLING AND PRESERVATION
LP in oral care products

- Toothpaste (Zendium, Biotene)
- Chewing gum (Biotene, Bio-Xtra)
- Mouth rinses (Oralbalance, Biotene)
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“Koppert’s” LP system?

- System based on:
  - 2 enzymes + 3 substrate components
  - adjuvant based on a vegetable oil

- Based on the formation of reactive oxygen species that inactivate proteins in micro-organisms
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Mode of Action LP-system

LACTOPEROXIDASE SYSTEM:

\[ \text{H}_2\text{O}_2 \xrightarrow{\text{LP}} \text{SCN}^- \xrightarrow{\text{LP}} \text{HOS-R} \]

\[ \text{H}_2\text{O} \xrightarrow{\text{LP}} \text{I}^- \xrightarrow{\text{LP}} \text{HS-R} \]
Mode of action

- Oxidation of sulfhydryl groups in proteins
- Membrane effects
- Active transports of amino acids and sugars disturbed
- Glycolytic enzymes inhibited
- Other metabolic functions disturbed
  → cell death
Activity (1)

- Activity based on contact
- Curative action
- Activity not translaminar nor systemic
- No preventive effect
- No residual effect
- Only active in water phase – High Volume spray
- not dependent on temperature and relative humidity
Activity (2)

- Activity on
  - fungi
  - yeast
  - bacteria
  - viruses
  - mycoplasma’s
- Kills spores, cells, mycelium
- No resistance development possible due to multiple-site activity
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Research

- Target 1: powdery mildew(s)
- Protected crops worldwide
- Research model:
  - cucumber/Sphaerotheca
- Composition of active system
- Formulation, incl adjuvant
- Product developed
KBV 99-01 in cucumber

Control of Powdery Mildew (*Sphaerotherca fuliginea*) in cucumber (M. Post, July 2003, NI)

![Bar graph showing efficacy % of control for Baycor flow, KBV 99-01 (1N), and KBV 99-01 (2N) 2 days and 8 days after treatment.](image-url)
Visible effect in cucumber
KBV 99-01 in tomato

Control of Powdery Mildew (*Oidium lycopersicum*) in tomato (2002 and 2003, NI)

<table>
<thead>
<tr>
<th>Grower</th>
<th>Efficacy % of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Dec '02)</td>
<td>Baycor flow</td>
</tr>
<tr>
<td>2 (May '03)</td>
<td>KBV 99-01 (1N)</td>
</tr>
<tr>
<td>3 (Aug '03)</td>
<td>KBV 99-01 (2N)</td>
</tr>
</tbody>
</table>
Visible effect in tomato
Research

- Target 2: Fusarium oxysporum
- Bulbs
- Research model: tulip/ “zuur”
- Other bulbs and diseases in research
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Research

- Target: bacterial diseases
- Lab trials on Erwinia, Clavibacter, Pseudomonas, Xanthomonas spp. positive
- Further research needed
Research

- Apple: fire blight
- Lab trials positive
- Field trial: result moderate
- Further research: formulation and application strategy
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• Compatibility
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LP against viruses

- Medical uses reported
- In vitro trials
  - TMV
  - PepMV
LP against viruses

Results:

- TMV → no effect
- PepMV → variable results → up to 95% effect
- More research needed
Patent

- Composition and application patented
- For use as an agricultural fungicide / bactericide
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<table>
<thead>
<tr>
<th>Natural enemy</th>
<th>Life stage</th>
<th>Direct / residue</th>
<th>Corrected mortality (%)</th>
<th>IOBC category</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amblyseius cucumeris</em></td>
<td>♀ ♂</td>
<td>Residue</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td><em>Amblyseius swirskii</em></td>
<td>♀ ♂</td>
<td>Residue</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Eggs</td>
<td>Direct</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td><em>Phytoseiulus persimilis</em></td>
<td>♀ ♂</td>
<td>Residue</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td><em>Typhlodromus pyri</em></td>
<td>Protonymphs</td>
<td>Residue</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reproduction reduction</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td><em>Aphidius colemani</em></td>
<td>♀ ♂</td>
<td>Residue</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Mummies</td>
<td>Direct</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td><em>Encarsia formosa</em></td>
<td>♀ ♂</td>
<td>Residue</td>
<td>56</td>
<td>3</td>
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<tr>
<td><em>Eretmocerus mundus</em></td>
<td>♀ ♂</td>
<td>Residue</td>
<td>20</td>
<td>1</td>
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<tr>
<td><em>Macrolophus caliginosus</em></td>
<td>Nymphs (L2)</td>
<td>Residue</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Direct</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td><em>Orius laevigatus</em></td>
<td>Nymphs (L1)</td>
<td>Direct</td>
<td>60</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Nymphs (L2-3)</td>
<td>Residue</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reproduction reduction</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Apis mellifera</em></td>
<td>Workers</td>
<td>Oral Contact</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Compatibility Enzicur with natural enemies

IOBC category reflects the compatibility with natural enemies.
## Compatibility Enzicur with pesticides

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Commercial name</th>
<th>Fungicides</th>
<th>Commercial name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abamectine</td>
<td>Vertimec</td>
<td>Azoxystrobin</td>
<td>Ortiva</td>
</tr>
<tr>
<td><em>Bacillus thuringiensis</em></td>
<td>Dipel</td>
<td>Bitertanol</td>
<td>Baycor</td>
</tr>
<tr>
<td>Cyromazin</td>
<td>Trigard</td>
<td>Captan</td>
<td>Captan</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>Admire</td>
<td>Chloorthalovit + prochloraz</td>
<td>Allure</td>
</tr>
<tr>
<td>Pirimicarb</td>
<td>Pirimoz</td>
<td>Imazalit</td>
<td>Fungaflor</td>
</tr>
<tr>
<td>Pymetrozin</td>
<td>Plenem</td>
<td>Bicarbonate</td>
<td>Milstop</td>
</tr>
<tr>
<td>Pyridaben</td>
<td>Asepta Carex</td>
<td>Triflumizole</td>
<td>Rocket</td>
</tr>
<tr>
<td>Pyriproxifen</td>
<td>Admiral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spiromesfen</td>
<td>Oberon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teflubenzaron</td>
<td>Nomoet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiacloprid</td>
<td>Calypso</td>
<td></td>
<td></td>
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<tr>
<td><em>Verticillium lecanii</em></td>
<td>Mycotal</td>
<td>Sulphur</td>
<td>Thiovit</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thiophanate-methyl</td>
<td>Tospin-M</td>
</tr>
</tbody>
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Advantages of LP system

- A unique natural product
- No resistance
- Safe to humans, plants, animals and the environment
- Safe to natural enemies and bumblebees
- Fits in Integrated Pest Management
- Fits in organic agriculture
Conclusions

- A new type of fungicide and bactericide
- New mode of action
- Contact activity
- Easy to use
- Potentially for a broad range of diseases and applications

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Thank you for your attention!

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