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To
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Report on microbial tests towards dust mite

In the framework of Chrisal's R&D strategy for the Probiotics In Progress (PIP) product line, LabMET performed a number of microbial tests to verify the repellent activity of sporulating bacteria towards dust mite. Three types of experiments were performed in triplicate and are presented below.

1) Repellent action of PIP product towards dust mite

Sealed and aerated plastic containers, provided with a textile bottom, were inoculated with dust mite and incubated at 28°C at a humidity of 70%. No additional feed was added. Treatment of the containers with Chrisal's PIP product was performed on the right half of the textile surface, with the other half being treated with an equal volume of sterile water. Treatment protocols were as follows :

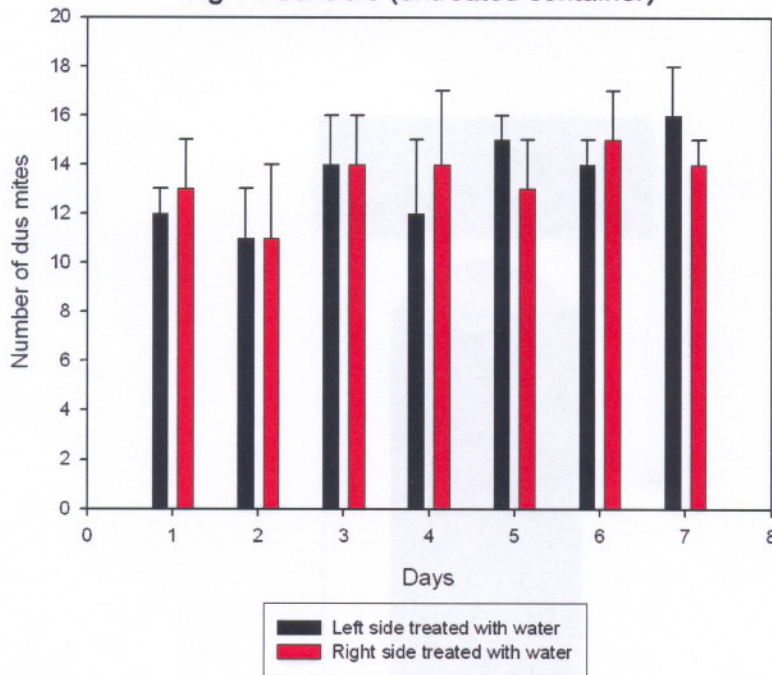
- Container 1 : Control (left and right side treated with water)
- Container 2 : Daily treatment of the right side with PIP product (during 8 days)
- Container 3 : 72 hours treatment of the right side with PIP product (during 2 weeks)
- Container 4 : Weekly treatment of the right side with PIP product (during 3 weeks)

Results :

Container 1 : Data are averaged over three replicate experiments. No significant differences were obtained between the dust mite counts on both halves of the surface. This indicates that with no treatment, the dust mites migrate randomly across the surface. The slight rise in absolute dust mite numbers also indicates that the lack of supplemented food is not detrimental to the population.

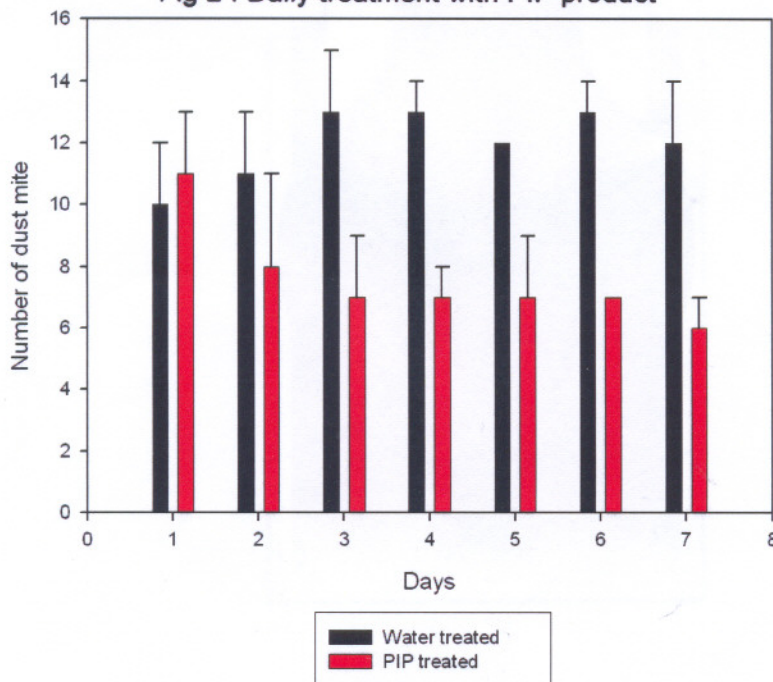
Fig 1 presents the results on dust mite counts of container 1.

Fig 1 : Controle (untreated container)

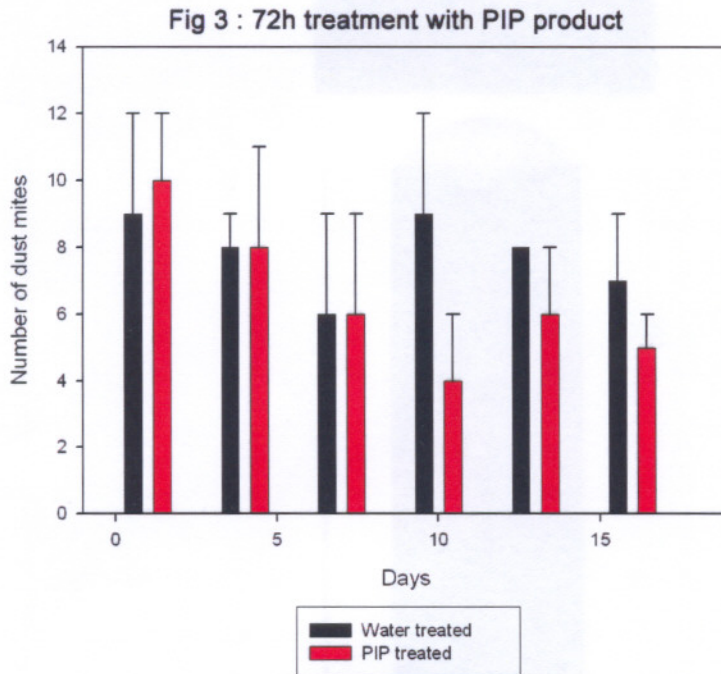


Container 2 : Daily treatment with the PIP product led to a significant drop in the numbers of dust mite on the treated half of the surface. Again, a slight rise on the untreated side was noticed, however, the total number of dust mites on the overall surface was lower compared to the control, indicating a possible stress on dust mite replication resulting from the PIP product. Results are presented in Fig 2.

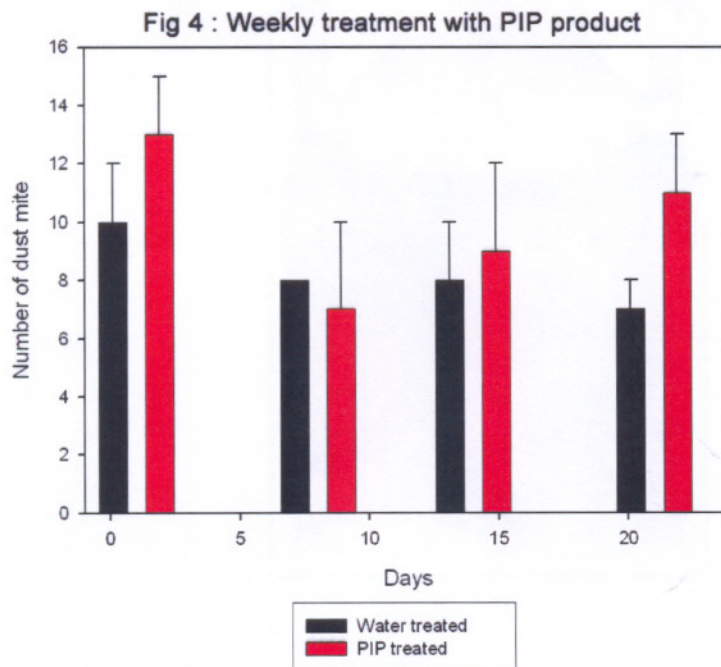
Fig 2 : Daily treatment with PIP product



Container 3 : Treatment every 3 days showed a lower level of dust mite on the treated half of the surface after 2 weeks; however statistical data based on three experiments indicate that these results are not significant. Results are presented in Fig. 3.



Container 4 : The weekly treatment did not result in any significant result on the dust mite distribution as presented in Fig 4.



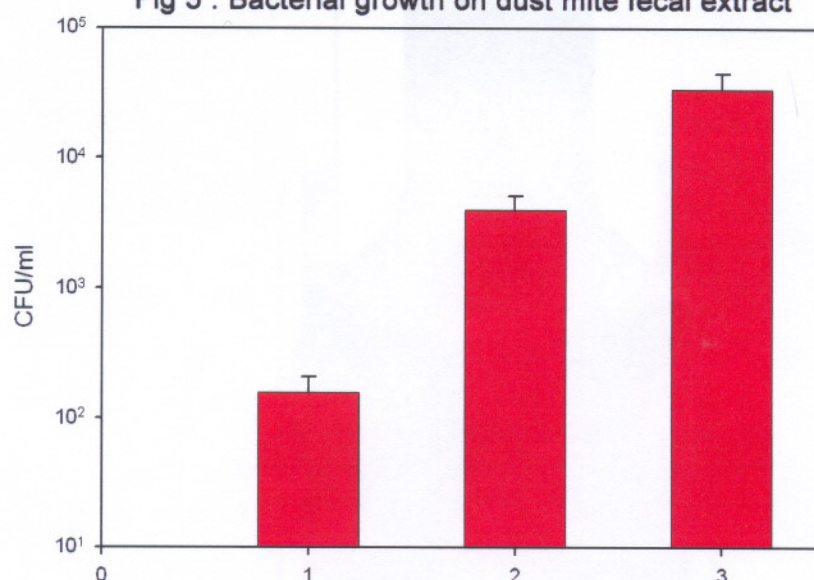
Conclusion : From the above results it can be concluded that the PIP product is effective in repelling dust mite when applied on a daily basis. Lowering of the treatment frequency after an initial intensive dose may result in a suppressed dust mite population.

2) Fecal deterioration :

A second experiment verified whether the spore forming bacteria present in the PIP product are able to grow on a faecal extract from dust mite. These extracts were obtained by removing dust mites from a 1-week populated container without additional food and suspending the remaining fractions in the container. This suspension was homogenised and filter sterilised, followed by its addition as a nutrient source to a watery suspension of the spore forming bacteria from the PIP product. Determination of the number of bacteria after 72 hours was performed by means of plate counts on Nutrient Agar at 37°C. Comparison was made to the control where sterile physiological solution instead of faecal extract was added to the bacterial suspension.

Results : As presented in Fig. 5, the PIP bacteria are able to grow on the faecal extract of dust mite. Even a 10-fold diluted extract resulted in a significant growth of the bacteria.

Fig 5 : Bacterial growth on dust mite fecal extract



1. Control; 2. 10-fold diluted fecal extract; 3. Fecal extract as such

Conclusion : The above results indicate that the bacteria present in the PIP product are able to grow and hence consume faecal extract from dust mite.

3) Biocidal activity

Using Live/dead staining on the flow cytometry, it was determined whether the filtrate of a 48h old bacterial suspension of the PIP product was able to kill *Staphylococcus aureus* and *Streptococcus faecalis*.

Results : Live/dead counts on *S. faecalis* and *S. aureus* are presented in table 1.

Table 1 : Viability counts on *Streptococcus faecalis* and *Staphylococcus aureus* to determine a possible biocidal action of the PIP product.

Control: *Streptococcus faecalis*

Live	Dead	Total	Live (%)	Dead (%)
9317	18	9335	99,81	0,19
9342	25	9367	99,73	0,27
9311	12	9323	99,87	0,13

Streptococcus faecalis + bacterial filtrate

Live	Dead	Total	Live (%)	Dead (%)
9338	95	9433	98,99	1,01
9365	43	9408	99,54	0,46
9349	45	9394	99,52	0,48

Control: *Staphylococcus aureus*

Live	Dead	Total	Live (%)	Dead (%)
8925	7	8932	99,92	0,08
8846	13	8859	99,85	0,15
8868	12	8880	99,86	0,14

Staphylococcus aureus+ bacterial filtrate

Live	Dead	Total	Live (%)	Dead (%)
9677	40	9717	99,59	0,41
9635	23	9658	99,76	0,24
9629	28	9657	99,71	0,29

Conclusion : No biocidal activity of the PIP product towards the tested bacteria was witnessed.

Overall conclusion : With a sufficiently high frequency of application, the PIP product was able to reduce the number of dust mite on the treated surface. Results further indicate that dust mite faeces can act as a nutrient source to the bacteria in the product. This may result in the suppression of dust mite allergy symptoms by application of the PIP product. No biocidal action of the PIP product towards *Streptococcus* and *Staphylococcus* was recorded.

Sincerely yours,

Prof. Willy Verstraete
Project promoter

Dr. Robin Temmerman
Project manager