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Pests and diseases

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# The use of plant extracts to control the major disease and pest in mushroom cultivation

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## Abstract

Dry bubble disease and its spread by insects represents a major problem in the cultivation of mushrooms. Prevention of dry bubble disease and its vectors usually involves chemical crop protection. However, the use of chemical crop protection is becoming less acceptable. We expect that, within a few years, prochloraz will no longer be available for use in mushroom cultivation in the European Union.

It is therefore necessary to develop alternative methods to control dry bubble disease. In a number of projects financed by the Dutch Product Board we investigated the efficacy of plant extracts and pure compounds as an alternative to chemical crop protection. We focused on the use of plant extracts to combat dry bubble disease and phorid flies.

With respect to dry bubble disease, we identified a plant extract that could reduce the incidence of dry bubbles by 80% after a single application in experimental cultivation of mushrooms. The efficacy of this plant extract compares favourably with the efficacy of prochloraz.

With respect to *Megaselia halterata* phorid flies, we identified plant extracts that (when used in a preventive manner) could reduce the number of flies by 60%. If used curatively, immediately after the flies produced eggs in the compost, some plant extracts could reduce the number of flies by 90%.

For both applications further research on plant extracts is aimed at developing crop protection agents that can be used in organic mushroom cultivation.

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## 1. Introduction

Pests and fungal diseases are a major problem in the Dutch mushroom industry. Currently, the phorid fly *Megaselia halterata* is the predominant important pest organism in The Netherlands. One of the most prominent fungal diseases is dry bubble disease, caused by *Verticillium fungicola*. A combination of these two organisms on mushroom farms represents a bigger problem, as the phorid flies can spread the spores of *Verticillium fungicola* and thereby act as vectors for the fungal disease (Geels et al. 1988).

In the near future, Dutch growers will mostly have to rely on preventive measures to control phorid flies and dry bubble disease. It is expected that a number of chemical crop protection agents will be withdrawn from use in mushroom cultivation, minimising the options of chemical control. Next to this, government and consumers are much more aware of food safety issues. This means that within EurepGap and QS certification much more attention is paid on preventing the maximum residue limits of chemical crop protection agents being exceeded. This may lead to a decision by the grower not to use a chemical crop protection agent. It could even be forbidden by a retail organisation to use certain chemical crop protection agents.

The need for alternative, non-chemical crop protection agents is therefore evident. With respect to controlling mushroom pests, there already is a non-chemical alternative. Sciarid mushroom flies (*Lycoriella auripila*, the main type and not a major problem in The Netherlands) are as effectively controlled by the entomopathogenic nematode *Steinernema feltiae* as by the chemical crop protection agent diflubenzuron. However control of phorid flies is at best variable (Scheepmaker et al. 1997). Therefore, for phorid flies, an alternative is needed.

With respect to controlling mushroom fungal diseases there are currently no non-chemical alternatives on the market. Essential oils derived from plants may provide such alternatives (Isman 2000). Sokovic and Van Griensven (2006) have shown growth-inhibiting effects of essential oils or their components on cultures of the fungal pathogens *Verticillium fungicola* var. *fungicola* and *Trichoderma harzianum* (Th1), *Trichoderma aggressivum* (Th2) and *Trichoderma atroviride* (Th3). Especially *Origanum vulgare* oil showed the highest and broadest activity. Its component carvacrol possessed the highest antifungal activity.

Also for the control of the sciarid flies *Lycoriella mali* (Choi et al. 2006) and *Lycoriella ingenua* (Park et al. 2006a,b) the use of plant essential oils has been investigated. Effects on the phorid fly *Megaselia halterata* have not been tested. This paper describes the use of plant essential oils as possible crop protection agents for the control of the mushroom pest *Megaselia halterata* and the fungal pathogen *Verticillium fungicola*.

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## **2. Materials and methods**

### **2.1. Plant extracts and essential oils**

Plant extracts and essential oils were supplied by Plant Research International (PRI) and for one experiment by Prof. Van Griensven. In order not to frustrate commercialisation of the relevant extracts or essential oils for the mushroom industry, the names are coded.

### **2.2. Experiments with dry bubble disease**

The effects of application of plant extracts on the incidence of dry bubble disease were tested in three independent experiments. Commercially available full-grown compost was used in all experiments. Compost was filled in 0.2 m<sup>2</sup> trays containing 15 kg of compost. After casing, the trays were infected by spraying the casing soils with fresh spore suspensions ( $3 \times 10^5$  spores/tray) of *Verticillium fungicola* var. *fungicola* strain V9503. This strain was maintained in our own collection. Ruffling was done on day 11 and venting-off started on day 14 after casing. Three flushes of mushrooms were harvested daily (except for the weekend). Yields of healthy mushrooms and numbers of spotted mushrooms and dry bubbles were recorded.

To test their effect in controlling dry bubble disease, plant extracts were applied both in different concentrations (2–6%) and at different times in the growing cycle (after casing or both after casing and after first flush). Each treatment was tested in five replicates. Plant extracts were dissolved either in 70% ethanol, sunflower oil or 1% Luxan H solution. Application of the solvent on infected trays was used as a control. Infected trays without treatment with plant extracts were used as a positive control on the success of infection. Non-infected trays were used as a positive control on yield. Statistical analysis of results (ANOVA) was performed using Genstat, 8th edition.

### **2.3. Experiments with phorid mushroom flies**

#### **2.3.1. Olfactometer experiments**

To test the response of phorid flies to plant extracts as a preventive action, a still-air olfactometer modified according to Van Tol et al. (2002) (Figure 1) was used. Ten phorid flies were released in the large dish on top of the olfactometer. The large dish had two exits, each leading to a different source of scent. The phorid flies were offered a choice between two scents: full grown compost (4 grams) with and without plant extracts. Plant extracts (10 µL) were pipetted onto a cotton tip and placed on top of the compost. A cotton tip without plant extract was used as a control. After 2 hours of incubation at 20°C in darkness, the number of phorid flies in each compartment was counted. Each experiment was performed in 10 replicates. Statistical analysis of the results was performed using a paired t-test (Sokal and Rohlf 1995).

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**2.3.2. Compost experiments**

Fully colonised compost was treated either before (preventive action) or after (curative action) egg deposition by the phorid mushroom flies. Egg deposition by phorid mushroom flies (*M. halterata*) was obtained by placing trays of full-grown compost in a growing room of a heavily infected commercial mushroom farm for one (preventive action) or four (curative action) days. To predict chances of a successful infection, the number of mushroom flies in the growing room on the commercial mushroom farm was estimated using glue-traps.



**Figure 1.** Still-air olfactometer. Phorid mushroom flies were released in the top dish and were offered a choice between two samples of fully colonised compost. One of these samples served as a control; to the other one, plant extracts were added (on a cotton stick placed on top of the compost). The hypothesis was that phorid flies are equally attracted to the two portions of fully colonised compost. Repellent action of plant extracts results in a lower number of flies attracted to the compost, as compared to the control.

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Severity of the subsequent infection was tested by mixing 0.5 kg of infected compost with 1 kg of non-infected fully colonised compost and, after casing, allowing the flies to hatch in a closed container. All treatments were incubated at an average air/compost temperature of 24°C. All flies that emerged from the compost were caught on the glue-traps. After a period of 3½ weeks, it was assumed that all flies had hatched and the number of flies was counted. Statistical analysis of results (ANOVA) was performed using Genstat, 8th edition.

### 2.3.3. Phytotoxicity tests

To test phytotoxicity effects of plant extracts on mushroom production, 15 kg of full-grown compost was filled into 0.2 m<sup>2</sup> trays and treated with plant extracts by watering the surface with 1 or 2% solutions of the extracts (using 1% Triton-X 100 as solvent). After casing, some trays received a second watering of 1 or 2% solutions of plant extracts (again using 1% Triton-X 100 as solvent) on the casing soil. The mushrooms were produced according to standard methods. Mushrooms were harvested from three flushes and yields were recorded. Each treatment was performed in five replicates. Statistical analysis of results (ANOVA) was performed using Genstat, 8th edition.

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## 3. Results

### 3.1. Effects on dry bubble disease

The effect of three different plant extracts on the incidence of dry bubble disease was tested in preliminary experiments. Plant extracts were applied after casing and at different times during the cultivation cycle. Extracts were dissolved either in 70% ethanol or sunflower oil. Extracts were either applied once after casing or at a number of times during cultivation (i.e. after casing and after first flush etc.).

At concentrations between 25 and 50 mL plant extract/m<sup>2</sup>, the number of dry bubbles could be suppressed by 90%. Application of less than 10 mL did not significantly lower the number of dry bubbles.

However, application of 25–50 mL of active extract also had large effects on mushroom yield (lowered by 50%). Application of plant extracts above 10 mL of active extract/m<sup>2</sup> of casing soil, produced a marked reduction of colonisation of casing soil by *Agaricus bisporus*.

A second set of 13 plant extracts was tested in two independent experiments (Figure 2). The plant extracts were dissolved in 1% Luxan H. All plant extracts significantly decreased the number of dry bubbles in infected cultures ( $p = 0.05$ ). However, some plant extracts were more effective than others. Effects of the tested plant extracts on mushroom yields were limited. Yields of mushrooms of the non-infected controls in the two experiments were 36.00 kg/m<sup>2</sup> and 32.29 kg/m<sup>2</sup>. Application of plant extracts resulted in yield reductions of 1 or 2 kg/m<sup>2</sup>. Thus, yield

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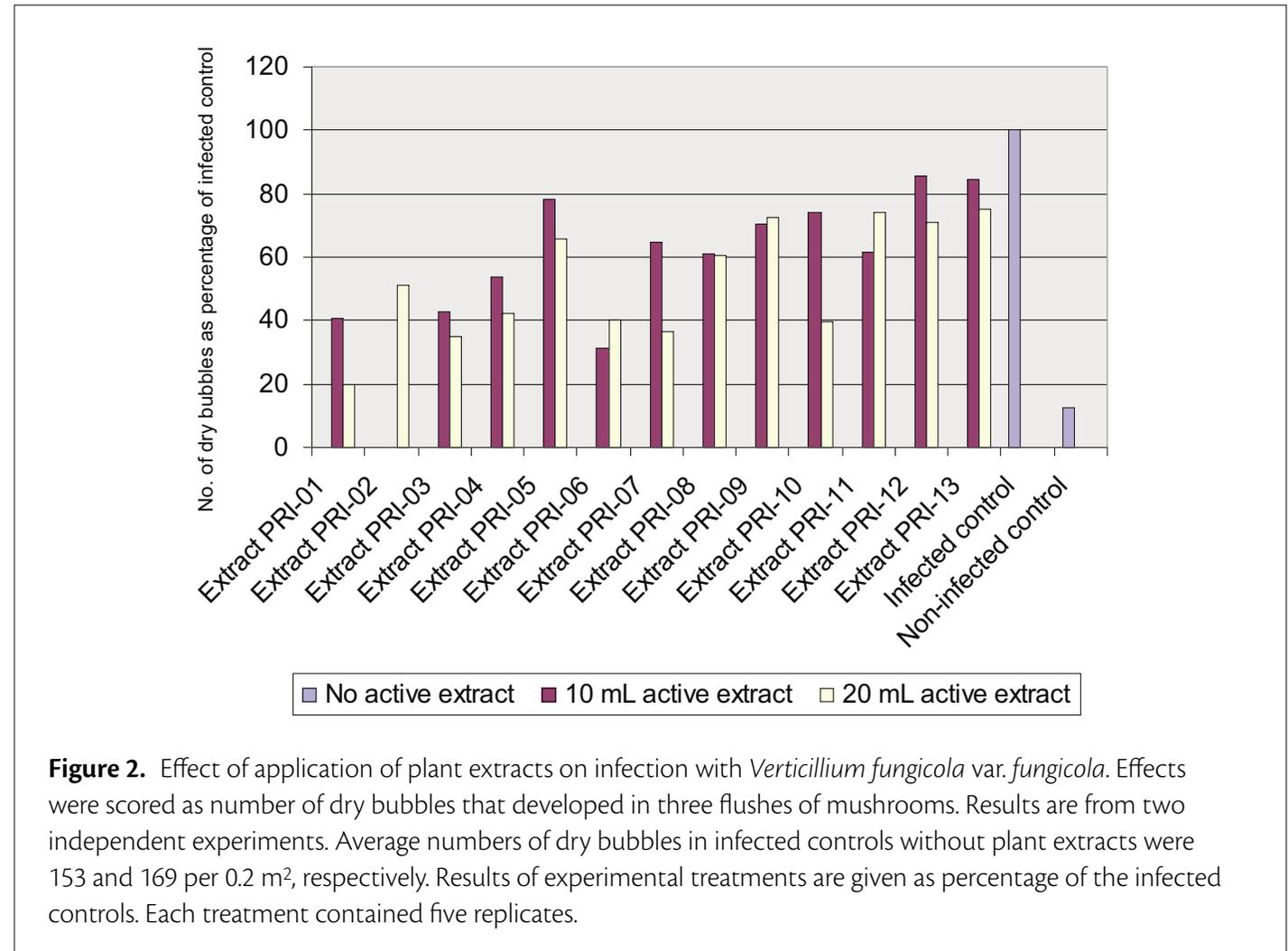
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reduction was quite limited and statistically not significant ( $p = -0.05$ ). It was noted, however, that application of plant extracts on the casing soil resulted in a statistically significant reduction of colonisation rate of casing soil by *A. bisporus* ( $p = 0.05$ ).

Plant extracts PRI-01, PRI-03, PRI-07 and PRI-10 were selected for an additional test, in which larger volumes of plant extracts were tested. Figure 3 shows marked reductions of the number of dry bubbles in infected cultures for all four plant extracts. It must be noted that in this experiment the number of dry bubbles in the control (no plant extract/



**Figure 2.** Effect of application of plant extracts on infection with *Verticillium fungicola* var. *fungicola*. Effects were scored as number of dry bubbles that developed in three flushes of mushrooms. Results are from two independent experiments. Average numbers of dry bubbles in infected controls without plant extracts were 153 and 169 per 0.2 m<sup>2</sup>, respectively. Results of experimental treatments are given as percentage of the infected controls. Each treatment contained five replicates.

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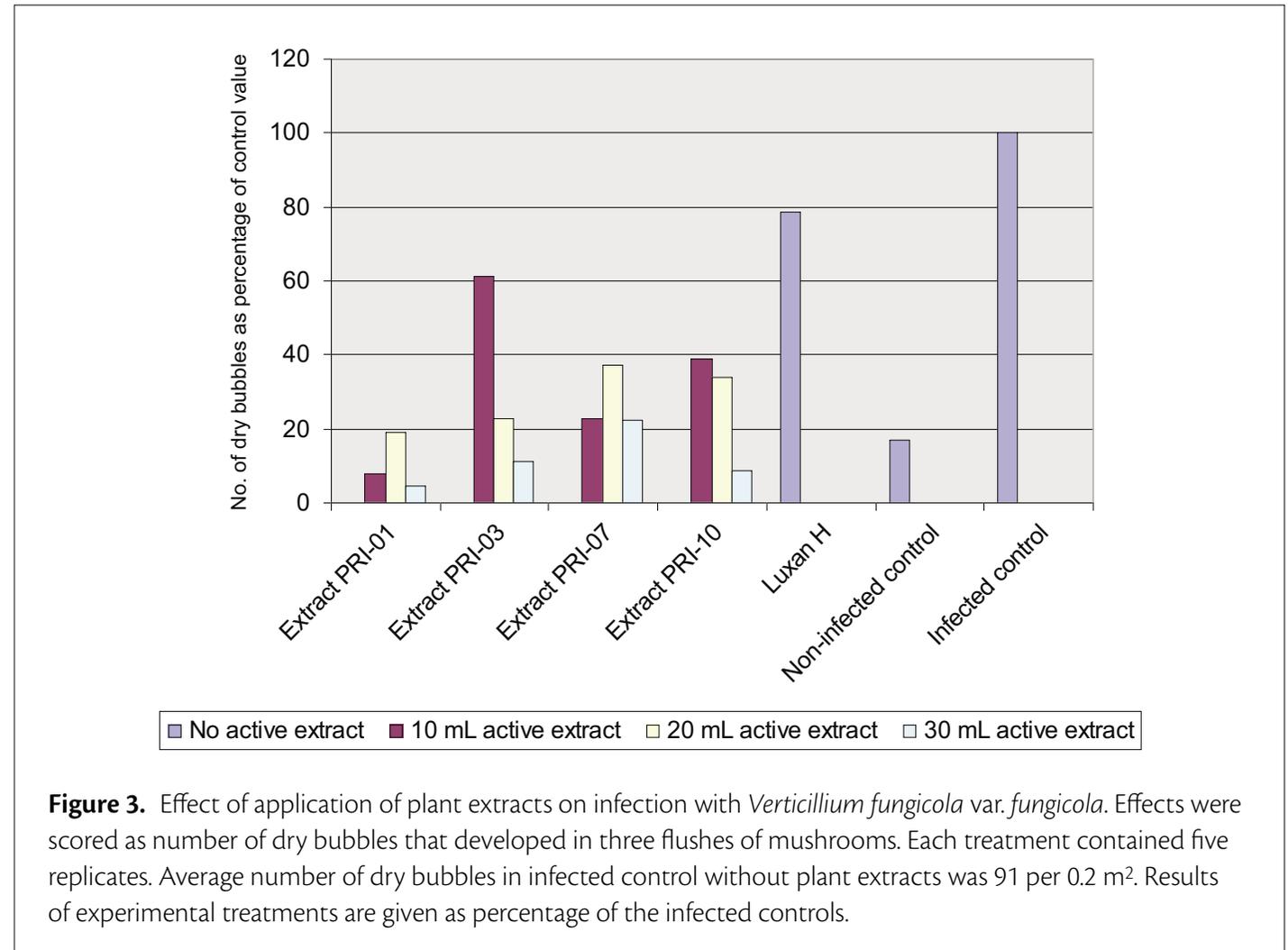
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infected) was much lower (91 per 0.2 m<sup>2</sup> tray) than in the previous experiments (153 and 169 per 0.2 m<sup>2</sup> tray). There is no clear dose-response relation but, in general, the highest dose of plant extracts reduced the number of dry bubbles best. It is also noteworthy that the treatment with only the solvent for the plant extracts showed a slight reduction in the number of dry bubbles.

Mushroom yield of the non-infected control was 31.9 kg/m<sup>2</sup>. Even at the higher concentrations of plant extracts no statistically significant reductions of yield were noted ( $p = 0.05$ ).



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### **3.2. Effects on infection with phorid mushroom flies**

#### **3.2.1. Olfactometer experiments**

In the olfactometer experiments a good response of the phorid mushroom flies towards the different plant extracts was obtained. On average, 71% of the phorid mushroom flies moved towards the origin of a scent. As a control, the phorid mushroom flies were given a choice between fully colonised compost in one container and fully colonised compost with plant extracts in the other container. It was expected that the phorid flies would be equally attracted to both sources of scent.

As a second control, the phorid mushroom flies were offered the choice between fully colonised compost in one container and an empty container. Here it was expected that the majority of the phorid mushroom flies would accumulate near the container containing the fully colonised compost. Results are summarised in Figure 4. In both control experiments, the phorid mushroom flies behaved as expected. No difference in the number of flies was found when they were offered a choice between two containers with fully colonised compost. In the second control experiment, a statistically significant majority of flies accumulated near the fully colonised compost.

When offering the phorid mushroom flies a choice between fully colonised compost and fully colonised compost containing plant extracts, it was noted that a statistically significant majority of phorid mushroom flies avoided fully colonised compost containing the plant extracts PRI-02, PRI-11 and PRI-13. The strongest repelling effect was obtained when using extracts PRI-11 and PRI-13. For these two extracts a fumigation effect was also noted. Of 11 phorid flies that visited the compost with plant extract PRI-11, nine did not survive. Apparently, the vapour of this extract is toxic to the phorid flies. Extracts PRI-01 and PRI-03 did not exhibit a repelling effect.

#### **3.2.2. Compost experiments**

Application of plant extracts for controlling infection of compost with phorid mushroom flies was tested in two ways. Plant extracts were tested for their usefulness in preventing infection of compost and in curing an already infected compost.

To test the preventive action of addition of plant extracts to compost, four trays of fully colonised compost were placed for one day in a growing room that was heavily infected with phorid mushroom flies. Two of these trays were treated with 20 mL active ingredient per m<sup>2</sup>. Extracts were applied on the surface of the compost using 1% Luxan-H as a solvent. The other two trays remained untreated.

The severity of the subsequent infection was tested by mixing 0.5 kg of infected compost with 1 kg of non-infected fully colonised compost and, after casing, allowing the flies to hatch in a closed container. After the flies had hatched, the numbers of flies on the glue-traps were counted. Some plant extracts were able to prevent infection with phorid

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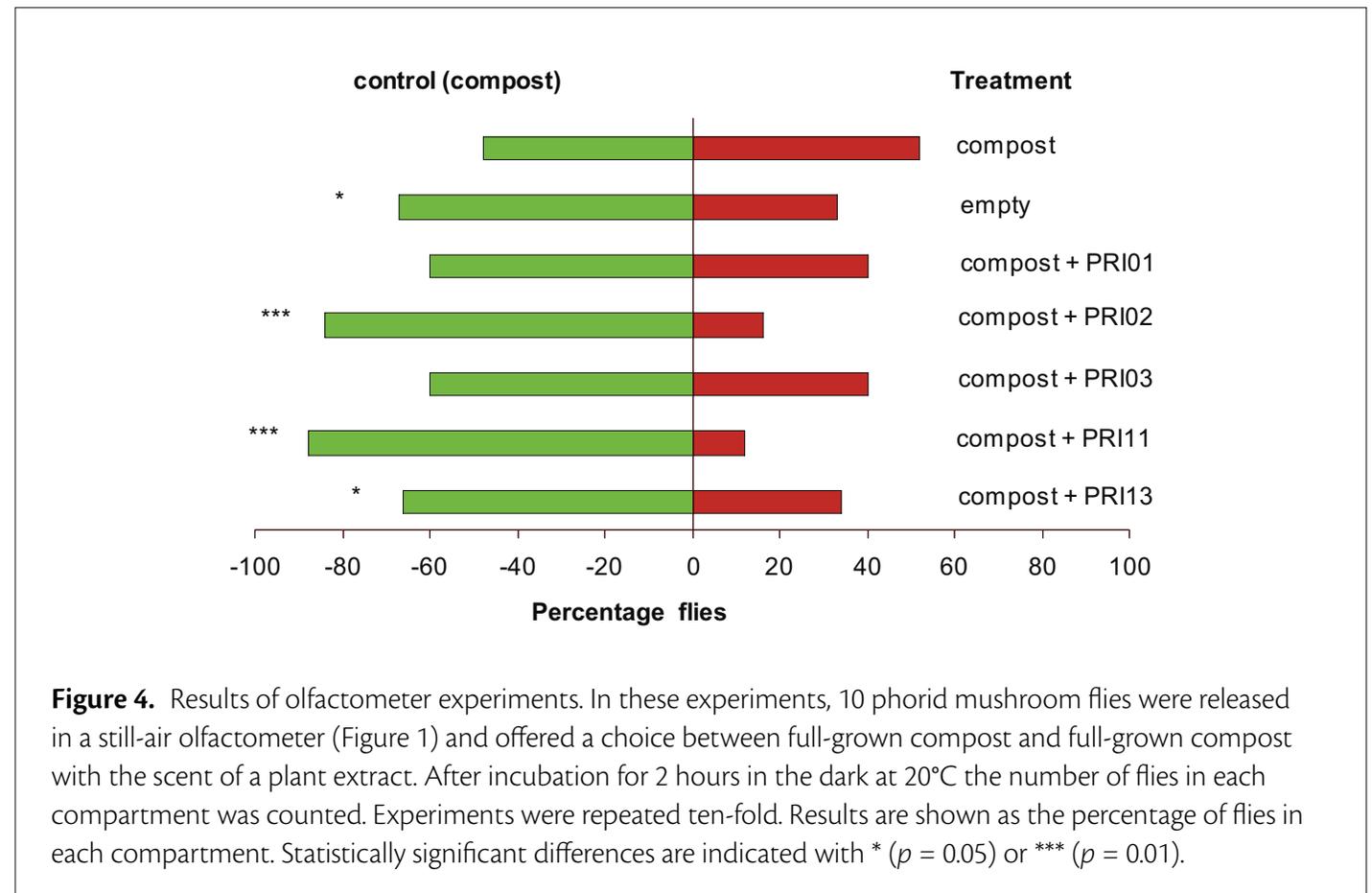
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mushroom flies to a considerable extent (Figure 5). Extracts PRI-02 and PRI-11 were most effective and reduced the number of phorid mushroom flies by 50–60%. It is also noteworthy that the solvent (1% Luxan-H), when used without plant extracts added, appeared to lower the number of flies caught.

To test if plant extracts were able to kill phorid mushroom flies in already infected compost, fully colonised compost was placed for 4 days in a growing room that was heavily infected with phorid mushroom flies. During these 4 days flies were allowed to deposit eggs in the compost. After this infection period, compost was treated with a solution of 1 or 2% of plant extract dissolved in Triton X100. Again, severity of the infection was tested by mixing 0.5 kg of infected compost with 1 kg of non-infected fully colonised compost and, after casing, allowing the flies to hatch in a closed container. After the flies had hatched, the numbers of flies on the glue-traps were counted. Some plant extracts were



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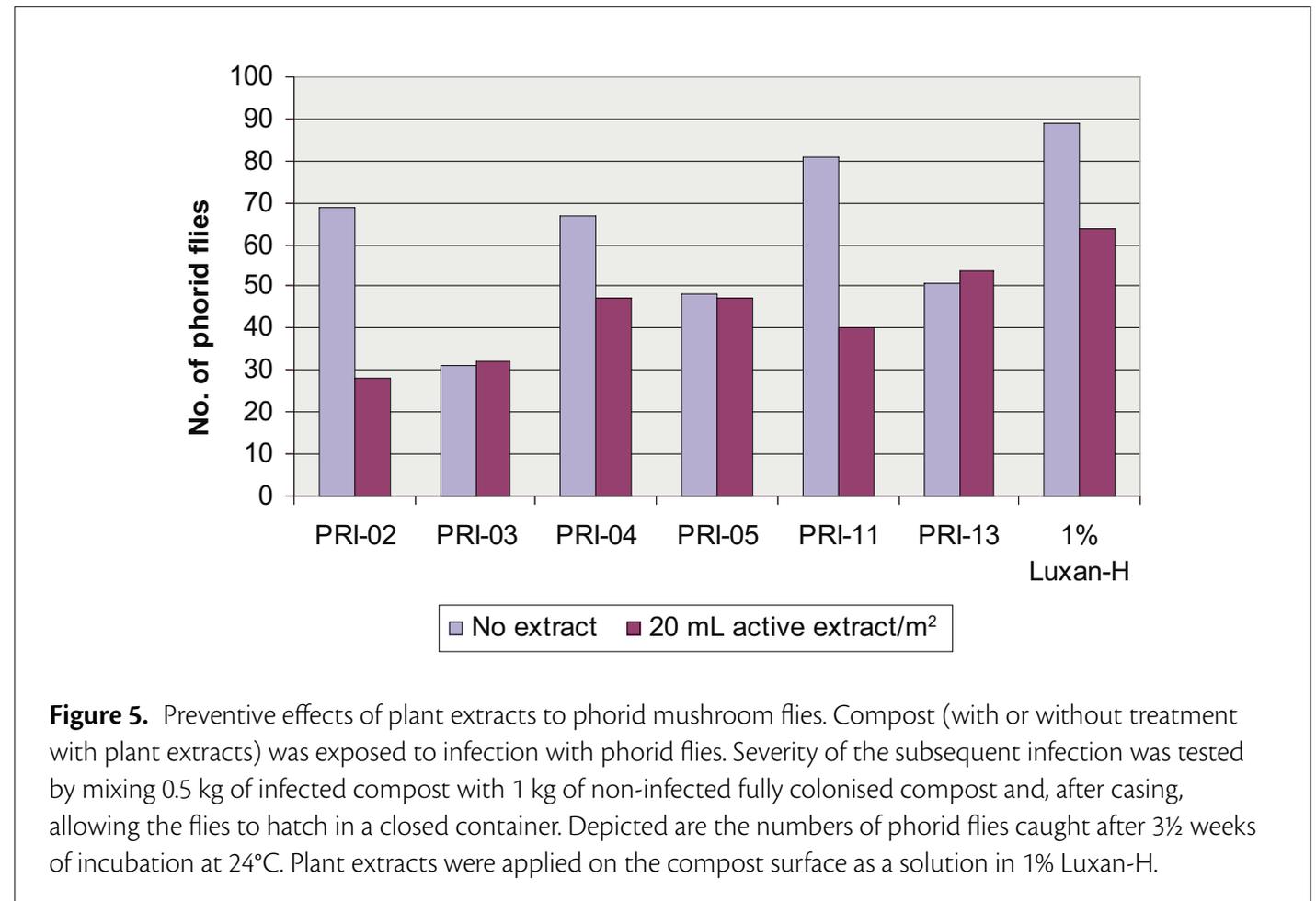
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able to cure infected compost to a large extent (Figure 6). A 1% solution of plant extract PRI-02 reduced the number of phorid mushroom flies that hatched by 89%. For all plant extracts tested, a dose-response effect was noted. Also Triton-X 100 by itself reduced the number of phorid mushroom flies.

**3.2.3. Phytotoxicity tests**

Experiments on phytotoxicity of plant extracts PRI-01, PRI-02, PRI-03, PRI-11 and PRI-13 showed that a single application did not harm mushroom production. Application of plant extracts PRI-02 and PRI-03 (8 mL active extract/m<sup>2</sup>) even resulted in a small but statistically significant ( $p = 0.05$ ) increases in productivity of 2 and 1.6 kg/m<sup>2</sup>,



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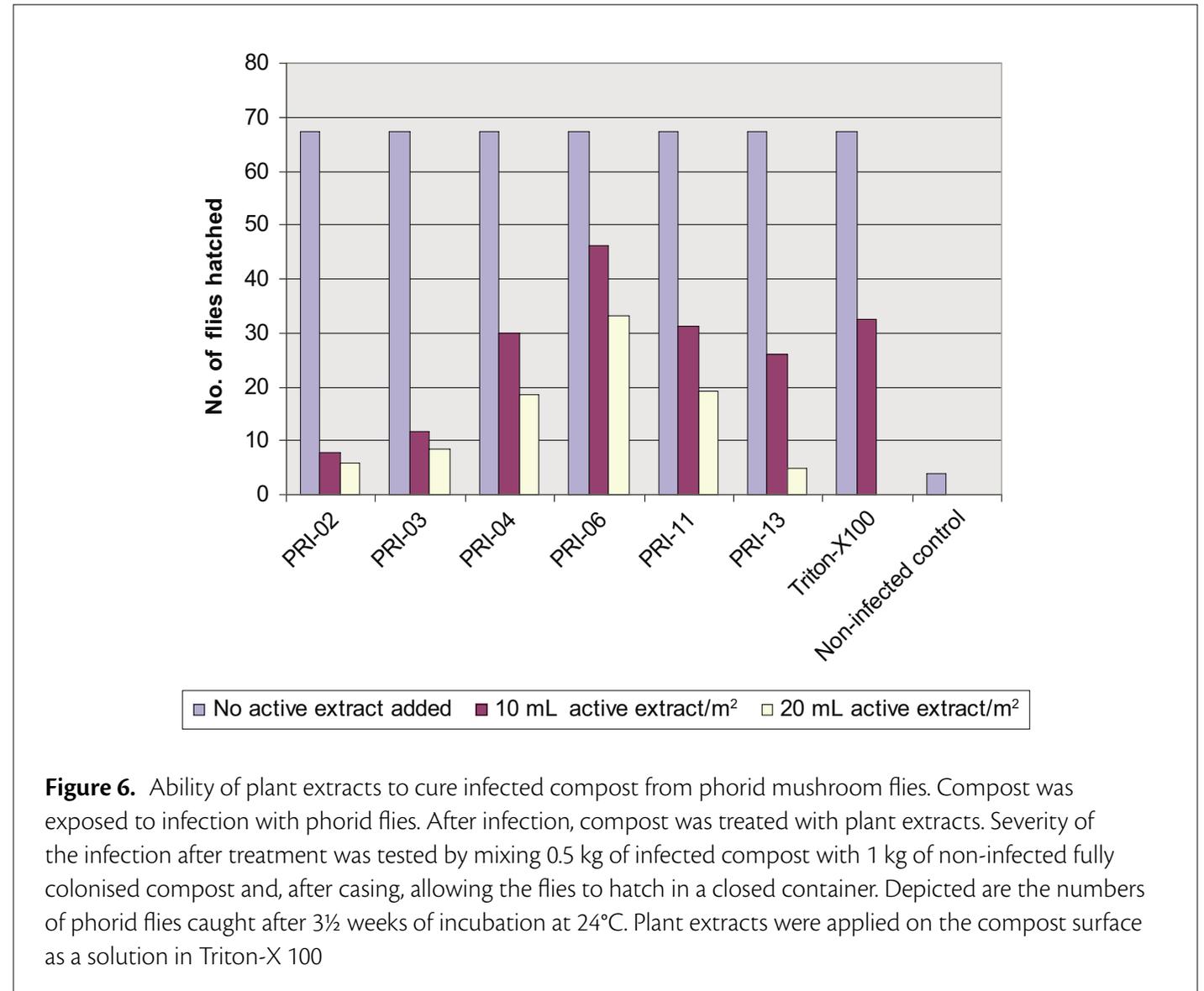
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respectively. Application of plant extract PRI-03 both on compost (8 mL active extract/m<sup>2</sup>) and casing soil (again 8 mL active extract/m<sup>2</sup>) also showed a small but statistically significant ( $p = 0.05$ ) increase of productivity of 1.7 kg/m<sup>2</sup>. All other plant extracts had no significant effect on productivity of the compost.



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## 4. Conclusion

The combination of the presence of phorid mushroom flies and dry bubble disease (caused by *V. fungicola*) causes problems on Dutch mushroom farms. Spores of *V. fungicola* are easily spread by the phorid mushroom flies (Geels et al. 1988). Therefore, both phorid mushroom flies and dry bubble disease need to be effectively controlled. In The Netherlands deltamethrin, diflubenzuron and malathion are available for pest control. For control of dry bubble disease, prochloraz-Mn is available. However, in the next few years it is expected that a number of these crop protection agents will no longer be available. On one hand, the manufacturing companies are expected to be reluctant to prolong registration of these chemicals for use in mushroom production. On the other hand, retail organisations will put more emphasis on produce that is free of residues of chemical crop protection agents. In addition to this, an increased tolerance of *V. fungicola* var. *fungicola* towards prochloraz-Mn is reported (Desrumeaux and Sedeyn 2001; Gea et al. 2005) so it is to be expected that control of this pathogen using prochloraz will become increasingly difficult. The need for development of alternative methods of crop protection is obvious.

The results described in this paper show that plant extracts can control both dry bubble disease and phorid mushroom flies, without negative effects on mushroom yield. For some plant extracts, even these preliminary results show levels of control that are at least comparable to chemical crop protection agents. For comparison, prochloraz-Mn reduces the incidence of dry bubbles by 73% after a single application (2 grams/m<sup>2</sup>). (No figures are known for deltamethrin.) Further optimisation of the application method might even improve their performance.

Plant extracts are considered to be low-risk crop protection agents (Isman 2006). Steam distillation of aromatic plants yields essential oils. These oils have long been used as fragrances and flavourings in the perfume and food industries. With respect to the use of plant extracts as insecticides, the broadest range of botanicals is allowed in the United States. However, within the European Union regulatory approval is still a barrier for use of plant extracts as crop protection agents. Currently, efforts are made in the European Union to facilitate the registration of plant protection products containing plant extracts.

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