

Optimal Efficacy of a Fungicide Preparation, Natamycin, in the Control of the House-Dust Mite, *Dermatophagoides pteronyssinus*

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ABSTRACT

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The efficacy of a fungicidal preparation, natamycin, for the effective control of *Dermatophagoides pteronyssinus* (Trouessart), was determined at different concentrations on mattress-dust microsites, including ticking. One treatment with the commercially available natamycin concentration (Tymasil) resulted in a 65% reduction of house-dust mite populations within 4 weeks of treatment. The acaricidal control could be proven despite the protective role of mattress fibres such as ticking. However, mite fecundity was much more affected after treatment with twice the commercial concentration, which then assured a long-term control. Concentrations below the commercially available one were not effective. In a maritime climate, six sequential treatments three times a year would be effective for an adequate long-term control because of the high growth of house-dust fungi and the close interaction between the mite and the mould *Aspergillus penicilloides*.

INTRODUCTION

The fungicide natamycin offers a tool for controlling populations of the most important house-dust mites involved in respiratory allergy, *Dermatophagoides pteronyssinus* (Trouessart) and *D. farinae* Hughes (De Saint Georges-Grیدهlet, 1981a). Natamycin interferes in the close interrelationship between the mites and endemic moulds of the xerophilic *Aspergillus* groups. Fungi are needed for mite food utilisation (Van Bronswijk and Sinha, 1973; Van de Lustgraaf, 1977). But antagonistic effects of xerophilic fungi on house-dust mites have been observed after abundant fungal growth (Van de Lustgraaf, 1978). Antagonism caused by the symbiotic fungus *Aspergillus penicilloides* is frequently observed on powdery glucidic culture media at the optimal relative humidity of 75% and higher. It is less marked on natural substrates, which are more

heterogeneous physically and chemically; here, the beneficial effects of the mould prevail since the mites control its growth more easily (De Saint Georges-Grèdelet, 1980, 1981b; Lebrun and de Saint Georges-Grèdelet, 1984).

The aim of this study was to determine the lowest concentration of nystatin needed to obtain sufficient and persistent control of pyroglyphid mites in mattresses.

MATERIAL AND METHODS

Culture of Dermatophagoides pteronyssinus

A test strain of *D. pteronyssinus* was obtained from mattress dust incubated at optimal mite conditions (25°C and 75% R.H.). Laboratory cultures were maintained on a mixture of human skin scales and yeast powder (1:1; Spieksma, 1967, 1969) in 50-ml glass vials.

Test rearing cages

Pooled mattress dust was used as the culture medium. After assembling and homogenizing with mounted needles, the dust was divided into 48 samples of 100 mg each and exposed for 72 h to a 30-watt U.V. light (germicidal max. 2537 Å) at 10 cm distance. Each dust sample was spread out over a 25-cm² surface and agitated three times a day to ensure complete U.V. exposure of all dust particles. This procedure reduces pre-existent microbiological infestation to a low level without changing the chemical nature of the substrate (Leach, 1971). To provide a good resemblance to the in vivo situation, each dust sample was then spread over a mattress ticking (5×5 cm) stretched between the cup and cover of a petri glass dish which was 5 cm in diameter; a vent of 12.5 cm² was left open in the lid of the cover (Fig. 1). The whole procedure ensures adequate drying and sufficient ventilation of the ticking from underneath as well as from above after the wet fungicidal treatment, as normally found under field conditions. To prevent the mites from escaping, the rims of the cup and cover were smeared with tanglefoot (Spieksma, 1967) and a 50-μm-mesh bolting nylon fabric was stretched just underneath the lid without being in contact with the dusted ticking.

Mite rearing

After the rearing cages had been pre-incubated for 72 h at 25°C and 75% R.H., 20 males and 20 females (young adults) of *D. pteronyssinus* were transferred to the center of each dusted ticking. According to preliminary studies, 20 pairs of adult mites per 100 mg dust ensures a rapid colonization of the

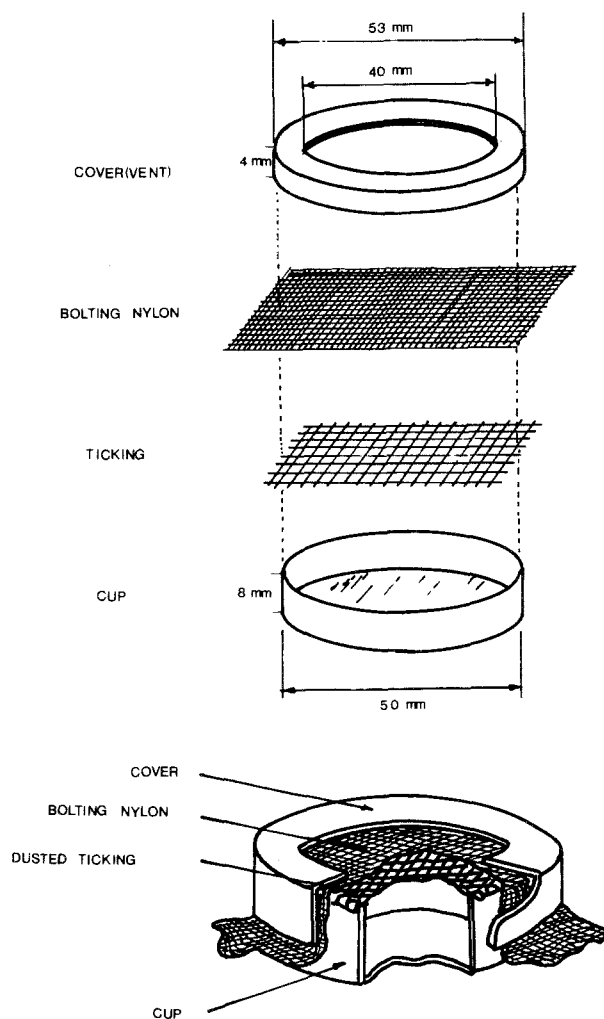


Fig. 1. Test rearing cages

substrate and there is no risk of overcrowding (Fig. 2). The rearing cages were then maintained under optimal thermohygrometric conditions.

Fungicidal test

Fungicidal suspensions in water were prepared according to the Tymasil formulation (Gist-Brocades n.v., Delft, The Netherlands). The four concentrations tested (0.4, 0.8, 1.6 and 3.2 mg/25 cm² ticking) represent one-half, one, two and four times the rate as recommended by the manufacturer for one treatment of a 180 × 90-cm mattress and 60 × 60-cm pillow (625 mg/19800 cm²

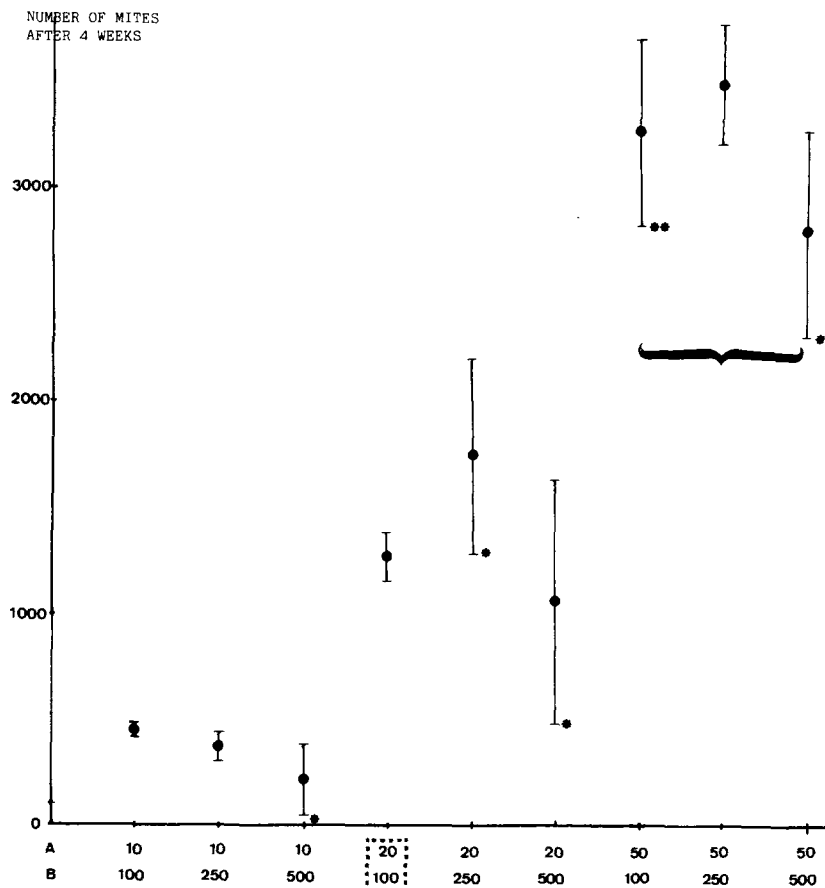


Fig. 2. Preliminary studies, rate of increase of populations of *D. pteronyssinus* on mattress-dust. Different combinations of initial mite population and quantity of dust. Constant climatic conditions (25°C; 75% R.H.) Means of 3 replicates $\pm$ standard deviation

A: pairs of mite adults

B: mg of dust substrate

—: excessive numbers of mites

*: dispersal of mites

* *: overcrowding and escape

— about 0.5 g/m² for a mattress surface; Lebrun and De Saint Georges-Grèdelet, 1984). For the test, each amount of natamycin was mixed with 1 ml water and then applied using an electric microsprayer (Spraymatic®, I.M.L., Italy). Spraying was carried out at a distance of 15 cm from the dusted ticking. Homogeneity of the water suspension was guaranteed during spraying with a magnetic agitator. Each dish was treated only once.

Treatment was applied according to the following protocol: A total of five series was studied, each consisting of three replicates. In four series, natamycin

was tested in the 4th week. One series was left untreated as a control. One, 2 and 4 weeks after treatment, mite numbers were assessed in the three replicates to determine impact of spraying on the growth of mite populations. In addition, just before the treatment of the four series, mite numbers in the control series were counted in all three replicates to examine the vitality of mite culture at this 4th incubation period.

Treatment assessment

An alcohol-suspension method was used to count the mites after the four different intervals. Mites from the pieces of ticking were counted as follows: each piece of fabric was immersed in 10 ml of ethanol 75% on the bottom of a 10-cm petri dish, and then gently scratched with a mounted needle to remove all mites from the fibres – individuals can easily be distinguished since they appear white against the dark background of the fabric. The fabric was then rinsed three to four times with 25 ml of ethanol 75% until the suspension was clear. Each aliquot was examined under a binocular stereomicroscope following the method of Gridelet and Lebrun (1973) and Van Bronswijk et al. (1978).

The data are presented in terms of reduction of mite populations, fecundity, and mortality rates. Fecundity was expressed as the percentage of gravid females and the number of eggs per live female. Larval counts permit evaluation of a possible lack of egg-hatching following treatment. *F*-tests (Dagnelie, 1975) were performed on total population densities and on fecundity values (gravid females) for the various series.

RESULTS

All concentrations tested appeared effective, with mite population regressions ranging from 30 to 75% depending on the intensity of treatment and the time elapsing after treatment (Table 1). However, the fecundity was generally lower at concentrations of 1.6/25 cm² ticking (Fig. 3). No large differences in efficacy levels were apparent on dusted ticking treated with the higher and most-effective 1.6 and 3.2 concentrations. On the other hand, a peculiar variability of results for the 0.4 and 0.8 mg/25cm² concentrations was observed at all incubation times.

On treated microsites as well as on non-treated ones, high proportions of adults, immature mites and eggs were observed on the fine rigid fibres of the ticking (Table 2); the presence of faecal pellets was also noted.

Despite the high fungicidal efficacy, mortality was low in all microsites compared to that recorded in previous, similar tests (De Saint Georges-Gridelet, 1981a,b).

One week after treatment, a distinct fungicidal effect was already observed in all of the series, marked by a population regression of 28.3–41.9%. Optimal

Growth of *D. pteronyssinus* populations on non-treated and treated mattress dust at different times following one application of four different natamycin concentrations (average numbers of three replicates)

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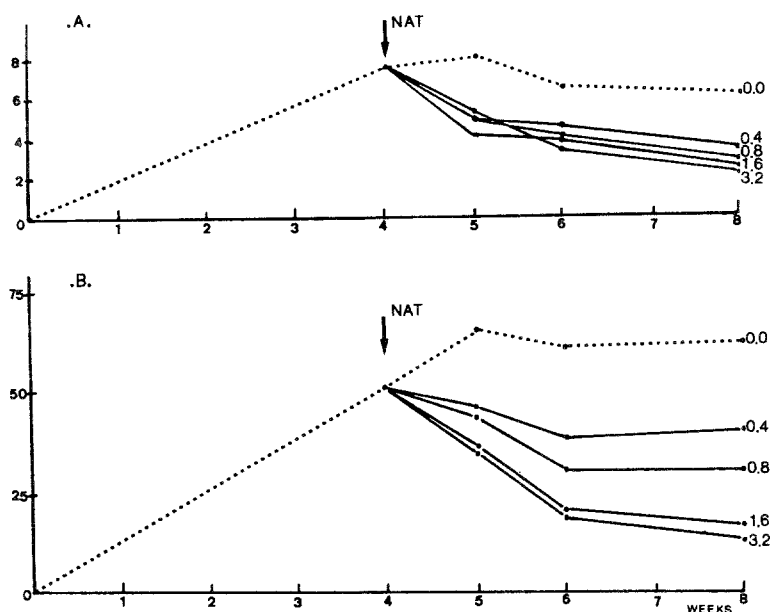


Fig. 3. Evolution of *D. pteronyssinus* fecundity on non-treated and treated mattress dust
 .A. Average number of eggs per female at different natamycin concentrations (in mg/25 cm²)
 .B. Average percentage of gravid females (nat. = time of treatment)

efficacy was observed with the 1.6 concentration. Despite the higher mean number of eggs per female in the 3.2 series, no improvement in acaricidal effect was noticed with the higher concentrations ($F=0.19$).

Two weeks after treatment, the percentages of gravid females appeared more reduced at concentrations 1.6 and 3.2 ($0.01 < P < 0.025$; Fig. 3). With this longer period of incubation, acaricidal activity on *D. pteronyssinus* populations appeared significantly higher in the 3.2 series ($P < 0.01$).

Four weeks after treatment, good acaricidal control (46–65%) persisted at the lowest concentrations but was consistent with the 1.6 concentration and the higher levels. This was evidenced by the distinct reduction in fecundity and the higher levels of mortality (Fig. 3 and 4). Only microsites treated with the 1.6 and 3.2 concentrations showed persistent activity, reflected in a continuous reduction of gravid females. Acaricidal activity appeared significantly higher in the 3.2 series, but somewhat less significant than that observed 2 weeks after treatment ($P < 0.025$). At this point after treatment, adult mite populations were mainly seen in ticking except in non-treated microsites (Table 2).

DISCUSSION

In spite of fungicidal activity at the lowest concentrations, some variability within the results indicates a lack of efficacy of the treatment. This could be

TABLE 2

Proportions (in %) of each stage of *D. pteronyssinus* in the ticking of non-treated and treated mattress microcosms (average of three replicates)

Treatment	Natamycin concentration (mg/25 cm ²)				
	0.0	0.4	0.8	1.6	3.2
5 incubation weeks and 1 week after treatment					
Adults	56.1	52.4	58.4	64.6**	64.7*
Nymphs	40.4	49.4	44.1	63.6**	62.3**
Larvae	31.1	31.9	34.6	48.8*	50.3**
Eggs	44.6	42.7	56.1*	63.8**	63.8**
6 Incubation weeks and 2 weeks after treatment					
Adults	70.8 ^a	55.3	56.5	60.7*	70.8**
Nymphs	68.1 ^a	47.3	45.4	60.2**	64.1**
Larvae	34.0	27.9	25.2	45.9**	50.7**
Eggs	62.3 ^a	42.0	50.2	62.6**	63.8**
8 Incubation weeks and 4 weeks after treatment					
Adults	62.6 ^a	42.9	52.6	54.5*	63.4**
Nymphs	55.5 ^a	31.8	37.1	40.1*	43.8**
Larvae	37.2	30.1	30.9	33.4	39.0*
Eggs	58.4 ^a	21.1	40.3*	44.2**	45.8**

*, **Significance of the difference between each of 0.8, 1.6, 3.2 concentrations and the 0.4 concentration was evaluated by Student's *t* test: **P* < 0.05; ***P* < 0.01

(a) 'overcrowding' effect in controls at 2 and 4 weeks after treatment

caused by an irregular distribution of the product at low concentrations. A high effect on mites requires absolute fungicidal activity. Few colonies of *Aspergillus penicilloides* are needed to optimize the growth of *Dermatophagoides pteronyssinus* (Van de Lustgraaf, 1978; De Saint Georges-Grédelet, 1981a). Insufficient fungicidal activity on the mattress dust could even result in a greater ability of the mite to control the symbiotic fungi at high relative humidities

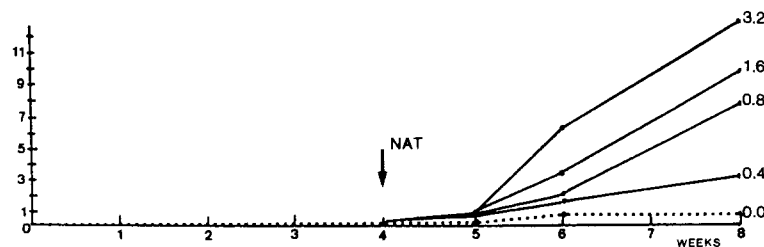


Fig. 4. Evolution of *D. pteronyssinus* mortality on non-treated and treated mattress dust (mortality in %)

(85% and higher). Such conditions can occur in temperate climates (Van Bronswijk, 1981). This control is effected to a certain extent by the secretion of antifungal pheromones by the mites (Kuwahara et al., 1980a,b).

Concentrations higher than 1.6 mg/25 cm² on ticking would ensure a more constant and longer-lasting control since, at this concentration, a distinct reduction in fecundity was observed. In this respect, 1 week after treatment the relatively high mean number of eggs per female at the highest concentration of natamycin (3.2) could be explained by egg accumulation due to a delay or even a lack of larval hatching, as attested by lower larval populations. On the other hand, 2 weeks after treatment, or after 6 weeks of incubation, the reduction in the average number of eggs per female in the controls was probably related to mite overpopulation. This depends on the unpredictable 'stimulating' effects of the ticking. The maintenance of a low mortality rate in treated mites and the high number of mite eggs, immature and adult populations in ticking should attest to the protective effects of such fibrous matter to mites in the mattress ecosystem. Four weeks after treatment, the greater reduction in the number of immature mites in the treated ticking may predict the limit of acaricidal efficacy.

Thus, assuming that surface fibres retain nutritive dander particles, fibres would constitute the more specific biotope of *D. pteronyssinus*. Such a distribution of house-dust mite populations in the ticking of the control units, which was the case in this experiment and in previous tests on fibrous dusts (data not presented), shows their essential role. In this experiment, activities such as feeding or reproduction in fibrous matter are proven by the presence of faecal pellets and egg accumulations. Other deeper mattress fibres are probably also implicated, since *D. pteronyssinus* usually finds shelter from unfavourable winter conditions, as can be deduced from the rapid increase in mite populations in the spring.

Therefore, to optimize natamycin's acaricidal efficacy and keeping in mind all environmental conditions, it seems necessary to apply adequate sequential treatment. The possible lack of acaricidal control in the 4th week following one treatment could indicate that treatments should be applied every 2 weeks. However, at least twice the commercial natamycin concentration is required to ensure a greater reduction in mite fecundity for long-term control. Moreover, to prevent a seasonal mite re-infestation, the recommended sequential treatment of six consecutive applications in the season (Lebrun and De Saint Georges-Grédelet, 1984) might be applied to all bedding twice a year. An initial preventive treatment at the end of the winter and a second treatment at the end (2/3) of the summer season would be advisable. It must be remembered that, from that time, the number of xerophilic fungi increases in the house-dust ecosystem, because of the more-favourable outdoor climate. Since relative humidity in a temperate maritime climate remains high in all seasons, mite

control might be ensured each year by applying a fungicidal agent at the beginning of March, June and September.

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