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International Journal of Pharmaceutics 256 (2003) 175–181
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Action of pyrethrum-based formulations against grain weevils

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Received 15 July 2002; received in revised form 13 November 2002; accepted 10 December 2002

Abstract

Pyrethrum extract, containing six insecticidal esters, has a long history of successful application in the control of stored products. Its low environmental hazard makes it an ideal pesticide for outdoor pre-harvest treatment. However the disadvantage of its low light stability then becomes apparent. This drawback can be overcome by the complexation of pyrethrum extract with gamma-cyclodextrin. Primary object of the conducted studies was to investigate the effect of complexation upon the insecticidal action against the grain weevil, an important storage pest in temperate climates. To slow down the quick metabolism of pyrethrum by the insects' microsomal system synergistic substances are added. Additional to the already well-known piperonyl butoxide two natural synergists, sesamol and tocopherol acetate, were combined with pyrethrum extract to investigate their synergistic activity. A complex of pyrethrum with gamma-cyclodextrin, with piperonyl butoxide as synergist, has a slightly enhanced action compared to a commercial product, which contained pyrethrum in its free form. Sesamol and tocopherol acetate also display a synergistic action, but to a much smaller degree, even if applied in larger amounts. The optimal concentration of pyrethrum was found to be 0.3% combined with 3% piperonyl butoxide.

Keywords: Pyrethrum; Gamma-cyclodextrin; Insecticide; Grain weevil; In vivo study

1. Introduction

Pyrethrum is the insecticidal principle extracted from flowers of *Chrysanthemum cinerariaefolium*. It is a toxic agent for pest insects with a century-long history of safe use (Katsuda, 1999). The active compounds of pyrethrum extract are six esters, commonly referred to as the pyrethrins (Fig. 1). The great advantages of pyrethrum are its action against a wide variety of different insect species (Silcox and Roth, 1994), a low mammalian toxicity (Satelle and Yamamoto, 1988), and a rapid metabolism (Yamamoto et al., 1969). Regarding the environmental fate of the pyrethrins their rapid metabolism is a point in their favour, while it is a drawback concerning the frequency of the application which has to be done very often. To overcome this disadvantage synergists are added to ensure an insecticidal effect of pyrethrum. The most widely used synergist in the last decades has been piperonyl butoxide (PBO). It combines the favourable aspects of having a low mammalian toxicity and of helping to reduce the amounts of pyrethrum which have to be applied (Kakko et al., 2000). Nevertheless PBO is a synthetic compound and trends in the last decades show a tendency towards natural products. The methylenedioxyphenyl group has been

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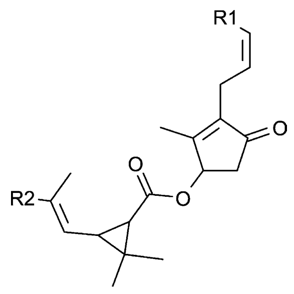
doi:10.1016/S0378-5173(03)00075-9



Pyrethrin I	R1: $CH{=}CH_2$	R2: CH_3
Pyrethrin II	R1: $CH{=}CH_2$	R2: $COOCH_3$
Cinerin I	R1: CH_3	R2: CH_3
Cinerin II	R1: CH_3	R2: $COOCH_3$
Jasmolin I	R1: $CH2\text{-}CH_3$	R2: CH_3
Jasmolin II	R1: $CH2\text{-}CH_3$	R2: $COOCH_3$

Fig. 1. Insecticidal compounds in pyrethrum extract.

recognised as the active structural feature of PBO (Yamamoto, 1973). The parent molecule also displaying this synergophor group is sesamol, a substance gained from roasted seeds of *Sesamum indicum*. The increased synergistic effect of roasted sesame oil has been attributed to its higher content of sesamol compared to unroasted oil (Yen and Shyu, 1989). Another possibly synergistic ingredient of sesame oil has been identified as tocopherol acetate (TA) (Yoshida and Tagaki, 1999). To study the synergistic potential of both sesamol and TA in comparison to PBO was therefore one of the aims of the conducted experiments.

Cyclodextrins (CDs) were added to slow down the degradation of pyrethrum induced by exposure to light. CDs offer a hydrophilic exterior and a hydrophobic cavity and can form inclusion complexes with hydrophobic guest molecules rendering them useful for a wide variety of purposes and also used for pesticides (Szejtli, 1985). One of their great advantages is furthermore that they are not toxic towards mankind, livestock, and environment. Calculations showed gamma-cyclodextrin to have the highest complex-formation constant with pyrethrum and was therefore selected for further studies. The stability of pyrethrum against light can be enhanced by the complexation with gamma-cyclodextrin (Biebel et al., 2000). The effect of the complexation on the action of pyrethrum was the main object of this investigation. Synergists may also profit from a complexation with CDs (Szejtli et al., 1984). Therefore, complexes of sesamol or TA, respectively, with γ-CD were prepared and analysed for their synergistic action. The grain weevil, *Sitophilus granarius*, was chosen as it is one of the major pest insects for stored products in temperate climates, like Western Europe and North America (Longstaff and Desmarchelier, 1983). Moreover it is easy to bread and handle.

2. Materials and methods

2.1. Materials

Pyrethrum extract was supplied by Serbios S.R.L., γ-CD (pharm. grade) was a gift of Wacker–Chemie GmbH. Sesamol and α-tocopherol acetate were purchased from Sigma. Granex® was supplied by F.Joh. Kwizda GmbH.

2.2. Methods

2.2.1. Preparation of pyrethrum γ-CD complexes

To a 10% aqueous solution of γ-CD pyrethrum extract containing about 70% pyrethrum was added dropwise with continuous stirring at room temperature. In order to ensure quantitative complexation this mixture was stirred for 24 h. To prevent any degradation by exposure to light the whole process was carried out in darkness. The precipitated complexes were then filtered under suction and dried in vacuum.

The complexes were then analysed by HPLC (Kasaj et al., 1999). The pre-treatment for the HPLC was done by extracting 10 mg of pyrethrum γ-CD complex three times with 100 ml of *n*-hexane. The suspensions were exposed to ultrasound for 1 h in each case and then centrifuged. The supernatant was flashed off and the remaining pyrethrum extract was taken up in a definite amount of acetonitrile for injection. HPLC was carried out on two ISCO 2350 pumps with an ISCO UV/Vis 205 detector. As analytical column Nucleosil 100-5C 18, 250 mm × 4 mm (Forschungszentrum Seibersdorf) was used. The mobile phase consisted of acetonitrile/water 58/42 at the beginning. This ratio was kept for 5 min and then the acetonitrile content was raised to 75% over 30 min. Afterwards the column was purged with 100% acetonitrile and the system was allowed an equilibration time of 10 min with acetonitrile/water 58/42. Samples were injected by means of an ISIS autoinjector system. The flow rate was 1 ml/min and the detection was performed at 230 nm. Each sample was injected twice. The chromatograms were integrated using ISCO JCL-6000 software. The fact that a real complex and not only a physical mixture is the result of the preparation process was determined by Differential Scanning Calorimetry on a Perkin Elmer DSC 7 calorimeter. The complexes were stored at 4 °C until further use.

2.2.2. *Preparation of sesamol γ-CD complexes*

γ-CD was treated with water (50% of the γ-CD weight) until a pasty mass was gained. To this highly viscous suspension a proportionate amount of a 10% solution of the synergist in acetone was added and the mixture was intensively kneaded until complete evaporation of the acetone. Afterwards the product was dried in vacuum. Since only small amounts of complex were produced the kneading was carried out manually using mortar and pestle. The fact of complexation was again established by DSC.

2.2.3. *Preparation of TA γ-CD complexes*

TA was added dropwise to a solution of 10% γ-CD in water. The mixture was then stirred at room temperature for 24 h to ensure complete complexation. Then, the precipitated complex was filtered off and dried in a vacuum.

2.2.4. *Preparation of the test formulations*

The pyrethrum content in the samples was varied to find the most economic concentration. Furthermore different synergists were added. The formulations were adjusted to the chosen content with talcum. The compositions of the formulations are shown in Table 1. To estimate the usual death rate of the grain weevils untreated wheat served as control. Granex®, a commercial product containing 0.3% pyrethrum and 3% PBO was chosen as reference. Additionally samples with γ-CD and all synergists alone were prepared to exclude an insecticidal action of these substances.

Table 1
Formulations for the activity studies

Pyrethrum (%)/γ-CD	Synergist (%)	Formulation
0.03/+	PBO/0.3	I
0.15/+	PBO/1.5	II
0.2/+	PBO/2	III
0.3/+	PBO/3.0	IV
0.3/+		V
2.0/+		VI
0.3/+	Sesamol/3	VII
0.3/+	Sesamol/6	VIII
0.3/+	Sesamol/15	IX
0.3/+	Sesamol γ-CD/3	X
0.3/+	Sesamol γ-CD/7	XI
0.3/+	Sesamol γ-CD/15	XII
0.3/+	TA/3	XIII
0.3/+	TA/5	XIV
0.3/+	TA γ-CD/5	XV
0.3/−		XVI
2.0/−		XVII
0.3/−	PBO/3.0	XVIII
0.3/−	PBO/3.0	Granex®

+, with γ-CD; −, without γ-CD.

2.3. *Conditions of the activity studies*

On 300 g of wheat with a corn humidity of 14% 25 weevils were put. The room temperature was kept at 25 °C and the humidity was adjusted to 50% rh. On the 2nd, 4th, 7th, and 14th day the insects were separated from the grain be sieving. After checking their condition (living, paralysed, dead) the dead weevils were thrown away and the others put back on the wheat. All experiments were carried out at least in quadruple.

3. Results and discussion

3.1. *Activity of pyrethrum γ-CD complexes with PBO or without synergist*

The aim of the activity studies was to establish the best concentration of pyrethrum extract as well as PBO. Starting from 0.03% pyrethrum and the 10-fold amount of PBO the concentration was increased up to 0.3% pyrethrum and 3% PBO. The activity of pyrethrum extract γ-CD complex without any synergist was also investigated.

Fig. 2 depicts the outcome of these experiments. The lowest concentration applied showed almost no

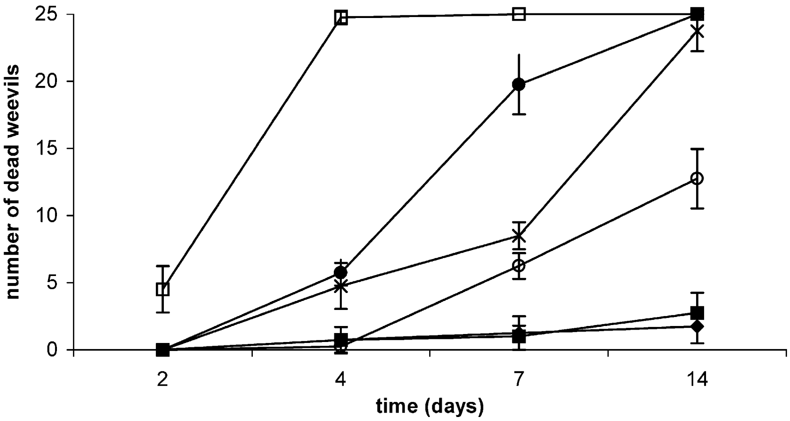


Fig. 2. Activity of pyrethrum γ-CD complexes with PBO as synergist or without synergist. I (◆), II (×), III (●), IV (□), V (■), VI (○) ($n = 4$).

insecticidal activity at all. After the whole test period nearly all the animals survived. This sample was also the only one containing PBO where any living and not damaged weevils were observed. If pyrethrum was used in amounts of 0.15% up to 0.3% a definite action was observed, increasing with rising pyrethrum concentrations. The highest efficacy was detected using 0.3% pyrethrum and 3% PBO. This formulation lead to an almost complete eradication of the weevils after 4 days. Using the formulation containing 0.2% pyrethrum and 2% PBO about one fifth of the insects were still not dead after 1 week. Only after the whole test period all animals were killed. About half of the weevils were dead after 7 days when applying the formulation which had been adjusted to 0.15% insecticide and 1.5% synergist. Therefore, the pyrethrum concentration in the following experiments was always adjusted to 0.3%. Without the addition of a synergist pyrethrum extract γ-CD complex only showed an insecticidal action if applied in higher amounts and even then the effect was strongly decreased with half of the insects surviving the application. The formulation which contained 0.3% pyrethrum γ-CD complex and no synergist had almost no insecticidal effect at all, once again underlining the essential addition of a synergistic component.

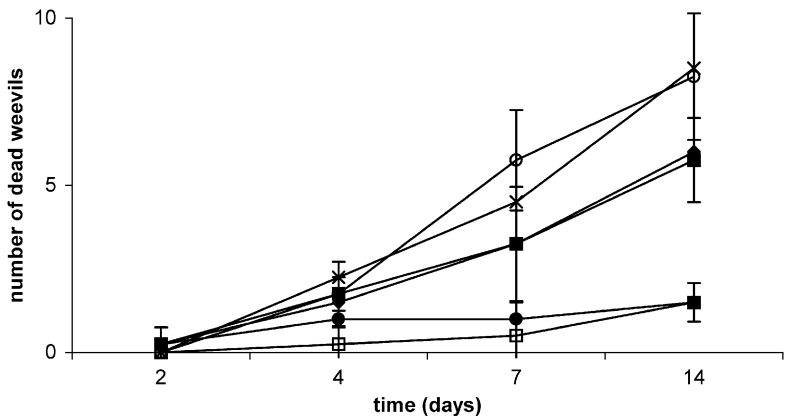


Fig. 3. Activity of pyrethrum γ-CD complexes with sesamol or sesamol γ-CD complexes as synergist. VII (◆), VIII (×), IX (■), X (○), XI (□), XII (●) ($n = 4$).

3.2. Activity of pyrethrum γ-CD complexes with sesamol or sesamol γ-CD complexes

In order to gain a completely "nature-derived" product PBO as synergist was substituted by sesamol. Different sesamol concentrations were added to the pyrethrum γ-CD complex, since there were no literature data found concerning the synergistic activity of sesamol against the grain weevil. Starting from a molar ratio of pyrethrum: sesamol from 1:20 (which relates to a sesamol concentration of 3%) the amount of sesamol was increased up to 15%. Because complexation had a beneficial effect on other synergists (Szejtli et al., 1984) studies were also carried out using the synergist after complexation with γ-CD.

The synergistic activity of sesamol can be regarded as rather small. In all investigated formulations about two thirds of the animals stayed alive (Fig. 3). The highest effect was observed with a sesamol concentration of 6%. Raising the sesamol concentration up to 15% did not increase the synergistic effect of this substance. On the contrary the effect was even reduced to a small extent. The complexation of sesamol did not have the desired effect. The rate of the surviving weevils was even higher than without complexation. Only the lowest sesamol concentration displayed a significant, if not too high, synergistic effect. The samples containing 7 or 15% sesamol as γ-CD complex had nearly no impact on the death rate of the insects. The reason may be that γ-CD slows down the release of sesamol and prevents it from interacting with the microsomal system of the grain weevils.

3.3. Activity of pyrethrum γ-CD complexes with TA or TA γ-CD complexes

As another natural synergist tocopherol acetate was tested starting from a 3% concentration up to 5%. Additionally a complex between TA and γ-CD was investigated.

Tocopherol showed very similar results to sesamol. Only an addition of 5% TA had a noticeable effect on the death rate of the insects. About one third of the insects were dead after 2 weeks. After complexation the observed effect was once again strongly reduced and the greatest part of the weevils survived the experiment undamaged. Fig. 4 shows the results of these studies.

3.4. Activity of pyrethrum extract with PBO or without synergist

To be able to estimate the effect of complexation of γ-CD on the activity of pyrethrum extract against grain weevils formulations were also prepared which contained pyrethrum extract without γ-CD and in part no synergist. A commercially available product, Granex®, was also included in the studies.

As shown in Fig. 5 the best formulation of the above shown experiments was, not surprisingly, the formulation with 0.3% pyrethrum and 3% PBO, which performed even a little bit better than Granex®. Pyrethrum extract without any synergist is also active against the insects. Formulations containing up to 2% pyrethrum extract resulted in a complete extinction of the weevils

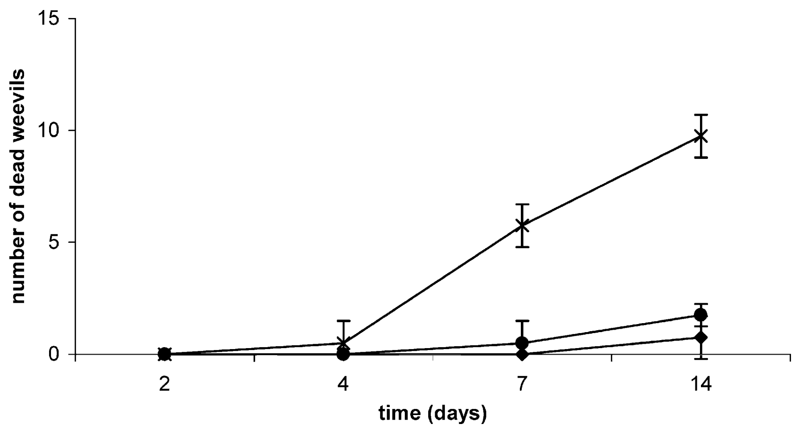


Fig. 4. Activity of pyrethrum γ-CD complexes with TA or TA γ-CD complex as synergist. XIII (◆), XIV (×), XV (●) ($n = 4$).

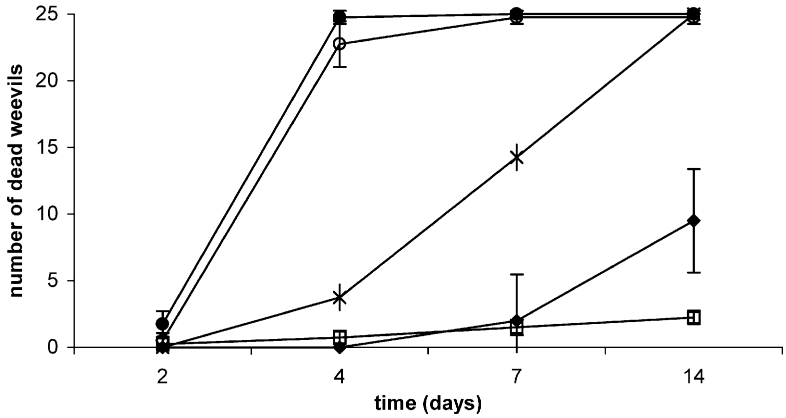


Fig. 5. Activity of pyrethrum extract with PBO as synergist or without synergist. XVI (◆), XVII (×), XVIII (●), Granex® (○), wheat (□) ($n = 4$).

after 2 weeks. If applied in a concentration of 0.3% the effect is strongly reduced. Half of the insects survived the test conditions during the whole test period. Complexation of pyrethrum extract with γ-CD had nonetheless a very small enhancing effect on insect death, which can be recognised on the second day.

3.5. *Influence of γ-CD and the synergists alone*

Neither PBO nor sesamol or TA displayed any insecticidal effect on its own. γ-CD alone had also no negative effects on the weevils. Therefore, it can be concluded that all observed insecticidal action is caused by pyrethrum extract.

4. Conclusion

In conclusion it can be said that the complexation of pyrethrum extract with gamma-cyclodextrin has a slightly enhancing effect when compared to the commercial product Granex®. The concentration for an optimal efficacy was established to be 0.3% pyrethrum and 3% PBO. If these amounts of insecticide and synergist are applied a satisfying insecticidal action can be obtained. By applying pyrethrum as a γ-CD complex, which increases the stability against light, it is therefore possible to use this valuable biocide also for outdoor applications. The search for a natural synergist lead to no promising results since both sesamol and tocopherol acetate have a much weaker synergistic potential than the synthetic piperonyl butoxide. Looking for other natural synergists will be research work in the future.

Acknowledgements

This study was conducted within the scope of the fourth frame programme of the European Commission, Directorate-General XII Contract FAIR.CT96. 1436.

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