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(54) **UTILISATION D'AU MOINS UN ACIDE DU CYCLE ACIDE
CITRIQUE EN COMBINAISON AVEC DE LA GLYCERINE,
COMME AGENT DE LUTTE CONTRE LES PARASITES**

(54) **USE OF AT LEAST ONE ACID FROM THE CITRIC ACID
CYCLE COMBINED WITH GLYCERINE AS A PESTICIDE**

(57) L'invention concerne l'utilisation d'un acide du cycle de l'acide citrique en combinaison avec de la glycérine pour lutter contre les parasites.

(57) The present invention relates to the use of at least one acid from the citric acid cycle combined with glycerine in order to control pests.

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Abstract

The present invention relates to the use of at least one acid of the citric acid cycle in combination with glycerol for pest control.

USE OF AT LEAST ONE ACID OF THE CITRIC ACID CYCLE IN COMBINATION WITH GLYCEROL AS A PEST REPELLENT

The present invention relates to a pest repellent against flying, creeping, stinging, biting and sucking pests and annoying animals of the order articulates (Arthropodes), in particular of the classes insects, spider animals, Chilopoda, Myriapoda, Diplopoda, and Isopoda.

Pest repellents are substances intended to get rid of pests and harmful organisms. Agents against the above-mentioned species are called insecticides and acaricides, respectively. Chemically, a distinction is made between natural and synthetic insecticides. Natural insecticides include rotenoids, such as deguelin, tephrosine, toxicarol, elliptone and sumatrol, which are obtained from tropical Derris, Lonchocarpus, Pachyrrhizus and Tephrosia species, but have the drawback to develop enormous toxic effects as furocumarines (arrow poison) also in warm-blooded animals. Further natural insecticides are alkaloids (e.g. nicotine) and endotoxins isolated from bacteria, which also present a high risk for warm-blooded users; furthermore, pyrethroids of Pyrethrum (pyrethrin, cinerin and jasmolin) which have also been discussed in recent years because of their toxicological risks.

The synthetic insecticides can be subdivided into inorganic and organic compounds. Inorganic insecticides are e.g. arsenic preparations, cryolite, fluorosilicates, Na fluoride, hydrogen cyanide, phosphorus hydride, and sulfuryl fluoride whose drawbacks upon application and whose health risks were already negatively and finally assessed a long time ago. Organic insecticides are e.g. chlorinated hydrocarbons, such as DDT, aldrin, dieldrin, chlordane, lindane, heptachlor and

toxaphene. These compounds are persistent and mostly accumulate in the fatty tissue of warm-blooded animals because of their high fat solubility. In the meantime, these substances have also been assessed in a mostly negative manner. Further known insecticides are phosphoric acid esters, such as E 605, which are disadvantageous because of their high toxicity for warm-blooded animals, carbamates, such as carbaryl, carbofuran and propoxur, as well as synthetic pyrethroids, such as cypermethrin, cyfluthrin, cyhalothrin, deltamethrin, fenpropathrin, permethrin, tralomethrin, phenothrin, resmethrin, tetramethrin, allethrin, fenvalerate, etofenprox, and many others, with all of the so far known side effects as notorious neurotoxins.

The disadvantages of all of the natural and synthetic insecticides that have so far been known are, on the one hand, their toxicity for humans and warm-blooded animals (mammals, birds) which varies, but is inherent to all substances. On the other hand, many pests are capable of developing resistances to the various active substances in a rapid alteration of generations because even with the most careful treatment method it can never be ruled out that individual specimens get insecticide doses that are not lethal. These specimens can rapidly detoxicate by way of enzymatic oxidation, and this capacity is passed on in the genetic information to their progeny. The successive populations are thus immune (resistant) to the insecticide.

This effect can be attenuated by adding so-called synergists, such as piperonyl butoxide, which is known to be cancerogenic, or highly toxic phosphoric acid esters or carbamates. Such a combination, however, still shows dangerous impacts on warm-blooded animals.

Various insecticides which are ecologically and toxicologically relatively safe have been proposed because of these drawbacks.

For instance, JP-A-63-297303, JP-A-04-250804, DE-A-4233806, WO-A-9531100, and US-A-5489433 disclose insecticidal compositions which are based on carboxylic acids, such as citric acid.

WO-A-9205699 and JP-A-02-142704 describe insecticidal compositions which contain polyvalent alcohols or monoesters of polyvalent alcohols as the active substance.

However, the insecticides which are based on toxicologically safe substances often show an inadequate efficiency in comparison with conventional substances.

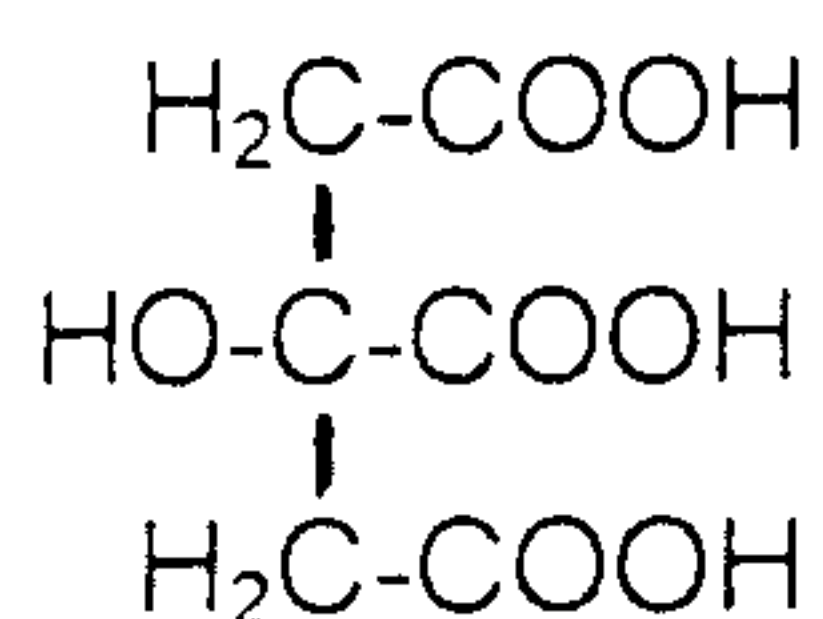
It is therefore the object of the present invention to provide an efficient pest repellent based on natural and/or nature-identical active substances with the lowest toxicological risk, which can be used without any harm for humans and their environment (pets, food, contaminated areas and articles) even if excessively misapplied, which is not persistent, i.e. is rapidly biodegradable without formation of harmful metabolites, and which in the target animals does not permit the development of any resistance even if non-lethal doses are applied, and which despite these prerequisites has a sufficiently long period of action to totally eradicate infestation by pests.

According to the invention this object is achieved by using at least one acid of the citric acid cycle in combination with glycerol.

The acids of the citric acid cycle are oxalacetic acid, glutaric acid, citric acid, oxalic acid, itaconic acid, succinic acid, fumaric acid and L-malic acid. Of these acids, citric acid is preferred.

The acids of the citric acid cycle shall just be called acids in the following.

Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid) with the structure



and the empirical formula $\text{C}_6\text{H}_8\text{O}_7$

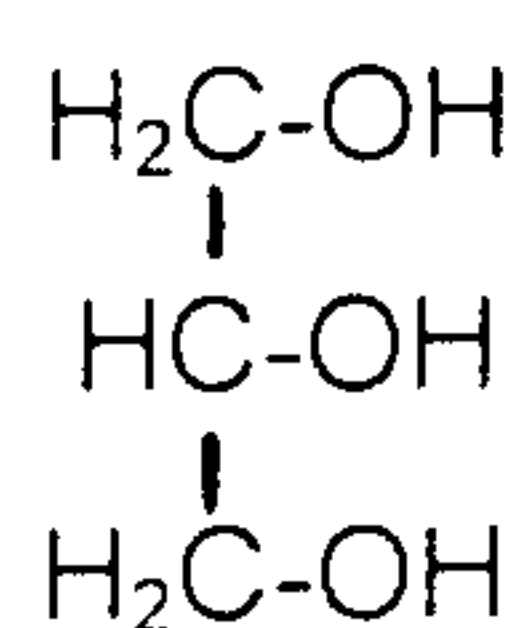
has a molecular weight of 192.12 in its water-free state and may be of natural or synthetic origin. Normally, citric acid is present with different crystallization water contents, ranging from monohydrate ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$) to 7-hydrate ($\text{C}_6\text{H}_8\text{O}_7 \cdot 7\text{H}_2\text{O}$). Natural citric acid is prepared from lemon juice by precipitation with lime milk and subsequent decomposition of the calcium citrate by means of sulfuric acid into calcium sulfate and free citric acid. The most common synthesizing method is the submerged-culture process in which citric acid is biotechnologically produced by microbial culture technique. Further methods of the citric acid synthesis are the surface method and other fermentation processes which in technical terms are now of minor importance.

Citric acid belongs to the most wide-spread plant acids and has been detected in numerous fruits, milk, coniferous trees, orchids, beet juice, mushrooms, tobacco leaves, wine, etc. Particularly rich is lemon juice which contains about 5 to 7% citric acid. Since citric acid regularly occurs in the citric acid cycle as an intermediate product in the oxidation of fats, carbohydrates and amino acids, it can be found in small amounts in all organisms. Its physiological and toxicological safety for humans and warm-blooded animals, such as birds and fish, is impressively documented by the fact that e.g. in the citric acid cycle of a grown human being a total amount of about 2000 g citric acid is formed as an energy-rich intermediate product every day and degraded again. A relatively high content of citric acid is permanently found in the bone system of all mammals and birds.

Evidently, humans, in particular, but also herbivorous species of mammals and birds, consume with their daily food (fruit, vegetables, fruit juices, etc.) and without any harm many times the amount of citric acid that could be taken up orally, dermally or inhalatively

no matter how high the ingestion is upon application of the pest repellent according to the invention. The LD₅₀ value (rats, oral route) of citric acid is above 20,000 mg/kg body weight, whereas it is 300 mg/kg in the case of carbaryl (1-naphthyl-methyl-carbamate), 88 mg/kg in the case of lindane and 56 mg/kg body weight in the case of dieldrin as a representative of the phosphoric acid ester. Moreover, phosphoric acid esters, such as azinphos, malathion or parathion, have the same structure as the warfare agents and neurotoxins sarin, tabun and soman and have been suspected to be carcinogenic and mutagenic.

Glycerol (1,2,3-propanetriol) with the structure



and the empirical formula C₃H₈O₃ is a clear, colorless, hardly mobile, odorless and sweet-tasting hygroscopic liquid with a molecular weight of 92. Glycerol may be of natural or synthetic origin.

Glycerol is extremely wide-spread in animal fats and vegetable oils and is separated therefrom by means of saponification. Synthetic glycerol is prepared, starting from propane across the intermediate stages allyl chloride or allyl alcohol. Glycerol is also found in the blood of some insects. On account of the antifreeze effect based thereon, arctic ground beetles, for instance, survive temperatures up to -85°C. The LD₅₀ value of glycerol is above 20,000 mg/kg body weight (rats, oral route). Glycerol can therefore be used without any toxic risks.

The above-mentioned risks of conventional insecticides can be completely ruled out in the inventive use of at least one acid of the citric acid cycle in combination with glycerol. The complete solubility of the combination in water turns out to be a further considerable difference and advantage over all of the other known insecticides because the use of

toxicologically and ecologically unsafe formulation constituents and adjuvants, such as solvents (organic, halogenated, etc.), hydrocarbons and emulsifiers, can thus be entirely dispensed with, which in particular in pest control in agriculture and horticulture yields the following advantages of the use according to the invention:

1. Total absence of phytotoxicity on chlorophyll-containing plant parts as is observed time and again in all other agents due to the action of emulsifiers, resulting in crop failure and dying off of perennial useful plants.
2. Totally safe use of citric acid without impairment and potential danger for air, earth and water by total rapid biodegradation without harmful metabolite formation.
3. Exclusion of any imperilment by contamination of field and garden fruits for fruit consumers, such as humans and animals.
4. No development of any resistance; therefore, the application of a minimum dosage is always possible, and no change in the active substance is required when resistant populations are found.

Just as novel is the mode of action on which the use of the combination according to the invention is based. Since it neither represents a neurotoxin (pyrethroids) nor an acetylcholinesterase inhibitor, such as phosphoric acid esters and carbamates, but as a contact and stomach poison directly acts on the citric acid cycle of the pests and thus inhibits the glyco and amino acid metabolism of the pests as a result of a rapid increase in the citric acid level, the use according to the invention leads to death very rapidly without any possibility of recovery (reanimation). In the synthesis and degradation products of citric acid which are present in the natural citric acid cycle in very small amounts in the pest bodies, a slight increase in the content of citric acid is already absolutely and reliably insecticidal and reliably kills off all pests. Surprisingly enough, the combination with glycerol intensifies this effect very strongly, which leads to a superior efficiency of the use according to the invention over the sole use of e.g. citric acid.

According to the invention any desired mixtures of at least one acid with glycerol may be used. The mixing ratio may here be in the range of 0.01 to 99.99 parts by weight of acid to 99.99 to 0.01 parts by weight of glycerol. Preferably, 10 to 80 parts by weight of acid and 80 to 10 parts by weight of glycerol are used. Particularly preferred is the use of equal parts of acid and glycerol.

Acid and glycerol can directly be mixed with each other, but it is also possible to add a suitable solvent. The mixture of acid and glycerol can thus be atomized, injected or sprayed. Suitable solvents are water and 1,2-propylenediol. By the addition of thickening agents the mixture of acid and glycerol can also be adjusted such that a spreadable mixture is obtained. Particularly preferred is a mixture which can be thickened such that a gel with a viscosity of 30 to 1,000,000 mPas is obtained.

Preferred thickening agents are agar-agar, Carrageenan, Traganth, Xanthan Gum, cellulose and whole-meal ether, polyacryl and polymethacryl compounds, polycarboxylic acid, polysilicic acid and clay minerals, such as montmorillonite, zeolite and silicic acids.

Particularly preferred is the use of acid and glycerol in the form of xerogels. The mixture of acid and glycerol is here dissolved in a suitable solvent and mixed with a thickening agent. The resulting lyogel can be applied by atomizing, spreading or spraying. A dried material, the xerogel, is obtained due to the escaping solvent. The insecticidal mixture can easily be applied just once onto surfaces to be treated and will maintain its effect at these places for a long time. Due to the absorption of moisture from the ambient atmosphere a renewed swelling is constantly started, which brings about a renewed effectiveness. This kind of use has the advantage that the insecticidal mixture can easily be applied, and places that are only accessible with difficulty can easily be reached. It is also ensured by the drying up that the insecticidal composition also remains, for instance, in vertical upright joints, for a long period of time and exhibits a long-lasting effect.

Surprisingly enough, the efficiency of the mixture of acid and glycerol can further be enhanced by adding at least one anionic surfactant.

Suitable anionic surfactants are e.g. soaps, alkyl benzene sulfonates, alkane sulfonates, methyl ester sulfonates, α -olefin sulfonates, alkyl sulfates and alkyl ether sulfates.

Preferred is the use of sodium lauryl sulfate.

The amount of the used anionic surfactant is not limited. Preferably, however, anionic surfactant and citric acid/glycerol are employed in a weight ratio of 1 : 100 to 100 : 1, particularly preferably in the weight ratio of 10 : 90 to 90 : 10, the range of 20 : 80 to 80 : 20 being most preferred.

The following examples will explain the invention:

1. Examples of formulations for the use of the citric acid (TP) according to the invention

- | | | |
|-----------|---------------|------------------------|
| (I) | 0,10% by wt. | citric acid, 7-hydrate |
| | 0,10% by wt. | glycerol |
| | 99,80% by wt. | water |
|
(II) | 0,08% by wt. | citric acid, 7-hydrate |
| | 0,08% by wt. | glycerol |
| | 0,04% by wt. | Na sodium sulfate |
| | 99,80% by wt. | water |
|
(III) | 1,00% by wt. | citric acid, 7-hydrate |
| | 1,00% by wt. | glycerol |
| | 98,00% by wt. | water |

- | | | |
|------|---------------|------------------------|
| (IV) | 0,80% by wt. | citric acid, 7-hydrate |
| | 0,80% by wt. | glycerol |
| | 0,40% by wt. | Na sodium sulfate |
| | 98,00% by wt. | water |
| | | |
| (V) | 20,00% by wt. | citric acid |
| | 10,00% by wt. | water |
| | 0,20% by wt. | carbopol |
| | 9,80% by wt. | Na lauryl sulfate |
| | 60,00% by wt. | glycerol |

1.A. Reference products used (REF)

- | | | |
|-------|---------------|------------------------|
| (I) | 0,20% by wt. | methomyl (carbamate) |
| | 99,80% by wt. | water |
| | | |
| (II) | 0,50% by wt. | chlorpyriphos |
| | 0,37% by wt. | neopynamin |
| | 1,95% by wt. | methoxychlor |
| | 97,18% by wt. | water |
| | | |
| (III) | 20,00% by wt. | deltamethrin |
| | 10,00% by wt. | water |
| | 0,20% by wt. | carbopol |
| | 69,80% by wt. | 1,2,3-propanetriol |
| | | |
| (IV) | 0,20% by wt. | citric acid, 7-hydrate |
| | 99,80% by wt. | water |
| | | |
| (V) | 2,00% by wt. | citric acid, 7-hydrate |
| | 98,00% by wt. | water |

2. Examples

2.a. TP (I) and TP (II) were compared with REF (I) and REF (IV). To this end 20 begonias heavily infested with spider mites (*Panonychus ulmi*) were respectively wetted over the whole surface with the corresponding products at an amount of 20 ml (1 ml each plant).

Results: Both in TP (I) and in TP (II) and upon application of REF (I) all of the treated plants were entirely free from infestation after 1, 4, 7 and 14 days. In REF (IV) the treated plants were only free from infestation for one day. After 4 days 10%, after 7 days 50% and after 100 days 100% of the initial infestation was counted. The evaluation of the phytotoxicity of the products after application (leaf edge discolorations, necroses, etc.) after 14 days revealed the value 0 (no visible changes) on a five-grade scale in TP (I) and TP (II); the value 1 (slight discoloration on leaves or blossoms) was determined in REF (I).

This shows that the combinations according to the invention have the same effect as the commercial product REF(I), but without the risks of the latter for the health of users and plants. The combinations were superior by about 50% to reference product (IV), which was based on an isolated agent.

2.b. The same products as in test 2.a. were tested in four glasshouses on 200 respective vegetables strongly infested with whiteflies (*Aleyrodes proletella*) and greenflies (Aphididae) of various species.

The amount of application was 200 ml each time, i.e. 1 ml each plant, over the whole surface and wet.

Results: Both in TP (I) and TP (II) and upon application of REF (I) all of the treated plants were free from infestation after 1, 4, 7 and 14 days, whereas upon application of REF (IV) the treated plants only remained free from infestation for 1 and 4 days. After 7 days 30% of the initial infestation and after 14 days 100% of the initial infestation was counted.

2.c. TP (III) and TP (IV) were used in equal amounts in a comparative way with respect to REF (II) and REF (V) in a laboratory test as spray agents for the control of the following pests (adults and metamorphoses):

- A) *Musca domestica* – common housefly
- B) *Blatella germanica* – German cockroach
- C) *Lasius niger* – black garden ant
- D) *Ixodes ricinus* – (wood) tick
- E) *Tineola bisselliella* – clothes moth

For the tests 30 specimens of the flying pests A) and E) were respectively placed in gauze-draped cages with the dimensions 1x1x1 m and each sprayed with 1 ml of the above-mentioned products. Evaluated were after 1, 5, 10 and 20 minutes animals lying on the bottom and unable to fly, and after 24 hours the mortality or reanimation rate (recovery).

Results in Percent:

Flies						Moths				
	1	5	10	20 min	24 h	1	5	10	20 min	24 h
TP (III)	100	100	100	100	100	100	100	100	100	100
TP (IV)	100	100	100	100	100	100	100	100	100	100
REF (II)	20	80	100	100	95	100	100	100	100	100
REF (V)	10	70	100	100	100	90	100	100	100	100

Of the creeping pests, 30 specimens of mixed metamorphoses were also placed in tubs having the dimensions 1x1x1 and each sprayed with 1 ml – treatment as above.

TP (III)						REF (V)				
Roaches	1	5	10	20 min	24 h	1	5	10	20 min	24 h
	100	100	100	100	100	60	90	100	100	100
Ants	1	5	10	20 min	24 h	1	5	10	20 min	24 h
	85	100	100	100	100	10	35	70	100	100
Ticks	1	5	10	20 min	24 h	1	5	10	20 min	24 h
	40	60	100	100	100	0	0	20	40	100

TP (IV)						REF (II)				
Roaches	1	5	10	20 min	24 h	1	5	10	20 min	24 h
	100	100	100	100	100	65	95	100	100	100
Ants	1	5	10	20 min	24 h	1	5	10	20 min	24 h
	100	100	100	100	100	10	30	85	100	100
Ticks	1	5	10	20 min	24 h	1	5	10	20 min	24 h
	55	100	100	100	100	0	25	60	40	100

Evaluation after the respectively expired time exclusively covered animals lying on their backs, which under the magnifying glass showed no leg or tentacle movements.

These experimental arrangements also show that upon application of the pest repellent according to the invention not only a comparable expected effect, but even an effect superior to the commercial comparative product (REF I) could surprisingly be achieved.

The combination products TP (III) and TP (IV) are especially superior to REF (V) in their effect. TP (III) shows a remarkably high efficiency in ticks.

2.d. In a long-term test over 6 months the efficiency of a dissolved permanent spread for cockroach control was compared between TP (V) and REF (II) in the laboratory. A flat tub of Nirosta with the dimensions L = 50, W = 30 cm, H = 2 cm was coated all around on the

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2-cm high side surfaces and on the bottom with 2 ml TP (V) and 2 ml REF (III), respectively, and put into one large acrylic glass tub 1x1x1 m. 10 mixed specimens (adults and nymphs) of

Blatella germanica – German cockroach

Periplaneta americana – American cockroach and

Blatta orientalis – oriental cockroach

were respectively put into the treated nitrosteel tubs once a month over a total of six successive months. Upon contact with the product these moved into the untreated acrylic tubs, whereupon the treated tubs were removed and stored for further tests.

The contaminated cockroaches were fed in the untreated tub with water and food and observed. After 5, 10, 30 minutes and after 24 hours the animals lying on their backs were counted and after a further 24-hour observation period the mortality rate was assessed in percent of the total number of cockroaches used.

Results in Percent of Mortality:

Blatella Germanica

	TP (V)					REF (III)				
	Minutes			Hours		Minutes			Hours	
	5	10	30	24	48	5	10	30	24	48
1 st month	100	100	100	100	100	100	100	100	100	100
2 nd month	100	100	100	100	100	90	100	100	100	100
3 rd month	100	100	100	100	100	85	100	100	100	100
4 th month	100	100	100	100	100	60	80	100	100	100
5 th month	100	100	100	100	100	20	65	80	90	100
6 th month	100	100	100	100	100	0	15	40	60	80

Periplaneta Americana

	TP (V)					REF (III)				
	Minutes			Hours		Minutes			Hours	
	5	10	30	24	48	5	10	30	24	48
1 st month	100	100	100	100	100	60	100	100	100	100
2 nd month	100	100	100	100	100	60	100	100	100	100
3 rd month	100	100	100	100	100	45	85	95	100	100
4 th month	100	100	100	100	100	20	50	80	100	100
5 th month	100	100	100	100	100	0	35	60	90	100
6 th month	100	100	100	100	100	0	30	60	75	90

Blatta Orientalis

	TP (V)					REF (III)				
	Minutes			Hours		Minutes			Hours	
	5	10	30	24	48	5	10	30	24	48
1 st month	100	100	100	100	100	100	100	100	100	100
2 nd month	100	100	100	100	100	100	100	100	100	100
3 rd month	100	100	100	100	100	90	100	100	100	100
4 th month	100	100	100	100	100	90	90	100	100	100
5 th month	100	100	100	100	100	60	80	90	100	100
6 th month	100	100	100	100	100	45	65	90	100	100

This long-term test demonstrates the surprisingly good and long-lasting effect of the use according to the invention, in particular with its 100% elimination rate of mixed species and stages of cockroaches (Blattodea) even after a storage time of 6 months in comparison with a pyrethroid-containing dissolved spreading agent.

2.e. For achieving a sufficient long-term effect, i.e. an elimination rate of the existing pest infestation of 100%, if possible, a repelling potential (repellency) which is as small as possible is needed together with a long-lasting kill rate to ensure that all of the pests gradually come into contact with existing spray, spread or powder coatings and are thus reliably destroyed.

For testing this repellency potential, product strips of a width of 3 cm were centrally positioned on the side walls to extend over the bottom in the acryl tubs having the dimensions 1x1x1 m; water and food were placed at one side of the tub and 10 test animals were placed at the other side and observed as to whether the pests would cross the product strip to get the food.

Tested were cockroaches of the species *B. germanica* and ants of the species *L. niger*, mixed stages, in a comparison of TP (III), TP (IV) and TP (V) with REF (I), REF (II) and REF (III).

Results:

Surprisingly enough, TEST PRODUCTS (III), (IV) and (V) did not show any signs of repellency. All test animals crossed the product coatings rapidly and determinedly to get the food. On the average of all 20 tests (5 tests each per test product), this took two minutes.

Of the reference products, REF (I) showed the lowest repellency; the product coatings were here crossed after 17 minutes on average in the 5 tests; in REF (II) the mean of the crossing time was 48 hours. REF (III) showed such a high repellency that the test animals never crossed the product coatings within a period of 96 hours; it is therefore entirely unsuited as a long-term repellent because pests definitely avoid surfaces treated with REF (III).

Surfaces treated according to the invention have absolutely no repelling potential (repellency) and are picked by pests without any difficulty, whereupon these are reliably killed off after contact. On account of the entirely missing negative effect of the pest repellent according to the invention on humans, warm-blooded animals (pets and birds) and its proven, excellent and entirely surprising mortality for pests, it can be used without any risk in pest control everywhere and at any time without limitation.

Patent Claims

1. Use of at least one acid of the citric acid cycle in combination with glycerol for pest control.
2. Use according to claim 1 **characterized in** that the acid used is citric acid.
3. Use according to claim 1 or 2, **characterized in** that a suitable liquid carrier is used in addition.
4. Use according to at least one of the preceding claims, **characterized in** that at least one anionic surfactant is used in addition.
5. Use according to claim 4, **characterized in** that the anionic surfactant is sodium lauryl sulfate.
6. Use according to claim 3, **characterized in** that the liquid carrier is water, alcohol or 1,2-propylene glycol, alone or in mixtures.
7. Use according to at least one of the preceding claims, **characterized in** that a suitable thickening agent is additionally used, the viscosity of the resulting mixture ranging from 30 to 1,000,000 mPas.
8. Use according to at least one of the preceding claims in flying, creeping, stinging, biting and sucking pests and annoying animals.