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Tribolium castaneum (Coleoptera: Curculionidae) sensitivity to repetitive applications of lethal doses of imidacloprid and extracts of *Clausena anisata* (Rutaceae) and *Plectranthus glandulosus* (Lamiaceae)

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ABSTRACT

Nowadays, chemical control strategies in crop protection are mostly based on biopesticides or on low persistence synthetic molecules. These tools are alternatives of some products exhibiting adverse effects on consumers and polluting environment. Biopesticides made of essential oils of aromatic plants are more and more advised as user and environmental friendly crop protectants. Few works reviewed consequences of their repetitive use on the capability of the pest to resist to their insecticidal efficiency. The present work was carried out to compare the variation in the efficiency of a synthetic insecticide, imidacloprid and essential oils of *Clausena anisata* (Rutaceae) and *Plectranthus glandulosus* (Lamiaceae) against the flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae), during 4 generations (F4). The lethal dose of these insecticides that causes 80% of mortality (LD_{80}) at the first generation killed at the 3rd generation, 60% of pest for the two essential oils and 25% for imidacloprid. At the 4th generation, 25% of the pest populations were killed by the essential oils and 5% by imidacloprid applications. Egg and post-ovum development time differed among generations ($P < 0.001$). However, the fecundity of sensitive females was greater than that of resistant ones treated with essential oils. Insects treated with imidacloprid exhibited important fecundity in advanced generations. The LD_{50} level of the F4 treated with imidacloprid is 10 times that of the parental generation; however, the increase doubled from the parent to the F4 while treated with the essential oils. This work showed that *T. castaneum* could acquire resistance to imidacloprid faster than to 2 tested essential oils.

Keywords: Chemical control, Essential oils, Imidacloprid, Susceptibility, Stored products, *Tribolium castaneum*.

INTRODUCTION

Pesticides are an important and regular component of most systems. The quality and the quantity of harvested foods in modern agriculture depend mostly on these inputs. Insecticides are in quantity the most important chemicals used to kill insect in houses, gardens, crops and in stores. Their success in insect pest control leads to continuous increase. Insecticide action is rapid. It usually takes effect within hours and alleviates a problem within few days. This success brought users to intensive use of insecticides and from there, many consequences appeared. After regular and systematic treatments, many insect pests became insensitive to the insecticides and therefore resistant (Müch, 1996), other resurgent pests replaced previous ones. The resistance of an insect occurs after exposure of several generations to an insecticide, applied to a given rate that renders the insecticide ineffective in subsequent application at that dose. Since the Second World War, the increasing use of insecticide has led to the development of a tens resistant species of insect pest in the early sixties. In the nineties, more than 600 species were resistant to insecticides (Haubruge, 2001). Among these resistant insects, 59% are major crop pests belonging to three main insects Orders (Anonym, 2002).

The problem of insecticide resistance is acute; many major pests are becoming resistant to one or more insecticides. An increasingly larger proportion of new records of resistance involves species that are already resistant to at least one insecticide group (Ramasubramanian & Regupathy, 2004). Unfortunately, when an insecticide begins to lose its effectiveness, the response of the users is to increase dosage and frequency of applications. This behaviour enhances the rate of resistance. To survive in their environment, insects developed various mechanisms to reduce the toxicity of substances. The possibilities to insect for the detoxification of insecticide depend on their enzymatic system, and can be achieved through three major mechanisms: biochemical resistance, physiological and behavioural resistance (Haubruge & Amichot, 1998).

Confronted by this problem, the researchers react by producing insecticide with low possibility of resistance for targeted pests. In this respect, essential oils of some aromatic plants of the Northern Cameroon are described to have effectiveness in the control of stored grain insect pests (Tapondjou et al., 2002, Ngamo et al. 2004). Essential oils of *Clausena anisata* (Rutaceaee) and *Plecthranthus glandulosus* (Lamiaceae) are among the most insecticidal ones towards the red flour weevil *Tribolium castaneum* (Coleoptera: Tenebrionidae). In spite of their insecticidal efficiency, essential oils are characterised by their low persistence (Essam, 2001; Regnault-Roger et al., 2002; Goudoum, 2006). To achieve a good protection of stored product using these tools, many applications must be carried out. This pest management tactic, known as environmentally friendly method, is highly advised to users. To avoid substitution of problems, the present work aimed at analysing the eventuality of the resistance acquisition to essential oils compared to that of an industrial molecule currently homologated in Cameroon as insecticide: imidacloprid.

The targeted pest for the study, *T. castaneum* is noxious to flour and animal food. It is also one of the most resistant insect to insecticides (Bughio & Wilkins, 2004). Moreover, in Cameroon, there is no homologated insecticide to treat flour. Methyl bromide is currently used in industrial storage to treat imported wheat used in flour industries. It is in 2010 that the noxious product is going to be completely banned (Fleurat-Lessard et al., 1996).

MATERIALS AND METHODS

The insect pest used

A strain of *T. castaneum* labelled 24Z/LN-LA/02 has been reared in the laboratory at the University of Ngaoundéré since 2003. This insect was collected in a peasant granary at Beka (Adamawa-Cameroon) and from that date, was kept in permanent rearing. This insect feeds on flour of maize in glass container put in a controlled incubator monitor at 30 °C and 65 ± 5.7% of hygrometry. From this rearing, adults of less than one month of age were taken for the tests.

Insecticidal products tested

The essential oils tested were obtained by hydrodistillation of plant leaves of *C. anisata* and *P. glandulosus* with a

Clevenger type apparatus during 4 hours. Before the distillation, leaves of these aromatic plants were dried out of sunlight during 24 hours and cut in pieces. A quantity of each essential oil ranging from 100, 200, 300, 400 µl to 500 µl was dropped and diluted in 10 ml of acetone to formulate the insecticidal solution. For each concentration, 350 µl were applied to filter paper placed in Petri dishes. Three minutes after, 20 insects were introduced. These concentrations of essential oils expressed mortality of insect from 0 to 100% mortality. From this, the LD_{80} was established and used for other tests on all successive offspring from the first generation to the fourth generation.

The industrial insecticide, imidacloprid purchased at Gembloux (Belgium), is produced by Bayer CropScience Inc. As it was done for the essential oils, the active doses were established by testing the formulation from 0.02 to 0.1%, and from this preliminary tests, the LD_{80} was determined for the tests on the first generation and the successive offspring till the fourth generation. For each insecticidal product, 4 replications were made and 20 adults considered per test.

Analysis of mechanism of the acquisition of the resistance

A parental generation of *T. castaneum* constituted by 15 couples (male and female) was reared for the establishment of the LD_{80}. In their first generation, among the insects obtained, 2 groups of at least 25 insects each were formed. One group was treated with each insecticidal product at the LD_{80}. And the other kept out of treatment. The insects surviving from the tested group were reared apart to have offspring of the treated group. Their offspring were also divided into 2 groups, one treated and the other kept. The same investigation on the LD_{80} was carried out for the second, third and fourth generations of surviving insects. Each time, 4 replications were made. Moreover, at each generation, 30 young females were removed and observed for the analysis of their daily egg hatching, the survival of their eggs, larvae and nymphs.

Statistical analysis

The experimental design used is a factorial 3 x 5; with 3 treatments (two essential oils and the imidacloprid) and 5 concentrations, with 4 repetitions. The data obtained were expressed as a percentage transformed probit and the effective concentrations obtained using the Proinra.2 Software. The values of death rates obtained were transformed into Arcsinus, then ANOVA analysis using the software Statgraphic 5.0. The average values were classified with the Duncan Multiple range test and Khi tests with the same software.

RESULTS AND DISCUSSION

Insecticidal efficiency of the products tested

The parental generation of the strain of *T. castaneum* considered for this study exhibited variation in its sensitivity to the active products tested. The LD_{80}, lethal dose killing 80% of the experimental population, changes from substance to another. With imidacloprid, it is 0.08%, 410 ppm for *C. anisata* and 270 ppm for *P. glandulosus* (Table 1).

Insecticidal efficiency of essential oils is related to their chemical composition (Kim et al., 2003). Some of their compounds such as 1,8-cineole, α and β-pinene, α-phellandrene, γ-terpene and limonene have insecticidal activities (Huang et al., 2000; Cimanga et al., 2002 ; Kouninki et al., 2007); they act alone or have synergistic activities ones upon others (Cimanga et al., 2002; Kouninki et al., 2007).

Sensitivity of the offspring to the insecticidal products tested

The sensitivity of *T. castaneum* was not the same from a generation to another. The possibility of the offspring to overcome the toxicity of the product decreases progressively. Concerning imidacloprid, the reduction of amount of surviving individual to the LD_{80} of the parental generation is important and significant: 95% of the parental tolerances are lost (P < 0.001). The reduction is not so important for the essential oils of *C. anisata* and *P. glandulosus* after 4 generations which cover the duration of one storage campaign. 75% of the parent tolerance are lost. Between the 2 losses of tolerance, that of imidacloprid is significantly higher than essential oils (F= 5.13; ndl= 2, 57). From the parents till the third generation, concerning the essential oils, only an average of half of the tolerance is lost (56% for *C. anisata* and 62% for *P. glandulosus*), for the industrial substance, 75% is already lost (Figure 1).

The reduction of tolerance to an insecticide from a generation to another is a preliminary fact, leading to the resistance (Champ & Dyte, 1976). This is in relationship with the insect and with the nature of the active product considered. In consequence of this loss of tolerance, sensitive individuals will disappear to be replaced by resistant ones (Champ, 1986; Subramanyam & Hagstrum, 1995; Mück, 1996).

Variation of the egg hatching among parental and successive generations

Females of *T. castaneum* laid eggs continuously independent of the treatment with insecticides. Nevertheless, repetitive applications of essential oils kept the daily egg hatching at an average (2 eggs) significantly lower than that observed with imidacloprid (4 eggs) which did not differ from the control, which is the egg hatching of insect kept out of any treatment (Figure 2). (F= 1.70 ; ndl = 4, 15).

The total amount of eggs laid by treated insects during 10 days period in strains treated with essential oils decreased significantly and reached a lower level of 7 eggs during 10 days at the fourth generation (Table 2). The strain treated with imidacloprid exhibited different behaviour, the amount of eggs laid during 10 days increased significantly from a generation to another. The higher value of 16 eggs was observed at the fourth generation.

Imidacloprid had no effect on the egg hatching in all generations, whereas essential oils reduced egg hatching progressively from a generation to another. Similar observations were made explaining the activities of some essential oils on female insects through the egg hatching reduction (Regnault-Roger & Hamraoui, 1995; Goudoum, 2006; Ngamo et al., 2007). Sidibé (1999) pointed out the same phenomenon with lindane, which had no inhibitory effect on the egg, but in some case increased the egg hatching of the resistant strains of *T. castaneum*. As it was observed with essential oils, Subramanyam & Hagstrum (1995) noted that while acquiring resistance, the fertility of resistant insect decreases.

Variation in the duration of the life cycle of parental and successive generations of *Tribolium castaneum* treated with active products

Repetitive treatment of parental and four successive generations of *T. castaneum* treated with imidacloprid and essential oils led to the augmentation of the duration of the generation as the treatment continued (Table 3). The duration of the parental generation is significantly shorter than that of the treated offspring (F = 0.94 ; ndl = 4, 15). Some insecticidal activities of essential oils are known; they have feedant deterrent action, and are growth regulators. These reasons and others may explain the increase of the duration of the life cycle of the treated population.

Offspring life cycle duration increased progressively from a generation to a new one. The increase was more important with imidacloprid than with the 2 essential oils. No change was observed in the control. As observed by (Subramanyam & Hagstrum, 1995 ; Arnaud et al., 2002) in the same species, resistant strains had their life cycle longer than that of sensitive ones. Moreover, testing the effectiveness of lindane towards *T.*

castaneum, Bhatia & Pradhan (1971) pointed out that resistant strains had their life cycle longer than that of sensitive ones. For the same insect treated with malathion, resistant strains had life cycle longer than sensitive ones (Haubruge, 1995; Arnaud, 1996; Sidibé, 1999). The increase of the duration of the biological cycle in resistant insect could be due to some of their chronic toxicity by inhibition of metabolic pathways reducing the welfare of the insect as feedant deterrent action or growth regulation (Ngamo et al., 2007).

Variation in the insecticidal effectiveness of the insecticidal products

The variation among the LD_{50} of the insecticidal products applied on the parental and the successive generations is presented in Table 4. The effectiveness of the product reduces from a generation to the offspring. With imidacloprid, the LD_{50} of the parental generation, LD_{50} = 0.44 became 10 times lower (LD_{50} = 0.04) after 4 generations and for the essential oils, the reduction is only 2 times (Table 4). The resistant factor (RF) established with the LD_{50} is more important

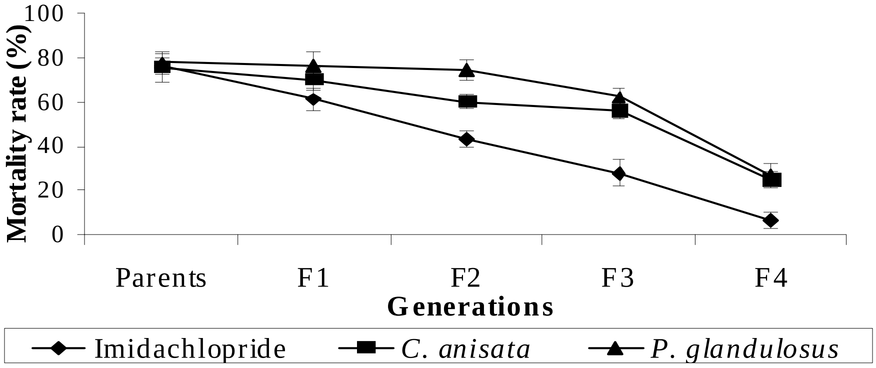

Figure 1: Sensitivity of successive generation of *Tribolium castaneum* to imidacloprid and essential oils treated with the parental LD_{80}.

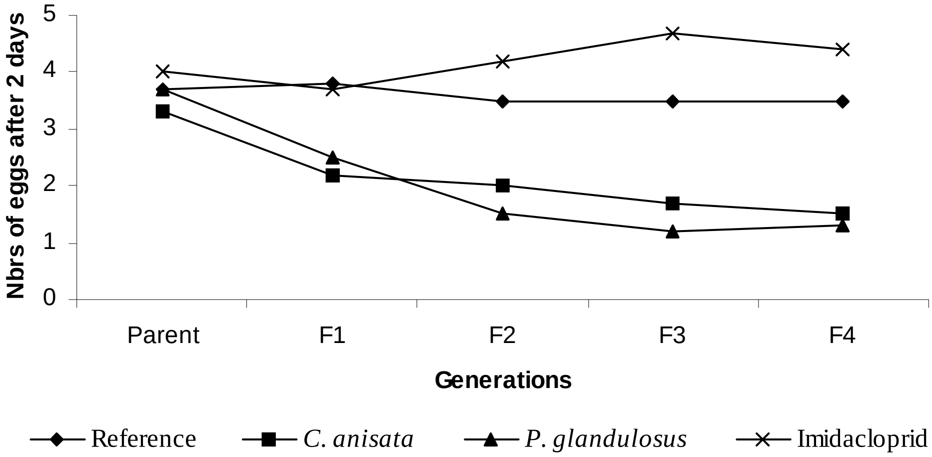

Figure 2: Oviposition of parental and successive generations of *Tribolium castaneum* treated with insecticidal products compared to the oviposition of the untreated control population.

Table 1: Sensitivity of the parental generation of *Tribolium castaneum* to imidacloprid and 2 essential oils.

	LD80	Confidence interval	Slope
Imidaclopride	0.08 %	0.06 <IC<0.10	2.10
C. anisata	410 ppm a	385<IC<435	3.41
P. glandulosus	270 ppm b	251<IC<289	2.67
X^2	28.82***		

****= Significant at 1 ‰. ; a, b : Mean values followed by the same letter in the same column do not differ significantly at P < 0.0001 (Duncan's test)*

Table 2: Cumulative amount of eggs laid by *Tribolium castaneum* treated with insecticidal products compared to that of control.

	Untreated	Insecticidal products		
Generations	Control	Imidacloprid	*C. anisata*	*P. glandulosus*
Parent	13.6	12.8	13.7 a	13.3 a
F1	15.0	14.3	9.4 b	11.2 ab
F2	14.8	14.7	8.0 b	9.5 b
F3	15.3	15.1	6.3 c	6.9 c
F4	14.4	16.1	6.6 c	6.6 c
X^2	**0.12 (NS)**	**0.48 (N S)**	**4.10***	**3.42***

**= Significant at 1 ‰; NS= Non significant.*
Mean values followed by the same letter in the same column do not differ significantly at P < 0.0001 (Duncan's test).

Table 3: Duration of the life cycle of parental and successive generations of *Tribolium castaneum* treated with insecticidal products compared to that of the non treated control population.

	Control	*C. anisata*	*P. glandulosus*	Imidacloprid	F (ndl : 3, 76)
Parent	32.2 ± 1.25	31.2 ± 1.25 a	32.5 ± 1.3 ab	30.7 ± 0.95 a	
F1	33 ± 0.81	33.5 ± 1.3 ab	31.7 ± 0.95 a	33.5 ± 1.3 b	
F2	32 ±1.41	35.5 ± 1.3 bc	34,2 ± 1.25 bc	34.7 ± 1.25 bc	
F3	33 ± 0.81	34.7 ± 2.06 bc	34.7 ± 1.70 c	36.5 ± 0.57 cd	
F4	32 ± 0.81	36.5 ± 1.73 c	35.5 ± 1.73 c	37.7 ± 1.70 d	
F (ndl : 4, 15)	**0.94 NS**	**6.75*****	**4.90****	**19.94*****	
Mean	**32.4 a**	**34.3 b**	**33.7 ab**	**34.6 b**	**4.25*****
Minimum	**32**	**31**	**31**	**30**	
Maximum	**33**	**36**	**35**	**37**	

****= Significant at 1 ‰; **= Significant at 1 %.*
a,b,c,d: Mean values followed by the same letter in the same column do not differ significantly at P < 0.0001 (Duncan's test)

for imidacloprid at the fourth generation [FR_{50}(F4) = 10.04] than with the essential oils at the same level [FR_{50}(F4) = 2.35].

It is evident from these results that the reactions to the different active matters used vary from a generation to another. These results are in conformity with those of Champ & Dyte (1976) who showed that among the stored product insects, the reaction to insecticide can vary from a population to another within the same species. According to the same authors, this variation can also exist according to the species of the insect and the insecticide tested.

The weak variation of the LD_{50} of the essential oils used between the different generations of insects can be attributed to the cocktail of bioactive compounds contained in these oils having some various insecticidal

Table 4: Variation among LD_{50} level and resistant factor (RF) observed with the LD_{50} of successive generations of *Tribolium castaneum* treated with insecticidal active products.

	LD_{50}			RF_{50}		
	Imidacloprid	***C. anisata***	***P. glandulosus***	**Imidacloprid**	***C. anisata***	***P. glandulosus***
Parent	0.04	155.95	106.80	/	/	/
F1	0.10	189.27	153.16	2.38	1.21	1.43
F2	0.13	232.81	200.52	3	1.50	1.87
F3	0.22	333.15	228.27	5.02	2.13	2.13
F4	0.44	368.48	251.26	10.04	2.36	2.35

effects (Goudoum et al., 2007). These compounds are notably the terpenoïds, that is, at a time insecticidal (Pungitore et al., 2005; Kouninki et al., 2007) and feedant deterrence action (Ducrot, 2002; Pungitore et al., 2005), the polyaromatic compounds and the alkaloids (Ducrot, 2002). On the other hand, the very significant variation observed between the different generations that acquired a tolerance to the imidacloprid is forcing due to the latter that contains only one active molecule.

These essential oils have suitable performance for an insect pest management delaying the apparition of the resistance and having low adverse effect on the environment.

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