African Journal of Food Science Vol. 5(6), pp. 366 – 375, June 2011
Available online http://www.academicjournals.org/ajfs
ISSN 1996-0794 ©2011 Academic Journals

Full Length Research Paper

Insecticidal effects of essential oils of *Pelargonium graveolens* and *Cymbopogon citratus* on *Sitophilus zeamais* (Motsch.)

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Accepted April 27, 2011

The evaluation of the effect of essential oils of *Pelargonium graveolens* and *Cymbopogon citratus* on maize weevil (*Sitophilus zeamais*) has assigned the most significant insecticidal activity against it with a maximum mortality rate of 100%. The essential oils were found to have high contact and ingestion toxicity than fumigant / respiratory poison. This is evident from 90% mortality of weevils that come in contact or ingest contaminated food and only 40% when inhaled. This observation clarifies that the non volatile active components are more efficient than the volatile compounds of these essential oils. The insecticidal activity was more intense but short for higher concentrations of these oils and low and long-lasting for average concentrations. In fact, insecticidal activity decreased with the concentration of essential oils and varnishes with very small concentrations (from a dilution of 1/80 in our case) while it decreased with an increase in contact distance. The statistical results showed that the difference between the insecticidal activity of essential oils of *P. graveolens* (geranium) and *C. citratus* (lemongrass) was not significant in the three tests. Given that the weevil is the most devastating insect of maize (post-harvest), this study showed that these essential oils can serve, in one way or another, as a means of conserving maize corns for a longer time.

Key words: *Cymbopogon citratus*, *Pelargonium graveolens*, essential oils, insecticidal activity, *Sitophilus zeamais*.

INTRODUCTION

In recent years, the use of synthetic insecticides in the fighting against agricultural pests has developed unintended damages both on human life and the environment. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non target species, air, water, bottom sediments, and food (Agrawal et al., 2011; Miller, 2004). Some chemical pesticides, including the famous DDT, aldrin, chlordane,dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, and toxaphene have the ability to volatilize and travel great distances through the atmosphere to become deposited in remote regions. These chemicals also have the ability to bioaccumulate and biomagnify, and can bioconcentrate (that is, become more concentrated) up to 70,000 times their original concentrations. They may continue to poison non-target organisms in the environment and increase risk to humans by disruption in the endocrine, reproductive, and immune systems; cancer; neurobehavioral disorders infertility and mutagenic effects, although very little is currently known about these chronic effects (Buchanan et al., 2010 ; Jurewicz et al., 2008; Ritter, 2007).

The chemical insecticides may lead to air and water pollution respectively when volatilized by the wind and carried away by the rain water (US Environmental Protection

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Figure 1. *Pelargonium graveolens* (left), *S. zeamais* (centre) *C. citratus* (right).

Protection Agency, 2007; Papendick et al., 1986) but they can also harm the soil. Worse again, the chemical insecticides have more serious damage on biota. Animals may be poisoned by insecticide residues that remain on food after spraying, for example when wild animals enter sprayed fields or nearby areas shortly after spraying (Palmer et al., 2007). The insecticides can enter the human body through inhalation of aerosols, dust and vapor that contain pesticides; through oral exposure by consuming food and water; and through dermal exposure by direct contact of pesticides with skin (Department of Pesticide Regulation, 2008; Lorenz et al., 2009). Therefore, the introduction of alternative harmless means, one of which is the use of plant-based insecticides has become a serious issue of research.

To counter this disaster, the essential oils from plants have been subjected to various tests and have been revealed to be active compounds against quite a few insects (Kabera, 2004). The main reason is that they show toxic properties to insects and have the advantage of being inexpensive. Taking into account their invaluable role, the evaluation of their effects against pests of plants-target of Rwanda should therefore be crucial. Longtime ago, various products (crude extracts, powder, essential oils, etc.) from plants were used in developing countries for controlling ravaging insects (Pandy et al., 1995; Dabiré, 1993). The most known plants are *Acorus calamus*, *Curcuma domestica*, *Cassia nigricans*, *Melia azadarach* and *Azadirachta indica* but now, different researches continue to identify others on the basis of the advantages they bring. This works lies within the framework of evaluating the insecticidal activity of essential oils of *Pelargonium graveolens* and *Cymbopogon citratus* against the maize weevil (*Sitophilus zeamais*) (Figure 1)

Corn is one of four national strategic crops for agricultural intensification amongst the rice, potatoes and beans (MINECOFIN, 2002) in Rwanda. However, its yield is still hindered by various natural facts like pests which cause serious pre- and post-harvest losses. The weevil, *S. zeamais,* is still the main devastator of maize post-harvest (Delobel et al., 1993). It is a harmful insect that eats and develops thanks to the cereals stocked as corns, mainly the maize (Adams, 1976). On the other hand, *P. graveolens* (Family: *Geraniaceae*) and *C. citratus* (Family: *Poaceae*) are plants grown for their miraculous essential oils and are now being grown in Rwanda, where they showed to produce better yields. For this research study, the main objective was twofold; to valorize essential oils from the aforesaid plants and reduce post-harvest losses of maize in rural regions of Rwanda. Experimentally, the essential oils were extracted from leaves of *P. graveolens* and *C. citratus* and tested on mature weevil by three alternatives: test by inhalation, ingestion and direct contact.

MATERIALS AND METHODS

Biological materials

After dew was removed by the morning sun, the leaves of *P. graveolens* and *C. citratus* were collected from the garden of the Medicinal and Essential oils Plants of the Institute for Scientific and Technological Research (IRST) located at Huye in the Southern Province of Rwanda. In turn, mature individuals of *S. zeamais* were purchased mixed with dry grains of maize in the main market of Huye.

Weevil farming

Given that the maize grains are the staple food of *S. zeamais*, the mature weevils were reared on intact maize grains in jars for three months, other conditions for survival being insured of course. At the beginning, the insects were fed 250 g of maize grains.

Extraction of essential oils

The extraction of essential oils was performed on 4 kg of dried leaves of each plant, *P. graveolens* and *C. citratus*, in the chemistry laboratory of the IRST in the Phytomedicine and Life Sciences Research Centre. In addition, the hydrodistillation method was used

Table 1. Mortality cases caused by the essential oils inhaled, ingested or put in contact with *S. zeamais*.

Dilution	Mortality by inhalation		Mortality by contact		Mortality by ingestion	
	P. graveolens	*C. citratus*	*P. graveolens*	*C. citratus*	*P. graveolens*	*C.citratus*
D1/5	21	9	40	40	40	36
D1/10	15	7	40	40	40	39
D1/20	11	4	40	32	40	32
D1/40	4	0	36	24	32	16
D1/60	4	3	28	14	28	20
D1/80	0	0	0	0	0	0
D1/100	0	0	0	0	0	0

to obtain better yield of the essential oils. Hydrodistillation is a chemical process for essential extraction from vegetative plant parts. In this process, the mixture of fresh water and plants vegetative parts is put in a flask and brought to boil. The steam passes upward through plant vegetative causing the evaporation of volatile and fragrant chemical compounds. In this case, the plant cells burst and release the chemical species (soluble or non soluble in water) which are driven by water vapor through a condensation coil and finally recovered in another container after the cooling process with water passing across the refrigerator. The obtained hydrodistillate is made of the aqueous and organic phases which are often clearly separated due to their different densities. In our study, we carried out the usual hydrodistillation to extract the essential oils. The leaves were washed with fresh water and introduced in a clean flask. The water was added so as to cover the mixture and the grains of pumice were introduced in order to regulate the boiling and homogenized temperatures inside the flask. The flask was connected to the distillation system and brought to boil. The hydrodistillate recover and separate the aqueous and organic phases by decantation method. The organic phase was the solution containing the essential oil that we looked for.

Test of *S. zeamais* resistance

Effect of fasting

During this test, 40 mature individuals of *S. zeamais* were selected and subjected to a period of fasting for 14 days. The evolution of insects' health was assessed after every 24 h and the mortality cases were recorded simultaneously. This test permitted us not to confuse death caused by starvation and that caused by other factors such as active compounds contained in essential oils during our experiments.

Effect of solvent

Given that the used essential oils must be diluted with a solution consisting glycerin, water and tween-20 in the proportions 2:1:1, the determination of the effect of this mixture was necessary. This test was an effort to pre-determine whether this solvent would affect the insect in order to know the peculiar effect of the essential oils. The observations were made after every 24 h for 14 days and the results were simultaneously recorded.

Effect of essential oils

These tests consist of subjecting mature individuals of *S. zeamais* to the effect of crude essential oils of *P. graveolens* and *C. citratus* and following the evolution of their health. Like the previous tests, observations were made each 24 h until the death of all insects and all results were obtained after 6 days and were recorded and statistically analyzed.

Preparation of solutions of essential oils

Stock-solutions were made of glycerin, water, Tween-20 and essential oils in the proportions 1:1:2:1 or the dilution of 1/5. To have day-solutions, dilutions 1/10, 1/20, 1/40, 1/60, 1/80 and 1/100 were made.

Test by inhalation

The insecticidal effect by inhalation was tested twice on 40 healthy weevils using containers with the bottoms on which are deposited filter paper that were soaked with the same amount of emulsified essential oils of different concentrations with the insects being put on a plate with holes in it placed at a small distance above of the soaked filter paper. Three replications were done and the average number of dead insects during these replications was used as the number of dead insects.

Test by ingestion

Mature and living insects were isolated from host grains and 40 healthy ones were subjected to a period of fasting for 48 h and then they were fed with maize grains previously impregnated with essential oils. This test was replicated three times under thesame conditions and parameters.

Test by direct contact

Adults *S. zeamais* were selected and 40 healthy ones were directly deposited on the filter papers previously soaked with essential oils of different concentrations. The test was repeated three times and the average results were considered.

Statistical analyses

Our statistical analyses were aimed at comparing the insecticidal effect of essential oils of *P. graveolens* and *C. citratus* on *S. zeamais*. In our analyses, we used the available data collected during the experiments, mainly the number of insects killed by every type of essential oils (Table 1).

With these results, we used the test of Wilks supported by

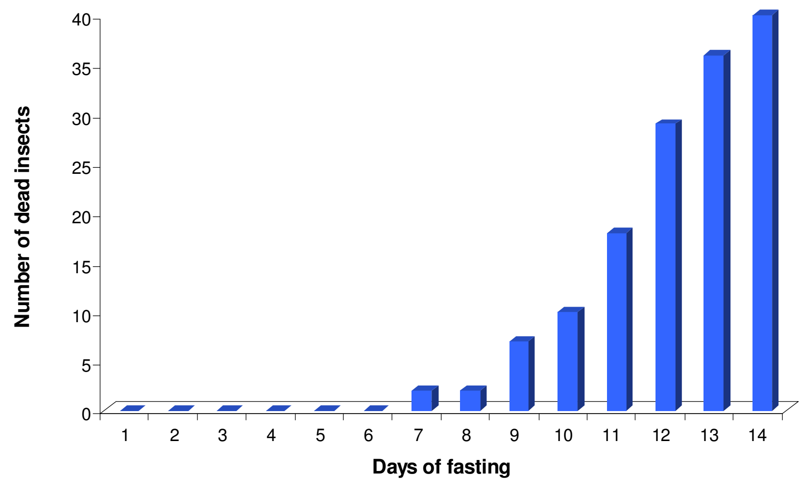

Figure 2. Mortality cases caused by the fasting (delete background).

Lambda (λ) of Wilks in order to test whether these essential oils are significantly with the three alternatives tests (inhalation, contact and ingestion) to kill the mature weevils. This statistic (Λ) is between 0 and 1. Its low value (close to 0) is an indication of well separated groups, whereas its high value (close to 1) is an indication of little or no separate groups.

RESULTS

S. zeamais farming

The test farming of *S. zeamais* allowed us to obtain an adequate number of mature individuals which were used in our experiments while the grains were processed into powder, an obvious sign of their consumption by the insect and a certain cause of their multiplication. After 3 months, enough weevils were multiplied for bioassays

Extraction of essential oils

By hydrodistillation method and from 4 kg of dried leaves of each plant, we obtained 10.6 ml of essential oils of *P. graveolens* and 8.8 ml of *C. citratus with* yield rates of 0.26 and 0.22%, respectively.

Effect of fasting

During this test, the submission of mature insects to the period of fasting showed that they can survive fasting for a maximum of 6 days. After this survival period, mortalities were recorded and are shown in Figure 2.

Effect of solvent

After this test, the solvent (mixture of glycerin, water and Tween-20) showed harmless effects on *S. zeamais* in the 6 days period which coincides with that of its survival to the fasting period. Similarly, the evaluation of the fasting effect revealed that the first insect was found dead on the seventh day. Thus, no effect of the solvent is noticed (Figure 3).

Effect of essential oils

The results of the evaluation of the effect of essential oils on the maize weevil are presented in the Figures 4 and 5. It should be noted that the insects were deprived of food.

Test by inhalation

Figure 5 represents the results of the tests by inhalation. The results from the tests by inhalation of essential oils of *P. graveolens* showed that the highest mortality rate of mature maize weevils (40%) was recorded on the 1st day of the test with a dilution of 1/5 (the lowest of the dilutions made). The insecticidal activity was intense, but less lasting (up to 2 days). For the dilutions 1/10 and 1/20, the insecticidal activity was very low (10%) but lasting (up to 5 days) while for dilutions 1/40 and 1/60, the activity was very low (10%), less lasting (1 day or less) and delayed (at least from the 2nd day). As for the highest dilutions (1/80 and 1/100), they did not show any insecticidal activity on the maize weevil. As far as the test by inhalation of essential oils of *C. citratus* is concerned, the dilutions 1/5, 1/10, 1/20 and 1/60 showed a very low insecticidal activity and a long delay of occurrence (only about 10% of the insects died on the 3rd day). Like the essential oils of geranium, we witnessed an insecticidal activity more or less lasting (up to 2 days) at lower dilutions (1/5 and 1/10) and a total lack of insecticidal activity at higher dilutions (1/80 and 1/100).

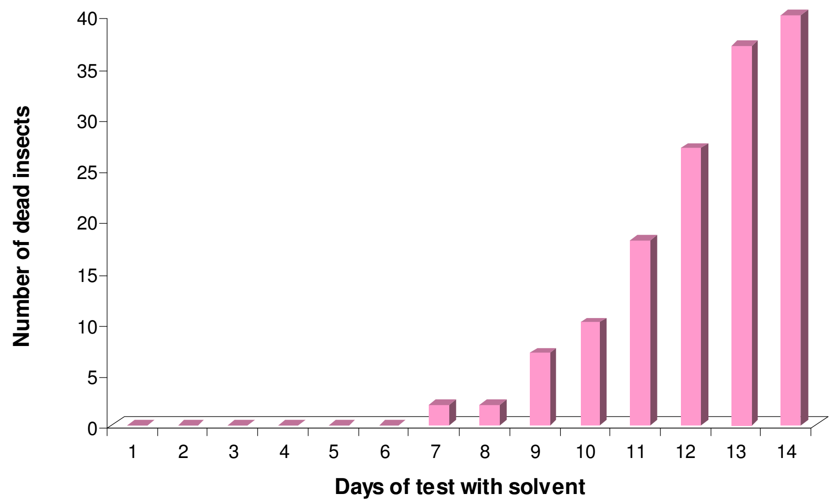

Figure 3. Mortality of maze weevils deprived of food and exposed to the solvent.

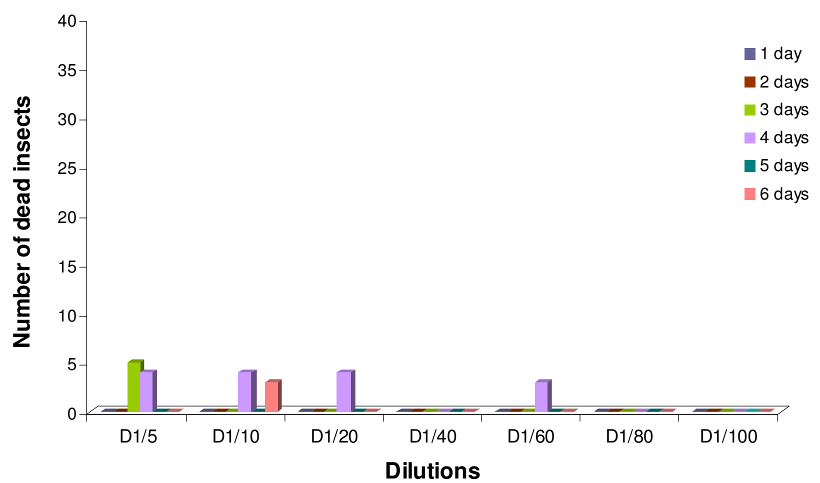

Figure 4. Inhalation toxicity of essential oils of *C. citratus* to maize weevil.

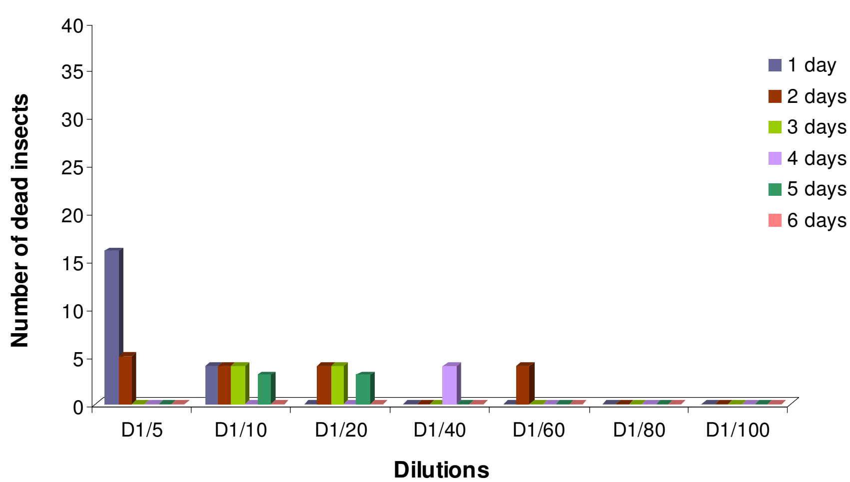

Figure 5. Inhalation toxicity of essential oils of *P. graveolens* to maize weevil.

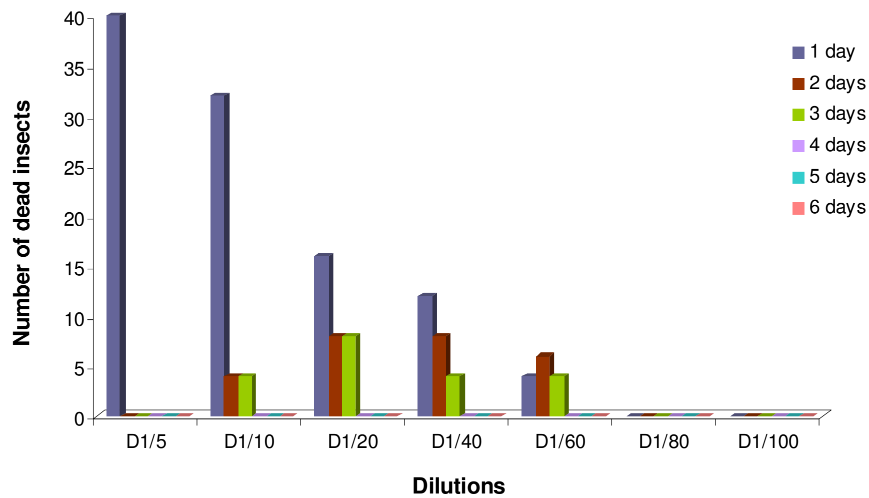

Figure 6. Contact toxicity of essential oils of *C. citratus* to maize weevil.

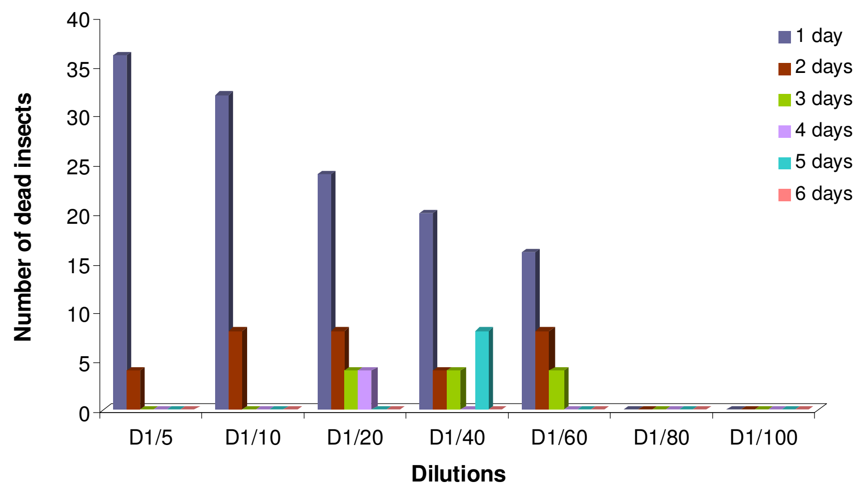

Figure 7. Contact toxicity of essential oils of *P. graveolens* to maize weevil.

Test by direct contact

Figures 6 and 7 present the test results after putting matured weevils in direct contact with the essential oils of the two plant species. Compared to the test by inhalation, the tests of putting the weevil (*S. zeamais*) in direct contact with the essential oils of *P. graveolens* and *C. citratus* gave more interesting results. It appears from Figures 6 and 7 that the insecticidal activity of these essential oils was very high (mortality rate of 100% with essential oils of *C. citratus* [lemongrass]) and persistent (up to 5 for days for the essential oils from *P. graveolens* [*geranium*]). With essential oils of *P. graveolens* at 1/5 dilution, 90% mortality was obtained on the first day of the test, whereas it was only 40% with the 1/60 dilution. Similarly, the essential oils of *C. citratus* dilutions 1/5 resulted in 100% mortality whereas it was 10% at a dilution of 1/60. With all these essential oils, we observed a mortality rate of 20% on day 2 (even on the 3rd day for *P. graveolens*) and the total lack of insecticidal activity when it came to higher dilutions (>1/ 80).

Test by ingestion

For this test, the observed cases of mortality are summarized in Figures 8 and 9 to clarify the effect of these essential oils according to their dilutions. The test by ingestion of essential oils from the two plants has led to results comparable to those obtained during the tests with direct contact, apart from some differences. The mortality rate caused by the ingestion of essential oils of *P. graveolens* on the first day are identical to those calculated when the weevils was put in direct contact (90,

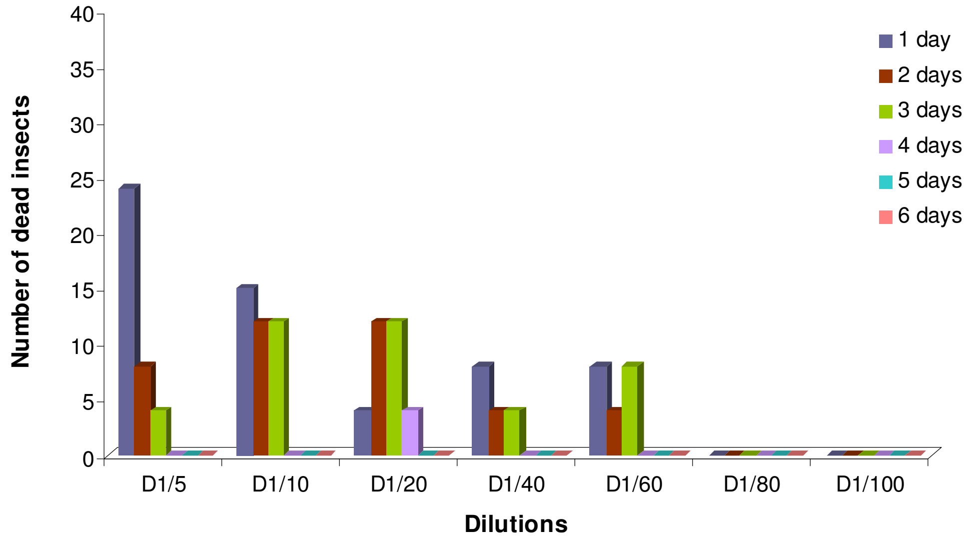

Figure 8. Ingestion toxicity of essential oils of *C. citratus* to maze weevil.

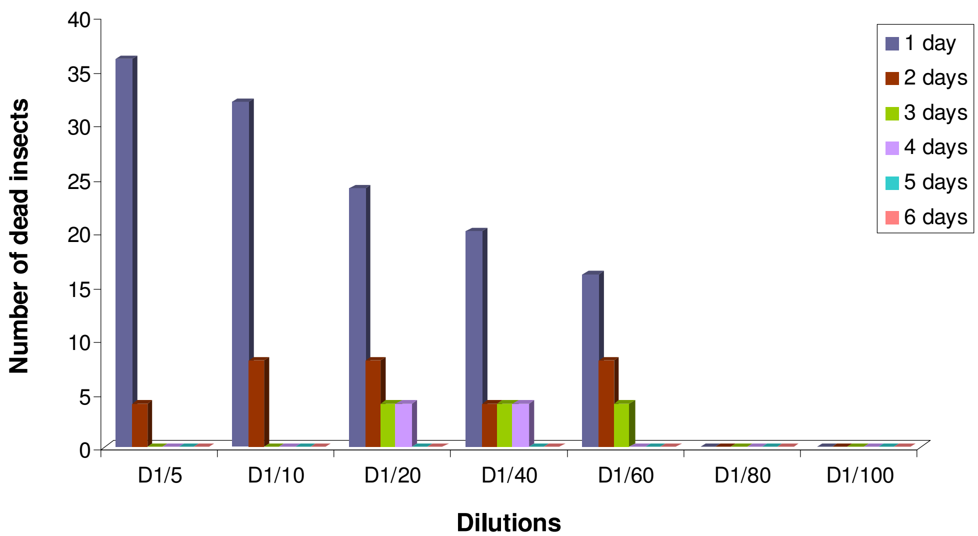

Figure 9. Ingestion toxicity of essential oils of *P. graveolens* to maze weevil.

80, 60, 50 and 40% for respective dilutions of 1/5, 1/10, 1/20, 1/40 and 1/60), while it is not the case for the essential oils of *C. citratus*. In addition, whatever the type of essential oil, this activity disappears completely with higher dilutions (1/80 and 1/100). However, some differences were recorded during tests by ingestion of essential oils from *C. citratus*. They include a lower maximum mortality rate (60% for ingestion vs. 100% observed during the test by direct contact), the lasting and high insecticidal activity (up to 30% on the 3rd day) for medium dilutions (1/40) but also the durability and reduced, albeit slight, insecticidal activity of essential oils of *P. graveolens* (up to the 4th day of test).

Statistical results

At a significance level of α= 0.05, this means that there is 95% surety of having made a good decision. The value of the test statistic was calculated using the data given in Table 1. According to calculations, the critical value (λ_{th}=0.846) is higher than the Wilk's test of significance (λ_{obs}=0.70). Based on this situation, the null hypothesis, H_0 stating that the same performance for the three tests is rejected and alternate hypothesis, H_a stating that the three tests do not show the same average in eliminating *S. zeamais* with essential oils of *C. citratus* and *P. graveolens* is accepted. In addition, since Lambda (λ)

Table 1. Mortality cases caused by the essential oils inhaled, ingested or put in contact with *S. zeamais.*

Dilution	Mortality by inhalation		Mortality by contact		Mortality by ingestion	
	P. graveolens	*C. citratus*	*P. graveolens*	*C. citratus*	*P. graveolens*	*C.citratus*
D1/5	21	9	40	40	40	36
D1/10	15	7	40	40	40	39
D1/20	11	4	40	32	40	32
D1/40	4	0	36	24	32	16
D1/60	4	3	28	14	28	20
D1/80	0	0	0	0	0	0
D1/100	0	0	0	0	0	0

equals the value of Wilk's test (0.846), a high value close to 1, it shows that the studied sample has little or no separate groups. Therefore, the statistical analysis leads to the conclusion that the essential oils of *C. citratus* and *P. graveolens* in the three tests have different efficiency in eliminating *S. zeamais* but the difference is not significant.

DISCUSSION

Insects are the world's most prolific organisms so they are able to populate all walks of life in the world (Gasogo, 2009, Kozár et al., 2009). At the end of breeding the weevil, all insects were mature and all grains were pierced with their major internal parts, beyond all doubt, being eaten by the insects to survive. So, it is this acquired capacity of proliferation and the good maintenance of growth conditions that have resulted in the successful breeding of *S. zeamais*. This successful insect breeding helped us to get a sufficient number of adult individuals needed for the experiments. As far as the essential oils extraction is concerned, the results were reliable. Essential oils are naturally present in very small quantities in plants. For example, the yield of extraction of essential oils of geranium is between 0.15 and 0.33% as reported by Sebagenzi in 2006 (unpublished observation). From this finding, the results we obtained in this study (yields of 0.26 and 0.22% for *P. graveolens* and *C. citratus,* respectively) were reliable. The results also reflect the good quality of leaves used as plant materials and show the effectiveness of the used extraction method (the hydrodistillation). Indeed, the yield were maximum because essential oils are naturally and abundantly located in the aerial parts of plants, particularly in the leaves and the method of hydrodistillation is better because of its potential to eliminate undesirable products by dissolution in water. When subjected to fasting periods, the insects showed remarkable resistance. The results showed that adult individuals of *S. zeamais* can at most spend six days alive without eating. This resistance to the periods of fasting throws more light in understanding how these insects manages to travel overland from one household to another, however far apart, in search of maize, their ideal food. Testing the effects of solvents on the insect allowed the identification and clarification of the insecticidal activity peculiar to the essential oils of *P. graveolens* and *C. citratus*. The results obtained in the first 7 days were identical to those observed in the determination of the effect of fasting. With this similarity in results, it is noticed that the death of the first insect occurring on the seventh day was not due to the solvent, but to lack of food. For that, this solvent was qualified to be harmless to matured *S. zeamais*. Therefore optimism was expressed that the effects that would be observed when the insect is subjected to essential oils, if any, would be attributed surely to the essential oils alone.

After the preliminary tests, the overall experiments and targeted beforehand were done and showed beneficial results for those persons who are interested in. The three tests carried out (inhalation, contact tests and ingestion) were all based on one parameter; the death caused by the active ingredients in most plant products including essential oils. For all these three tests, the first two days gave different but very interesting results. Mortality during the inhalation experiment, albeit weak for the essential oils of *C. citratus*, indicated that the toxic compounds exhibited absolutely its volatility properties since during this test the insects were kept at a distance from the paper soaked in essential oils. The same results were found in testing the insecticidal activity of the essential oils of *Artemisia mongolica* against the maize weevil (Liu, 2010). This should also be true because the essential oils are natural substances with a very marked volatility. On the other hand, the mortality of insects in direct contact with the filter paper was higher for small dilutions and for all essential oils. This rise in mortality rates should be due to two major factors. The insects have been in direct contact with the toxic products and, since they were deprived of their usual food, they surely consumed, in one way or another, a certain amount of essential oils contained in the filter paper. This assertion explains itself because the ingestion test of essential oils gave the same results as those of the test by contact. As far as the essential oils of *P. graveolens* are concerned, the test by ingestion led to the same results as the contact test. For the test by inhalation of essential oils of *C. citratus*, the insecticidal activity was delayed, so it appears just for the first day of other tests. This means that the essential oils of *C. citratus* are less volatile, less toxic or act in large quantities/high concentrations of its active compounds. The high insecticidal activity observed on the first day of the test is justified by the fact that the active components of the essential oils used are still concentrated while its durability for some dilutions and absence at other dilutions demonstrate the existence of a threshold concentration at which the insecticidal activity of essential oils of these two plants appears.

The insecticidal activity of essential oils of *P. graveolens* and *C. citratus* inhaled is weak and more or less stable. However, this activity was directly proportional to the concentration of essential oils ingested or put in contact with the insects. This is obvious because, in the first case, insects were placed at a distance from the essential oils in general and in particular the active molecules while in the second case they were in direct contact with these molecules. Globally, the essential oils of the two plants studied in this research showed remarkable insecticidal characteristics against maize weevil and most grain borers (Bruneton, 1997). From this and some other information on the chemical compounds in essential oils, it is deduced that some of their compounds mainly geraniol and citronellol were chief perpetrators of this action but also their derivatives could also be responsible. In fact, according to previous studies, the anorectic activity against the larvae of the caterpillar *Lymantria dispar*, devastator of oak forests has been attributed to geraniol (McEwan et al., 2008). Similarly, insecticidal activity (insect repellent and fungicide) were observed in using citronellol, citronellyl formiate and citronellyl acetate (Yusufoglu et al., 1996) while nematicidal activity against *Caenorrhabditis elegans* and *Pratylenchus penetrans* were attributed to geraniol and citronellol, respectively (Tsao et al., 2000; Reeves et al., 2010). Finally, antioxidant activities were entrusted to isomenthone and citronellol while acaricidal activity against *Psoroptes cuniculi* has been observed in using linalool and citronellol (Keszei et al., 2008; Perruci et al., 1997). Most of these compounds are present in the essential oils of *P. graveolens* and *C. citratus* and should have acted alone or in synergy in one way or another on mature individuals of the maize weevil *S. zeamais*. By way of comparison, results from previous researches show that the essential oils of *P. graveolens* and *C. citratus* exhibit similar chemical composition but differ in their volatility, the major parameter which influences the toxicity of a product (Bruneton, 1969). This information permitted us to understand and confirm the results of our experiment. Indeed, for the test by inhalation, the essential oil of *P. graveolens* was more effective than of *C. citratus* whereas they have the same effect when put in direct contact or ingested. On the basis of the results of our study, it is possible to put adequate mechanisms in place for use of these essential oils to protect all sorts of grains attacked by *S. zeamais* and to reduce post-harvest losses.

ACKNOWLEDGEMENTS

We are greatly indebted to the National University of Rwanda, the Institute of Scientific and Technological (IRST) for the moral as well as material support shown to us. The authors also wish to thank their colleagues for their priceless contribution to the study by way of sharing their experiences with us.

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