J. Pest Cont. & Environ. Sci. 12 (1/2): 13-26 (2004).

Safe alternatives of pesticides for pest management of *Tribolium confusum*

Kawther, El-Gendy [1], A. Kenawy [2] A. El-Bakary [1], and A. H. El-Sebae[1]
[1] Pesticide Chemistry Dept., Fac. of Agric. Alex. Univ., Egypt.
[2] Central Agric. Pesticide Lab., Agric. Res. Center, Sabahia, Alex, Egypt.

ABSTRACT

In the laboratory experiments, adult stage of the confused flour beetles *Tribolium confusum* was exposed to treated Petri dishes with three conventional insecticides (malathion , pirimiphos - methyl and cyhalothrin) and one biocide (abamectin) separately. Pirimiphos-methyl proved to be the most potent of the three synthetic insecticides, followed by cyhalothrin then malathion. The biocide abamectin comes after malathion, but its potency was obvious. Also, the relative toxicity of some monoterpenoids and three essential oils to *T. confusum* adults was assessed using contact and fumigation techniques. Cinnamic aldehyde was the most effective followed by trans anithole, cinnamic alcohol and menthol, while pulegone and α-terpineol were the least toxic compounds when compared using the residual film method. Again, in the fumigation assay, cinnamic aldehyde proved to be the most toxic compound with LC_{50} value 11 mg / L air / 48 hours followed by rosemary oil, while menthol was the least effective fumigant. In addition, the results indicated that heat treatment up to 60 °C for 1 hour will get rid of *T. confusum* and exposure to the microwave radiation for 25 sec was successful in causing 100 % mortality of the confused flour beetles. Combinations of these methods are suggested as new alternatives within the IPM program to combat the infestation of various insect pests.

INTRODUCTION

Insects are a problem in stored grain throughout the world because they reduce the quantity and quality of grain (Evans, 1987 and Madrid *et al.*, 1990). Thus, control of stored product insects requires adequate and continuous attention (Essa, 1995). Synthetic residual insecticides and fumigation are the main methods of grain protection and pest control. However, increased public concern over the residual toxicity of insecticides applied to stored grain, phytotoxicity to the grain, the development of insecticide resistant insect strains, and the precautions necessary to work with conventional insecticides calls for new approaches to control stored-product pests, (Talukder and Howse, 1993 and Sorour *et al.*, 2004).

Essential oils, are potential source of alternative compounds and were currently tested as fumigants. Essential oils have low toxicity to warm-blooded animals, high volatility, and toxicity to stored-grain insect pests (Regnault-Roger and Hamraoui, 1995, Shaaya *et al.*, 1997 and Wahaba 2003). Their major constituents, monoterpenes, are also of interest to industrial markets because of their potential biological activities in addition to their toxicity to insects (Kubo *et al.*, 1994; Selim, 2003 and Kalinovic and Rozman, 2004).

Physical methods such as moisture content, temperature, micro-wave radiation, gamma radiation, infrared irradiation and radio frequency treatment are attractive for post harvest commodities because they possess low mammalian toxicity with a broad spectrum of activity against insects and mites, Moreover, they do not leave b chemical residues on food (Wilkin and Hurlock, 1986).

The objective of the present study is suggesting and evaluating new alternatives which are much safer to man and the environment, within the framework of national program for the integrated pest management (IPM) of the confused flour beetle *Tribolium confusum*, which is one of the most destructive insect pests causing serious damage to stored food products and wheat flour. This includes the toxicity of some conventional insecticides; malathion, pirimiphos methyl, and cyhalothrin in addition to biocide; abamectin. Examine the contact and fumigant activity of some plant extracts such as essential oils and monoterpenoids. Finally, the objective extended to evaluate the effect of heat treatment and microwave radiation as physical methods to control *T. confusum*.

MATERIALS AND METHODS

Tested insect

Adults of the confused flour beetle; *Tribolium confusum* were reared in the laboratory on whole wheat flour and yeast medium, at 30 °C ± 2 and 70 % ± 5 relative humidity. Adults used for the present work were 2-3 weeks old.

Tested compounds

1- Synthetic conventional insecticides: 95 % malathion was produced by American Cyanamid Company, 95 % Pirimiphos-methyl and 95 % Cyhalomethrin were obtained from ICI company, Australia.

2- Biocide: abamectin (95 %) was produced by Novertis Agro-Switzerland.

3- Essential oils: Anise, Lavander and Rosemary were obtained from Faculty of Pharmacy, Alexandria University.

4- Monoterpenoids: Cinnamic aldehyde, trans-anithole, α-terpineol, Cinnamic alcohol, pulegone and menthol were gifted from the Eco Smart Co., USA through Prof. E. Enan.

Methods of application:-

a. Residual film technique:-

The dry film of each compound was prepared by applying 1 ml acetone containing the desired concentration of each compound (mg / L) on 9 cm diameter Petri dish followed the method of Moustafa *et al.*, (1980). After the solvent was evaporated, ten adults of *T. confusum* were introduced in each Petri dish. Three replicates were carried out for each concentration and control. Mortality counts were recorded 48 hrs after initial exposure.

b.Fumigation technique:-

Susceptibility of *T. confusum* adults to the fumigant effect of the monoterpenoids and essential oils was investigated, according to the method of Rice and Coats (1994). Twenty adult beetles were placed in a 50 ml beaker covered with 60-mech cloth. Glass chambers (1 liter) was treated with different concentrations of the tested compounds (mg compound/L. of the air in the fumigation chamber), and the beakers with the adult beetles were placed in the glass chamber. The chambers were then sealed tightly with lid, and mortality counts were recorded after 48 hrs of exposure to each compound.

C. Physical methods

1. Effect of temperature

Twenty adults of *T. confusum* were exposed to different degrees of temperatures; 35, 40, 50 and 60 °C for five time intervals (1, 6, 12, 24 and 48 hours) in Petri dish. Insect mortality at each temperature and time exposure were recorded.

2. Effect of microwave radiation

Twenty adult beetles were placed in Petri dish and exposed to microwave radiation (obtained from Galancy microwave oven, operating at a frequency of 2.45 GH_Z and at a power level of 900w) for different periods; 5, 10, 15, 20, 25 and 30 sec. The mortality was recorded directly after each time interval. Each treatment was replicated three times.

Statistical analysis:

Toxicity data were analysed by Finney method (1970) and expressed as LC_{50} , upper and lower confidence limits and slope values. The results of the physical methods were statistically analysed by one or two ways analysis of variance (ANOVA) using Student Newman–Keuls Test for comparison (Sokal and Rphlf, 1969). Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

1. Effect of conventional insecticides and biocide against *T. confusum*:-

The toxicity data of malathion, pirimiphos-methyl, cyhalothrin and the biocide abamectin against *T. confusum* after 48 hours using the surface deposit application on Petri dishes were presented in Table (1). Among the conventional insecticides pirimiphos-methyl was the most effective insecticide with the lethal concentration (LC_{50}) 0.58 mg / l . Cyhalothrin was the next most effective one with LC_{50} 0.95 mg / l . While the biocide abamectin was the least toxic compound (LC_{50} 14.2 mg / l). Malathion was the intermediate between that of pirimiphos- methyl and abamectin. Although, malathion remains the most commonly applied insecticide to stored products for the control insect attack, however, the superiority of pirimiphos-methyl as the most toxic insecticide is in agreement with the findings of Wahaba (2003).

Malathion, abamectin and cyhalothrin showed (Table 1) the low slope values, thus indicating a high degree of genetic variability to the action of these pesticides. This low slope indicates also high level of tolerance, which leads to the build up of resistance.

Table (1) : Toxicity of three conventional insecticides and one biocide against *T. confusum* after 48 hours exposure using residual film technique.

Compound	LC_{50} (mg / l)	Confidence limits of LC_{50}	Slope
Control	0	0	0
Conventional insecticides			
Cyhalothrin	0.95	0.8-1.1	1.8
Malathion	7.7	6.3-1.2	1.2
Pirimiphos-methyl	0.58	0.55-0.62	3.8
Biocide			
Abamectin	14.2	11.9-17.0	1.6

2. Effect of the natural plant products against *T. confusum*.

a) Using residual film technique

Table (2) illustrates the contact effect of six monoterpenoids and three essential oils against *T. confusum* adults after 48 hrs using residual film technique. It is clear that the monoterpenoid cinnamic aldehyde was the most toxic material with LC_{50} 147 mg / l , followed by trans anithole, cinnamic alcohol and menthol. While, pulegone and α-terpineol showed the least toxic effect with LC_{50}'s 808 and 1232 mg / l , respectively.

In the case of essential oils, the data showed that lavander and anise had little effect against *T. confusum* adults. On the other hand, rosemary oil was the most effective tested essential oil with LC_{50} 335 mg / l .

Table (2): Toxicity of six monoterpenoids and three essential oils against *T. confusum* after 48 hours exposure using residual film technique.

Treatments	LC_{50} mg / l	Confidence limits of LC_{50}	Slope
Control	0	0	0
Monoterpenoids			
trans Anithole	303	283 – 318	4.5
Cinnamic alcohol	516	482 – 553	4.4
Cinnamic aldehyde	147	132 - 163	2.7
Menthol	547	437 – 686	1.5
Pulegone	808	779 - 838	7.3
α-Terpineol	1232	1125 – 1350	2.7
Essential oils			
Anise	1491	1330 - 1671	2.4
Lavander	1123	1054 – 1196	4.6
Rosemary	335	303 - 361	4.0

b) Using fumigation technique

This method is another established method for testing stored product insects susceptibility to volatile compounds . Besides, it is an indication of the actual method practiced in stored products to protect stored grains from insect pests attack .

Table (3) illustrates the fumigant toxicity of some monoterpenoids and essential oils against *T. confusum.* It is clear that, cinnamic aldehyde was the most active monoterpenoid (LC_{50} 11 mg / l air) followed by trans anithole, while menthol was the least effective fumigant with LC_{50} 576 mg / l air.

Table (3) Toxicity of some monoterpenoids and essential oils against *T.confusum* after 48 hours exposure using fumigation technique .

Treatments	LC_{50} (mg / l air)	Confidence limits of LC_{50}	Slope
Control	0	0	0
Monoterpenoids			
trans Anithole	155	143 – 168	3.8
Cinnamic aldehyde	11	10 – 12	4.8
Menthol	576	480 – 621	3.4
Essential oils			
Anise	219	176 – 273	1.5
Lavander	116	104 – 130	2.3
Rosemary	35	30 – 41	2.6

The most effective essential oil as fumigant was rosemary followed by lavander. Anise had relatively low potency. This result is in agreement with the result of Shaaya *et al.*, (1997) who concluded that rosemary and lavander were the most active as fumigants against *R. dominica*. Based on the LC_{50} values in the two methods of application (Tables 2 & 3), it is found that the tested monoterpenoids and essential oils were more effective when used as fumigants than as contacts , except in the case of menthol. Lavander and rosemary were more than 10 fold active as fumigant toxicity than as contact toxicity, Huang *et al.*, (2000) published similar results.

Fumigation is playing a significant role in controlling stored- product insects, and alternatives will be needed in the future as replacements, e.g. for phosphine and methyl bromide. The biological activities of natural plant products as insecticides against stored-product insects has been studied by many researchers; Talukder and Howse (1993); Xie *et al.*, (1995) and Lee *et al.*, (2003). Monoterpenoids and essential oils are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions.

Although, non of the monoterpenoids tested here had activities comparable to conventional insecticides (Smith and Krischick, 2000), it is possible that some monoterpenoids have sufficient potencies to replace the more problematic fumigants and insecticides. The large volume of literature devoted to the insecticidal properties of monoterpenoids and the attention paid by researchers to these compounds are indicative of current attitudes and of the desire to find potentially safer, yet effective, pest mangement stragegies. Further investigations are needed to increase our understanding the effective use of these technologies.

3. Efficacy of physical methods

a) Effect of temperature

The results in Table (4) showed that mortality of *T. confusum* adults was increased with increasing temperature and also with increasing exposure time interval. The exposure of insects to temperature degrees with combined time of exposure, (50 °C / 48 hrs) and (60 °C / 1 h) were very effective where 100 % mortality was achieved. At 35 °C for 48 hrs did not increase mortality relative to insects in control. These data show a similarity with those of Zewar (1993); El-Disouky (2002) and Mbata and Philips (2001) who concluded that increasing the temperature and exposure time, significantly increased the mortality percentages.

Increasing temperatures had a profound effect on lowering the exposure times to reach desired levels of mortality. Reducing the required exposure time would be advantageous when commodities are being received in large volumes at the processors, and for reducing the number and size of treatment facilities.

The heat sterilization technique uses high target temperature ranging from 50 to 60 °C to kill stored product insects by inducing dehydration and / or protein denaturation or enzyme destruction.

Our data agree with Beckett and Wright (2004) who reported that heat disinfestation has potential for high market acceptance as an alternative to methyl bromide because it is potentially as rapid as methyl bromide fumigation, it is completely non chemical and it has great versatility of scale.

Table (4): Effect of temperature and time of exposure on mortality of *T. confusum* adults .

Temperature (°C)	Exposure time (hour)	% Mortality (mean ± SD)
Control	48	0
35	48	0.0 ± 0.0
40	48	26.6 ± 5.7
50	12	83.3 ± 5.7
	24	96.6 ± 5.8
	48	100 ± 0.0
60	1	100 ± 0.0

Control was exposed to room temperature
L.S.D. $_{0.05}$ (temperature) = 0.169
L.S.D. $_{0.05}$ (exposure time) = 0.169
L.S.D. $_{0.05}$ (interaction) = 0.071

b) Effect of microwave radiation:-

The effect of microwave irradiation at 2450 M H_z on mortality of *T. confusum* adults was recorded at six exposure time intervals : 5, 10, 15, 20, 25 and 30 sec (Table 5). The data showed that mortality of insects increased with increasing the exposure periods where it reached 98.3 ± 2.9 % after 25 sec. The treatment at 5 sec did not significantly different than control. Mortality was a function of exposure time and insect moisture content.

The obtained results are paralleled with the results of Halverson *et al.*, (1996); El-Disouky (2002); Plarre *et al.*, (1997) and Wahaba (2003) who found that the high power of microwave and source operating at a frequency of > 2.45 GHz gave mortality percentage ≥ 94 % for many insects of stored products.

Therefore, the insects were probably damaged from overheating while immersed in the hot medium. The observed movements towards the surface indicated avoidance behavior to high heat, but this also increased the probability of direct microwave absorption. The direct absorption of microwaves would also be highly effective in killing because of the heat generated due to the high frequency oscillation of the dielectric molecules such as water in the body fluid of the insects.

Table (5): Effect of microwave radiation against *T. confusum* adults at different exposure periods

Exposure period (Sec.)	% Mortality ** (mean ± SD)
Control *	0.0
5	20.0 ± 0.0 [a]
10	71.7 ± 12.6 [b]
15	86.7 ± 18.9 [c]
20	91.7 ± 10.0 [cd]
25	98.3 ± 2.9 [d]
30	98.3 ± 3.0 [d]

* Control was exposed to room temperature

** Values having the same letters are not significantly different from each other.

Finally; it can be concluded that the present study had suggested introduction of new biocide abamectin to replace the conventional insecticides, which is expected not to suffer from resistance or cross-resistance because it is not an inhibitor of the acetyl cholinesterase. The use of some monoterpenoids and essential oils as fumigants to replace partially the ozone depleting methyl bromide. Further testing of these compounds under storage conditions in the field is necessary to demonstrate their usefulness. Besides, the use of physical methods; heating and microwave radiation which are considered the most promising alternatives.

REFERENCES

Beckett, S. J. and E. J. Wright (2004). Increasing heating rate and decreasing disinfestation temperature for more affordable heat disinfestation of grain . In Proccedings of the 15th International Plant Protection Congress, China, May 11-16, 2004: 623.

El-Disouky, M. I. (2002). Efficacy of insecticide formulations and alternative methods against certain insects. Ph.D. Thesis. Faculty of Agriculture, Alexandria Univ. Egypt.

Essa, I. S. (1995). Insect and animal pests of stored products and its control methods in Egypt and Arab countries. Arabic Company and Distribution, Cairo, Egypt.

Evans, D. E. (1987). Stored products. In Integrated Pest Management. Burn, A.J.; Coaker, T.H., and Jepson, P.C. (eds.), Academic Press, London, U.K, pp. 425-461.

Finney, D. J. (1971): Probit Analysis 3rd Edition. Cambridge Univ. Press, London, UK. *pp*: 318.

Halverson, S. L.; W. E. Burkholder; T. S. Bigelow; E. V. Nordheim and M. E. Misçnheimer (1996). High power microwave radiation as an alternative insect control method for stored products. J. Econ. Entomol., 89 (6): 1638-1648.

Huang, Y.; L. Si and H. Sh (2000). Bioactivities of essential oil from *Elletaria cardamomum* (L.) Maton to *Sitophilus zeamais* (M.) and *Tribolium castaneum* (Herbst). J. Stored Prod. Res., 36 (2): 107-117.

Kalinovic, I. and V. Rozman (2004). Essential oil vapours as protectants against stored product insects. In Proceedings of the 15th International Plant Protection Congress, China, May 11-16, p. 622.

Kubo, I.; H. Muroi and A. Kubo (1994). Naturally occurring antiance agents. J. Nat. Prod., 57: 9-17.

Lee, S.; C. J. Peterson and J. R. Coats (2003). Fumigation toxicity of monoterpenoids to several stored product insects. J. Stored Prod. Res., 39: 77-85.

Madrid, F. J.; N. D. G. White and S. R. Loschiavo (1990). Insects in stored cereals, and their association with farming practices in southern Manitoba. Canadian Entomologist., 122: 515-523.

Mbata, G. N. and T. W. Phillips (2001). Effect of temperature and exposure time on mortality of stored-product insects exposed to low pressure. J. Econ. Entomol., *94* (5): 1302-1307.

Moustafa, F.; A. H. El-Sebae; N. S. El-Hawashi and M. I. Zeid (1980). Toxicity of seven organophosphrus insecticides to the confused flour beetle; *Tribolium confusum* (Duv) applied by two different methods. Alex. J. Agric. Res., 38 (3): 273-277.

Plarre, R.; S. Halverson; W. Burkholder; T. Bigelow; M. Misenheimer; J. Booske and E. Nardheim (1997). Effects of high power microwave radiation on *Sitophilus zeamais* at different frequencies. Int. Conf. on Pests in Agric. 6-8 January, 1997, at Le corum, Montpellier, France, 3: 819-828.

Regnault-Roger, C. and A. Hamraoui (1995). Fumigant toxic activity and reproductive inhibition induced by monoterpenes on Acanthoscelides obtectus (Say) (Coleoptera), abruchid of kidney bean (phaseolus Vulgaris L.) . J. Stored Prod. Res., 31: 291-299.

Rice, P. J. and J. R. Coats (1994). Insecticidal properties of several monoterpenoids to the house fly (Diptera : Muscidae) , red flour beetle (Coleoptera : Tenebrionidae) and the southern cron rootworm (Coleoptera : Chrysomelidae). J. Econ. Entomol., 87: 1172-1179.

Selim, Sh. A. (2003). Studies on pesticide chemistry " Chemistry and bioactivity of certain natural products". M. Sc. Thesis, Faculty of Agriculture, Alexandria University, Egypt.

Shaaya, E.; M. Kostjukovski; J. Eilberg and C. Sukprakarn (1997). Plant oils as fumigants and contact insecticides for the control of stored – product insects. J. Stored Prod. Res., 33 (1): 7 – 15.

Smith, S. F. and V. A. Krischick (2000). Effect of biorational pesticides on flour coccinellid species (Coleoptera: Coccinellidae) having potential as biological control agents in interiorscapes. J. Ecom. Entomol., 93 (3): 732-736.

Sokal, R. R. and F. Rphlf (1969) Single classification analysis of variance. In Biometry W.H. Freeman, San Francisco. *pp* : 204.

Sorour, H. T. Uchino and D. Hamanaka (2004). The effect of sterilization by using infrared irradiation and storage on wheat respiration. In Proccedings of the 15^{th} International Plant Protection Congress, China, May 11-16, p. 620.

Talukder, F. A. and P. E. Howse (1993). Deterrent and insecticidal effects of extracts of pithraj, Aphanamixis polystachya (Meliaceae), against *Tribolium castaneum* in storage. Chemical Ecology, 19: 2463-2471.

Wahaba, T. F. (2003). Control of some dried fruit pests. M.Sc. Thesis, Faculty of Agric., Alex. Univ., Egypt.

Wilkin, D. R. and E. R. Hurlock (1986). Stored grain pests and their management. International Biodeterioation Supp., 22: 1-6.

Xie, Y. S.; P. G. Fields and M. B. Isman (1995). Repellency and toxicity of azadiractin and neem concentrations against three stored – product beetles. J. Econ. Entomol., 88 (4): 1024-1031.

Zewar, M. .M. (1993). The use of high temperature for disinfecting wheat from *Sitophlus granarius* (L.) and cowpea *Callosobruchus maculatus* (F.). Egypt. J. Agric. Res., 71 (3): 1993.

Received 27 / 4 / 2004
Accepted 31 / 5 / 2004

بدائل آمنة للمبيدات للمكافحة المتكاملة لخنفساء الدقيق المتشابهة

د.كوثر الجندى[1]، د. عنتر قناوى[2]، د. احمد البقرى[1]، أ.د. عبد الخالق السباعى[1]
[1] قسم كيمياء المبيدات – كلية الزراعة – جامعة الإسكندرية – مصر.
[2] المعمل المركزى للمبيدات – مركز البحوث الزراعية – الصباحية – الإسكندرية.

تم دراسة تأثير بعض المبيدات التقليدية وهى الملاثيون والبيريمفوس ميثيل والسيهالوثرين وكذلك احد المركبات البيولوجية (الابامكتين) على حساسية الحشرات الكاملة لخنفساء الدقيق المتشابهة بطريقة التعرض لمتبقى المبيد وكان اكثر المبيدات تأثيرا مبيد البيريميفوس – ميثيل بينما كان اقلها فى التأثير مركب الابامكتين.

كما تم اختبار حساسية حشرة خنفساء الدقيق المتشابهة لمجموعة من المونوتربينيويدات وثلاث من الزيوت الطيارة بطريقة التعرض لمتبقى المبيد والتدخين- واتضح من النتائج ان السيانميك الدهيد اشد المركبات فاعلية وكان اقلها فاعلية مركب الالفا-تربيتيول باستخدام طريقة التعرض لمتبقى المبيد. وكذلك كان مركب السيانيك الدهيد هو اكثر المركبات فاعلية باستخدام طريقة التدخين وكانت قيمة التركيز القاتل لـ ٥٠ % هى ١١مجم / لتر هواء / ٤٨ ساعة يليه حصا اللبان بينما كان المنثول اقل المركبات فاعلية . وتعتبر هذه البدائل اكثر اختيارية من المبيدات التقليدية لانها تستهدف مستقبل الاوكتوبامين شديدة الحساسية والاهمية فى الجهاز العصبى للحشرات بينما ليس له نفس الاهمية فى الانسان .

بالاضافة إلى ذلك فقد وجد ان استخدام درجة حرارة ٦٠ºم والتعرض لاشعة الميكروويف لمدة ٢٥ ثانية كانت كافية لقتل جميع الحشرات. ونجاح هذه الوسائل يجعل من الممكن اقتراح تطبيقها عمليا فى المكافحة المتكاملة لحشرات الحبوب المخزونة.