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Identification of main constituents of date odour and their behavioural activity against *Ephestia cautella* in white sticky traps

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Abstract: The relative percentage, molecular weight and chemical structure of the main constituents of date odour were carried out by using Gas Chromatography /Mass Spectrometry (GC/MS) analysis. The identified compounds of date odour extract accounted 94.83 % of the total constituents. The major compounds were identified as oleic acid followed by butylated hydroxytoluene and linoliec acid. They accounted 76.46%; while the other identified compounds accounted about 18.37 % only.

Both sexes of *E. cautella* moth could be attracted and captured in the white sticky traps baited with extracted date odour. Bating traps with oleic acid (an active component of date) also captured both sexes of the moths, but in much less numbers. In both cases, females were highly more attracted to baited traps than to control ones.

Traps baited with semiochemicals or food odours can be used for catching both sexes of the insect pests; and can successfully and safely integrated with other insect control measures in IPM programs.

Key words: GC-MS analysis, oleic acid, almond moth, baited traps.

Introduction

The Almond moth, *Ephestia* [*Cadra*] *cautella* (Walker) (Lepedoptera: Pyralidae) is a well-known cosmopolitan indoor pest active during night. The moths feed as larvae, whereas all other stages are non-feeding. Hence, most damage associated with stored product moths is due to the larvae. The Almond moth is a primary pest on many stored products[1].

Many problems are associated with the use of synthetic insecticides as grain protectant, in addition to the upset of ecological balance, insect resistance and increased pollution hazards. Therefore, the use of natural pest control agents, especially those derived from plants, may offer better promise in grain storage[2, 3, 4,5,6,7,8].

Food volatiles are those substances or mixtures of substances which emanate from food and disperse easily through air, due to their high volatility. These compounds consist of a chain of 5-20 carbon atoms and a variety of functional groups, e.g. ketones, aldehydes, esters and alcohols. The volatile compounds can be discriminated by their effect on the receiving insect; attractants, causing oriented locomotion towards the source of the chemical, and repellents, causing oriented locomotion away from the source[9].

In a study of volatile components in Zahadi dates, 27 neutral compounds and 11 organic acids were separated and identified from a date extract which possessed date aroma[10]. The date aroma and flavor of the accurate compound mixture was not detected. Palmitic acid, stearic acid and oleic acid are common fatty acid moieties of triglycerides, which could induce both aggregation and feeding for *Tribolium confusum* when being presented as individual free fatty acids[11,12 ,1]. On the other hand, 36 volatile components from three date varieties were identified[13]. Recently, 78 volatile compounds were extracted and separated from date palm fruits,and only 35 compounds could be identified[14]. These included esters, alcohols, lactones, aldehydes and ketones.

Food-derived oils, when applied both in pheromone traps and physical traps, increased the number of captured insects[1]. By comparing traps without grain odour to traps with grain odour, he concluded that the trap efficacy for *T. castaneum* was increased two-fold when wheat odour was present. The results confirmed the multispecies function of the food-baited traps and showed that the simplicity of this monitoring system made it an economically good alternative. Food-baited traps can possibly trap both sexes; several species, both adult and larval insects and they are cheap[15,16]. For trapping female moths, the progress of food odour attractants was studied [17]. In flight tunnel experiments, *E. cautella* and *P. interpunctella* males and mated females were attracted to three different chocolate products odours.

The present work aims to identification the main constituents of date odour and their behavioural activity against *Ephestia cautella* moths in white sticky traps .

Experimental

Insect rearing

The larvae of *E. cautella* (Walker) (Lepedoptera: Pyralidae) were reared on a standard diet comprised of 100 g wheat germ, 10g Brewer's yeast and 20g glycerol **,**all thoroughly mixed to obtain homogeneous diet[17]. Larvae were introduced into glass jars covered with muslin cloth, tied with rubber bands and kept in an incubator adjusted at 27±1ºC, 65±5 R.H. % and a photoregime L:D14:10 hr[18].

Emerged adults were confined in an oviposition cage whose bottom was a sieve. Eggs laid through the sieve were collected in a dish every day and kept till hatching so all the larvae obtained from these eggs had approximately the same age[19].

Date odour extraction procedure

Extract of date was prepared by maceration of 500 g date with diethyl ether for one week. The mixture was filtrated with filter paper and the residue was remacerated with diethyl ether again for another one week. After instillation, the filtrates of each substance were condensed by rotary vacuum evaporator and then adjusted by the solvent to 50 ml in a volumetric tube[20] .

Identification of main constituents of extracted date odours

The moth of *E. cautella* gave the highest attractive reaction to date odour extract[15]. So, the constituents of the extract of date was subjected for Gas Chromatography /Mass Spectrometry (GC/MS) analysis. A sample from the date extract was separately subjected to GC/MS investigation adopting the following procedure.All samples were analyzed at the Institute of Plant Diseases, Agriculture Research Centre, Ministry of Agriculture.

Gas Liquid Chromatography (GLC):

- Apparatus: Hewlett Packard 5890 series II.
- Detector: Flame ionization detector.
- Column: Carbowax 20 M capillary column (50m x 0.2mm).
- Oven temperature: 60-200°C increased by 3°C/mm.
- Detector temperature: 250°C.
- Injector temperature: 200 °C.
- Carrier Gas: Helium, with flow rate of lml/min.

GC/MS:

- Apparatus: Hewlett Packard Mass spectrometry 5970.

- Detector: Total Ion Current (TIC)
- Column: Carbowax 20M capillary column (50m x 0.2mm).
- Temperature program: 60-200°C increased by 3°C/mm.
- MS ionization voltage: 70 v.

Qualitative and quantitative identification of the extract constituents were carried out by comparing retention times and mass fragmentation patterns with those stored in the library of GC/MS and more conformation was carried out with the MS of these compounds of the available published data.

Behavioural activity of extracted date odour and its active compound against *E. cautella* in white sticky traps:

To assess the most attractive food odour and its active compound a simple assay using a wooden storage bin (100 cm × 50 cm × 50 cm) was developed.

The experiments were carried out in the wooden bin covered with black muslin cloth on its top and its two opposite sides (50 cm × 50 cm). On the bin bottom, packages of peanut were introduced.

Commercially available white trap cards coated with adhesives substance designed to capture flying insects were used in these experiments. This trap type is triangle shaped with a sticky surface on the interior walls (15 × 22.5 = 337.5 cm^2 trapping surface). The trap was suspended at the centre of the top of the bin. The trap was either containing the tested material and acted as treatment or containing the solvent only and acted as the control. Each trap septum containing the tested material was hanged from the center by a wire.

The pervious experiments[4] showed that the extracted date odour was the most attractive odour to the almond moth and the identified active compound of date odour was oleic acid. Half gram of oleic acid was dissolved in 5 ml of diethyl ether and the attractant activity of 3.6 µL and 7.2 µL of this solution was tested.

In these experiments the attractant activity of extracted date odour and its active compound (oleic acid) was tested.

A group of 40 laboratory-reared almond moth adults of mixed sex were released into each bin and kept at 27±1 ºC, 65±5 R.H. % in dark. The number of adult moths that were caught in the white sticky traps baited with tested materials was counted after 2 days. Female moths were discriminated from males by the sex-specific genitalic structures prominent from the distal end of the abdomen. Mated females or males (2-3 days old) were experimented with four replicates for each treatment and the test was repeated two times.

The odds ratio was calculated according to[21].

Odds ratio = $P\text{t} \times (100-P\text{c}) / (100-P\text{t}) \times P\text{c}$

Where Pt is the attractant activity (%) for the treatment and Pc is the attractant activity (%) for the control.

The higher the odds ratio, the better the discrimination between treated and control samples.

Statistical Analysis:

The difference between treated and control samples was statistically analyzed by Duncan t test in SPSS and measured by the odds ratio according to[21].

Results and Discussions

Identification of main constituents of extracted date odours

Identification of food odour emitted by date that attract tested insect could lead to the development of a lure in monitoring system of stored product insects.

According to the pervious results[4], the most attractive and favorable food odour was date . So that constituents of the extract of date was subjected for Gas Chromatography/ Mass Spectrometry (GC-MS) analysis.

The relative percentages, molecular weights and the structures of the main constituents of the ether extract of date as identified by GC-MS are shown in Table (1). The identified compounds accounted 94.83 % of the total compounds. The major compound was identified as oleic acid (9-Octadecenoic acid) and reached 27.54% followed by butylated hydroxytoluene which accounted 27.26% and then linoliec acid (9, 12-Octadecadienoic acid) 21.66%. These three compounds reached 76.46%, while the other 16 identified compounds accounted about 18.37 % only. The three major compounds of date odour extract are the only oxygenated compounds among the 19 identified compounds. Most of the activity may be referred to the oxygenated compounds[22,23]. For this reason pure authentic sample from the fatty acid (oleic acid) was subjected for biological activity study as insect attractants.

Table (1): The main constituents of date odour as identified by Gas Chromatography/ Mass Spectrometry (GC-MS).

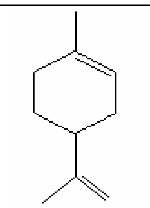
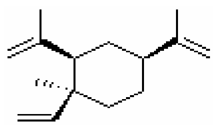
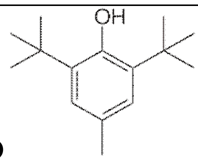
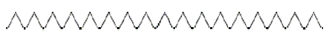
pk	RT (min)	Area %	Compound	M.W	Structure	Base Peak
1	8.270	3.21	d1-Limonene	136	 $C_{10}H_{16}$	68
2	22.975	0.79	beta Elemene	204.35	 $C_{15}H_{24}$	93
3	27.284	1.82	Non identified compound			
4	27.947	27.26	Butylated Hydroxytoluene	220.35	 $C_{15}H_{24}O$	205
5	31.129	0.45	Hexadecane (CAS)	226.45	 $C_{16}H_{34}$	57
6	32.805	0.52	Heneicosane (CAS)	296.57	 $C_{21}H_{44}$	57
7	32.914	0.46	Pentatriacontane	492.95	 $C_{35}H_{72}$	57

Table (1) : continued

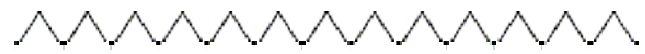
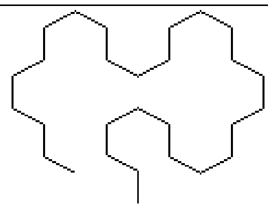
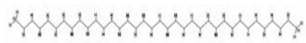
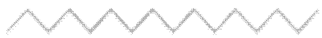
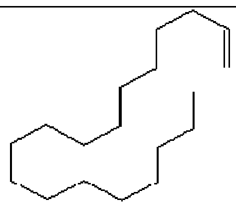
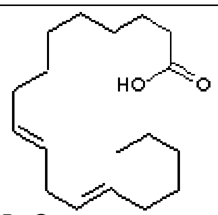
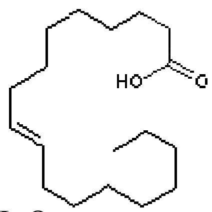
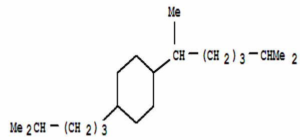
pk	RT (min)	Area %	Compound	M.W	Structure	Base Peak
8	33.469	0.54	Hexadecane,2-methyl-	240.4677	 $C_{17}H_{36}$	57
9	33.738	0.47	Heptacosane	380.73	 $C_{27}H_{56}$	57
10	34.814	0.77	Heptadecane	240.47	 $C_{17}H_{36}$	57
11	35.020	0.59	Octacosane (CAS)	394.76	 $C_{28}H_{58}$	57
12	36.353	1.07	Hexatriacontane	506.97276	 $C_{36}H_{74}$	57
13	36.633	0.46	Non identified compound			
14	36.845	0.42	Non identified compound			
15	37.303	0.46	Hexadecane (CAS)	226.45	 $C_{16}H_{34}$	57
16	38.321	0.48	Octadecane	254.49	 $C_{18}H_{38}$	57
17	38.476	0.54	Non identified compound			
18	38.602	1.19	1-Octadecene	252.48	 $C_{18}H_{36}$	57
19	39.746	1.12	Heneicosane (CAS)	296.58036	 $C_{21}H_{44}$	57
20	39.906	0.31	Non identified compound			
21-22	40.256	0.83	Non identified compound			

Table (1) : continued

pk	RT (min)	Area %	Compound	M.W	Structure	Base Peak
23-26	40.978	21.66	9,12-Octadecadienoic acid (Linoleic acid)	280.45	 $C_{18}H_{32}O_2$	55
27-29	41.457	27.54	9-Octadecenoic acid(Z)-(CAS) (Oleic acid)	282.46 14	 $C_{18}H_{34}O_2$	55
30-31	43.085	6	Cyclohexane,1-(1,5-dimethylhexyl)-4-(4-methyl pentyl)	280	 $C_{20}H_{40}$	57
32	44.410	0.78	Non identified compound			
33	46.103	0.25	Tridecane	184.36	 $C_{13}H_{28}$	57

It is known that food volatiles are those substances or mixtures of substances that emanate from food and disperse easily through air, due to their high volatility. These compounds consist of a chain of 5-20 carbon atoms and variety of functional groups as ketones , aldehydes , esters and alcohols[9]. They can be differentiated by their effect on the receiving insects, either attractants or repellents.

In this respect, 27 neutral compounds and 11 organic acids from date extract were separated and identified[10]. On the other hand, 36 volatile components from three date varieties were identified[13]. Recently, 78 volatile compounds were extracted and separated from date palm fruits; only 35 compounds could be identified. These included esters, alcohols, lactones, aldehydes and ketones[14].

Behavioural activity of extracted date odour and its active compound against *E. cautella* in white sticky traps

Previous results, indicated that extracted date odour was the most favorable and had the highest attractive activity to *E. cautella* moths either males or females[4]. Oleic acid was the major compound of date as identified by GC-MS. So, the behavioural response of *E. cautella* towards the extracted date odour and oleic acid was tested in white sticky traps. In case of date odour, captured moths recorded high percentage in case of tested concentrations in comparison to controls (Table 2). Traps baited with either 0.1 or 0.2 ml captured more female and male moths (30% and 22.5 %) than controls (6.3% and 5%), respectively. The lower concentration (0.1 ml) attracted more moths than the higher one. The baited traps captured about five times females more than males.

Table (2): Capture percentage of *E. cautella* moths in white sticky traps baited with extracted date odour.

Insect sex	Date odour concentration (ml)							
	0.1				0.2			
	Treated (%)	Control (%)	Odds ratio	Capture (%)	Treated (%)	Control (%)	Odds ratio	Capture (%)
Female	24.4	4.4	7.0	20	16.3	2.5	7.6	13.8
Male	5.6	1.9	3.1	3.7	6.2	2.5	2.6	3.7
Both sexes	30	6.3	6.4	23.7	22.5	5	5.5	17.5

Traps baited with the active compound of date odour (oleic acid) followed nearly the same trend in case of baited traps with extracted date odour concerning the effect of bait concentration as well as its attraction to both moth sexes (Table 3). However, the efficiency of extracted date odour trap was much higher than that of the active component (oleic acid) trap. This is probably because extracted date odour has more active components which add to its attractivity. This supports the findings by[24] who find that the single fatty acids can be used as attractant with less attractive effect than the crude extract.

Table (3): Capture percentage of *E. cautella* moths in white sticky traps baited with oleic acid.

Insect sex	Oleic acid concentration (µL)							
	3.6				7.2			
	Treated (%)	Control (%)	Odds ratio	Capture (%)	Treated (%)	Control (%)	Odds ratio	Capture (%)
Female	13.7	4.4	3.5	9.3	11.2	1.3	9.6	9.9
Male	3.8	3.1	1	0.7	4.4	3.1	1.4	1.3
Both sexes	17.5	7.5	2.6	10	15.6	4.4	4	11.2

In both cases, the odds ratio values of females trapped by either extracted date odour or oleic acid were higher than those of males. This indicated that more females than males of *E. cautella* were attracted to baited traps with date odour extract. Therefore, female catching in baited traps will reduce the egg production and so the insect population greater than catching of males in pest control management[25]. Similarly, field trapping experiments for indoor pyralids with food-derived substances have shown the possibility of trapping females[21].

In this respect, food derived substances attracting females have been identified for many lepidopteran pests. For example, *Amyelois transitella* , a moth infesting almonds[27], *Ostrinia nubilalis*[28,29,30,31] , *E. cautella* and *P. interpunctella* [17]and *P. interpunctella*[32] **.** Also, attraction of lepidopteran males to food odours has been reported for *O.nubilalis* [28,29,33] and for *E. cautella* and *P. interpunctella*[17]**.**

Date odour extract induce higher catches and are more attractive as trap baits than its constituent (oleic acid) to *E. cautella*((key pest of peanut). This makes date odour as good baits used to improve the trap catch of tested insect in peanut storage. Catching more females, as resulted in the present study, reduces the egg deposition and hence the insect population greater than catching males in pest control strategies. Results emanating from the present study may pave the way for further investigations that can be of great value in the use of semiochemicals with other control methods in programs of stored product insect management.

References

1. Olsson, P.O.C. , The function of food volatiles: insect behaviour and pest control, Tryck: Reprocentralen Lunds Universitet, 2001, 1-32.
2. Abd-El-Aziz, Shadia E., Control Strategies of Stored Product Pests. Journal of Entomology, 2011, 8(2), 101 -122.
3. Abd El-Aziz ,Shadia E., Persistence of some plant oils against the Bruchid beetle, *Callosobruchus maculates* (F.) (Coleoptera: Bruchidae) during storage. Arab Universities Journal of Agricultural Sciences, Ain Shams , Cairo, 2001, 9 (1), 423-432 .

4. Abd El-Aziz, Shadia E.,Abdel Rahman, H., Omar, E.A., Abdel-Hakim, E.A. and Fahim, Sh.F., Olfaction cues of food odours influencing behavior response of the almond moth , *Ephestia cautella* (Walker) (Lepidoptera : Pyralidae). Egyptian Journal of Biological Pest Control, 2012 , 22(1), 45-50.
5. Abdel Rahman,H., Abd-El-Aziz, Shadia E. , Omar, E.A. , Abdel-Hakim, E.A. and Fahim, Sh.F., Olfaction cues of food odours influencing behavior response of the red flour beetle, *Tribolium castanium* (Herbst)(Coleoptera: Tenebrionidae). Bulletin of the National Research Centre, 2014, 39 (1), 1-11.
6. Sharaby, A., Abdel Rahman, H., Abd El- Aziz, Shadia E. and Moawad, S., Susceptibility of different potato varieties to infestation by potato tuber moth and role of the plant powders on their protection .IOSR Journal of Agriculture and Veterinary Science, 2014, 7 (4), 71-80.
7. Sabbour,M.M. and Abd-El-Aziz, Shadia E., Evaluation of some bioinsecticides and packaging materials for protecting broad bean against *Callosobruchus maculatus* (Coleoptera: Bruchidae) infestation during storage . Bulletin of the Entomological Society of Egypt, Economic Series, 2007, 33, 129-137.
8. Sabbour,M.M. and Abd El-Aziz, Shadia E., Efficiency of some bioinsecticides against broad bean beetle, *Bruchus rufimanus* (Coleoptera : Bruchidae). Research Journal of Agriculture and Biological Sciences, 2007, 3(2), 67-72.
9. Dethier, V. G.; Brown, L. B. and Smith, C. W., The designation of chemicals in terms of the responses they elicit from insects. Journal of Economic Entomology, 1960, 53, 134–136.
10. Jaddou, H., Mhaisen, M.T. and Al-Hakim, M., Flavor volatile analysis of Zahdi dates by gas- liquid chromatography. Date Palm Journal, 1984, 3 (2), 367-379.
11. Loschiavo, S. R., The chemosensory influence of some extracts of Brewer´s yeast and cereal products on the feeding behavior of the confused flour beetles, *Tribolium confusum* (Coleoptera: Tenebrionidae). Annals of the Entomological Society of America, 1965,58,576-588.
12. Starratt, A.N. and Loschiavo, S.R., Aggregation of the confused flour beetle, *Tribolium confusum*, elicited by mycelial constituents of the fungus *Nigrospora sphaerica*. Journal of Insect Physiology, 1971, 17, 407-414.
13. Reynes, M.; Lebrun, M. and Shaw, P. E., Identification of volatile date components and use of multivariate analysis to distinguish date varieties. Journal of Food Quality, 1996, 19,505–514.
14. Narain, N., Volatile compounds in date palm fruit.International Society for Horticultural Science, III International Date Palm Conference, ISHS Acta
15. Horticulturae, 2007, 736, Vol.1.
16. Chambers, J., Overview on stored-product insect pheromones and food attractants. Journal of the Kansas Entomological Society,1990, 63, 490-499.
17. Pinniger, D. B., 1990 Food-baited traps: Past, present and future. Journal of the Kansas Entomological Society,1990, 63(4), 533- 538.
18. Olsson, P.O.C., Anderbrant, O. and Löfstedt, C., Flight and Oviposition Behavior of *Ephestia cautella* and *Plodia interpunctella* in Response to Odors of Different Chocolate Products. Journal of Insect Behavior, 2005, 18 (3), 363-380.
19. Bell, C.H. and Bowley, C.R., Effect of photoperiod and temperature on diapause in a florida strain of the tropical warehouse moth *Ephestia cautella*. Journal of Insect Physiology, 1980, 26,533-538.
20. Bell, C.H. and Walker, D.J., Diapause induction in *Ephestia elutella* (Hubner) and *Plodia interpunctella* (Hubner) (Lepidoptera, Pyralidae) with a dawn-dusk lighting system. Journal of Stored Products Research, 1973, 9,149-158.
21. Seifelnasr, Y. E., Hopkins, T. L. and Mills, R. B., Olfactory responses of adult *Tribolium castaneum* (Herbst), to volatiles of wheat and millet kernels, milled fractions and extracts. Journal of Chemical Ecology, 1982, 8 (12), 1463-1472.
22. Morgan, C., Sherington, J., Gudrups, I. and Bowden, N.S., The assessment of potential attractants to beetle pests: improvements to laboratory pitfall bioassay methods. Journal of Stored Products Research, 1998, 34 (1), 59–74.
23. Omer, E.A., Ouda, H.E. and EL-Bazza, Z.E., Chemical composition and aflatoxigenic activity of the essential oils of *Mentha longifolia* L. and *Laurus nobilis* L. *Egyptian Journal of Applied Science*, 1991, 6(2) ,32-37.
24. Abd El-Aziz, Shadia E., Omer, E.A. and Sabra, A.S., Chemical composition of *Ocimum americanum* essential oil and its biological effects against *Agrotis ipsilon* (Lepidoptera: Noctuidae). Research Journal of Agriculture and Biological Sciences, 2007, 3 (6), 740-747.
25. Baker, T.C. and Phelan, P.F., Ovipositional disruption of the navel orange worm with fatty acids. United States Patent, Patent, 1992, 104, no.5, 654.

26. Nansen, C., Konemann, C., Phillips, T.W. and Dillwith, J.W., Attractant for monitoring or controlling female stored product moths. United States, Patent Application Publication, 2007 pp 1-7.
27. Toth, M., Repasi, V. and Szocs, G., Chemical attractants for females of pest pyralids and phycitids (Lepidoptera: Pyralidae, Phycitidae). Acta Phytopathologica et Entomologica Hungarica, 2002, 37,375–384.
28. Phelan, P. L., Roelofs, C. J.; Youngman, R. R. and Baker, T. C., Characterization of chemicals mediating ovipositional host-plant finding by *Amyelois transitella* females. Journal of Chemical Ecology, 1991, 17(3), 599–613.
29. Cantelo, W. W. and Jacobson, M., Phenyl acetaldehyde attracts moths to bladder flower and to black light traps. Environmental Entomology, 1979,8(3), 444 - 447.
30. Maini, S. and Burgio, G., Influence of trap design and phenylacetaldehyde upon field capture of male and female *Ostrinia nubilalis* (Hb.) (Lepidoptera: Pyralidae) and other moths. Boll. 1st. Entomol. Univ. Stud. Bologna, 1990, 45,157–165.
31. Binder, B. F., Robbins, J. C. and Wilson, R. L., Chemically mediated ovipositional behaviors of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Journal of Chemical Ecology, 1995, 21(9), 1315–1327.
32. Binder, B. F. and Robbins, J. C., Effect of terpenoids and other related compounds on the oviposition behavior of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Journal of Agriculture and Food Chemistry, 1997, 45(3), 890-984.
33. Uechi, K., Matsuyama, S. and Suzuki, T., Oviposition attractants for *Plodia interpunctella*(Hübner) (Lepidoptera: Pyralidae) in the volatiles of whole wheat flour. Journal of Stored Products Research, 2007, 43,193–201.
34. Maini, S. and Burgio, G., *Ostrinia nubilalis* (Hb.) (Lep., Pyralidae) on sweet corn: Relationship between adults caught in multibaited traps and ear damages. Journal of Applied Entomology, 1999, 123, 179–185.

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