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A NEW DESIGN FOR SIMULTANEOUS CHOICE TEST TO STUDY THE RESPONSE OF ORIENTAL HORNETS TO SOME NATURAL SUBSTANCES UNDER LABORATORY CONDITONS

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Abstract: The present investigation was carried out in the Apiary of Plant Protection Department, Faculty of Agriculture, Assiut University during the peak of oriental hornet, from the first of September to the end of October, 2006. A new technique (with four to eight-way) for simultaneous choice test was used in the present investigation to study the attraction of oriental hornets to certain natural substances under laboratory conditions. The evaluation of n-butanol, ammonia and acetic acid (absolute) showed that the n-butanol gave the highest attractiveness (59.3%), resulting in a 0.59 response index, towards the tested hornets compared to the others. Of the three tested concentrations of n-butanol (100, 50, 25%) the 50% gave the highest attractiveness (55.7%), inducing 0.56 response suggested index to tested hornets as compared with others, which were unaffected. The concentration of 5% of ammonia was highly attractive (42.7%) than others (25, 10%, control), resulting in a 0.43 response index. The 0.5% concentration of acetic acid indicated only high attractiveness (45.7%) to the hornets than others (2, 1%, control), inducing a 0.46 response. Testing of essential oils, eucalyptus, fennel and anise, in their pure forms, indicated that the eucalyptus oil gave the most attractive effect (48.7%). Of the different concentrations of eucalyptus oil (50, 25, 10%), the 50% concentration resulted in the greatest attractiveness (49%). The test of the greatest attractive concentrations of tested substances, n-butanol (50%), acetic acid (0.5%), ammonia (5%) and eucalyptus oil (50%) showed that the n-butanol (50%) induced the greatest attractiveness at all to oriental hornets (45.7%), resulting in a 0.46 response index.

Key words: oriental hornet, *Vespa orientalis*, simultaneous choice, attractiveness, natural substances.

Introduction

The oriental hornet, *Vespa orientalis* L. is the main and most important pest confronting Egyptian beekeepers, especially in Assiut region. This pest attacks and causes serious damages to many fruits before and after the harvesting, especially grape, date and pear trees (Ibrahim and Mazeed, 1967 and Wafa *et al*., 1968). The hornets hovere near bee hives, and attack and prey guard bees at the hive

entrances, capture returning foragers and also catch foragers from the flowers (Sihag, 1992 and Abrol, 1994). The hornet would capture bees and take them to nearby stand to cut the head and abdomen from the prey keeping only the thorax for the short flight back to the nest (Caron, 1986). Sometimes, the hornets enter bee hives and carry off both larvae and pupae, and fly back to their nest to feed hornet colony. So, the hornets cause weakness and destruction to bee colonies, and decrease their productivity (Matheson *et al*., 1989 and Sihag, 1992). The activity of oriental hornets is low in winter, spring and early summer, but increases to reach the maximum population in the autumn particularly during October. It starts to fall off during November and disappears in December (Khater *et al*., 2001; Gomaa and Abd El-Wahba, 2006). At Assiut governo-rate in Dirut location, the oriental hornets start to appear in the first week of April and gradually decrease to the minimum levels during June and July. Then the activity of hornets increases gradually from second week of August to the fourth week of September and reaches its peak in October (Gomaa and Abd El-Wahab, 2006).

The present study focused on the oriental hornet, in two major themes. The first, to use a new technique with four to eight-way (four-eight choices) designed to test four substances simultaneously. The second, to study the response of oriental hornet to some natural substances under laboratory conditions, in order to use them or their combinations in the future for capturing the oriental hornet in the apiaries.

Materials and Methods

Substances used for bioassay tests:

In laboratory test, six substances were used in the present study as follows:

Tested substances and their concentrations:

- Acetic acid glacial (100, 2, 1, 0.5% co-ncentrations).
- n-Butanol (100, 50, 25% concentr-ations).
- Ammonia (100, 25, 10, 5% concentr-ations)
- Eucalyptus oil (100, 50, 25, 10% conc-entrations)
- Fennel oil (100%)
- Anise oil (100%)

In the application method, 100 µl solution of tested substance and solvent were used for three times (replicates) for each treatment and the control.

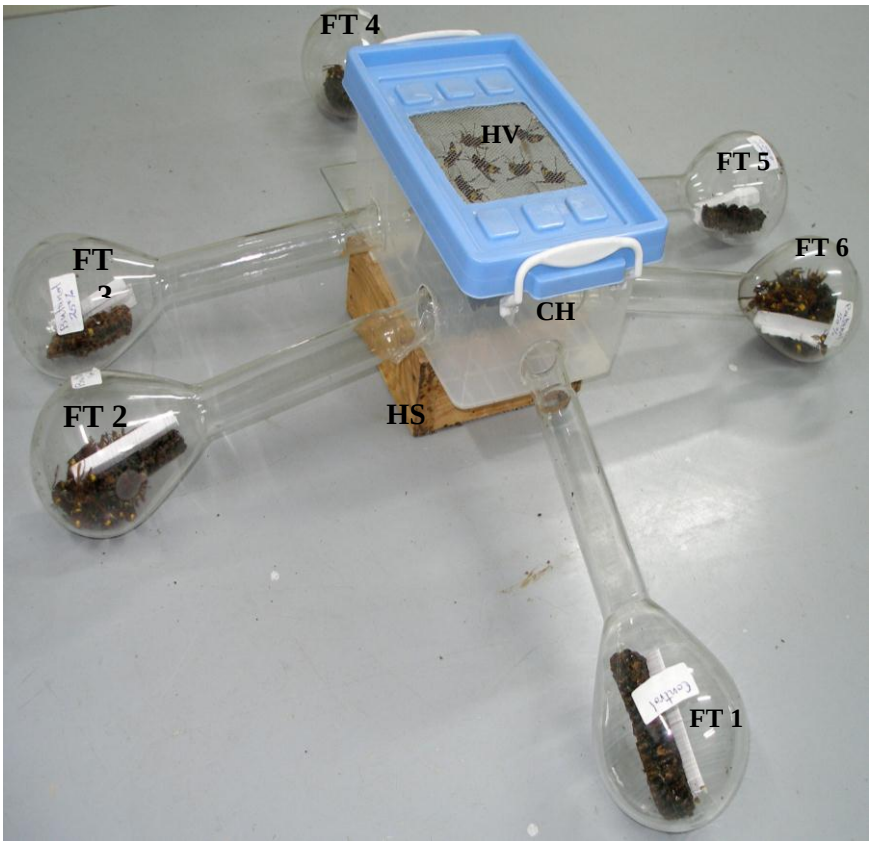
New technique for bioassay tests:

In order to test the attractiveness and repellency of certain natural materials a simultaneous choice test for oriental hornets was used. A new technique for performing the tests was designed. The device useded (Figure 1) which consisted of two main units, the first is a choice test unit and the second is a hornet individuals supply unit.

The choice test-unit consisted of a plastic box (30x20x15 cm dimen-

sions) with four to eight circular holes, one or two in each side of the box. Each hole (3 cm in diameter) fixed to a glass spherical flask with a long neck (25 cm). Inside the flask, a small piece of wax comb provided with bee honey was placed inside the bottom of each flask. On top of the box, there was a hole (20x10 cm dimensions), rectangular in shape provided with hornet proof wire netting for ventilation. At the bottom there was also rectangular hole for hornet supply.

The hornet individuals supply unit consisted of a wooden cage (12x12x5 cm dimensions) and a glass door to control hornets supply. This unit was put on attached to the bottom of choice test unit.



HS: Hornet supply Unit; CH: Choice test Unit; FT: Field of Test (4-8); HV: Hole Ventilation

Fig.(1): New Design for performing attractiveness and repellency simultaneous choice tests for oriental hornets under laboratory conditions.

Hornet individuals collection and bioassay tests:

In the Apiary of Assiut University, the oriental hornets during their active season (October, 2007) were trapped and collected alive using modified wooden traps (45x65x80 cm dimensions) attached to the top of hive box contains dead brood and honey combs to attract the oriental hornets. The trap and the hive box were fitted over four small legs to allow the hornet individuals to enter through the bottom. Three traps were used and placed in the apiary, thereafter the individuals of hornets caught in each trap were collected alive and were deposited inside wooden cages until their use.

At the beginning of the choice tests, 100 individuals of oriental hornet were placed in each hornet supply unit, then positioned below the choice test unit. A piece of filter paper was wetted with either a 100 µl solution of the substance to be tested or only 100 µl of the solvent used (distilled water or acetone) and placed inside the flask at the bottom. Three replications were used for each treatment. All replications were held at room temperature in the laboratory for the experimental period. Two hours later, the distribution of the hornets in the four directions was determined. At the end of choice test, the four flasks were washed and the hornet individuals renewed before the new test. The strength of response was calculated using the following suggested equation:

$$\textbf{Response index} = \frac{\textbf{Number of hornet in any field}}{\textbf{Total number of tested hornet}}$$

- an index value of 1.0 means that the tested substance is extremely attractive.

- an index value of 0.0 means that the tested substance is extremely repellent.

- an index value of 0.25 means that the tested substance has no effect.

Also, the increase or the decrease of the hornet numbers in any of the four-way compared to control in relation to the total number of tested hornets was calculated using the following suggested equation:

$$\textbf{Increase or decrease in hornets to control (\%)} = \frac{\textbf{Hornet n. in any field} - \textbf{Hornet n. in control}}{\textbf{Total n. of tested honet}} \times 100$$

Statistical analysis:

The statistical analysis were conducted using the SAS general linear models procedure. Differences among means were determined by L.S.D.Significant differences were determined at $P<0.05$ (SAS Institute, 1990).

Results and Discussions

n-Butanol, ammonia and acetic acid:

The new technique was designed to evaluate the attractiveness and repellency of different substances in simultaneous choice tests in laboratory. The evaluation of n-butanol, ammonia and acetic acid (absolute) compared to control showed that 59.3, 38.7, 0.0 and 2% of the tested hornets were attracted to these materials, respectively, inducing 0.59, 0.39, 0.0 and 0.02 response index. These values as well as the index indicate that the n-butanol showed highly significant attractiveness, moderate for ammonia and repellent for acetic acid towards the tested hornets (Table 1 and Fig. 2).

Table(1): Effects of different substances and their concentrations on distribution of oriental hornet in simultaneous choice tests, in laboratory.

Response index and change compared to control (%)	Different substances and concentrations			
	Absolute substances			
	n-Butanol	Acetic acid	Ammonia	Control
Response index	0.59	0	0.39	0.02
Change compared to control (%)	57.3	-2	36.7	-
	n-Butanol (concentrations)			
	100%	50%	25%	Control
Response index	0.25	0.56	0.17	0.02
Change compared to control (%)	22.4	53.4	15.0	-
	Ammonia (concentrations)			
	25%	10%	5%	Control
Response index	0.19	0.30	0.43	0.08
Change compared to control (%)	11.3	22	34.7	-
	Acetic acid (concentrations)			
	2%	1%	0.5%	Control
Response index	0.21	0.26	0.46	0.08
Change compared to control (%)	12.6	17.7	37.7	-
	Essential oils (pure)			
	Eucalyptus	Fennel	Anise	Control
Response index	0.49	0.29	0.03	0.19
Change compared to control (%)	30.0	10.6	-15.4	-
	Eucalyptus oil (concentrations)			
	50%	25%	10%	Control
Response index	0.49	0.26	0.17	0.08
Change compared to control (%)	40.7	17.7	8.3	-
	Different substances			
	n-Butanol (50%)	Acetic acid (0.5%)	Ammonia (5%)	Eucalyptus oil (50%)
Response index	0.46	0.25	0.16	0.13

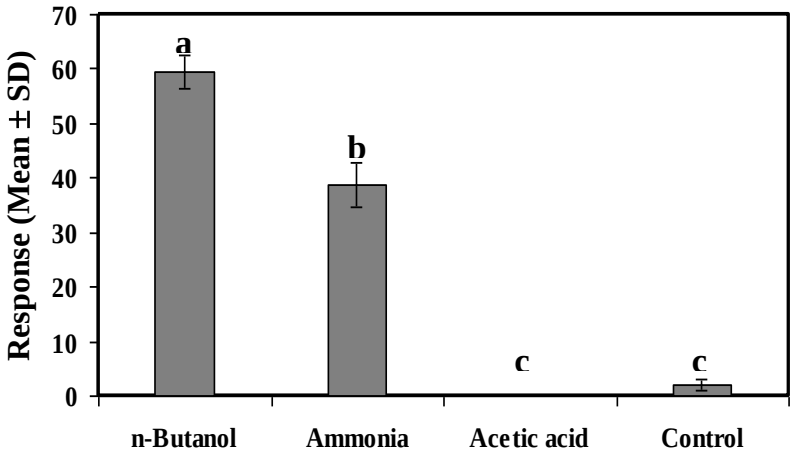


Fig.(2): Attractive effect of n-butanol, ammonia and acetic acid on response of oriental hornets. Treatments followed by the same letters are not significantly different at 0.05 level of probability, Vertical lines denote standard errors.

In the simultaneous choice test among three concentrations (100, 50, 25%) of n-butanol compared to control, 55.7, 24.7, 17.3 and 2.3% hornets were found in the fields of 50, 100, 25% concent-rations and control, respectively, inducing 0.56, 0.25, 0.17 and 0.02 response index, respect-ively. These results indic-ate that the concentration of 50% n-butanol only gave the highest signi-ficantly attractiveness (55.7 %) to hornets as compared with the others which were unaffected (Table 1 and Fig. 3).

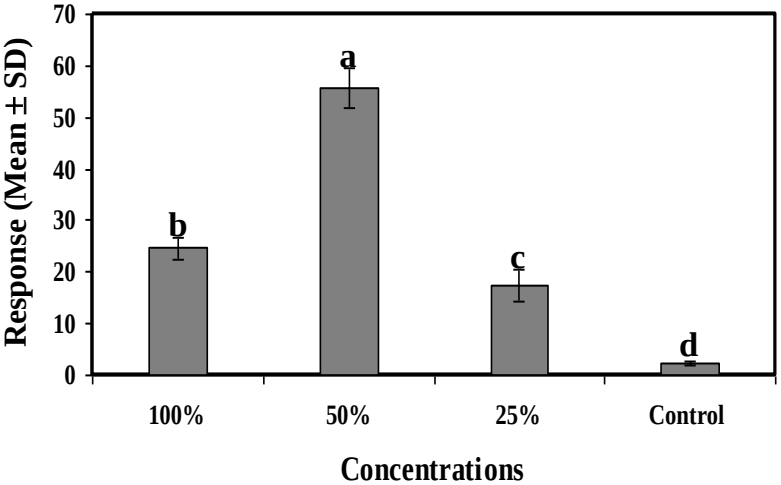


Fig.(3): Attractive effect of different concentrations of n-butanol on response of oriental hornets. Treatments followed by the same letters are not significantly different at 0.05 level of probability, Vertical lines denote standard errors.

The tests with three concentrations (25, 10, 5%) of ammonia compared to control, showed the following results: the concentration of 5% of the tested substances was highly attractive (0.43 response index) at a very high significance level. Whereas, the 10% concentration gave moderate attractiveness (0.30 response index) to hornets as compared to others (Table 1 and Fig. 4).

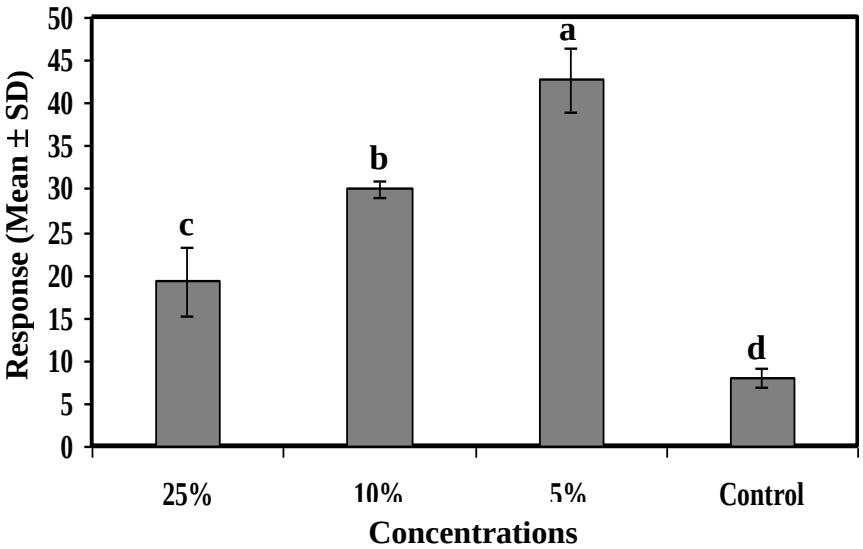


Fig. (4): Attractive effect of different concentrations of ammonia on response of oriental hornets. Treatments followed by the same letters are not significantly different at 0.05 level of probability, Vertical lines denote standard errors.

In case of using the acetic acid in the concentrations of 2, 1, 0.5% in choice test, the 0.5% concentration only indicated highly significant attractiveness to hornets as compared to others, inducing 0.46 response index (Table 1 and Fig. 5).

The combination of acetic acid and isobutanol was the first chemical attractant found to be useful for traping European hornet (Landolt, 1998) and the first chemical attractant for any species of *Polistes* (Landolt, 1999). Also, this combination was reported to be attractive to different species of Vespidae in different areas of the United States (Landolt *et al*., 1999). Nineteen compounds with three to six carbon chains or branched chains and with a hydroxy functional group were selected for testing as baits for trapping the different types of hornets. Based on their similarity to isobutanol with

acetic acid, which is a known hornet attractant, traps baited with the racemic mixture of 2-methyl-1-butanol in combination with acetic acid captured similar numbers of hornets compared with isobutanol with acetic acid (Landolt *et al*., 2000).

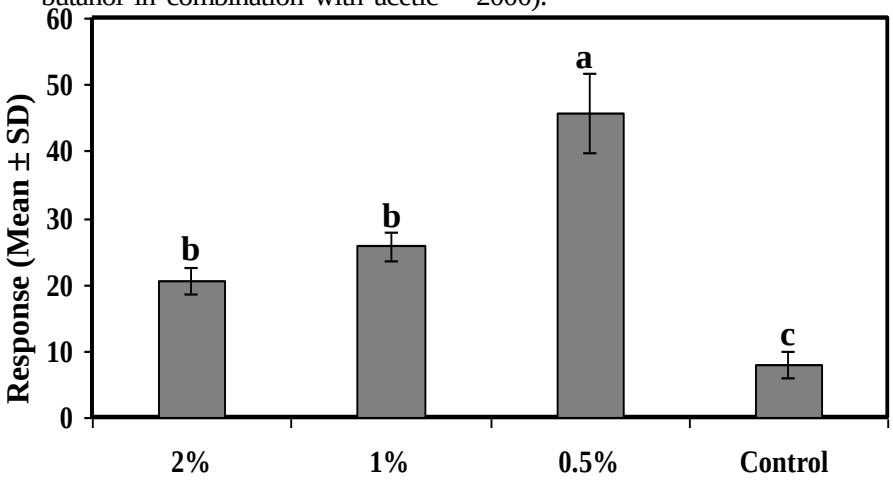


Fig. (5): Attractive effect of different concentrations of acetic acid on response of oriental hornets. Treatments followed by the same letters are not significantly different at 0.05 level of probability, Vertical lines denote standard errors.

A wide variety of food baits are attractive to some hornet species Spurr (1995, 1996), indicating strong chemotaxis response of hornets to food materials. Heptyl butyrate and octyl butyrate are strongly attractive to some of *Vespa* spp. Gomaa and Abd El-Wahab (2006) studied the efficiency of various concentrations of liquid culture of yeast, *Candida tropicalis* (25, 50 and 100%) as an attractive agent and bait to the hornets. The traps baited with 100% captured the highest numbers of the hornet, whereas the application of 25% of yeast recorded the lowest captured hornets in the traps.

This chemical blend is probably a feeding attractant because the compounds were isolated from fermented molasses solutions which are attractive to many insects (Landolt, 1995), including Vespi-dae (Thomas, 1960). Various fruits juices and syrups were tested as feeding attractants for oriental hornet, for use in bait traps. Grape juice, fermented for 3 days gave the most favourable results (Lim *et al*., 1989). Shoreit (1998) used fermen-ted honey or sugar syrup in traps to control hornets in apiaries at Cairo and Assiut. Several authors reported capturing the hornets when used organic chemicals or fruit baits in the traps (Aihara, 1980 and Mishra *et al*., 1989).

Essential oils:

In the four-way simultaneous choice test with essential oils, eucalyptus, fennel and anise in their pure forms, the results indicated that the most significantly attractive effect (48.7%) was caused by eucalyptus oil, resulting 0.49 response index. Followed by fennel oil (0.29), whereas no clear affect was found for both anise and control (without oils) (Table 1 and Fig. 6).

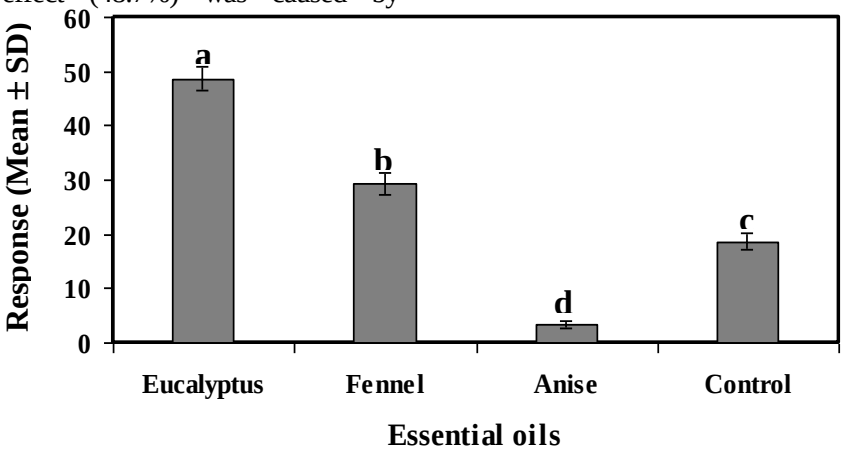


Fig. (6): Attractive effect of different essential oils on response of oriental hornets. Treatments followed by the same letters are not significantly different at 0.05 level of probability, Vertical lines denote standard errors.

Using the concentrations of 50, 25 and 10% of eucalyptus oil in choice test resulted in the highest signify-cant attractiveness value that was 49%, inducing 0.49 response index by the 50% concentration (Table 1 and Fig. 7).

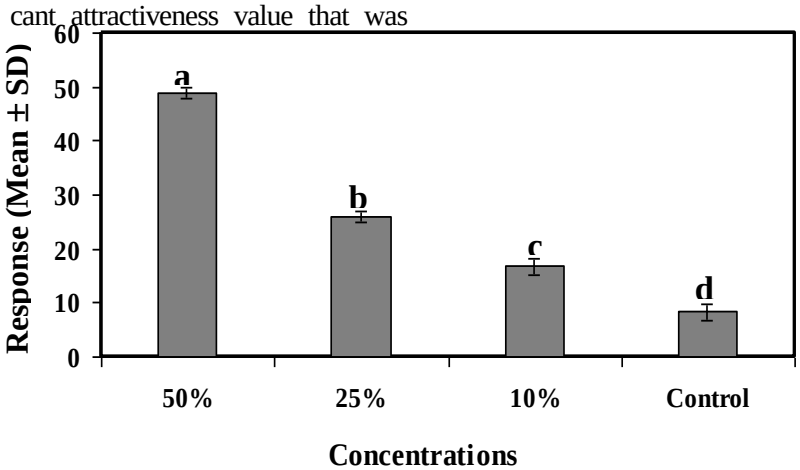


Fig.(7): Attractive effect of different concentrations of eucalyptus oil on response of oriental hornets. Treatments followed by the same letters are not significantly different at 0.05 level of probability, Vertical lines denote standard errors.

The test with the highest attractive concentrations of the tested substances, n-butanol (50%), acetic acid (0.5%), ammonia (5%) and eucalyptus oil (50%) showed that the n-butanol was the highly attractive to oriental hornet (45.7%) at all, resulting in a 0.46 response index as compared with the other substances (Table 1 and Fig. 8).

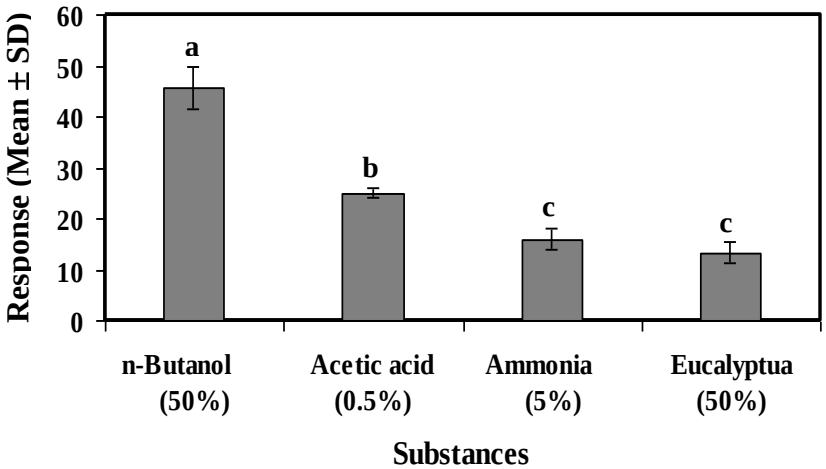


Fig.(8): Attractive effect of the highest effective concentrations of different substances on response of oriental hornets. Treatments followed by the same letters are not significantly different at 0.05 level of probability, Vertical lines denote standard errors.

These results are confirmed by Abo-Sheaesha and Serag El-Dien (2004) tested the volatiles, citronellol, linalool and geraniol as attractive agents to oriental hornet. They found that the hornets were captured as the highest numbers in traps lured by saturated sugar syrup, particularly when mixed with paraffin oil. The tested volatiles attracted the hornet where citronellol was the most efficient one.However, these volatile usually became more efficient when provided with paraffin oil. The tested volatile oils proved to attract the hornet to some extent; since the superior one was citronellol, followed by geraniol and then linalool. The difference may be due to the modifications in behavioral response of the oriental hornets to these compound according to their chemical structure and physical properties (Schoonhoven, 1992). There are other different factors effect on the attractiveness of oriental hornets such as weather factors, seasonal variation, the orientations, colours and others. Klein and Adler (1996) found that baiting the traps with meat can improve the capability of the traps in capturing the hornets.

Based on the present findings and previous information, it can be concluded that using a new technique with four to eight-way design to test four to eight substances in simultaneous choice test, gave a wide choice to test different substances towards the hornets and wasps in the same time. It can be concluded also that using n-butanol (50%), acetic acid (0.5%), ammonia (5%) and eucalyptus oil (50%) alone or in a combination play an important role as baits agent to improve the trapping of oriental hornets with several other factors. The behavioral chemotaxis responsiveness of oriental hornet to these attractant substances suggest their usage as a good baits for trapping the different types of hornets.

References

Abo-Sheaesha, M.A. and F.S. Serag El-Dien. 2004. Attractiveness of volatile oils and paraffin oil to the oriental wasp (*Vespa orientalis* Fab.) attacking apiaries. J. Agric. Sci. Mansoura Univ., 29: 417-423.

Abrol, D.P. 1994. Ecology, behavior and management of social wasp *Vespa vulutina* Smith (Hymenoptera: Vespidae), attacing honey bee colonies. Korean J. Apic., 9: 5-10.

Aihara, S. 1980. Control of gaint hornets by mixture of honey and agrichemical. Honey bee Science, 1: 23-24.

Caron, D.M. 1986. Social wasps as bee pests. American Bee Journal, 126: 269-271.

Gomaa, A.M. and T.E. Abd El-Wahab. 2006. Seasonal abundance and the efficiency of yeast liquid culture (*Candida trapicalis*) as bait for capturing the oriental wasps (*Vespa orientalis* L.) under Egyptian environment. J. Appl. Sci. Res. 2: 1042-1046.

Ibrahim, M.M. and M.M. Mazeed. 1967. Studies on the oriental hornet, *Vespa orientalis* F. Agric. Res. Rev., Cairo, 45: 163-180.

Khater, A.M., I.M.A. Ebadah and S.I. Yousif-Khalil. 2001. The seasonal activity of oriental wasps, *Vespa orientalis* L. populations attacking honey bee colonies. Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 9: 447-455.

Klein, Z. and H. Adler. 1996. Wasps and their control in Israel.Abstracts of papers presented at the Joint International Conference of FAOPMACEPA on pest control in the 21st Century. Tel. Aviv. Israel.

Landolt, P.J. 1995. Attraction of *Mocis latipes* (Lepodoptera: Noctuidae) to sweet baits in traps. Florida Entomol., 78: 523-530.

Landolt, P.J. 1998. Chemical attarctants for trapping yellow jackets *Vespula germanica* and *Vespula pensylvanica* (Hymenotpera: Vespidae). Env-iron. Entomol., 27: 1229-1234.

Landolt, P.J. 1999. A chemical attractant for the golden paper

wasp, *Polistes aurifer* Saussure (Hymenoptera: Vespidae). J. Kansas Entomol. Soc., 71: 69-73.

Landolt, P.J., C.S. Smithhisler, H.C. Reed and L.M. McDonough. 2000. Trapping social wasps (Hymenoptera: Vespidae) with acetic acid and saturated short chain alcohols. Bio One Online Journals, 93: 1613-1618.

Landolt, P.J., H.C. Reed, J.R. Aldrich, A.L. Antonelli and C. Dickey. 1999. Social wasps (Hymenoptera: Vespidae) trapped with acetic acid and isobutanol.Florida Entomol., 82: 609-614.

Lim, B.H., M.L. Lee and K.S. Woo. 1989. Studies on the control of hornets (*Vespa* spp.) by means of feeding attractants. Korean J. Apic. 4: 19-33.

Matheson, A., K. Clapperton, H. Moller and P. Alspach. 1989. The impact of wasps on New Zealand bee-keeping – the 1986/1987 wasps survey. New Zealand Beekeeper, 203: 28-31.

Mishra, R.C., J. Kumar and J.K. Gupta. 1989. A new approach to the control of predatory wasps (*Vespa* spp.) of the honey bee (*Apis mellifera* L.). J. apic. Res., 28: 126-130.

SAS Institute (1990). SAS/STAT. User's Guide: Release 6.04. SAS Institute, Inc., Cary, N.C.

Schoonhoven, L.M. 1992. Insect-Plant interactions. IV; 7-77, CRC Press Boca Raton Ann Arbor London Tokyo.

Shoreit, M.N. 1998. Field observations on the seasonal abundance and control of *Vespa orientalis* F. attacking honey bee colonies in Egypt. J. Appl. Sci., 13: 250-256.

Sihag, R.C. 1992. The yellow banded brown wasp, *Vespa orientalis* L. 1- A predator and colony robber of honey bee (*Apis mellifera* L.). Korean J. Apic., 7: 32-34.

Spurr, E.B. 1995. Protein bait preferences of wasps (*Vespula vulgaris* and *V. germanica*) at Mt Thomas, Canterbury, New Zealand. New Zealand J. Zool. 22: 281-289.

Spurr, E.B. 1996. Carbohydrate bait preferences of wasps (*Vespula vulgaris* and *V. germanica*) (Hymenoptera: Vespidae) in New Zealand. New Zealand J. Zool. 23: 315-324.

Thomas, C.R. 1960. The European wasp (*Vespula germanica* Fab.) in New Zealand. New Zealand Department of Scientific and Industrial Research Information Series, 27: 74 pp.

Wafa, A.K., F.F. El-Borolossy and S.G. Charkawi. 1968. Studies on *Vespa orientalis* F. Bull. Ent. Soc. Egypt, Econ. Ser, 2: 9-27.

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تصميم جديد لإمكانية الاختبار المتزامن لدراسة إستجابة الدبور الشرقى لبعض المواد الطبيعية تحت ظروف المعمل

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أجريت هذه الدراسة بمنحل قسم وقاية النبات بكلية الزراعة جامعة أسيوط خلال ذروة نشاط الدبور الشرقى فى الفترة من الأول من شهر سبتمبر إلى نهاية أكتوبر 2006م . وذلك لغرض استخدام تكنيك جديد لإمكانية الاختبار المتزامن لدراسة استجابة الدبور الشرقى لبعض المواد الطبيعية تحت ظروف المعمل . وكانت المواد المستخدمة بتركيزاتها المختلفة هى : كحول البيوتانول (100 ، 50، 25%) وحامض الخليك الثلجى (100 ، 2 ، 1 ، 0.5%) والأمونيا (100 ، 25 ، 10 ، 5%) والزيوت العطرية الكافور (100 ، 50 ، 25 ، 10%) وزيت الشمر وزيت الينسون (100%). هذا وقد أوضحت النتائج أنه فى حالة استخدام كحول البيوتانول وحامض الخليك والأمونيا فى صورة مركزة وجد أن كحول البيوتانول أعطى أعلى تأثيراً جاذباً للدبور الشرقى (59.3%) مسجلاً قيمة 0.59 لدليل الإستجابة المقترح مقارنة بالآخرين. فى حالة إختبار ثلاث تركيزات (100، 50، 25%) من كحول البيوتانول ، أحدث التركيز 50% أعلى تأثير جاذب (55.7%) ، مسجلاً 0.56 دليل إستجابة وذلك بالمقارنة بالآخرين والتى كانت جاذبة بصورة قليلة . وأحدث تركيز 5% من الأمونيا أعلى تأثيراً جاذباً (42.7%) وذلك مقارنة بالتركيزات الأخرى (25، 10%) والمقارنة ، مسجلاً 0.43 دليل استجابة . أما فى حالة استخدام تركيز 0.5% من حامض الخليك الثلجى فإنه أحدث أعلى معدل جذب للدبور (45.7%) مقارنة بتركيزات الحامض الأخرى، مسجلاً 0.46 دليل إستجابة. وفى حالة إختبار زيوت الكافور والشمر والينسون فى صورتها المركزة أظهرت النتائج أن زيت الكافورأعطى أعلى معدل لجذب الدبور (48.7%) ، بينما فى حالة استخدام تركيزات 50 ، 25، 10% لزيت الكافور ، أحدث التركيز 50% أعلى معدل جذب (49%). بينما فى حالة إختبار تركيزات المواد المختلفة التى أنتجت أعلى تأثير جاذب للدبور وهى: كحول البيوتانول (50%) ، حامض الخليك (0.5%) ، الأمونيا (5%) وزيت الكافور (50%)، أظهرت النتائج أن كحول البيوتانول بتركيز 50% أحدث أعلى معدل جذب على الإطلاق للدبور الشرقى (45.7%) مسجلاً 0.46 دليل استجابة ، مقارنة بجميع المواد بتركيزاتها المختلفة.