EFFICACY OF SOME REPELLENT CHEMICALS AGAINST TWO HONEYBEE SPECIES, *Apis mellifera* L. AND *Apis florea* F. IN FIELD TRIALS

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Summary

Fifteen chemicals belonging to three different groups, viz., phenols, aldehydes and ketones at 0.5% concentration were tested for 3 hours for their repellency against two honeybee species viz. *Apis mellifera* and *Apis florea* under field conditions on mustard crop (*Brassica juncea*). Out of these, only three chemicals viz. p-bromophenol, m-bromoacetophenone and 3, 4, 5-trimethoxyacetophenone showed the repellency close to the desired level of 80% for 3 hours against both the honeybee species. These chemicals are recommended for their use as repellents in the fields for the safety of honeybees against pesticides.

Keywords: *Apis florea*, *Apis mellifera*, pollination, pesticides, repellents.

INTRODUCTION

Honeybees are one of the excellent pollinators of several crops and help increase the seed/fruit yield of several entomophilous crops (McGregor, 1976; Sihag, 1986, 2001; Free, 1993). But, the crops are treated with pesticides to protect them from pests and diseases (Hill and Waller, 1982; Fenemore, 1984). These pesticides cause large scale honeybee mortality (Johansen, 1977). As a consequence, the poor beekeepers have to lose their colonies and the crop growers their pollinating agents (Kevan, 1975; Johansen, 1977). The economic losses in terms of decreased crop yields due to the mortality of honeybees are enormous (Kevan and Collins, 1974; Kevan, 1977; Kevan and LaBerge, 1979; Kevan and Opperman, 1980; Sihag, 1988). Use of repellent chemicals is one of the alternatives to provide functional protection to honeybees against pesticides under field conditions.

A repellent is important as a means of making sure the bees avoid an area with pesticides. When the area is safer for the bees to return the repellent will have worn off. This is because, the repellents are highly volatile chemicals and their effect is short lived (Laere and Gillard, 1960). That is why several essential oils have been identified as efficient repellents and included as a part of integrated pest management (Wood et al., 1970). O-aminoacetophenon is a compound produced by the virgin queen honeybee and is released with faeces. It has been shown to repel bees (Page et al., 1988). Oil of bitter almond has also been reported to be effective repellent (Collins and Hellmich, 1988). Henning et al. (1992) indicated methyl salicylate and 3-octanone as good repellents. Several chemicals have exhibited repellent effect in small concentration in the laboratory, but none has been found to be of practical use in the field (Harpaz and Lensky, 1959). A repellent can be considered effective if it repels ≥ 80% of honeybees from the pesticide treated fields (Sihag, 2008). In our earlier study, we tested 25 chemicals for their repellency against two honeybees (i. e. *Apis mellifera* and *Apis florea*) under semi-field conditions (Mishra and Sihag, 2009). Out of these 25 chemicals, 15 chemicals at 0.5% concentration were effective in showing repellency ≥ 80% against these two honeybee species. However, their repellency under field conditions has not been tested. The aim of this study is to test the efficacy of these 15 chemicals for their repellency against two honeybee species under field conditions.

MATERIAL AND METHODS

These experiments were carried out at the Research Farm of CCS Haryana Agricultural University, Hisar (India). Fifteen chemical compounds which showed 95% repellency at 0.5% concentration in the semi-field trials (Mishra and Sihag, 2009) were tested for their efficacy directly in the field on mustard crop (*Brassica juncea*) against two honeybee species i.e. i) *Apis mellifera* - a domesticated species used for honey production and managed pollination of crops (Sihag, 1989, 1993, 1997) and ii) *Apis florea* - a wild species and an excellent pollinator of many crops (Sihag, 2000). Six, 1 x 1 m randomly selected plots were marked for each chemical when the crop was in full bloom during December / January. In order to avoid the mixing up of different chemical vapours, a minimum distance of 3 m was kept between any two plots. Three plots were sprayed uniformly with a 100 ml homogenized chemical water (CW) solution using a 1 liter hand spray pump. The other three plots were sprayed with the same quantity of distilled water (DW) taken as control. The number of honeybees of *Apis mellifera* and *Apis florea* visiting each plot for a five minute duration were counted at hourly intervals. The experiment was started at 11:00 a.m. and continued until 2:00 p.m. The % repellency was calculated by using the following mathematical model suggested by Sihag (2008):

$$\%R = \left[1 - \frac{n_1}{n_2}\right] \times 100$$

where:
R = Repellency
n_1= Number of bees on repellent treated plots
n_2 = Number of bees on control plot

The data so recorded for all the experiments were subjected to angular transformation. To compare the means of different treatments these data were analyzed in Completely Randomized Design following Snedecor and Cochran (1989).

RESULTS

The results of 15 chemicals screened for their repellency at 0.5% concentration against two honeybee species viz. *Apis mellifera* and *Apis florea* are presented in Tab. 1-6. All the chemicals at this concentration showed 100% repellency immediately after their spray (i.e. at 0 h), as not even a single bee visited the treated plots at this stage. After some time, however, there was a decline in the repellency of all the chemicals and subsequent trends were variable for each group of chemicals. For proper comparisons of biological response, therefore, each group of chemicals has been dealt with separately as under:

Table 1

Periodic level of repellency of different phenols against *Apis mellifera* under field conditions

Chemicals	Per cent repellency for different durations (h) of treatments*			
	0	1	2	3
o-Cresol	100.00 (84.23)	80.90 (64.13)	77.65 (61.76)	72.07 (58.07)
p- Cresol	100.00 (84.23)	79.75 (73.30)	75.37 (60.22)	67.44 (55.18)
m- Cresol	100.00 (84.23)	77.63 (61.76)	73.97 (59.31)	66.64 (54.69)
o-Ethoxyphenol	100.00 (84.23)	85.57 (67.67)	80.59 (63.85)	72.40 (58.30)
p-Bromophenol	100.00 (84.23)	91.64 (73.28)	84.90 (67.11)	79.90 (63.13)
CD ($P \leq 0.05$)	Chemical = 3.06, Time duration = 2.74, Chemical × Time duration = 6.12			

*Mean of three replications. Figures within the parentheses are transformed values (angular transformation)

Table 2

Periodic level of repellency of different phenols against *Apis florea* under field conditions

Chemicals	Per cent repellency for different durations (h) of treatments*			
	0	1	2	3
o-Cresol	100.00 (84.23)	81.67 (64.66)	75.57 (60.37)	72.76 (58.53)
p- Cresol	100.00 (84.23)	79.68 (63.19)	74.24 (59.48)	68.67 (55.94)
m- Cresol	100.00 (84.23)	79.16 (62.82)	71.70 (57.84)	68.13 (55.63)
o-Ethoxyphenol	100.00 (84.23)	86.36 (68.35)	81.36 (64.43)	74.93 (59.57)
p-Bromophenol	100.00 (84.23)	92.18 (73.84)	83.48 (66.03)	79.79 (63.26)
CD ($P \leq 0.05$)	Chemical = 3.03 , Time duration = 2.71 Chemical × Time duration = Non-significant			

*Mean of three replications. Figures within the parentheses are transformed values (angular transformation)

Group I: Phenols

After one hour of chemical spray, three phenols showed the desired repellency (of 80% and above) against both the honeybees (Tab. 1, 2). These were: p-bromophenol (with 91.64% repellency), o-ethoxyphenol (85.57%) and o-cresol (80.90%). Two phenols viz. p-cresol (79.75%) and m-cresol (77.63%), however, failed to show the desired repellency of ≥ 80% (Tab. 1, 2). Two hours after spraying, p-bromophenol (84.90% repellency) and o-ethoxyphenol (80.59% repellency) were found to show the desired repellency of ≥ 80%. With a further lapse of time i.e. after 3 hours, none of the tested phenols could show the desired repellency, although the repellency of p-bromophenol (79.90 and 79.79% against two honeybees, respectively) was very close to the desired repellency of 80% and statistically similar to this ($p \leq 0.05$, ANOVA).

Group II: Aldehydes

The repellent chemicals, phenylacetaldehyde and 4-nitrobenzaldehyde showed the desired repellency of ≥ 80% (91.21 and 89.0%, respectively, Tab. 3, 4) one hour after they were sprayed. However, the other three aldehydes i.e. 3-methoxybenzaldehyde, 2-methoxybenzaldehyde and 2, 4-dimethoxybenzaldehyde failed to show the desired repellency. Only phenylacetaldehyde showed the desired repellency (82.84% repellency) two hours after it was sprayed to repel *A mellifera.* Activity shown by 4- nitrobenzaldehyde (79.60%) was, however, very close to the desired threshold level of 80% (Tab. 3). On the other hand, both these chemicals showed the desired repellency against *A. floea* 2 hours after they were sprayed (Tab. 4). The other three aldehydes i.e. 3-methoxybenzaldehyde (76.52%

Table 3

Periodic level of repellency of different aldehydes against *Apis mellifera* under field conditions

Chemicals	Per cent repellency for different durations (h) of treatments*			
	0	1	2	3
2-Methoxybenzaldehyde	100.00 (84.23)	76.71 (61.13)	72.61 (58.46)	66.40 (54.56)
3-Methoxybenzaldehyde	100.00 (84.23)	77.71 (61.82)	76.52 (61.01)	70.80 (57.26)
2,4-Dimethoxybenzaldehyde	100.00 (84.23)	74.20 (59.46)	71.77 (57.89)	67.88 (55.45)
4-Nitrobenzaldehyde	100.00 (84.23)	89.00 (70.86)	79.60 (62.66)	72.52 (58.37)
Phenylacetaldehyde	100.00 (84.23)	91.21 (72.91)	82.84 (65.54)	78.06 (62.11)
CD ($P \leq 0.05$)	Chemical = 2.81, Time duration = 2.51 Chemical × Time duration = Non-significant			

*Mean of three replications. Figures within the parentheses are transformed values (angular transformation)

Table 4

Periodic level of repellency of different aldehydes against *Apis florea* under field conditions

Chemicals	Per cent repellency for different durations (h) of treatments*			
	0	1	2	3
2-Methoxybenzaldehyde	100.00 (84.23)	75.49 (60.32)	72.13 (58.14)	66.02 (54.32)
3-Methoxybenzaldehyde	100.00 (84.23)	77.77 (61.84)	73.64 (59.08)	70.75 (57.23)
2,4-Dimethoxybenzaldehyde	100.00 (84.23)	75.37 (60.23)	73.61 (59.09)	69.11 (56.22)
4-Nitrobenzaldehyde	100.00 (84.23)	90.57 (72.18)	81.13 (64.26)	73.49 (59.01)
Phenylacetaldehyde	100.00 (84.23)	90.83 (72.49)	81.73 (64.71)	76.17 (60.77)
CD (P≤ 0.05)	Chemical = 2.83 , Time duration = 2.53 Chemical × Time duration = 5.67			

*Mean of three replications. Figures within the parentheses are transformed values (angular transformation)

repellency), 2-methoxybenzaldehyde (72.61% repellency) and 2, 4-dimethoxybenzaldehyde (71.77% repellency) did not show the desired repellency of ≥ 80% two hours after they were sprayed. After 3 hours, none of the tested aldehyde chemicals showed the desired repellency of ≥ 80% (Tab. 3, 4).

Group III: Ketones

One hour after all the tested ketones were sprayed, they showed the desired repellency of ≥ 80% (o-hydroxypropiophenone showed 79.26 and 78.66% repellency against *A. mellifera* and *A florae* respectively which were statistically not significantly different from the desired repellency of 80%; respective C.D. (p ≤ 0.05) = 3.24 and 3.30) ; m-bromoacetophenone showed the maximum repellency followed by 1-acetonaphthone, p-ethoxyacetophenone and 3,4,5-trimethoxyacetophenone (Tab. 5,6). Two hours after they were sprayed, only two ketones were found to show the desired repellency i.e. m-bromoacetophenone and 3, 4, 5-trimethoxyacetophenone (83.38% and 83.88%) against both the species. However, p-ethoxyacetophenone showed 78.11% repellency against *A. mellifera* and 80.99% repellency against *A. florea* and had statistically similar effects (Tab. 5, 6). Other two ketones (i.e. 1-Acetonaphthone and o-hydroxypropiophenone) did not show the desired repellency of ≥ 80% and thus were not considered to be effective 2 hours after they had been sprayed (Tab. 5, 6). Three hours after the spraying, none of the tested chemicals were found to show the desired 80% repellency; m-bromoacetophenone and 3, 4, 5-trimethoxyacetophenone, however, still showed a 78% repellency making them statistically similar to the desired repellency of 80% (p ≤ 0.05, ANOVA).

From the foregoing text it is clear that one phenol (i.e. p-bromophenol) and two ketones (i.e. m-bromoacetophenone and

Table 5

Periodic level of repellency of different ketones against *Apis mellifera* under field conditions

Chemicals	Per cent repellency for different durations (h) of treatments*			
	0	1	2	3
m-Bromoacetophenone	100.00 (84.23)	91.64 (73.28)	83.38 (65.93)	79.86 (62.76)
o-Hydroxypropiophenone	100.00 (84.23)	79.26 (62.53)	69.48 (56.47)	62.71 (52.36)
p-Ethoxyacetophenone	100.00 (84.23)	87.70 (70.16)	78.11 (62.11)	71.68 (57.87)
3,4,5-Trimethoxy- acetophenone	100.00 (84.23)	85.51 (67.65)	83.88 (66.32)	78.33 (62.22)
1-Acetonaphthone	100.00 (84.23)	89.65 (71.31)	74.02 (59.35)	70.06 (56.82)
CD ($P \leq 0.05$)	Chemical = 3.24, Time duration = 2.90 Chemical × Time duration = Non-significant			

*Mean of three replications. Figures within the parentheses are transformed values (angular transformation)

Table 6

Periodic level of repellency of different ketones against *Apis florea* under field conditions

Chemicals	Per cent repellency for different durations (h) of treatments*			
	0	1	2	3
m-Bromoacetophenone	100.00 (84.23)	92.75 (73.12)	83.92 (66.38)	78.02 (62.35)
o-Hydroxypropiophenone	100.00 (84.23)	78.66 (62.45)	65.01 (53.71)	58.33 (49.79)
p-Ethoxyacetophenone	100.00 (84.23)	87.01 (68.94)	80.99 (64.22)	73.48 (59.00)
3,4,5-Trimethoxy- acetophenone	100.00 (84.23)	86.74 (68.68)	83.38 (65.93)	78.47 (62.37)
1-Acetonaphthone	100.00 (84.23)	89.34 (71.01)	73.15 (58.78)	70.88 (57.33)
CD ($P \leq 0.05$)	Chemical = 3.30, Time duration = 2.95 Chemical × Time duration = Non-significant			

*Mean of three replications. Figures within the parentheses are transformed values (angular transformation)

3,4,5-trimethoxy acetophenone) showed the repellency close to the desired value of ≥ 80% up to 3 hours after their application in a mustard field. These three chemicals were, therefore, considered to qualify as effective. This is because, in all the cases, the values of C.D. for the chemicals was > 2.8 (Tab. 1-6), and a difference of 78 and 80% repellency is statistically non-significant. Under field conditions, all other tested chemicals failed to reach this threshold and, therefore, proved to be ineffective.

DISCUSSION

The efficacy of aliphatic straight chain ketones as bee repellents was assessed by some earlier researchers (Ahlawat et al., 1997). They observed that ethyl propyl ketone and ethylbutyl ketone showed repellent activity for 5 hours and ethylpentyl ketone for 4 hours. Four carbonyl compounds at a 0.5% concentration as repellents to *Apis florea* under field conditions were also tested (Singh et al., 1993). Out of these, acetylacetone exhibited maximum repellency for 4 hours. But, the earlier reseachers used an erroneous model to determine the repellency of those chemicals. They took 60% repellency as the lowest threshold of efficacy. That repellency was, in fact, equal to 20% if derived in the new model (Sihag, 2008). In the present studies, 80% repellency has been taken as the lowest threshold of an effective level (Sihag, 2008; Mishra and Sihag, 2009). This difference in the threshold levels of repellency derived from the two models has excluded many of the earlier recommended chemicals from the list of effective and potential bee repellents. Besides this, all the chemicals earlier recommended were too expensive for poor, small, and marginal farmers (Sihag, 1995). Earlier recommendations made on the repellent chemicals were neither economically viable nor ecologically feasible and were rejected.

The present experiments were performed using a 0.5% concentration of the chemicals. In our earlier studies carried out under semi-field conditions, this was the maximum concentration which was tested under semi-field conditions. In our earlier study, all these 15 chemicals showed desirable repellency (Mishra and Sihag, 2009). In this study also, the instant repellency of all these chemicals was 100% (Tab. 1-6). However, there was a variable temporal decline in the repellency depending upon the nature of the chemical. A direct relationship between the molecular weight of the chemical and its repellency seemed to prevail. In general those chemicals showing greater persistence had a higher molecular weight than the others (Mishra and Sihag, 2009).

Out of fifteen chemicals tested at a 0.5% concentration in the fields for their repellency against two honeybee species, only three chemicals i.e. p-bromophenol, m-bromoacetophenone and 3, 4, 5-trimethoxyacetophenone showed > 78% repellency (close to the desired 80% repellency) for the three hours after their being sprayed in the fields. This three hour duration has great significance as far as the safety of bees in the pesticide treated fields is concerned. This duration can be very effective for the protection of the bees if the pesticide sprays are made in the evening when there is the least amount of bee activity (Sihag and Rathi, 1995). Therefore, these chemicals have been characterized as the potential and effective honeybee repellents. These chemicals are cheap and readily available. Even the small and marginal farmers can afford them. Therefore, their use in the field is recommended for the safety of honeybees against pesticides.

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ANALIZA WYDAJNOŚCI WYBRANYCH ŚRODKÓW CHEMICZNYCH ODSTRASZAJĄCYCH DWA GATUNKI PSZCZÓŁ *Apis mellifera* L. I *Apis florea* F. W DOŚWIADCZENIACH POLOWYCH

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Streszczenie

Piętnaście repelentów należących do trzech różnych grup, tj. fenole, aldehydy i ketony, zastosowanych w stężeniu 0,5% przez 3 godziny badano w aspekcie ich właściwości odstraszających w stosunku to dwóch gatunków pszczoły: *Apis mellifera* i *Apis florea*, w warunkach polowej uprawy gorczycy sarepskiej (*Brassica juncea*). Spośród badanych repelentów, jedynie trzy - tj. p-bromofenol, m-bromoacetofenon oraz 3, 4, 5-trimetoksyacetofenon, wykazywały właściwości odstraszające bliskie pożądanemu poziomowi 80% przez 3 godziny wobec obu analizowanych gatunków pszczół. Na podstawie uzyskanych wyników można rekomendować te środki jako repelenty stosowane na polach w celu ochrony pszczół miodnych przed działaniem pestycydów.

Słowa kluczowe: *Apis florea*, *Apis mellifera*, zapylanie, pestycydy, repelenty.