EFFICACY OF SOME CHEMICALS AS REPELLENTS AGAINST TWO HONEY BEE SPECIES, *Apis mellifera* L. AND *Apis florea* F. IN SEMI-FIELD TRIALS

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Summary

The objective of this research was to devise methods to provide functional protection to the honeybees in pesticide treated fields. Twenty five chemical compounds belonging to these five different groups - amine, ester, phenol, aldehyde and ketone, at six different concentration levels ranging from 0.05 to 0.5% were tested for their repellency. They were tested for their repellency against the two honey bee species, *Apis mellifera* and *Apis florea* under semi-field conditions. Out of these, fifteen compounds belonging to three groups i.e. phenol, aldehyde and ketone exhibited the desired level of ≥ 80% repellency. Ketones showed desired repellency at even low concentrations followed by aldehydes and phenols. Among these fifteen compounds, p-ethoxyacetophenone, m-bromoacetophenone and 3, 4, 5-trimethoxyacetophenone at a 0.2% concentration; phenylacetaldehyde at a 0.3% concentration and; 4-nitrobenzaldehyde, p-bromophenol and p-cresol at a 0.4% concentration exhibited the desired level of 80% repellency.

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

INTRODUCTION

Honeybees are one of the most valuable pollinators and help yield gain in several entomophilous crops (McGregor 1976, Sihag 1986, Free 1993). Conversely, several pests and diseases cause severe damage to the crops resulting in heavy yield losses. About a 6-7 times higher seed yield was reported in crops protected from various pests and diseases than those where no such operations were applied (Sihag 1988). Use of pesticides is still the best recourse in plant protection, especially in the developing countries where the masses are still largely uneducated. However, the indiscriminate uses of pesticides cause large scale mortality of honeybees and other bees (Anderson et al. 1971, Johansen 1972, Kevan and Collins 1974, Kevan and Laberge 1979, Sihag and Rathi 1995). Most bees poisoning occurs when pesticides are applied to the crops during the blooming periods. An option to protect honeybees from the hazards of pesticides is to repel them from the fields during the period of pesticide activity. Alcohols, aldehydes, ketones, acids, acid anhydrides, amines and many essential oils have been tested as honey bee repellents (Bharadwaj 1974, Atkins et al. 1975a, b, Kumari 1976, Goyal 1977, Gupta 1985, 1987a, b, c, d, Gupta and Mohla 1986, Patyal and Kumar 1989, Rani 1989, Malhotra 1998 , Ahlawat et al. 1997, Kasana et al. 1997, 1998e, 2000a, b, Gill 2000). The problem is that

none of these chemicals are cheap or readily available and they are not stable as bee repellents. Moreover, their recommended concentrations have not proved effective in providing functional protection to honeybees in the pesticide treated fields. Keeping this in mind, the present work was formulated with the objective of testing the efficacy of some cheap and easily available chemicals as bee repellents. This was done for the two honeybee species, *Apis mellifera* L. and *Apis florea* F. under semi-field conditions.

MATERIAL AND METHODS

For the present investigations, a colony of *Apis mellifera* L. was brought and placed in the Horticulture Garden of CCS Haryana Agricultural University, Hisar. The bees were kept for the entire experimentation period. *Apis florea* F. colonies were brought from the wild to the

Table 1

Chemical compounds, their molecular formulae, boiling point and molecular weights.

S. No.	Compound	Molecular formulae	Boiling/Melting point (°C)	Molecular weight
Amines				
1	*o*-Chloroaniline	C_6H_6NCl	208-210	127.57
2	*p*-Chloroaniline	C_6H_6NCl	232	127.57
3	*m*-Anisidine	C_7H_9NO	251	123.20
4	2,4-Dinitroaniline	$C_6H_5N_3O_4$	176	183.12
5	Diphenylamine	$C_{12}H_{11}N$	302	169.23
Esters				
1	Ethyl 2-methylacetoacetate	$C_7H_{12}O_3$	187	144.17
2	Ethyl diacetoacetate	$C_8H_{12}O_4$	209-211	172.18
3	Ethyl benzoylacetate	$C_{11}H_{12}O_3$	265-270	192.21
4	Ethyl 4-chloroacetoacetate	$C_6H_9O_3Cl$	115	164.59
5	Allyl acetoacetate	$C_7H_{10}O_3$	194-195	142.16
Phenols				
1	*o*-Cresol	C_7H_8O	1 91	108.10
2	*m*-Cresol	C_7H_8O	203	108.10
3	*p*-Cresol	C_7H_8O	202	108.10
4	*o*-Ethoxyphenol	$C_8H_{10}O_2$	216-217	138.17
5	*p*-Bromophenol	C_6H_5OBr	235-236	173.01
Aldehydes				
1	2-Methoxybenzaldehyde	$C_8H_8O_2$	238	136.15
2	3-Methoxybenzaldehyde	$C_8H_8O_2$	143	136.15
3	2,4-Dimethoxybenzaldehyde	$C_9H_{10}O_3$	165	166.18
4	4-Nitrobenzaldehyde	$C_7H_5NO_3$	105-108	151.12
5	Phenylacetaldehyde	C_8H_8O	195	120.15
Ketones				
1	*m*-Bromoacetophenone	C_8H_7OBr	110	199.05
2	*o*-Hydroxypropiophenone	$C_9H_{10}O_2$	115	150.18
3	*p*-Ethoxyacetophenone	$C_{10}H_{12}O_2$	268-269	164.20
4	3, 4, 5-Trimethoxy-acetophenone	$C_{11}H_{14}O_4$	173-174	210.23
5	1-Acetonaphthone	$C_{12}H_{10}O$	302	170.21

Horticulture Garden of the University whenever required. Twenty five compounds belonging to five different groups; amine, ester, phenol, aldehyde and ketone were evaluated to determine their repellent properties (Table 1) at six concentration levels; 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5%. Each concentration was applied 3 times.

A 30% sugar-water (SW) solution was prepared by dissolving 30g of sugar in distilled water to reach a volume of 100ml. Concentrations of SW and of various compounds were based on a weight/volume proportion for solids and a volume/volume proportion for liquids. The SW solution served as the solvent for the preparation of various compound concentrations. For preparing the stock solution, one gram of chemical was dissolved in a minimum amount of acetone (1-10ml) and later on, the volume of 100ml was reached using SW as the diluent.

For training bees, a feeder made of a Plexiglas plate designed by Renner (1959) was filled with a 30% sugar solution (SW) and was laid on an inverted pot placed near the entrance gate of the colony. The feeder had radial slits of 10mm in length and 1mm in depth. Three feeding stations were placed 0.5 meter above the ground and 1 meter away from each other in a row. The bees drank solutions with ease from these channels. Once the bees started visiting this feeder, the distance between the colony and feeder was gradually increased to 10m. These trained bees were used for testing the repellency of various chemicals. The bees were offered a free choice of what to feed on. They could feed on a : 30% SW (control 1), acetone-sugar water (control 2) and the test chemical-sugar water solution. The position of the test chemical and sugar solution was interchanged after every observation recorded at 30 min intervals till the end of each experiment. The reason for this was so the bees would not get adapted to a particular kind of food (sugar water in this case).The number of bees visiting the feeding station was counted for 1 minute. The repellency% was calculated using the following formula modified from Sihag (2008):

$$\%R = [1 - \frac{3n_1}{(n_1 + n_2 + n_3)}] \times 100 \ldots\ldots (l)$$

Where,

R = Repellency

n_1 = Number of bees on chemical solution

n_2 = Number of bees on acetone solution

n_3 = Number of bees on sugar solution

The data recorded were subjected to angular transformation and were analyzed in a completely random manner in order to compare the means of the different treatments according to Snedecor and Cochran (1989). Earlier workers considered a 60% repellency of the chemical as its effective threshold which, in fact, is equal to 20% in the present formula. In former cases, 80% of the bees visiting the feeding station/field were at the risk of exposure to the pesticides. To minimize this risk, we have taken ≥ 80% repellency of the chemical as its effective threshold level. At this level, only 20% of the forager bees are expected to be under the risk of their exposure to the pesticides.

RESULTS

In order to properly compare the biological responses of the bees, each chemical group was dealt with separately for each species. The results obtained on two honey bee species are as follows:

Group I: Amines

Apis mellifera

At low concentrations, p-chloroaniline was found to be the most effective repellent against *Apis mellifera* among the tested amines and m-anisidine was the least effective; others showed an efficacy range

Table 2

Repellency of five amines at different concentrations against *Apis mellifera* under semi-field conditions.

Chemicals	Repellency under different concentrations(%)*					
	0.05	0.10	0.20	0.30	0.40	0.50
o-Chloroaniline	6.11 (13.94)	31.17 (33.90)	46.94 (43.22)	57.17 (49.11)	62.62 (52.30)	69.70 (56.58)
p-Chloroaniline	49.67 (44.79)	53.19 (46.81)	59.22 (50.29)	63.57 (52.88)	69.68 (56.58)	75.16 (60.10)
m-Anisidine	11.29 (19.42)	18.67 (25.51)	24.97 (29.91)	38.31 (38.19)	53.87 (47.21)	60.59 (51.10)
2,4-Dinitroaniline	28.30 (32.08)	29.17 (32.63)	42.44 (40.63)	56.22 (48.57)	69.58 (56.54)	72.70 (58.50)
Diphenylamine	27.33 (31.49)	32.56 (34.75)	50.62 (45.33)	60.52 (51.06)	65.16 (53.80)	72.03 (58.05)
CD ($P \leq 0.05$)	Chemical = (1.69), Concentration = (1.85), Chemical × Concentration = (4.13)					

* Mean of three replications
[Values within parenthesis are transformed values (angular transformation)]

in between these two compounds (Table 2). According to their repellency at lower concentrations, the order of efficacy of different amines was: p-chloroaniline = diphenylamine = 2,4-dinitroaniline> o-chloroaniline> m-anisidine. But, as the concentration was increased to 0.5, this order changed to: p-chloroaniline >

Table 3

Repellency of five amines at different concentrations against *Apis florea* under semi-field conditions.

Chemicals	Repellency under different concentrations (%)*					
	0.05	0.10	0.20	0.30	0.40	0.50
o-Chloroaniline	7.07 (15.31)	24.15 (29.30)	41.87 (40.29)	58.34 (49.78)	68.99 (56.14)	70.91 (57.34)
p-Chloroaniline	45.39 (42.33)	54.84 (47.75)	59.27 (50.33)	60.65 (51.13)	70.00 (56.77)	75.88 (60.61)
m-Anisidine	13.62 (21.58)	23.47 (28.94)	43.37 (41.07)	37.39 (37.58)	58.79 (50.04)	70.96 (57.39)
2,4-Dinitroaniline	28.37 (32.16)	41.14 (39.84)	59.65 (50.56)	69.95 (56.75)	71.81 (57.94)	76.65 (61.13)
Diphenylamine	29.53 (32.84)	31.96 (34.38)	44.83 (42.01)	58.54 (49.91)	69.34 (56.39)	72.85 (58.59)
CD ($P \leq 0.05$)	Chemical = (2.05), Concentration = (2.25), Chemical ×Concentration = (5.02)					

* Mean of three replications
[Values within parenthesis are transformed values (angular transformation)]

2,4-dinitroaniline > diphenylamine > o-chloroaniline > m-anisidine.

Apis florea

Unlike *A. mellifera*, here 2,4-dinitroaniline was the most effective bee repellent for *A. florea*. However, like *A. mellifera*, m-anisidine was the least effective among the tested amines (Table 3). At lower concentrations, the order of efficacy of different amines was: 2,4-dinitroaniline > p-chloroaniline > diphenylamine > o-chloroaniline > m-anisidine. At a 0.5% concentration, this order changed to: 2,4-dinitroaniline = p-chloroaniline > diphenylamine = o-chloroaniline = m-anisidine.

Group II: Esters

Apis mellifera

Among the esters, ethyl 4-chloroacetoacetate was the most effective repellent against *A. mellifera* whereas allylacetoacetate was the least effective. The order of efficacy of different esters tested was: ethyl 4- chloroacetoacetate > ethyl benzoylacetate = ethyl 2-methylacetoacetate > ethyl diacetoacetate >allyl acetoacetate (Table 4). However, with an increase in concentration (i.e. at a 0.5% concentration) the order of efficacy of esters changed to: ethyl 4-chloroacetoacetate = ethyl benzoylacetate > ethyl diacetoacetate = ethyl 2-methylacetoacetate > allyl acetoacetate.

Table 4

Repellency of five esters at different concentrations against *Apis mellifera* under semi-field conditions.

Chemicals	Repellency under different concentrations (%)*					
	0.05	0.10	0.20	0.30	0.40	0.50
Ethyl 2-methylaceto- acetate	18.22 (25.07)	43.23 (41.08)	59.21 (50.29)	62.83 (52.41)	68.61 (55.90)	71.20 (57.58)
Ethyl diacetoacetate	19.03 (25.83)	29.55 (32.88)	38.19 (38.14)	54.07 (47.32)	65.52 (54.03)	71.58 (57.81)
Ethyl benzoylacetate	21.43 (27.56)	33.14 (35.12)	60.74 (51.18)	65.25 (53.87)	68.01 (55.54)	74.49 (59.65)
Ethyl 4-chloroaceto- acetate	14.51 (22.26)	37.75 (37.87)	52.91 (46.65)	67.69 (55.37)	70.43 (57.06)	74.67 (59.77)
Allyl acetoacetate	4.60 (11.90)	8.00 (16.37)	22.55 (28.32)	30.88 (33.72)	42.17 (40.47)	58.65 (49.97)
CD ($P \leq 0.05$)	Chemical = (1.49), Concentration = (1.63), Chemical × Concentration = (3.65)					

* Mean of three replications

[Values within parenthesis are transformed values (angular transformation)]

The repellency of 2,4-dinitroaniline differed significantly from diphenylamine, of p-chloraniline from o-chloroaniline and of diphenylamine from m-anisidine. However, the repellency of all the amines at different concentrations could not reach the desired level of 80%.

Apis florea

Unlike, *A. mellifera*, here ethyl benzoylac effective whereas, like *A. mellifera,* allyl acetoacetate was the least effective repellent. The effectiveness of other esters fell in between these two. The

order of efficacy of different esters tested against *Apis florea* at lower concentrations was: ethyl benzoylacetate > ethyl 4-chloroacetoacetate > ethyl 2-methylacetoacetate > ethyl diacetoacetate > allyl acetoacetate (Table 5). However, at a 0.5% concentration the order changed to : ethyl benzoylacetate > ethyl 4-chloroacetoacetate > ethyl 2-methylacetoacetate = ethyl diacetoacetate > allyl acetoacetate.

Like amines, the% repellency of all the esters also, at all concentrations, could not reach the desired threshold of 80%. Therefore, none of the compounds of this group are recommended for further testing.

Table 5

Repellency of five esters at different concentrations against *Apis florea* under semi-field conditions.

Chemicals	Repellency under different concentrations (%)*					
	0.05	0.10	0.20	0.30	0.40	0.50
Ethyl 2-methylaceto- acetate	18.14 (25.16)	50.77 (45.42)	58.03 (49.60)	63.52 (52.83)	67.30 (55.10)	71.75 (57.90)
Ethyl diacetoacetate	19.94 (26.49)	29.42 (32.70)	42.85 (40.86)	52.64 (46.48)	65.07 (53.75)	70.65 (57.25)
Ethyl benzoylacetate	17.31 (24.56)	43.87 (41.46)	60.21 (50.87)	64.56 (53.47)	68.27 (55.69)	75.04 (60.03)
Ethyl 4-chloroaceto- acetate	21.65 (27.71)	31.37 (34.02)	58.02 (49.60)	63.52 (52.82)	68.78 (56.00)	72.98 (58.66)
Allyl acetoacetate	18.50 (25.41)	23.07 (28.58)	38.29 (38.20)	46.85 (43.17)	58.70 (49.99)	68.21 (55.66)
CD ($P \leq 0.05$)	Chemical = (1.68) , Concentration = (1.83), Chemical × Concentration = (4.10)					

* Mean of three replications
[Values within parenthesis are transformed values (angular transformation)]

Group III: Phenols

Apis mellifera

The% repellency of all the tested phenols at lower concentrations i.e. 0.05, 0.1 and 0.2 was below the desired level of 80%. When the concentration was increased to 0.3%, p-bromophenol and p-cresol showed the desired repellency (≥ 80%), however, the% repellency of o-cresol, o-ethoxyphenol and m-cresol was still less than the desired one (Table 6). With a further increase in concentration to 0.4%, repellency of all the tested phenols crossed the threshold level of efficacy. The order of repellency was: p-bromophenol = o-cresol > o-ethoxyphenol = p-cresol > m-cresol. With a further increase in concentration to 0.5%, except for o-ethoxyphenol and m-cresol, all other tested phenols showed 100% repellency; the order of repellency was: p-bromophenol = o-cresol = p-cresol > o-ethoxyphenol > m-cresol.

Apis florea

The repellency of all the chemicals at lower concentrations i.e. 0.05, a 0.1 and 0.2 concentration was below the desired repellency level of 80%. At a 0.3% concentration, only p-bromophenol could produce the desired repellency (84.68) (Table 7). In *Apis mellifera*, p-cresol and

Table 6

Repellency of five phenols at different concentrations against *Apis mellifera* under semi-field conditions.

Chemicals	Repellency under different concentrations (%)*					
	0.05	0.10	0.20	0.30	0.40	0.50
o-Cresol	22.15 (28.00)	45.55 (42.42)	64.59 (53.49)	77.04 (61.38)	93.54 (75.41)	100.00 (84.22)
m-Cresol	29.03 (32.52)	52.99 (46.69)	66.31 (54.52)	73.12 (58.75)	80.71 (64.15)	95.99 (78.59)
p-Cresol	41.45 (40.05)	59.28 (50.36)	71.89 (58.00)	82.95 (65.61)	90.15 (71.80)	100.00 (84.22)
o-Ethoxyphenol	1.06 (5.91)	34.38 (35.87)	57.74 (49.43)	75.20 (60.13)	90.72 (72.62)	98.39 (81.90)
p-Bromophenol	55.27 (48.01)	64.44 (53.38)	74.34 (59.56)	81.86 (64.81)	93.16 (75.00)	100.00 (84.22)
CD ($P \leq 0.05$)	Chemical = (1.81), Concentration = (1.98), Chemical × Concentration = (4.44)					

* Mean of three replications
[Values within parenthesis are transformed values (angular transformation)]

p-bromophenol showed the desired efficacy at a 0.3% concentration. At a 0.4% concentration, all the compounds showed the desired repellency, and the order of efficacy was: p-bromophenol > o-ethoxyphenol = o-cresol > m-cresol = p-cresol. As the concentration of the chemical was further increased to 0.5%, the repellency of p-bromophenol and o-cresol each reached 100% ,and the repellency of

Table 7

Repellency of five phenols at different concentrations against *Apis florea* under semi-field conditions.

Chemicals	Repellency under different concentrations (%)*					
	0.05	0.10	0.20	0.30	0.40	0.50
o-Cresol	4.40 (10.62)	33.04 (35.06)	49.25 (44.55)	70.86 (57.34)	89.53 (71.50)	100.00 (84.22)
m-Cresol	26.46 (30.84)	52.08 (46.17)	64.86 (53.64)	74.95 (59.93)	85.14 (67.41)	97.92 (81.31)
p-Cresol	33.76 (35.49)	44.91 (42.05)	61.03 (51.36)	77.40 (61.63)	83.53 (66.10)	97.44 (80.77)
o-Ethoxyphenol	2.99 (9.84)	34.75 (36.10)	65.15 (53.80)	74.52 (59.67)	90.12 (71.71)	97.08 (79.99)
p-Bromophenol	54.84 (47.76)	62.01 (51.94)	73.46 (58.96)	84.68 (66.98)	93.68 (75.60)	100.00 (84.22)
CD ($P \leq 0.05$)	Chemical = (2.03), Concentration = (2.22), Chemical × Concentration = (4.97)					

* Mean of three replications
[Values within parenthesis are transformed values (angular transformation)]

the other three phenols was also very high (>97%). The order of repellency was: P-bromophenol = o-cresol > m-cresol = p-cresol = o-ethoxyphenol.

Group IV: Aldehydes

Apis mellifera

The repellency of the tested aldehydes against *Apis mellifera* at a 0.05 and a 0.1 concentration was below 80%. At a 0.2% concentration, phenylacetaldehyde and 4-nitrobenzaldehyde showed the desired repellency (Table 8). The repellency of 2-methoxybenzaldehyde, 2,4-dimethoxybenzaldehyde and 3-methoxybenzaldehyde was less than the desired level at this concentration. At a 0.3% concentration, except for 3- methoxybenzldehyde, all other tested aldehydes showed the desired repellency (≥ 80%). Phenylacetaldehyde had the highest repellency followed by 2-methoxybenzaldehyde, 4-nitrobenzaldehyde and 2,4-dimethoxybenzaldehyde. At a 0.4% concentration, phenylacetaldehyde showed 100% repellency and all other chemicals showed > 90% repellency. At a 0.5% concentration, however, 4-nitrobenzaldehyde too showed 100% repellency whereas the others showed >98% repellency.

Apis florea

The repellency of all the tested aldehydes against *Apis florea* at a 0.05 and a 0.1% concentration was below the desired level of 80% (Table 9). At a 0.2% concentration, phenylacetaldehyde could show the desired repellency of ≥ 80%. At a 0.3% concentration, there was an increase in the repellency% and the desired level was achieved by phenylacetaldehyde, 2-methoxybenzaldehyde and 4-nitrobenzaldehyde. 2,4-dimethoxybenzaldehyde and 3-methoxybenzaldehyde could not attain the desired level at this concentration. At a 0.4% concentration, the repellency of the tested chemicals further increased and the trend of efficacy of aldehydes as bee repellents changed to this order: phenylacetaldehyde > 2,4-dimethoxybenzaldehyde > 2-methoxybenzaldehyde =

Table 8

Repellency of five aldehydes at different concentrations against *Apis mellifera* under semi-field conditions.

Chemicals	Repellency under different concentrations (%)*					
	0.05	0.10	0.20	0.30	0.40	0.50
2-Methoxybenzaldehyde	57.44 (49.28)	62.44 (52.19)	75.99 (60.65)	86.21 (68.21)	94.51 (76.42)	98.33 (81.83)
3-Methoxybenzaldehyde	54.19 (47.38)	62.25 (52.08)	67.71 (54.84)	78.79 (62.58)	91.77 (73.49)	98.33 (81.83)
2,4-Dimethoxybenzaldehyde	55.02 (47.86)	63.72 (52.96)	73.08 (58.73)	82.58 (65.35)	97.37 (80.04)	98.08 (81.90)
4-Nitrobenzaldehyde	57.96 (48.65)	68.71 (55.96)	80.71 (63.97)	84.64 (66.92)	94.47 (76.37)	100.00 (84.22)
Phenylacetaldehyde	65.92 (54.27)	78.61 (61.94)	82.92 (65.59)	95.25 (77.38)	100.00 (84.22)	100.00 (84.22)
CD (P≤ 0.05)	Chemical = (3.57) , Concentration = (3.91), Chemical × Concentration = (N.S.)					

* Mean of three replications

[Values within parenthesis are transformed values (angular transformation)]

Table 9

Repellency of five aldehydes at different concentrations against Apis florea under semi-field conditions.

Chemicals	Repellency under different concentrations (%)*					
	0.05	0.10	0.20	0.30	0.40	0.50
2-Methoxybenzaldehyde	50.97 (45.54)	65.11 (53.79)	76.63 (61.10)	89.22 (70.86)	95.07 (77.14)	98.39 (81.90)
3-Methoxybenzaldehyde	56.89 (48.94)	59.65 (50.55)	68.45 (55.84)	78.35 (62.27)	94.45 (76.35)	98.33 (81.83)
2,4-Dimethoxybenzaldehyde	55.61 (48.20)	61.77 (51.79)	71.72 (57.85)	78.63 (62.50)	96.36 (79.05)	96.75 (79.54)
4-Nitrobenzaldehyde	63.72 (52.95)	68.38 (55.79)	79.06 (62.75)	86.89 (68.82)	94.75 (76.73)	100.00 (84.22)
Phenylacetaldehyde	65.43 (53.97)	73.79 (59.19)	82.68 (65.38)	95.70 (78.00)	100.00 (84.22)	100.00 (84.22)
CD ($P \leq 0.05$)	Chemical = (1.43), Concentration = (1.57), Chemical × Concentration = (3.51)					

* Mean of three replications
[Values within parenthesis are transformed values (angular transformation)]

Table 10

Repellency of different ketones at different concentrations against *Apis mellifera* under semi-field conditions.

Chemicals	Repellency under different concentrations (%)*					
	0.05	0.10	0.20	0.30	0.40	0.50
m-Bromoacetophenone	80.32 (63.64)	89.84 (71.41)	95.96 (78.51)	100.00 (84.22)	100.00 (84.22)	100.00 (84.22)
o-Hydroxypropiophenone	76.07 (60.70)	83.36 (65.90)	89.83 (71.40)	95.88 (79.11)	100.00 (84.22)	100.00 (84.22)
p-Ethoxyacetophenone	80.61 (63.89)	88.42 (70.11)	94.78 (76.89)	100.00 (84.22)	100.00 (84.22)	100.00 (84.22)
3,4,5-Trimethoxy-acetophenone	78.85 (62.60)	88.78 (70.45)	94.76 (76.86)	100.00 (84.22)	100.00 (84.22)	100.00 (84.22)
1-Acetonaphthone	77.57 (61.73)	82.60 (65.33)	90.71 (72.28)	100.00 (84.22)	100.00 (84.22)	100.00 (84.22)
CD ($P \leq 0.05$)	Chemical = (0.97), Concentration = (1.06), Chemical × Concentration = (2.38)					

* Mean of three replications
[Values within parenthesis are transformed values (angular transformation)]

4-nitrobenzaldehyde = 3-methoxybenzaldehyde.

Phenylacetaldehyde showed 100% repellency at a 0.4% concentration while all other chemicals showed >94% repellency at this concentration.

At a 0.5% concentration phenylacetaldice and 4- nitrobenzaldehyde

Table 11

Repellency of different ketones at different concentrations against *Apis florea* under semi-field conditions.

Chemicals	Repellency under different concentrations (%)*					
	0.05	0.10	0.20	0.30	0.40	0.50
m-Bromoacetophenone	77.86 (61.93)	88.50 (70.16)	94.73 (76.87)	100.00 (84.22)	100.00 (84.22)	100.00 (84.22)
o-Hydroxypropiophenone	77.82 (61.91)	84.74 (66.98)	89.15 (70.78)	94.07 (76.18)	100.00 (84.22)	100.00 (84.22)
p-Ethoxyacetophenone	79.52 (63.06)	89.13 (70.75)	93.06 (74.76)	100.00 (84.22)	100.00 (84.22)	100.00 (84.22)
3,4,5-Trimethoxy-acetophenone	77.04 (61.40)	89.47 (71.09)	95.64 (79.03)	100.00 (84.22)	100.00 (84.22)	100.00 (84.22)
1-Acetonaphthone	75.46 (60.31)	84.74 (66.99)	93.13 (74.77)	100.00 (84.22)	100.00 (84.22)	100.00 (84.22)
CD ($P \leq 0.05$)	Chemical = (0.94), Concentration = (1.03), Chemical × Concentration = (2.29)					

* Mean of three replications
[Values within parenthesis are transformed values (angular transformation)]

showed a 100% repellency. The remaining aldehydes showed a repellency > 96%. At a 0.5% concentration the different aldehydes tested against *A. florea* for their repellency are arranged in the order: phenylacetaldehyde = 4-nitrobenzaldehyde = 2-methoxybenzaldehyde = 3-methoxybenzaldehyde > 2,4- dimethoxybenzaldehyde.

Group V: Ketones

Apis mellifera

Two chemicals i.e. p-ethoxyacetophenone and m-bromoacetophenone, showed the desired repellency against *Apis mellifera* even at a 0.05% concentration (Table 10). This shows that these chemicals are the most active repellents of all the tested chemicals. As the concentration was increased to 0.1, the remaining chemicals too showed the desired activity. The order of repellency of different ketones was: m-bromoacetophenone > p-ethoxyacetophenone = 3, 4, 5-trimethoxyacetophenone > o-hydroxypropiophenone = 1-acetonaphthone. All the tested ketones showed the desired repellency ≥ 80% at a lower concentration (i. e. 0.1%). With a further increase in concentration i.e. at a 0.2% concentration, the order of repellency was: m-bromoacetophenone > p-ethoxyacetophenone = 3,4,5-trimethoxyacetophenone > 1-acetonaphthone = o-hydroxypropiophenone. At a 0.3% concentration, m-bromoacetophenone, p-ethoxyacetophenone, 3,4,5-trimethoxyacetophenone and 1-acetonaphthone showed 100% repellency; o-hydroxypropiophenone could do so at a 0.4% concentration.

Apis florea

Unlike *A. mellifera*, none of the tested ketones could show the desired repellency against *Apis florea* at a 0.5% concentration (Table 11). As the concentration was increased to 0.1%, all the tested ketones, however, showed the desired repellency.

The order of repellency of the different ketones was:
3,4,5-trimethoxyacetophenone = p-ethoxyacetophenone = m-bromoacetophenone > 1-acetonaphthone = o-hydroxypropiophenone. At 2% cocentration, the order of efficacy of the different ketones was :
m-bromoacetophenone > p-ethoxyacetophenone = 3,4,5-trimethoxyacetophenone > 1-acetonaphthone = o-hydroxypropiophenone. However, at a 0.3% concentration all the tested ketones, except for o-hydroxypropiophenone, showed 100% repellency; the latter compound could do so at a 0.4% concentration.

DISCUSSION

The previous researchers considered 60% as the effective and safe threshold level of repellency (Woodrow et al. 1965, Atkins et al. 1975a, b, Bharadwaj 1974, Kumari 1976, Goyal 1977, Gupta 1985, 1987b, c, d, e, Gupta and Mohla, 1986, Patyal and Kumar 1989, Rani 1989, Gill 2000). In the earlier studies, repellency was calculated with the help of a faulty mathematical model which gave 50% repellency even for an inert compound. In that model, 60% repellency was equal to 20% repellency in the improved model (Sihag 2008). Therefore, in the earlier studies, 80% of the bees were at a risk of exposure to pesticides while in the new model only 20% of bees are at such a risk. There is a great difference in the risk factor. This is because, in the present investigation, 80% repellency of a chemical has been considered as the effective threshold level (Sihag 2008). This means that only 20% of bees will be at a risk for exposure to the pesticides

Of the five groups tested only three groups namely phenols, aldehydes and ketones could show the desired repellency of ≥ 80%. The two groups which did not show the desired repellency at all the tested concentrations were the amines and esters. On the basis of the repellency of the different groups tested, they can be arranged in the following order of efficacy: ketones > aldehydes > phenols > esters > amines.

Among the twenty five compounds tested for their repellency, ten compounds belonging to the groups amines and esters did not show the desired ≥ 80% repellency even at a 0.5% concentration. The remaining three groups i.e. phenols, aldehydes and ketones, however, gave the desired repellency of ≥ 80% in semi-field trials against both species of honey bees. All the latter compounds showed either 100% or greater than 95% repellency at a 0.5% concentration. However, with a decrease in concentration, the repellency also decreased. Phenols showed the effective repellency at a 0.3% concentration which, in the case of aldehydes, could be observed at a 0.2% concentration. Ketones, however, showed desired repellency at all the concentration levels except for o-hydroxypropiophenone, 1-acetonaphthone and 3,4,5-trimethoxyacetophenone; the latter could not show effective repellency at a 0.05% concentration. Among these fifteen compounds, p-ethoxyacetophenone, m-bromoacetophenone,3,4,5-trimethoxyacetophenone at a 0.2% concentration, phenylacetaldehyde at a 0.3% concentration, 4-nitrobenzaldehyde, p-bromophenol and p-cresol at a 0.4% concentration exhibited ≥ 80% repellency. Therefore, either or all these latter compounds can prove to be effective repellents against these two honey bee species. The repellents are meant to protect the bees from the pesticides. However, their efficacy when used in the field needs

to be further investigated.

The repellency of all the tested ketones was > 80% even at a lower concentration (0.1%). However, among all the tested chemicals, p-ethoxyacetophenone seemed to be the most effective repellent followed by m-bromoacetophenone and 3,4,5-trimethoxyacetophenone. Aldehydes ranked second, just behind the ketones, in their repellency. All the ketones showed 100% repellency at a 0.3 to a 0.4% concentration which, in the case of aldehydes, could be observed only at a 0.4 or a 0.5% concentration. Among all the aldehydes, phenylacetaldehyde was the most effective repellent followed by 4-nitrobenzaldehyde. Phenols ranked third in the order of repellency. None of the tested phenols showed 100% repellency at a 0.4% concentration.

The chemicals selected for this study are cheap and easily available. Some of these chemicals have shown the desired repellency under semi-field conditions. These are needed to be further tested under field conditions.

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SKUTECZNOŚĆ WYBRANYCH ZWIĄZKÓW CHEMICZNYCH JAKO ŚRODKÓW ODSTRASZAJĄCYCH DWA GATUNKI PSZCZÓŁ *Apis mellifera* L. ORAZ *Apis florea* F. I. DOŚWIADCZENIA PÓŁ-POLOWE

Mishra S., Sihag R. C.

S t r e s z c z e n i e

Celem badań było opracowanie metod zapewniających funkcjonalną ochronę pszczół na polach opryskiwanych pestycydami. Zbadano dwadzieścia pięć związków chemicznych należących do pięciu różnych grup, tj., aminy, estry, fenole, aldehydy i ketony, w sześciu różnych stężeniach w zakresie od 0,05 do 0,5% pod względem ich skuteczności odstraszania dwóch gatunków pszczoły miodnej, tj. *Apis mellifera* oraz *Apis florea*, w warunkach pół-polowych. Spośród badanych związków, piętnaście należących do trzech grup, tj., fenole, aldehydy i ketony, wykazywało pożądany poziom odstraszania wynoszący ≥ 80%. Ketony wykazywały pożądany poziom odstraszania nawet w niższych stężeniach niż aldehydy i fenole. Spośród tych piętnastu związków, p-etoksyacetofenon, m-bromoacetofenon i 3,4,5-trimetoksyacetofenon w stężeniu 0,2%; aldehyd fenylooctowy w stężeniu 0,3% oraz aldehyd 4-nitrobenzoesowy, p-bromofenol i p-krezol w stężeniu 0,4% wykazywały pożądany 80% odstraszania.

Słowa kluczowe: pszczoły miodne, *Apis florea*, *Apis mellifera*, zapylanie, pestycydy, środki odstraszające