

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

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### S u m m a r y

Fifteen compounds belonging to three different groups, viz., phenols, aldehydes and ketones along with two additives i.e. ethylene glycol and glycerol were tested at three concentrations for their repellency against two honeybee species viz. *Apis mellifera* L. and *Apis florea* F. under semi-field conditions. The addition of ethylene glycol and glycerol to all the fifteen compounds enhanced their repellency. All the ketones with additives showed the desired repellency of  $\geq 80\%$  at 0.1% concentration, followed by aldehydes which at 0.2% concentration showed the desired threshold of effective activity. Phenyl acetaldehyde showed the desired repellency at 0.1% concentration. Most of the phenols showed the desired repellency at 0.3 and 0.4% concentrations whereas p-bromophenol and p-cresol did this at 0.2% concentration. The effect of glycerol as an additive in enhancing the repellency of chemicals was much higher than ethylene glycol.

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

### INTRODUCTION

Bee pollination of crops is reported to give higher yields as well as better quality of seeds and fruits (McGregor, 1976; Free, 1993; Sihag, 1986, 1997). It has been estimated that more than an 80% pollination of fruits and seed crops is accomplished by honeybees world over. However, mass scale mortality of bees owing to wide spread use of pesticides affect the pollination and subsequently seed yield of agricultural crops (Sihag, 1988, 1991). Bee poisoning occurs mainly when insecticides are applied to the crops during the blooming period and by the drifting of toxic sprays or dusts into neighbouring crops. Application of chemical repellents on pesticide treated crops is one alternative to protect honeybees from the pesticide hazards. But the efficacy of these compounds is limited due to their short persistence on the crop under field conditions. It is desirable to

keep the affectivity of repellents for 2-3 hours. This is because, the effect of the pesticides lessens on the next day if the sprays are made in the evening when there is low bee activity on the crop (Sihag and Rathi, 1995). To increase the duration of activity of some repellents, certain other chemicals were earlier used as additives to those repellents by some workers (Kasana et al., 1997). However, the usefulness of these certain other chemicals could not be ascertained for two reasons: - (i) economically the chemicals were very costly, and (ii) the mathematical model used for the derivation of % repellency by the earlier workers was erroneous and the recommendations thus made were faulty. A new mathematical model for the derivation of % repellency was proposed by Sihag (2008). However, efficacy of earlier tested chemicals needs to be enhanced in order to make them cheaper. This study is a continuation of earlier ones (Mishra and

Sihag, 2009a,b). The objective being to test some chemicals as additives to enhance the efficacy and duration of activity of different repellent chemicals against two honeybees viz. *Apis mellifera* and *Apis florea* under semi-field conditions.

## MATERIAL AND METHODS

The chemicals which showed >80% repellency in semi-field trials (Mishra and Sihag, 2009a) were tested with the two additives, glycerol and ethylene glycol, for their repellency under semi-field conditions. The experiment was carried out on the same pattern as described by Mishra and Sihag (2009a). All the chemicals and additives were tested in a 1:1 ratio and at three concentrations. The lowest concentration of the chemical was adjusted to give 80% repellency. The bees were trained in a similar manner as in the semi-field experiment (Mishra and Sihag, 2009a). The bees were given a free choice to feed on a 30% sugar solution (which served as the control) or chemical + additive. The observations on number of bees present on the test chemical and the control for each concentration were repeated three times. The bees were counted for a duration of 1 minute. The two feeders were interchanged after every two observations, until the end of each experiment. This was done to keep the bees from adapting to a particular kind of food (sugar water in this case). The % repellency was calculated by the following formula as suggested by Sihag (2008):

$$\%R = \left[ 1 - \frac{2n}{N} \right] \times 100$$

Where,

n = Number of bees on a mixture of chemical solution and additive.

N = Number of bees on a sugar water solution.

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

## RESULTS

For proper comparison of biological response, results of each group have been presented separately with each species.

### GROUP I: PHENOLS

The efficacy of all the phenols tested in the semi-field experiment at a 0.5% concentration showed a >95% repellency (Mishra and Sihag, 2009a) Therefore, the 0.2, 0.3 and 0.4% concentrations were tested with additives to enhance the percent repellency of the tested chemicals. All the phenols at a 0.4% concentration gave a >80% repellency but at 0.2% none of these could do so. However, with additives, at a 0.2% concentration p-cresol and p-bromophenol could show the desired repellency in *A. mellifera* whereas in *A. florea* only p-bromophenol could do so. All other phenols failed to meet the repellency standards at this concentration. The effect of glycerol was better than ethylene glycol (Tab. 1 and Tab. 2).

#### (i) *Apis mellifera*

The data on % repellency of different concentrations of phenols against *Apis mellifera* with or without additives under semi-field conditions are given in Tab. 1. The repellency of o-cresol alone at 0.2, 0.3 and 0.4% concentrations was 64.66, 77.04 and 93.54%, respectively. It was observed that the addition of ethylene glycol to o-cresol enhanced the activity to 70.70, 83.79 and 97.53%, whereas, with the addition of glycerol activity was enhanced to 71.63, 90.46 and 97.78%, respectively. Though the repellency at a 0.2% concentration was increased by both the additives, none could reach the desired level of  $\geq 80\%$ . The activity of this chemical, however, was enhanced to the desired level by the addition of both the additives at a 0.3 and 0.4% concentration.

The repellency of p-cresol at the above concentrations was 71.89, 82.95 and 90.15% which was enhanced by the addition of ethylene glycol to 80.51, 86.94 and 93.18%. Glycerol enhanced repellency to 88.33, 92.93 and 100.00%, respectively. Here, the repellency was enhanced to the

Table 1

Repellency (%) of different concentrations of phenols against *Apis mellifera* with or without additives under semi-field conditions

| S. No.               | Chemicals                        | *Concentration (%)   |                  |                   |
|----------------------|----------------------------------|--|------------------|-------------------|
|                      |                                  | 0.2  | 0.3              | 0.4               |
| 1                    | o-Cresol                         | 64.66<br>(53.54)   | 77.04<br>(61.39) | 93.54<br>(75.41)  |
| 2                    | o-Cresol + Ethylene glycol       | 70.70<br>(57.30)   | 83.79<br>(66.26) | 97.53<br>(80.87)  |
| 3                    | o-Cresol + Glycerol              | 71.63<br>(57.83)   | 90.46<br>(72.09) | 97.78<br>(81.15)  |
| 4                    | p-Cresol                         | 71.89<br>(58.01)   | 82.95<br>(65.61) | 90.15<br>(71.81)  |
| 5                    | p-Cresol + Ethylene glycol       | 80.51<br>(63.86)   | 86.94<br>(68.79) | 93.18<br>(74.83)  |
| 6                    | p-Cresol + Glycerol              | 88.33<br>(70.16)   | 92.93<br>(74.55) | 100.00<br>(84.23) |
| 7                    | m-Cresol                         | 66.30<br>(54.52)   | 73.12<br>(58.76) | 80.71<br>(64.41)  |
| 8                    | m-Cresol + Ethylene glycol       | 72.56<br>(58.40)   | 82.96<br>(65.86) | 86.22<br>(68.24)  |
| 9                    | m-Cresol + Glycerol              | 77.31<br>(61.53)   | 86.85<br>(68.71) | 91.50<br>(73.13)  |
| 10                   | o-Ethoxyphenol                   | 57.74<br>(49.44)   | 75.20<br>(60.14) | 90.71<br>(72.61)  |
| 11                   | o-Ethoxyphenol + Ethylene glycol | 65.07<br>(53.75)   | 79.68<br>(63.19) | 94.86<br>(77.31)  |
| 12                   | o-Ethoxyphenol + Glycerol        | 69.11<br>(56.22)   | 85.98<br>(67.99) | 100.00<br>(84.23) |
| 13                   | p-Bromophenol                    | 74.34<br>(59.56)   | 82.03<br>(64.94) | 93.16<br>(75.01)  |
| 14                   | p-Bromophenol + thylene glycol   | 82.67<br>(65.38)   | 88.44<br>(70.21) | 97.78<br>(81.15)  |
| 15                   | p-Bromophenol + Glycerol         | 88.10<br>(69.80)   | 95.40<br>(77.90) | 100.00<br>(84.23) |
| <b>CD (P≤ 0.05):</b> |                                  | Concentration = 1.20<br>Chemical = 2.69<br>Concentration Chemical = 4.65 |                  |                   |

\* Mean of three replicates

Figures within parentheses are transformed values (angular transformation)

desired level by both the additives at all three concentrations.

In the case of m-cresol at the above concentrations, the repellency was 66.30, 73.12 and 80.71%, which, with the addition of ethylene glycol was 72.56, 82.96 and 86.22%, respectively. The addition of glycerol, on the other hand, could enhance

the repellency to 77.31, 86.85 and 91.50%, respectively. At a 0.2% concentration, addition of both the additives could not enhance the % repellency to the desired level of 80%. However, the activity of the chemical was enhanced to the desired level by both the additives at 0.3 and 0.4% concentrations.

Similarly, the repellency of o-ethoxyphenol (57.74, 75.20 and 90.71%) at three concentrations i.e. 0.2, 0.3 and 0.4% was enhanced with the addition of both ethylene glycol (65.07, 79.68 and 94.86%) and glycerol (69.11, 85.98 and 100.00%, respectively). Here, at a 0.2% concentration repellency was not enhanced to the desired level (i.e. 80% and above) by use of both the additives. However, at 0.3 and 0.4% concentrations, both the additives enhanced the % repellency to the desired level of 80%.

The % repellency of p-bromophenol alone at 0.2, 0.3 and 0.4% concentrations was 74.34, 82.03 and 93.16%, respectively. The addition of ethylene glycol enhanced the activity to 82.67, 88.44 and 97.78%. The addition of glycerol could increase the activity to 88.10, 95.40 and 100.00%, respectively. Here, the desired repellency was achieved at all the three concentrations by the addition of both the additives.

All these results clearly indicate that the addition of glycerol increased the % repellency of phenolic compounds against *A. mellifera* more than that obtained with the addition of ethylene glycol. The order of efficacy of these chemicals according to their % repellency with the addition of additives was as follows:

p-bromophenol>p-cresol>o-ethoxyphenol  
>o-cresol>m-cresol.

(ii) *Apis florea*

The data on % repellency of different concentrations of phenols against *Apis florea* with or without additives under semi-field conditions are given in Tab. 2. The repellency of o-cresol alone at 0.2, 0.3 and 0.4% concentrations was 49.25, 70.86 and 89.53%, respectively. It was observed that the addition of ethylene glycol enhanced the activity to 55.35, 77.36 and 93.48% which, however, in the case of glycerol was 59.03, 83.63 and 97.78%, respectively. Here, the activity of the chemical was enhanced to the desired level by glycerol at a 0.3% concentration whereas ethylene glycol could do so at a 0.4% concentration. The repellency of p-cresol at the above concentrations was

61.03, 77.40 and 83.53% respectively. Repellency was enhanced by the addition of ethylene glycol to 68.05, 83.17 and 91.05% and by glycerol to 73.92, 90.08 and 100.00%, respectively. Both the additives could increase the repellency of p-cresol at a 0.2% concentration but none could reach the desired level of 80% repellency. However, both the additives could cross this target at 0.3% concentration.

In the case of m-cresol alone at the above concentrations, the repellency was 64.86, 74.95 and 85.14% respectively which, with the addition of ethylene glycol, was 69.83, 83.41 and 90.69%, respectively. The addition of glycerol, however, increased the repellency to 74.54, 89.91 and 92.91%, respectively. At a 0.2% concentration, none of the additives could increase the repellency to the desired 80% level. However, this target could be achieved at 0.3% concentration.

The repellency of o-ethoxyphenol alone was (65.15, 74.52 and 90.12%) at three concentrations 0.2, 0.3 and 0.4% respectively. The repellency was enhanced with the addition of both ethylene glycol to 72.16, 80.13 and 93.48% and with glycerol to 77.09, 86.30 and 100.00% respectively. Here too, the desired repellency (i.e. 80% and above) was achieved at 0.3% concentration.

The repellency of p-bromophenol, alone at 0.2, 0.3 and 0.4% concentrations was 73.52, 84.68 and 93.68%, respectively. The addition of ethylene glycol enhanced the activity to 80.77, 87.18 and 97.78%, which in case of glycerol was 86.75, 93.38 and 100.00%, respectively. Here, the desired % repellency was achieved at a 0.2% concentration by the addition of both the additives.

These results clearly reveal that the addition of glycerol increased the % repellency of phenolic compounds against *A. florea* much more than that obtained with the addition of ethylene glycol. The order of efficacy of these chemicals according to their % repellency with the addition of additives was as follows: p-bromophenol>p-cresol>o-ethoxyphenol

Table 2

Repellency (%) of different concentrations of phenols against *Apis florea* with or without additives under semi-field conditions

| S. No.                | Chemicals                        | Concentration (%)  |                  |                   |
|-----------------------|----------------------------------|--|------------------|-------------------|
|                       |                                  | 0.2  | 0.3              | 0.4               |
| 1                     | o-Cresol                         | 49.25<br>(44.55)   | 70.86<br>(57.34) | 89.53<br>(71.50)  |
| 2                     | o-Cresol + Ethylene glycol       | 55.35<br>(48.05)   | 77.36<br>(61.59) | 93.48<br>(76.05)  |
| 3                     | o-Cresol + Glycerol              | 59.03<br>(50.19)   | 83.63<br>(66.17) | 97.78<br>(81.15)  |
| 4                     | p-Cresol                         | 61.03<br>(51.37)   | 77.40<br>(61.63) | 83.53<br>(66.10)  |
| 5                     | p-Cresol + Ethylene glycol       | 68.05<br>(55.57)   | 83.17<br>(65.83) | 91.05<br>(72.71)  |
| 6                     | p-Cresol + Glycerol              | 73.92<br>(59.27)   | 90.08<br>(71.81) | 100.00<br>(84.23) |
| 7                     | m-Cresol                         | 64.86<br>(53.64)   | 74.95<br>(59.93) | 85.14<br>(67.42)  |
| 8                     | m-Cresol + Ethylene glycol       | 69.83<br>(56.67)   | 83.41<br>(66.04) | 90.69<br>(72.39)  |
| 9                     | m-Cresol + Glycerol              | 74.54<br>(59.71)   | 89.91<br>(71.81) | 92.91<br>(74.54)  |
| 10                    | o-Ethoxyphenol                   | 65.15<br>(53.81)   | 74.52<br>(59.68) | 90.12<br>(71.72)  |
| 11                    | o-Ethoxyphenol + Ethylene glycol | 72.16<br>(58.14)   | 80.13<br>(63.52) | 93.48<br>(75.18)  |
| 12                    | o-Ethoxyphenol + Glycerol        | 77.09<br>(61.39)   | 86.30<br>(68.26) | 100.00<br>(84.23) |
| 13                    | p-Bromophenol                    | 73.52<br>(59.01)   | 84.68<br>(66.98) | 93.68<br>(75.61)  |
| 14                    | p-Bromophenol + Ethylene glycol  | 80.77<br>(63.98)   | 87.18<br>(69.00) | 97.78<br>(81.15)  |
| 15                    | p-Bromophenol + Glycerol         | 86.75<br>(68.64)   | 93.38<br>(75.06) | 100.00<br>(84.23) |
| <b>CD (P ≤ 0.05):</b> |                                  | Concentration = 1.27<br>Chemical = 2.52<br>Concentration × Chemical = 4.37 |                  |                   |

\* Mean of three replicates

Figures within parentheses are transformed values (angular transformation)

>o-cresol>m-cresol.

**GROUP II: ALDEHYDES**

The efficacy of all the aldehydes tested in our earlier study (Mishra et al., 2009a) revealed that at 0.4 and 0.5% concentrations these showed greater than 95% repellency against two honeybees viz. *Apis mellifera* and *Apis florea*. Therefore,

their lower concentrations i.e. 0.1, 0.2 and 0.3% were tested with additives to enhance their repellency.

(i) *Apis mellifera*

The data on repellency of different concentrations of aldehydes against *Apis mellifera* with or without additives under semi-field conditions

are given in Tab. 3. The repellency of 2,4-dimethoxybenzaldehyde alone at 0.1, 0.2 and 0.3% concentrations was 63.72, 73.08 and 82.58%, respectively. The addition of ethylene glycol enhanced the activity to 67.26, 77.41 and 88.80% which, in the case of glycerol, was 71.95, 83.09 and 95.31%, respectively. The repellency of 2, 4-dimethoxybenzaldehyde at a 0.1% concentration was increased considerably by the addition of both the additives but none could reach the desired 80% repellency. As the concentration was increased to 0.2%, the activity of the chemical was enhanced to the desired level (i.e. 80% and above) by glycerol only. At a 0.3% concentration, addition of even ethylene glycol was able to enhance the repellency to the desired level of  $\geq 80\%$ .

The repellency of 2-methoxybenzaldehyde at the three concentrations tested above was 62.44, 75.99 and 86.21% which was enhanced by the addition of ethylene glycol to 67.37, 80.69 and 90.89% and by glycerol to 71.18, 87.71, 92.91%, respectively. Here, the repellency was enhanced to the desired level (i.e. 80% and above) by both the additives at a 0.2% concentration. It could not reach 80% at a 0.1% concentration even after the addition of two additives.

Further, in the case of 3-methoxybenzaldehyde alone, at the above concentrations, the repellency was 62.25, 67.70 and 78.79%, respectively which, with the addition of ethylene glycol, was 65.24, 74.41 and 87.71%, respectively. With the addition of glycerol, however, it was 69.67, 80.29 and 90.89%, respectively. At a 0.1% concentration the repellency was increased by both the additives but none could reach the desired level of 80%. However, the activity of the chemical was enhanced to the desired level (i.e. 80% and above) by glycerol at a 0.2% concentration and by ethylene glycol at a 0.3% concentration.

Similarly, the % repellency of 4-nitrobenzaldehyde at these three concentrations (i.e. 0.1, 0.2 and 0.3%) was 68.71, 80.71 and 84.64%, respectively which was enhanced with the addition of both ethylene glycol (72.69, 84.64 and

88.67%, respectively) and glycerol (77.06, 90.86 and 95.47%, respectively). Here, none of the additives enhanced the % repellency to the desired level (i.e. 80% and above) at the 0.1% concentration but both the additives at a 0.2% concentration enhanced the % repellency to the desired level.

The % repellency of phenylacetaldehyde alone at 0.1, 0.2 and 0.3% concentrations was 78.61, 82.92 and 95.25%, respectively. The addition of ethylene glycol to it enhanced the activity to 86.30, 93.00 and 100.00%. The addition of glycerol enhanced the activity to 92.91, 97.62 and 100.00%, respectively. Here, the desired % repellency was achieved at 0.1 concentrations by both the additives.

These results, therefore, indicate that the addition of glycerol increased the % repellency of aldehyde compounds against *A. mellifera* much more than that obtained with the addition of ethylene glycol. The order of efficacy of these chemicals according to their % repellency with the addition of additives was as follows:

phenylacetaldehyde>  
4-nitrobenzaldehyde>  
2,4-dimethoxybenzaldehyde>  
2-methoxybenzaldehyde>3-  
methoxybenzaldehyde.

(ii) *Apis florea*

The data on % repellency of different concentrations of aldehydes against *Apis florea* with or without additives under semi-field conditions are given in Tab. 4. The repellency of 2, 4-dimethoxybenzaldehyde alone at 0.1, 0.2 and 0.3% concentrations was 61.77, 71.72 and 78.63%, respectively. The addition of ethylene glycol enhanced the activity to 67.77, 77.57 and 85.45%. The addition of glycerol enhanced the activity to 71.47, 85.74 and 89.01%, respectively. Though, the % repellency of this chemical at a 0.1% concentration was increased by both the additives, none could reach the desired level of 80%. Here, the activity of the chemical was enhanced to the desired level by glycerol at a 0.2% concentration and by ethylene glycol at a 0.3% concentration.

Table 3

Repellency (%) of different concentrations of aldehydes against *Apis mellifera* with or without additives under semi-field conditions

| S. No.               | Chemicals                                    | *Concentration (%)   |                  |                   |
|----------------------|--|--|------------------|-------------------|
|                      |  | 0.1  | 0.2              | 0.3               |
| 1                    | 2-Methoxybenzaldehyde                        | 62.44<br>(52.20)   | 75.99<br>(60.65) | 86.21<br>(68.21)  |
| 2                    | 2-Methoxybenzaldehyde + Ethylene glycol      | 67.37<br>(55.22)   | 80.69<br>(64.01) | 90.89<br>(72.54)  |
| 3                    | 2-Methoxybenzaldehyde + Glycerol             | 71.18<br>(57.59)   | 87.71<br>(69.74) | 92.91<br>(74.53)  |
| 4                    | 3-Methoxybenzaldehyde                        | 62.25<br>(52.08)   | 67.70<br>(55.36) | 78.79<br>(62.58)  |
| 5                    | 3-Methoxybenzaldehyde + Ethylene glycol      | 65.24<br>(53.86)   | 74.41<br>(59.64) | 87.71<br>(69.64)  |
| 6                    | 3-Methoxybenzaldehyde + Glycerol             | 69.67<br>(56.57)   | 80.29<br>(63.73) | 90.89<br>(72.54)  |
| 7                    | 2, 4-Dimethoxybenzaldehyde                   | 63.72<br>(52.97)   | 73.08<br>(58.73) | 82.58<br>(65.36)  |
| 8                    | 2, 4-Dimethoxybenzaldehyde + Ethylene glycol | 67.26<br>(55.12)   | 77.41<br>(61.62) | 88.80<br>(70.61)  |
| 9                    | 2, 4-Dimethoxybenzaldehyde + Glycerol        | 71.95<br>(58.07)   | 83.09<br>(65.74) | 95.31<br>(77.80)  |
| 10                   | 4-Nitrobenzaldehyde                          | 68.71<br>(55.97)   | 80.71<br>(63.98) | 84.64<br>(66.92)  |
| 11                   | 4-Nitrobenzaldehyde + Ethylene glycol        | 72.69<br>(58.48)   | 84.64<br>(67.04) | 88.67<br>(70.44)  |
| 12                   | 4-Nitrobenzaldehyde + Glycerol               | 77.06<br>(61.36)   | 90.86<br>(72.68) | 95.47<br>(77.99)  |
| 13                   | Phenylacetaldehyde                           | 78.61<br>(62.45)   | 82.92<br>(65.60) | 95.25<br>(77.38)  |
| 14                   | Phenylacetaldehyde + Ethylene glycol         | 86.30<br>(68.26)   | 93.00<br>(74.63) | 100.00<br>(84.23) |
| 15                   | Phenylacetaldehyde + Glycerol                | 92.91<br>(74.53)   | 97.62<br>(80.98) | 100.00<br>(84.23) |
| <b>CD (P≤ 0.05):</b> |  | Concentration = 1.17<br>Chemical = 2.60<br>Concentration × Chemical = Non- significant |                  |                   |

\* Mean of three replicates

Figures within parentheses are transformed values (angular transformation)

The repellency of 2-ethoxybenzaldehyde at the above three concentrations was 65.11, 76.63 and 89.22% which was enhanced by the addition of ethylene glycol to 72.25, 85.11 and 92.40% and by glycerol to 78.44, 87.74 and 95.48%, respectively. Here, the repellency was enhanced to the desired level (i.e. 80% and above) by glycerol at a

0.1% concentration and by ethylene glycol at a 0.2% concentration.

Likewise, the repellency of 3-methoxybenzaldehyde at three concentrations, was 59.65, 68.45, 78.35% which with the addition of ethylene glycol was 62.09, 73.06 and 83.71%, respectively. With the addition of glycerol, repellency

Table 4

Repellency (%) of different concentrations of aldehydes against *Apis florea* with or without additives under semi-field condition

| S. No.               | Chemicals                                    | *Concentration (%)   |                  |                   |
|----------------------|--|--|------------------|-------------------|
|                      |  | 0.1  | 0.2              | 0.3               |
| 1                    | 2-Methoxybenzaldehyde                        | 65.11<br>(53.80)   | 76.63<br>(61.10) | 89.22<br>(61.92)  |
| 2                    | 2-Methoxybenzaldehyde + Ethylene glycol      | 72.25<br>(58.20)   | 85.11<br>(67.61) | 92.40<br>(66.60)  |
| 3                    | 2-Methoxybenzaldehyde + Glycerol             | 78.44<br>(62.32)   | 87.74<br>(69.58) | 95.48<br>(69.97)  |
| 4                    | 3-Methoxybenzaldehyde                        | 59.65<br>(50.55)   | 68.45<br>(55.85) | 78.35<br>(56.22)  |
| 5                    | 3-Methoxybenzaldehyde + Ethylene glycol      | 62.09<br>(51.98)   | 73.06<br>(58.75) | 83.71<br>(58.97)  |
| 6                    | 3-Methoxybenzaldehyde + Glycerol             | 66.60<br>(54.68)   | 78.29<br>(62.28) | 90.03<br>(62.89)  |
| 7                    | 2, 4-Dimethoxybenzaldehyde                   | 61.77<br>(51.79)   | 71.72<br>(57.86) | 78.63<br>(57.38)  |
| 8                    | 2, 4-Dimethoxybenzaldehyde + Ethylene glycol | 67.77<br>(55.40)   | 77.57<br>(61.71) | 85.45<br>(61.62)  |
| 9                    | 2, 4-Dimethoxybenzaldehyde + Glycerol        | 71.47<br>(57.70)   | 85.74<br>(67.81) | 89.01<br>(65.44)  |
| 10                   | 4-Nitrobenzaldehyde                          | 68.38<br>(55.79)   | 79.06<br>(62.75) | 86.89<br>(62.46)  |
| 11                   | 4-Nitrobenzaldehyde + Ethylene glycol        | 72.21<br>(58.20)   | 86.03<br>(68.03) | 91.98<br>(66.59)  |
| 12                   | 4-Nitrobenzaldehyde + Glycerol               | 77.40<br>(61.60)   | 92.39<br>(73.96) | 93.08<br>(70.10)  |
| 13                   | Phenylacetaldehyde                           | 73.79<br>(59.20)   | 82.68<br>(65.38) | 95.70<br>(68.50)  |
| 14                   | Phenylacetaldehyde + Ethylene glycol         | 78.74<br>(62.59)   | 90.53<br>(72.20) | 100.00<br>(73.01) |
| 15                   | Phenylacetaldehyde + Glycerol                | 85.00<br>(67.65)   | 97.70<br>(81.07) | 100.00<br>(77.65) |
| <b>CD (P≤ 0.05):</b> |  | Concentration = 1.11<br>Chemical = 2.48<br>Concentration × Chemical = 4.30 |                  |                   |

\* Mean of three replicates

Figures within parentheses are transformed values (angular transformation)

was 66.60, 78.29 and 90.03%, respectively. At a 0.1% concentration, the % repellency was increased by both the additives but none could reach the desired level of 80%. The activity of the chemical was enhanced to the desired level by glycerol at 0.2% concentration and by ethylene glycol at a 0.3% concentration. Similarly, the

repellency of 4-nitrobenzaldehyde (68.38, 79.06 and 86.89%) at three concentrations i.e. 0.1, 0.2 and 0.3% was enhanced with the addition of both ethylene glycol (72.21, 86.03 and 91.98%) and glycerol (77.40, 92.39 and 93.08%, respectively). Here, the repellency could not be enhanced to the desired level of 80% by any of the additives

at a 0.1% concentration. The repellency of 4-nitrobenzaldehyde could be enhanced to the desired level of 80% by both the additives at a 0.2% concentration. In the case of phenylacetaldehyde, the repellency at 0.1, 0.2 and 0.3% concentrations was 73.79, 82.68, and 95.70%, respectively. The addition of ethylene glycol enhanced the activity to 78.74, 90.53 and 100.00% which, in the case of glycerol, increased to 85.00, 97.70 and 100.00%, respectively. Here, the desired repellency of 80% was achieved even at the lowest of the three concentrations by the addition of glycerol. The desired repellency was achieved at a 0.2% concentration by the addition of ethylene glycol.

These results, therefore, clearly reveal that the addition of glycerol increased the % repellency of the aldehyde chemicals as repellents against *A. florea* much more than with the addition of ethylene glycol. The order of efficacy of the aldehyde chemicals according to their repellency with the addition of additives was as follows:

phenylacetaldehyde>  
4-nitrobenzaldehyde>2-  
methoxybenzaldehyde>2,  
4-dimethoxybenzaldehyde>  
3-methoxybenzaldehyde.

### GROUP III: KETONES

All the ketones tested in the semi-field experiment for their efficacy as honeybee repellents at 0.3, 0.4 and 0.5% concentrations showed 100.00% repellency (Mishra and Sihag, 2009a). Therefore, lower concentrations of these chemicals at 0.05, 0.1 and 0.2% were tested with additives to enhance their repellency.

#### (i) *Apis mellifera*

The data on % repellency of different concentrations of ketones against *Apis mellifera* with or without additives under semi-field conditions are given in Tab. 5. The repellency of 1-acetonaphthone alone at 0.05, 0.1 and 0.2% concentrations was 77.57, 82.59 and 90.71%, respectively. The addition of ethylene glycol enhanced the activity to 80.63, 90.56 and 95.32% which, in case of glycerol, was 86.20, 92.64 and 100.00%, respectively.

Thus, both the additives enhanced the activity of the chemical to the desired level i.e. above 80%, at the lowest concentration of 0.05%.

The repellency of p-ethoxyacetophenone at three concentrations was 80.58, 88.42 and 94.78%. The repellency was enhanced by the addition of ethylene glycol to 84.90, 92.84 and 98.00%, respectively. It was found that the addition of glycerol enhanced the activity of the chemical compound to 86.86, 95.40 and 100.00%, respectively which was much greater than that obtained with the addition of the ethylene glycol.

The repellency of o-hydroxypropiophenone at 0.05, 0.1 and 0.2% concentrations was 76.07, 83.36 and 89.83%, respectively. Here also the addition of glycerol enhanced the activity of the chemical much more than that obtained with the addition of ethylene glycol. With ethylene glycol it was 80.89, 86.18, and 93.02% which however, in the case of glycerol was 87.18, 92.91 and 100.00%, respectively.

Likewise, the repellency of m-bromoacetophenone as 80.32, 89.84, and 95.96% at three concentrations (*viz.* 0.05, 0.1, and 0.2%) was enhanced with the addition of both ethylene glycol (85.05, 92.65, and 100.00%) and glycerol (91.29, 97.78 and 100.00%, respectively).

The repellency of 3, 4, 5-trimethoxyacetophenone at 0.05, 0.1 and 0.2% concentrations was 78.85, 88.78, 94.76%, respectively. Addition of ethylene glycol enhanced the activity to 83.25, 92.59 and 100.00%, respectively. In the case of glycerol the repellency increased to 88.46, 97.78 and 100.00%, respectively.

From these results it is evident that the addition of glycerol increased the % repellency of ketone compounds against *A. mellifera* much more than that obtained with the addition of ethylene glycol. Also all the chemicals showed a % repellency above 80% even at the lowest test concentration with the addition of both the additives. The order of efficacy according to their % repellency with the addition of additives was as follows:

Table 5

Repellency (%) of different concentrations of ketones against *Apis mellifera* with or without additives under semi-field conditions

| S. No.                | Chemicals                                      | *Concentration (%)  |                   |                   |
|-----------------------|--|---|-------------------|-------------------|
|                       |  | 0.05  | 0.1               | 0.2               |
| 1                     | m-Bromoacetophenone                            | 80.32<br>(63.65)  | 89.84<br>(71.42)  | 95.96<br>(78.512) |
| 2                     | m-Bromoacetophenone + Ethylene glycol          | 85.05<br>(67.26)  | 92.65<br>(74.25)  | 100.00<br>(84.23) |
| 3                     | m-Bromoacetophenone + Glycerol                 | 91.29<br>(72.93)  | 97.78<br>(81.15)  | 100.00<br>(84.23) |
| 4                     | o-Hydroxypropiophenone                         | 76.07<br>(60.71)  | 83.36<br>(65.90)  | 89.83<br>(71.40)  |
| 5                     | o-Hydroxypropiophenone + Ethylene glycol       | 80.89<br>(64.08)  | 86.18<br>(68.15)  | 93.02<br>(74.65)  |
| 6                     | o-Hydroxypropiophenone + Glycerol              | 87.18<br>(69.00)  | 92.91<br>(74.538) | 100.00<br>(84.23) |
| 7                     | p-Ethoxyacetophenone                           | 80.58<br>(63.87)  | 88.42<br>(70.115) | 94.78<br>(76.89)  |
| 8                     | p-Ethoxyacetophenone + Ethylene glycol         | 84.90<br>(67.16)  | 92.84<br>(74.46)  | 98.00<br>(81.42)  |
| 9                     | p-Ethoxyacetophenone + Glycerol                | 86.86<br>(68.72)  | 95.40<br>(77.901) | 100.00<br>(84.23) |
| 10                    | 3,4,5-Trimethoxyacetophenone                   | 78.85<br>(62.61)  | 88.78<br>(70.45)  | 94.76<br>(76.87)  |
| 11                    | 3,4,5-Trimethoxyacetophenone + Ethylene glycol | 83.25<br>(65.87)  | 92.59<br>(74.17)  | 100.00<br>(84.23) |
| 12                    | 3,4,5-Trimethoxyacetophenone + Glycerol        | 88.46<br>(70.33)  | 97.78<br>(81.15)  | 100.00<br>(84.23) |
| 13                    | 1-Acetonaphthone                               | 77.57<br>(61.73)  | 82.59<br>(65.32)  | 90.71<br>(72.29)  |
| 14                    | 1-Acetonaphthone + Ethylene glycol             | 80.63<br>(63.87)  | 90.56<br>(72.21)  | 95.32<br>(77.81)  |
| 15                    | 1-Acetonaphthone + Glycerol                    | 86.20<br>(68.17)  | 92.64<br>(74.24)  | 100.00<br>(84.23) |
| <b>CD (P ≤ 0.05):</b> |  | Concentration = 0.97<br>Chemical = 2.18<br>Concentration × Chemical = Non-significant |                   |                   |

\* Mean of three replicates

Figures within parentheses are transformed values (angular transformation)

m-bromoacetophenone>  
3,4,5-trimethoxyacetophenone>  
p-ethoxyacetophenone>1-acetonaphthone  
=o-hydroxypropiophenone  
(ii) *Apis florea*

The data on % repellency of different concentrations of ketones against *Apis florea* with or without additives under

semi-field conditions are given in Tab. 6. The repellency of 1-acetonaphthone at 0.05, 0.1 and 0.2% concentrations was 75.46, 84.73 and 93.13%, respectively. The addition of ethylene glycol enhanced the activity to 80.33, 87.69 and 95.78%. In the case of glycerol activity was enhanced to 86.66, 93.24 and 98.00%, respectively.

Table 6

Repellency (%) of different concentrations of ketones against *Apis florea* with or without additives under semi-field conditions

| S. No.        | Chemicals                                      | *Concentration (%)   |                  |                   |
|---------------|--|--|------------------|-------------------|
|               |  | 0.05   | 0.1              | 0.2               |
| 1             | m-Bromoacetophenone                            | 77.86<br>(61.93)   | 88.50<br>(70.17) | 94.73<br>(69.66)  |
| 2             | -Bromoacetophenone + Ethylene glycol           | 79.46<br>(63.05)   | 92.20<br>(73.75) | 100.00<br>(73.68) |
| 3             | m-Bromoacetophenone + Glycerol                 | 84.65<br>(66.99)   | 95.48<br>(77.99) | 100.00<br>(76.40) |
| 4             | o-Hydroxypropiophenone                         | 77.82<br>(61.91)   | 84.74<br>(66.99) | 89.15<br>(66.56)  |
| 5             | o-Hydroxypropiophenone + Ethylene glycol       | 82.26<br>(65.14)   | 87.06<br>(68.90) | 93.13<br>(69.61)  |
| 6             | o-Hydroxypropiophenone + Glycerol              | 88.56<br>(70.39)   | 93.31<br>(74.99) | 100.00<br>(76.53) |
| 7             | p-Ethoxyacetophenone                           | 79.52<br>(63.07)   | 89.13<br>(70.75) | 93.06<br>(68.61)  |
| 8             | p-Ethoxyacetophenone + Ethylene glycol         | 80.26<br>(63.10)   | 92.88<br>(74.51) | 98.00<br>(72.94)  |
| 9             | p-Ethoxyacetophenone + Glycerol                | 85.08<br>(67.23)   | 93.31<br>(74.99) | 100.00<br>(75.50) |
| 10            | 3,4,5-Trimethoxyacetophenone                   | 77.04<br>(61.41)   | 89.47<br>(71.10) | 95.64<br>(70.13)  |
| 11            | 3,4,5-Trimethoxyacetophenone + Ethylene glycol | 82.44<br>(65.28)   | 93.01<br>(74.64) | 100.00<br>(74.72) |
| 12            | 3,4,5-Trimethoxyacetophenone + Glycerol        | 88.66<br>(70.49)   | 97.78<br>(81.15) | 100.00<br>(78.62) |
| 13            | 1-Acetonaphthone                               | 75.46<br>(60.31)   | 84.73<br>(66.99) | 93.13<br>(68.28)  |
| 14            | 1-Acetonaphthone + Ethylene glycol             | 80.83<br>(64.02)   | 87.69<br>(69.48) | 95.78<br>(70.61)  |
| 15            | 1-Acetonaphthone + Glycerol                    | 86.66<br>(68.55)   | 93.24<br>(75.81) | 98.00<br>(75.26)  |
| CD (P≤ 0.05): |  | Concentration = 1.08<br>Chemical = 2.42<br>Concentration × Chemical = 4.19 |                  |                   |

\* Mean of three replicates

Figures within parentheses are transformed values (angular transformation)

Thus, both the additives enhanced the activity of the chemical to the desired level i.e. above 80%.

The repellency of p-ethoxyacetophenone at the above concentrations was 79.52, 89.13, and 93.06% which was enhanced by the addition of ethylene glycol to 80.26, 92.88 and 98.00% and by

glycerol to 85.08, 93.31 and 100.00%, respectively. At the above concentrations, o-hydroxypropiophenone showed 77.82, 84.74 and 89.15% repellency, which, with the addition of ethylene glycol increased to 82.26, 87.06 and 93.13%. However, in the case of glycerol activity was enhanced to 88.56, 93.31 and 100.00%, respectively.

Similarly, the repellency of m-bromoacetophenone at three concentrations i.e. 0.05, 0.1 and 0.2% was 77.86, 88.50 and 94.73%. The repellency of m-bromoacetophenone was enhanced with the addition of both ethylene glycol (79.46, 92.20 and 100.00%) and glycerol (84.65, 85.48, and 100.00%, respectively). In the case of 3, 4, 5-trimethoxyacetophenone, the repellency at 0.05, 0.1 and 0.2% concentrations was 77.04, 89.47 and 95.64%, respectively. The addition of ethylene glycol enhanced the activity to 82.44, 93.01 and 100.00%. In the case of glycerol the activity increased to 88.66, 97.78 and 100.00%, respectively.

These results also clearly reveal that the addition of glycerol increased the % repellency of ketones as repellents against *A. mellifera* much more than that obtained with the addition of ethylene glycol. Also, all the tested ketone chemicals showed the repellency >80% with the addition of both the additives even at 0.05%. The order of efficacy of the tested ketonic chemicals according to their % repellency with the addition of additives was as follows:

3,4,5-trimethoxyacetophenone  
 >m-bromoacetophenone>  
 p-ethoxyacetophenone>  
 o-hydroxypropiophenone=  
 1-acetonaphthone.

## DISCUSSION

Formulation of two repellents with four different chemical fixatives was tried by Mahavir (1982). Six different proportions of o-aminoacetophenone and ethyl benzyl ketone were mixed and tested in six different proportions with four different fixatives-benzophenone, benzyl benzoate, benzyl alcohol and diethyl phthalate at three different temperatures (25, 30 and 35°C). The proportion of o-aminoacetophenone / ethyl benzyl ketone with benzophenone (1:1) remained active for 8 hours at 25°C, 6 hours at 30°C and 4 hours at 35°C. The proportion of o-aminoacetophenone/ ethyl benzyl ketone with benzyl alcohol (1:1)/ benzyl benzoate (1:1) showed effectivity for 6 hours at 25°C and for less than 4 hours

at 30°C and 35°C.

Ahlawat et al. (1997) tested four carbonyl compounds at 0.5% concentrations individually and with additives for repellency to *Apis florea* under field conditions. On the basis of maximum repellency and effective duration observed, the mixture of benzyl benzoate+acetyl acetone (6 hours) was the best followed by dioctyl phthalate+methyl ethyl ketone (5 hours). However, 60% repellency was, in fact, equal to 20% repellency in the new model (Sihag, 2008). The new model was made to show how necessary it was to be safe, as 80% bees were still at the risk of exposure to the pesticides. That is why; we took 80% as the safe repellency, a difference of 60% in the minimum threshold of effective level. This is very significant from the honey bee safety point of view.

Many chemicals were tested earlier for their repellency against different honeybee species (Bhardwaj, 1974; Kumari, 1976; Goyal, 1977; Mahavir, 1982; Rani, 1989; Malhotra, 1998; Gill, 2000). The effect of all the chemicals, however, was short lived. Furthermore, the method of calculation of % repellency also suffered from a drawback which resulted in erroneous inferences of the results. Sihag (2008) rectified the error in the basic repellency formulae. Therefore, there was a need to test new chemicals for their prolonged enhanced repellency.

The results of this study revealed that all the three groups i.e. phenols, ketones and aldehydes showed an increase in their repellency after the addition of chemical additives. The pattern of increase was almost similar for the two honeybee species viz. *A. mellifera* and *A. florea*. However, the addition of glycerol to the compounds enhanced their activity much more as compared to when the addition of ethylene glycol was used.

Ketones were way ahead of all the tested chemicals in their efficiency as honeybee repellents. This is because, with the addition of both the additives, the activity of all the ketone compounds even

at 0.05 concentration, which is the lowest among all the concentrations tested, the repellency increased to the desired level of 80%. The efficacy of aldehydes with additives was next in line to the ketones. This is because, unlike ketones the aldehydes showed the desired repellency of 80% only at a 0.2% concentration and in a few cases like phenylacetaldehyde at a 0.1% concentration. The phenols with additives were at the bottom of the list and just behind aldehydes in their repellency. All the phenols showed desired repellency at a 0.3% concentration and only in a few cases e.g. p-bromophenol and p-cresol at a 0.2% concentration. Here also, the activity was increased by both the additives.

In our earlier studies, glycerol was used as a food softener in the soybean based artificial diet prepared for feeding the *Apis mellifera* colonies during dearth period (Sihag, 1990). However, this diet was much less accepted by the workers honeybees. The reason is evident now when the properties of glycerol are known. Glycerol in the diet, in fact, acted as a repellent that led to the poor acceptability of the diet.

In this study glycerol and ethylene glycol were taken as additives for the repellent molecules. The repellents are highly volatile and their molecules are released in air. Ambient conditions influence their volatility. Normally the repellent wears off quickly and hence the effective time of repellency is low. Glycerol or glycol is able to hold the repellent molecules by H-bonding and their release into the atmosphere slows down. Hence the effective duration of repellent should increase. Glycerol should be more effective than glycol because the former is trihydric alcohol and form more H-bonds than the latter which is dihydric alcohol.

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## SKUTECZNOŚĆ WYBRANYCH ZWIĄZKÓW CHEMICZNYCH Z DODATKAMI JAKO ŚRODKÓW ODSTRASZAJĄCYCH DWA GATUNKI PSZCZÓŁ *Apis mellifera* L. ORAZ *Apis florea* F. W DOŚWIADCZENIACH PÓŁ-POLOWYCH

**Mishra S., Sihag R. C.**

Zbadano wpływ dodatku glikolu etylenowego i glicerolu do 15 związków chemicznych należących do trzech różnych grup tj. fenole, aldehydy i ketony na ich właściwości odstrasżające w stosunku do dwóch gatunków pszczoł: *Apis mellifera* L. i *Apis florea* F. Dla każdego związku badania wykonano w trzech różnych stężeniach, w warunkach pół-polowych. Dodatek glikolu etylenowego i glicerolu do wszystkich piętnastu związków zwiększył ich właściwości odstrasżające. Wszystkie ketony z dodatkiem obu tych substancji wykazywały właściwości odstrasżające bliskie pożądanemu poziomowi  $\geq 80\%$  przy stężeniu 0,1%. Aldehydy wykazały pożądaną poziom przy stężeniu 0,2%, natomiast fenyloacetaldehyd przy stężeniu 0,1%. Większość fenoli wykazała pożądaną właściwości odstrasżające przy stężeniu 0,3% i 0,4%, podczas gdy p-bromofenol i p-krezol przy stężeniu 0,2%. Wpływ dodatku glicerolu na wzrost właściwości odstrasżających badanych związków chemicznych był dużo większy niż glikolu etylenowego.

**Słowa kluczowe:** pszczoła miodna, *Apis mellifera* L., *Apis florea* F., zapylanie, pestycydy, repelenty.