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# Safety Assessment of Certain Newer Insecticides to Western Honey Bee, *Apis mellifera* L.

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***Abstract*:** The widespread use of insecticides in agriculture has raised concerns about their potential impacts on non-target pollinators, particularly the European honey bee, *Apis mellifera* L which plays a key role in ecosystems and pollination services. The current study aimed to evaluate newer generation insecticides including neonicotinoids such as sulfoxaflor, flupyradifurone and pyrifluquinazon with respect to their effects on the honey bees. Laboratory bioassays were conducted to assess both acute and sublethal effects on worker bees including mortality. The results revealed that acephate and sulfoxaflor were highly toxic to honey bees causing the highest mortality in less time followed by chlorantraniliprole.

***Keywords*:** Mortality, Honey bee, Safety, Insecticides, Toxicity

Excessive use of pesticides in modern agriculture, though vital for reducing crop losses and ensuring food security, poses a critical threat to pollinators. This has resulted not only in the contamination of agroecosystems and agricultural produce but also in the contamination of nectar and pollen, causing heavy losses to honey bees and other pollinators. Unfortunately, crop protection practices and pollinator conservation are not always compatible, as honey bees are susceptible to many commonly used pesticides (Sundararaju 2003, Brittain et al., 2010). Pollinators provide ecosystem services that are crucial for crop production and wild plant biodiversity. Hence, the conservation of honey bees for crop pollination is vital for sustaining agricultural productivity. The honey bee, a major pollinator, is experiencing global declines, raising concerns about ecological impacts, food security and human welfare. Multiple studies have focused on honey bees because their biochemistry and neurophysiology are better understood than those of other pollinators and bees can also serve as models for assessing pesticide risks to other insect pollinators. Among the major stressors affecting bee health, pesticides remain paramount particularly under prolonged or multiple exposures leading to increased mortality. Although neonicotinoids have been extensively studied, their use is increasingly restricted due to harmful effects on bees and rising pest resistance. Consequently, newer pesticides such as flupyradifurone, sulfoxaflor, pyrifluquinazon, fenpyroximate, and afidopyropen have recently entered the market, yet evidence indicates that these too may be detrimental to bees. A planned pollination strategy integrating the use of bee attractants and safer insecticides is crucial for enhancing crop productivity. Hence, the present study was undertaken to investigate the safety of novel insecticides to *Apis mellifera* L.

## MATERIAL AND METHODS

The safety of certain newer insecticide molecules *viz*, sulfoxaflor 21.8% SC, flupyradifurone 17.09% SL, pyrifluquinazon 20% WG, fenpyroximate 5% EC, afidopyropen 4.89% DC, chlorantraniliprole 18.5% SC, flonicamid 50% WG and acephate 75% SP was evaluated against *A. mellifera* at Agricultural college, Bapatla (15.9039° N and 80.4671° E) Andhra Pradesh, India. The experiment was carried out under laboratory conditions by exposing a definite number (n=10) of bees to thin dry film of insecticide inside the plastic jars.

**Bioassay using dry film method:** The toxicity of the test insecticides to honey bees was assessed using the dry film method. Recommended doses of the insecticide solutions were prepared by diluting the required quantity of each commercial formulation in one liter of distilled water. From this, one ml of each of the insecticide solutions was applied as a thin film to the inner surface of clean dry rearing jar of size 10 x 7 cm diameter and the jar was gently rotated and left for drying so that a thin dry film of the insecticide was formed inside the jar. Forager honey bees were collected at the entrance of the hive with a cone-type muslin hand net (30 cm diameter) and transferred into plastic jars and were immobilized by refrigeration for approximately 5 min. Ten forager bees were released into each treated jar at intervals *i.e.*,0, 12 and 24 hours after dry film formation following the method of Ratnakar (2015). Since bees were exposed to insecticides any time after their spray in the field, these intervals were considered to understand the residual toxicity of the insecticides. A cotton pad soaked in sugar solution (20%) was provided inside the jar as food for bees and the jars were covered using muslin cloth. Each treatment was replicated thrice and a jar with a dry film of distilled water alone was served as untreated control.

## RESULTS AND DISCUSSION

**Mortality of honey bees exposed to insecticides immediately (zero hours) after treatment**: Mortality data of *A. mellifera* workers exposed to insecticide dry films (Plate 2) at different intervals (Table 1). Immediately (zero hours) after the treatment, no mortality was observed in any treatment. However, 2 h after exposure, significant differences were evident among treatments. Acephate (56.66%) and sulfoxaflor (53.33%) caused the highest mortality, followed by chlorantraniliprole and flupyradifurone . Pyrifluquinazon and afidopyropen caused equal mortality (13.33%), while fenpyroximate (10.00%) and flonicamid (6.66%) recorded the lowest values. The order of toxicity was acephate > sulfoxaflor > chlorantraniliprole > flupyradifurone > pyrifluquinazon = afidopyropen > fenpyroximate > flonicamid.

At 4 h, acephate increased mortality to 76.66%, followed by sulfoxaflor (53.33%), chlorantraniliprole (30.00%), flupyradifurone (23.33%) and were statistically on par with pyrifluquinazon and fenpyroximate. Afidopyropen and flonicamid recorded the lowest mortalities. The toxicity order remained similar to the 2 h observations. At 6 h, acephate further increased mortality to 96.29%, followed by sulfoxaflor, chlorantraniliprole, caused moderate mortality. The flupyradifurone and pyrifluquinazon and fenpyroximate were on par. afidopyropen and flonicamid. By 12 h, acephate reached 100% mortality, followed by sulfoxaflor and chlorantraniliprole. Pyrifluquinazon, flupyradifurone, fenpyroximate and afidopyropen were statistically comparable. The lowest mortality was recorded in flonicamid (19.44%).

At 24 h, acephate and sulfoxaflor both caused 100% mortality. Chlorantraniliprole (59.72%) followed, while pyrifluquinazon (43.98%), fenpyroximate (46.75%), and flonicamid (43.17%) were statistically on par. Flupyradifurone and afidopyropen (31.94% each) recorded the lowest values.

At 48 h, acephate and sulfoxaflor continued to cause 100% mortality. Chlorantraniliprole recorded 95.23% mortality, followed by pyrifluquinazon (71.42%). Fenpyroximate (56.66%), afidopyropen (63.33%), and flonicamid (64.28%) were on par, while flupyradifurone recorded the lowest mortality (35.71%).

Overall, Acephate proved highly toxic, causing 100% mortality within 12 h, followed by sulfoxaflor which reached 100% mortality at 24 h. Chlorantraniliprole exhibited delayed but high toxicity (95.23% at 48 h). Pyrifluquinazon, fenpyroximate, and flonicamid showed a gradual increase in mortality, while flupyradifurone and afidopyropen consistently caused lower mortality across all intervals.

**Mortality of honey bees exposed after 12 hours of insecticide treatment:** After 2 h of release, Sulfoxaflor caused the highest mortality (33.33%), followed by chlorantraniliprole and acephate (Table 2). Flupyradifurone resulted in 13.33% mortality, while pyrifluquinazon, fenpyroximate, afidopyropen, and flonicamid caused no mortality. The order of toxicity was sulfoxaflor > acephate = chlorantraniliprole > flupyradifurone > pyrifluquinazon = fenpyroximate = afidopyropen = flonicamid.

After 4 h, sulfoxaflor again caused the highest mortality

**Table 1.** Effect of insecticides at recommended dose on mortality of *A. mellifera* exposed immediately after dry film formation

| Treatment | Dose (ml or g/l) | 0 h | 2 h | 4 h | 6 h | 12 h | 24 h | 48 h |
|---|---|---|---|---|---|---|---|---|
| Sulfoxaflor 21.8% SC | 0.75 ml/l | 0.0 (0.0) | 53.33 (32.25) | 53.33 (32.25) | 66.64 (41.87) | 79.16 (52.36) | 100 (90.05) | 100 (90.05) |
| Flupyradifurone 17.09% SL | 0.5 ml/l | 0.0 (0.0) | 23.33 (13.50) | 23.33 (13.50) | 25.92 (15.03) | 27.77 (16.13) | 31.94 (18.64) | 35.71 (20.93) |
| Pyrifluquinazon 20% WG | 1.0 g/l | 0.0 (0.0) | 13.33 (7.66) | 13.33 (11.54) | 25.92 (15.03) | 36.11 (21.18) | 43.98 (21.10) | 71.42 (45.60) |
| Fenpyroximate 5% EC | 0.6 ml/l | 0.0 (0.0) | 10.00 (5.74) | 10.00 (11.54) | 22.22 (12.84) | 27.77 (16.13) | 46.75 (27.89) | 56.66 (34.53) |
| Afidopyropen 4.89% DC | 2.0 ml/l | 0.0 (0.0) | 13.33 (7.66) | 13.33 (7.66) | 14.81 (8.52) | 24.07 (13.93) | 31.94 (18.64) | 63.33 (39.31) |
| Chlorantraniliprole 18.5% SC | 0.3 ml/l | 0.0 (0.0) | 26.66 (15.47) | 26.66 (17.47) | 33.33 (19.48) | 47.68 (28.49) | 59.72 (36.69) | 95.23 (72.27) |
| Flonicamid 50% WG | 0.3 g/l | 0.0 (0.0) | 6.66 (3.82) | 6.66 (3.82) | 7.40 (4.25) | 43.51 (25.80) | 43.98 (25.59) | 64.28 (40.02) |
| Acephate 75% SP | 1.2 g/l | 0.0 (0.0) | 56.66 (34.53) | 56.66 (50.07) | 96.29 (74.38) | 100 (90.05) | 100 (90.05) | 100 (90.05) |
| Untreated Control | - | 0.0 (0.0) | 0.0 (0.00) | 0.0 (0.0) | 10.00 (5.74) | 13.33 (7.66) | 13.33 (7.66) | 26.66 (15.47) |
| CD (p=0.05) | | - | 10.43 | 14.76 | 17.59 | 16.20 | 22.21 | 22.10 |

Figures in parentheses are angular transformed values

(33.33%), followed by chlorantraniliprole and acephate which were statistically similar. No mortality was recorded in bees treated with pyrifluquinazon, fenpyroximate, and afidopyropen. The order of toxicity was sulfoxaflor > chlorantraniliprole = acephate > flupyradifurone > flonicamid > pyrifluquinazon = fenpyroximate = afidopyropen.

After 6 h, sulfoxaflor caused the highest mortality (53.33%), followed by acephate, chlorantraniliprole and flupyradifurone. No mortality was observed in pyrifluquinazon and fenpyroximate. The order of toxicity was sulfoxaflor > acephate > chlorantraniliprole = flupyradifurone > flonicamid > afidopyropen > pyrifluquinazon = fenpyroximate. After 12 h, similar trend was observed The order of toxicity was sulfoxaflor > acephate > chlorantraniliprole > flupyradifurone > flonicamid > afidopyropen > fenpyroximate > pyrifluquinazon.

After 24 h, sulfoxaflor recorded the highest mortality (91.66%), followed by acephate and flupyradifurone, pyrifluquinazon, chlorantraniliprole and afidopyropen with non-significant differences. Fenpyroximate recorded the lowest (11.57%). The order of toxicity was sulfoxaflor > acephate > flupyradifurone > pyrifluquinazon > chlorantraniliprole = afidopyropen > flonicamid > fenpyroximate.

After 48 h, cent percent mortality was recorded in sulfoxaflor and acephate treatments. Chlorantraniliprole caused 91.66% mortality, followed by flupyradifurone, pyrifluquinazon and fenpyroximate. Afidopyropen caused the least mortality (37.50%). The order of toxicity was sulfoxaflor = acephate > chlorantraniliprole > flupyradifurone > pyrifluquinazon = fenpyroximate > flonicamid > afidopyropen.

Overall, sulfoxaflor and acephate consistently showed high toxicity, with 100% mortality at 48 h. Chlorantraniliprole was moderately toxic, while flupyradifurone and flonicamid caused delayed but considerable mortality. Pyrifluquinazon, fenpyroximate, and afidopyropen exhibited negligible effects up to 6-12 h but showed moderate mortality after 24-48 h.

**Mortality of honey bees exposed after 24 hours of insecticide treatment:** No mortality was observed immediately (0 h) after bee release in any treatment. At 2 h after release, acephate caused the highest mortality (43.33%), whereas chlorantraniliprole (3.33%) and flupyradifurone (6.66%) were the least toxic (Table 3). Pyrifluquinazon, fenpyroximate, afidopyropen, and flonicamid caused no mortality. At 4 h, acephate remained the most toxic (43.33%). Chlorantraniliprole and flupyradifurone (6.66% each) and afidopyropen (10%) showed low mortality, while pyrifluquinazon, fenpyroximate, and flonicamid recorded no mortality. After 12 h, acephate caused the highest mortality (59.25%), followed by pyrifluquinazon and flonicamid (33.33%). Fenpyroximate (29.62%) and sulfoxaflor (25.92%) showed moderate effects, whereas afidopyropen and chlorantraniliprole (11.11% each) were comparatively low. Flupyradifurone recorded the least mortality (7.44%). At 24 h after release, acephate again recorded the highest mortality (90.47%), followed by flonicamid, pyrifluquinazon, fenpyroximate and sulfoxaflor.

**Table 2.** Effect of insecticides at recommended dose on mortality of *A. mellifera* exposed 12 hours after dry film formation

| Treatment | Dose (ml or g/l) | 2 h | 4 h | 6 h | 12 h | 24 h | 48 h |
|---|---|---|---|---|---|---|---|
| Sulfoxaflor 21.8% SC | 0.75 ml/l | 33.33 (19.48) | 33.33 (19.48) | 53.33 (32.25) | 77.77 (51.08) | 91.66 (66.47) | 100.00 (90.05) |
| Flupyradifurone 17.09% SL | 0.5 ml/l | 13.33 (9.40) | 16.66 (9.60) | 26.66 (15.47) | 33.33 (19.48) | 44.44 (25.39) | 76.18 (49.65) |
| Pyrifluquinazon 20% WG | 1.0 g/l | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) | 39.81 (23.47) | 66.66 (41.83) |
| Fenpyroximate 5% EC | 0.6 ml/l | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) | 3.70 (2.12) | 11.57 (4.52) | 66.66 (41.83) |
| Afidopyropen 4.89% DC | 2.0 ml/l | 0.0 (0.0) | 0.0 (0.0) | 10.00 (5.74) | 11.11 (6.38) | 36.11 (21.18) | 37.50 (22.04) |
| Chlorantraniliprole 18.5% SC | 0.3 ml/l | 23.33 (13.50) | 26.66 (15.47) | 26.66 (15.47) | 29.62 (17.24) | 36.11 (50.24) | 91.66 (66.47) |
| Flonicamid 50% WG | 0.3 g/l | 0.0 (0.0) | 13.33 (7.66) | 16.66 (9.60) | 18.51 (10.67) | 19.07 (11.00) | 45.83 (27.29) |
| Acephate 75% SP | 1.2 g/l | 0.0 (0.0) | 26.66 (15.47) | 33.33 (19.48) | 37.03 (21.75) | 67.59 (42.55) | 100.00 (90.05) |
| Untreated Control | - | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) | 10.00 (5.74) | 23.33 (9.60) | 20.00 (11.54) |
| CD (p=0.05) | | 8.08 | 10.94 | 20.62 | 17.20 | 25.02 | 20.69 |

Figures in parentheses are angular transformed values

**Table 3.** Effect of insecticides at recommended dose on mortality of *A. mellifera* exposed 24 hours after dry film formation

| Treatment | 2 h | 4 h | 6 h | 12 h | 24 h | 48 h |
|---|---|---|---|---|---|---|
| Sulfoxaflor 21.8% SC | 13.33 (7.66) | 16.66 (9.60) | 16.66 (11.54) | 25.92 (15.03) | 30.35 (17.68) | 42.85 (25.39) |
| Flupyradifurone 17.09% SL | 6.66 (3.82) | 6.66 (3.82) | 6.66 (3.82) | 7.40 (4.25) | 17.26 (9.94) | 33.32 (19.47) |
| Pyrifluquinazon 20% WG | 0.0 (0.0) | 0.00 (0.0) | 0.0 (0.0) | 37.03 (21.75) | 38.69 (22.77) | 61.90 (38.26) |
| Fenpyroximate 5% EC | 0.0 (0.0) | 0.00 (0.0) | 0.0[d] (0.0) | 29.62 (17.24) | 34.52 (20.20) | 61.89 (38.26) |
| Afidopyropen 4.89% DC | 0.0 (0.0) | 10.00 (5.74) | 10.00 (5.74) | 11.11 (6.38) | 19.90 (11.48) | 66.66 (41.83) |
| Chlorantraniliprole 18.5% SC | 3.33 (1.91) | 6.66 (3.82) | 6.66 (3.82) | 11.11 (6.38) | 17.26 (9.94) | 42.85 (25.39) |
| Flonicamid 50% WG | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) | 29.67 (17.27) | 39.28 (23.14) | 61.89 (38.26) |
| Acephate 75% SP | 43.33 (25.69) | 43.33 (25.69) | 43.33 (25.69) | 59.25 (36.35) | 90.47 (64.82) | 100 (90.05) |
| Untreated Control | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) | 0.0 (0.0) | 23.33 (13.50) | 30.00 (17.47) |
| CD (p=0.05) | 6.60 | 6.60 | 6.60 | 8.20 | 14.39 | 34.02 |

Figures in parentheses are angular transformed values

Flupyradifurone and chlorantraniliprole were the least toxic (17.26% each).

At 48 h after release, cent percent mortality was observed in acephate-treated bees followd by flupyradifurone, afidopyropen, pyrifluquinazon fenpyroximate and flonicamid. Chlorantraniliprole and sulfoxaflor recorded the lowest mortalities (42.85% each).

Overall, acephate consistently caused the highest mortality, reaching 100% by 48 h. Flupyradifurone and afidopyropen showed delayed but substantial toxicity. Sulfoxaflor induced moderate mortality over time, whereas chlorantraniliprole, pyrifluquinazon, fenpyroximate, and flonicamid showed lower or delayed effects. These results indicate that residual toxicity varies considerably among newer insecticides and exposure timing is a critical factor in honey bee safety. Among organophosphates, acephate was found to be moderately to highly toxic, causing 37.05-100% mortality within 4-12 h of exposure. These findings are consistent with observations of Reddy and Reddy (2006), and Stanley et al. (2015). Chlorantraniliprole, a diamide, indicated low to moderately toxic, with mortality ranging from 3.33-95.23% at 2–48 h and is likely due to limited interaction with honey bee ryanodine receptors when in aqueous solutions (Dinter et al., 2010). Similar observations were reported by Hasanab et al. (2013), Stanley et al. (2015), Dinter and Samel (2015), Dai et al. (2017), Wade (2019) and Anwar et al. (2022) indicating diamides are generally safer for bees.

Flonicamid (pyridine group) exhibited low toxicity in the present study, with 6.66-64.28% mortality at 6-48 h. Anwar et al. (2022) and Meikle and Weiss (2022) also reported minimal effects on colony growth, behavior and worker longevity under field-realistic exposures. Pyrifluquinazon (pyridine azomethine derivative) was considered non-toxic to low-moderately toxic with mortality ranging 0-71.42% after 12-48 h. Wilson et al. (2019) also reported low toxicity of this compound to *A. mellifera*. Fenpyroximate (mitochondrial complex I electron transport inhibitor) showed moderate toxicity, with 0-66.66% mortality over 6-48 h. Similar trends were reported by Leite et al. (2018) and Dahlgren et al. (2012) indicating acaricides of this class can affect worker bee survival. Afidopyropen (pyropene group) caused moderate toxicity with mortality of 0-66.66% within 6-48 h. Recent studies indicate chronic exposure can result in mortality and nutritional deficiency in bees (Peng et al., 2023) and moderate toxicity to silkworms and earthworms (Wei et al., 2023, Hu 2008). Sulfoxaflor (sulfoximine) was highly toxic, causing 20-100% mortality over 6-48 h which in agreement with Li et al. (2021) and Chakrabarti et al. (2020), highlighting severe risk to honey bees. Flupyradifurone (butenolide group) exhibited low toxicity, with 6.66-71.98% mortality at 24-48 h. Chakrabarti et al. (2020) also reported limited lethality following contact exposure.

## CONCLUSIONS

The study demonstrates that the toxicity of newer insecticides to *Apis mellifera* varies with chemical class and residual exposure time. Acephate and sulfoxaflor exhibited the highest toxicity, whereas flupyradifurone, pyrifluquinazon, fenpyroximate, flonicamid, and

afidopyropen were relatively safer, showing low to moderate effects on honey bee mortality. These findings highlight the importance of selecting pollinator-friendly insecticides in integrated pest management programs and emphasize the need for further studies to develop sustainable strategies that minimize risks to honey bees and other essential pollinators.

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