Compatibility and synergistic interactions of fungi, *Metarhizium anisopliae*, and insecticide combinations against the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae)

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Aphids are major pests affecting cereals, vegetables, fruit, forestry and horticultural produce. A multimodal approach may be an effective route to controlling this prolific pest. We assessed the individual and combined effect of eight insecticides and the entomopathogenic fungi, *Metarhizium anisopliae* (Metschin.) against the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), under laboratory conditions. Six of the insecticides tested were found to be highly compatible (flonicamid, imidacloprid, nitenpyram, dinotefuran, pyriproxyfen and spirotetramat), showing positive integration with the fungus and were selected for bioassays. The combination mixtures (1:1 ratio of *M. anisopliae*: insecticide) were significantly more toxic to *A. gossypii* than individual treatments. Maximum mortality (91.68%) of *A. gossypii* was recorded with combination of flonicamid and *M. anisopliae* (2.4 × 10⁶ cfu/ml) 72 h after application. While minimum mortality (17.08%) was observed with the individual treatment of *M. anisopliae* (2.4 × 10⁶ cfu/ml). The insecticides revealed toxicity consistent with their compatibility with *M. anisopliae*, ranking for efficacy exactly as they did for compatibility. In addition, the synergy factor (SF) and co-toxicity coefficient (CTC) values indicated synergistic interactions at different time intervals. The synergistic efficacy revealed the potential of fungus-insecticide integration against sucking insect pests.

**Abbreviations**

IPM  Integrated pest management  
EPF  Entomopathogenic fungi  
PDA  Potato dextrose agar  
CFU  Colony forming unit  
CTC  Co-toxicity coefficient  
SF  Synergy factor

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Aphids are small sap-sucking insects. Among the 5000 described species, 450 aphid species cause intense damage to crop and ornamental plants around the world. They are distributed globally but most commonly found in temperate zones where species diversity is also much higher compared to the tropics. Aphids are considered serious pests because they reach a high population density and can develop resistance to insecticides in a short period of time. The cotton aphid, *Aphis gossypii* (Glover) (Hemiptera: Aphididae), is a highly polyphagous pest. It causes serious damage like leaf curling, leaf deformation and transmits at least 76 viral diseases including potyvirus, cucumber mosaic virus and zucchini yellow virus to a wide range of crops. Aphid nymphs and adults deplete photo assimilates through their feeding and devitalize the plant in the process. Aphids also secrete honeydew which attracts black sooty mould that stains cotton fiber and blocks photosynthesis. The honeydew also causes sticky cotton during mechanical harvesting, ginning, and processing. Several control measures including host plant resistance, cultural, biological and chemical control are utilized to keep the pest population below economic injury level. Sucking insect pests like aphids and whiteflies can be controlled by using neonicotinoids. Neonicotinoids act as inhibitor on nicotinic acetylcholine receptors in the central nervous system. The intensive use of insecticides to control cotton aphids has led to populations that are now resistant to several classes of insecticides. In addition, pesticides can cause serious problems of environmental contamination and adverse effects on beneficial insects such as bee populations. Biosticides offer a route to protecting the crop while reducing the reliance on synthetic insecticides. Entomopathogenic fungi (EPF) have been found to be effective as a biopesticide and have potential to minimize the target pest populations on multiple crops. Moreover, 750 species of EPF are known to inoculate insect pests. One commonly used entomopathogenic fungus is *Metarhizium anisopliae* (Metsch.), which has been shown to be effective for control against 200 insect species including *Aphis gossypii*. More than 150 insect biocontrol products based on fungal entomopathogens have been commercialized with over 75% of these products based on the hypocrealean fungi *M. anisopliae*, *Beauveria bassiana*, *Isaria fumosorosea*, and *B. brongniartii*. However, this number is expected to have increased since the last major market evaluations were conducted. Entomopathogenic fungi are generally considered slow-acting, taking longer than conventional methods to achieve sufficient insect mortality. The technique of combining EPF into a management strategy with faster-acting materials may be the solution to this problem. The synergistic action of mycoinsecticides with chemical insecticides can increase mortality and reduce the time until death in insects. The combined use of fungal pathogens and the full, or reduced, dose of chemical insecticides is a promising pest-control option. The application of synergists can effectively enhance the cost-effectiveness and eco-friendliness of insecticides by reducing the required quantity and extending the residual activity. By attacking the pest through a different mode of action, they are equally important as an alternative for resistance management. The data is lacking regarding the compatibility of EPF with insecticides and synthetic insecticide combinations with mycoinsecticides are rarely evaluated against aphids. In this study we gauge the compatibility of different insecticides with *M. anisopliae* and assess their toxicity to a prominent aphid pest.

**Materials and methods**

*M. anisopliae* culture. Potato Dextrose Broth (PDB) media was used in a 1000 ml Erlenmeyer flask and autoclaved at 121 °C for 20 min as previously described. A disc of the cultured fungi approximately 5 mm in diameter was taken from its Petri dish and added into the prepared media under a laminar air flow chamber and kept at 25 ± 1 °C for 5 days before being transferred to a shaking incubator (Firstek Scientific, Tokyo, Japan) at 180 rpm for 48 h at 28 ± 1 °C. An optical density of 0.5 was measured with an OD meter (BIOLOG MODEL-21907; BIOLOG INC.) at λ 600 nm. This was achieved by dilution to maintain uniform conidia density (10^6 CFU mL⁻¹) prior to application. Inoculum and saline buffer (0.85% NaCl w/v) at ratios 1:9 and 2:18 were mixed to prepare *M. anisopliae* suspensions containing 10^6 CFU mL⁻¹. To achieve these populations, OD 0.4 and 0.3 samples were adjusted prior to application.

Insecticides compatibility with *M. anisopliae*. To assess compatibility, the effect of different insecticides (flonicamid, imidacloprid, nitenpyram, pyriproxyfen, spirotetramat and matrine) on the radial growth of *M. anisopliae* was evaluated. The recommended field doses of insecticides were added to potato dextrose agar (PDA) in an Erlenmeyer flask before solidification. After mixing thoroughly, was evaluated. The recommended field doses of insecticides (flonicamid, imidacloprid, nitenpyram, dinotefuran, pymetrozine, pyriproxyfen, spirotetramat and matrine) that exhibited good compatibility with *M. anisopliae* mixture (+ insecticide) were prepared for each treatment (Table 1). After sterilization with sodium hypochlorite (0.5% v/v), detached cotton leaves were washed three times with distilled water, air dried and placed on 1.5% agar (non-nutritive) in 90 × 20 mm² plastic Petri dishes. The 1.5% agar supplied moisture to maintain relative humidity during the test. Around 25 aphids (mixed adult and nymph population) were collected and allowed to settle for 1 day before treatment. A topical spray method was used to treat the aphids with individual and combined applications of insecticides and *M. anisopliae* applied using a hand atomizer (WIRE-
LESS ATOMIZER SPRAYER, A7-01). Three replicates were completed for each treatment. Mortality data were recorded 24, 48 and 72 h post treatment.

Determination of synergistic effect. The toxicity of combined and isolated treatments was calculated based on LC50 and LC90 of insecticides and combination treatments with EPF using probit analysis. The co-toxicity coefficient and synergy factor for mixed formulation were calculated utilizing the LC50 and LC90 identified for each treatment.

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\text{Co-toxicity coefficient} = \frac{\text{Toxicity of insecticide (alone)}}{\text{Toxicity of insecticide with fungal extract}} \times 100
\]

\[
\text{Synergy factor (SF)} = \frac{\text{Toxicity of insecticide (alone)}}{\text{Toxicity of insecticide with fungal extract}}
\]

Within this system, a SF value > 1 indicates synergism and an SF value < 1 indicates antagonism.

Statistical analysis. Percentage mortality of aphids was calculated by Abbot's Formula. The experiment was carried out under controlled condition inside the incubator (POL-EKO_APARATURA SPJ. S02ADF 180665) and collected data were checked for normality and homogeneity of variance using Shapiro–Wilk test. The P value obtained was larger than probability value of 5% which indicated that distribution of data was normal. Mortality data were recorded daily after treatment and analyzed using the Statistix software version 8.1. Percentage corrected mortality data were analyzed by main effects one way ANOVA through Multivariate General Linear Model (MGLM) Technique, using a STATISTICA software version 10.0 to determine the parameters of significance and mean values for different treatments and followed by a Tukey’s honestly significant difference (HSD) test with significant differences recognized when p < 0.05. Regression between aphid’s mortality and concentrations of insecticides was also established, using linear regression and Pearson correlation analysis at 5% level of probability. Scattered diagrams for concentration of each insecticides (alone or in combination) and mortality of aphid were also drawn to construct fitted simple regression line of mortality on concentrations.

Results

In vitro study on compatibility of insecticides with *M. anisopliae*. Effects of the insecticides on *M. anisopliae* vegetative growth showed that all tested formulations significantly inhibited the fungal growth. However, insecticides did not all inhibit *M. anisopliae* growth to the same extent. The greatest radial growth of the fungi with any insecticide treatment was observed with flonicamid with a colony diameter of 4.74 mm at the lowest concentration. The mean diameters of colonies based on 3 replicates were 4.65, 4.37, 3.96, 3.79, and 3.69 mm for imidacloprid, nitenpyram, dinotefuran, pyriproxyfen, and spirotetramat respectively. The pymetrozine and matrine treatments led to the lowest radial growth (Fig. 1).

### Table 1. Insecticides and entomopathogenic fungi individual and combined application with different doses used for laboratory bioassays.

| S. no | Treatment          | Concentrations                       |
|-------|--------------------|--------------------------------------|
|       | Individual and combined molecules | Sub lethal concentration (C1) | Lethal concentration (C2) | Super lethal concentration (C3) |
| T1    | Flonicamid         | 0.03%                                | 0.06%                    | 0.12%                      |
| T2    | Imidacloprid       | 0.125%                               | 0.25%                    | 0.5%                       |
| T3    | Nitenpyram         | 0.02%                                | 0.04%                    | 0.08%                      |
| T4    | Dinotefuran        | 0.0375%                              | 0.075%                   | 0.15%                      |
| T5    | Pyriproxyfen       | 0.23%                                | 0.45%                    | 0.9%                       |
| T6    | Spirotetramat      | 0.062%                               | 0.125%                   | 0.25%                      |
| T7    | *M. anisopliae*    | 2.4 × 10⁶ cfu/ml                     |                          |                           |
| T8    | Flonicamid + *M. anisopliae* | 0.03% + 2.4 × 10⁶ cfu/ml            | 0.06% + 2.4 × 10⁶ cfu/ml | 0.12% + 2.4 × 10⁶ cfu/ml |
| T9    | Imidacloprid + *M. anisopliae* | 0.125% + 2.4 × 10⁶ cfu/ml            | 0.25% + 2.4 × 10⁶ cfu/ml | 0.5% + 2.4 × 10⁶ cfu/ml   |
| T10   | Nitenpyram + *M. anisopliae* | 0.02% + 2.4 × 10⁶ cfu/ml            | 0.04% + 2.4 × 10⁶ cfu/ml | 0.08% + 2.4 × 10⁶ cfu/ml  |
| T11   | Dinotefuran + *M. anisopliae* | 0.0375% + 2.4 × 10⁶ cfu/ml           | 0.075% + 2.4 × 10⁶ cfu/ml | 0.15% + 2.4 × 10⁶ cfu/ml  |
| T12   | Pyriproxyfen + *M. anisopliae* | 0.23% + 2.4 × 10⁶ cfu/ml            | 0.45% + 2.4 × 10⁶ cfu/ml | 0.9% + 2.4 × 10⁶ cfu/ml |
| T13   | Spirotetramat + *M. anisopliae* | 0.062% + 2.4 × 10⁶ cfu/ml           | 0.125% + 2.4 × 10⁶ cfu/ml | 0.25% + 2.4 × 10⁶ cfu/ml |
| T14   | Control            | Water                                |                          |                            |
Efficacy of treatments alone and in combination against cotton aphid. Percentage mortality of the cotton aphid after 24, 48 and 72 h post treatment were found significantly different (Fig. 2). M. anisopliae alone was least effective among all treatments, leading to 5.26, 11.76 and 17.08% mortality after 24, 48 and 72 h post exposure respectively. All insecticide-only treatments showed dose and time dependent toxicity. Flonicamid was most toxic followed by imidacloprid, nitenpyram, dinotefuran, pyriproxyfen, and spinotetramat presented at the lower doses. The combination mixtures of M. anisopliae and insecticides were significantly more toxic than individual treatments. The combined application of M. anisopliae with flonicamid exhibited the greatest mortality in A. gossypii after 72 h (91.68%), followed by mixtures of the EPF with imidacloprid (88.59%), Nitenpyram (85.45%), Dinotefuran (79.69%), Pyriproxyfen (68.73%), and Spirotetramat (64.63%) (Fig. 2c). The correlation coefficient values (r) demonstrate a positive correlation with mean percent mortality of the pest (Fig. 3).

Synergetic effects of M. anisopliae and insecticides on A. gossypii. The LC50 and LC90 values of each insecticide and their mixture with M. anisopliae were inversely proportional with time. These values were used to determine the SF (Tables 2, 3, 4). Overall, it was observed that LC50 and LC90 values were lower in combination treatments than individual applications for the insecticides despite half the insecticides studied indicating an antagonistic effect with the EPF at the initial 24 h point.

The LC50 of flonicamid against cotton aphid was 0.439 ppm at 24 h and 0.010 ppm at 72 h. The LC90 was 7.61 ppm at 24 h and 0.383 ppm at 72 h. The mixture of flonicamid with M. anisopliae showed synergistic interaction against A. gossypii (Table 2), dropping those values significantly for both 24 h (LC50 = 0.2173, LC90 = 3.50) and 72 h (LC50 = 0.008, LC90 = 0.10) mortality counts. The time dependent co-toxicity coefficient (CTC) oscillated from 202.02 to 125 and 217.4 to 450 for LC50 and LC90 respectively. The SF of the combination treatment varied at different time points but remained above 1 for both LC50 and LC90. Imidacloprid showed antagonistic interaction with M. anisopliae for LC90 after 24 h, however, thereafter showed a synergistic interaction (Table 2). At 72 h of exposure, CTC (133.3) and SF (1.33) values were reduced for LC50 while they increased to 554.23 and 5.542 for LC90 respectively. For dinotefuran, it was found that a combination with the EPF resulted in a synergistic interaction in all samples except for the LC90 at 24 h where antagonism was observed (SF = 0.754).

Pyriproxyfen showed synergistic interactions with M. anisopliae at all levels of data analysis (Table 4). The LC50 values of pyriproxyfen and M. anisopliae were 4.70, 1.04 and 0.18 ppm and LC90 values were 40.12, 9.13 and 7.83 ppm after 24, 48 and 72 h post exposure, respectively.

Spirotetramat showed an antagonistic interaction with M. anisopliae for LC90 (CTC = 84.85, SF = 0.848) after 24 h, however, all other time points showed synergistic interactions (Table 4). For evaluation using the LC50, synergistic interactions were observed for all time points (SF > 1).

Discussion
Insecticides have the potential to affect the various developmental stages of entomopathogenic fungi. The effect of an insecticide on conidial germination is the most important factor in determining fungus-insecticide compatibility. We found that the insecticides tested did reduce vegetative growth and sporulation compared
to the control but not always to the extent that would preclude compatibility of the insecticides tested, flonicamid, imidacloprid, nitenpyram, dinotefuran, pyriproxyfen, and spirotetramat exhibited good compatibility with M. anisopliae. Significantly reduced fungal colony diameter was observed for pymetrozine and matrine treatments. The insecticides caused different levels of inhibition of germination, vegetative growth, and sporulation of M. anisopliae. This is dependent on compounds present that block conidia metabolic functions as well as concentrations of the active compounds40,41. Oliveira42 reported that, molecules analogous to prosthetic groups diffuse to the cytoplasm where they bind to specific receivers affecting membrane permeability and enzymatic synthesis, consequently affecting metabolic processes. The same mechanism of inhibition is likely to be responsible for conidial germination and vegetative growth differences in M. anisopliae.

M. anisopliae have been employed effectively to control several insect pest species, including other aphid species such as Lipaphis erysimi43. Variation in interaction modalities (synergistic, antagonistic or neutral) of EPF

Figure 2. (a) Percentage mortality of cotton aphid 24 h post individual and combined treatment applications. Letters above the bars indicate differences between treatments as determined by ANOVA followed by Tukey HSD. Those not sharing a letter are significantly different (p < 0.05). The combined applications show significantly greater mortality than individual treatments and control. (C1 (F = 23.0; df = 13, 28; p < 0.000), C2 (F = 37.1; df = 13, 28; p < 0.0000), C3 (F = 75.0; df = 13, 28; p < 0.0001). (b) Percentage mortality of cotton aphid 48 h post individual and combined treatment applications. Letters above the bars indicate differences between treatments as determined by ANOVA followed by Tukey HSD. Those not sharing a letter are significantly different (p < 0.05). The combined applications show significantly greater mortality than individual treatments and control. (C1 (F = 93.1; df = 13, 28; p < 0.000), C2 (F = 163; df = 13, 28; p < 0.0000), C3 (F = 80.8; df = 13, 28; p < 0.0001). (c) Percentage mortality of cotton aphid 72 h post individual and combined treatment applications. Letters above the bars indicate differences between treatments as determined by ANOVA followed by Tukey HSD. Those not sharing a letter are significantly different (p < 0.05). The combined applications show significantly greater mortality than individual treatments and control. (C1 (F = 173; df = 13, 28; p < 0.000), C2 (F = 288; df = 13, 28; p < 0.0000), C3 (F = 321; df = 13, 28; p < 0.0001).
with insecticides have been previously documented with species *B. bassiana* and *M. anisopliae*. *A. gossypii* has developed high resistance to numerous common insecticides, such as neonicotinoids, carbamates, organophosphates, and pyrethroids. Our study indicates that *M. anisopliae* has the potential to control *A. gossypii* within short period of time when combined with insecticides. The combined insecticide-*M. anisopliae* were consistently more toxic than individual treatments. Of the combinations tested, maximum mortality (91.68%) of *A. gossypii* was recorded with a mixture of flonicamid and *M. anisopliae* (2.4 × 10⁶ cfu/ml). Dayakar have previously found that the combination of insecticides with *M. anisopliae* can lead to a 1.19–1.42-fold increase in virulence over the sole treatment for Lepidoptera pests. The enhanced efficiency of combined application of fungal and chemical agents under laboratory conditions or field conditions has been reported in several studies. Looking at the mustard aphid, *Lipaphis erysimi*, Purwar and Sachan also observed enhanced efficiency through an insecticide-EPF combination.

**Figure 3.** Correlation coefficient (r), linear regression equation (Ŷ = bx ± a), coefficient of determination (100 R²) and scatter plot showing a fitted simple regression lines of Ŷ (% mortality of *Aphis gossypii* in laboratory conditions) on X (concentration of insecticides alone and in combinations with *Metarhizium anisopliae*).
The present study utilized co-toxicity coefficients and synergy factors to calculate the efficacies of different insecticides + *M. anisopliae* formulations. The toxicity of insecticides, based on their LC50 and LC90 values increased when mixed with *M. anisopliae*. The mixture of insecticides and *M. anisopliae* as a 1:1 ratio demonstrates synergistic effects against *A. gossypii* (Tables 2, 3, 4) The antagonistic effect observed for imiacloprid, dinotefuran, and spriotetramat at 24 h post exposure may be related to issues of compatibility, particularly suppression of EPF activity before the colony fully establishes, especially given that this antagonism is not observed at later time points. Ultimately, the combined treatments proved to be more effective than individual applications of all compounds tested (insecticides and *M. anisopliae*). The high values of co-toxicity coefficients, which were accompanied by insect mortalities > 90% for some treatments, illustrate the effectiveness of this dual-attack method of insect pest control. This finding is supported by previous studies, such as Quintela and McCoy\(^53,54\) which found that *B. bassiana* and *M. anisopliae* combined with sublethal doses of imidacloprid as a contact or oral treatment increased the mortality synergistically in the weevil, *Diaprepes abbreviatus*. Or the additive effect that has been observed with aphid species when *B. bassiana* is combined with a botanical pesticide, showing efficacy enhanced even in lower concentrations\(^55\).

**Conclusion**

The combination of *M. anisopliae* with insecticides showed a synergistic effect and led to higher mortality of the cotton aphid, *A. gossypii*. If laboratory evidence for synergistic effects of *M. anisopliae* and insecticides against *A. gossypii* applies under greenhouse or field conditions, this control solution could mitigate potential issues related to environmental contamination, non-target impacts and pesticide resistance. However, further studies on the mechanism of toxicity of these combinations are needed.

| Treatments | Ratio | Exposure period (hours) | Regression equation | Chi-square | LC50 ± SE (fiducial limits) (ppm) | SF | CTC | Type of action | LC90 ± SE (fiducial limits) (ppm) | SF | CTC | Type of action |
|------------|-------|-------------------------|---------------------|------------|---------------------------------|----|-----|---------------|---------------------------------|----|-----|---------------|
| Flonicamid |       |                         |                     |            |                                 |    |      |               |                                 |    |      |               |
|            | 24    |                         | y = 133x + 17.85    | 0.008      | 0.439 ± 0.415 (0.161–13.852)    |    |      |               |                                 |    |      |               |
|            | 48    |                         | y = 160.67x + 32.74 | 0.150      | 0.122 ± 0.052 (0.071–17.320)   |    |      |               |                                 |    |      |               |
|            | 72    |                         | y = 147.24x + 60.8  | 0.250      | 0.010 ± 0.007 (0.00–0.04)      |    |      |               |                                 |    |      |               |
| Flonicamid + *M. anisopliae* | 1:1   |                         |                     |            |                                 |    |      |               |                                 |    |      |               |
|            | 24    |                         | y = 136.95x + 24.58 | 0.091      | 0.2173 ± 0.118 (0.112–56.531) | 2.020 | 202.020 | Synergistic | 3.500 ± 0.875 (0.563–3378) | 2.174 | 217.400 | Synergistic |
|            | 48    |                         | y = 190.76x + 44.64 | 0.124      | 0.030 ± 0.011 (0.003–0.052)   | 4.066 | 406.660 | Synergistic | 0.943 ± 0.166 (0.280–6340) | 5.797 | 579.710 | Synergistic |
| Imidacloprid |       |                         |                     |            |                                 |    |      |               |                                 |    |      |               |
|            | 24    |                         | y = 29.737x + 16.8  | 0.011      | 1.933 ± 0.833 (0.700–20.021)   |    |      |               | 29.460 ± 5.374 (3.040–36.550) |    |      |               |
|            | 48    |                         | y = 34.457x + 30.97 | 0.151      | 0.611 ± 0.303 (0.331–20.652)  |    |      |               | 24.261 ± 4.920 (2.530–89.100) |    |      |               |
|            | 72    |                         | y = 36.354x + 57.61 | 0.110      | 0.040 ± 0.0381 (0.000–0.100)  |    |      |               | 3.271 ± 0.405 (0.900–16.590)  |    |      |               |
| Imidacloprid + *M. anisopliae* | 1:1   |                         |                     |            |                                 |    |      |               |                                 |    |      |               |
|            | 24    |                         | y = 33.777x + 21.781| 0.039      | 1.400 ± 0.218 (0.561–63.011)  | 1.378 | 137.800 | Synergistic | 35.81 ± 8.585 (3.080–91.351) | 0.822 | 82.261 | Antagonistic |
|            | 48    |                         | y = 44.914x + 41.700| 0.040      | 0.180 ± 0.0480 (0.031–0.270)  | 3.381 | 338.880 | Synergistic | 4.850 ± 5.641 (1.310–84.150)  | 5.002 | 500.200 | Synergistic |
|            | 72    |                         | y = 45.263x + 67.020| 0.063      | 0.031 ± 0.021 (0.001–0.070)   | 1.333 | 133.300 | Synergistic | 0.590 ± 0.193 (0.380–2.720)   | 5.542 | 554.231 | Synergistic |

Table 2. Toxicity of insecticides (Flonicamid and Imidacloprid) with and without *M. anisopliae* for *A. gossypii*. CTC co-toxicity coefficient, SF synergy factor.
| Treatments | Ratio | Exposure period (hours) | Regression equation | Chi-square | LC50 ± SE (fiducial limits) (ppm) | SF | Type of action | LC90 ± SE (fiducial limits) (ppm) | SF | Type of action |
|------------|-------|------------------------|---------------------|------------|----------------------------------|----|----------------|----------------------------------|----|----------------|
| Nitenpyram | 1:1   | 24                     | y = 0.010           | 0.100      | 0.394 ± 0.433 (0.121–3.030)      |    |                | 6.49 ± 6.325 (0.531–8.120)        |    |                |
|            |       | 48                     | y = 0.090           | 0.106 ± 0.048 (0.061–15.420)   |    |                | 2.26 ± 3.899 (0.360–5040)         |    |                |
|            |       | 72                     | y = 0.070           | 0.009 ± 0.006 (0.000–0.020)     |    |                | 0.45 ± 0.572 (0.140–1468)         |    |                |
| Nitenpyram + M. anisopliae | 1:1 | 24                     | y = 0.053           | 0.210 ± 0.168 (0.090–78.200)   | 1.876 | Synergistic | 3.80 ± 0.859 (0.441–18.640)       | 1.707 | Synergistic |
|            |       | 48                     | y = 0.001           | 0.031 ± 0.008 (0.002–0.050)     | 3.533 | Synergistic | 1.20 ± 0.802 (0.240–3136)         | 1.883 | Synergistic |
|            |       | 72                     | y = 0.151           | 0.004 ± 0.003 (0.000–0.012)     | 2.250 | Synergistic | 0.12 ± 0.057 (0.071–1.730)        | 3.750 | Synergistic |
| Dinotefuran |       | 24                     | y = 0.040           | 0.806 ± 0.823 (0.260–7904)      |    |                | 6.59 ± 2.866 (0.850–4.422)        |    |                |
|            |       | 48                     | y = 0.010           | 0.331 ± 0.216 (0.150–1995)      |    |                | 5.64 ± 0.548 (0.772–12.521)       |    |                |
|            |       | 72                     | y = 0.190           | 0.076 ± 0.018 (0.020–0.171)     |    |                | 2.65 ± 0.153 (0.500–30.373)       |    |                |
| Dinotefuran + M. anisopliae | 1:1 | 24                     | y = 0.020           | 0.570 ± 0.547 (0.211–10.720)   | 1.414 | Synergistic | 8.73 ± 19.301 (0.900–26.251)      | 0.754 | Antagonistic |
|            |       | 48                     | y = 0.080           | 0.100 ± 0.028 (0.005–2.95)      | 3.310 | Synergistic | 4.13 ± 0.788 (0.59–22.76)         | 1.365 | Synergistic |
|            |       | 72                     | y = 0.001           | 0.020 ± 0.009 (0.004–0.040)     | 3.810 | Synergistic | 0.36 ± 0.176 (0.190–3.371)        | 7.361 | Synergistic |

Table 3. Toxicity of insecticides (Nitenpyram and Dinotefuran) with and without M. anisopliae for A. gossypii. CTC co-toxicity coefficient, SF synergy factor.

| Treatments | Ratio | Exposure period (hours) | Regression equation | Chi-square | LC50 ± SE (fiducial limits) (ppm) | SF | Type of action | LC90 ± SE (fiducial limits) (ppm) | SF | Type of action |
|------------|-------|------------------------|---------------------|------------|----------------------------------|----|----------------|----------------------------------|----|----------------|
| Pyriproxyfen |       | 24                     | y = 0.560           | 0.574 ± 0.618 (1.731–12.52)     |    |                | 40.35 ± 8.072 (5.030–2041)        |    |                |
|            |       | 48                     | y = 0.002           | 3.100 ± 0.300 (1.181–17.270)    |    |                | 54.332 ± 21.337 (5.390–91.060)    |    |                |
|            |       | 72                     | y = 0.060           | 0.62 ± 0.178 (0.36–5.14)        |    |                | 23.940 ± 5.335 (3.541–27.920)     |    |                |
| Pyriproxyfen + M. anisopliae | 1:1 | 24                     | y = 0.010           | 4.701 ± 0.807 (1.580–2122)      | 1.185 | Synergistic | 40.122 ± 6.413 (5.070–85.02)      | 1.065 | Synergistic |
|            |       | 48                     | y = 0.010           | 1.040 ± 0.305 (0.690–4.173)     | 2.980 | Synergistic | 9.530 ± 9.242 (2.951–175.85)      | 5.700 | Synergistic |
|            |       | 72                     | y = 0.030           | 0.184 ± 0.087 (0.000–0.321)     | 3.444 | Synergistic | 7.83 ± 9.709 (2.06–6581)          | 3.057 | Synergistic |
| Spirotetramat |     | 24                     | y = 0.060           | 1.791 ± 0.912 (0.522–13.02)     |    |                | 11.153 ± 2.596 (1.380–29.950)     |    |                |
|            |       | 48                     | y = 0.001           | 1.00 ± 0.963 (0.363–11.070)     |    |                | 14.670 ± 3.280 (1.532–30.81)      |    |                |
|            |       | 72                     | y = 0.040           | 0.260 ± 0.116 (0.141–5.280)     |    |                | 11.590 ± 2.129 (1.221–107.50)     |    |                |
| Spirotetramat + M. anisopliae | 1:1 | 24                     | y = 0.007           | 1.622 ± 0.870 (0.451–13.24)     | 1.104 | Synergistic | 13.14 ± 7.889 (1.48–25.20)        | 0.848 | Antagonistic |
|            |       | 48                     | y = 0.171           | 0.360 ± 0.140 (0.220–3.551)     | 2.777 | Synergistic | 3.934 ± 4.729 (0.973–8866)        | 3.732 | Synergistic |
|            |       | 72                     | y = 0.004           | 0.060 ± 0.027 (0.000–0.110)     | 4.333 | Synergistic | 3.790 ± 0.605 (0.721–22.010)      | 3.058 | Synergistic |

Table 4. Toxicity of insecticides (Pyriproxyfen and Spirotetramat) with and without M. anisopliae for A. gossypii. CTC co-toxicity coefficient, SF synergy factor.
Data availability
The data used and analyzed during this project are available from the corresponding author on reasonable request.

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Author contributions
A.N. and F.R. planned and designed the research experiments. F.R. and A.R. performed the experiments and wrote the research article while A.I facilitated for execution of experiments and revision process. M.D.G. helped in statistical analysis, G.M.F.G. and M.J.A. reviewed and edited the article, M.T., M.A.A. and M.S. guided the students for preparation of formulations, M.R.S. and MAQ helped in aphid identification and collection, MN provided facility for lab culturing of entomopathogenic fungi. All authors reviewed the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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