Research Article

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New slow release mixture of (*E*)-*β*-farnesene with methyl salicylate to enhance aphid biocontrol efficacy in wheat ecosystem

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Abstract

Semiochemical use is a promising way to reduce damage from pests by improving natural control in agro-ecosystems. The aphid alarm pheromone (*E*)-*β*-farnesene (E*β*F) and herbivore-induced methyl salicylate (MeSA) are two volatile cues to induce changes in aphid behavior with functional significance. Because of limitations related to the volatility and oxidization of E*β*F and MeSA under natural conditions, slow-release and antioxidant techniques should be developed and optimized before application. Here, a slow-release alginate bead of E*β*F mixed with MeSA was first designed and manufactured. We hypothesized that a mixture of these two semiochemicals could be effective in controlling *Sitobion miscanthi* in wheat crops. Both MeSA and E*β*F in alginate beads were released stably and continuously for at least 15 days in the laboratory, whereas E*β*F in paraffin oil and pure MeSA were released for only 2 and 7 days, respectively. In 2018 field experiments, E*β*F and MeSA alone or in association significantly decreased the abundance of alate and apterous aphids. An increased abundance of mummified aphids enhanced by higher parasitism rates was observed when using E*β*F and MeSA in association, with a significant reduction of apterous abundance, more so than E*β*F or MeSA alone. In 2019, plots treated with a mixture of E*β*F and MeSA showed significantly decreased abundance of alate and apterous aphids with higher parasitism rates compared with the control. The new slow-release alginate bead containing a mixture of E*β*F with MeSA could be the most efficient formulation to control *S. miscanthi* population by attracting parasitoids in the wheat agro-ecosystem.

Supporting information may be found in the online version of this article.

Keywords: *Triticum aestivum*; wheat aphid; behavioral manipulation; biological control; semiochemical release; ecological service

1 INTRODUCTION

Environmentally friendly pest management strategies are needed to reduce conventional chemical insecticide applications that lead to many well-known problems, such as target pest resistance and chemical residue occurrence in agro-ecosystems inducing ecological disruption.[1–3] Therefore, pest control approaches should fit within integrated pest management strategies.[4] Interactions between natural enemies, herbivores and host plants are important in integrated pest management development where natural enemies could be manipulated by semiochemical release for improved biological control.[5]

The aphid alarm pheromone, (*E*)-*β*-farnesene (E*β*F), is emitted from cornicles when aphids are attacked by their natural enemies, warning others of the presence of immediate danger.[6] When other aphids detect this cue, they remove their stylets from the host plant and fall, jump or walk to escape potential danger.[7, 8] Numerous laboratory studies have shown that E*β*F can attract a variety of natural enemies, such as lady beetles,[9] hoverflies,[10] lacewings,[11] and parasitoids.[12] However, the persistence of this alarm pheromone in the environment is reduced by its ease of oxidation due to the presence of several double bonds in the pheromone molecule.[13]

When attacked by herbivores, plants can release specific volatile blends that attract predators and/or parasitoids.[14–16] Herbivore-induced plant volatiles (HIPVs) have a key role in the tritrophic interactions among plants, herbivores and their natural enemies.[17] Methyl salicylate (MeSA), an important HIPV induced by aphids, is a key signal in the plant immune response[18] that can repel wheat aphids[19,20] and attract aphid predators such as *Syrphus corollae* Fabricius (Diptera: Syrphidae),[21] *Coccinella septempunctata* L. (Coleoptera: Coccinellidae),[22,23] *Chrysopa nigricornis*

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Burmeister (Neuroptera: Chrysopidae)[24] and aphid parasitoids (Hymenoptera: Braconidae).[25,26]

Herbivore-induced semiochemicals have long been considered as potential alternative pest control elements that are nontoxic and harmless to beneficials and could be applied in an efficient and sustainable way to control insect pests in the field.[27] Semiochemical cues from host-damaged plants or hosts themselves are of crucial importance in host location by natural enemies. Several studies on semiochemicals under natural conditions have demonstrated their applicability for enhancing natural enemy abundance on strawberry, *Fragaria* × *ananassa* Duch (Rosidae: Rosstarae),[28] cotton, *Gossypium* spp. (Malvales: Malvaceae),[29] hops, *Humulus lupulus* Linn (Urticales: Moraceae) and grapes, *Vitis vinifera* (Rhamnales: Vitaceae),[30] and for reducing pest populations in wheat, *Triticum aestivum*[31] and barley, *Hordeum vulgare* (Poales: Poaceae).[20]

Wheat is the most important food crop worldwide impacted by wheat aphid, major global pest.[32] *S. miscanthi*, a phloem feeder and vector of plant viruses, damages approximately 14.6 million km^2 of wheat per year and causes up to 40% wheat yield loss in China[33,34] Aphid parasitoids such as *Aphidius avenae* Haliday and *A. gifuensis* Ashmead (Hymenoptera: Braconidae) have recently shown considerable potential as biological control agents against wheat aphids.[35]

Some aphid natural enemies could use E*β*F or MeSA to locate their aphid prey.[32] Although a few E*β*F carriers have been shown to provide a slow-release system in biological control,[12,32] challenges related to E*β*F's instability and easy oxidation in nature have not been well considered.[13, 36] Moreover, a type of MeSA alginate bead carrier has been found to maintain continuous release for up to 25 days in laboratory experiments. This could optimize wheat aphid control in the field by manipulating syrphid fly abundance.[21] Therefore, we hypothesize that this bead carrier system, with a mixture of the aphid alarm pheromone E*β*F and the HIPV MeSA might enhance aphid control efficacy in agro-ecosystems. Our experiments were in four parts. First, we manufactured alginate beads containing E*β*F, MeSA and a mixture of E*β*F and MeSA to increase the volatility and stability for field application. Second, the release amount and duration of the alginate beads containing E*β*F, MeSA and a mixture of E*β*F and MeSA were measured. Third, field experiments were conducted to evaluate the ecological effects of alginate beads containing E*β*F, MeSA and a mixture of E*β*F and MeSA in wheat fields. Finally, a second-year field experiment was performed to investigate the functional significance of the optimum beads for use in the wheat ecosystems.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

Sodium alginate (CAS: 9005-38-3) (≥ 99%) used in the bead formulation was purchased from Tianjin Fuchen Chemical Reagents Factory. MeSA (CAS: 119-36-8; analytical reagent) and calcium chloride (CAS: 10043-52-4) were purchased from Tianjin Kaitong Chemical Reagents Co., Ltd. E*β*F was provided by F. Francis (Liege University, Belgium). The concentration of E*β*F was 1 mg ml^{-1} diluted in paraffin oil. The food additive *tert*-butylhydroquinone (CAS: 1948-2033-0) was used as an antioxidant and was purchased from Tengzhou Tianyi Industrial Trade Co., Ltd.

2.2 Alginate bead formulation

E*β*F, MeSA and E*β*F + MeSA alginate beads were manufactured separately. The doses of E*β*F and MeSA were recommended by Xu *et al.*[32] and by Wang *et al.*,[21] respectively: 20 ml (1.5% w/v) of sodium alginate solution added to 4.3 ml of sunflower oil, 0.2 g of food additive *tert*-butylhydroquinone (antioxidant), and 0.1 ml of E*β*F, or 10 ml of MeSA, or 0.1 ml of E*β*F + 10 ml of MeSA. The components were mixed using an Ultra-Turax system (GF-1 timed and adjusted high-speed disperser; Haimen Kylin-Bell Lab Instruments Co., Ltd) at 24 000 rpm for 40 s to obtain a thin and homogeneous emulsion. A peristaltic pump (BS100-1A; Baoding Signal Fluid Technology Co., Ltd) was then used to extrude the emulsion into a 3-mm diameter tube, and droplets fell into 200 ml (5% w/v) of $CaCl_2$ solution stirred using an agitator (85–2 constant temperature magnetic stirrer; Changzhou Putian Instrument Manufacturing Co., Ltd, Changzhou) at 20°C and 600 rpm to give alginate beads containing the semiochemical components. To give spherical beads, the distance between the tube of the peristaltic pump and the $CaCl_2$ solution was 20 cm. To stabilize the synthesis, beads remained in the $CaCl_2$ solution for 2 h. The beads were drained on a filter paper for 5 min before use as slow-release devices.

2.3 Release rate measurement

The release rates for E*β*F and MeSA emitted from the three types of beads were analyzed and measured separately using headspace-gas chromatography mass spectrometry (HS-GC–MS). Five treatments were used: (i) four alginate beads containing MeSA; (ii) 16 μl of pure MeSA (the amount used in the four MeSA alginate beads); (iii) four alginate beads containing E*β*F; (iv) 0.23 μl of pure E*β*F (the amount used in the four alginate beads of E*β*F); and (v) four alginate beads containing E*β*F + MeSA. In accordance with previous research,[12] four different alginate beads (approximate 100 mg) were transferred to different headspace vials, which were then sealed and placed in a thermally controlled room at 20 ± 2°C and 30 ± 2% relative humidity. One vial was selected daily for 15 days to measure the volatile content; each vial was measured only once. The cumulative volatiles were calculated, and the measurements were repeated three times.

The vial was incubated at 60°C for 5 min in a 7697A Headspace Sampler (Agilent Technologies). The injection pressure was 1723.75 Pa, and the injection time was 0.5 min. The transfer line was set at 80°C.

Chromatographic analysis was carried out on an Agilent 7890A gas chromatograph (Agilent Technologies) and a 7000D mass spectrometer (Agilent Technologies) using an HP-5 MS 5% diphenyl–95% dimethyl polysiloxane capillary column (30.0 × 250 × 0.25 μm, Agilent Technologies). The column temperature regime was 40°C for 2 min, followed by a 30°C min^{-1} ramp-up to 95°C for 2 min, a 35°C min^{-1} ramp-up to 155°C for 2 min, and a 40°C min^{-1} ramp-up to 280°C maintained for 2 min. Helium (99.9% purity) was used as the carrier gas at a column flow rate of 1 ml min^{-1} and a precolumn pressure of 7.0699 psi. The split ratio was 1:100. The ion-source temperature was 250°C. To increase the resolution of the E*β*F and MeSA analyses, MS detection was performed in the SIM acquisition mode. The *m/z* values were 15.00, 29.00, 39.00, 64.00, 65.00, 92.00, 120.00, 121.00 and 152.00 for MeSA, and 69.05, 91.06, 133.06, 161.12, 204.14, 256.26, 313.00, 373.41 and 460.15 for E*β*F.[38]

To obtain a standard curve, six E*β*F (dissolved in liquid paraffin) and MeSA (dissolved in ethyl acetate) gradient standard samples were selected. Both E*β*F and MeSA standard samples were as follows: 0.03125, 0.0625, 0.125, 0.25, 0.5 and 1 μg μl^{-1}. The samples were dissolved in 10 μl of siphon and transferred to the headspace under conditions similar to those described above. E*β*F and MeSA were tentatively identified by comparing their mass spectra with spectral data from the National Institute of Standards and Technology Library. Peak areas and corresponding concentrations were recorded, and the regression equation of the peak area and concentration was obtained by linear regression. The cumulative volatiles of EβF and MeSA were calculated from the regression equation.

2.4 Field experiments

Field studies were conducted on experimental farmland of the Weifang Academy of Agricultural Sciences, Shandong Province, China (119°22′E, 36°82′N), over a period of 2 years. In 2018, four treatment plots were used as follows: (i) control, beads free of MeSA and EβF; (ii) MeSA beads; (iii) EβF beads; and (iv) MeSA + EβF beads plots. Each plot measured 10 × 10 m, and the space with wheat between each plot was 10 m. In accordance with the experimental results in 2018, two treatments were used in 2019: (i) control plots, treated with beads free of EβF and MeSA; and (ii) plots treated with MeSA + EβF beads. The plots measured 10 × 10 m, and the space with wheat between each plot was 50 m. A completely randomized design was used both in 2018 and in 2019. Each treatment was replicated three times.

Wheat ('Lumai 21') was planted in rows with a row space of 20 cm and was sown on 15 October 2017 and 16 October 2018. No herbicide or insecticide was used in the experimental plots during the growing season.

Ten grams of alginate beads were placed in a dispenser (8.5 cm in diameter and 5 cm in depth) made of stainless steel with a rainproof lid and 30 small holes (1 mm in diameter). The dispenser was located over the wheat canopy at the center of each experimental plot and was 90 cm above the ground. The alginate beads were renewed every 2 weeks according to the release duration of the slow-release formula. The first application of the slow-release beads was on 5 April 2018 and 16 April 2019 for the two study years.

2.5 Sampling for *Sitobion miscanthi* and mummified aphids

The numbers of apterous and mummified aphids were sampled every week on five sampling dates. Each time, five sampling points were randomly selected along the bidiagonal lines in each plot, and 20 tillers in each point were selected. The alates were collected using yellow pan traps (27 cm in diameter and 10 cm in depth). Traps were made of polyethylene, and were located at the center of each plot and fixed 20 cm above the wheat canopy. Water was poured into the pan trap to two-thirds of the total volume, and an appropriate amount of detergent was added to prevent aphids from escaping. Traps were emptied and refilled weekly, and alate were transferred to a tube containing 70% ethanol, and brought back to the laboratory for identification. The parasitism rate was calculated by the formula [mummies/(aphids + mummies)].

2.6 Statistical analysis

In the 2018 field experiment, the effects of the mixture of EβF with MeSA on aphids and parasitoids were analyzed by analysis of variance (ANOVA) and least- significant difference test (LSD). In 2019, data were analyzed using the independent samples *t*-test. The statistical analysis software used was SPSS v. 19.0 for Windows.

3 RESULTS

3.1 External standard linear regression

Both EβF and MeSA show a significant linear relationship between the concentration of the standard sample and peak area. The linear regression equation between concentration and peak area for EβF was $y = 760\ 271x + 686\ 203$ (where x is the EβF concentration and y is the peak area; $R^2 = 0.9948$). For MeSA, the linear regression equation was $y = 31\ 132x + 1464.9$ (where x is the MeSA concentration and y is the peak area; $R^2 = 0.9997$).

3.2 Retention period and release rate of semiochemical carriers

EβF in the control (diluted in paraffin oil) was rapidly released within 2 days, whereas EβF from the slow-release beads was released over 15 days (Figure 1A). Pure MeSA showed stable homogenous release initially and was completely released within a week, whereas for MeSA and EβF + MeSA alginate beads release was over a period of 15 days (Figure 1B). According to the

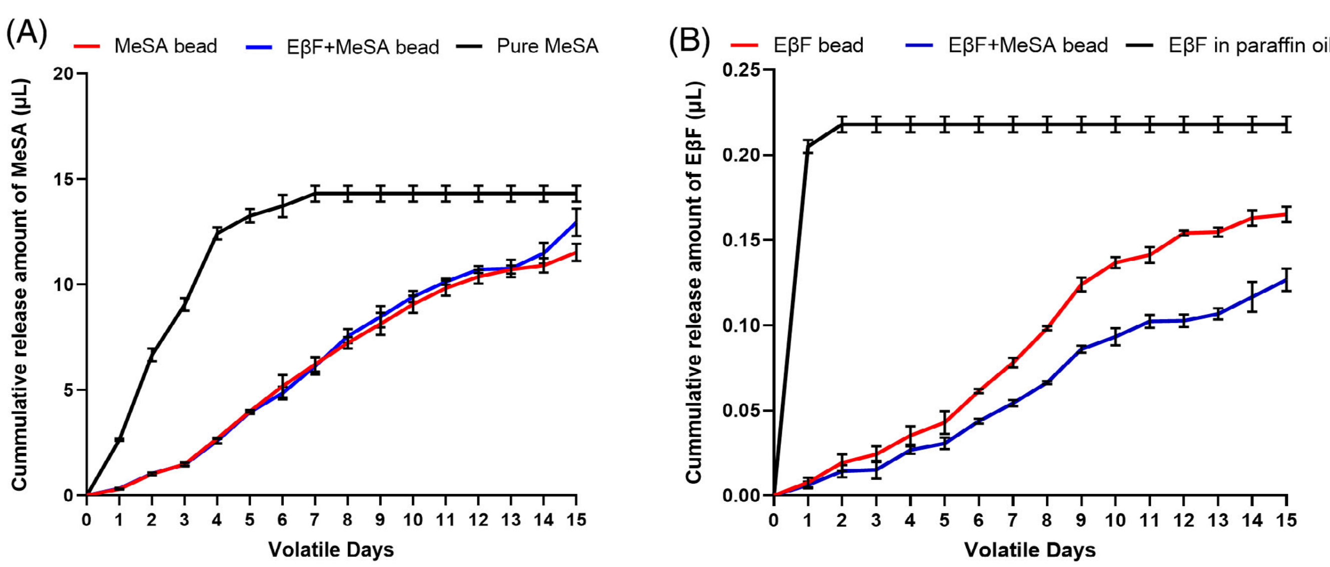

FIGURE 1. Cumulative amounts of methyl salicylate (MeSA) (A) and (*E*)-*β*-farnesene (EβF) (B) released from alginate beads, alone (pure or in paraffin oil) or in association over 15 days under laboratory-controlled conditions. Data are mean ± SE (n = 3).

variance analysis, alginate beads with EβF alone ($t = 8.07$, df = 28, $P < 0.001$) or with EβF + MeSA ($t = 7.13$, df = 28, $P < 0.001$) had a significantly slower release rate compared with the control. MeSA alone ($t = 4.30$, df = 28, $P < 0.001$) or EβF + MeSA alginate bead ($t = 4.21$, df = 28, $P < 0.001$) had significantly slower release rate than pure MeSA. Therefore, compared with EβF in paraffin oil or pure MeSA these slow-release beads can decrease the release rate and extend the volatilization period (Figure 1).

3.3 Effects of EβF and MeSA alone and in association on *Sitobion miscanthi* abundance

The population trends for alate and apterous *S. miscanthi* in the treatments were similar to the control conditions (Figure 2A,B). Alates occurred earlier than apterous aphids; peaks for alates were in week 2 (heading stage) and week 4 (flowering stage), whereas for apterous aphids a peak was seen was only in week 5 (filling stage). The treatment significantly affected the peak abundance of alates (week 2: $F = 24.9$, df = 3, $P < 0.001$; week 4: $F = 17.48$, df = 3, $P = 0.002$) and apterous aphids (week 5: $F = 17.56$, df = 3, $P = 0.001$). At the first peak of alates (week 2), numbers in bead-treated plots were significantly lower than in the control ($P < 0.05$). At the second peak (week 4), MeSA- and EβF + MeSA-treated plots significantly decreased alate abundance ($P < 0.05$) (Figure 2A). Moreover, MeSA- and EβF + MeSA-treated plots significantly decreased the peak abundance of apterous aphids at week 5 ($P < 0.05$) (Figure 2B).

Total abundances of alate and apterous aphids were significantly affected by treatment across the sampling period (alate: $F = 23.364$, df = 3, $P < 0.001$; apterous: $F = 74.631$, df = 3, $P < 0.001$). Alate and apterous aphid total abundances were significantly decreased in bead-treated plots compared with control plots ($P < 0.05$). Among the treatments, MeSA and EβF + MeSA significantly decreased the abundance of alate ($P < 0.05$) and apterous ($P < 0.001$) aphids compared with EβF. No significant difference in alate aphids was found between MeSA- and EβF + MeSA-treated plots ($P = 0.58$). Plots treated with EβF + MeSA beads showed a significant reduction in apterous *S. miscanthi* compared with the other treatments ($P < 0.001$) (Figure 3).

3.4 Effect of EβF and MeSA alone and in association on mummified aphid abundance

The abundance of mummified aphids and the parasitism rate were significantly affected by treatments (mummified aphids: $F = 4.94$, df = 3, $P = 0.032$; parasitism rate: $F = 16.38$, df = 3, $P = 0.001$). The abundance of mummified aphids was significantly increased in the bead-treated plots compared with untreated plots across the sampling period ($P < 0.05$) (Figure 4A). The parasitism rate under treatments was significantly higher than in the control ($P < 0.05$) (Figure 4B). Plots treated with EβF + MeSA beads had a significantly higher parasitism rate than plots treated with EβF beads ($P = 0.01$). No significant difference in parasitism rates was found between plots treated with EβF + MeSA and MeSA beads ($P = 0.08$), or between MeSA and EβF beads ($P = 0.27$).

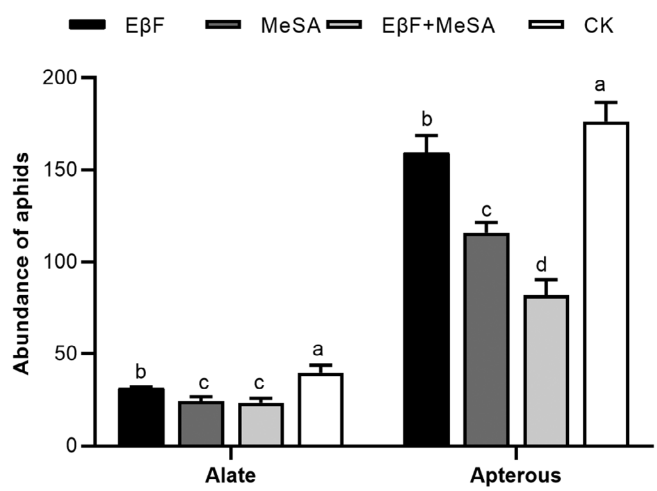

FIGURE 3. Effects of methyl salicylate (MeSA) and (*E*)-β-farnesene (EβF) alone and in association in slow release beads on *Sitobion miscanthi* total abundance for the entire duration of the experiment in 2018. Data are mean ± SE ($n = 3$). Different letters on bars indicate significant differences (one-way ANOVA, $P < 0.05$). CK, beads free of MeSA and EβF.

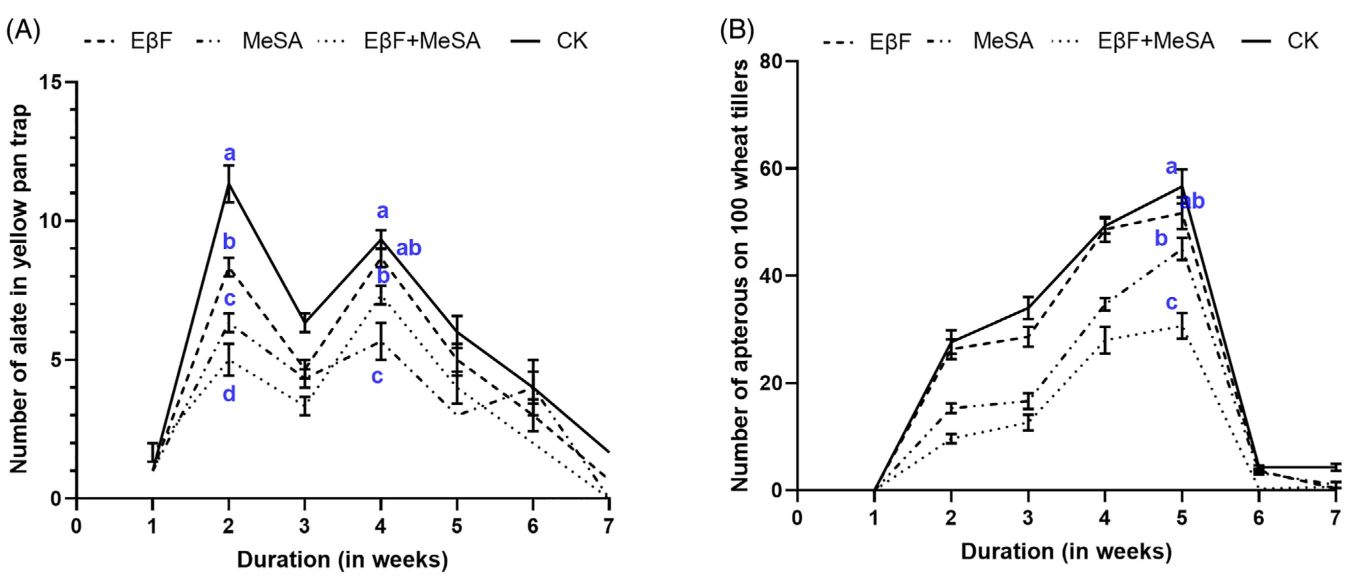

FIGURE 2. Effects of methyl salicylate (MeSA) and (*E*)-β-farnesene (EβF) alone and in association in slow release beads on the abundance of *Sitobion miscanthi* in 2018. (A) Alate aphids. (B) Apterous aphids. Data are mean ± SE (n = 3). Different letters indicate significant differences (one-way ANOVA, $P < 0.05$). CK, beads free of MeSA and EβF.

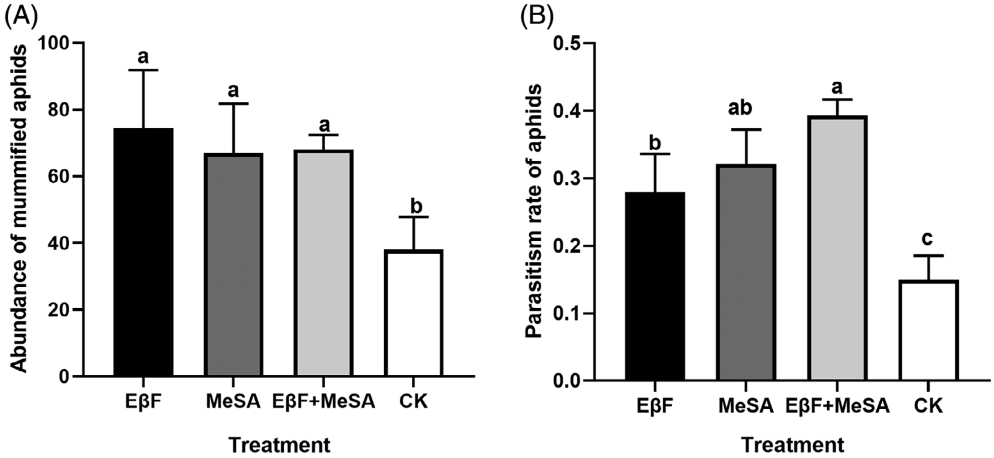

FIGURE 4. Effects of methyl salicylate (MeSA) and (*E*)-β-farnesene (EβF) alone and in association in slow release beads on the total abundance of (A) mummified aphids and (B) parasitism rate for the entire duration of the experiment in 2018. Data are mean ± SE ($n = 3$). Different letters on bars indicate significant differences (one-way ANOVA, $P < 0.05$). CK, beads free of MeSA and EβF.

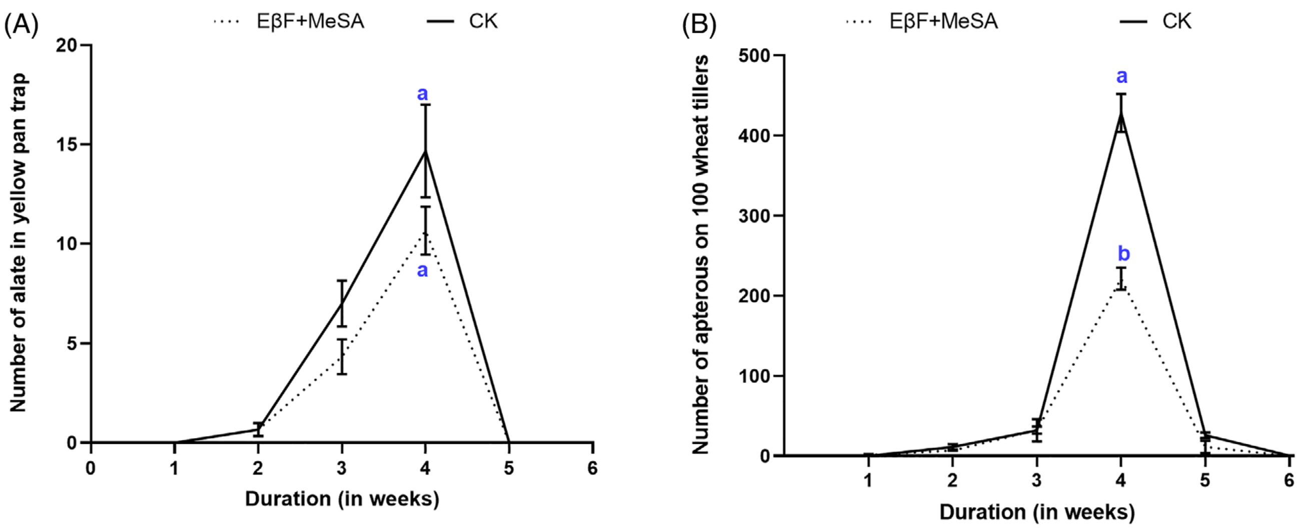

FIGURE 5. Effects of a mixture of (*E*)-β-farnesene (EβF) with methyl salicylate (MeSA) slow release beads on the abundance of *Sitobion miscanthi* in 2019. (A) Alate aophids. (B) Apterous aphids. Data are mean ± SE ($n = 3$). Different letters on bars indicate significant differences (independent samples t-test, $P < 0.05$). CK, beads free of MeSA and EβF.

3.5 Effect of EβF and MeSA mixture on *Sitobion miscanthi* and mummified aphid abundance

From the 2018 field test results, the EβF + MeSA bead was selected as the optimal formulation for the second year of the field test in 2019. Population trends for alate and apterous aphids in plots treated with EβF + MeSA beads were similar to the control (Figure 5). The peak abundance of apterous aphids in EβF + MeSA-treated plots was significantly lower than that of the control ($t = 7.532$, df = 3, $P = 0.002$). No significant difference in alate aphids was found between EβF + MeSA and the control ($t = 1.737$, df = 3, $P = 0.157$).

The total abundance of alates and apterous aphids significantly decreased in the EβF + MeSA-treated plot compared with the untreated plot across the sampling period (alate: $t = 3.55$, df = 4, $P = 0.024$; apterous: $t = 9.52$, df = 4, $P = 0.01$) (Figure 6). This formulation significantly increased the number of mummified aphids ($t = 9.34$, df = 4, $P = 0.01$) and improved the parasitism rate ($t = 9.73$, df = 4, $P = 0.001$) in wheat fields (Figure 7).

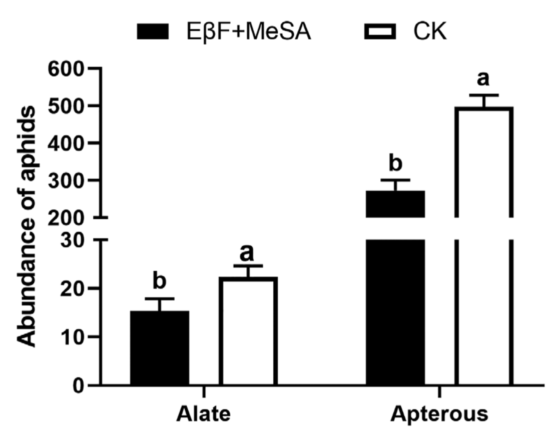

FIGURE 6. Effects of a mixture of (*E*)-β-farnesene (EβF) with methyl salicylate (MeSA) on the total abundance of *Sitobion miscanthi* in 2019. (A) Aalate aphids. (B) Apterous aphids. Data are mean ± SE ($n = 3$). Different letters on bars indicate significant differences (independent samples t-test, $P < 0.05$). CK, beads free of MeSA and EβF.

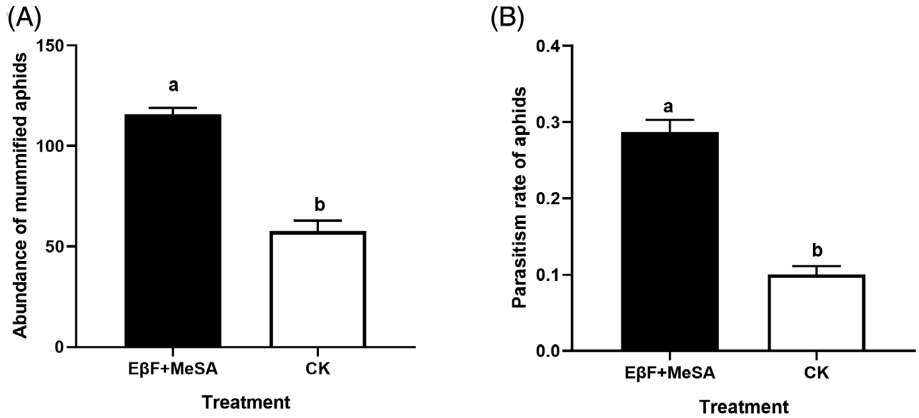

FIGURE 7. Effects of mixture of (*E*)-*β*-farnesene (E*β*F) with methyl salicylate (MeSA) slow release beads on the total abundance of (A) mummified aphids and (B) the parasitism rate in 2019. Data are mean ± SE (n = 3). Different letters on bars indicate significant differences (independent samples *t*-test, $P < 0.05$). CK, beads free of MeSA and E*β*F.

4 DISCUSSION

Understanding the nature of semiochemicals and their ecological role in trophic interactions is essential for the design of more sustainable strategies for pest management, including biological control.[39] Our laboratory experiment showed that the alginate beads that we manufactured containing E*β*F and MeSA either alone or in association had a slow-release effect compared with the control. Both E*β*F and MeSA in the beads exhibited stable and continuous release for at least 15 days, whereas E*β*F in paraffin oil and MeSA only remained volatile for 2 and 7 days, respectively. Furthermore, we optimized the formulation of the alginate beads and added antioxidants to reduce the likelihood of E*β*F and MeSA oxidation. In field experiments, an E*β*F + MeSA alginate bead showed enhanced efficacy in reducing the abundance of *S. miscanthi* and increasing the parasitism rate. Therefore, we suggest that alginate beads containing a mixture of E*β*F and MeSA could be the most effective candidate to control cereal aphids under the push–pull strategy.

E*β*F has ecological significance in its behavioral manipulation to aphids and their natural enemies in tritrophic interactions.[40] Several factors could contribute to the low abundance of *S. miscanthi* in the E*β*F-release plots. First, E*β*F may directly prevent aphid settling.[41] Second, E*β*F can disrupt feeding to prevent the aphid population growing.[42] Lastly, E*β*F-release plots may have developed larger populations of parasitoids that killed more aphids. Plant defense responses to sucking herbivores activated the salicylic acid signaling pathway[43] and these pests were generally repelled by MeSA. Several studies have shown that MeSA in crop fields other than wheat can be effective in repelling pests and attracting their natural enemies.[21, 23] The improved control shown using alginate beads with a combination of E*β*F with MeSA in this study might have been due to several factors. First, E*β*F can increase aphid mobility, enhancing their exposure to parasitoids attracted by MeSA.[44, 45] Second, parasitoids can use MeSA to locate wheat habitats infected by *S. miscanthi* and then use E*β*F to locate *S. miscanthi*,[46–49] Plots treated with E*β*F + MeSA may have attracted larger populations of parasitoids that parasitized more aphids than E*β*F or MeSA alone.

In the 2-year experiments, the E*β*F + MeSA treatment significantly increased the rate of winged aphids, whereas E*β*F or MeSA alone had no significant effects compared with the control (Figure S1). Although E*β*F could stimulate winged morph differentiation in the laboratory,[14] the field test was conducted under an open and complicated environment and the yellow pan trap could not capture all alates because of their high mobility. Moreover, a minimum density of aphids might be required for winged morph differentiation under E*β*F exposure. In the 2018 field test, maximum aphid density was below 70 per 100 plants (Figure 2), which might be too low to meet the demands of winged morph differentiation in E*β*F-treated plots. By contrast in the E*β*F + MeSA-treated plot, significant interactions between E*β*F and MeSA might increase the rate of alates. On the other hand, the large population of parasitoids might also induce the production of alates.[50] So the E*β*F + MeSA treatment could significantly increase the rate of winged aphids. In the experimental fields, a 2-m-wide strip of wildflowers such as *Cirsium setosum* Willd (Asterales: Asteraceae), *Cnidium monnieri* Linn (Apiales: Apiaceae) and *Lagopsis supina* Stephan ex Willd (Lamiales: Lamiaceae) could form a natural enemy bank in the surrounding area. Nectar and pollen from wildflowers could provide supplementary nutrients that increase the longevity and reproduction of parasitoids, thus enhancing the parasitic rates. A combination of wildflowers and slow-release alginate beads could increase the control efficacy against cereal aphids.

E*β*F was found to be sensitive to air and oxygen; a previous study showed that 77% of E*β*F was degraded after 24 h, and only traces were left after 48 h under normal temperature and sunlight conditions.[51] This instability and easy oxidization in nature restrict its application in the field. We optimized the formulation of the E*β*F and MeSA alginate beads and added an antioxidant, the food additive *tert*-butylhydroquinone, to reduce the possibility of E*β*F and MeSA oxidation. Moreover, the manufactured slow-release beads are nontoxic and cheap. The bead has a polyporous structure, and its chemical information is enclosed within a solid polymeric network. This polyporous structure is between a solid and a liquid, so volatile chemicals can be released fully.[12] Our field experiments showed that a combination of E*β*F and MeSA in an alginate bead was the most efficient formulation to control wheat aphids.

In the 2-year field experiments, the number of predators (for example, coccinellids, syrphid flies and green lacewings) was too small to have any significant effect on the aphids. Nevertheless, the MeSA alginate bead was found to increase the abundance of syrphid fly, *S. corollae*.[21] Previous studies have reported that the treatment with MeSA or EβF in other crops also increased the abundance of predators.[22, 52, 53] Alginate beads with a mixture of EβF with MeSA should be retested in crop fields to demonstrate their contribution to the biocontrol of aphid pest populations based on both predator and parasite combined activities.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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