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A Comprehensive Review on Entomovectoring in Agroecosystem

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Review Article

ABSTRACT

Protecting beneficial arthropods is essential, as they provide crucial services beyond pollination, including disease and insect pest management. The combination of several ecosystem services for agricultural sustainability requires the recognition that biodiversity is coupled with bio-complexity, productivity, resilience, and ecosystem functionality. Insects such as bumblebees, mason bees, and honey bees have long been employed professionally for pollination, likewise microbial biocontrol agents are frequently employed in pest management. A key aspect of pollination ecology is entomovectoring technology that utilizes managed bees to disseminate biocontrol agents to

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flowering crops. This can enhance crop yields by providing non-chemical protection against pests and diseases as well as enhancing pollination efficiency. This technology is based on bee management, manipulation of bee behaviour, components of cropping system, plant-pathogenvector-antagonist system which can be a trend-breaking pest management system in agriculture and will give double benefit to agriculture i.e. crop pollination and crop protection acting together for increased crop yield and quality.

Keywords: Pollination; biocontrol; entomopathogens; crop protection; bees; yield.

1. INTRODUCTION

Creating the next generation of its own is the ultimate goal of every living organism including plants [1]. Approximately 80 percent of flower producing plant species are dependent on animals, mostly insects for pollination [2]. Therefore, pollination is an important aspect of crop production, and awareness about this essential ecosystem service is important for everyone including general public, school children, farmers and also decision-makers from local, national to international levels as suggested by Food and Agriculture Organization (FAO) [3] (Fig. 1).

Pollination, a crucial biological process, serves as the cornerstone of agriculture by facilitating the reproduction of flowering plants and the important players in pollination are bats and various types of bees, along with other insects *viz.,* moths, hoverflies, birds, butterflies, wasps, thrips, and beetles, play a crucial role in pollination, impacting the economy significantly [4]. They inadvertently distribute pollens as they visit flowers in search of the nectar. Pollination is traditionally carried out by vectors like wind, water, and animals, underscoring the need for diverse pollinators in this process [5].

However, several issues, including diseases, pesticide usage, habitat loss, and climate change, have resulted in decrease of pollinator numbers, thus presenting serious obstacles to agricultural output [6]. To get rid of these problems, researchers have been looking at pollination alternatives such as entomovectoring. Farmers may increase crop yields and pollination rates by carefully placing pollinating insects in agricultural fields [7]. Since entomovectoring research is still in its infancy, more work is required to evaluate its methods, optimizing them, and determining their economic and scalability. Nonetheless, initial findings are encouraging, suggesting that entomovectoring is playing a crucial role in sustainable agriculture [8].

Fig. 1. A diagrammatic representation of role of pollinators and biological control agents in agroecosystem [9]

The crop quality and quantity are always enhancing due to bee pollination which provides its excellent value thus, improving global economic and dietary outcomes [10]. The pollinators like bumblebees, mason bee and honey bees act as entomovectors of microbial control agents (MCA) which happens by employing dispensers outside the bee outlet whose surface has been inoculated with biocontrol agents of desirable properties, which can be frequently employed in pest management [11].

Entomovectoring is more environmentally sustainable compared to other pollination methods as it relies on natural processes and does not introduce foreign substances into the ecosystem [12]. Unlike chemical pollinators or mechanical pollination techniques that have unpropitious effects on the environment and thus causes damage. This makes it an attractive option for eco-conscious farmers seeking to minimize their environmental footprint. Our present review highlights and emphasize the role played by bees in pollination and the utilization of pollination technology in agriculture for crop protection and crop production.

2. ENTOMOVECTORING: A SCOPE FOR BIOLOGICAL CONTROL

The integration of the technologies *viz*., pollination and biological control gives rise to an integrative technique that not only enhances the crop production but in addition, more precisely also gives protection to the crops thus comes the terminology *i.e.* entomovectoring technology [13]. To control the pest level of harmful insects and pathogens, natural enemies, such as parasitoids, predators are introduced into the environment of a crop pest [14]. If natural enemies are already present, they are encouraged to multiply and become more effective in reducing the number of pest organisms [15]. The biological control of potential pest insects can be increased by conserving current natural enemies, introducing new ones, and establishing stable populations. Additionally, mass rearing and periodic releases of natural enemies, seasonally or inundatively, amplify control efforts against potential pests, promoting ecological balance and reducing reliance on chemical pesticides [16].

A pollinating insect that is employed as a vector to disperse a substance for the biocontrol of plant diseases and pests is known as an entomovector [17]. The choice of vector species is decided by a combination of native species in the area to be pollinated, the plant species to be treated, and the ease of care of the vector species [18]. The substance is typically a powdered substance containing a virus, bacterium, or fungus to be used in protecting the host plant from a given disease or pest [19] (Table 1).

The insect, or vector, is typically exposed to this material by placing a tray containing this powder at the exit of hive or by blowing fans into the hive. This technology is nowadays employed in different crops of apples (storage rot disease), blueberries (mummification, grey mould), coffees (coffee berry borer), pears (fire blight), raspberries, tomatoes (grey mould), sweet peppers (plant bug, western flower thrips), strawberries (grey mould), rapeseed (plant bug), and sunflowers (Sclerotinia rot), flower thrips as a sustainable alternative to pests and diseases preventive management [20] (Table 2).

Contemporary agriculture must prioritize pollination to enhance crop protection and production. By embracing biodiversity and biocomplexity, agricultural sustainability can be achieved through improved productivity, resilience, and ecosystem performance. This holistic approach is essential for maximizing the benefits of pollination technology and ensuring a sustainable future for agriculture.

3. HISTORY OF ENTOMOVECTORING

In 2006, Heikki Hokkanen at University of Helsinki initiated a pioneering pilot study in Finland, marking the inception of entomovectoring [1]. This innovative approach to agricultural management utilized insects as vectors for beneficial microorganisms, aiming to enhance crop health and yield. Over the years, the success of this pilot study paved the way for significant developments in the field.

In Finland, by 2012 this application of entomovectoring had transitioned from experimental to practical, with large commercial farms incorporating this technique into their operations. Buoyed by the promising results in Finland, efforts to expand the scope of entomovectoring were undertaken in other regions too. Slovenia and Turkey became focal points for further experimentation and implementation of entomovectoring techniques, showcasing its potential as a viable agricultural practice beyond national borders. *Gliocladium catenulatum* (Prestop ® Mix) along with its entomovector were used in a total of 26 field tests on strawberries, with five further trials being done on raspberries. The field investigations have yielded good efficacy findings. According to the results, crop protection is on par with or better than that offered by a comprehensive chemical fungicide programme in all-weather scenarios and throughout a sizable geographic area (from Finland to Turkey). Entomovectoring reduced diseases by 47% on an average under conditions of high disease pressure, which was equivalent to many fungicide treatments [1].

In May 2019, the University of Belgrade's Faculty of Biology in Serbia took a significant step forward by hosting the First International Advanced Course on Entomovectoring. This event brought together a diverse cohort of 20 participants hailing from various countries, facilitated by instructors representing 10 nations [21]. Such international collaboration not only underscored the growing interest in underscored the growing interest in entomovectoring but also fostered knowledge exchange and expertise sharing on a global scale. Building upon this momentum, another milestone was reached with the organization of the International Workshop on Entomovectoring (November 3-5, 2021) and Curated by Heikki Hokkanen and Ingeborg Menzler-Hokkanen. This

SI. No.	Infected Crop	Name of microbial agent (Biopesticide)	Plant diseases	Entomovectors used
1.	Strawberry	Clonostachys rosea	Grey mould	Honey bee, Bumblebee
2.	Pome	1. Pseudomonas fluorescens 2. Pantoea agglomerans a.k.a. Erwinia herbicola + Enetrobacter agglomerans	Fire blight	Honey bee
3.	Raspberry, Strawberry	Clonostachys rosea and Trichoderma harzianum	Grey mould	Honey bee and/ or bumblebee
4.	Greenhouse cucumber	Binab-T® (Trichoderma harzianum and T. polysporum)	Cucumber rot (Didymella byoniae)	Bumblebee
5.	Lowbush blueberry	Clonostachys rosea	Botrytis blight	Bumble bee
6.	Cherry	Clonostachys catenulatum	Monolinia brown rot	Honey bee
7.	Rabbiteye blueberry	Streptomyces griseoviridis and Gliocladium catenulatum	Blueberry blossom blight	Bumblebee
8.	Lowbush blueberry	Clonostachys rosea	Mummyberry	Bumblebee
9.	Sunflower	Clonostachys rosea + Bacillus thuringiensis	Sunflower head rot + banded sunflower moth	Bumblebee
10.	Greenhouse	Trichoderma harzianum + Gliocladium virens	None indicated	Bumblebee
11.	None indicated	Trichoderma atroviride + Hypocrea parapilulifera	None indicated	Bumblebee
12.	Strawberry	Gliocladium catenulatum	Grey mould	Bumblebee
13.	Alfalfa	Coniothyrium minitans and Trichoderma atroviride	Alfalfa blossom blight	Alfalfa leafcutting bee
14.	Sunflower	Trichoderma spp. incl. harzianum $Sout < \cdot Sma 0 < 2020$	Sunflower head rot	Honey bee

Table 1. The list of plant diseases controlled by entomovectoring technology

Source : Smagghe, 2020

Source: Smagghe, 2020

workshop convened 23 experts from 11 different countries at University of Eastern Finland but
also served as a forum for in-depth served as a forum for in-depth discussions, research presentations, and strategic planning aimed at further advancing, understanding and applications of entomovectoring in agriculture.

4. MECHANISM OF ENTOMOVECTORING

When a honey bee exits from its hive through a specialized dispenser that dispenses the Microbial control agent (MCA), covering itself with thin powder coating. A portion of this biological control agent is left behind when it lands on a flower. The powder is also spread throughout the leaves as it soars across the field, returning its its colony 'clean' to discharge its collected nectar and pollen [22]. The MCA left behind on the blossoms and foliage may begin to fight insects and diseases right away. It may also colonize the flower and serve as a preventative measure for the fruit that will eventually form and spread [12] (Fig. 2).

5. FACTORS RESPONSIBLE FOR SUCCESSFUL ENTOMOVECTORING

This section consists of the role of microbial control agents (MCAs) like fungi, bacteria, and viruses, and the utility of vectoring by pollinating insects in managing economically significant diseases and pests in agriculture and horticulture are discussed [23]. However, the success of entomovectoring hinges on the suitable interactions among vector, control agent, formulation, and dispenser. It must also ensure environmental and human health safety. The formulation of MCAs is crucial for their transport by the entomovector to its target [15]. While many commercial powdery MCA formulations are designed for water-spray application, enhancements can be made to optimize their transport by vectors. This underscores the importance of refining formulations to maximize the efficacy of entomovectoring in pest and disease control.

However, the acquisition of the product on the body of the vector is not only affected by the

Fig. 2. A diagrammatic representation of Mechanism of entomovectoring (8, designed using Biorender online software *Source[:https://www.biorender.com/](https://www.biorender.com/))*

formulation, but also the dispenser needs to be appropriate. Over the past 20 years, multiple authors have reported on the development of different dispenser systems to allow the loading of vectors with a biocontrol agent. In entomovectoring studies, dispensers can be categorized into two types: one-way dispensers and two-way dispensers. In one-way dispensers, the entrance and exit chambers for vectors are either the same or not fully separated, causing the vectors to pass through the powder both when they leave and return to the hive. Conversely, two-way dispensers have completely separated entrance and exit chambers, ensuring that only the vectors exiting the nest come into contact with the powder [23]. To date, eight dispenser types and a few modified versions have been designed for *A. mellifera*. In parallel, six dispensers were developed for bumble bees, whereof two consisted of modified version for *B. impatiens*, three of *B. terrestris*, and one of *O. cornuta* for the solitary orchard pollinator [24].

The environmental and human safety of the entomovector strategy [8] must be addressed as a final aspect. MCAs used in practice have been isolated from the environment, present in the target ecosystem, but rarely come into contact with vectors under natural conditions as they mainly residing in soil or foliage. Evaluating their safety towards vectors is crucial. So far, only a few MCAs have been employed, warranting a

discussion on their efficacy and limitations at a broader level. Vectoring studies have primarily noted mortality when disseminating insecticidal MCAs, yet caution is required as sublethal effects may not be immediately apparent [25]. Therefore, understanding the broader implications of entomovector strategies necessitates considering both their effectiveness and potential risks.

Basic studies on the senses of olfaction and taste in insects are essential building blocks for developing functional entomovectoring systems. It was necessary to consider the knock-on impacts of the current climate change, including weather extremes and how they affect entomovectoring systems. The contributions to the virtual special issue of Arthropod-Plant Interactions titled "Pollinator-plant interactions as the basis for entomovectoring" represent an effort to gather data that highlights some of the obstacles and bottlenecks in this field of study [26].

6. SUCCESS STORIES ON ENTOMOVECTORING

A potential and positive alternative for chemical pesticide dependency and plant disease and insect pest management is the use of microbial biocontrol agents. The uneven effectiveness of these agents in field settings, which is frequently brought on by the poor establishment and restricted dissemination of microorganisms, presents a serious obstacle to their application. One potential answer to these problems is entomovectoring, a method in which pollinators distribute microbial biocontrol substances to crops.

6.1 Japanese Orchard Bee, *Osmia cornifrons*

6.1.1 Testing of modified bee hive for entomovectoring

Osmia cornifrons [27] used in entomovectoring for fire blight management BR using Serenade® MAX, *Bacillus subtilis* strain QST 713 formulation which contained a minimum of 7.3 x 10⁹ CFU/g placed in the grooves at the exit of the nest dispenser. The design contained a simple wooden structural having an exit ramp design made up of transparent plastic and then a shallow station sitting at the base of the exit ramp designed to hold the biocontrol product in fine powdered or granular form, and the results showed that on an average, the bees were using the right exit 95% of the time and were exposed to the biological control product *B. subtilis*, Serenade. However, only about 50% of bees under observation returned via the lower entry tubes of the dispenser nest. The remaining bees in this observation re-entered via the exit grooves of the dispenser nest.

6.1.2 Testing of total quantity of formulation loaded by entomovectors

A study [27] conducted to quantify the biological control agent collected by bees, *O. cornifrons*, from dispensers. Bees were captured and placed in glass vials containing 2 mL of sterile 0.1 M potassium phosphate buffer and 0.1 mL of Tween-20 surfactant per litre. After vigorous shaking and sonication, the bacteria adhering to the bees were removed. After serial dilution, the samples were plated on NYDA medium and incubated at 28°C. Bees from automats without the product served as a control. Results showed that *O. cornifrons* bees from the redesigned dispensers carried approximately 20 times more Serenade than the previous methods.

6.1.3 Testing on secondary transmission of primary inoculum

In a carefully designed experiment, *B. subtilis* was strategically introduced into the ecosystem

as a biological control to combat fire blight. The process involved the transfer of the bacteria by bees from the flowers to the crabapple trees. These trees, initially exposed to the treated bees, were then relocated to an isolated area. Only the flowers visited by the treated bees were selected
for the subsequent transfer experiment. for the subsequent transfer Meanwhile, another group of trees that had not been touched by the bees or the treatment served as a control group nearby. It is noteworthy that after exposure to new, untreated bee nests, previously uninfected flowers showed successful secondary transfer of bacteria. The significant increase in bacterial colonies on these flowers underlines the self-sustaining nature of the system. These results offer promising prospects for effective control of fire blight in commercial orchards by promptly controlling the pathogen, *Erwinia amylovora* [27].

6.2 Indian Bee, *Apis cerana cerana*

6.2.1 Study on acute and oral toxicity to entomovectors

The study conducted to investigated the acute oral and contact toxicity of *Trichoderma. harzianum* spore powder in Indian honey bees (approx. 100 forager bees). In this experiment, the forager bees were fed with 50% sucrose solution containing spores and contact toxicity tests were conducted by applying the spore powder directly to the bees. Interestingly, in the oral toxicity test, no bee mortality was observed after 24 and 48 hours in both the experimental and control groups. However, in the contact toxicity test, a mortality rate of 13.3% was observed in the experimental group after 48 hours. These results highlight the importance of understanding the potential effects of biological control agents such as *T. harzianum* on nontarget organisms such as honey bees and emphasize the need for further research in this area to ensure environmental safety [28].

6.2.2 Efficacy of dispersants for dilution of spore powder

Furthermore, a screening process was conducted to determine the efficacy of three different substances as alternative dispersants for dilution of spore powder: wheat flour, starch, and sugar powder. The spore powder was mixed with *T. harzianum* in a mass ratio of 1:1 and then spread on the bottom of large test tubes. The results showed that the wheat flour mixture with *T. harzianum* spore powder facilitated the transmission of the most significant amount of spore powder, reaching 2.81×10⁷ CFU/ bee [28].

6.2.3 Optimization of dispersant and biopesticide dilute

Afterward, the greenhouse evaluation of the optimal dilution factor of *T. harzianum* spore powder was studied using wheat flour and *T. harzianum* spore powder dilutions in 8:1, 4:1, 2:1, 1:1, 1:2, and 1:4, respectively. when dilution was 1:1, the number of spores on strawberry flowers visited by spore carrying bees's was between 3.25×10^5 and 2.85×10^6 CFU/ flower, with an average spore count of 1.31 \times 10⁶ CFU/flower. This result confirmed that the use of spore dispensers facilitated efficient up-take, transmission, and release of spore powder by Indian bee (*Apis cerana indica*) [28].

6.3 Bumble bee, *Bombus terrestris*

6.3.1 Efficacy of biocontrol by entomovectoring

The bee-vectored *Aureobasidium pullulans* for biocontrol of gray mold in strawberry was studied by employing *Bombus terrestris* along with Flying Doctors® system (containing a queen and 100- 110 workers) with an integrated product dispenser. For conducting trials, the fungal strains, *A. pullulans* (AP-SLU6) and *Bacillus cinerea* (B05.10) formulations were prepared using wheat-bran based powder for *A. pullulans* and a control (without *A. pullulans*) was also prepared. Along with above material an existing Prestop® Mix (*Clonostachys rosea f. catenulata*) was also used. The results showed that bumblebee exiting the hives carried, an average of 3.31 × 10⁵ and 4.38 × 10⁵CFUs/bee of *A. pullulans* and *C. rosea, f. catenulata* (PrestopVR Mix), respectively. The difference between *A. pullulans* and Prestop Mix was not found to be significant [22].

6.3.2 Estimation of *A. pullulans* **CFUs delivered to strawberry flowers**

Similarly, to study the estimation of *A. pullulans* CFUs delivered to strawberry flowers, the flowers were excised and number of CFU's were counted using serial dilution technique. It was estimated that all flowers sampled plants which were visited by bees were loaded with *A. pullulans* and contained considerable amounts of this biocontrol agent (between 1.2×10^2 and 2.1 \times 10³ CFU/flower) [22].

6.3.3 Bio-efficacy of *A. pullulans* **by entomovectoring**

The Biocontrol of grey mould using *A. pullulans* was performed in a greenhouse, which consisted of six treatments i.e. (T1) control formulation vectored by bees, (T2) *A. pullulans* powder formulation vectored by bees, (T3) Prestop® Mix vectored by bees, (T4) control (i.e. no bees or formulations), (T5) spray application of *A. pullulans* liquid formulation and (T6) spray application of water only. The results showed that the plants treated with *A. pullulans* vectored by bumblebees reduced the disease score on freshly harvested fruits (day 0) by 69% as compared with the control treatments *A. pullulans* and Prestop Mix, vectored by bees, significantly lowered the gray mold infection on fruits compared with control treatment; this reduction corresponded to 73 and 50%, respectively [22].

6.3.4 Study on shelf life of fruit

To study the effects of *A. pullulans* on grey mould infection and strawberry shelf life, the above-mentioned biocontrol treatments (T1 – T6) was tested by harvesting strawberries. The punnets of fruits were stored at 4 °C for 3 weeks and it showed a significantly prolonged shelf life (100%) compared with to control [22].

6.3.5 Potential of entomovectoring

It demonstrated that *Lactiplantibacillus plantarum* (AMBP214) formulation is a potential biocontrol agent when loaded bumblebees were released into a greenhouse of strawberry plants. *L. plantarum* AMBP214 was effectively dispersed to flowers, resulting in high bacterial abundance (an average of 1 \times 105 CFUs per flower) and consistent coverage across all sampled flowers [29].

7. ENTOMOVECTORING IN INSECT PEST MANAGEMENT

7.1 Management of western flower thrips (*Frankliniella occidentalis***) using entomovectors**

The evaluation of biocontrol strategies such as apivectoring was done using bumblebees (*Bombus impatiens)* in greenhouse strawberry production facility, where they determined how well the conidia of entomopathogen, *Beauveria bassiana*, would be disseminated for control of crop pests such as the western flower thrips (*Frankliniella occidentalis)* and results showed that the bumblebees effectively dispersed the formulation of *B. bassiana* throughout the greenhouse crop and had minimal impacts on bumblebee populations, with under 16% mortality attributed to infection by *B. bassiana* and with up to 75% of *Frankliniella occidentalis* collected from some treatment zones testing positive for infection by *B. bassiana* [30].

7.2 Whitefly (*Trialeurodes vaporariorum***) and Tarnished Plant Bug**

In 2008, it was explored bumble bee pollinators' effectiveness in co-vectoring two fungi, *Beauveria bassiana* and *Clonostachys rosea*, in greenhouse tomato and sweet pepper crops. They aimed to control insect pests like whitefly (*Trialeurodes vaporariorum*) and tarnished plant bug (*Lygus lineolaris*), as well as grey mould (Botrytis cinerea). The experiment involved three groups: one with active inoculum vectored by bees, another with heat-inactivated inoculum vectored by bees, and a control with no inoculum or bees. Results showed the active inoculum reduced *T. vaporariorum* by 49% in tomatoes and *L. lineolaris* by 73% in sweet peppers. Grey mould suppression rates ranged from 46% to 59% across the crops, suggesting promising pest management potential with bee-mediated fungal transmission [31].

7.3 Biosafety of Biopesticide Formulations towards Entomovector

However, it is important to quantify the hazards posed by the microbial biocontrol agent to the vectoring insect before using such an entomovectoring system. It investigated the impact of a biocontrol agent on entomovectors (*Bombus terrestris*) using a powder containing 10⁷ spores of *Metarhizium anisopliae*. Bumblebees carried $9.3 \pm 1 \times 10^6$ spores/bee after passing through a dispenser with 107 spores/gram for 60 seconds. The uptake on the bumblebee's body was 2.5 times higher. This indicates the potential toxicity of the agent. Future research could explore the entomovector system's efficacy in flower protection against *M. anisopliae*, ensuring vector safety while sufficiently loading it for effective inoculation and protection [32].

8. ENTOMOVECTORING IN INDIA

8.1 Utilization of Honey Bees as Entomovector for *Helicoverpa armigera* **NPV (HaNPV)**

In a study conducted at Anand Agricultural University, Anand, Gujarat to assess the effectiveness of honey bees as entomovector of *Helicoverpa armigera* NPV (HaNPV) in pigeonpea, the activity of the entomovector bees ranged from 1.19 bee/5 min/m^2 to 2.78 bee/5 min/m² , with the highest activity recorded at a distance of 10 m; the lowest activity was observed at 50 m and the lowest at 100 m from the dispenser [33].

Similarly, a tendency of 4.00×10⁵ POB for the total mean HaNPV load carried by bees was also noted in particular applications. In various applications, the bees' mean HaNPV load varied between 1.70×10^5 and 5.95×10^5 POB. On flower,
the average HaNPV load was the average HaNPV load was 13.9×10^{3} ±53.2 $\times10^{3}$ POB. When the flower was 10 meters away from the dispenser, the total mean HaNPV load was much higher than when it was 50 and 100 metres away [33].

8.2 Study on Efficacy of Entomovectoring in Relation to Distance between Dispenser and Crops

The *H. armigera* larval mortality in both bioassays declined as the distance from the dispenser increased. The *H. armigera* larval population was found to be the lowest at 10 m from the dispenser (2.22 larvae/10 twigs), compared to 2.42 larvae/10 twigs at 50 m and 2.63 larvae/10 twigs at 100 m. The *H. armigera* population varied considerably between treatments both during and after application [33].

9. CONCLUSION

To sum up, the amalgamation of pollination ecology with entomovectoring technology is poised to revolutionize contemporary agriculture by encompassing both crop production and protection as an integrated process. Adopting entomovectoring is a big step towards environment friendly farming practices that ensure a healthy environment. Furthermore, apiculture will become more prevalent courtesy to this new technology, which will build an ecology for farming that is both balanced and sophisticated in its design. Contrary to these conventional readings, it makes more sense to see the most recent management system as a means of achieving predictable results. This is a more sustainable and productive method of farming. This new perspective of combining pollination technology and pest management is beneficial, and this change will help to resolve food defense challenges while maintaining the vital equilibrium of the atmosphere as an entire system.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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