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Scope of Entomopathogenic Fungi in the Mustard Ecosystem: A Review

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ABSTRACT

Mustard (*Brassica juncea*) is a versatile and widely cultivated plant in the Brassicaceae family. Among the primary factors impeding mustard yield, insect pest damage stands prominent. Insect pests such as mustard aphid (*Lipaphis erysimi*), mustard sawfly (*Athalia lugens proxima*), painted bug (*Bagrada hilaris*), diamondback moth (*Plutella xylostella*) and cabbage butterfly (*Pieris brassicae*) cause significant damage to mustard production. The excessive reliance on chemical insecticides to control these insect pests may negatively affect non-target organisms such as beneficial insects, pollinators and natural enemies of pests, thus disrupting ecosystem balance and posing risks to biodiversity. In pursuit of sustainable alternatives to broad-spectrum chemical pesticides, entomopathogens play a pivotal role in safer pest management strategies. These biological control agents, including various fungi, bacteria, viruses and nematodes, specifically target insect pests while being safer for non-target organisms and the environment. With more widespread commercialization, entomopathogenic fungi hold significant promise in bolstering integrated pest management practices.

Key words: Biological control, Entomopathogenic fungus, Entomopathogens, Management, Mustard, Pest.

Oilseeds constitute the second most significant sector in India's agricultural economy after cereals, with a consistent growth rate of 4.1 per cent annually over the past three decades (Jat *et al.,* 2019). Mustard (*Brassica juncea* L.) (Brassicaceae) plays a significant role in the total oilseed production of India, contributing approximately 28.6% of the total edible oil output. The distinct pungency of mustard seeds and oil is due to the glycoside known as "Sinigrin" ($C_{10}H_{16}O_9NS_2K$), which makes it ideal for use in condiments and suitable for making pickles, curriesand vegetable dishes (Parihar *et al.,* 2014). It holds the second position among the most important edible oilseeds in India, right after soybean, in terms of area (24%) and production (25%) (Singh *et al.,* 2013; Pradhan *et al.,* 2020a). In India, mustard covers around 6.69 million hectares of area with an annual production of 10.11 million tonnes, yielding about 1511 kg/ha (Anonymous, 2021). Despite the availability of advanced production technology, mustard crops fail to achieve their potential yield in the country, primarily due to significant losses caused by various biotic and abiotic factors. Among oilseed crops, mustard is susceptible to a diverse array of insect pests, spanning from sowing to harvest. In India, 24 distinct species of crucial insect pests affect mustard and rapeseed crops, leading to substantial damage during various stages of the crop's growth. Notably, among these pests, the ones with a significant impact on mustard cultivation include the mustard aphid, mustard sawfly, painted bug and leaf miner (Pradhan *et al.,* 2020b; Yadav and Rathee, 2020).

Over-reliance on chemical insecticides to manage insect pests in mustard crops disrupts the balance of beneficial organisms like parasitoids and predators within the mustard ecosystem and thereby leads to the adoption of biological control methods, which ensure sustainable mustard production by effectively managing sucking pests. Besides beneficial insects, in the biological control of insect pests, entomopathogens have a crucial role in the bio-intensive IPM approach, providing an eco-friendly path of insect pest suppression (Gurulingappa *et al.,* 2011). Entomopathogens are microorganisms that can infect insects and utilize them as hosts to complete their life cycle (Mora *et al.,* 2018; Litwin *et al.,* 2020). These microorganisms help to reduce insect pest populations to levels that do not cause significant economic damage to crops (Beemrote *et al.,* 2024). Furthermore, they serve as an effective means of controlling and reducing the populations of disease vectors (Scholte *et al.,* 2004). Around 80 per cent of insect diseases are caused by fungal pathogens (Mora *et al.,* 2018). Worldwide several fungal species like *Beauveria bassiana* (Al Mazra'awi *et al.,* 2006;

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Ujjan and Shahzad, 2014; Janu *et al.,* 2018; Javed *et al.,* 2019; Shaalan *et al.,* 2021); *Lecanicillium lecanii* (Rana and Singh 2002; Janu *et al.,* 2018), *Metarhizium anisopliae* (Ahmed, 2013; Duraimurugan and Sujatha, 2023); *Hirsutella thompsonii* (Ramanujam *et al.,* 2014; El-Sharabasy, 2015) and *Paecilomyces fumosoroseus* (Priyatno and Ibrahim, 2004; Pineda *et al.,* 2007) have been reported pathogenic to aphids (*Aphis gosyppi, Myzus persicae, Lipaphis erysimi*); leafhoppers (*Amrasca devastans, Nephotettix virescenes, Empoasca sp.*), whitefly (*Bemisia tabaci; Trialeurodes vaporariorum*), bugs (*Bagrada hilaris*, *Lygus lineolaris*).

The development of commercial formulations of entomopathogenic fungi (EPF) for crop pest management has developed diverse products, with *B. bassiana* and *M. anisopliae* being most prevalent at 33.9 per cent each, followed by *I. fumosorosea* (5.8%) and *B. brongniartii* (4.1%) (Moorhouse *et al.*, 1992; Hajek and Leger, 1994; de Faria and Wraght, 2007). In nature, the most common taxa are those of the Ascomycota and Entomophtoromycota (Litwin *et al.,* 2020).

With approximately 90 genera and 700 species of insect-infecting fungi (Ramanujam *et al.,* 2014), the potential of EPF is vast, indicating sustainable and environmentally friendly pest management strategies. Therefore, keeping these facts in view, this topic discusses the effective use of entomopathogenic fungus against different insect pests of mustard.

Mode of action of entomopathogenic fungi

Entomopathogenic fungi invade insects by directly penetrating their cuticle. This differs from bacteria or viruses, as the fungi do not require ingestion by the insect to initiate infection (Sevim *et al.,* 2015; Bilgo *et al.,* 2018). According to Clarkson and Chamley (1996), this process is partially physical and partially enzymatic. The mode of action of the entomopathogenic fungi initiates with the fungal spores adhering to the insect's cuticle, where they germinate and enter the cuticle by creating an appressorium. Apressorium exerts strong mechanical pressure on the insect's cuticle, while also secreting lytic enzymes such as proteases, lipases and chitinases to break down the insect's epicuticle. Once inside the insect's body cavity (hemocoel), fungal hyphae grow and may produce blastospores that further spread the infection within the host's tissues. Secondary metabolites released by the fungi induce paralysis and suppress the insect's immune responses (Donzelli and Krasnoff, 2016; Altinok *et al.,* 2019; Litwin *et al.,* 2020). This infection process typically spans around 14 days, with initial symptoms appearing approximately 7 days post-infection. Eventually, the infected insect's body becomes stiff due to fluid absorption by the fungus. After consuming all nutrients and killing the insect, fungal hyphae emerge from the cadaver through natural openings (oral opening, anal opening, spiracles) and produce resting or infective spores, facilitating further spread to other individuals (Skinner *et al.,* 2014). Asexual spores can spread by saprophytic development on these dead individuals and cause permanent sexual and asexual cycles (Altinok *et al.,* 2019) (Fig 1).

Use of entomopathogenic fungi for managing insect pests of mustard

One of the significant contributors to the low yield of mustard is the damage caused by various insect pests. In India, over 43 species of insect pests have been documented to affect mustard crops with approximately 12 of these species classified as major pests (Patel *et al*., 2019; Pradhan

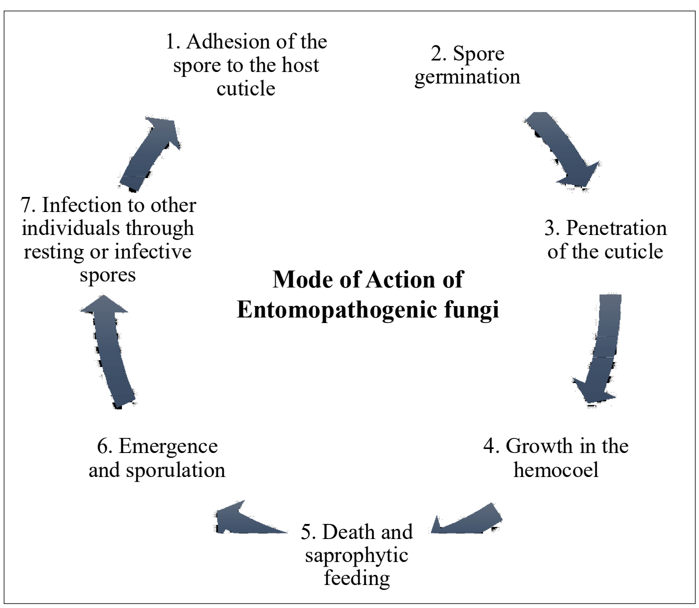

Fig 1: Steps of infection by Entomopathogenic fungi (Source: Sinha *et al.,* 2016).

et al., 2020a). Out of various (24) insect pests (Rai *et al.,* 1976), sawfly (*Athalia lugens proxima* Klug.), leaf miner (*Chromatomyia horticola* Gorreau), painted bug (*Bagrada cruciferarum* Kirk.), flea beetle (*Phyllotreta cruciferae* Goeze), diamondback moth (*Plutella xylostella* L.), cabbage butterfly (*Pieris brassicae* L.), mustard aphid (*Lipaphis erysimi* Kalt.), cabbage aphid (*Brevicoryne brassicae* L.) and green peach aphid (*Myzus persicae* Sulzer) are considered important to cause considerable losses (Table 1).

Mustard aphid

Mustard aphid (*L. erysismi*) is one of the most destructive insect pests in mustard ecosystem, inflicting staggering losses of up to 96 per cent throughout the growth cycle, from seedling to maturity (Gautam *et al.,* 2019; Pradhan *et al.,* 2020a). Nymphs and adults suck the cell sap from different plant parts like inflorescence, shoots, pods and underside of the leaves, impairing growth, causing flower wiltingand disrupting pod development, resulting in about 35-90 per cent yield loss depending upon the seasons (Biswas and Das, 2000; Rohilla *et al.,* 2004; Gautam *et al.,* 2019). Their excretion of honeydew onto the leaves fosters the proliferation of black sooty mold, thereby hindering the photosynthetic activity of the leaves. Mustard aphid, *L. erysimi* is prevalent and reported to be a major pest of rapeseed and mustard. *Brassica juncea* experiences yield losses from 10-90 per cent (Rana, 2005), alongside a 5-6 per cent decrease in oil content (Shylesha *et al.,* 2006). Moreover, Patel *et al.* (2014) reported a significant reduction in yield of up to 97.6 per cent under field conditions when compared to protected environments.

Deka *et al.* (2017) found varying degrees of susceptibility among mustard aphids exposed to spore suspensions of *M. anisopliae*, *N. releyi*, *V. lecanii* and *B. bassiana*, with all fungal isolates at the highest spore concentration (10^8 spores/ml) resulting in high mortality. *M. anisopliae* exhibited the highest virulence, followed by *N. releyi*, in controlling mustard aphids, as indicated by LC_{50} and LT_{50} values. In certain isolates of *M. anisopliae*, a substantial 64 per cent level of virulence was recorded against mustard aphid populations within a brief infection period of 3.8 days (Araujo *et al*., 2009). However, Ujjan and Shahzad (2012) documented the effectiveness of *L. lecanii*, *M. anisopliae* and *B. bassiana*, observing mortality rates of 98, 72 and 88 per cent respectively among mustard aphid populations within 3 days at a spore concentration of 10^7 spores/ml. Pradhan *et al.* (2020b) reported the highest mortality of mustard aphids due to *L. lecanii* (85.90%) followed by *B. bassiana* (82.39%) and *M. anisopliae* (78.71%). Similarly, Hayden *et al.* (1992) assessed the efficacy of *L. lecanii* and *B. bassiana* against aphids, revealing *L. lecanii* to be the most virulent with LT_{50} of 2.4 days, while *B. bassiana* exhibited a longer LT_{50} of 9.5 days.

Kumar (2021) observed the combined application of azadirachtin 5000 ppm @ 5 ml/liter of water followed by *B. bassiana* @ 2 g/liter of water, as an effective control measure against *L. erysimi*. It was concluded by Rahul *et al.* (2020) that the timely application of bio-insecticide *V. lecanii* can protect mustard against aphid and helps in increasing crop yield. These results were aligned with the findings of Sundria *et al.* (2019) found *V. lecanii* (1×10^8) @ 5.0 g/l as most effective against mustard aphid and followed by *M. anisopliae* (1×10^8) @ 5.0 g/l and *B. bassiana* (1×10^8) @ 5.0 g/l. Conversely, Deka *et al.* (2017) reported that *M. anisopliae* was most effective in causing the highest mortality of mustard aphid compared to *B. bassiana* and *V. lecanii*. Additionally, it was suggested that 25 per cent (5.39×10^8 CFU) of *M. anisopliae* induced mortality rates ranging from 19 to 83 per cent, whereas 25 per cent of *B. bassiana* (4.78×10^8 CFU) caused 16 to 78 per cent mortality of mustard aphids, highlighting the potential of *M. anisopliae* as a promising entomopathogenic fungi for integrated pest management against mustard aphid due to its field efficacy (Sajid and Zia, 2017). Patel *et al.* (2021) observed that a sequential application of Flonicamid 50 WG at 0.02 per cent, followed by *B. bassiana* 1.15 WP at 0.006

Table 1: Important insect pests of mustard ecosystem and their associated entomopathogenic fungi.

Insect pest	Family	Entomopathogenic fungi	Doses	Reference
Mustard aphid, *L. erysimi*	Hemiptera: Aphididae	*B. bassiana* *V. lecanii* *M. anisopliae*	2 g/ltr. 5 g/ltr.	Kumar (2021) Sundria *et al.* (2019)
Mustard sawfly, *A. lugens proxima*	Hymenoptera: Tenthredinidae	*B. bassiana* *Lecanicillium lecanii*	10^{13} conidia/ml 0.59 larvae/plant	Rabha (2009) Vinyas *et al.* (2022)
Painted bug, *B. hilaris*	Hemiptera: Pentatomidae	*B. bassiana* *L. lecanii* *N. rileyi*	2.5 kg/ha 2.0 kg/ha 2.5 kg/ha	Kalasariya and Parmar (2019)
Diamondback moth, *P. xylostella*	Lepidoptera: Plutellidae	*B. bassiana* *M. anisopliae*	1.78×10^4 conidia /ml 2.78×10^4 conidia/ml	Shehzad *et al.* (2021)
Cabbage butterfly, *P. brassicae*	Lepidoptera: Peiridae	*B. bassiana* MTCC 4495	10^9 conidia/ml	Dhawan and Josi (2017)

per cent, *V. lecanii* 1.15 per cent WP at 0.006 per cent and Azadiractin 0.15 EC, proved most effective in controlling mustard aphids in all crop growth stages.

Mustard sawfly

Over the years, the mustard sawfly, *A. lugens proxima*, has emerged as a significant pest of mustard, spreading to various regions of India, including north-east India (Narayanan and Gopalakrishnan, 2003; Chowdhury, 2009), causing havoc from December to March (Pandey *et al.,* 2023). The incidence of mustard sawfly infestation occurs during the initial growth phase, typically when seedlings reach an age of 3-4 weeks. The pest undergoes six larval instars, with its entire life cycle typically spanning approximately 30-39 days (Thigale and Pawar, 2021; Pradhan *et al.,* 2020b). The larvae are more destructive, feeding leaves from the margins to inward, resulting formation of holes in young leaves and subsequent skeletonization. Under certain circumstances, the impact of *A. proxima* infestation can result in a total yield loss; however, on average, the reduction in yield ranges from 34.62 to 59.33 per cent (Sahoo, 2016). Beyond leaves, sometimes, they also feed on other plant parts like the epidermis of tender shoots, flowers and fruits (Chowdhury, 2009).

Rabha (2009) investigated the effectiveness of *B. bassiana* against sawfly larvae, finding the fungal conidial solution at a concentration of 10^{13} conidia/ml was highly efficacious, resulting in mortalities of 63.33, 86.67, 93.33 and 100 per cent after 48, 72, 96 and 120 hours of treatment, respectively. However, Pradhan *et al.* (2020a) reported that *L. lecanii* was the most effective in controlling the sawfly population with 80.40 per cent mortality than *B. bassiana* (67.11%) and *M. anisopliae* (61.48%). But Vinyas *et al.* (2022) found that the average larval population of sawfly was effectively controlled by *B. bassiana* (0.44 larvae/plant), followed by the application of *L. lecanii* (0.59 larvae/plant) and Azadirachtin 10000 ppm (0.61 larvae/plant).

Painted bug

The painted bug, *B. hilaris* is the most devastating pest of cruciferous crops throughout India. Both nymphs and adults attack the crop at the seedling stage and suck the cell sap, resulting in plant withering and a subsequent reduction in overall plant population (Divya *et al.,* 2015; Patel *et al.,* 2017). The damage caused by the pest adversely affects both the quality and quantity of mustard seeds.This feeding behavior induces the formation of white spots on young plant leaves. The bug poses a significant threat to rapeseed in both the seedling stage (October-November) and harvest stage (March-April). Severe infestations during the seedling stage can result in plant mortality and a characteristic wilted appearance. Losses attributed to painted bug attacks during the seedling stage range from 26.8 to 70.8 per cent. The damage is more alarming during pod formation and maturity stages, resulting in yield losses of 30.1 per cent and a reduction of 3.4 per cent in oil content (Patel *et al*., 2017). Additionally, there is a substantial loss of protein (3.56%) and sugar content (1.11%), as reported by Singh *et al.* (1980).

Studies on the efficacy of different insecticides (Singh and Sarvesh, 2010; Singh *et al.,* 2011; Ratnoo *et al.,* 2018; Kalasariya and Parmar, 2019) against painted bugs have been reported enormously so far. However, few studies are being done on the application of entomopathogens against painted bug.

B. bassiana was applied @ 2.5 kg/ha, leading to a mortality of 60.25 per cent; *L. lecanii* @ 2.0 kg/ha recorded 51.16 per cent mortality while, the application of *N. rileyi* @ 2.5 kg/ha showed 44.41 per cent mortality, during the flowering stage (Kalasariya and Parmar, 2019). The brown marmorated stink bug (*Halyomorpha halys* Stål), an exotic pest damaging fruits and vegetables was treated with *B. bassiana* and resulted in 100 per cent mortality after 12 days of treatment (Gouli *et al.,* 2012). Among *B. bassiana*, *M. anisopliae* and *L. lecanii* and their respective combinations with Neem oil @1:1, the combination of *M. anisopliae* + Neem oil was found most effective against *B. hilaris* (Halder *et al.,* 2017).

Diamondback moth

In recent years, the diamondback moth (DBM), *P. xylostella*, has emerged as the predominant pest inflicting severe damage upon cruciferous crops such as cabbage, cauliflower, radish, knol khol, turnip, beetroot, mustardand rapeseed throughout the world (Lingappa *et al.,* 2004), resulting in yield loss varying 31-100 per cent (Lingappa *et al.,* 2004). The Mediterranean region, where the majority of cruciferous plants have their roots, is thought to be the origin of the diamondback moth. Though the pest is observed throughout the entire year, the prevalence of incidence typically escalates from February to September. However, Shaila *et al.* (2022) observed the incidence of this pest during the *Rabi* season in the mustard crop.

Diamondback moth larvae, feed voraciously on the leaves of mustard plants, creating holes on the leavesand they feed on the soft leaf tissues between the veins, thereby, leaving behind skeletonized leaves. In severe infestations, the persistent feeding by the caterpillars can result in the formation of loose webbing or silken threads amidst the affected leaves, consequently impeding the growth of mustard plants. This webbing serves as a protective shelter for the larvae as they pupate and transform into adult moths. Diamondback moth damage can significantly affect mustard plants, but the severity of the infestation and resulting symptoms may vary based on factors like plant age, environmental conditions and moth population density. Early detection and appropriate pest management strategies can help minimize the damage caused by diamondback moths on mustard crops (Dosdall *et al.,* 2011; Utthamasamy *et al.,* 2011).

P. xylostella has developed resistance to almost every group of insecticides but, excessive use of them will create a resurgence and cause environmental pollution

(Uthamasamy *et al.,* 2011). Therefore, the exploration of entomopathogenic fungi can be taken as a safer alternative to control this destructive pest. There are several studies on the use of entomopathogens against DBM which are mentioned below.

Loc and Chi (2007) identified different isolates such as *M.a (OM3-STO)*, *B.b (OM2-SDO)*, *M.a* (*OM1-R*) and *B.b (VL1-SCL)* of *B. bassiana* and *M. anisopliae*, as effective for controlling *P. xyllostella*, demonstrating mortality rates ranging from 38.6 to 52.4 per cent three days post-treatment. Additionally, the yield of cauliflower increased by 73.2, 68.2 and 66.7 per cent, respectively, in the *M.a (OM3-STO)*, *B.b (OM2-SDO)* and *M.a (OM1-R)* treatments as compared to the untreated control. Shehzad *et al.* (2021) assessed the efficacy of two entomopathogenic fungi, *B. bassiana* and *M. anisopliae* against the second and third larval instars of *P. xylostella*, revealing *B. bassiana* to be more effective compared to *M. anisopliae.* As per the result, a corrected mortality rate of 77.80 per cent was recorded for the second instar larvae, with an LC_{50} of 1.78×10^4/ml observed on the 6th DAT for *B. bassiana*. Conversely, for *M. anisopliae*, an LC_{50} of 2.78×10^4/ml was accompanied by a mortality rate of 70 per cent. Moreover, the influence of various conidial concentrations (5×10^4, 3.5×10^5, 2.5×10^6, 1.2×10^7 conidia/ml) of *M. anisopliae* resulted in a notable decrease in pupation and adult emergence (Hasibuan *et al.,* 2009).

Sáenz-Aponte *et al.* (2020) assessed the combined application of *Heterorhabditis bacteriophora* (strain HNI0100), *B. bassiana* (strain Bb9205) and *M. anisopliae* (strain Ma9236) and found it effective in controlling *P. xyllostella*, both in green house and field conditions. Moreover, the joint action of DDVP 0.025%+*B. bassiana* 4 per cent was determined to be effective in controlling *P. xylostella* under laboratory conditions, resulting in a net larval mortality of 80 per cent when compared to the sole utilization of DDVP 0.05 per cent (Alexander *et al.,* 2012). Additionally, it was observed that 10 per cent *B. bassiana* exhibited the highest efficacy in controlling *P. xylostella* within the cabbage ecosystem (Alexander *et al.,* 2018). In another investigation conducted by Batta (2018), it was illustrated that *M. anisopliae* (strain MA1) exhibited significant biocontrol efficacy against both susceptible and Bt-tolerant *P. xylostella* larvae, with LC_{50} values of 1.34×10^5 and 7.70×10^6 conidia/ml, respectively.

Cabbage butterfly

Cabbage butterfly, *P. brassicae* is a pest of regional significance in crops of brassica (crucifers) family (Choudhury and Pal, 2006; Bhati *et al.,* 2016; Pradhan *et al.,* 2020b), posing a threat during the vegetative stage to pod bearing stage of mustard (Patel *et al.* 2019). About 69 per cent yield loss is reported due to this lepidopteran pest in cabbage crop (Rai *et al.,* 2014). It is necessary to know about its safer and sustainable management through entomopathogens. Dhawan and Josi (2017) assessed the virulence of different fungal isolates of *B. bassiana viz.* against third instar larvae of *P. brassicae*, with *B. bassiana* MTCC 4495 (10^9 conidia/ml) resulting in the highest mortality (86.66%) and *B. bassiana* MTCC 6291 (10^7 conidia/ml) showing the lowest mortality (30.00%) after ten days of treatment.

The synergistic effect of *M. anisopliae* with *Satureja hortensis* extracts improved the efficacy of the entomopathogenic fungus and gave more effective control against *P. brassicae* pupa under laboratory conditions (Khorrami *et al.,* 2018).

CONCLUSION

In conclusion, entomopathogenic fungi have emerged as a promising and sustainable solution for pest management. These naturally occurring fungi offer several advantages over conventional chemical pesticides, making them an environment friendly and effective alternative. Additionally, the mode of action of these fungi is highly specific to insects, minimizing potential harm to humans and other non-target organisms. Moreover, these fungi can evolve and develop resistance against pest populations, reducing the likelihood of resistance development in pests. Another significant advantage is their compatibility with integrated pest management (IPM) strategies. This integration promotes a holistic approach to pest management, reducing reliance on synthetic chemicals and fostering sustainable agricultural practices. Overall, using entomopathogenic fungi for pest management offers many benefits, including environmental sustainability, selective targeting, adaptabilityand compatibility with IPM strategies. As we seek safer and more sustainable solutions to pest control, these fungi hold great promise in helping us achieve ecologically sound and economically viable pest management practices.

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Conflict of interest

The authors declare that they have no competing interests.

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