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Pathogenicity of entomopathogenic fungi against the aphid and the whitefly species on crops grown under greenhouse conditions in India

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Abstract

The aphids, *Myzus persicae* (Sulzer) and *Aphis gossypii* (Glover) (Hemiptera: Aphididae) and the whiteflies, *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) are the most damaging pests of greenhouse crops, which cause vector-borne viral diseases and its damage includes chlorosis, necrosis, and fruit abortion. This review article addresses the protected cultivation of vegetable crops (cucumber, capsicum, tomato, and gerbera), important insect pests of greenhouse crops in India along with its management by entomopathogenic fungi (EPF) and increase in the virulence by different genetically modified techniques. Due to excessive and indiscriminate use of insecticides over the years, these insect pests became resistant to these insecticides. So, there is a need for the effectual substitutes to manage these pests. Biological control is a foundation of integrated pest management (IPM) that plays a key role in the repression of arthropod pests. Among different IPM program, the uses of different microbial formulations are ecofriendly and safe for life and proven a boon for the farmers and entrepreneurs. EPF are the most effective in reducing aphid and whitefly populations on vegetable crops recommending its organic production under greenhouse conditions and could be a part of IPM. In the future, these insect pests will become more resistant to entomopathogens. Some novel techniques such as genetic engineering of fungal formulations will be required to increase the efficiency of various entomopathogens as these techniques are well adopted by countries like the USA, China, and European countries but its use in India and needs to be improved in the near future.

Keywords: Protected cultivation, Aphid, Whitefly, Entomopathogenic fungi, Genetic engineering

Background

In the changing scenario of increasing human population, decreasing cultivable land and water resources, there is a need to produce more food from available land and water. One technology being used in this direction is growing crops under protected cultivation, which has shown enormous potential during the last few decades and is well adopted for vegetable and ornamental crops. Protected cultivation of vegetable crops hold good for manifold increase in productivity compared to open-field conditions (Singh 2013; Pekkeriet et al. 2015, and Choudhary 2016). In the recent past, there has been a tremendous increase in area under protected cultivation (around 20 million hectares area all over the world). In India, the area under protected cultivation is around 46,000 hectares with productivity of 327 thousand tons, which is mainly confined to Andhra Pradesh, Maharashtra, Karnataka, Himachal Pradesh, Punjab, Haryana, and Rajasthan (Sabir and Singh 2013; Senthilkumar et al. 2018). The

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farmers are growing vegetables, flowers, and other high-value crops under the protected environment and earn high profit. Among the vegetables, capsicum, chili, cucumber, tomato, and brinjal are the main crops grown under protected environment. The productions of these crops are greatly influenced by the insect pests (Vashishth 2009 and Sood et al. 2012a). The whitefly, *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius); red spider mite, *Tetranychus ludeni* (Zacher) and *T. urticae* (Koch); serpentine leaf miner, *Liriomyza trifoli* (Burgess)*;* *Polyphagotarsonemus latus* (Banks); thrips, *Franklinella ocidentalis* (Pergande); *Scirtothrips dorsalis* (Hood); tobacco caterpillar, *Spodoptera litura* (Fabricius) and aphid*, Aphis gossypii* (Glover) *and Myzus persicae* (Sulzer) are economic pests infest crops under protected cultivation (Sood et al. 2018; Singh and Joshi 2020). Among these, the aphids, *M. persicae* and *A. gossypii* (Hemiptera: Aphididae) and the whitefly, *T. vaporariorum* and *B. tabaci* (Hemiptera: Aleyrodidae) are the major insect pests, which cause vector borne viral diseases and their damage includes chlorosis, necrosis, wilting, stunting, flower and fruit abortion, leaf distortion, and defoliation (Sayed et al. 2019). Due to the availability of favorable environmental conditions under protected cultivation, the management of these pests is quite challenging. It is mainly done by chemical pesticides but due to excessive and indiscriminate use of these pesticides, insect pests become resistant against them. Additionally, inauspicious effects of chemical insecticide residues on the crop (Van Lenteren 2000), killing of non-target organisms (Pilkington et al. 2010), and development of resistance to pesticides (Sood and Sood 2005; Pappas et al. 2013) are linked with its excessive use.

Various parasitoids, predators, pathogens, and botanicals are being exploited for the management of these pests (Ali et al. 2017 and Ullah et al. 2019). Entomopathogenic fungi (EPF) have been identified as potential control agents against aphids and the whitefly species under protected conditions (Saito and Sugiyama 2005). *Beauveria bassiana* (Balsamo) Vuillemin, *Lecanicillium lecanii* (Zimmerman), *Metarhizium anisopliae* (Metsch.), and *Paecilomyces fumosoroseus* (Wize) are the key tools for the management of various agricultural insect pests, including whiteflies, mealy bugs, aphids and thrips in outdoor and greenhouse crops (Wraight et al. 2000; Daniel and Wyss 2010; Shah and Shukla 2014).

This review will elucidate the fundamental information on the crops grown under protected conditions; discuss major insect pests of crops grown under protected cultivation in India along with its management with the use of different commercial and indigenous fungal formulations and genetically engineered entomopathogens.

Protected cultivation of vegetable crops in India

The concept of protected cultivation came into existence in seventeenth century when natives of Netherlands and England used artificial structures (similar to polyhouse structures) to produce the crop under adequate climatic conditions (Janick et al. 2007). In today's world, the Netherlands is forefront in the production of large-capacity polyhouses to enhance the crop yield and quality (Muijzenberg and Erwin 1980). The adoption of new agricultural techniques is foremost in the agriculture leading countries to produce the crop with high quality and revenue from low-cost investment. The uses of various agricultural approaches put India out of frontier (Navale et al. 2003 and Kanzaria et al. 2017). The protected cultivation includes growing crops under polyhouse, greenhouse, net house which are made up of materials like acrylic, polycarbonate, polyethylene which help to protect the crops from environmental factors. Nowadays, India is becoming popular for protected cultivation among the farmers and entrepreneurship because of its profitability and sustainability. Protected cultivation is the most important way to produce a large variety of horticultural crops, including tomato, capsicum, cucumber, and other vegetable crops (Sabir and Singh 2013). Under protected conditions, various factors like temperature, humidity, light, soil, and water are controlled. Additionally, the economics of capsicum under protected cultivation was studied by Sreedhara et al. (2013) and they revealed that India contributes one-fourth of world production of capsicum with an average annual production of 0.9 million tons from an area of 0.885 million hectares with a productivity of 1266 kg per hectare and out of total production; Andhra Pradesh stands first with production of 748.5 thousand tons followed by Karnataka from an area of 76 thousand hectares with a productivity of 131 thousand tons. Weintraub (2007) explained that when IPM is employed properly, it had the advantage of eliminating some open field pests, thereby results in increasing fruit yields. The performance of crops grown under open and protected cultivation was studied and observed that capsicum grown under protected conditions enhanced the yield of a crop by 49.8 MT/ha than open-field cultivation (18.34 MT/ha) (Ngullie and Biswas 2016). The performance and economics of crop varieties are almost thrice in protected conditions as compared to open field conditions (Singh et al. 2011) due to the protective ability of greenhouses.

Abundances of aphid and whitefly species on crops grown under protected cultivation in India

Whiteflies

Whiteflies are the devastating pests of vegetables, ornamentals, and field crops throughout the tropical, subtropical, and temperate regions of the world. More than 1420 species of whiteflies associated with agricultural crops throughout the world. Among different species of whiteflies, *B. tabaci* being one of the most widely distributed pests found in tropical and subtropical regions of the world, where it infests over 600 different cultivated and wild plant species from 63 plant families (Oliveira et al. 2001). In India, *B. tabaci* was first recorded on cotton at Pusa (Bihar) in 1905 (Misra and Lamba 1929). Seventeen plant whitefly species belonging to widely separate families were reported in the Kalyani area of West Bengal. In Andhra Pradesh, *B. tabaci* was found to survive on 14 off-season hosts (Verma et al. 1989). Later, (Arneja 2000) from Punjab reported *B. tabaci* from 16 different host plants comprising field crops, vegetables, and weeds. Konar during (1997) also observed the three species of Aleyrodidae associated with *Citrus reticulate* (mandarin) orchards in the Darjeeling district of West Bengal. Whereas, in Lakshadweep (Dubey et al. 2004) reported the occurrence of 12 species of whiteflies representing 11 genera. From Himachal Pradesh, (Bhalla and Pawar 1977) recorded 10 species of whiteflies belonging to 6 genera. *Bemisia tabaci* was recorded on crops such as *Capsicum annum*, *Cucumis sativus*, *Lycopersican esculentum*, *Gerbera jamesonii*, and *Solanum melongena* in different regions of India (Reddy and Kumar 2006; Sood and Sharma 2010; Ibrahim et al. 2011; Kumar et al. 2017; and Padhi et al. 2017; Khanzada et al. 2018) (Table 1). In India, the incidence of *T. vaporariorum* was recorded first at Thummantty in the Nilgiri hills of Tamil Nadu and Himachal Pradesh on crops such as potato, capsicum, tomato, and cucumber (David 1971; Sharma et al. 2006; Sood et al. 2006; Vashisth 2009; Sood et al. 2012b; Sood and David 2012). So far, It has been reported on 120 host plants belonging to 38 plant families, mainly Solanaceae (15 species), Compositae (17 species), Labiatae (8 species), Acanthaceae (7 species), Leguminosae and Onagraceae (6 species each), and Malvaceae and Verbenaceae (5 species each) from different parts of India (Mohan et al. 1988; Krishnan and David 1999, and Bakshi et al. 2003).

The wide range of aleyrodid fauna, *B. tabaci* and *T. vaporariorum* has drawn the maximum attention due to their wider host range and the losses incurred. *Bemisia tabaci* is distributed in the warmer regions Byrne and Bellows (1991) whereas *T. vaporariorum* is a serious pest in temperate region under glasshouses and field crops with summer region (Hill 1987). It is a serious pest in temperate regions under protected cultivation situations and in field crops where the summers are warm enough. It is considered as a "New World" species having distribution throughout Europe, parts of Africa, Asia, Australasia, North America, and South America (Hill 1987). Moreover, the infestation of greenhouse whitefly occurs in 249 host plants from 84 families of different crops and ornamental plants in temperate regions from six continents Russell (1977). The whitefly breeds were found throughout the year and it completes 13 generations in a year under protected environment in India (Sood et al. 2014). Adults of *T. vaporariorum* are tiny (1 mm long) snowy whiteflies with a covering of white waxy powder on wings. They are mostly found on the under surface of the leaves (Hill 1987). One hundred and fourteen virus species are transmitted by whiteflies (Aleyrodidae). Whiteflies, *B. tabaci*, and *T. vaporariorum* transmits 111 and 3 species, respectively. Of the whitefly-transmitted virus species, 90% belongs to the *Begomovirus* genus, 6% belongs to the *Crinivirus* genus, and the remaining 4% belongs to the *Closterovirus*, *Ipomovirus*, and *Carlavirus* genera (Jones 2003). The host plants were found to have a pronounced effect on the selection, feeding, oviposition, and development of whitefly. Overall, the yield loss due to various insect pests ranged from 30-40% among different vegetable crops in India (Sharma et al. 2017). However, loss due to whitefly under protected conditions varies among different vegetables. In capsicum, whitefly, *B. tabaci* caused 13.60% yield loss (Singh H and Joshi N 2020) whereas, on cucumber plant yield loss was approximately 26% (Ghongade 2020). Moreover, whitefly caused 54% yield loss in okra plant (Dhandapani et al. 2003). The development of the whitefly was seen higher on eggplant and cucumber, followed by tomato whereas slow on capsicum crop (Kamp and Lenteren 1981). Research indicated

Table 1 List of aphid and whitefly species recorded under protected conditions in different regions of India

Species	Host plants	Location	Author and year
Aphis gossypii	*Capsicum annum*, *Cucumis sativus*, and *Lycopersican esculentum*	Punjab, New Delhi	Kaur (2005), Ibrahim et al. (2011), Khanzada et al. (2018)
Myzus persicae	*Capsicum annum*, *Cucumis sativus*, and *Gerbera jamesonii*	Punjab, Maharashtra, and Himachal Pradesh	Singh et al. (2003), Singh et al. (2004), Gavkare et al (2014), Ibrahim et al. (2011), Weintraub (2007), Javed et al. (2019)
Bemisia tabaci	*Capsicum annum*, *Cucumis sativus*, *Lycopersican esculentum Gerbera jamesonii*, and *Solanum melongena*	Karnataka, Punjab, Himachal Pradesh, Uttar Pradesh, and West Bengal	Singh et al. (2003), Reddy and Kumar (2006), Ibrahim et al. (2011), Khanzada et al. (2018), Kumar et al. (2017), Padhi et al. (2017)
Trialeurodes vaporariorum	*Capsicum annum*, *Cucumis sativus*, *Lycopersican esculentum*, and *Gerbera jamesonii*	Tamil Nadu and Himachal Pradesh	Vashisth (2009), Sood et al. (2012), Sharma et al. 2006, Chinniah et al. 2016

that the whitefly prefers tobacco, cucumber, broad bean, and cowpea significantly more than paprika with more probing time on preferred hosts (Xu et al. 1994).

Aphids

The aphid species, *M. persicae* and *A. gossypii* are the most damaging insect pests of crops grown under protected conditions across the world because of their ability to transmit viruses to the plants. Their damage includes chlorosis, necrosis, fruit abortion, and stunted growth (Perdikis et al. 2008). *Myzus persicae* caused 19.43% yield loss on vegetable crops under protected cultivation in India (Singh and Joshi 2020). However, on an average, damage caused by insect pests under protected conditions on various crops ranged from 15-37% (Dhandapani et al. 2003; Rathee and Dalal 2018). Damage caused by aphid species on chili crops was recorded as 38.85% (Chinniah et al. 2016). More than 500 species of *M. persicae* are known to kill various host plants all around the world. In India, *M. persicae* is recorded in various regions of Punjab, Himachal Pradesh, and Maharastra on crops capsicum, cucumber, tomato, and Gerbera (Singh et al. 2004; Weintraub 2007; Vashisth 2009; Ibrahim et al. 2011; Sood et al. 2012; Sabir et al. 2013; Gavkare et al. 2014; and Javed et al. 2019) (Table 1). As reported by Vasicek et al. (2001), among the most important pests of pepper (*Capsicum annuum*) crop in Argentina, the aphids stand out, of which *M. persicae* is the key pest, followed by *Aphis solani* and *Macrosiphum euphorbiae*. The glasshouse provides the best condition for the development of aphid population. Greenhouse crops are susceptible to infestation by no. of insects like aphid, whitefly, mites, and thrips that cause yield loss and crop damage. In India, capsicum is an important vegetable crop. *Capsicum annum* and *C. frutescens*, family Solanaceae are the two capsicum species extensively cultivated in tropic and sub tropic regions. The population dynamics of sucking pests and their correlation with weather on capsicum was studied and result showed an incidence of aphid *A. gossypii* on capsicum crop under protected conditions (Meena et al. 2013). *Aphis gossypii* is the most abundant in Punjab and New Delhi regions on *Capsicum annum*, *Cucumis sativus*, and *Lycopersican esculentum* (Singh et al. 2004; Kaur 2005; Ibrahim et al. 2011, and Nagamandla et al. 2017) It was observed in West Bengal region along with other insect pests on tomato under protected conditions and cause various vector-borne diseases. It is observed that the majority of the aphids and whiteflies species are observed in the northern and southern regions of India.

Biological control by entomopathogenic fungi

Entomopathogenic fungi (EPF) are bioinsecticides with an ability to infect and kill arthropods. They are isolated from the naturally occurring soil and arthropod carcasses (Behie and Bidochka 2014; Litwin et al. 2020). These fungi belong to 6 fungal classes: *Basidiomycota*, *Entomophtoromycota*, *Oomycetes*, *Chytridiomycota*, *Microsporidia*, and *Ascomycota*. Until now, 238 species of *Basidiomycota*, 474 species of *Entomophtoromycota*, 12 species of *Oomycetes*, 65 species of *Chytridiomycota*, 339 species of *Microsporidia*, and 476 species *Ascomycota* were reported (Araujo and Hughes 2016). However, species of *Ascomycota* and *Entomophtoromycota* are more often in natural habitat. *Metarhizium* spp. (Metschnikoff) Sorokin are ubiquitous naturally occurring soil-inhabiting fungi (Meyling et al. 2011). In 1888, Elie Metchnikoff, a Russian microbiologist firstly discovered the EPF and named it as *Metarhizium anisopliae*. In 1965, Boverin, a *Beauveria bassiana* based mycoinsecticide was developed to control the Colorado potato beetle and codling moth (Litwin et al. 2020). *Verticillium lecanii* (Zimmerman) Viégas (reclassified as *Lecanicillium lecanii* (Petch) Zare and Gams as an insect pathogen that is used commercially to control greenhouse pests (Cuthberston and Walter 2005).

Strains of entomopathogenic fungi are concentrated in the following orders: Hypocreales (various genera), Onygenales (*Ascosphaera* genus), Entomophthorales, and Neozygitales (Entomophthoromycota). Entomopathogens are the most important biological agents that can occur as epizootic or enzootic levels in their host populations (Mora et al. 2017). Entomopathogens are being reported to control various crops insect pests. These EPF viz. *Beauveria bassiana* (Hypocreales: Cordycipitaceae), *M. anisopliae* (Hypocreales: Clavicipitaceae), *Lecanicillium* spp., (previously *Verticillium lecanii* (Hypocreales: Cordycipitaceae), and *I. fumosorosea* (Hypocreales: Clavicipitaceae) (previously *Paecilomyces fumosoroseus)* are reported to kill insect by nutritional deficiency, the release of adhesions (MAD1 and MAD2), secondary metabolites, tissue degradation, and release of toxins. The EPF contain cuticle degrading enzymes like protease; lipase and chitinase which degrade the insect cuticle, followed by penetration of fungal germ tube into insect body and thereby release of several mycotoxins such as Beauvericin, Beauverolides, Bassianolide to kill the insect (Gabarty et al. 2014; Skinner et al. 2014; Lacey et al. 2015). It has been reported that *B. bassiana* and *M. robertsii* provides plant with nitrogen during insect parasitization, thus encourages plant growth (Behie and Bidochka 2014).

Virulence of entomopathogenic fungi against whitefly

All biopesticides containing insect pathogen *B. bassiana*, *V. lecanii*, and *M. anisopliae* were found effective in reducing the pest population. But the organic products and mineral oils were comparatively less effective. Therefore, a study was conducted to evaluate EPF against *B. tabaci* and *T. vaporariorum*, and obtained result showed that *L. lecanii* was more effective against *B. tabaci* and *T. vaporariorum* causing 89.3 to 96 and 79.3 to 95.6% mortality, respectively under greenhouse conditions. In addition to this, the infectious and epizootic potential of *P. fumosoroseus* on the susceptibility of *B. argentifolii* were not affected by the host vegetable species of tomato (Vidal et al. 1998). However, mortality was > 70%, the first week after treatment and increased further in the second week. *Lecanicillium lecanii* caused 86% mortality of *B. tabaci* on brinjal at 5 days after treatment (Phadke and Phadke 2000). Besides this, *B. bassiana* and *P. fumosoroseus* have strong potential for microbial control of nymphal whiteflies infesting cucurbit crops (Wraight et al. 2000). Thirty-five strains of *L. lecanii* originated from different hosts and geographical locations were tested as potential biocontrol agents against silver leaf whitefly, *B. argentifolii* (Gindin et al. 2000). Pathogenicity of *B. bassiana* (strain GHA, lot TGAI 97-10-1) and *P. fumosoroseus* (strain 612, lot 940917) with conidial concentration of 1.4×10^{11} and 1.8×10^{11} conidia/ml were tested against the 3rd-instar nymphs of *T. vaporariorum* reared on cucumber and tomato plants. Nymphs were highly susceptible to infection by both fungi after a one-time application of conidia onto cucumber plants. In contrast, insects reared on tomato plants were significantly less susceptible to infection (Poprawski et al. 2000). The whitefly mycoinsecticide products based on *L. lecanii*, *P. fumosoroseus*, and *B. bassiana* have the capacity to suppress and in some instances, provide good control of whiteflies in both greenhouse and field crops. Development of methods and strategies to enhance mycoinsecticide efficacy has been attempted and primarily focus on initiating treatments against the early stages of the pest to prevent population build up, targeting pest populations developing under moderate environmental conditions (e.g., during spring or fall growing seasons), selecting crops amenable to multiple, highly efficient spray applications and applying fungi asynchronously with incompatible fungicides (Faria and Wraight 2001). Pathogenicity of commercial EPF *L. muscarium* (Mycotal, Koppert Biological Systems Ltd, UK) was evaluated on sweet potato whitefly *B. tabaci* under laboratory and glasshouse cultivation and recorded that the application of *L. muscarium* against *B. tabaci* resulted in a significant increase in the mortality of *B. tabaci* under glasshouse cultivation and also no plant damage was recorded during the experiment (Cuthbertson and Walters 2005). Furthermore, the EPF *L. lecanii* caused 26.6 to 76.6% mortality against *T. vaporariorum* 7 days post treatment when applied with the concentration of 1×10^7 conidia/ml as reported by (Scorsetti et al. 2008). The efficacy of different bio-pesticide was reported against whitefly *B. tabaci* on tomato plant and result showed that biopesticides viz. Bio Magic (91.64%), Mealikil (93.55%), and Biopower (88.91) were highly effective in killing the nymphal whitefly population over control after 3rd spray (Budha et al. 2015). Some of the commercially available biopesticides are shown in Table 2. The most effective EPF that reduced the pest population were *B. bassiana*, *V. lecanii*, and *M. anisopliae* that was evaluated at three different concentrations (1×10^7, 1×10^8, and 1×10^9 spores/ml) against *B. tabaci* in laboratory and field cultivation, respectively (Abdel-Rahim and Ahmed 2017). They reported that the highest concentration (1×10^9 spores/ml) of the 3 EPF was highly toxic to adults of *B. tabaci* than the others. Under field cultivation also highest concentration (1×10^9 spores/ml) of *V. lecanii* was the best in managing the adult whitefly population.

Table 2 Commercial bioformulations for the management of aphid and whitefly species under protected conditions in India

Active ingredient and concentration	Brand name	Target pests	Crops	Manufacturer
Beauveria bassiana 1.50% [1×10^8 CFU/ml]	Bio-Power	Aphids, whiteflies, mites, and thrips	Cabbage, Capsicum, Tomato	T. Stanes and Company Limited, India
Lecanicillium lecanii 1.50% [1×10^8 CFU/ml]	Bio-Catch	Whiteflies, aphids, mealybugs	Several crops	T. Stanes and Company Limited, India
Metarhizium anisopliae 1.50% [1×10^8 CFU/ml]	Bio-Magic	Borer, termites, leaf hoppers, and aphids	Rice, Capsicum	T. Stanes and Company Limited, India
Beauveria bassiana 2.0% strain IPL/BB/MI/01 [2×10^8 CFU/ml]	Daman	Sucking pests, caterpillars	Greenhouse crops	International Panaacea Limited, India
Verticillium lecanii 2.0% strain IPL/VL/05 [2×10^8 CFU/ml]	Varunastra	Whiteflies, aphids, thrips, and scale insects	Capsicum, cucumber, tomato and brinjal	International Panaacea Limited, India
Metarhizium anisopliae 1.0% strain IPL/KC/44 [1×10^8 CFU/g]	Kalichakra	Beetles, grasshoppers, and aphids	All crops	International Panaacea Limited, India
Beauveria bassiana strain 63428-82-0 [1×10^9 CFU/g]	Biosoft	Sucking pests	Capsicum, cabbage, cotton	Agriland Biotech Limited, India
Beauveria bassiana NCIM/1216 ATCC 26851 [1×10^8 CFU/ml]	Racer	*Spodoptera litura*, caterpillars, mealybugs, aphids	Several horticultural crops	Agrilife Biosolutions for soil & crops, India

Data source: (Ramanujam et al. 2014; Chinniah et al. 2016; Mascarin and Jaronski 2016 and Ruiu L 2018; Singh and Joshi 2020)

Virulence of entomopathogenic fungi against aphid

Twelve different strains of EPF, viz., *L. lecanii*, *P. farinosus*, *B. bassiana*, *M. anisopliae*, *Cordyceps scarabaeicola*, and *Nomuraea rileyi* (Hypocreales: Clavicipitaceae) were evaluated against aphids on cabbage and cucumber crops grown under greenhouse conditions. Among these EPF's, *L. lecanii* 41185 strain was highly virulent than all other strains against *M. persicae* and *A. gossypii* because it germinates and grew well under a wide range of temperature and humidity as reported by Vu et al. (2007). *Beauveria bassiana* was used for the control of *M. persicae* on cabbage and it caused 76-83% mortality in 4 weeks after spray (Filho et al. 2011). While native isolate of *B. bassiana* caused 100% mortality 7 days post treatment against *M. persicae* (Ibrahim et al. 2011). Aphids are the most destructive pests in crop production such as pepper and cucumber (Kim et al. 2013). They conducted a bioassay with 47 fungal culture filtrates (three isolates of *Isaria* spp., and *Lecanicillium* spp., 20 isolates of *B. bassiana* and 20 isolates of *Cordeceps* spp.) in order to evaluate the potential of secondary metabolites produced by entomopathogenic fungi for aphid control. Among 47 culture filtrates cultured potato dextrose broth, filtrate of *B. bassiana* Bb08 at 1 ml concentration showed the highest mortality (78%) against green peach aphid 3 days post treatment. Filtrate of Bb08 cultured in Adamek's medium showed a toxicity of 100% to 3rd instar nymphs of the aphid compared with 7 other filtrates cultured in different broths and results indicated that the fungal culture fluid or culture filtrate of *B. bassiana* Bb08 cultured in Adamek's medium had potential for development as a mycopesticide for aphid control. Interestingly, among different tested conidial bioformulation against *A. gossypii* and *M. persicae*, maximum percent reduction was found by *B. bassiana* isolate JW-1with 98-100% mortality (Jandricic et al. 2014). Additionally, it was observed that 2 strains of *B. bassiana* and 1 strain of *L. lecanii* against *M. persicae* caused maximum mortality after 10 days post treatment with maximum of 95% and minimum of 87% with *B. bassiana* and *L. lecanii*, respectively (Nazir et al. 2019). Reseach studies on the effect of EPF showed that mortality caused by *L. lecanii* formulation is significantly better than *M. anisopliae* and *B. bassiana* against *M. persicae* and *A. gossypii* under laboratory and greenhouse cultivations. Four procured native isolates of *B. bassiana*, *M. anisopliae*, *L. lecanii*, and *Chaetomium globosum* and recorded for efficacy against the aphids *M. persicae* and *A. gossypii* and results showed that *L. lecanii* showed higher mortality than all 3 isolates against *M. persicae* and *A. gossypii* under laboratory as well as under greenhouse cultivations, when applied at the concentration of 1×10^8 conidia/ml (Mohammed et al. 2018). *Beauveria bassiana* and *L. lecanii* were more virulent entomopathogens for the management of *M. persicae* (Javed et al. 2019).

Increased virulence by genetically engineered methods

The release of the cuticle enzyme was low during the initial penetration t and high during the degradation of protein (St. Leger et al. 1996). *Beauveria bassiana* and *M. anisopliae* caused infection against insect pests through their cuticle by release of cuticle degrading enzymes.

By the use of phage display technology, hydrophobins were isolated from the culture of *B. bassiana*. Furthermore, cDNA library was built up by using RNA, in the presence of insect pests. Hydrophobins appears should be selectively enriched by melibiose agarose beads, which further yield two (*hyd 1* and *hyd 2*) different hydrobhobin proteins. Results revealed that *hyd 1* gene expressed well in aerial conidia, in vitro blastospores, submerged conidia, and cells sporulating on chitin and insect cuticle in contrast to *hyd 2* gene (Cho et al. 2007). Additionally, modification of cuticle degrading and adhesion toxin intestine-specific virulence factor (Vip3Aa1) into *B. bassiana* isolate BbV28 in the cabbage transgenic plant against *S. litura* aids in digestion in the cytoplasm of insect after 18 to 36 h of conidial infection (Qin et al. 2010).

Systematically disruption of *Cag8* gene is one of the alternative ways to increase the expression of gene *Mrku70* (Xu et al. 2014) that helps to increase the pathogenicity of *M. robertsii* ARSEF2575 by 93% as compared to wild type (7%). Insect cuticle is mainly composed of chitin fibrils, to penetrate this barrier fungus produce chitin degrading enzyme such as proteases and chitinases (Zhao et al. 2016). Over expression of *Pr1A* enzyme in *M. robertsii* and *CDEP1 Bbchit1* in *B. bassiana* can lead to increase the virulence of these fungi against major crop pests. Genes along with metabolic pathways to increase the virulence are described in Table 3.

Conclusion

Protected cultivation or greenhouse cultivations would be the modern approach to produce vegetable crops qualitatively and quantitatively in India. Due to the availability of favorable environmental conditions, various insect pests cause acute to severe crop damages under

Table 3 Modified genes along with their metabolic pathways to increase the pathogenicity

Modified genes	Metabolic pathway	Fungal species
Pr 1A	Protease	*Metarhizium robertsii*
CDEP 1	Subtilism-like protease	*Beauveria bassiana*
Bbchit 1	Chitinase	*Beauveria bassiana*
Mr-Npc 2a	Sterol carrier	*Metarhizium robertsii*
ATM 1	Trehalase	*Metarhizium acridium*
BbBqrA	Benzoquinone oxidoreductase	*Beauveria bassiana*

Data source (Zhao et al. 2016 and Romeis et al. 2019) protected cultivations. Aphids and whiteflies are the major insect pests of crops grown under protected cultivations. The use of entomopathogenic fungi (EPF) is one of the alternatives to control various insect pests in India over a few decades. In the future, some novel techniques are required to increase the efficiency of various entomopathogens. Techniques like genetically engineering and cDNA probe will be one of the appropriate ones to decrease pest resistance. These techniques are well adopted by countries like the USA, China, and European countries but its use is limited in India and need to be improved in the near future.

Abbreviations
EPF: Entomopathogenic fungi; GE: Genetically engineered; IPM: Integrated pest management; CFU: Colony forming unit

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Authors' contributions
The first author, H S collected the information mentioned in the tables from different sources, edited the paper and remove errors and grammatical mistakes, and written the manuscript. T K prepared Table 2 and topic with heading increase virulence by genetically engineered methods and made necessary corrections. All authors read and approved the final manuscript.

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