International Journal of Tropical Insect Science (2022) 42:1639–1647
https://doi.org/10.1007/s42690-021-00685-3

ORIGINAL RESEARCH ARTICLE

Toxicological behavior of entomopathogenic fungi with insecticides: *in vitro* growth efficacies and conidial processes on mite cuticle

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Received: 15 September 2021 / Accepted: 30 October 2021 / Published online: 26 November 2021

Abstract
The Crop protection system is dominated by the use of synthetic insecticides however incorporation of fungal biocontrol agents (FBA) at lower doses is considered to consolidate the integrated pest management (IPM) program. The success of IPM is delimited and relies on the understanding of how its performance is affected by adverse effects on FBA by agrochemicals deployed for its management as well as of other pests and diseases. In this study, laboratory grade actives of three different insecticides used for the control of *Tetranychus urticae* were tested at different concentrations to determine their effects on germination, mycelial growth and sporulation of eight entomopathogenic fungal isolates under in vitro conditions. The fungal isolate with the most adverse effects on the biological index was further studied for underlying reasons of antagonism. This paper reports for the first time changes in conidial surface morphology, its germination and penetration capacity on mite cuticle, post insecticide treatment. Bioassays showed that all insecticide actives at their lowest concentration (12.5% MC) were most toxic to *Beauveria bassiana* P isolate. Ethion and chlorpyriphos were compatible with *Hirsutella thompsonii* PDBC-1 at 12.5% MC while propargite showed compatibility with *B. bassiana* MTCC 6097, *Metarhizium anisopliae* MTCC 4104 and *Cordyceps fumosorosea* MTCC 4636 at the same concentration. Further, SEM studies showed that post-insecticide treatments, there were structural deformations on the conidial surface with a decrease in its germination and germ tube penetration capacity on the cuticle of *T. urticae*. Future studies in this area will help in improving IPM along with overcoming insecticide resistance problems.

Keywords Biological index · Compatibility · Conidial processes · Entomopathogenic fungi · Insecticides

Introduction

In India, a large proportion of the population (56.7%) is associated with agriculture and is under exposure to insecticides at large (Banerjee et al. 2014; Gupta 2004). Harmful effects of insecticides are not unprecedented and include residue problems in food products and water sources, deleterious effects on livestock and microbial control agents (MCA), resistance in pest insects (Johnsen et al. 2001; Aktar et al. 2009; Widenfalk et al. 2008; Saxena et al. 2002). Among pest insects, *Tetranychus urticae* has been reported as 'most resistant' with > 500 cases of insecticide resistance and against 94 active substances (Michigan State University 2017; IRAC 2017). *T. urticae* is a polyphagous mite which infects > 900 different plant types (Mondal and Ara 2006) and the injudicious use of synthetic insecticides for its control has led to the development of uncontrolled resistance (Yucel 2021). Nevertheless, pest management operations rely largely on the extensive use of insecticides as other practices might not lead to instant results as desired by farmers (Sharma et al. 2019; Koli and Bhardwaj 2018; Tawfiq and Isra 2013; Kumral et al. 2010).

Entomopathogenic fungi have shown immense potential over the years in controlling many economically crucial insect pests of the agro-ecosystem (Dolinski and Lacey 2007; Lacey and Shapiro-Ilan 2008; Lacey et al. 2015; Qasim et al. 2018, 2021b). Previous studies have recommended higher inoculation rates of fungal biocontrol agents (FBA) for the control to be efficacious (> 90%) (Younas et al. 2017; Rivero-Borja et al. 2018; Meyling et al. 2018). However, the use of FBA at lower inoculation rates in

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combination with low doses of synthetic insecticides (SI) has replaced the sole use of either two to protect the environment as well as conservation of beneficial insects in the agro-ecosystem (Khun et al. 2021). This combinatorial process makes the insect pest more vulnerable for fungal attachment and penetration via various mechanisms viz. incapacitation of target pest mobility by paralysis, weakening of insect cuticle or removal of fungal conidia from pest body surface via grooming behaviour or gustatory and olfactory signals (Yanagawa et al. 2018; Brito et al. 2008). Workers have reported many insecticides as compatible with EPF (Younas et al. 2017; Rivero-Borja et al. 2018; Meyling et al. 2018) while others have been shown to be antagonistic (Alves et al. 2016; Asi et al. 2010; Akbar et al. 2012). To our knowledge, the underlying cause of this antagonism has not been studied or identified so far. However, few studies have demonstrated the role of some components from emulsifiable concentrates (toluene and similar aromatic solvents) as responsible agents for such adverse effects on bacterial (Morris 1977) and fungal entomopathogens (FE) (Anderson and Roberts 1983) while limited preliminary studies on active ingredients (AI) of pesticides as causative agents for antagonism is documented in the literature (Khun et al. 2021; Chakravarty and Sidhu 1987). In this study, we evaluated the impact of the active ingredient of three insecticides (used against *T. urticae*) on the germination, mycelial growth and sporulation of entomopathogenic fungi and sought to identify the underlying cause of antagonistic effects on fungal growth parameters by identifying changes in conidial structure. Also the effect of insecticide actives on conidial germination and germ tube penetration of the mite cuticle were observed.

Materials and methods

Strains and preparation of entomopathogenic fungal strains

The fungal isolates viz. *Beauveria bassiana* (MTCC 6097, MTCC 6291), *Metarhizium anisopliae* MTCC 4104, *Cordyceps fumosorosea* (*Paecilomyces fumosoroseus*) MTCC 4636, *Akanthomyces lecanii* (*Lecanicillium lecanii*) MTCC 956 and *Cladosporium cladosporioides* MTCC 3872 were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India. *Beauveria bassiana* P isolate was isolated from the cadavers of *T. urticae* while *Hirsutella thompsonii* PDBC-1 was isolated in the laboratory previously.

B. bassiana isolates were cultured on malt extract agar (MEA) while all other isolates were cultured on Sabouraud dextrose agar (Sigma Ltd.) supplemented with yeast extract (1% w/v) [SDAY]. These culture media are routinely used for culturing the entomopathogenic fungi and the isolates used in the present study grew best on them, ensuring appropriate response to the insecticide supplemented media in the in vitro study while taking all precautionary measures to avoid any negative effects due to suboptimal media. All these isolates were incubated at 25 ± 1 ºC in the dark. From 14 days old sporulating cultures, conidia were harvested for experimentation. The viability of conidia was evaluated at > 90% RH, and exceeded 90% for all isolates (Zhang et al. 2014, 2016).

Response of eight fungal isolates to insecticide-supplemented media (ISM)

Among the insecticides used for the control of *T. urticae*, the tested insecticides in the present study are listed in Table 1. These insecticides were chosen at random among the large number of insecticides used in the pest management operation against the mite. In this study, the protocols were adopted from pre-established methods for determining the effects of insecticides on EPF (Coremans Pelseneer 1994). Commercial formulations of insecticides were not used in order to determine that the effect produced is due to the active ingredient in the insecticide. Analytical standards (Laboratory-grade) of propargite, ethion and chlorpyriphos were obtained from Sigma-Aldrich and all these had purity levels in the range of 96.3% to 99.9%. These active ingredients (AI) at 12.5%, 25%, 50% and 100% of their respective mean concentrations (MCs) along with negative control (no insecticide) and vehicle control (0.5% acetone) were analyzed for their response against the eight EPF isolates. Five replications were maintained for the

Table 1 Insecticide treatments used against *Tetranychus urticae* and evaluated in the study

Trade Name	Active Ingredient	Formulation	Chemical Group	MC (ml)	ppm
Omite	Propargite 57% EC	EC	Organosulfite	300	3000
Fosmite	Ethion 50% EC	EC	Organophosphate	400	4000
Classic	Chlorpyriphos 20% EC	EC	Organophosphate	1000	10,000

MC: Mean concentration of commercial product for application in 100 L of water per acre, *EC:* Emulsifiable concentrate, *ppm:* parts per million

experiment at 24 h interval and from each of the five plates of replication for each isolate, conidial suspensions were prepared independently.

The methods used in the study of Oliveira and Neves (2004) were slightly modified and used in the present study. Acetone (3% v/v) has been reported to have a negative impact on EPF (Anderson and Roberts 1983). So in the present study, acetone at lower concentrations was tested for its impact on EPF in preliminary studies prior to evaluation of laboratory-grade analytical standards. Each of the AI was dissolved in acetone (HPLC grade, ≥ 99.8%, Sigma-Aldrich) to prepare 200X MC stock solution (SS). It was further diluted in sterile distilled water to achieve 50X of its MC. This diluted SS was filter-sterilized and added to the warm media at 1/400, 1/200, 1/100 and 1/50 times of the total media volume in order to obtain AI concentrations of 12.5%, 25%, 50% and 100% of its MC in the ISM. The acetone concentration was only 0.25%, 0.125%, 0.0625% in the ISM with 50%, 25% and 12.5% of its MC respectively, so acetone (25%) was added in each of these to obtain a uniform concentration of acetone (0.5%) in each treatment.

From 14 days old sporulating cultures, conidia were harvested from the plates using a sterile spatula and were dispersed in sterile distilled water containing 0.05% tween 20 (Sigma-Aldrich). It was vortexed for 5 min to obtain a homogenized suspension. The conidial concentration was determined using a haemocytometer (Bright-Line™ Hemacytometer, Sigma-Aldrich) and compound microscope (Olympus BX53, 400X) equipped with a digital camera (DP74, Olympus). The concentration of the conidial suspension was adjusted to 1×10^4 conidia mL^{-1} using Tween 20 (0.05% v/v).

To determine mycelial growth, conidial suspension (10 µL, 1×10^4 conidia mL^{-1}) was inoculated in the centre of ISM. The petri-plates were doubled sealed with parafilm M (Sigma-Aldrich) and incubated (25 ± 2 °C; 15 days). Vegetative growth in terms of two orthogonal diameters was recorded for 7 days (Neves et al. 2001).

For sporulation, the mycelial mat was harvested from the entire surface of the colony with a sterile spatula. The conidia were dispersed in sterile distilled water with Tween 20 (0.05% v/v) and vortexed (5 min) for homogenisation. Using a haemocytometer, conidial concentration was determined as described previously.

To determine conidial germination, a uniform spread of conidial suspension (20 µL, 1×10^4 conidia mL^{-1}) on a SDAY or MEA block (4 cm^2) on a sterile glass slide was made. The slides of each treatment and replication were placed in separate moistened filter paper-lined sterile petri plates and incubated (25 ± 2 °C) for 18 h under dark conditions. With 100–200 conidial counts on each slide, conidial germination (%) was determined. If the germ tubes were 2X the diameter of the propagule, the conidia were regarded as germinated.

Rearing of red spider mite, *Tetranychus urticae*

Brinjal (*Solanum melongena*) nursery was established and French beans (*Phaseolous vulgaris* Linn.) seeds were sown in earthen pots and maintained at 25 ± 2 °C, 60 ± 10% RH, and 16 h light photoperiod in screen house at the Department of Entomology, PAU. The leaves (5–6 leaf stage onwards) of these crops were used for rearing *Tetranychus urticae* adults, collected from various *P. vulgaris* fields. The mite culture obtained after the second generation was used for experimentation.

Treatment of *Tetranychus urticae* with *Beauveria bassiana* P isolate

Under laboratory conditions, petri dishes containing mulberry leaves were sprayed with 2.5 mL of conidial suspension (1×10^6 conidia mL^{-1}) of *B. bassiana* P isolate (obtained from ISM plates) in a laminar airflow cabinet. Control leaves were treated with Tween 20 (0.3% v/v). The adult mites were released on mulberry leaves damped underneath with moist cotton wool and the petiole of the leaf remained immersed in damped cotton to remain hydrated. The leaf disc was surrounded by a Tanglefoot® barrier to prevent mites from escaping to the lower side of the leaf. The petri dishes were incubated at 25 ± 2 °C, 70 ± 5% RH and observed daily for seven days for mite mortality. All the treatments were replicated thrice, with 20 mites in each replication. In preliminary experiments (data not shown), the infectivity of *B. bassiana* P isolate (obtained from untreated media plates) at the same conidial concentration against *T. urticae* was determined and showed high mortality (Dash et al. 2018).

Effect of insecticides

Scanning electron microscopy (SEM)

SEM studies were used to observe the morphological changes in the conidia and also to determine the tendency of the conidia to germinate and penetrate the cuticle of *T. urticae*, post insecticide treatment.

Conidial surface morphology of *Beauveria bassiana* P isolate

Dried conidial samples from control (untreated) and all the treatments were covered with evaporated platinum. Possible morphological changes on the conidial surface were observed under SEM (SEM; Hitachi S4800, Ibaraki, Hitachi) (Shan et al. 2010).

Germination and penetration capacity of *Beauveria bassiana* P isolate conidia

Adult mites were treated with *B. bassiana* P isolate suspension (2 mL; 1×10^6 conidia mL^{-1}) for 5 s and each replication was reared separately by the method described above. After 12, 24, 36, 48 and 60 h, the treated mites were removed and fixed with glutaraldehyde (10%) and dehydrated using gradient series of ethanol and hexamethyldisilazane. Under a high vacuum evaporator, dried samples were sputter-coated with gold and observed under SEM (Zhang et al. 2018).

Statistical Analysis

The compatibility study between EPF and laboratory-grade analytical standards of insecticides was determined using the biological index (BI), as proposed by Rossi-Zalaf et al. (2008) and used by Ribeiro et al. (2012), da Silva et al. (2013), Alves et al. (2016) and Khun et al. (2021) in their studies, calculated as:

$$BI = \frac{(47 * VG) + (43 * SP) + (10 * GER)}{100}$$

where VG, SP and GER represent radial growth of fungal colony (%), colony sporulation (%) and conidia germination (%), respectively. Compatibility level is indicated by the value of BI, where the value of 0–41, 42–66 and > 66 indicates toxic, moderately toxic and compatible, respectively. All subsequent analyses were performed in Minitab version 19.2020.2.0.

Analysis of the biological indices for the entomopathogenic fungal isolates

For the determination of normality and homogeneity of variance, the Anderson–Darling test (Anderson 2011) and Levene's test (Erjavec 2011) using Minitab version 19.2020.2.0 were applied, respectively. As the data observed conformation to the assumption of normality, two-way analysis of variance (ANOVA) using a general linear model from Minitab version 19.2020.2.0 was used. Significant differences between treatments were determined with Tukey adjustment for multiple comparisons using the Lsmeans (Least-Squares means) (Lenth 2016) using Minitab 19.2020.2.0.

Results

Response of entomopathogenic fungal isolates to ISM

The biological index of the tested insecticides was found to vary in all the fungal treatments ($P < 0.05$, Table 2). These differences were primarily due to the species/strain of EPF, chemical nature of the insecticide and concentration of the insecticide tested. Propargite at 12.5% of its MC was compatible with *B. bassiana* MTCC 6097, *M. anisopliae* MTCC 4104 and *C. fumosorosea* MTCC 4636. Ethion was compatible with *H. thompsonii* PDBC-1 at 12.5% and 25% of its MCs. Similar to ethion, chlorpyriphos recorded compatibility with *H. thompsonii* PDBC-1 at 12.5% and 25% of its MCs. An increase in the concentration of insecticides in the media from 12.5% to 100% of their respective MCs showed a resultant decrease in their biological index and it was true for all the tested insecticides ($P < 0.05$). Propargite, ethion and chlorpyriphos even at their lowest concentration were very toxic to *B. bassiana* P isolate, *A. lecanii* MTCC 956 and *C. cladosporioides* MTCC 3872; *B. bassiana* P isolate and *A. lecanii* MTCC 956; *B. bassiana* MTCC 6097, *B. bassiana* P isolate and *C. cladosporioides* MTCC 3872, respectively (Table 2). This signifies differential antagonistic behaviour of fungal strains in response to the same chemical stress. The maximum was recorded by *B. bassiana* P isolate among all the insecticides at their minimal concentration tested (12.5% MC).

Effect of insecticides on the structure and infectivity of fungal conidia

The surface morphology of EPF conidia and its penetration into the mite cuticle after exposure to insecticides was measured as a function of the mortality of the exposed mites. Intriguingly, SEM observations showed that insecticide-treated conidia of *B. bassiana* P isolate were deformed or having structural aberrations (Fig. 1B–D) in comparison to the control conidia (Fig. 1A). In all the treatments, most of the conidia shrivel and shed from the surface of conidia within 48 h. This might be due to the reason that following insecticide treatment, and their cell walls become more fragile and vulnerable. Further in control, the conidia germinated on the cuticle of *T. urticae* and germ tube penetration of the cuticle was also observed (Fig. 1E). However, compared to control, propargite pre-treated conidia showed germination on the conidial surface but didn't penetrate the cuticle after 24–36 h (Fig. 1F). Ethion pre-treatment led to larger conidia formation in comparison to control and non-penetration of the cuticle was also observed by these larger conidia (Fig. 1G), while hollow tubular structures were observed in chlorpyriphos pre-treated conidia. Also, miniature germ tubes erupted in the latter, which did not show cuticle penetration after 48 h (Fig. 1H).

Discussion

The present study emphasises the importance of interaction between agrochemicals and fungal biocontrol agents (FBA) as well as its impact on the latter, for their use in the soil

Table 2 Summary of the responses of eight entomopathogenic fungal isolates to laboratory- grade insecticides used against *Tetranychus urticae*. Data are biological index (BI). MC, Mean concentration. Pink cells, highly toxic (BI ≤ 41); Yellow cells, moderately toxic (BI = 42–66); green cells, compatible (BI ≥ 66)

		Insecticide Concentrations			
Insecticide	Isolates	12.5% of MC ± SE	25% of MC ± SE	50% of MC ± SE	100% of MC ± SE
Propargite[a]	*Beauveria bassiana* MTCC 6097	70.98 ± 5.36 aB	51.64 ± 6.82 bC	48.93 ± 2.20 cAB	42.55 ± 1.91 dB
	B. bassiana P isolate	36.04 ± 5.85 aG	32.11 ± 11.09 bF	27.06 ± 6.77 cF	24.54 ± 5.45 dE
	B. bassiana MTCC 6291	58 ± 5.58 aD	47.79 ± 3.52 bD	42.45 ± 3.12 cC	35.73 ± 2.63 dC
	Metarhizium anisopliae MTCC 4104	67.37 ± 8.78 aC	57.42 ± 1.83 bB	47.63 ± 1.51 cB	34.42 ± 1.09 dC
	Cordyceps fumosorosea MTCC 4636	74.51 ± 7.27 aA	63.83 ± 1.97 bA	50.48 ± 1.56 cA	46.84 ± 5.42 dA
	Akanthomyces lecanii MTCC 956	39.07 ± 3.90 aF	37.43 ± 1.92 abE	36.88 ± 6.92 bD	33.33 ± 1.71 cC
	Cladosporium cladosporioides MTCC 3872	40.34 ± 2.92 aF	36.36 ± 8.36 bE	31.63 ± 5.14 cE	28.26 ± 7.30 dD
	Hirsutella thompsonii PDBC-1	49.76 ± 6.64 aE	37.51 ± 1.57 bE	22.57 ± 9.37 cG	21.46 ± 6.34 cF
Ethion[b]	*B. bassiana* MTCC 6097	54.73 ± 12.31 aD	32.3 ± 9.08 bG	27.07 ± 10.21 cD	25.72 ± 6.39 cD
	B. bassiana P isolate	30.57 ± 6.51 bF	35.62 ± 6.67 aF	25.66 ± 7.11 cD	20.19 ± 4.65 dE
	B. bassiana MTCC 6291	57.07 ± 6.67 aC	54.06 ± 4.80 bC	51.97 ± 7.20 bA	48.49 ± 7.44 cA
	M. anisopliae MTCC 4104	53.01 ± 5.07 aD	50.73 ± 4.85 bD	46.49 ± 4.44 cB	44.53 ± 4.26 dB
	C. fumosorosea MTCC 4636	79.36 ± 7.34 aA	70.38 ± 4.92 bA	53.88 ± 3.76 cA	42.96 ± 3.00 dB
	A. lecanii MTCC 956	39.89 ± 8.03 aE	37.97 ± 3.96 abE	36.61 ± 6.39 bcC	35.79 ± 6.85 cC
Chlorpyriphos[c]	*C. cladosporioides* MTCC 3872	41.22 ± 6.88 aE	36.36 ± 3.49 bEF	25.4 ± 2.44 cD	23.03 ± 2.21 dDE
	H. thompsonii PDBC-1	68.04 ± 10.40 aB	59.61 ± 7.95 bB	37.36 ± 5.68 cC	33.38 ± 6.26 dC
	B. bassiana MTCC 6097	30.94 ± 6.76 aF	30.36 ± 12.40 aE	27.65 ± 6.72 bE	25.72 ± 6.92 bC
	B. bassiana P isolate	36.18 ± 9.51 aE	30.71 ± 5.57 bE	29.03 ± 6.75 bE	24.26 ± 4.54 cC
	B. bassiana MTCC 6291	51.97 ± 7.11 aC	42.92 ± 3.81 bC	35.49 ± 9.18 cD	29 ± 6.34 dB
	M. anisopliae MTCC 4104	59.21 ± 7.58 aB	50.24 ± 4.80 bB	45.84 ± 4.38 cB	38 ± 3.63 dA
	C. fumosorosea MTCC 4636	46.84 ± 7.29 aD	38.59 ± 2.69 bD	35.19 ± 2.46 cD	31.31 ± 2.18 dB
	A. lecanii MTCC 956	45.9 ± 7.33 aD	42.89 ± 4.48 bC	40.71 ± 4.25 bcC	38.52 ± 7.32 cA
	C. cladosporioides MTCC 3872	38.48 ± 6.11 aE	37.48 ± 9.29 aD	30.26 ± 4.84 bE	19.67 ± 5.57 cD
	H. thompsonii PDBC-1	79.8 ± 8.10 aA	70.27 ± 5.44 bA	58.66 ± 4.54 cA	37.67 ± 8.56 dA

Table 2 (continued)

[a]$F_{(7, 64)} = 47.19$, $P < 0.001$ (for isolate factor), $F_{(3,64)} = 68.63$, $P < 0.001$ (for concentration factor), $F_{(21,64)} = 3.06$, $P > 0.001$ (for interaction). Means followed by different uppercase letters in columns and lowercase letters in rows indicate significant differences (LSMEANS test with Tukey adjustment, $\alpha = 0.05$)

[b]$F_{(7, 64)} = 36.28$, $P < 0.001$ (for isolate factor), $F_{(3,64)} = 43.49$, $P < 0.001$ (for concentration factor), $F_{(21,64)} = 2.76$, $P > 0.05$ (for interaction). Means followed by different uppercase letters in columns and lowercase letters in rows indicate significant differences (LSMEANS test with Tukey adjustment, $\alpha = 0.05$)

[c]$F_{(7, 64)} = 35.50$, $P < 0.001$ (for isolate factor), $F_{(3,64)} = 33.34$, $P < 0.001$ (for concentration factor), $F_{(21,64)} = 1.90$, $P > 0.05$ (for interaction). Means followed by different uppercase letters in columns and lowercase letters in rows indicate significant differences (LSMEANS test with Tukey adjustment, $\alpha = 0.05$)

ecosystem. The fungi are ubiquitous, and most of them are largely studied as biocontrol agents and are imperative components of integrated pest management (IPM) programs (Yadav et al. 2019). The agro-chemicals in the agro-ecosystem, either used in integration with FBA or elsewhere, greatly influence fungi and their functions (Meena et al. 2020; Aktar et al. 2009). This signifies to determine the most suitable FBA to be used in combination with synthetic agro-chemical(s) against insect pest(s) in Integrated Pest Management (IPM). Also, the underlying reasons for fungal antagonism when used with these chemical pest control agents becomes imperative to understand the better protection and sustainability of the environment as a whole.

The agrochemicals, which tend to either have a positive or least effect on the growth parameters of entomopathogenic fungi, should be adopted for their use in IPM (Oliveira et al. 2003; Qasim et al. 2021a). The use of azadirachtin inhibited the growth of *Glomus etunicatum* strain while carbendazim, hampered colonization as well as community structure of indigenous arbuscular mycorrhizal fungi (Ipsilantis et al. 2012). The application of organophosphate insecticides has been reported to impact various soil fungal populations and nitrogen mineralization rates (Pandey and Singh 2004). These findings suggest that these chemicals depreciated fungal growth efficacy and functions and resulted in structural aberrations in the EPF. There might be a possibility that if their use remained uncontrolled and injudicious, the deformations in the fungal structure might become inherent with generations and affect its pest infectivity rate over time.

To the best of our knowledge, no literature is found on the direct effect of insecticide active ingredients on the structure and infectivity of insecticide-treated conidia against *T. urticae*. However, preliminary compatibility studies of different insecticides against several pests have been reported (Wari et al. 2020; Abidin et al. 2017; da Silva et al. 2013; Mikunthan and Manjunatha 2010; Oliveira et al. 2003). Our results found agreement with Khun et al. (2021), who reported high toxicity of diazinon active ingredient to germination and growth of *M. anisopliae* QS155 and *B. bassiana* B50 at all tested concentrations, except 25% of full-field concentration for later. Yadav et al. (2019) also found that tolfenpyrad, spirotetramat, fipronil were highly toxic to *B. bassiana* (Bals.) and significantly reduced its vegetative growth and conidia production while emamectin benzoate (@ 0.5, 1 and 1.5FR [field recommendation] dose), imidacloprid (@ 0.5FR and 1FR dose), clothianidin (@ 0.5FR and 1FR dose) and buprofezin (@ 0.5FR and 1FR dose) showed high compatibility. Pelizza et al. (2018) reported that *B. bassiana* LPSC 1067 grown in the presence of gamma-cyhalothrin (52 ppm), showed 83.13% reduction in conidia production. In contrast, a study by Rashid et al. (2010) reported that tested insecticides inhibited conidial germination of *M. anisopliae* DEMI 001 independent of their concentrations.

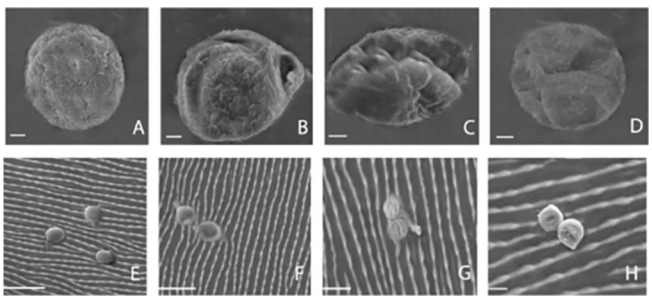

Fig. 1 SEM views of the surface changes of treated *Beauveria bassiana* P isolate conidia. **A**. No insecticide and acetone (Control); **B-D**. Insecticide treated conidia (**B**. Propargite; **C**. Ethion; **D**. Chlorpyriphos). *Scale bars* = 0.5 µm. Conidial attachment, germination and penetration of *B. bassiana* P isolate on the cuticle of *Tetranychus urticae* post treatment with, **E**. No insecticide and acetone (control); **F**. Propargite (24–36 h); **G**. Ethion (48 h); **H**. Chlorpyriphos (48 h). *Scale bars* = 10.0 µm

At the concentration of 50 ppm, 100%, 28.2% and 3.31% conidial germination reduction were found with hexaflumuron, fipronil and pyriproxyfen. Contrary, the work of Niassy et al. (2012) showed that there was no deleterious effect of imidacloprid on vegetative growth and conidia production of *M. anisopliae* ICIPE 69. Imidacloprid used with *M. brunneum* showed similar results during the studies of Paula et al. (2011). This synergistic growth effect is due to the potential of certain fungi to metabolize specific compounds and use them as secondary nutrients (Moino and Alves 1998).

Limited studies have reported adverse effects of chemicals besides pesticides on the structure of EPF. Shan et al. (2010) observed that rodlet layers on the conidial surface of *B. bassiana* Bb2860 and *M. anisopliae* Ma456 were removed after treatment with formic acid (FA) and trifluoroacetic acid (TFA). They reported that hydrophobins, the proteins associated with adhesion, antigenicity and morphogenesis of the conidial surface, got dissociated after exposure to FA and TFA. These chemicals tend to neutralize hydrophobicity and thus binding of conidia to insect cuticle (Boucias et al. 1988). Exposure to detergents and other chemicals which alters pH sensitize the process of conidial attachment to insect cuticle and inhibits adhesion by 80–90% and 30%, respectively (Holder and Keyhani 2005). The biochemical basis of insecticides killing fungi has been documented by certain workers. NADH oxidoreductase complex-I has been reported to be inhibited by the action of Tolfenpyrad (List FC 2018). Toxicity of Spirotetramat to *M. brunneum* (Petch) and *B. bassiana* (Bals.) (Yadav et al. 2019) is attributed to its potential to inhibit acetyl CoA carboxylase, which affects lipid synthesis in fungi (IRAC 2018).

Conclusions

Obtained results showed that propargite, ethion and chlorpyriphos all significantly altered the infectivity potential of *Beauveria bassiana* P isolate towards *Tetranychus urticae*. So, there is an urgent need to monitor the potential antagonistic effects of agrochemicals on microbial control agents (MCA), especially in the industries where crop protection programs are reformed by the integration of entomopathogens with synthetic agrochemicals. The development of crop protection calendars will further consolidate these programs, which will not only provide data on interactions between different agrochemicals and MCA but timely profile of chemicals usage in the fields also. More detailed studies need to be conducted concerning the mortality response of *T. urticae* to the combination treatment of EPF and insecticides that have the potential for efficient mite control together with the reduction in adverse effects on EPF and amounts of insecticide usage as well as prevention or delay in the development of insecticide resistance. Future studies can be explored to advance formulation engineering of insecticide active ingredients to obtain more compatibility with EPF.

Acknowledgements Department of Microbiology and Department of Entomology, PAU are thanked for all the research facilities and funding. Dr. Neelam Joshi and Dr. Manpreet Brar Bhullar are thanked for their expert guidance, assistance and support for the research work regarding *Tetranychus urticae*. We are also thankful to Dr. B. K. Kang for his assistance with concentrations of insecticide actives. We are grateful to Guru Nanak College, Ferozepur Cantt for timely support and facilities.

Funding Department of Microbiology and Department of Entomology, PAU are thanked for all the associated funding.

Declarations

Ethics approval Not Applicable.

Consent to participate Not Applicable.

Consent for publication Not Applicable.

Conflicts of interest/Competing interests The authors declare that they have no conflicts of interest/competing interests.

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