Efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* (Ascomycota: Hypocreales) against *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) under controlled and open-field conditions on bitter gourd

Abdul Mannan Hamzah[1*] , Ata ul Mohsin[1], Muhammad Naeem[1] and Muhammad Azam Khan[2]

Abstract

Background: *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) is the most destructive pest of bitter gourd. Conventionally, it is controlled by chemical insecticides despite their hazardous impacts. Entomopathogenic fungi are considered as eco-friendly and safer alternative of chemical. This study aimed to evaluate the efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* against *B. cucurbitae* on bitter gourd.

Results: The study revealed that *B. bassiana* and *M. anisopliae* were efficient against *B. cucurbitae* in controlled as well as field conditions. Both mode of application (i.e., contact and oral) were found to be effective; however, high efficacies of them were observed through contact application (73.43 and 59.72%), respectively. Pathogenicity of both fungi increased with increase in concentration and time intervals. Under field conditions, significantly low fruit infestations and significantly a high population reduction of *B. cucurbitae* were observed at 30 DAT when both fungi were applied with 10^8 CFU/ml concentrations.

Conclusions: *Beauveria bassiana* and *M. anisopliae* had the potential to be used against *B. cucurbitae;* however, the former showed high efficiency comparatively.

Keywords: *Bactrocera cucurbitae*, Biological control, *Beauveria bassiana*, *Metarhizium anisopliae*

Background

Bactrocera cucurbitae (Coquillett) (Diptera: Tephritidae) is a destructive pest of all cucurbits; however, bitter gourd is preferred one (Qazzaz et al. 2015). Depending upon the crop and environmental conditions, serious economic losses are caused by this pest every year (Dhillon et al.

2005). Despite the hazardous impacts of chemical insecticides, these are still being used to manage *B. cucurbitae* on a large scale (Abbas et al. 2017). Due to the over-use of insecticides, insect pest resistance, toxic effects on non-targeted organisms and residual effects in soil, water and crops are some of the major issues that have been faced by human beings (Hsu and Feng 2006). Therefore, it is a need of time to explore those pest management strategies which are eco-friendly and easy to adopt.

Entomopathogenic fungi (EPF) are gaining importance as an alternative to chemical insecticides. These are

*Correspondence: abdulmanan_uaf@yahoo.com
[1] Department of Entomology, Faculty of Crop and Food Sciences, Pir Mehr Ali Shah - Arid Agriculture University, Rawalpindi, Punjab, Pakistan
Full list of author information is available at the end of the article

Hamzah *et al. Egyptian Journal of Biological Pest Control*   (2021) 31:144

Page 2 of 8

considered natural enemies of a broad range of insects and exist in diverse environments around the globe (Vega et al. 2009). EPF are eco-friendly, and the insect pests' resistance against them has not been reported yet (Hadi et al. 2013).

Fruit flies are susceptible to various EPF; however, *Beauveria bassiana* and *Metarhizium anisopliae* are the most efficient ones (Ekesi et al. 2002). These are capable of targeting a wide range of arthropod species and have a good potential to be utilized in IPM programs (Daniel and Wyss 2009). *B. bassiana* and *M. anisopliae* have a high pathogenicity against adult fruit flies (Sookar et al. 2008). It is the need of time to check their virulence through different methods against *B. cucurbitae* (Ortu et al. 2009). In view of the devastating potential of melon fruit flies to infest cucurbits and to reduce the over usage of insecticides, the current study was planned to effectively control *B. cucurbitae* by the application of EPF under laboratory and field condition. The ultimate goal of the current study is to develop a sound strategy for the management of melon fruit fly through tested the effect of *B. bassiana* and *M. anisopliae* against *B. cucurbitae*.

Methods
Research experiments were conducted in bio-control laboratory and open field during the years of 2017 and 2018.

Rearing of *Bactrocera cucurbitae*
Rearing of the melon fruit fly was started with infested bitter gourds, collected from local vegetable markets. Transparent plastic cages ($45 \times 40 \times 40$ cm) fitted with muslin cloth were used for fruit fly rearing. Autoclaved sand and soil mixture (5-cm-thick layer) was placed in the cages at the bottom, and infested fruits with emerging maggots were placed on it for pupation. Cages were observed on daily basis, and as soon as the pupation completed, sand and soil mixture was sieved out to separate pupae. Collected pupae were then placed in another cage for adult emergence. Emerged adults of *B. cucurbitae* were identified (on the basis of morphological characteristics) and separated. *B. cucurbitae* adults were then fed on artificial diet made-up of banana, egg white, yeast, molasses, nutrients, vitamins and honey. After 2 weeks, males and females were released ($\male/\female$, 1:1) in rearing cages for mating. After 24 h, females were again separated for experimental studies. Laboratory conditions were maintained at 27 ± 2 °C, $65 \pm 5\%$ RH and photoperiod of 14:10 (L/D) hours.

Entomopathogenic culture and formulations
Already established culture of EPF (i.e., *B. bassiana* and *M. anisopliae*), which was developed from the commercial products Racer® (NCIM 1216) and Pacer® (NCIM

1311), was obtained from Department of Plant Pathology. Obtained fungal culture was streaked on freshly prepared growth media (PDA). The new culture plates were incubated under dark conditions at 28 ± 2 °C for 15 days. The highest concentration (10^8 CFU/ml) was made by adding freshly harvested conidia in to distilled water. Hemocytometer was used to determine the required conidial concentration. Surfactant (0.05% Tween®80) was also mixed in each conidial suspension to increase the adhesiveness of fungal spores. Remaining 2 concentrations (i.e., 10^7 and 10^6 CFU/ml) were made by adding more distilled water in previously described concentration, and again hemocytometer was used to quantify the required conidia. All these formulations were tested against *B. cucurbitae*.

Laboratory experiments
Two types of bioassays (i.e., contact and oral) were performed under laboratory conditions.

Contact bioassay
Three concentrations (10^8, 10^7 and 10^6 CFU/ml) of each fungi (*B. bassiana* and *M. anisopliae*) were prepared in distilled water (Ain et al. 2021). Thirty randomly selected adult flies (15 days old) were anaesthetized by placing in refrigerator at -4 °C for 40 s (so that the handling would be easy). These 30 flies were placed in a test tube containing 4 ml conidial suspension from each concentration of both fungi. The test tubes containing flies and conidial suspension were shaken for 30 s until each fly attained some treatment. Two types of controls were maintained, i.e., a positive control with Malathion (3 ml/l of water) and a negative control with distilled water (containing 0.05% Tween®80). After the application of treatment, the tubes were placed vertically to allow the run off of excess suspension. Two hours after treatment, the flies were placed in plastic cages for further studies. Data regarding adult mortality were taken every 2 days after treatment (DAT) and consecutively up to 14 days. Factorial design (3-factors) with 5 replications was followed. Percent mortality was corrected using formula provided by Schneider-Orelli (1947).

$$\text{Corrected mortality} \% = \left(\frac{\text{Mt} - \text{Mc}}{100 - \text{Mc}} \right) \times 100$$

where Mt = mortality (%) in treatment and Mc = mortality (%) in control.

Oral bioassay
Three concentrations of each fungus (2×10^8, 2×10^7 and 2×10^6 CFU/ml) were estimated through hemocytometer. These three fungal concentrations were then mixed

Hamzah *et al. Egyptian Journal of Biological Pest Control*   (2021) 31:144

Page 3 of 8

with artificial diet in (1:1) ratio resulting in 10^8, 10^7 and 10^6 CFU/ml, respectively (Beris et al. 2013). One milliliter of this (suspension and diet mixture) was placed in small plate (3.5 cm diameter) in cage sized ($27 \times 25 \times 25$ cm). Fifteen -day-old 30 fruit flies, which were starved for 36 h prior to use in oral bioassay, were released in the cage for feeding on diet and conidial suspension mixture for 12 h and after this mixture was removed and pure diet was given as described by Beris et al. (2013). Two types of controls were maintained, i.e., a positive control with Malathion (1 ml/3 l of water) and a negative control with distilled water (containing 0.05% Tween®80). Data regarding adult mortality were taken every 2 days after treatment (DAT) and consecutively up to 14 days. After recording of each mortality observation, all dead flies were removed and transferred into sterile Petri dishes and wet filter paper was placed at the bottom of Petri plates. Then, these Petri dishes were sealed by using Parafilm, kept at 25 °C in dark and were examined daily for symptoms of mycosis. Factorial design (3-factors) with 5 replications was followed. Mortality was corrected using Schneider-Orelli (1947) formula (as mentioned above).

Field experiments

During laboratory experiments, the highest mortality causing concentration (i.e., 10^8 CFU/ml) of each EPF was identified and selected for further trials under field conditions. Each EPF was sprayed directly (Ain et al. 2021) on the crop at rate of 100 l/acre with the help of the knap sack sprayer when bitter gourd fruiting started. Spray was applied on the whole crop, but the soil beneath the plants and bitter gourd fruits were targeted specially. Bitter gourd crop was sown in March at farmers' field, for 2 consecutive years (i.e., 2017 and 2018). 'Faisalabad Long,' an indigenous and approved variety, was used. Priming was done by dipping seeds into water for 24 h. Plant-to-plant and row-to-row distances about 2 and 5ft were maintained, respectively. Recommended fertilizers and agronomic practices were applied, following Nawab and Mahmood (2014). When plants started to develop into vine, bamboo poled along with supporting nets were installed. In each unit plot, 10 plants were maintained and each treatment was replicated 5 times with 2 controlled treatments, i.e., positive control and negative control. In positive control, Malathion 57% EC (330 ml/acre), while in negative control only distilled water with 0.05% Tween®80 was used. Yellow sticky traps were hanged in each treatment to note the population of fruit flies before and after treatment. Data regarding fruit infestation (%) and population reduction (%) were taken every 3 days after treatment (DAT) and consecutively up to 30 days.

Randomized complete blocked design (RCBD) with 5 replications was followed. Fruit infestation was calculated by the following formula:

$$\text{Fruit infestation (\%)} = \frac{\text{No of infested fruits}}{\text{Total no of fruits}} \times 100$$

Population reduction was calculated following Henderson and Tilton (1955) equation:

$$\text{Population reduction \%} = \left(1 - \frac{\text{Ta} \times \text{Tb}}{\text{Ca} \times \text{Cb}}\right) \times 100$$

where Ta = number of flies stuck on yellow trap after treatment, Tb = number of flies stuck on yellow trap before treatment, Ca = number of flies stuck on yellow trap in control plot after treatment, Cb = number of flies stuck on yellow trap in control plots before treatment.

Statistical analysis

In laboratory experiments, recorded mortality data in each experiment were subjected to analysis of variance (ANOVA), following Tukey–Kramer honest significant difference (HSD) test ($\alpha = 0.05$). Percent mortality in laboratory experiments was corrected, following Schneider-Orelli (1947) formula, while percent population reduction was calculated in field conditions, following Henderson and Tilton (1955) formula. Analysis of the all the collected data was computed using Statistix 8.1 software (McGraw-Hill 2008), and graphical representations were created on Microsoft Excel (2010).

Results

Laboratory experiments

Contact pathogenicity of B. bassiana and M. anisopliae against B. cucurbitae

Contact pathogenicity of *B. bassiana* and *M. anisopliae* differed significantly (DF = 18, F = 3.99, P < 0.05) against *B. cucurbitae* under 3 different concentrations (10^8, 10^7 and 10^6 CFU/ml) and 7 time intervals (Fig. 1). As shown in the figure, among both the tested fungi *B. bassiana* induced significantly high pathogenicity (73.42%) 14 days after treatment (DAT) at 10^8 CFU/ml. However in case of *M. anisopliae*, maximum mortality (59.72%) was observed under the same time interval and concentration. Furthermore, it can also be seen that mortality induced by both of the fungi was time and concentration dependent. The minimum mortality caused by *B. bassiana* (5.33%) occurred 4DAT when 10^6 CFU/ml was applied, while in case of *M. anisopliae* the lowest mortality induced at the same time and concentration was (2.66%).
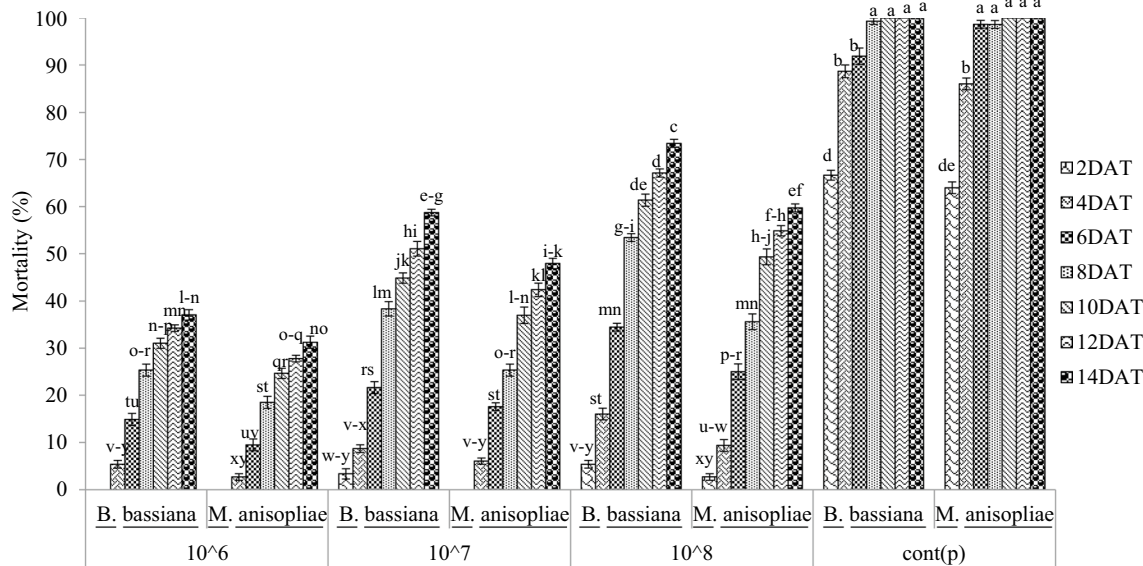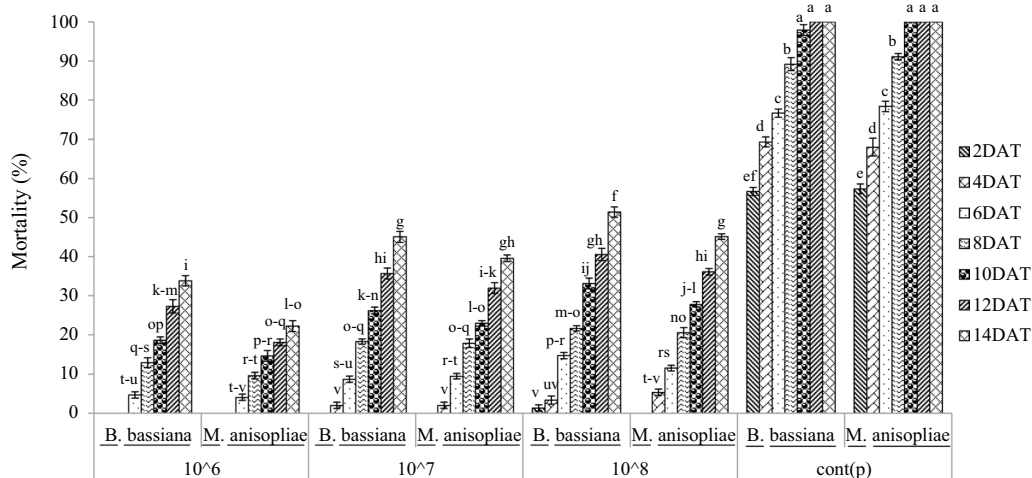
Fig. 1 Contact pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* against *Bactrocera cucurbitae* at three concentrations (10^6, 10^7 and 10^8 CFU/ml)

Oral pathogenicity of B. bassiana and M. anisopliae against B. cucurbitae

The pathogenicity of entomopathogenic fungi, i.e., *B. bassiana*, *M. anisopliae*, was also evaluated by adopting oral bioassay procedure. The virulence posed by both fungi was again concentration and time dependent. It can be seen from Fig. 2 that a significant difference existed among the virulent effect imposed by each fungi in melon fruit fly (DF = 18, F = 2.44, $P < 0.05$). The highest mortality (51.41%) was recorded when *B. bassiana* was applied

at the rate of 10^8 CFU/ml 14DAT and in case of *M. anisopliae* at the same time and concentration the peak lethality induced was (45.14%). Furthermore, the lowest mortality induced by *B. bassiana* and *M. anisopliae* was 4.66 and 4.06% 4 DAT, respectively, when 10^6 CFU/ml were offered to *B. cucurbitae* orally.

In case of *B. bassiana*, comparative study of contact and oral bioassay against melon fruit fly showed a significant difference (DF = 18, F = 19.32, $P < 0.05$). In contact application, when 10^8 CFU/ml were applied, *B. bassiana*
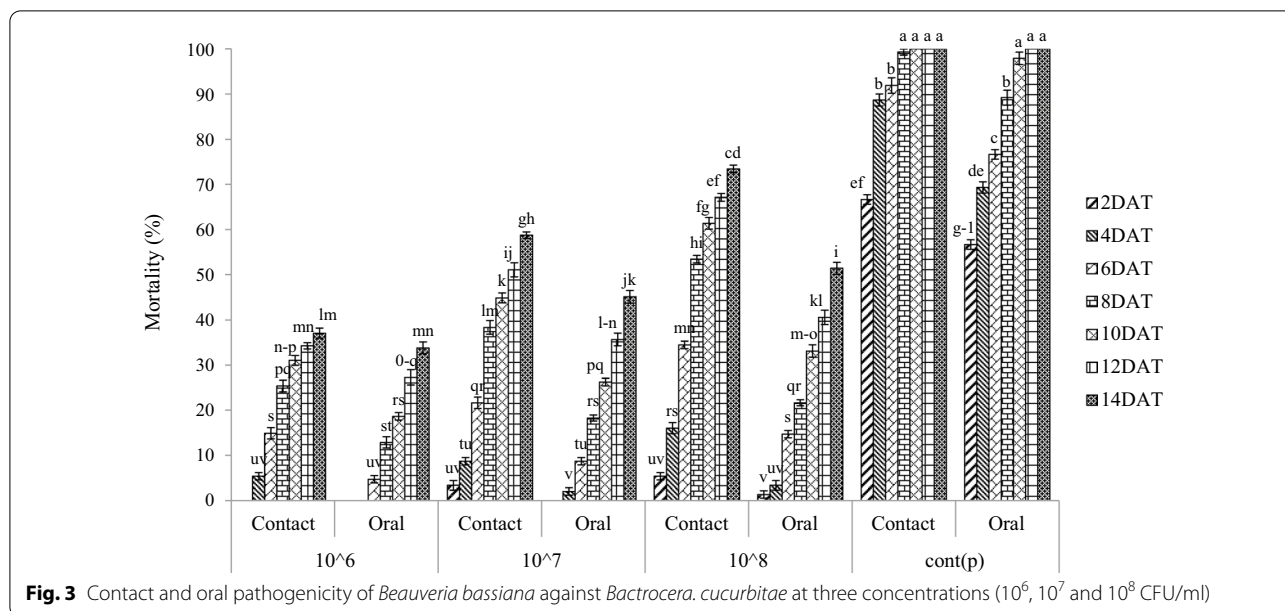

Fig. 2 Oral pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* against *Bactrocera cucurbitae* at three concentrations (10^6, 10^7 and 10^8 CFU/ml)

Fig. 3 Contact and oral pathogenicity of *Beauveria bassiana* against *Bactrocera. cucurbitae* at three concentrations (10^6, 10^7 and 10^8 CFU/ml)

was more pathogenic (73.42% mortality) 14 DAT, while in case of the oral bioassay, mortality was (51.41%) as shown in Fig. 3. On other hand, when *M. anisopliae* was used as an oral bioassay and contact bioassay differed significantly (DF = 18, F = 18.89, P < 0.05) against the melon fruit fly (Fig. 4). Contact bioassay of *M. anisopliae* gave significantly a high mortality (59.72%) 14 DAT under the application 10^8 CFU/ml suspension whereas it was significant low (45.14%) in case of oral bioassay.

Field experiments

Impacts of B. bassiana and M. anisopliae on fruit infestation by B. cucurbitae

Effect of the EPF on percent fruit infestation caused by *B. cucurbitae* is presented in Table 1. Fruit infestation against the application of *B. bassiana* and *M. anisopliae* differed significantly (DF = 3, F = 25.27, P < 0.05) 3DAT. Results revealed that percent fruit infestation due to melon fruit fly 3DAT was significantly high (43.25,
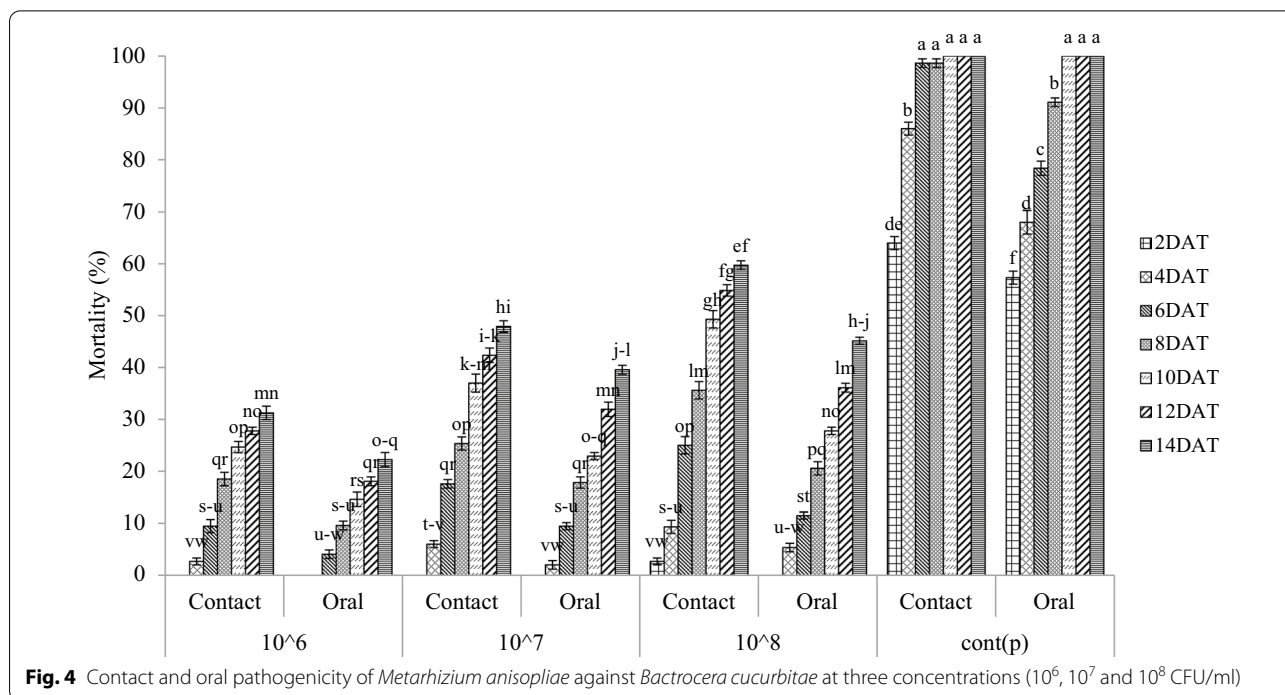

Fig. 4 Contact and oral pathogenicity of *Metarhizium anisopliae* against *Bactrocera cucurbitae* at three concentrations (10^6, 10^7 and 10^8 CFU/ml)

Hamzah *et al. Egyptian Journal of Biological Pest Control*   (2021) 31:144

Page 6 of 8

Table 1 Impact of entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) on fruit infestation (%) by *Bactrocera cucurbitae* under field conditions (pooled data 2017 and 2018)

Treatments	3 DAT	6 DAT	9 DAT	12 DAT	15 DAT	18 DAT	21 DAT	24 DAT	27 DAT	30 DAT
B. bassiana	43.25b	39.24b	34.27c	26.67c	19.97c	15.72d	12.03c	9.98c	8.12d	7.11c
M. anisopliae	49.71ab	46.72a	41.92b	36.31b	31.88b	25.56c	21.28b	19.46b	17.44c	15.14b
Malathion	31.67c	17.69c	13.66d	24.04c	35.09b	42.01b	48.71a	51.68a	52.64a	51.09a
Control	55.91a	51.65a	55.09a	51.79a	53.42a	49.38a	48.85a	47.98a	43.67b	49.44a

Means followed by the same letter in each column are not significantly different according to HSD ($P = 0.05$) (DAT: days after treatment)

49.71%) when plants were exposed to *B. bassiana* and *M. anisopliae,* respectively, while infestation was relatively low in the control. However, as shown in Table 1 that at this time interval Malathion effectively reduced the fruit infestation (31.67%). Furthermore, the time interval increased the effectiveness of both EPF started to increase, while Malathion lost its efficacy as time lapsed. 12DAT results showed that fruit infestation in Malathion treated plant started to increase and reached 24.04%; however, in case of *B. bassiana M. anisopliae* the fruits' infestation was (26.67, 36.31%) significantly lower than 3DAT. After 30 days of treatment, application EPF treated fruits showed significantly difference (DF = 3, $F = 495.11$, $P < 0.05$), high reduction in percent fruit infestation as, 7.11% fruit infestation recorded under *B. bassiana,* while *M. anisopliae* was able to reduce infestation about 15.14% and for Malathion it was 51.09%.

Impacts of B. bassiana and M. anisopliae on population reduction of B. cucurbitae

Effect of both EPF at 10^8 CFU/ml concentration was also examined on percent corrected population reduction at different time intervals under field conditions (Table 2). Three days after treatments application, a population reduction was significantly different (DF = 2, $F = 48.40$, $P < 0.05$) as 39.1% was observed in Malathion treated plot, while in case of EPF population reduction was significantly high (26.47%), when *B. bassiana* was sprayed, whereas it was significantly low (21.08%) in case of *M. anisopliae.* Population reduction against the application of Malathion remained higher than EPF up to 12DAT. Anyhow, 18 DAT population reduction (%) in EPF

treated plots increased by Malathion treated plot (DF = 2, $F = 96.82$, $P < 0.05$). It was significantly high (56.68%) in case of *B. bassiana,* and significantly low (41.34 and 30.62%) under treatment of *M. anisopliae* and Malathion, respectively. Maximum population reduction (68.59%) was recorded 30 DAT (DF = 2, $P < 0.05$), when *B. bassiana* was applied, while at the same time interval against the application of *M. anisopliae* and Malathion, it was (48.60 and 13.13%), respectively.

Discussion

EPF have a wide range of hosts, and their mode of action may be different (Sujeetha and Sahayaraj 2014). Unlike the chemical insecticides, EPF are safe for human being (Zimmermann 2007). Due to their unique and pests controlling features, they can be used as one of the most potential bio-control agent and can also be used efficiently in organic farming (Litwin et al. 2020).

This study was conducted to evaluate the efficacy of *B. bassiana* and *M. anisopliae* against *B. cucurbitae* under controlled and field conditions. Results showed that both fungi had the potential to be used against *B. cucurbitae*. The study conducted by Gul et al. (2015) and Qazzaz et al. (2015) also strengthens the above statement as they also found that these fungi could be used against fruit flies efficiently. However, in the present study, it was examined that *B. bassiana* had more virulent effect than *M. anisopliae* against *B. cucurbitae.* Finding regarding the more pathogenicity of *B. cucurbitae* in this study is also supported by the research work of (Mehdi and Al-Fadili 2020) who reported that under laboratory conditions isolates of *B. bassiana* were more virulent than the isolated of *M. anisopliae*.

Table 2 Impact of entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) on corrected population reduction (%) of *Bactrocera cucurbitae* under field conditions (pooled data 2017 and 2018)

Treatments	3 DAT	6 DAT	9 DAT	12 DAT	15 DAT	18 DAT	21 DAT	24 DAT	27 DAT	30 DAT
B. bassiana	26.47b	31.58b	35.63b	44.41b	51.23a	56.58a	60.06a	64.85a	66.71a	68.59a
M. anisopliae	21.08c	23.62c	28.13c	32.66c	36.85b	41.34b	43.9b	45.09b	46.94b	48.60b
Malathion	39.1a	58.83a	63.8a	53.07a	41.53b	30.62c	20.87c	16.24c	15.09c	13.13c

Means followed by the same letter in each column are not significantly different according to HSD ($P = 0.05$) (DAT: days after treatment)

Hamzah *et al. Egyptian Journal of Biological Pest Control*   (2021) 31:144

Page 7 of 8

During the present studies, contact and oral mode of applications were also tested and it was found that the contact mode was more suitable comparatively. Similar findings were also reported by Konstantopoulou and Mazomenos (2005) that the isolates of *B. bassiana* were more virulent against adult fruit flies than the isolates of other fungi. Obtained findings were also supported by Gul et al. (2015), who evaluated the pathogenicity of EPF against fruit flies (*B. zonata*) through different application methods and they found that these fungi were more virulence in contact method than the oral method. Mainly these fungi caused pathogenicity by adhering to the hosts and then penetrate in their bodies (Litwin et al. 2020). Conventionally, it was considered that EPF are pathogenic only in contact application methods, but during recent studies it has been found that other application methods can be used (Mannino et al. 2019). Anyhow the reason of low pathogenicity of fungi in oral bioassay may be that these fungi mostly kill their host arthropods by penetrating their cuticle as described by Mora et al. (2018) that in most cases EPF spores adhere with the cuticle of an insect, then germination start and ultimately by penetrating in the cuticle, spores disseminate in the body of insects.

Mode of application, dose rate, environmental conditions and isolates of fungi are important factors for causing pathogenicity in host pests (Rizvi et al. 2009). In this study, a maximum pathogenicity was attained at the highest concentration 1×10^8. Similar findings were revealed by Mar and Lumyong (2012). Three concentrations (10^6, 10^7 and 10^8 CFU/ml) were used in this study, and their efficacy was checked after 2 days up to 2 weeks in laboratory experiment and the most promising results were given by the highest concentration. Trinh et al. (2020) also used the same concentration against aphid, and his results were supportive to the obtained findings that the efficacy of EPF has increased by increasing the concentration. Time interval is also an important for EPF, as the maximum mortality was observed at 14 DAT. This result also corroborates with the results of Nazir et al. (2019) who reported that after applying the EPF, with the passage of time mortality of insects was increased.

Conclusions

Both EPF (i.e., *B. bassiana* and *M. anisopliae*) had the potential to be used against *B. cucurbitae*. However, *B. bassiana* showed a high efficacy than *M. anisopliae*. Moreover, contact application method was found to be more effective than the oral application.

Declarations

Author details
[1]Department of Entomology, Faculty of Crop and Food Sciences, Pir Mehr Ali Shah - Arid Agriculture University, Rawalpindi, Punjab, Pakistan. [2]Department of Horticulture, Faculty of Crop and Food Sciences, Pir Mehr Ali Shah - Arid Agriculture University, Rawalpindi, Punjab, Pakistan.

References
Abbas HS, Sultan AA, Hamad BS (2017) Pathogenicity of *Metarhizium anisopliae* isolates on adults of the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae) under laboratory condition. Int J Entomol Res 2(6):5–10

Ain Q, Mohsin AU, Naeem M, Shabbir G (2021) Effect of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, on *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) populations in different onion cultivars. Egypt J Bio Pest Cont 31(1):1–8

Beris EI, Papachristos DP, Fytrou A, Antonatos SA, Kontodimas DC (2013) Pathogenicity of three entomopathogenic fungi on pupae and adults of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). J Pest Sci 86(2):275–284

Daniel C, Wyss E (2009) Susceptibility of different life stages of the European cherry fruit fly, *Rhagoletis cerasi*, to entomopathogenic fungi. J Appl Entomol 133(6):473–483

Dhillon MK, Singh R, Naresh JS, Sharma HC (2005) The melon fruit fly, *Bactrocera cucurbitae*: a review of its biology and management. J Insect Sci 5(1):1–16

Ekesi S, Maniania NK, Lux SA (2002) Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Biocontrol Sci Technol 12(1):7–17

Hamzah *et al. Egyptian Journal of Biological Pest Control*   (2021) 31:144

Page 8 of 8

Gul HT, Freed S, Akmal M, Malik MN (2015) Vulnerability of different life stages of *Bactrocera zonata* (Tephritidae: Diptera) against entomogenous fungi. Pak J Zool 47(2):307–317

Hadi MS, Himawan T, Aini LQ (2013) The effectiveness of entomopathogenic fungi *Beauveria bassiana* with the addition of insect growth regulator lufenuron for controlling *Bactrocera carambolae*. J Trop Life Sci 3(3):187–192

Henderson CF, Tilton EW (1955) Tests with acaricides against the brown wheat mite. J Econ Entomol 48(2):157–161

Hsu JC, Feng HT (2006) Development of resistance to Spinosad in oriental fruit fly (Diptera: Tephritidae) in laboratory selection and cross-resistance. J Econ Entomol 99(3):931–936

Konstantopoulou MA, Mazomenos BE (2005) Evaluation of *Beauveria bassiana* and *B. brongniartii* strains and four wild-type fungal species against adults of *Bactrocera oleae* and *Ceratitis capitata*. Biocontrol 50(2):293–305

Litwin A, Nowak M, Różalska S (2020) Entomopathogenic fungi: unconventional applications. Rev Environ Sci Biotechnol 19:23–42

Mannino MC, Huarte-Bonnet C, Davyt-Colo B, Pedrini N (2019) Is the insect cuticle the only entry gate for fungal infection? Insights into alternative modes of action of entomopathogenic fungi. J Fungi 5(2):33

Mar TT, Lumyong S (2012) Evaluation of effective entomopathogenic fungi to fruit fly pupa, *Bactrocera* spp. and their antimicrobial activity. Chiang Mai J Sci 39(3):464–477

McGraw-Hill C (2008) Statistix 8.1 (Analytical Software, Tallahassee, Florida). Maurice/Thomas

Mehdi NS, Al-Fadili AW (2020) Laboratory evaluation of *Beauveria bassiana* and *Metarhizium anisopliae* isolates against rust-red flour beetle *Tribolium castanum* (Herbst) (Coleoptera: Tenebrionidae). Ann Trop Med Public Health 23:231–808

Mora MA, Castilho AM, Fraga ME (2018) Classification and infection mechanism of entomopathogenic fungi. Arq Inst Biol. https://doi.org/10.1590/1808-1657000552015

Nawab NN, Mahmood K (2014) 'Faisalabad long', a high-yielding bitter gourd variety. Int J Veg Sci 20(4):322–328

Nazir T, Basit A, Hanan A, Majeed MZ, Qiu D (2019) In vitro Pathogenicity of some entomopathogenic fungal strains against green peach aphid *Myzus persicae* (Homoptera: Aphididae). Agron 9(1):7–17

Ortu S, Cocco A, Dau R (2009) Evaluation of the entomopathogenic fungus *Beauveria bassiana* strain ATCC 74040 for the management of *Ceratitis capitata*. Bull Insectol 62(2):245–252

Qazzaz FO, Al-Masri MI, Barakat RM (2015) Effectiveness of *Beauveria bassiana* native isolates in the biological control of the Mediterranean fruit fly (*Ceratitis capitata*). Adv Entomol 3(2):44–55

Rizvi PQ, Choudhury RA, Ali A (2009) Recent advances in biopesticides. In: Khan MS, Zaidi A, Musarrat J (eds) Microbial strategies for crop improvement. Springer, pp 185–203

Schneider-Orelli O (1947) Entomologisches Praktikum. Sauerlander HR Aarau Switzerland.

Sookar P, Bhagwant S, Awuor OE (2008) Isolation of entomopathogenic fungi from the soil and their pathogenicity to two fruit fly species (Diptera: Tephritidae). J Appl Entomol 132(9–10):778–788

Sujeetha JAR, Sahayaraj K (2014) Role of entomopathogenic fungus in pest management. In: Sahayaraj K (ed) Basic and applied aspects of biopesticides. Springer, pp 31–46

Trinh DN, Ha TKL, Qiu D (2020) Biocontrol potential of some entomopathogenic fungal strains against bean aphid *Megoura japonica* (Matsumura). Agriculture 10(4):114

Vega FE, Goettel MS, Blackwell M, Chandler D, Jackson MA, Keller S, Pell JK (2009) Fungal entomopathogens: new insights on their ecology. Fungal Ecol 2(4):149–159

Zimmermann G (2007) Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Sci Technol 17(6):553–596

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.