

Efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* against wheat aphid

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Abstract

In the recent study, the entomopathogenic fungus, *Beauveria bassiana* and *Metarhizium anisopliae* against wheat aphid species in field conditions to check the insecticidal effect of entomopathogenic fungi were used to evaluate their pathogenicity against adults of different aphid species i.e., *Schizaphis graminum*, *Rhopalosiphum padi*, *Brevicoryne brassicae* and *Lipaphis erysimi*, and their natural enemies of crops. *Beauveria bassiana* and *Metarhizium anisopliae* were found effective at all concentrations i.e., 1×10^6 , 1×10^7 and 1×10^8 cfu/ml on all aphid species, but the uppermost concentration (1×10^8 cfu/ml) provided maximum control within a short period of time. *B. bassiana* concentration (1×10^8 cfu/ml) proved to be lethal to wheat aphid species after the 3rd day. Mortality of wheat aphids were observed at maximum highest concentration of 1×10^8 cfu/ml were in range of 100% at 8th day of treatment for different aphid species treated at various concentrations. *M. anisopliae* (1×10^8 cfu/ml) showed excellent mortality 85% and 84% at 5th and 6th day respectively, against wheat aphid species. In conclusion, the use of *Beauveria bassiana* and *Metarhizium anisopliae* as biopesticides in agriculture is a sustainable and environmentally friendly approach to control insects' pests.

Keywords: aphids, biological control, *Beauveria bassiana*, *Metarhizium anisopliae*, pathogenic fungi

Introduction

Wheat is the most important second crop after rice, feeds almost half of the total population and contributes 20% of the daily protein to 4.5 billion people [1]. The weightage of wheat crop in GDP of Pakistan is 3.1 percent (GoP, 2010). In Pakistan, major food of Pakistan is wheat and grown as a cereal crop, but other plant materials like straw, seed, and bran is used to feed livestock and also used in industries. Wheat is the 2nd largest cereal crop and contributes more than 12% value added in agriculture of Pakistan and about 2.6% to GDP [2]. The natural equilibrium was perfect between its natural enemies and aphid population up to 19's [3]. But in few decades, this balance was disturbed by the extensive use of chemicals and indiscrimination of non-selective pesticides on cereal crops to kill the insect population [4]. Recorded seven aphid's species on wheat from various districts of Punjab, Pakistan, i.e., *R. padi*, *Sitobion avenae* (F.), *Metopolophum dirhodum*, *Schizaphis graminum* (Rond.), *S. miscanthi*, *R. maidis* (Fitch) and *Rhopalosiphum rufiabdominalis* (Sasaki) [5]. Plants are damaged by aphid population that feed on cell sap directly and indirectly damaging plants by transmitting different plant viruses and act as a vector of different diseases and excrete honeydew where sooty mold grows and reduce the photosynthetic process of plant [6]. 70 and 50% grains weight may reduce during spring and wheat crop respectively due to aphid infestation [7].

Wheat aphid is the pest that drastically affects the wheat crop in Pakistan by sucking sap from the wheat plant resulting in direct and indirect crop damages. Its rapid spread stimulated research funding standing the aphid's biology, ecology, and potential for control [8]. More than 20 aphid species have been reported that resistance showed to several organophosphates, carbamate and pyrethroid [9]. Entomopathogenic fungi being a substitute to chemicals plays an important role in suppressing the insect population and

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reducing resistance development in insects [10]. They have less mammalian toxicity, less residual activity and easily to formulate [11]. In Pakistan, there are 92 species of aphids that damaging wheat crop seasonally [12]. In last few years, population of wheat aphids increasing and attaining the regular pest status on various cereal crops in Pakistan [3]. *Rhopalosiphum padi* and *S. graminum* cause severe loss to wheat crop throughout the world including Pakistan wheat growing areas [13]. Intensive use of agro-based insecticides to fulfil the needs of population food throughout the world causes socio-economic problems. For example, many species have showed resistance to all groups of insecticides like organophosphate, carbamate and pyrethroid [14]. Entomopathogenic fungi on the other hand provide an environmentally friendly substitute to the use of pesticides. No mammalian toxicity, easy to formulate, with less chance and no residual activity to develop resistance [10] [11]. *Metarhizium anisopliae* and *B. bassiana* can easily be isolated from the insect dead bodies and distributed all over the world [10].

Biological control, cultural practices, and host plant resistance of arthropod pests in Integrated pest management (IPM) plays an important role [15]. Intercropping, varietal mixtures and the treatment of stem cuttings in cultural practices to make sure pest-free planting material [15]. It is very difficult to use host plant resistance across many crops although it is an environmentally and economical approach [15] [16]. Bio-pesticides are generally natural, can be formulated, have approximately a little side effect to mammals and almost least residual impacts, hence; the likelihood of developing resistance is minimum [10] [11]. Entomopathogenic fungal sp., such as *Beauveria bassiana* and *Metarhizium anisopliae* have broad-spectrum relative host specificity [28], easily procured from insects or other resources like vegetation phylloplanes, soil etc. and are diversified with respect to habitat selection all around the globe [17].

Metarhizium anisopliae and *Beauveria bassiana* are broadly spread in all regions of the world and have a wide host range that can easily be isolated from insects [17] [19]. Biological control agents like predators and parasitoids show potential control for lettuce aphids and syrphids [20]. Several techniques have been used in target plants to establish the *B. bassiana* as an endophyte. At two leaf or whorl stage in maize crop *B. bassiana* showed endophytic effect [21]. Main radicle of seedlings in cocoa and coffee when inoculated with *B. bassiana* also showed endophytic effect [22]. *Beauveria bassiana* recognized as endophyte in coffee seedlings with highest endophytic healing by using foliar sprays, stem injections and soil drenches [23]. *B. bassiana* could be established as endophyte through foliar sprays, seed dressing and soil inoculation in different sorghum tissues [24].

Both fungal species exhibited greater efficacy against cowpea aphid (Ekesi et al., 2000), White fly (Javed et al., 2019), and against wheat aphids (Haron et al. 2020). Therefore, the current study was conducted to evaluate the potential of *Beauveria bassiana* and *Metarhizium anisopliae* against wheat aphid. What inspired you to conduct research on the efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* against wheat

aphid?

As, the use of *Beauveria bassiana* and *Metarhizium anisopliae* as biopesticides in agriculture is need for sustainable and environmentally friendly pest control methods. Both *Beauveria bassiana* and *Metarhizium anisopliae* are entomopathogenic fungi, meaning they are naturally occurring fungi that can infect and kill insects. 1. Environmental Sustainability 2. Targeted Pest Control, 3. Compatibility with Organic Farming and 4. Safety for Farmers and Consumers.

Materials and Methods

Location

Research was carried out to assess the efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* against wheat aphid in wheat fields during the cropping season of wheat 2018-2019. Research was carried out in the entomological research area young wala, Department of Entomology, University of Agriculture, Faisalabad, area is under cultivation with several crops e.g., wheat, cotton, maize, sugarcane, and other vegetables. The farm area is enriched with biodiversity of several insect pests and natural predators. In university of Agriculture different research areas present (Agronomy field, Horticulture fields and Entomology research fields), my experiment conducted in the Entomology research area young wala.

Plant material

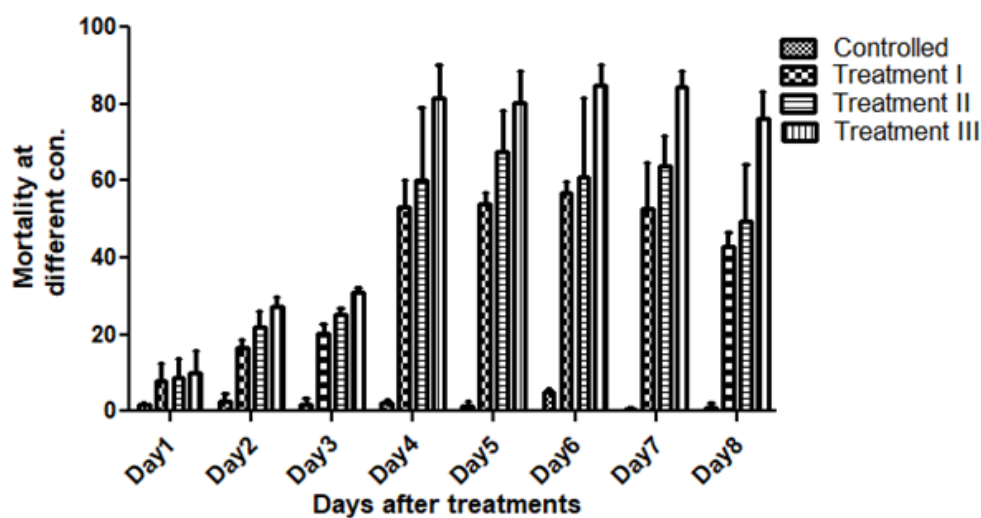
Galaxy 2017 variety of seed taken from Ayub Agriculture research institute. The seed was sown in the Research Area of Entomology Young wala on December 12, 2018. They were sown by line sowing method. The recommended agronomic practices were followed for the wheat crop

Mode of Action

A typical mode of infection of various entomopathogenic fungi involves several steps to complete its life cycle that depends on different steps. First step is the conidial attachment to the insect cuticle, second is the germination of hyphae, third is the penetration of hyphae through cuticle, fourth is the growth of fungi within the host body, fifth is the emergence of fungi outside the insect body and the last is conidiogenesis. Enzymes play an important role after the attachment of fungal pathogen to host's body.

Mode of Application

Concentration of *B. Bassiana* and *Metarhizium anisopliae* at least 1×10^8 cfu/ml, 1×10^7 cfu/ml, 1×10^6 cfu/ml. For the activity of *B. Bassiana* and *M. anisopliae* high humidity is required. Solar radiation causes fungal spores' mortality. Fungal application is very effective when it is sprayed in cool temperature. Spray should be efficient due to the short life span of fungus germination. Its life span is round about 10 days, after 10 days its effect ended. It is very difficult to control insects that bore into the plants. During early stages of insect growth, application of *B. Bassiana* and *M. anisopliae* become effective to manage the in-



sect pest population. Mortality of insects depends on the viability and number of spores contacting the insect, susceptibility, insect age and environmental condition.

Both fungal species with same or different concentration had been tested against cowpea aphid (Ekesei et al., 2000), White fly (Javed et al., 2019), and against wheat aphids (Haron et al. 2020).

Experimental layout

When wheat plants reach at 6-8 leaves stages (6-8 weeks old) or after first incidence of aphids, different concentration of *Beauveria bassiana* and *Metarhizium anisopliae* (1×10^8 cfu/ml, 1×10^7 cfu/ml, 1×10^6 cfu/ml with control treatment) were applied by using hand sprayer and foliar application method (Ekesei et al., 2000). The different applications of both fungi have been done after 2-3 weeks of interval depending on the rate of infestation. The experiment was laid out Randomize complete block design (RCBD) with control treatment. The 16 blocks in the field of wheat had been selected for each treatment and each treatment was applying 1 time in each block. In each block there are four treatments and each treatment replicated four times. The *Beauveria bassiana* and *Metarhizium anisopliae* were applied 3 times during the trial, with 3 concentration 1×10^8 cfu/ml, 1×10^7 cfu/ml, 1×10^6 cfu/ml after the 10 days intervals. Spray should be efficient due to the short life span of fungus germination. Its life span is round about 10 days, after 10 days its effect ended. The samplings were collected from aphid's arrival to harvesting of wheat crop. Data was recorded 5 times after applied treatment. The collected specimens brought to lab and checked under microscope to confirm the infection and mortality data were recorded after regular interval of time, and arranged in excel for further analysis.

Statistical analysis:

The collected data will be analysed statistically by using statistical software 8.1. Analysis of variance was followed Two-Factorial RCBD design and Means was computed by using Turkey-HSD test using 5% level of significance (LS) (Tukey, 1949).

Results and discussion

In the current study, treatments Controlled, T1, T2 and T3 were designed, and observations were made from 1st day to onward. Means of mortality were calculated with different concentrations of *Metarhizium anisopliae* as 1×10^6 , 1×10^7 and 1×10^8 , higher mortality mean was measured at T₃ on 6th day after treatment (84.67 ± 5.51) and lowest mortality mean was measured at T1 1st day (8.00 ± 4.36). day 1st, 2nd and 3rd day mortality level significant to other days 4th 5th 6th 7th and 8th day. At T₂ highest mortality calculated at 5th day after treatment mortality mean was (67.67 ± 10.59) and lowest mortality was measured at day 1st (8.67 ± 5.03) and day 1st, 2nd, 3rd and 8th significant to other days 4th, 5th, 6th and 7th day. At treatment T₃ the highest mortality was observed at day 6th (84.67 ± 5.51) and lowest mortality at 1st day after treatment (10.00 ± 5.56). day 1st significant to other days. Day 2nd significant to day 4th, 5th, 6th, 7th, and 8th day after treatment.

In the current study, treatments Controlled, T1, T2 and T3 were designed, and observations were made from 1st day to onward. Means of mortality were calculated with different concentrations of *Beauveria bassiana* as 1×10^6 , 1×10^7 and 1×10^8 , higher mortality mean was measured at T3 8th day after treatment (100.00 ± 8.88). and lowest at T1 day 1st (8.00 ± 4.35). T1 highest specimen of aphids die at day 4th after treatment (48.33 ± 1.15). at T2 highest level of aphids die at day 5th after treatment (75.00 ± 7.21) and lower level of mortality of aphids was at 1st day of treatment (8.67 ± 5.03) and T3 highest mortality

Efficacy of *Metarhizium anisopliae* against wheat aphid species

Days	Controlled	Treatment I	Treatment II	Treatment III
1 st	1.67±0.57 ^a	8.00±4.36 ^a	8.67±5.03 ^a	10.00±5.56 ^b
2 nd	2.67±2.08 ^a	16.67±2.08 ^a	22.00±4.00 ^a	27.33±2.31 ^a
3 rd	1.67±1.53 ^a	20.33±2.52 ^a	25.33±1.53 ^a	31.00±1.00 ^{bc}
4 th	2.00±1.00 ^a	53.33±6.81 ^c	60.33±18.58 ^c	81.66±8.38 ^d
5 th	1.33±1.15 ^a	54.00±3.00 ^c	67.67±10.59 ^c	80.33±8.08 ^d
6 th	5.00±1.00 ^b	57.00±2.64 ^c	61.00±20.42 ^c	84.67±5.51 ^d
7 th	0.333±0.57 ^a	52.67±11.93 ^c	64.00±7.81 ^c	84.33±4.16 ^d
8 th	1.00±1.00 ^a	43.00±3.46 ^{bc}	49.33±15.04 ^b	76.33±7.02 ^{cd}

Treatment I= MA (1×10⁶) = *Metarhizium anisopliae* (1×10⁶) Dead specimens

Treatment II= MA (1×10⁷) = *Metarhizium anisopliae* (1×10⁷) Dead specimens

Treatment III= MA (1×10⁸) = *Metarhizium anisopliae* (1×10⁸) Dead specimens

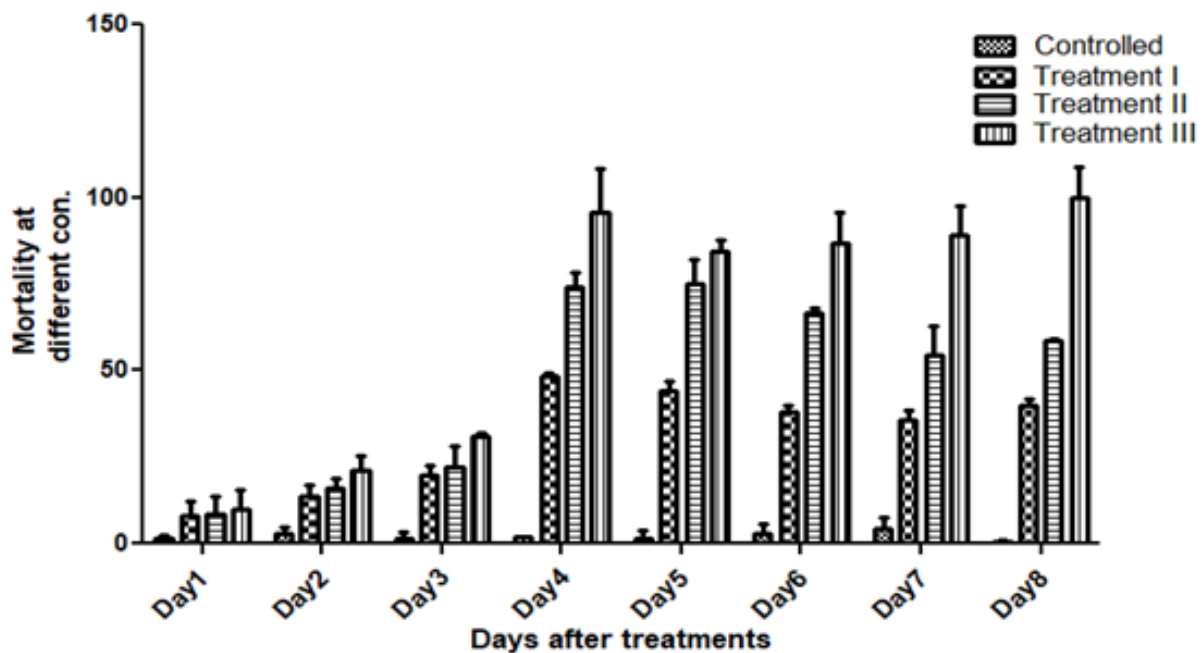
Efficacy of *Beauveria bassiana* against wheat aphid species

Days	Controlled	Treatment I	Treatment II	Treatment III
1 st	1.66 ± .57 ^a	8.00 ± 4.35 ^a	8.67 ± 5.03 ^a	10.00 ± 5.56 ^a
2 nd	2.66 ± 2.08 ^a	13.66± 3.21 ^{ab}	16.00± 2.64 ^a	21.33 ± 4.04 ^a
3 rd	1.67 ± 1.52 ^a	19.66 ± 3.05 ^b	22.00± 6.24 ^a	31.00 ± 1.00 ^{ab}
4 th	2.00 ± 0.00 ^a	48.33 ± 1.15 ^b	74.00± 4.35 ^b	95.67 ± 12.50 ^{cd}
5 th	1.33 ± 2.30 ^a	44.00 ± 3.00 ^b	75.00± 7.21 ^b	84.67 ± 2.88 ^c
6 th	2.67 ± 2.88 ^a	38.00 ± 1.73 ^b	66.67± 1.15 ^b	87.00 ± 8.88 ^c
7 th	4.33 ± 3.22 ^b	35.67 ± 3.05 ^b	54.67± 8.08 ^b	89.33 ± 8.08 ^c
8 th	0.67 ± 0.57 ^c	40.00 ± 2.00 ^b	58.66± 0.57 ^b	100.00 ± 8.88 ^{cd}

Treatment I= MA (1×10⁶) = *Beauveria bassiana* (1×10⁶) Dead specimens

Treatment II= MA (1×10⁷) = *Beauveria bassiana* (1×10⁷) Dead specimens

Treatment III= MA (1×10⁸) = *Beauveria bassiana* (1×10⁸) Dead specimens



Efficacy of *Metarhizium anisopliae* and *Beauveria bassiana* compare effect at wheat aphid species.

DAYS	Controlled		Treatment I		Treatment II		Treatment III	
	MA	BB	MA (1×10 ⁶)	BB (1×10 ⁶)	MA (1×10 ⁷)	BB (1×10 ⁷)	MA (1×10 ⁸)	BB (1×10 ⁸)
1 st	1.67±0.57a	1.66 ± 0.57a	8.00±4.36a	8.00 ± 4.35a	8.67±5.03a	8.67 ± 5.03a	10.00±5.56b	10.00 ± 5.56a
2 nd	2.67±2.08a	2.66 ± 2.08a	16.67±2.08a	13.66±3.21ab	22.00±4.00a	16.00±2.64a	27.33±2.31a	21.33±4.04a
3 rd	1.67±1.53a	1.67 ± 1.52a	20.33±2.52a	19.66 ± 3.05b	25.33±1.53a	22.00±6.24a	31.00±1.00bc	31.00±1.00ab
4 th	2.00±1.00a	2.00 ± 0.00a	53.33±6.81c	48.33 ± 1.15b	60.33±18.58c	74.00±4.35b	81.66±8.38d	95.67±12.50cd
5 th	1.33±1.15a	1.33 ± 2.30a	54.00±3.00c	44.00 ± 3.00b	67.67±10.59c	75.00±7.21b	80.33±8.08d	84.67±2.88c
6 th	5.00±1.00b	2.67 ± 2.88a	57.00±2.64c	38.00 ± 1.73b	61.00±20.42c	66.67±1.15b	84.67±5.51d	87.00±8.88c
7 th	0.333±0.57a	4.33 ± 3.22b	52.67±11.93c	35.67 ± 3.05b	64.00±7.81c	54.67±8.08b	84.33±4.16d	89.33±8.08c
8 th	1.00±1.00a	0.67 ± 0.57c	43.00±3.46bc	40.00 ± 2.00b	49.33±15.04b	58.66±0.57b	76.33±7.02cd	100.00±8.88cd

Treatment I = MA (1×10⁶) = *Metarhizium anisopliae* (1×10⁶) Dead specimens

Treatment I = BB (1×10⁶) = *Beauveria bassiana* (1×10⁶) Dead specimens

Treatment II = MA (1×10⁷) = *Metarhizium anisopliae* (1×10⁷) Dead specimens

Treatment II = BB (1×10⁷) = *Beauveria bassiana* (1×10⁷) Dead specimens

Treatment III = MA (1×10⁸) = *Metarhizium anisopliae* (1×10⁸) Dead specimens

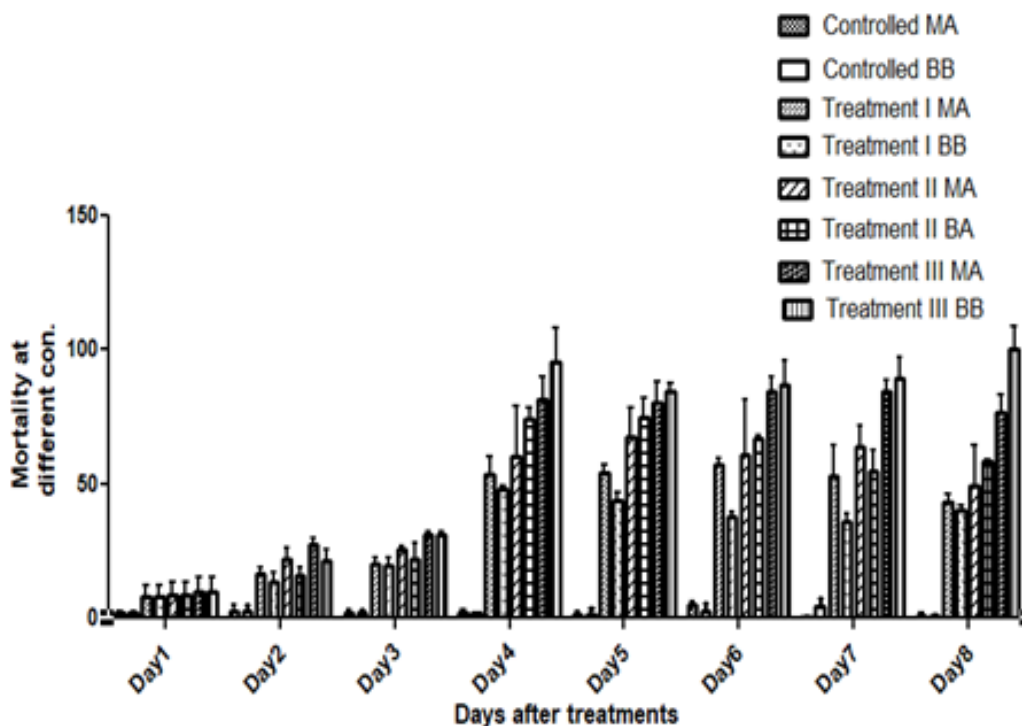
Treatment III = BB (1×10⁸) = *Beauveria bassiana* (1×10⁸) Dead specimens

level at 8th day of treatment and lowest at 1st day of treatment. In the current study, treatments controlled, T1, T2 and T3 were designed, and observations were made from 1st day to onward. Means of mortality were calculated with different concentrations of *Beauveria bassiana* and *Metarhizium anisopliae* as 1×10⁶, 1×10⁷ and 1×10⁸ concentrations. Both fungus applied at same concentrations and same way but in treatment T1 *M. anisopliae* shows more effective results against aphids as compared to *B. bassiana*. After the 4th day *M. anisopliae* show mortality of aphids (53.33±6.81) and at 4th day *B. bassiana* show mortality level (48.33 ± 1.15). On the 5th day of treatment mortality level was measured (54.00±3.00) by *M. anisopliae* and (44.00 ± 3.00) mean was measured by *B. bassiana*. At T2 *B. bassiana* shows more mortality as compared to *M. anisopliae*. On the 4th day of treatment were show means of mortality level of aphids (60.33±18.58) by *M. anisopliae* and mortal-

ity of aphids (74.00 ± 4.35) by *B. bassiana*. On the 5th day of treatment (67.67±10.59) were mean of mortality by *M. anisopliae* and (75.00 ± 7.21) mortality of aphids by *B. bassiana*. On the 6th day of treatment mean of mortality were observed (61.00±20.42) by *M. anisopliae* and (66.67 ± 1.15) mortality by *B. bassiana*. At 7th day of of treatment mean of mortality were measured (64.00±7.81) by *M. anisopliae* and mortality of aphids (54.67± 8.08) by *Beauveria bassiana* and at 8th day of treatment mortality mean were observed (49.33±15.04) by *M. anisopliae* and (58.66 ± 0.57) mortality of aphids was measured by *B. bassiana*. At T3 highly mortality was measured by *B. bassiana* as compared to *M. anisopliae*, (95.67±12.50) mortality level was measured by *B. bassiana* and 81.66±8.38) mortality level was observed by *M. anisopliae* at 4th day after treatment applied. 80.33±8.08 mortality was observed by *M. anisopliae* and (84.67 ± 2.88) mortality level calculated by *Beauveria bas-*

siana at 5th day of treatment. At 6th day of treatment specimens of aphids died was observed (84.67±5.51) by *M. anisopliae* and (87.00 ± 8.88) mortality was measured by *B. bassiana*. On the 7th day of treatment mortality was observed (76.33±7.02) by *M. anisopliae* and (100.00±8.88) mortality calculated by *B. bassiana*. Overall results of observation *B. bassiana* showed a higher mortality level as compared to *M. anisopliae*. *B. bassiana* and *M. anisopliae* showed significant results with controlled and

in laboratory as a biological control agent. *Beauveria bassiana* and *M. anisopliae* kills arthropods as a result of the insect encountering the conidia (fungal spores) contact is made in several ways. The most common and effective is the spray droplets landing on the pest or by walking on a treated surface. Once the fungal spores attach to the insect's cuticle, the fungus spores germinate sending out threaded hyphae which penetrate the insect's body and proliferate. It takes 3 to 5 days for an infected



non-significant results to each other's.

Discussion

Wheat is the most important second crop after rice, feed almost half of the total population and contributes 20% of the daily protein to 4.5 billion people Up to the natural equilibrium was perfect between and its natural enemies and aphid population. However, during recent past, this balance was disturbed by the extensive use of chemicals and indiscrimination of non-selective pesticides on cereal crops to kill the insect population. The shift in sowing time of wheat, availability of relatively photo insensitive varieties, temperature tolerant genotypes and higher fertilizer input have led changes in pest complex of wheat and elevated the status of aphids from occasional to regular pests on wheat crop.

Biological control, cultural practices, and host plant resistance of arthropod pests in Integrated pest management (IPM) plays an important role. Fungal entomopathogens, including *Metarhizium anisopliae* and *B. bassiana* and have been tested against many cassava pests of various crops in greenhouse and

insect to die. The dead insect may serve as a source of spores for secondary spread of the fungus. An infected adult male will also transmit the fungus during mating. *B. bassiana* and *M. anisopliae* had harmful impacts on *Aphis gossypii* directly and indirectly and decreased pest population. The present research work was carried out to assess the efficacy of *Beauveria bassiana* and *M. anisopliae* against wheat aphid in wheat fields during the cropping season of wheat 2018-2019. Research was carried out in the entomological research area young wala, Department of Entomology, University of Agriculture, Faisalabad.

In my research results, efficacy of *Metarhizium anisopliae* and *B. bassiana* against wheat aphid were assessed by applying different concentrations of *M. anisopliae*. The fungus was applied by using concentrations 1×10^8 , 1×10^7 and 1×10^6 respectively with a control treatment. Effectiveness of *M. anisopliae* and *B. bassiana* against different aphid species. The fungi causing pathogenicity in insects have been observed to cause mortality in insect pest populations and therefore studied for their use as biological control agents. *M. anisopliae* caused 84.6% mortality respectively of wheat aphid at 1×10^7 spores ml^{-1} . Similar findings were recorded by [25] who have been stated that, at the

concentration of 10^7 spores ml^{-1} , *M. anisopliae*, *L. lecanii*, and *B. bassiana* caused 83.3, 100 and 61.5% mortality after 7 days respectively of *Aphis craccivora* Koch (cowpea aphid).

At concentration 1×10^8 of *Metarhizium anisopliae*, maximum population reduction was recorded on day 6 when there was 84.7% *B. bassiana* caused mortality 100% after day 8. whereas [26] have stated that at concentration of 10^7 spore ml^{-1} and after 4.4 days, *B. bassiana* caused 90% mortality. The difference in population reduction is due to the virulence of fungal species applied. The results show that *M. anisopliae* caused 25% virulence against wheat aphid population after 3 days 67% mortality after 6th day at 1×10^7 . [26] also described that after 3.8 days, some *M. anisopliae* isolates caused 64% virulence against the population of mustard aphid. The findings of the current study show that, *M. anisopliae* caused 81% % mortality within 4 days and *B. bassiana* shown 95% mortality after 5th day of treatment against *Diuraphis noxia* when used at 10^8 spore ml^{-1} whereas [27] stated that, at the concentration of 10^5 spore ml^{-1} , *L. lecanii* showed 77.16% reduction against *L. erysimi* after 10 days. Differences are due to different fungal species and their different concentrations applied to different aphid species. In my research *M. anisopliae* caused approximately 85% mortality in *D. noxia* *B. bassiana* shown 84% mortality against wheat aphids whereas [28] illustrated that after 4 days, different strains of *B. bassiana* and *M. anisopliae* showed 100% mortality in *Myzus persicae* and *L. erysimi*. The difference in virulence of fungi is due to the aphid species.

The results of my research shows that *Metarhizium anisopliae* with different concentration exhibited 53 to 85% mortality in wheat aphid and different concentration of *B. bassiana* shows 48% to 100% mortality of wheat aphids (*Diuraphis noxia*) whereas [29] have been stated that strains of *B. bassiana*, *L. lecanii*, and *Paecilomyces* sp., showed 75 to 100% mortality in *Aphis gossypii* (cotton aphid). The difference in population reduction percentage may be due to different fungal species and concentrations at which they were applied and due to different aphid species. In the current findings, *Metarhizium anisopliae* was applied to wheat aphid (*Diuraphis noxia*) at concentration of 10^8 spores ml^{-1} caused 85% mortality and *B. bassiana* shows 95, 100% mortality of aphid in wheat crop. After day 4 to onward at day 8. These results of present research are also comparable to some previous scientists as described by [30] that after 3 days, *M. anisopliae* and *B. bassiana*, have been significant and contagious for the control of mustard aphid as they caused 88% mortality.

In the current research, 80% mortality of wheat aphid after 4 days with high concentration of *M. anisopliae* (10^8 spore per ml) *B. bassiana* 90% mortality against wheat aphid species was provided while [27] have been illustrated that, at the concentration of 10^7 spore/ml and after 4.4 days treatment, 90% mortality was caused by *B. bassiana*. Differences in results may be due to different concentrations of the fungus applied. Mortality of *Diuraphis noxia* was recorded from 70 to 85 percent by *M. anisopliae* at high concentration after four to five days interval of fungus application up to four days. However,

[25] reported mortality percentage with 12-hour interval up to seven days and concluded that mortality of aphid was increased with the increase in concentration; at high concentration the mortality was obtained after 72 hours, ranging between 53 to 60 percent. In the present research, 85% mortality of wheat aphid caused by *M. anisopliae* at concentration of 10^8 spore/ml and 68% mortality was caused by *M. anisopliae* at concentration of 10^7 spore/ml 95% to 100% mortality of wheat aphids by *B. bassiana* after day 4 to day 8, while [31]

Reported that mortality is also dependent on exposure time, temperature, and the concentration of conidial suspension [32]. The vulnerability of the same species of aphid is variable to different strains of fungi. Even various colonies of the same aphid species or biotypes have variable sensitivity to fungal infection [33] [34] that corroborates well with the present studies. The mortality of wheat aphid after the application of all concentrations and effect of various bio-pesticides exhibited that at the highest concentration (1×10^8), all the bio-pesticides showed the maximum mean mortality percentage.

The research work of [35] evaluated the pathogenicity of *B. bassiana* against different aphid species and *B. brassicae*, against aphid species, but with the high concentration maximum control within short time periods. *Beauveria bassiana* proved to be lethal against *L. erysimi* with LC50 of 1.36×10^6 at high concentration of 1×10^8 spores/ml, while at various concentrations of *B. bassiana* that used for different aphid species, the range of LC50 was 2.19-3.73 days. The results of these relate to our findings as the entomopathogenic fungi *B. bassiana* and *M. anisopliae* at different concentration levels in the experiment proved for the significant reduction of aphid population

The research work based on the integrated pest management of [36] against lettuce aphid to study the pathogenic effect of *B. bassiana* (Mosley) in laboratory conditions. They observed that GHA strain of entomopathogenic fungus *B. bassiana* has been effective against all life stages of aphid under lab conditions and affects aphid's fecundity. Leaf position has no effect against *Beauveria bassiana* persistence on lettuce foliage. 38% conidial population declined within 5 days which increased from 92% to 99% of post-spraying after 11 and 20 days. Our field experiment relates to their lab experiment as in both the aphids are controlled by the entomopathogenic effect of *Beauveria bassiana* and *M. anisopliae* with any use of pesticides effectively results in the reduction of population of target pest.

Conclusion

The research on the efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* against wheat aphids has provided valuable insights into the potential of these biopesticides for sustainable pest management in wheat crops. Through a series of carefully designed experiments, we have explored the impact of these fungal agents on aphid populations and their effectiveness under varying environmental conditions. Our findings indicate that both *Beauveria bassiana* and *Metarhizium anisopliae* show promise as effective biological control agents against

wheat aphids. However, the *Metarhizium anisopliae* significantly reduced the aphid population as compared to *Beauveria bassiana*. The observed reduction in aphid populations following application suggests a potential role for these fungi in integrated pest management strategies for wheat cultivation. In conclusion, the use of *Beauveria bassiana* and *Metarhizium anisopliae* as biopesticides in agriculture is a sustainable and environmentally friendly approach to control insects' pests.

Future interest

It is crucial to acknowledge the complexity of factors influencing the efficacy of these biopesticides. Environmental conditions, application methods, and the specific aphid species present can all influence the outcomes. Further research is needed to refine application protocols, optimize concentrations, and assess long-term effects on both target and non-target organisms.

To understanding the potential for aphids to develop resistance against *Beauveria bassiana* and *Metarhizium anisopliae*. To Studying the impact of *Beauveria bassiana* and *Metarhizium anisopliae* on non-target organisms for evaluating their environmental safety. Impact of these fungi on humans and other vertebrates, who contact with the field where *Beauveria bassiana* and *Metarhizium anisopliae* are applied.

Challenges faced

1. Factors like concentration, timing, and frequency of application need optimization.
2. Designing a well-controlled experiment is crucial but can be challenging. Variables such as environmental conditions, aphid populations, and application methods need careful consideration to ensure reliable results.
3. The effectiveness of *Beauveria bassiana* and *Metarhizium anisopliae* can be influenced by various environmental factors, such as temperature, humidity, and sunlight. Researchers must account for these variables to understand how the fungi perform under different conditions.
4. Different species of aphids may respond differently to the biopesticides.

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