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Biological control of pulse beetle *Callosobruchus chinensis* L. (Bruchidae: Coleoptera) in stored chickpea grains using entomopathogenic fungus *Beauveria bassiana* Balsamo

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Abstract
The entomopathogenic fungus *Beauveria bassiana* was used as biological control agent to control pulse beetle *Callosobruchus chinensis* in chickpea grains at different temperatures. Five concentrations (1×10^6, 1×10^7, 1×10^8, 1×10^9 and 1×10^{10} spores/ml) of commercially available conidia of *B. bassiana* were prepared by adding the sterile 0.02% tween 80 in distilled water. Haemocytometer was used for counting spores. Mortality of pulse beetle was directly proportional to concentrations of *B. bassiana*. *B. bassiana* was less effective at 25 ºC as compared to 30 ºC. At 25 ºC, highest mortality was recorded at concentration of 1×10^{10} spores/ml after 5, 10 and 15 days and vice versa. At 30 ºC, *B. bassiana* showed better results and all pulse beetles were died after 15 days at each concentration. This effective control strategy has significant contribution towards development of commercial microbial formulations of *B. bassiana* and is recommended to be a part of integrated pest management of pulse beetle.

Keywords: *Callosobruchus chinensis, Beauveria bassiana,* mortality, concentrations and temperature

1. Introduction
Chickpea (*Cicer arietinum*) is the major leguminous grain crop and is cultivated on large scale in the rain-fed areas of the world including Pakistan, Europe, Syria, India, Spain, Mexico, Algeria, Iran, Australia and Turkey [1-3]. Its grains are highly nutritious having 25% protein, 45% starch, 6% crude fiber, 5% fat, 6% sugar, 3% ash, 0.19% calcium and other minerals and vitamins are up to 0.01% [4-6]. It is deliberated as a good source of lowering cholesterol level [7]. The chickpea is liable to both quantitative and qualitative losses in storages. Quantitative damage results loss in seed weight and qualitative loss decrease aesthetic and nutritional value [8]. Biological (insects, birds and rodents etc.) and physical (temperature, humidity and moisture etc.) factors are mainly responsible for such losses. Insects cause severe damages to stored grains, which are about 20-35% and 5-10% in tropical and temperate zones, respectively [9].

Temperature plays a vital role in the development of stored grain insect pests and high temperature is mostly used to manage them. Lethal high temperature is 40 ºC for most of the stored insects [10]. Lethal low temperature is also effective in controlling stored grain insect pests. At higher temperature, metabolism rate is increased and food reserves are decreased. At lower temperature, insect development is slow and fecundity is reduced [11]. Temperatures below 14 ºC are lethal to stored grain insects, however; their development and fecundity are at climax between 25 ºC to 30 ºC [12].

Many insect pests including lesser grain borer, red flour beetle, Grainary weevil damage chickpea in storages however, pulse beetle *Callosobruchus chinensis* L. is the most damaging one. Being cosmopolitan, it also damages lentil, cowpea, mung, sorghum and maize [13]. It enters inside grains by making holes and start feeding until full damage. Normally infestation starts in the field because adult beetles can easily fly and lay eggs on the chickpea pods. Infestation is caused by grubs as well as adults [14].

Male and female pulse beetles can easily be distinguished on the basis of their antennae. Males have pectinate and females have serrate antennae [15]. Its female is larger than male [16]. Normally 6-8 overlapping generations are observed in a year [17, 18].

At present, mostly fumigants and synthetic insecticides are used in grain storages to manage insect pests that lead to issues like resistance, resurgence and poisoning of food [19]. There is a need of biological control measures such as entomopathogenic fungi, which are good alternatives of conventional pesticides being safer for the environment, animals and plants [20]. The fungus *Beauveria bassiana,* discovered by Bassi in 1835 from domesticated silkworm, is very effective in managing populations of stored grain insect pests causing white muscardine disease. This fungus is naturally occurring in soil having extensive host range [21].

Various studies have revealed that *B. bassiana* are effective against *Rhyzopertha dominica, C. maculatus, Sitophilus oryzae* and other stored pests [22-24]. *B. bassiana* are extensively used against many crop pests throughout the world under both laboratory and field conditions [25]. *B. bassiana* are important for controlling stored grain insects as well as sucking and chewing pests of animals and plants [26, 27]. *B. bassiana* differ from some other insect pathogens as they transmit a disease through the host integument. When spores of *B. bassiana* contact with insect body, spore germinate, perforate the cuticle and kill them within a few days by producing toxins in insect body and causing muscardine disease [28, 29].

The specific objective of the study was to assess the aptness/effectiveness of various concentrations of *B. bassiana* against *C. chinensis* in stored chickpea grains.

2. Materials and Methods

2.1 Collection and maintenance of pulse beetle culture

The research was executed during 2014-15. Chickpea samples infested with pulse beetle were collected from different storages and research stations. The incubator at temperature of 30 ± 2ºC and 70 ± 5% relative humidity was used to maintain *C. chinensis* culture in laboratory of Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan and given name as "Pulse Beetle Rearing Cell."

2.2 Chickpea cultivar for bioassays

For bioassays, chickpea cultivar CM-2000 was obtained from National Agriculture Research Center, Islamabad. Agtoxin was applied for fumigation to kill already existing insect pests, followed by Shaheen *et al*. [17].

2.3 Fungal source and maintenance of its culture

Commercially available conidia of *B. bassiana* were utilized in experiments. Single spore culturing method was used for sub-culturing of fungus and conidia were grown on potato dextrose agar (PDA) medium that contains potato starch 20g, dextrose 20g, agar 20g and distilled water 1000 ml. Another growth medium oat agar was also used for in vitro growth of fungus. Both media were sterilized at temperature of 121 °C and pressure of 15 Pascal for 20 minutes. The cooled media were poured in sterilized petri plates in laminar flow. After 24 hours, conidia of *B. bassiana* were placed on the media. The inoculated plates were incubated at 25 ± 2 °C temperature for 5-7 days.

Various concentrations of spores were developed by adding the sterile 0.02% Tween 80 in sterile distilled water. Haemocytometer was used for counting the conidial concentrations in serial dilutions. Five different conidial concentrations *viz.,* 1×10^6, 1×10^7, 1×10^8, 1×10^9 and 1×10^{10} spores/ml were prepared for trial. Then Petri dishes were kept in the incubator for fungi mass culturing. After drying the culture in oven, rubber scalpel was used to harvest the conidia from culture.

2.4 Bioassays

B. bassiana was evaluated against pulse beetle to check its effectiveness at different temperatures. Bioassay was carried out at two temperatures (25 ºC and 30 ºC). Chickpea samples of 100g grains were treated with different conidial concentrations of *B. bassiana*. All samples were placed in plastic jars. Each treatment had 3 replications. Five pairs of 1-4 days old adult pulse beetle were released into each jar, treated and untreated. The jars were covered and tightened with muslin cloth and then placed in incubators at 25 ºC and 30 ºC. Adult mortality was assessed after 5, 10 and 15 days of exposure to fungal concentrations. Percent mortality was calculated using the formula:

$$\textit{Percent mortality} = \frac{\textit{Number of dead insects}}{\textit{Number of released insects}} \times 100$$

2.5 Statistical analysis

Statistical analysis was done using SPSS-21 for Windows and Microsoft Excel was used for graphical work.

3. Results and Discussions

3.1 Effectiveness of *Beauveria bassiana* against adults of *Callosobruchus chinensis* at temperature of 25 ºC

3.1.1 Percent mortality of *C. chinensis* adults treated with different concentrations of *B. bassiana* after 5 days

Figure 1 illustrates the mortality percentages of *C. chinensis* at different concentrations of *B. bassiana*. All the concentrations showed significantly different mortalities of *C. chinensis* than the control except concentration of 1×10^6 conidia/ml. The maximum mortality (56.7%) was observed at concentration of 1×10^{10} conidia/ml and the lowest mortality of 10% was observed in the control treatment. Results indicated that mortality of *C. chinensis* was increasing with increasing concentrations of *B. bassiana*.

3.1.2 Percent mortality of *C. chinensis* adults treated with different concentrations of *B. bassiana* after 10 days

After 10 days (Figure 2), the highest mortality (76.66%) was observed at concentration of 1×10^{10} conidia/ml and the lowest mortality of 40% was observed at concentration (1×10^6 conidia/ml). With increasing concentrations of *B. bassiana*, mortality percentages of *C. chinensis* were also increased ranged from 40% to 76.66%. According to results, all concentrations showed significantly higher mortalities than the untreated control.

3.1.3 Percent mortality of *C. chinensis* adults treated with different concentrations of *B. bassiana* after 15 days

According to Figure 3, the maximum mortality (100%) was observed in jars treated with the higher concentration of 1×10^{10} conidia/ml compared to the lowest mortality (70%) for concentration of 1×10^6 conidia/ml.

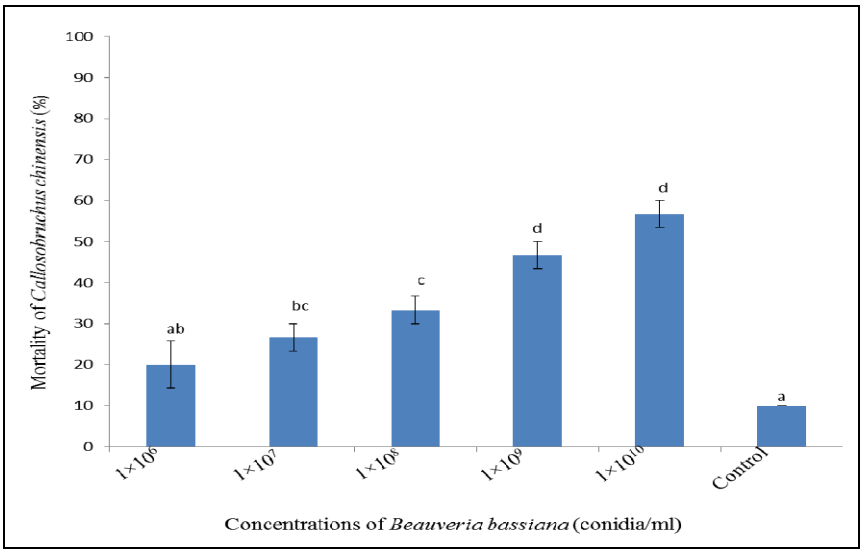

Fig 1: Percent mortality (Mean ± SE) of *Callosobruchus chinensis* adults treated with different concentrations of *Beauveria bassiana* at 25 °C after 5 days**.**

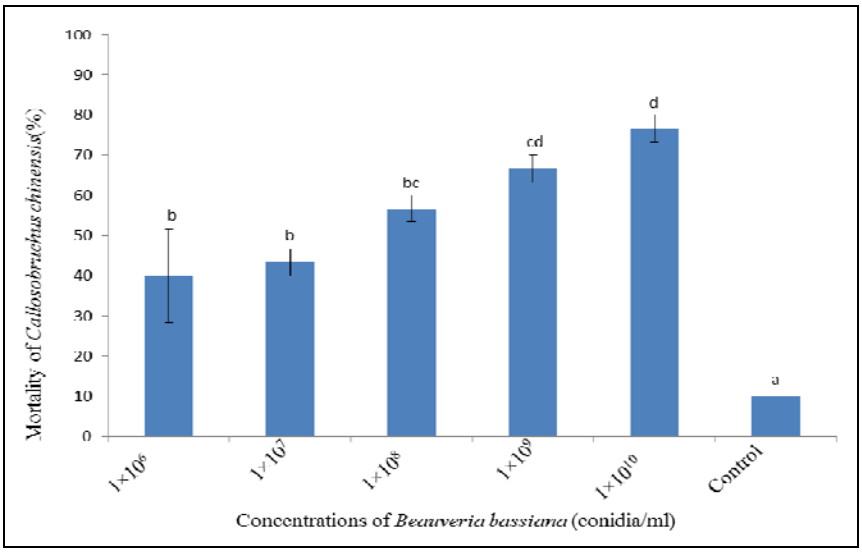

Fig 2: Percent mortality (Mean ± SE) of *Callosobruchus chinensis* adults treated with different concentrations of *Beauveria bassiana* at 25 °C after 10 days**.**

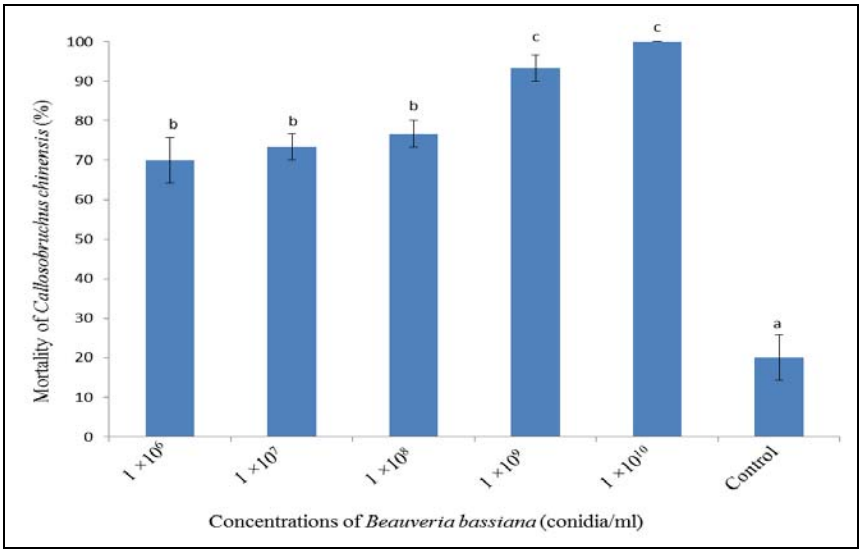

Fig 3: Percent mortality (Mean ± SE) of *Callosobruchus chinensis* adults treated with different concentrations of *Beauveria bassiana* at 25 °C after 15 days.

3.2 Effectiveness of *Beauveria bassiana* against adults of *Callosobruchus chinensis* at temperature of 30 ºC

3.2.1 Percent mortality of *C. chinensis* adults treated with different concentrations of *B. bassiana* after 5 days

The maximum mortality was 100% at 1×10^{10} conidia/ml concentration whereas 60% was the lowest observed in jars treated with concentration of 1×10^{6} conidia/ml (Figure 4). In control jars, only 10% mortality was observed. A non-significant difference was observed among concentrations of 1×10^{7}, 1×10^{8} and 1×10^{9} conidia/ml, however; the concentration of 1×10^{6} was significantly different with all other concentrations of *B. bassiana*.

3.2.2 Percent mortality of *C. chinensis* adults treated with different concentrations of *B. bassiana* after 10 days

Figure 5 showed that the minimum mortality was 76.66% at concentration of 1×10^{6} conidia/ml as compared to 20% that was observed in the control. The highest mortality (100%) was caused by concentrations of 1×10^{9} and 1×10^{10} conidia/ml. The control conditions were significantly different from all the concentrations of *B. bassiana*. Non-significant difference was observed among concentrations of 1×10^{7}, 1×10^{8}, 1×10^{9} and 1×10^{10} conidia/ml.

3.2.3 Percent mortality of *C. chinensis* adults treated with different concentrations of *B. bassiana* after 15 days

After 15 days exposure of the pulse beetle to *B. bassiana*, the mortality percentages were 100 at all concentrations (Figure 6). The control treatment with 20% mortality showed significant difference with all the fungal concentrations. However, there was non-significant difference among all the concentrations of *B. bassiana.*

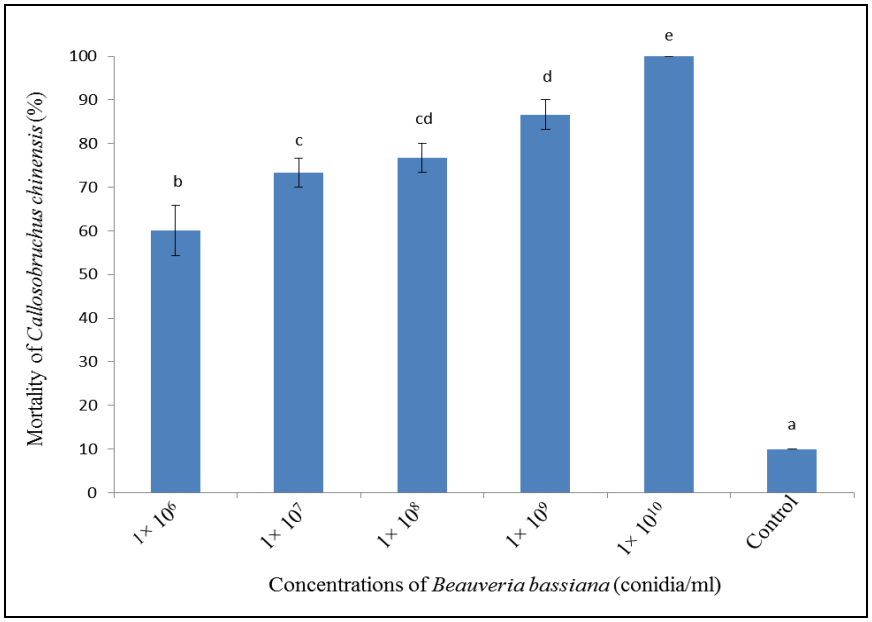

Fig 4: Percent mortality (Mean ± SE) of *Callosobruchus chinensis* adults treated with different concentrations of *Beauveria bassiana* at 30 °C after 5 days**.**

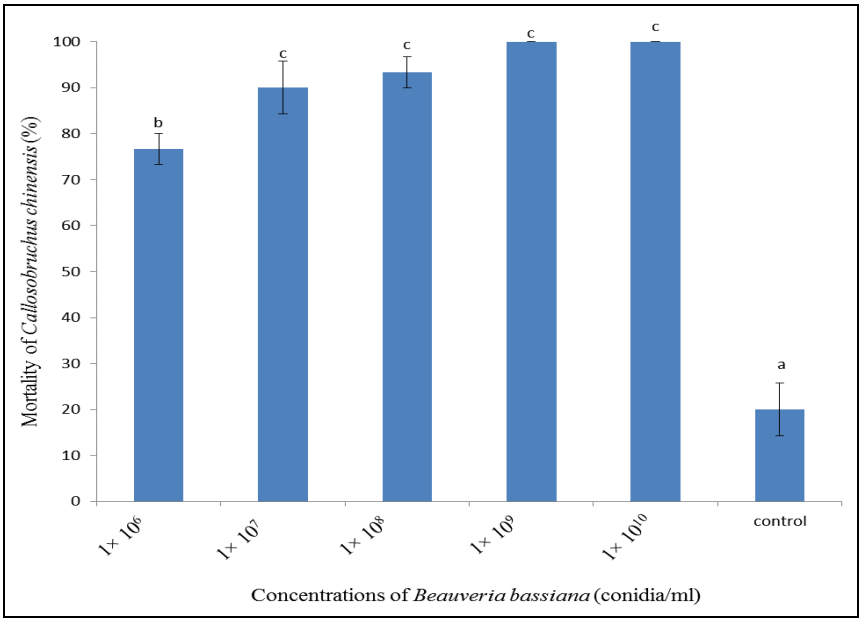

Fig 5: Percent mortality (Mean ± SE) of *Callosobruchus chinensis* adults treated with different concentrations of *Beauveria bassiana* at 30 °C after 10 days.

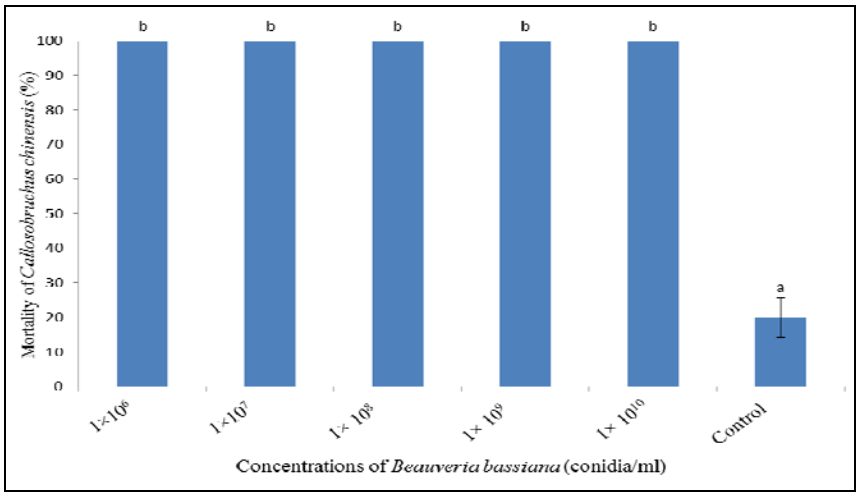

Fig 6: Percent mortality (Mean ± SE) of *Callosobruchus chinensis* adults treated with different concentrations of *Beauveria bassiana* at 30 °C after 15 days.

3.3 Comparison of *Callosobruchus chinensis* mortality using different concentrations of *Beauveria bassiana* at different temperatures

3.3.1 *C. chinensis* mortality at 25 ºC and 30 ºC after 5 days

After 5 days, results of different concentrations of *B. bassiana* when compared at temperatures 25 ºC and 30 ºC showed that mortality remained the highest at 30 ºC for each treatment as compared to 25 ºC (Figure 7). It was also observed that as the concentration of *B. bassiana* increased, the mortality of *C. chinensis* was also increased at both temperatures. At concentration of 1×10^6 conidia/ml, mortality was 20% at 25 ºC and 60% at 30 ºC having a significant difference. At concentration 1×10^7 conidia/ml, mortality was 73.33% at 30 ºC and 26.66% at 25 ºC, also with significant difference with each other. Mortality was 33.33% and 76.66% at temperature 25 ºC and 30 ºC, respectively at concentration of 1×10^8 conidia/ml. Mortality of 86.66% at 30 ºC and 46.66 at 25 ºC at the concentration of 1×10^9 conidia/ml was observed. At 1×10^{10} conidia/ml, mortality was observed 100% at 30 ºC and 56.66 at 25 ºC. The lowest mortality was observed at control treatment both at 25 ºC and 30 ºC where mortality was only 10%.

3.3.2 *C. chinensis* mortality at 25 ºC and 30 ºC after 10 days

Figure 8 showed the comparison of pulse beetle mortality at two temperatures with respect to fungal concentrations. After 10 days, the mortality of pulse beetle was the highest at 30 ºC as compared to 25 ºC. At concentration of 1×10^6 conidia/ml, the highest mortality (76.66%) and the lowest (40%) were recorded at 30 ºC and 25 ºC, respectively. For *B. bassiana* concentration (1×10^7 conidia/ml), mortality was 43.33% and 90% at 25 ºC and 30 ºC, respectively. The graph also indicated that at higher temperature, efficacy of *B. bassiana* was also higher. At concentration of 1×10^8 conidia/ml, mortality was 56.66% at 25 ºC whereas at 30 ºC, mortality was 93.33%. Mortality was 66.66% and 100% at temperatures 25 ºC and 30 ºC, respectively at concentration of 1×10^9 conidia/ml. At concentration of 1×10^{10} conidia/ml, the maximum mortality of 76.66% at 25 ºC and 100% at 30º C was observed. In control jars, the mortality was 10% at 25 ºC and 20% at 30 ºC.

3.3.3 *C. chinensis* mortality at 25 ºC and 30 ºC after 15 days

According to Figure 9, the mortality was 70% at 25 ºC and 100% at 30 ºC at the lowest concentration of 1×10^6 conidia/ml. After 15 days, mortality was 100% for all concentrations at 30 ºC. However, for the highest concentration of 1×10^{10} conidia/ml, mortality was 100% at both temperatures of 25 ºC and 30 ºC. For control treatments, mortality was 20% at both temperatures of 25 ºC and 30 ºC.

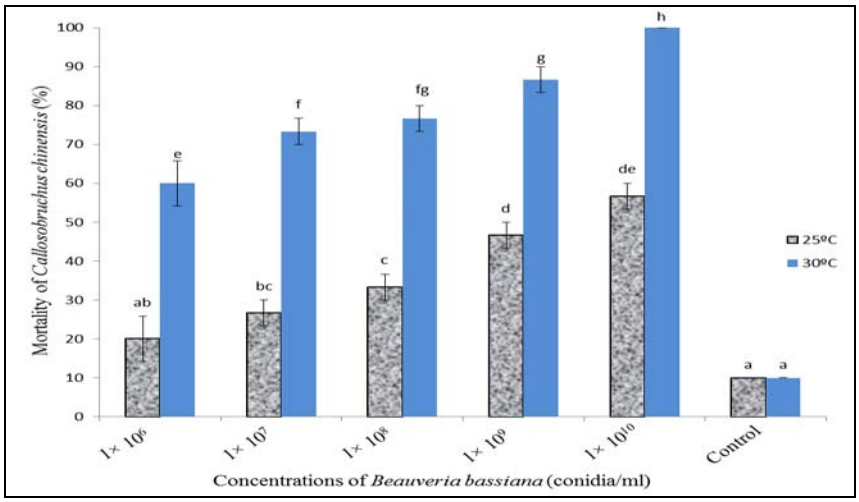

Fig 7: Comparative mortality of *Callosobruchus chinensis* after 5 days, using different concentrations of *Beauveria bassiana* at 25 ºC and 30 ºC

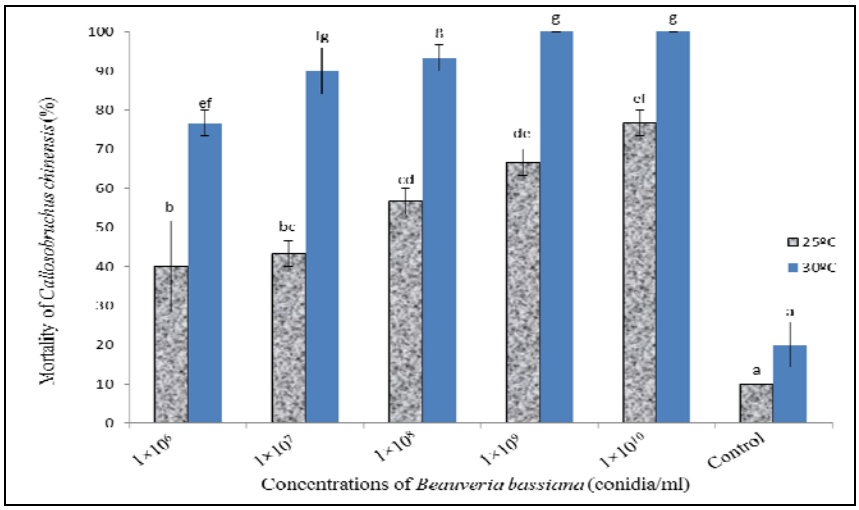

Fig 8: Comparative mortality of *Callosobruchus chinensis* after 10 days, using different concentrations of *Beauveria bassiana* at 25 ºC and 30 ºC

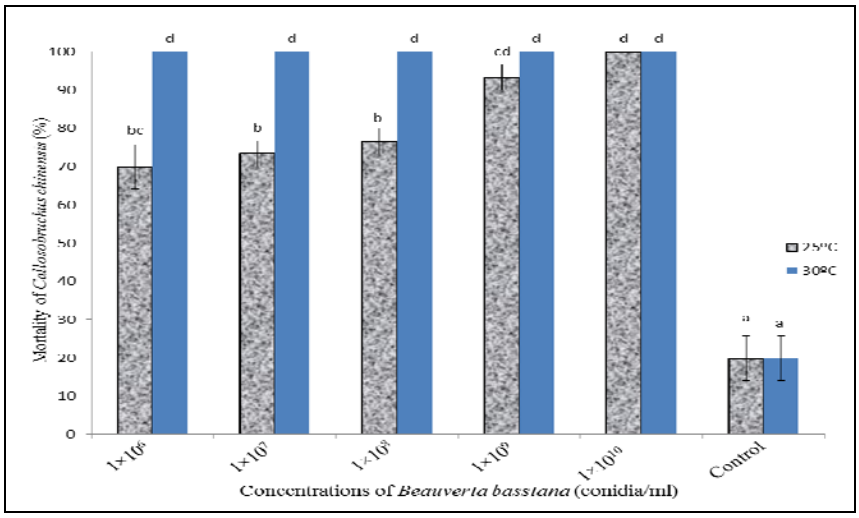

Fig 9: Comparative mortality of *Callosobruchus chinensis* after 15 days, using different concentrations of *Beauveria bassiana* at 25 ºC and 30 ºC

Results of this study showed that mortality of *C. chinensis* was increased with increased concentrations of *B. bassiana*. These results are in conformity with those reported by Vanmathi *et al*. [30] where they revealed that insect mortality is directly proportional to fungal concentrations. The results of the present study were also in agreement with junior *et al.* [31]. They found that *B. bassiana* was highly effective for the control of *C. maculatus* in lab conditions. Shams *et al.* [32] worked on the efficacy of five different concentrations of *B. bassiana* against stored product insects at 65 ± 5% relative humidity and 27 ± 2 °C temperatures. Comparisons between LC_{50} values and insect mortalities were made and it was concluded that *B. bassiana* was less virulent for *Sitophilus granarius* as compared to *C. maculatus*.

B. bassiana differ from some other insect pathogens as they transmit a disease through the host integument. When spores of *B. bassiana* contact with insect body, spores germinate, perforate the cuticle and kill them within a few days by producing toxins in insect body and causing muscardine disease [28, 29]. Kavallieratos *et al.* [33] revealed effective control of *S. oryzae* by using different suspensions of *B. bassiana, Isaria fumosorosea* and *Metarhizium anisopliae*. To control another important stored grain insect pest *R. dominica,* Wakil *et al*. [34] used different concentrations of *B. bassiana* mixed with the diatomaceous earth formulation enhanced with bitterbarkomycin (DEBBBM) under controlled laboratory conditions. Mortality of *R. dominica* was calculated after 5, 10 and 15 days interval of exposure. They concluded that *B. bassiana* along with DEBBBM provided better control against *R. dominica.* Besides stored grain insect pests, these entomopathogenic fungi also have insecticidal potential against insect pests of field crops. Akmal *et al.* [35] studied the pathogenicity of *B. bassiana* against different adults of aphid species like *Brevicoryne brassicae, Rhopalosiphum padi, Lipaphis erysimi and Schizaphis graminum.* They observed that *B. bassiana* was very effective against aphids at concentrations of 1×10^6, 1×10^7, 1×10^8spores/ml but it provided better control at higher concentration of 1×10^8 spores/ml.

4. Conclusion

The findings of this study have significant contribution towards development of commercial microbial formulations of *B. bassiana* and this effective control strategy is recommended to be a part of integrated pest management of pulse beetle *C. chinensis* for shorter and longer storages of chickpea grains.

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