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Efficacy of entomopathogenic fungi for control of walnut blue butterfly (*Chaetoprocta odata* [Hewitson, 1865]) in walnut (*Juglans regia* L.) under laboratory conditions

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Efficacy of entomopathogenic fungi for control of walnut blue butterfly (*Chaetoprocta odata* [Hewitson, 1865]) in walnut (*Juglans regia* L.) under laboratory conditions

Abstract: Biological control nowadays is rapidly growing to reduce the incessant use of chemical insecticides for control of various insect pests. In the present study, entomopathogenic fungi are used to determine insecticidal activity against walnut blue butterfly under laboratory conditions. The experimental setup was completely randomized design (CRD) with two treatments along with control with different concentrations of entomopathogenic fungi. The bioassay was carried out by spraying second larval instar of *Chaetoprocta odata* [Hewitson 1865] with 1, 2, 3 & 4 % conidial concentration of *Beauveria bassiana* (Balsamo) Vuill. (1912) and *Isaria fumosorosea* Wize (1904). The results of this study showed that all the concentrations showed remarkable pathogenic activity but *I. fumosorosea* was highly pathogenic and recorded the highest mortality rate of 93.33 % after 144 hours compared to *B. bassiana* where 73.33 % mortality was reported. LC_{50} values for *B. bassiana* (4.15) was higher than that of *I. fumosorosea* (3.34) which indicates that *I. fumosorosea* was more effective against *C. odata* population. Among different concentrations of *I. fumosorosea*, 4 % concentration was the most effective with lowest LC_{50} values.

Key words: *Juglans regia*; biological control; *Chaeotoprocta odata*;, virulence; *Beauveria bassiana*; *Isaria fumosorosea*

Učinkovitost entomopatogenih gliv za zatiranje gosenic orehovega modrina (*Chaetoprocta odata* [Hewitson 1865]) na orehu (*Juglans regia* L.) v laboratorijskih razmerah

Izvleček: Biotično varstvo rastlin danes pridobiva na pomenu, kar vpliva na manjšo rabo kemičnih insekticidov pri zatiranju različnih vrst škodljivih žuželk. V pričujoči raziskavi sta bili uporabljeni dve vrsti entomopatogenih gliv za določitev njunega insekticidnega delovanja na orehovega modrina v laboratorijskih razmerah. Poskus je bil zasnovan kot popolni naključni poskus z dvema obravnavanjema in kontrolo. Poskus z glivama je bil izveden s škropljenjem druge razvojne stopnje gosenic modrina z 1, 2, 3 & 4 % koncentracijo konidijev gliv *Beauveria bassiana* (Bals.-Criv.) Vuill. (1912) in *Isaria fumosorosea* Wize (1904). Rezultati raziskave so pokazali, da so vse koncentracije konidijev imele opazno patogeno aktivnost, a je bila gliva *I. fumosorosea* bolj patogena in je bila v obravnavanjih z njo dosežena največja smrtnost gosenic, 93,33 % po 144 urah, v primerjavi z glivo *B. bassiana*, kjer je bila smrtnost 73,33 %. LC_{50} vrednosti so bile za glivo *B. bassiana* večje (4,15) kot pri glivi *I. fumosorosea* (3,34), kar kaže, da je bila gliva *I. fumosorosea* bolj učinkovita pri zatiranju gosenic orehovega modrina. Med različnimi koncentracijami konidijev glive *I. fumosorosea* je bila 4 % koncentracija najbolj učinkovita z najmanjšimi vrednostmi LC_{50}.

Ključne besede: *Juglans regia,* biotično varstvo rastlin**,** *Chaeotoprocta odata,* učinkovitost, *Beauveria bassiana, Isaria fumosorosea*

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1 INTRODUCTION

The caterpillars of walnut blue butterfly, *Chaetoprocta odata* [Hewitson 1865] Lepidoptera: Lycaenidae are severe leaf defoliators which feed on parenchyma tissue of leaves resulting in scleretonizing of new leaflets. The neonate larvae feed on the succulent leaves and are voracious feeders (Butani, 1979; Masoodi & Trali, 1987; Abbas, 2013). It is one of the pests feeding on walnut trees of Kashmir Valley with devastating potential to defoliate the whole host tree (Mir & Wani, 2005). Chemical insecticides are being used since ages to protect plants due to which insects are developing resistance against them. One of the environmentally friendly techniques is biological control especially, the use of entomopathogenic fungi, forming a key part of integrated pest management (IPM) program (Ren & Chen, 2012). They cause cuticular infection resulting in toxin formation in pest after invasion. Additionally, they can infect any developmental stage of pests (Wang et al., 2010). Around 750 species of fungi are highly effective against various insect pests and offer great potential to manage insects with least hazard effects on environment and human health (Rabindra & Ramanujam, 2007; Laznik et al., 2012). This is the first attempt to use two strains of entomopathogenic fungi, *Beauveria bassiana* and *Isaria fumosorosea* for controlling pests on walnut trees.

2 MATERIALS AND METHODS

2.1 BIOLOGICAL MATERIAL

C. odata proceeding larvae were collected from the walnut orchards of Central Kashmir *Viz.,* Srinagar (34° 04' 54.36" N, 74° 48' 33.00" E, 1587 m), Budgam (34° 01' 2.05" N, 74° 43' 6.71" E, 1610 m) and Ganderbal (34° 13' 39.11" N, 74° 46' 19.78" E, 1619 m) and rearing was done in glass jars placed in an incubator maintained at temperature of 25 (± 1) °C and relative humidity of 65 (± 5) %. Two fungal strains *viz.*, *Beauveria bassiana* and *Isaria fumosorosea* in four different concentrations (1 %, 2 %, 3 % & 4 %) prepared from a stock solution of 1×10^9 conidiaml^{-1}by diluting with double distilled water obtained from Green Life Biotech Laboratory, Somanur, Coimbatore, India were tested against them to know the efficacy.

2.2 BIOASSAYS

In the experiment, freshly cut leaves of walnut trees were placed in 11 cm diameter petri- dishes and leaf petiole was covered with water swabbed cotton. Second instar larvae of *C. odata* were inoculated by immersing them in different conidial suspensions for 30 seconds and in case of the control, larvae were dipped in the distilled water. *C. odata* larvae were relocated to these leaf discs and all the petri-dishes were covered with the white muslin cloth tied with a rubber band for proper ventilation. The experiment design was a randomized complete block where three replicates were taken and each replicate had 10 larvae. Mortality was observed daily for six days at 23 (± 2) °C and 65 (± 5) % RH with a photoperiod of 12:12h (Irigaray et al., 2003). Mycosis test was done on the dead larvae and was transferred to petri-dishes in which moist filter paper was placed for 10 days. Microscopic examination confirmed the cause of mortality by fungal entomopathogens (Cherry et al., 2005).

2.3 MYCOSIS TEST

To know whether insect mortality had occurred due to fungal infection, mycosis test was done on the dead cadavers of insects. In this experiment, three petri plates were taken; two containing distilled water and one having 70 % ethanol. The process started by dipping cadavers of insects one by one in water followed by ethanol and then again in water to kill fungus present on the insect cuticle. Different isolates were dipped in separate Petri dishes. The procedure was repeated for all replicates of different isolates. Further, if fungi showed their growth again, the conidia of different isolates would penetrate the insect cuticle which subsequently enables us to know that death has occurred due to fungal infection (Grund & Hirch, 2010). The collected insect specimens were examined under a Leica M205A stereo zoom trinocular microscope and were photographed with a Leica DFC295 camera having automontage software version 4.10 (Leica Microsystems, Germany).

2.4 STATISTICAL ANALYSES

Experimental data was analysed by using Origin Pro software (Version 15). The data derived on means of percentage mortality and corrected mortality of *M. fotedari* adults in different treatments were analysed by ANOVA at 0.05 % level of significance. Tukey's Honest Square Difference test was used to separate means of treatment. Regression Analysis was done to estimate Lethal Concentration 50 (LC50) values at different concentrations. Abbott's formula (Abbott, 1925) was used for correction mortality data with that in control.

$$CM\ (\%) = T\ (\%) - C\ (\%) / 100 - C\ (\%)$$

Where, *CM* (%) - Corrected mortality
T - Mortality in treatment
C - Mortality in control

3 RESULTS

On treating larvae with *B. bassiana* at 1% concentration, it was found that mortality of larvae occurred on the 4th day after treatment. The corrected mortality after 120 hrs and 144 hrs was 3.33 % and 7.03 % respectively (Fig. 6 and Fig. 1). One way ANOVA depicted that mortalities after 96 hrs, 120 hrs and 144 hrs were statistically similar ($p > 0.05$) with each other. The regression equation for the suspension was calculated as Y=2.09X - 3.999 having a regression coefficient (R^2) value of 0.864 while LC_{50} value of the *B. bassiana* at 1 % concentration was 25.78 whereas on treating larvae with *I. fumosorosea* at 1 % concentration, the average corrected mortality after 120 hrs and 144 hrs was 13.70 % and 17.77 % respectively (Fig. 6 & Fig. 1). The calculated regression equation was Y=5.141X - 9.109 having a regression coefficient (R^2) value of 0.880. On subjecting data to one way ANOVA, insignificant difference was between the mortality rate at 96 hrs and 144 hrs while significant difference was between 144 hrs and 120 hrs at $p \leq 0.05$. LC_{50} value of *I. fumosorosea* at 1 % concentration was 11.49 %

At 2 % concentration, the corrected mortality by Abbott's formula after 120 hrs and 144 hrs was 41.11 % and 52.96 % respectively (Fig. 7 & Fig. 2) for *B. bassiana* with LC_{50} value 5.65 (Fig. 5). On subjecting the data to one way ANOVA, the results showed that the larvicidal activity at 72 hrs, 96 hrs, 120 hrs and 144 hrs was significantly different with $p \leq 0.05$ (Fig. 2). The regression equation was intended as Y=12.38X - 19.997 with an evaluated regression coefficient value (R^2) 0.950 (Fig. 5). On treating with 2 % *I. fumosorosea* the corrected mortality after 120 hrs and 144 hrs was 54.8 % and 71.47 % respectively (Fig. 7& Fig. 2). The evaluated LC_{50} value at 2 % concentration was 4.69. The regression equation at 2 % concentration was Y=15.808X - 24.221 having a regression coefficient (R^2) value of 0.966. From the data analysis, it was revealed that mortalities at 72 hrs, 96 hrs, 120 hrs and 144 hrs were significantly different among themselves as shown by ANOVA at $p \leq 0.05$.

When larvae were inoculated with a spore concentration of 3 %, larvicidal activity of *B. bassiana* against 2nd larval instar depicted that average mortality increased from 72 hrs to 144 hrs. The toxicity of *B. bassiana* was recorded showing the corrected mortality percentages at 120 hrs and 144 hrs were 58.51 and 64.07 respectively (Fig. 8) while LC_{50} value at 3 % was 4.71. Linear regression equation (Y=15.142X - 21.332) showed the value of regression coefficient (R^2) equal to 0.961 (Fig. 5 & Fig. 3). One way ANOVA results of the data showed that mortality rates at 3 % concentration were statistically different at 72 hrs, 96 hrs and 120 hrs although, percent mortalities at 120 hrs and 144 hrs were significantly similar among

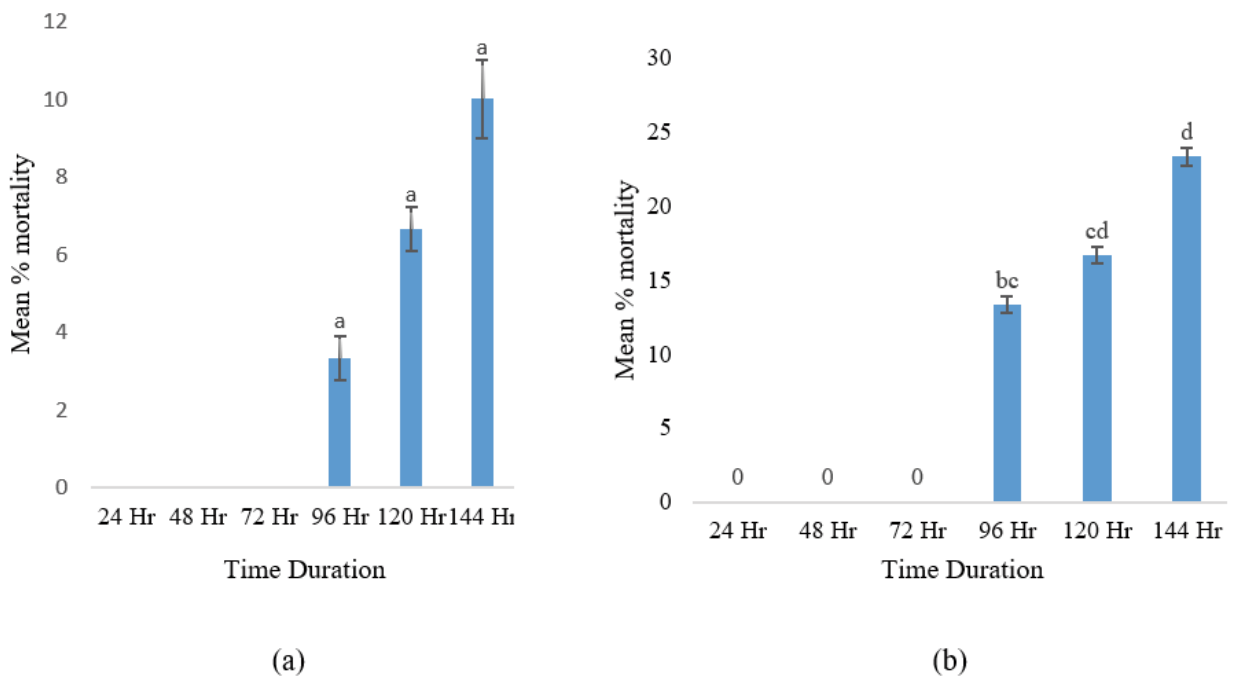

Figure 1: Mean percent mortality (± SD) at 1% concentration of (a) *Beauveria bassiana* (b) *Isaria fumosorosea*

themselves at $p \leq 0.05$. Similarly,when larvae were treated with 3 % concentration of *I. fumosorosea*, the corrected mortalities at 120 hrs and 144 hrs were recorded as 75.92 % and 82.21 % (Fig. 3 & Fig. 8). One way analysis of variance depicted that mortalities at 48 hrs, 72 hrs, 96 hrs and 120 hrs were statistically significant with each other although, mortality at 144 hrs was insignificant to mortality shown at 120 hrs. The obtained regression equation was Y=17.999X - 20.222 with regression coefficient (R^2) value 0.983 while the calculated LC_{50} value was 3.90.

At 4 % concentration, the larvicidal activity of *B. bassiana* was observed soon after 48 hours f treatment.

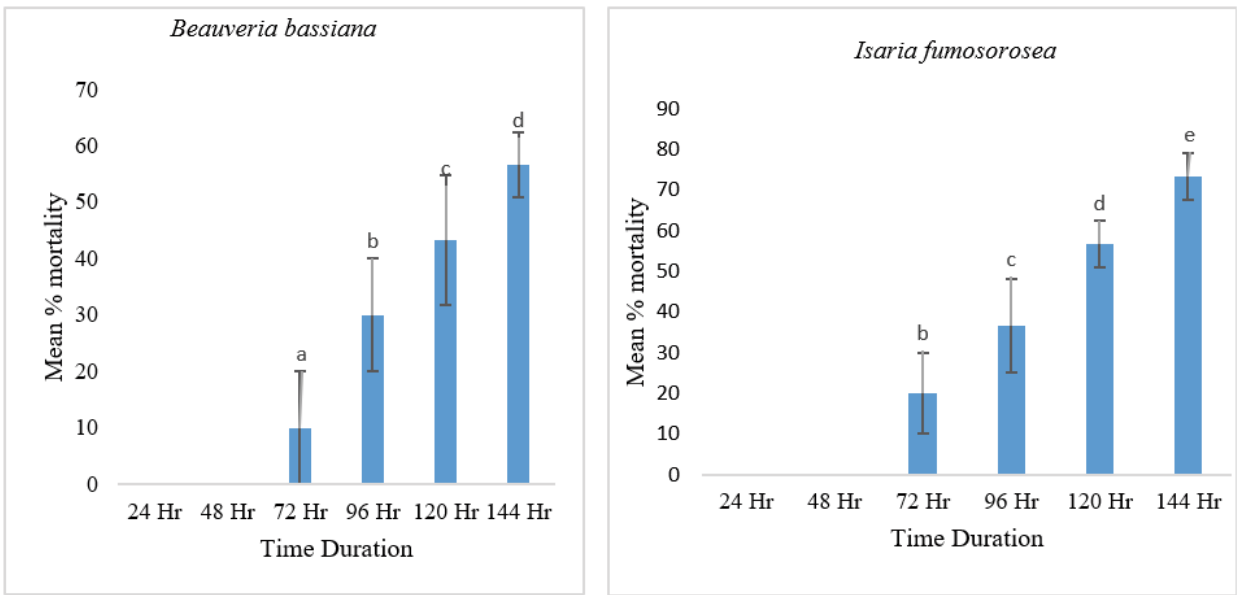

Figure 2: Mean percent mortality (± SD) at 2 % concentration of (a) *Beauveria bassiana* (b) *Isaria fumosorosea*

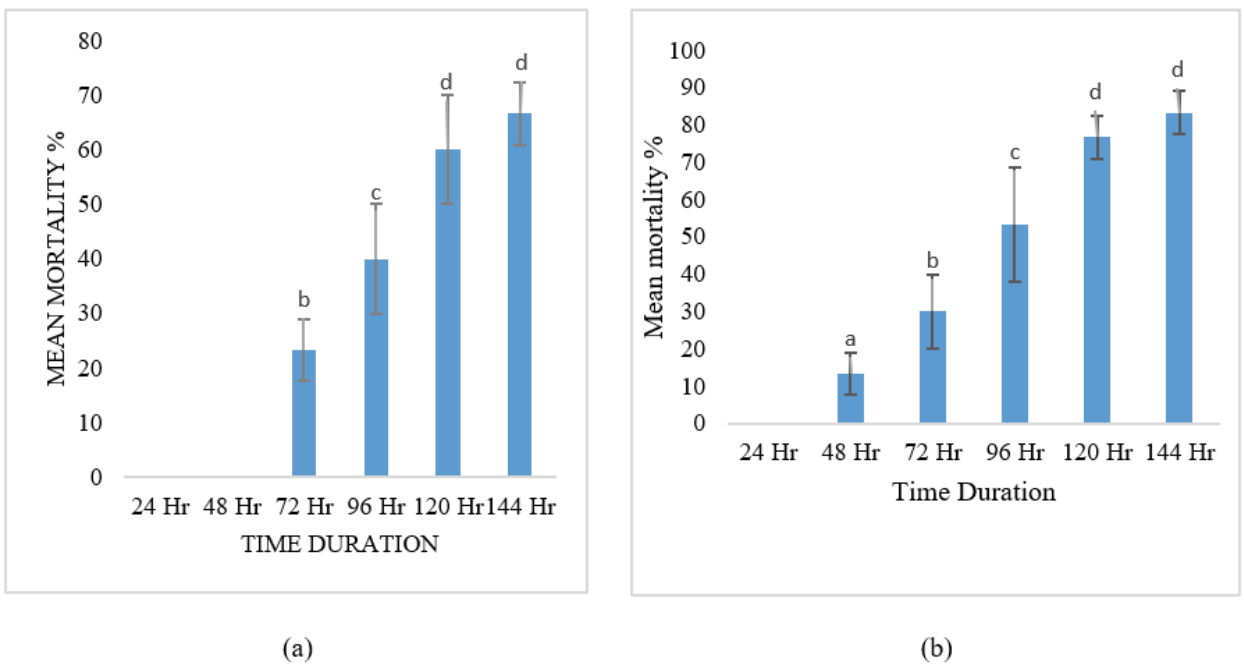

Figure 3: Mean percent mortality (± SD) at 3 % concentration of (a) *Beauveria bassiana* (b) *Isaria fumosorosea*

The mortality percentages at 120 hrs & 144 hrs corrected by Abbott's formula were 65.55 and 71.10 correspondingly (Fig. 8 & Fig. 4) whereas the calculated regression equation was Y=16.095X - 16.889 having a regression coefficient (R^2) value of 0.960 (Fig. 5) in contrast to *I. fumosorosea* at 4 % concentration, the corrected mortality percentages acquired through Abbott's formula at 120 hrs and 144 hrs were 86.29 and 92.96 respectively (Fig. 9). One way ANOVA results exhibited that mortality at 24 hrs was significant to the mortalities at 48 hrs, 72 hrs, 96 hrs, 120 hrs and 144 hrs. The mortality at 48 hrs and 72 hrs was insignificant with each other but significant to

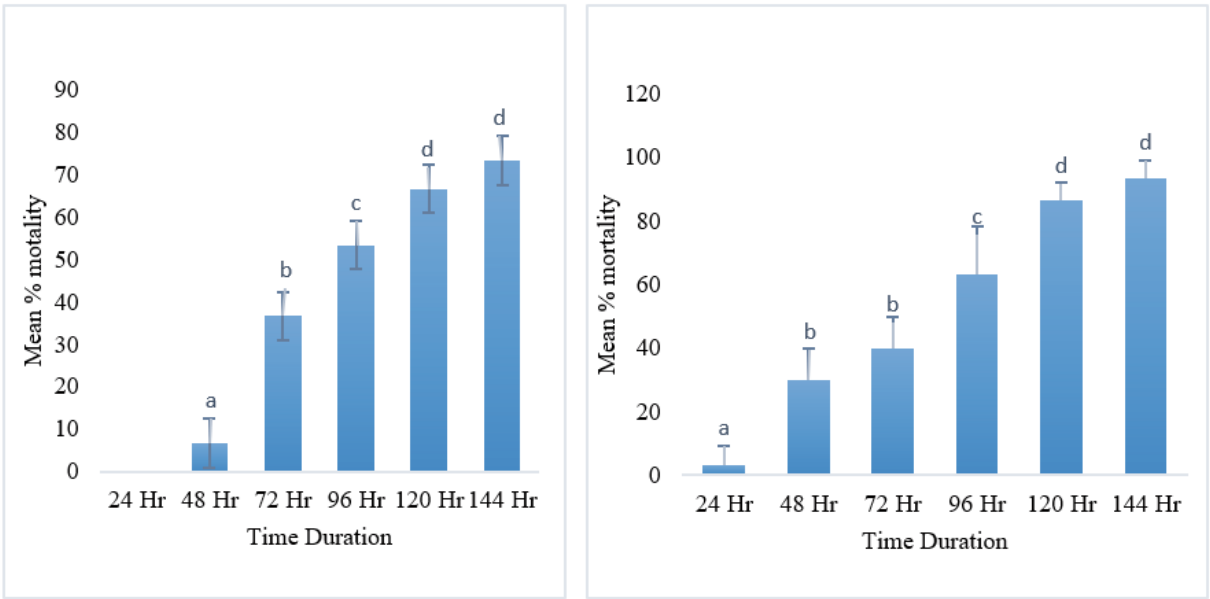

Figure 4: Mean percent mortality (± SD) at 4 % concentration of (a) *Beauveria bassiana* (b) *Isaria fumosorosea*

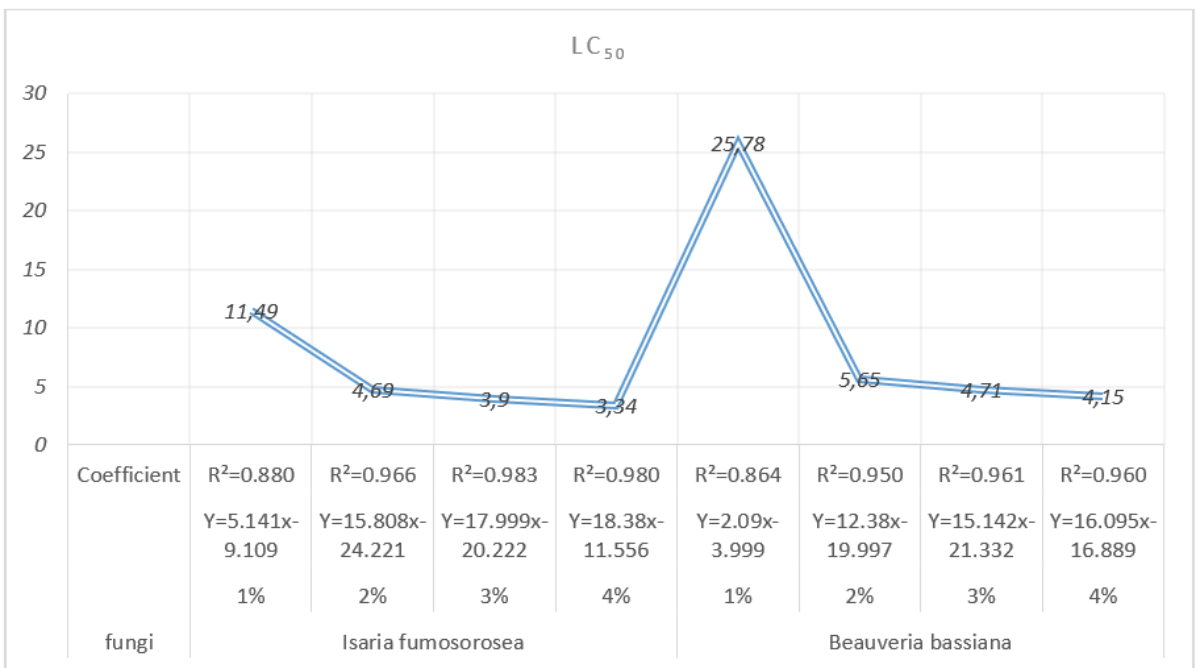

Figure 5: LC_{50} of second larval instar of *Chaetoprocta odata* treated with different suspensions of entomopathogenic fungi

others while the mortalities at 120 hrs and 144 hrs were statistically similar at $p \leq 0.05$. The calculated regression equation was Y=18.38X - 11.556 with a regression coefficient (R^2) value of 0.980. LC_{50} value at 4 % concentration was 3.34 (Fig. 5).

The calculated F values for 1, 2, 3 and 4% concen-

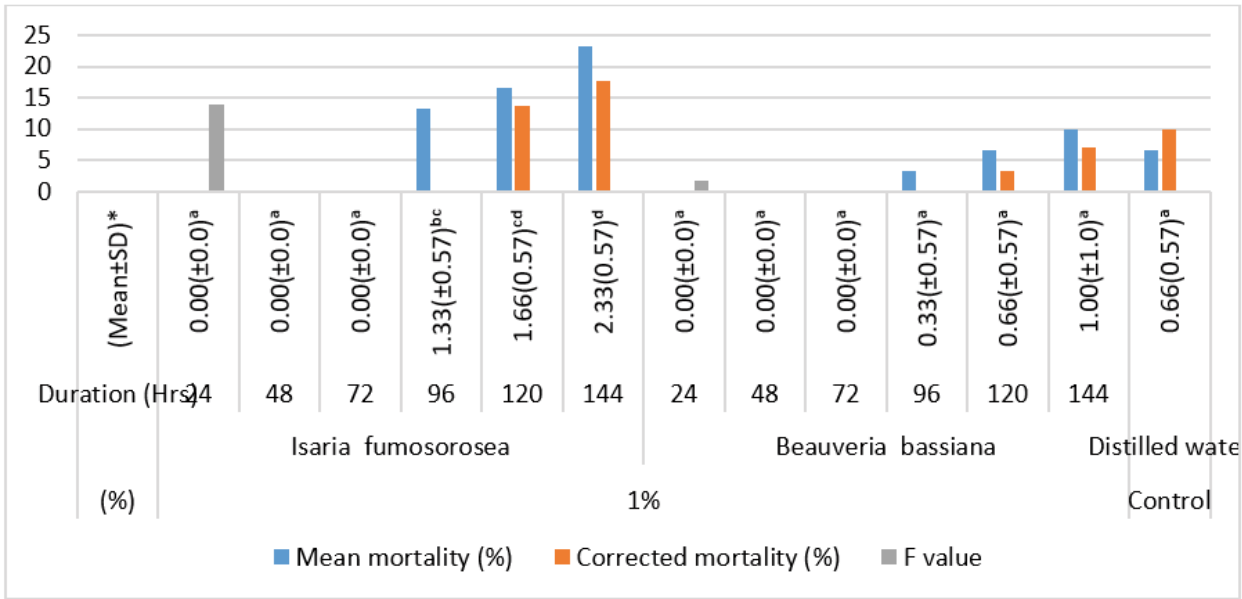

Figure 6: Corrected mortality percent and toxic effects of entomopathogenic fungi (1 % suspension) against second larval instar of *Chaetoprocta odata*\

*Mean of 10 second instar larvae of *Chaetoprocta odata*/ replication/ treatment; means followed by identical letters in lower case each column are not significantly different by Duncan's test at 5 %; Mean mortality % of individuals at the end of experiment corrected for mortality in control using Abbott formula

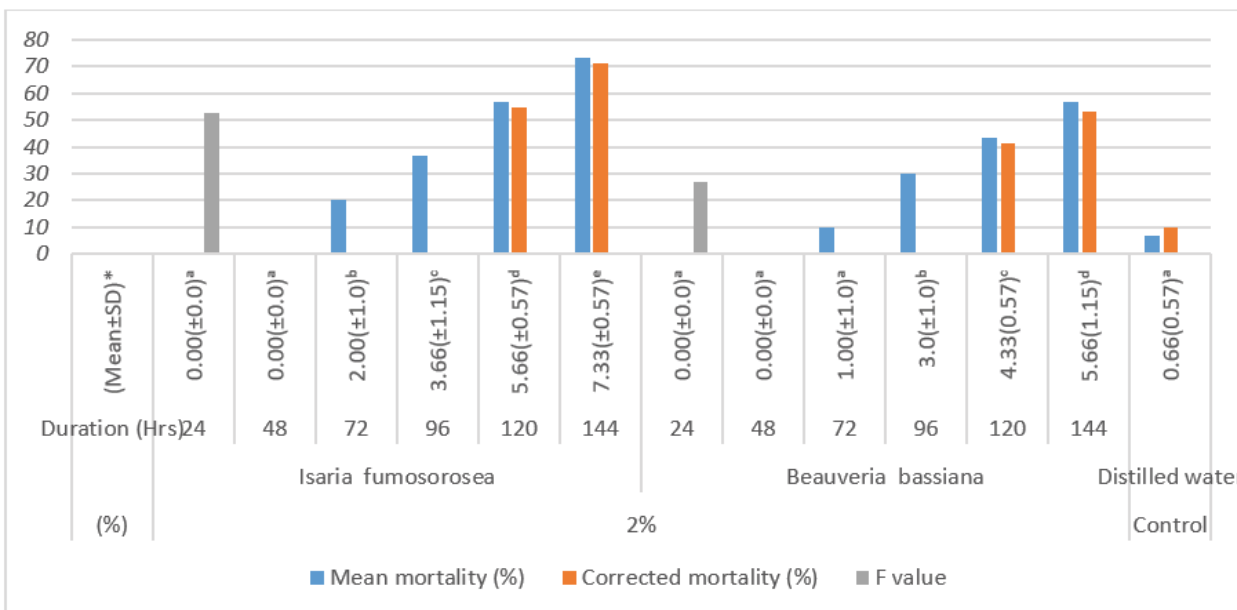

Figure 7: Corrected mortality percent and toxic effects of entomopathogenic fungi (2 % suspension) against second larval instar of *Chaetoprocta odata*

*Mean of 10 second instar larvae of *Chaetoprocta odata*/ replication/ treatment; means followed by identical letters in lower case each column are not significantly different by Duncan's test at 5 %; Mean mortality % of individuals at the end of experiment corrected for mortality in control using Abbott formula

tration was high for 4% concentration which means significant mortality had occurred at this concentration predicting that the variance between different mortalities isn't due to the random chance of all the variables used. The calculated regression equation for each concentration of *B. bassiana* demonstrated positive correlation

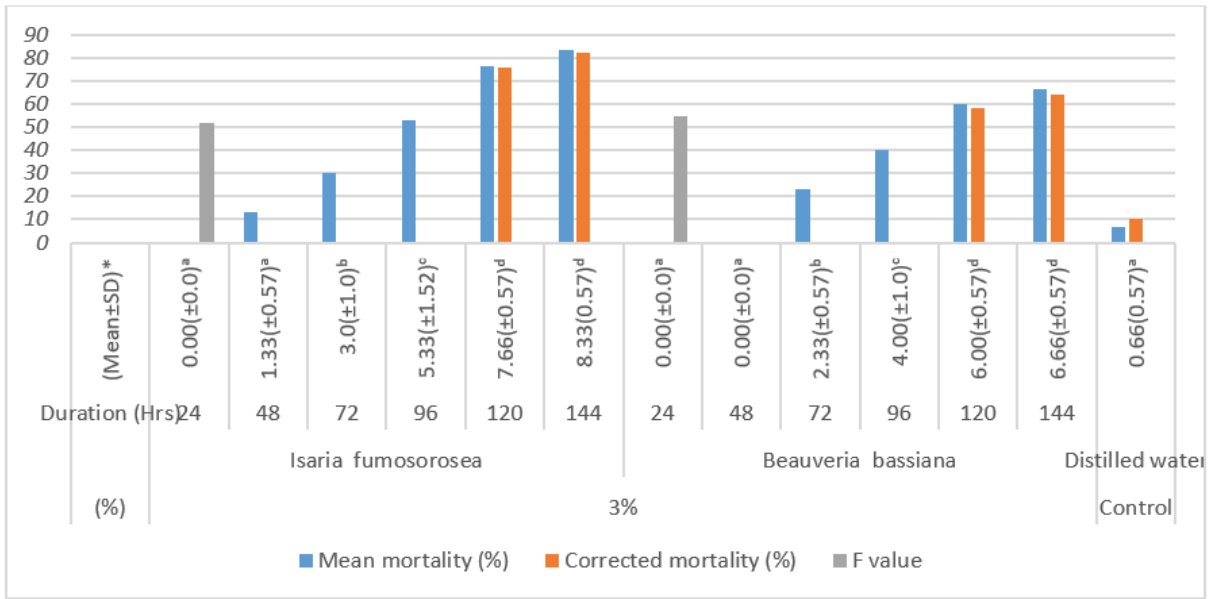

Figure 8: Corrected mortality percent and toxic effects of entomopathogenic fungi (3 % suspension) against second larval instar of *Chaetoprocta odata*

*Mean of 10 second instar larvae of *Chaetoprocta odata* / replication/ treatment; means followed by identical letters in lower case each column are not significantly different by Duncan's test at 5 %; Mean mortality % of individuals at the end of experiment corrected for mortality in control using Abbott formula

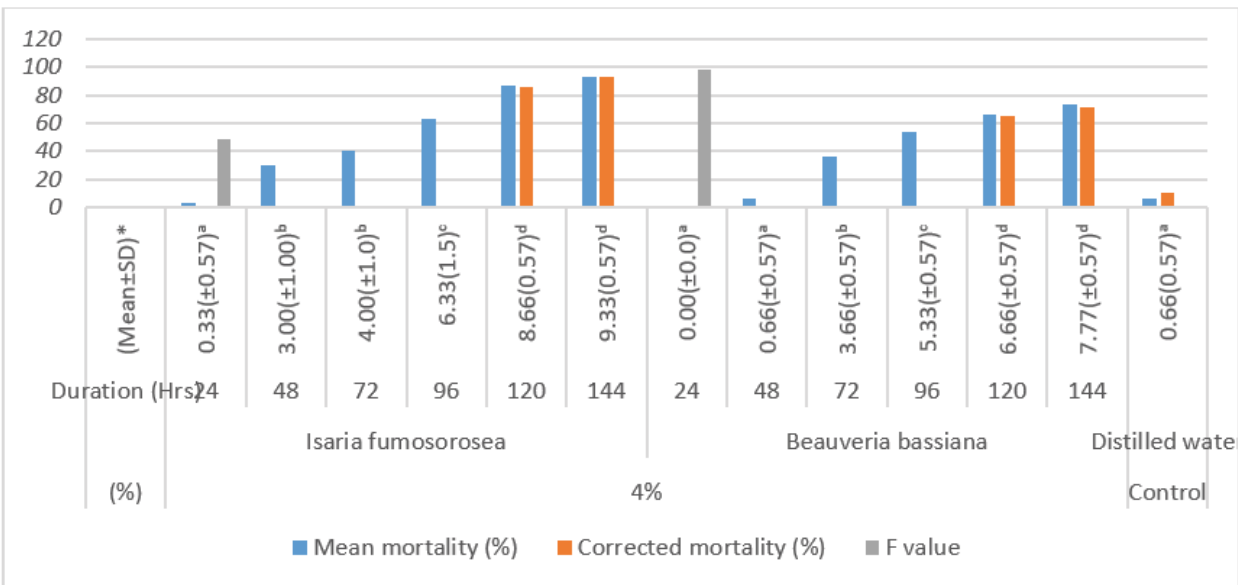

Figure 9: Corrected mortality percent and toxic effects of entomopathogenic fungi (4 % suspension) against second larval instar of *Chaetoproct aodata*

*Mean of 10 second instar larvae of *Chaetoprocta odata* /replication/ treatment; means followed by identical letters in lower case each column are not significantly different by Duncan's test at 5 %; Mean mortality % of individuals at the end of experiment corrected for mortality in control using Abbott formula

between concentration and mortality i.e., with an increase in the independent variable, the dependent variable also increases. The derived R^2 values range from 0 to 1 which signifies 0 to 100 % and the derived values in the experiment were close to 1 which depicted that increasing concentration of *B. bassiana* caused more larvicidal activity. The results of LC_{50} values calculated through Probit analysis are given in Fig. 5. Among the two entomopathogenic fungi *viz.,I. fumosorosea* and *B. bassiana*, *I. fumosorosea* was recorded to have maximum mean percent mortalities at different concentrations (Fig. 5). The lowest LC_{50} value (3.34) was calculated for *I. fumosorosea* at 4 % concentration which indicated its high toxicity to kill the larvae as lower LC_{50} values show acute pathogenicity. All the concentrations caused significantly higher mortality rates compared to control (distilled water). However, during the present study, no concentration of both entomopathogens caused 100 % mortality to larvae although; there was an upsurge in the mortality rates when concentration increased.

4 DISCUSSION

C. odata is one of the potential pests defoliating walnut trees in Kashmir valley (Abass, 2013). The present work is the first attempt to know the pathogenicity caused by two entomopathogenic fungi *viz., I. fumosorosea* and

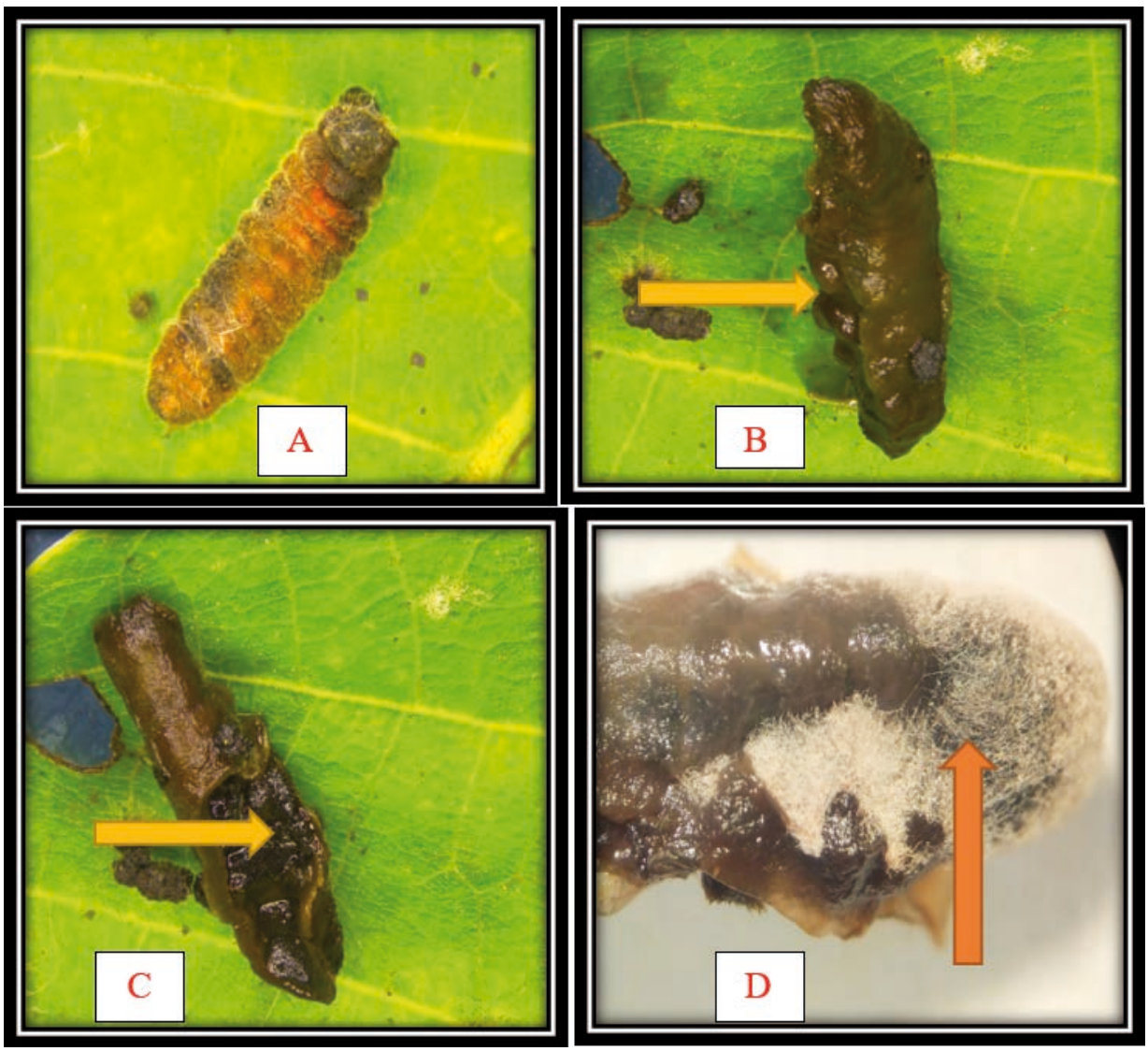

Figure 10: Entomopathogenic fungi *Isaria fumosorosea* and *Beauveria bassiana infesting* larvae of *Chaetoprocta odata* (A and B) Disruption of insect body tissue due to mycelial growth after infestation (C) Infestation by *Beauveria bassiana* (D) Infestation by *Isaria fumosorosea*

B. bassiana against second larval instar of *C. odata* (Fig. 10). Various entomopathogens have been used to control Lepidopteran pests especially *I. fumosorosea, B. bassiana* and *Metarhizium robertsii* (Metchnikoff) Sorokin (1883) (Hussain et al., 2009). Present results were in line with the observations of Gopalakrishnan & Narayan (1988) who evaluated the mortality rate of different larval instars of *Helicoverpa armigera* (Hübner, 1808), at different concentrations ranging from 1×10^{10} to 1×10^{7} with a mortality rate ranging from 60-100 %. During the experimental study, no emergence of adult took place from the treated larvae which was in agreement with the study carried by Hafez et al.(1994) who observed various life stage parameters in potato tuber moth (*Phthorimaea operculella* [Zeller, 1837] when treated with *B. bassiana* and found no emerge of adults at a concentration from 1×10^{4} to 1×10^{10} sporesml^{-1}.Entomopathogens are larvicidal as they contain extra-cellular secondary metabolites that have biocidal properties (Vey et al., 1985;Omura, 2011). As reported by other workers, the metabolites of entomopathogens use glycogen and lipid reserves of insects resulting in disruption of insect tissue due to mycelial growth that further leads to loss of appetite in them (Thomas et al., 1997) (Fig. 5). Similar observations were recorded during the experimental study when larvae were treated with entomopathogenic fungi and they refrained from the food as no notches were observed on the fresh walnut leaves. Our results were further affirmed with the findings of Shelton et al. (1998) who treated 2nd larval instar of diamondback moth, *Plutella xylostella* [Linnaeus, 1758] with entomopathogenic fungi, *Beauveria* spp. And found similar results. In addition, Hatting (2012) determined the pathogenicity of three entomopathogens when treated against ball worm, *Helicoverpa armigera* and found that *Nomuraea rileyi* (Farl.) Samson showed the highest toxicity followed by *I. fumosorosea* and *B. bassiana* respectively.

During the present investigation, it was observed that mortality due to entomopathogens was time and dose dependent. Initially, mortality was low after treatment which then gradually augmented from day 3 to day 6. Similarly, the finding of Wright et al. (2005) found that pathogenicity of the infected insect is dose and time dependent. Further, the study conducted by Bashir et al. (2018) reported that B. bassiana leads to 79 % mortality of Corcyra cephalonica [Stainton, 1866] larvae in in-vitro conditions. Likewise, our observations concurred with the finding of Nguyen et al. (2007) who found that the treatment of Helicoverpa armigera with M. anisopliae, B. bassiana and P. fumosoroseus, resulted in 68 to 100 % mortality,in laboratory investigations. Besides, it was observed that increased concentration showed higher mortality rates with amplified conidial growth which was in line with the findings of Safaviet al. (2007) who found that the virulence of entomopathogenic fungi is dependent on concentration, conidial growth and mostly on the nutritional content of pest especially carbon: nitrogen content. Thus, the availability of host food is one of the prime factors for the development of fungal pathogens (Tefera & Pringle, 2004). On the other hand, pathogenicity of I. farinosa against larval stages of Harmonia axyridis [Pallas, 1773] was studied by Steenberg & Harding (2009) and found that larval stages were most vulnerable and resulted in high mortality rates. In the current study, it was recorded that the highest mortality occurred due to I. fumosorosea which was in corroboration with findings of Zimmermam (2008) who found that I. fumosorosea has a broad host range and is highly infective to several insect orders especially Lepidoptera. Sabbour (2015) revealed that I. fumosorosea played a significant role in controlling the pests of the corn crop. Likewise, Schemmer et al. (2016) found I. fumosorosea as most pathogenetic fungi with a median lethal concentration of 0.09×104 conidiaml-1 against Camerariao hridella (Deschka & Dimic, 1986) (Lepidoptera: Gracillariidae). Thus from the above inferences and the results of the current study, it can be concluded that I. fumosorosea contains pathogenic characters that can be used as a bio-control agent against larvae of C. odata and may provide a practicable alternative to commercial insecticides used to control larvae in the early season.

5 CONCLUSION

Chemical insecticides are being used since ages to protect plants from pest attacks which resulted in insect resistance, environmental pollution and various health issues. Nowadays emphasis on biological control methods are increasing to manage insect pest population mostly entomopathogenic fungi. Current study demonstrated that, most important percent mortality was found in *I. fumosorosea* followed by *B. Bassiana* at all concentrations although both could be utilized as alternate tools to control population of *C. odata*. Among different concentrations of *I. fumosorosea*, 4 % concentration was the most effective with the lowest LC_{50} values. Therefore, further field study is necessary to evaluate the effectiveness of entomopathogenic fungal formulations in the management of *C. odata* in walnut orchards that can prove the most effective strategy in integrated pest management programs.

5.1 CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

5.2 ACKNOWLEDGEMENT

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