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## Efficacy of different concentrations of Green-Guard (*Metarhizium anisopliae* Var. *Acridum*) against desert Locust (*Schistocerca gregaria*) under lab and field conditions

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Desert Locust is considered as serious pests to vegetation worldwide. The recent plague hit the Africa and Asia very hard. Desert locust is mainly controlled by Chemical application which as adverse effect on nature and organisms. The study was carried out to test the efficacy of different concentrations of Green Guard (*Metarhizium anisopliae* Var. *acridum*) against locust nymphs in field and laboratory conditions. Four different concentration was applied against 1<sup>st</sup> and 2<sup>nd</sup> nymphal instar of desert locust. A significant higher mortality was recorded after 8, 10 days and 12 days in all concentrations compared to control. Higher concentration/dose should significantly higher mortality than recommended doses. Under field conditions maximum mortality above 70% was recorded in T3 and T4 after 10 and 12 days. In lab trial temperature and humidity was constant so all the higher doses showed significantly higher mortality than recommended dose at all intervals. In lab experiments all the high doses showed above 70% mortality after 8 days. Higher doses at optimum temperature and humidity showed higher mortality. Further studies should be carried out in different localities and concentrations/doses. Synergetic effect of oils should also be studied.

**Keywords:** Nymph, mortality, *Schistocerca gregaria*, *Metarhizium anisopliae*

### INTRODUCTION

Desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) has worldwide distribution. It is commonly called short horned grasshopper, a devastating pest of vegetation in Africa and Asia

(Song, 2004; Lovejoy et al. 2006; Cheseto et al. 2015). A total of 700 locust species recorded worldwide. Among them 50 species belong to genus *Schistocerca*. About four species *S. gregaria*, *S. piceifrons*, *S. cancellata* and *S. interrita*

having swarm formation habitat while the remaining *Schistocerca* species are sedentary grasshopper (Song 2004; Song et al. 2017). There are two further subspecies of *S. gregaria*, *S. gregaria gregaria* and *S. gregaria flaviventris*. The former has phase changing species, having swarm spreading across Africa and Asia. The later rarely undergoes phase changing (Chapuis et al. 2017). The species that were reported from Pakistan during 2019-2020 locust plague were *S. gregaria gregaria*. This species mainly reported from African and Asian countries (Ahmad et al. 2020) *Schistocerca gregaria* has incomplete metamorphosis. The main three life stages are egg, nymph and adult. In nymphal stage it has further instars. The life stage has great dependency on environmental conditions. It takes from weeks to months to get fully matured (Steedman, 1990). It remains calm when in solitary phase but can cause huge losses to crops in gregarious form, swarms are formed in gregarious phase (Meinzingen, 1993). The color of desert locust also varies in solitary and gregarious phase. In solitary phase the color remains greenish to brownish while in gregarious they color change remain orange and blackish (Pener, 1991; Ayali, 2019). A wide range of chemicals were mainly used to control desert locust plague. The chemicals have not only adverse effect on environment but also causes threat to the population of other non-target organisms as well (Lecoq., 2010). It is not easy to find alternative control measure for the management of Desert locust as the pest attack in swarm and having limited time for management. Recently some bio pesticides such as *Metarhizium anisopliae* Var. *acridum* were widely used against desert locust in Africa and Australia. FAO also recommended the use of *Metarhizium anisopliae* Var. *acridum* for their field operation in latest plague in African countries (Abdelatef, 2005). Botanical extracts also showed encouraging results, as they are non-toxic to other animals but cannot be apply on large scale (Koul et al. 2008). In Egyptian environmental conditions *M. anisopliae* Var. *acridum* showed some significant result in management Desert locust and grasshopper, but their action was found comparatively slow compared to other control methods (Abdelatef 2005). The present studies were carried out to evaluate different concentrations of Green Guard (*M. anisopliae* Var. *acridum*) against first and second nymphal instar under laboratory and field conditions in Dera Ismail Khan and Lakki Marwat districts of Khyber Pakhtunkhwa.

## MATERIALS AND METHODS

### Location of the study:

The experiments were carried out during March-May 2020 at District Dera Ismail Khan and Lakki Marwat. The laboratory experiments were carried out Agriculture Research Institute, Dera Ismail Khan, while the field experiments were carried out at different localities of District Lakki Marwat.

### Concentration of Green Guard:

Green Guard (*M. anisopliae* Var. *acridum*) imported from Australia was used in the recommended and high doses. The bottle has expiry date of The following treatment were applied according to the Table-1.

**Table 1: Recommended concentrations of the Green-Guard**

Treatment	Dose /2 acre	Remarks
T1	1 liter / 200 Liters of water	Recommended and standard dose by company
T2	1.3 liter / 200 Liters of water	Slightly higher than recommended
T3	1.5 liter/ 200 liters of water	One and half higher dose than recommended
T4	2 litters / 200 Liters of water	Double dose than recommended\ by company
Control	No treatment or application	

### Desert Locust selection:

The 1<sup>st</sup> Instar and 2<sup>nd</sup> instar of nymphs was selected and were brought to the laboratory for the experiments (Fig.2 a and b). Field cages were prepared and 1<sup>st</sup> Instar and 2<sup>nd</sup> instar were caged under field conditions (Fig.2 a & b) and were sprayed with concentrations mentioned earlier. A total of 100 nymphs were selected for each concentration. For each concentration three different locations in same field were selected for randomization. Control cage having no treatment was also kept for comparison.



**Figure 1: Field cages a) Field cage having nymphs b) Close view of the cage**



**Figure 2: Lab Experiment a) different treatments b) close view of lab cage having nymphs**

#### Data Recording:

The data was with different intervals, 4 days, 8 days 10 and 12 days. The number of dead and alive nymphs were counted in the cage and their number were recorded. Environmental conditions i.e temperature, humidity and sun shine was also recoded in field experiment. The average temperature during the field experiment ranged from 28-33°C with average humidity range from 30-40%. The weather conditions were generally sunny during the trial period. In Lab experiment the temperature were kept constant (26-27°C) having 50% humidity.

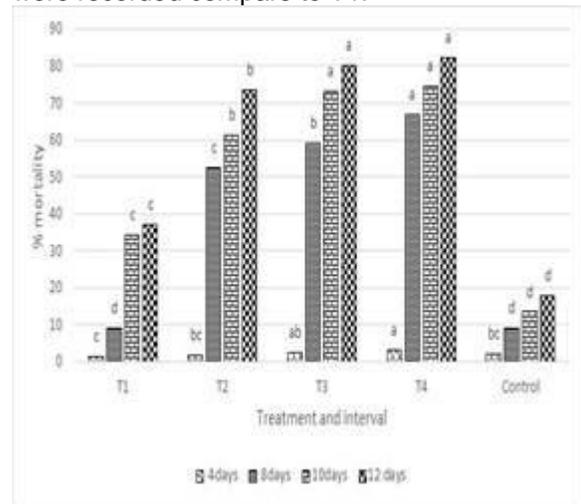
#### Statistical Analysis:

The recoded data were then subjected to statistical analysis using computer program Statistix. Least significant difference (LSD) recorded at <0.05.

### RESULTS AND DISCUSSION

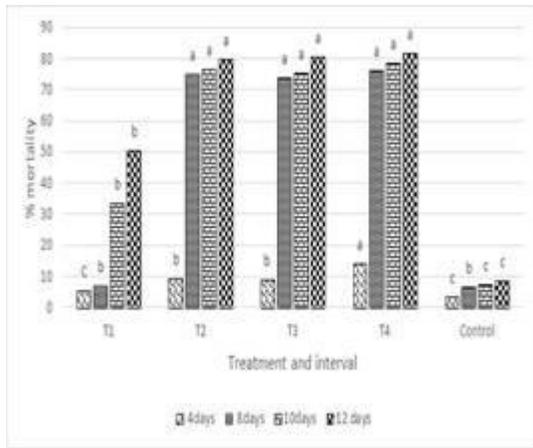
Efficacy of different concentration and its effect on 1<sup>st</sup> and 2<sup>nd</sup> instar under field conditions is presented in Fig-1. The results show that there was less than 5 % mortality recorded after 4 days in all treatments. A significant mortality was recoded after 8, 10 days and 12 days in all concentrations

compared to control. A significant different were recorded among the treatments after 8 days. Highest mortality was recorded in T4 (2 litters /200 Liters of water) and lowest was recorded in control and T1 (1 liter /200 Liters of water). No significant differences were recorded in T4 and T3 after 10days. However, they were significant difference in mortality with T2 (1.3 liter / 200 Liters of water) and T1. A significant lower mortality was recorded in control after 10 days of treatments under field conditions. After 12 days 80% and above mortality was recorded in T4 and T3 (1.5 liter/ 200 liters of water), significantly higher than T2 and T1. Furthermore, significantly lower mortality in control were recorded compare to T1.



**Figure 3: Percent nymphal mortality at different concentrations and intervals under Field conditions**

The efficacy of different concentration of Green Guard under lab condition is presented in Fig. 2. Less than 10% mortality in all treatments including control were recorded after 4 days. A significant higher mortality was recorded in T4 compared to T3 and T2. However, T1 and control showed similar mortality, significantly lower than T2 and T3. After 8 days of treatment T2, T3 and T4 showed same mortality, significantly higher than T1 and control. At 10 days interval no significant differences were recorded in T2, T3 and T4. A significantly lower mortality was recorded in control compared to control. Approximately 80% and above mortality was recoded in T2, T3 and T4 significantly higher than T1 after 12 days. The lowest mortality (9%) after 12 days were recorded in control.



**Figure 4: Percent nymphal mortality at different concentrations and intervals under Lab conditions**

Our findings are in close association with earlier results of Mohamed et al. (2014). They used *M. anisopliae* var. *acridum* against nymphs and found significant mortality under lab and field conditions after few days. Further they reported that toxic effect of fungi can be increase with addition of Abamectin or D- limonene. In our result we found that the significantly mortality increased with passage of time and reached similar observation were also recorded by Abdelatef. (2005). He reported that *M. anisopliae* is an effective biological control agent of desert locust nymphs and grasshoppers under the agro climatic conditions of Egypt. He notices that action of the biocontrol agent is relatively slow, which could be their only disadvantage. In another study Abdelatti and Hartbauer (2020) reported that the toxicity can be enhanced by the adding winter green oils a which act as a synergistic. Further, they notice that there is no adverse effect on these on non-target beneficial organism or insects.

In the current studies we used *Metarhizium anisopliae* Var. *acridum* against Desert locust (*Schistocerca gregaria*) which belong to grasshopper family and found mortality under lab and field conditions in early instars. Tounou et al. (2008) reported that these biocontrol agent has good effect on other grasshoppers as well. Further they reported that the efficacy is high in early instar compare to the late instars in grasshopper. Moreover, they also reported that wheat bran mixed with *M. anisopliae* increases the efficacy against early instars of grasshoppers. In the current study only *Metarhizium anisopliae* Var. *acridum* spores were sprayed under lab and field without adding any oils or other additives.

High mortality in desert locust nymphs were

recorded in laboratory trial compare to field trial in the current studies in early days at different doses. This could be related to the constant temperature and humidity under lab conditions. These findings are supported by some earlier studies of Blanford and Thomas (2001). They studied that at constant temperature the fungal pathogen showed a rapid and high mortality. Further they reported the mortality also effected by application method, inoculation time, nighttime temperature regime and dose of the fungal pathogen. This also concludes that the effectiveness of the fungal pathogen against the desert locust and grasshopper will be good in areas having long constant temperature humidity. Peng et al. (2008) also reported that *Metarhizium anisopliae* var. *acridum* were effective and showed high mortality against *Locusta migratoria*. The mortality was effected by the vegetation type and weather conditions (Temperature & Humidity). In current studies we also reported high mortality after 10 and 12 days in both laboratory and field conditions and these were similar to the findings of Blanford and Thomas (2001) and Peng et al. (2008). They reported above 80% mortality in field treated with fungal pathogen after 11-15 days.

In the current study the used Green Guard (*Metarhizium anisopliae* var. *acridum*) were used in higher concentrations than the recommended dose. Significantly lower mortality was recorded in the recommend dose treated nymphs under laboratory and field conditions compared to high doses. The reason could be the storage of the Green guard not at optimum temperature, as it was imported from Australia. Second reason was the short expiry of the product, as only three months were remaining to the expiry when reached for application. The third reason could be the environmental condition of Southern districts of Khyber Pakhtunkhwa, Pakistan are usually dry, having high temperature with low humidity. The average temperature during the field experiment ranged from 28-33°C with average humidity range from 30-40%. This could be also the reason of low mortality in recommended dose by company. Peng et al. (2008) also reported that environment and different doses also effect the mortality in *Locusta migratoria* treated with *Metarhizium anisopliae* var. *acridum*. Haroon et al. (2011) also reported different mortality in different concentrations of *Metarhizium acridum* against the tree locust *Anacridium melanorhodon*. Further they reported that with addition of neem oil 1.0% concentration increased the mortality in locut and also its efficacy without harming the fungus spores.

## CONCLUSION

It is concluded from the studies that higher concentration/doses than recommended showed significantly higher mortality compare to standard dose in both field and laboratory conditions. At laboratory conditions the mortality was the temperature was constant so higher mortality was recorded after 8,10 and 12 days. In field trail maximum mortality was recorded in higher doses after 10 and 12 days. Further studies should also be carried out in different location and with different doses. Synergetic effect of neem oil or other organic oils should also be studied.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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## AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: Hussain Ali<sup>1\*</sup>,

Analyzed the data: Bitanni Salahuddin

Contributed materials/ analysis/ tools: Hayat Zada and Fazal Said,

Wrote the paper: Asad Ali Zahid Saleem, Fazal Said, Irfan Ullah Khan, Toheed Iqbal, Kiran Shahjeer and Ihtishamul Haq

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