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Nitin V Parjane
Ph.D. Scholar, Post Graduate
Institute, Department of
Entomology, Mahatma Phule
Krishi Vidyapeeth, Rahuri,
Ahmednagar, Maharashtra,
India

Ghanasham B Kabre
Professor of Entomology, College
of Agriculture, Dhule, Mahatma
Phule Krishi Vidyapeeth,
Rahuri, Ahmednagar,
Maharashtra, India

Chidanand S Patil
Head, Department of
Entomology, Post Graduate
Institute, Mahatma Phule Krishi
Vidyapeeth, Rahuri,
Ahmednagar, Maharashtra,
India

Mahesh R Patil
Associate Professor of Statistics,
Post Graduate Institute,
Mahatma Phule Krishi
Vidyapeeth, Rahuri,
Ahmednagar, Maharashtra,
India

Corresponding Author:
Nitin V Parjane
Ph.D. Scholar, Post Graduate
Institute, Department of
Entomology, Mahatma Phule
Krishi Vidyapeeth, Rahuri,
Ahmednagar, Maharashtra,
India

Pathogenicity of *Metarhizium anisopliae* against *Spodoptera frugiperda* larvae under laboratory conditions

Nitin V Parjane, Ghanasham B Kabre, Chidanand S Patil and Mahesh R Patil

Abstract

The present investigation on “Pathogenicity of *Metarhizium anisopliae* against *Spodoptera frugiperda* larvae under laboratory conditions” was carried out at Biocontrol Laboratory, Department of Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri during January 2021. In order to study the pathogenicity of *Metarhizium anisopliae* against *Spodoptera frugiperda* second and third instar homogenous larvae of *Spodoptera frugiperda* were selected. The bioassay was conducted by larval dip method (Ramanujam *et al.*, 2020). Different doses of *Metarhizium anisopliae* 1.15% WP formulations *viz.*, 0 g/litre, 2.5 g/litre, 5 g/litre, 7.5 g/litre and 10 g/litre were evaluated against second and third instar larvae of *Spodoptera frugiperda* in laboratory. The results revealed that highest mortality was obtained in second instar larvae with mortality ranging from 0% to 81.38% whereas, third instar larvae showed lower mortality than second instar larvae ranging from 0 to 72.77% using different doses of *Metarhizium anisopliae* 1.15% WP.

Keywords: Bioassay, *Metarhizium anisopliae*, *Spodoptera frugiperda*, mortality, pathogenicity

Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is a devastating maize pest that is difficult to manage with a single pest management strategy. Fall armyworm causes significant economic losses in a range of crops including maize, soybean and cotton (Nagoshi *et al.*, 2007; Bueno *et al.*, 2011)^[20, 71]. Caterpillars cause serious harm to plant leaves during vegetative growth. In 2018, *Spodoptera frugiperda* was noticed for the first time in India. During the months of July and August, 2018 it was widely spread in Karnataka locales like Chikkaballapur, Shivamogga, Hassan, Davanagere, Bangalore and Chitradurga (Shylesha *et al.*, 2018)^[27].

Early crop assessment indicate that insect causes production losses of between 45 to 100 percent (Blanco *et al.*, 2014)^[5]. Larvae destroy developing tassels and cobs as well as leaves by puncturing them with shot holes, elongated irregular holes and other damages to the cobs (Day *et al.*, 2017)^[8]. In order to reduce crop losses, prompt action is required to manage the pest at an early stage. Around the world a variety of management strategies including chemical, biological and botanical methods have been used to control insect pests.

Chemicals (insecticides) are the most effective insect pest control methods yet, they are harmful to the environment, natural enemies and encourage insect pest resistance. On the other side, excessive use of chemical pesticides had a severe influence on soil health, environment as well as beneficial flora and fauna such as earthworms, natural predators and parasites (FAO, 2018). Given the dangers of pesticides, biological control holds immense promise for pest management. Using bio-pesticides for the control of pests is an ideal alternative as it has long term effect without any harmful effect on environment and animals

Entomopathogenic fungi (EPFs) are important bioagents due to their vast range of hosts, pathogenicity and ability to manage insect pests (Akutse *et al.*, 2019)^[1]. Herlinda *et al.* (2021)^[16] explored the efficiency of EPF *Metarhizium anisopliae* (Metchnikoff) Sorokin against FAW. Use of entomopathogenic fungi is a popular method to control fall armyworm because EPF and synthetic pesticides have different mode of action (Hajek, 1989; Fargues *et al.*, 1994)^[15, 12]. When EPF spores acquire contact with the arthropod host, they induce infection. Under optimal conditions, fungal spores germinate and penetrate the insect cuticle by enzymatic breakdown and mechanical pressure to achieve entry into the insect body.

After accessing the insect tissues, the EPF multiply rapidly and emerge from the deceased insect to create additional fungal spores (Dara, 2017; Altinok *et al.*, 2019; Ebani and Mancianti, 2021)^[8, 2, 10]. However, it is worth noting that these EPF serve to reduce crop damage by infecting host pests, causing a decrease in eating, egg laying, growth and mating as well as disrupting pest physiological function (Thomas *et al.*, 1997)^[29]. Isolates of EPF caused severe mortality of FAW eggs and neonate larvae (Akutse *et al.*, 2019)^[11] and greatly impaired larval feeding efficacy (Qadir *et al.*, 2021)^[22].

Keeping all facts in view, the experiment was conducted to test the efficacy of different doses of microbial bio-pesticides *Metarhizium anisopliae* against the 2nd and 3rd instar larvae of FAW *Spodoptera frugiperda* under laboratory condition.

Material and Methods

Collection and mass multiplication of *Spodoptera frugiperda* culture

In order to study the pathogenicity of *Spodoptera frugiperda*, egg masses of *Spodoptera frugiperda* were obtained from the infested maize fields of Entomology Research Farm, PGI, MPKV, Rahuri.

Laboratory rearing of *Spodoptera frugiperda* culture

The larvae were collected and brought to the culture room where they were given time to grow till emergence in order to maintain the *Spodoptera frugiperda* culture. After emergence of adults, male and female moths were separated and placed in clear jars with fine muslin cloth covers and rubber bands to facilitate mating. Each jar was filled with a 10% honey solution on a cotton swab for moth feeding. A black paper sheet surrounded the interior of the transparent jars making the eggs on the surface clearly visible. Eggs of fall armyworm laid on black paper sheet were collected for further multiplication and use. These eggs were used as nucleus culture of *Spodoptera frugiperda* to check the pathogenicity of *M. anisopliae* against the second and third instar larva.

Preparation of fungal cultures of *Metarhizium anisopliae* 1.15% WP

The pure culture of *Metarhizium anisopliae* IPL/KC/44, Strain No. ITCC 6895 was obtained from the Biocontrol laboratory, Department of Entomology, Post Graduate Institute, MPKV, Rahuri. Different doses of *Metarhizium anisopliae* 1.15% WP formulations were prepared in one litre of water. The amount of WP suspensions was prepared *viz.*, 0, 2.5, 5, 7.5 and 10 g/ litre to check the pathogenicity of *Metarhizium anisopliae* against FAW.

Pathogenicity of *Metarhizium anisopliae* against larvae of *Spodoptera frugiperda*

The newly hatched larvae were collected and placed in plastic containers containing fresh maize leaves for immediate feeding. Second and third instar homogenous larvae of *S. frugiperda* were selected for pathogenicity study. The bioassay was conducted by larval dip method (Ramanujam *et al.*, 2020). The selected larvae were dipped in the suspension of *Metarhizium anisopliae* with different dose for 20 s. The treated larvae were then transferred into plastic containers and supplied with fresh detached maize leaves. The treated larvae were maintained under controlled conditions of 25 ± 2 °C temperature and 70 ± 5 % relative humidity for 10 days. Fresh maize leaves were provided as a food to the larvae in 24 h interval. Controls were treated with sterile water containing 0.01% Tween 80.

Statistical analysis was carried out by analyzing the available data in Completely Randomised Design (CRD). The data was subjected to arc sin transformation prior to analysis.

Result and Discussion

Pathogenicity of *Metarhizium anisopliae* 1.15% WP against fall armyworm *Spodoptera frugiperda* in laboratory

Mortality of fall armyworm larvae using *Metarhizium anisopliae* 1.15% WP formulation at different doses with regular time interval of 6, 8 and 10 days was recorded. Different doses of *Metarhizium anisopliae* 1.15% WP formulations *viz.*, 0 g/litre, 2.5 g/litre, 5 g/litre, 7.5 g/litre and 10 g/litre were evaluated against second and third instar larvae of *Spodoptera frugiperda* under laboratory conditions.

Bioefficacy of different doses of *Metarhizium anisopliae* 1.15% WP against second instar larvae of fall armyworm *Spodoptera frugiperda* under laboratory condition.

Mortality of second instar *Spodoptera frugiperda* larvae after 6 days of treatment

Data from the average percent corrected mortality (Table 1) after 6 days of treatment revealed that average percent mortality of *Spodoptera frugiperda* larvae ranged from 0 to 58.89 percent.

The entomopathogenic fungi *Metarhizium anisopliae* when used at a dose of 10 g/l caused highest (58.89%) average mortality and it was at par with treatment with dose of 7.5 g/l (51.11%). The lowest average percent larval mortality was observed in treatment by using a dose of 2.5 g/l (20.56%).

Table 1: Mortality of second instar *Spodoptera frugiperda* larvae at 6, 8 and 10th day after treatment

Treatment	Dose	Percent Mortality		
		6 th Day	8 th Day	10 th Day
T ₁	0	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)
T ₂	2.5	20.56 (26.58)	33.33 (35.25)	43.05 (40.97)
T ₃	5	46.11 (42.76)	69.44 (56.55)	75.83 (60.60)
T ₄	7.5	51.11 (45.64)	71.94 (58.22)	78.33 (62.26)
T ₅	10	58.89 (50.19)	76.94 (61.38)	81.38 (64.92)
S.E.±		2.05	1.88	1.86
CD at 5%		6.18	5.69	5.63

Note: * Figures in parentheses are arc sin transformed values.

Mortality of second instar *Spodoptera frugiperda* larvae after 8 days of treatment

Data from the average percent corrected mortality (Table 1) after 8 days of treatment revealed that average percent mortality of *Spodoptera frugiperda* larvae ranged from 0 to 76.94 percent.

The entomopathogenic fungi *Metarhizium anisopliae* when used at a lethal dose of 10 g/l caused highest average percent larval mortality (76.94%) and it was at par with the treatment using lethal dose of 7.5 g/l (71.94%) and with the treatment using lethal dose of 5 g/l (69.44%). The lowest average percent larval mortality was observed in treatment by using a lethal dose of 2.5 g/l (33.33%).

Mortality of second instar *Spodoptera frugiperda* larvae after 10 days of treatment

Data from the average percent corrected mortality (Table 1) after 10 days of treatment revealed that average percent mortality of *Spodoptera frugiperda* larvae ranged from 0 to 81.38 percent.

The entomopathogenic fungi *Metarhizium anisopliae* when used at a lethal dose of 10 g/l caused highest average percent larval mortality (81.38%) and it was at par with the treatment using lethal dose of 7.5 g/l (78.33%) and with the treatment using lethal dose of 5 g/l (75.83%). The lowest average percent larval mortality was observed in treatment by using a lethal dose of 2.5 g/l (43.05%).

Bioefficacy of Different Doses of *Metarhizium anisopliae* 1.15% WP against Third Instar Larvae of Fall Armyworm *Spodoptera frugiperda* Under Laboratory Condition

Mortality of third instar *Spodoptera frugiperda* larvae after 6 days of treatment

Data from the average percent corrected mortality (Table 2) after 6 days of treatment revealed that average percent mortality of *Spodoptera frugiperda* larvae ranged from 0 to 48.61 percent.

The entomopathogenic fungi *Metarhizium anisopliae* when used at a lethal dose of 10 g/l caused highest average percent larval mortality (48.61%). The lowest average percent larval mortality was observed in treatment by using a lethal dose of 2.5 g/l (17.78%).

Mortality of third instar *Spodoptera frugiperda* larvae after 8 days of treatment

Data from the average percent corrected mortality (Table 2) after 8 days of treatment revealed that average percent mortality of *Spodoptera frugiperda* larvae ranged from 0 to 64.44 percent.

The entomopathogenic fungi *Metarhizium anisopliae* when used at a lethal dose of 10 g/l caused highest average percent larval mortality (64.44%) and it was at par with the treatment using lethal dose of 7.5 g/l (56.66%). The lowest average percent larval mortality was observed in treatment by using a lethal dose of 2.5 g/l (30.83%).

Table 2: Mortality third instar *Spodoptera frugiperda* larvae at 6, 8 and 10th day after treatment

Treatment	Dose	Percent Mortality		
		6 th Day	8 th Day	10 th Day
T ₁	0	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)
T ₂	2.5	17.78 (24.79)	30.83 (33.72)	40.55 (39.53)
T ₃	5	38.33 (38.18)	51.66 (45.95)	67.50 (55.25)
T ₄	7.5	41.11 (39.88)	56.66 (48.83)	70.27 (57.03)
T ₅	10	48.61 (44.20)	64.44 (53.63)	72.77 (58.69)
S.E.±		1.47	2.06	1.47
CD at 5%		4.45	6.22	4.43

Note: * Figures in parentheses are arc sin transformed values.

Mortality of third instar *Spodoptera frugiperda* larvae after 10 days of treatment

Data from the average percent corrected mortality (Table 2) after 10 days of treatment revealed that average percent mortality of *Spodoptera frugiperda* larvae ranged from 0 to 72.77 percent.

The entomopathogenic fungi *Metarhizium anisopliae* when used at a lethal dose of 10 g/l caused highest average percent larval mortality (72.77%) and it was at par with the treatment using lethal dose of 7.5 g/l (70.27%). The lowest average percent larval mortality was observed in treatment by using a lethal dose of 2.5 g/l (40.55%).

Dose Mortality Response of *Metarhizium anisopliae* 1.15% WP against Second and Third Instar *Spodoptera frugiperda* Larvae

The results on the Dose Mortality response of *Metarhizium anisopliae* against second and third instar larvae of fall

armyworm *Spodoptera frugiperda* is presented in the (Table 3)

The mortality from different doses of *Metarhizium anisopliae* against second and third instar larvae of fall armyworm *Spodoptera frugiperda* at different days after treatment was recorded and was subjected to estimation of LD₅₀ values. From the data obtained from the estimation of LD₅₀ of cumulative mortality of second instar LD₅₀ value of 3.80 g/litre was recorded which is less in comparison with LD₅₀ value of 4.10 g/litre of third instar cumulative mortality. Hence, the results clearly indicates that second instar larvae of fall armyworm *Spodoptera frugiperda* are more susceptible to *Metarhizium anisopliae* than third instar larvae. However, it also indicates that as instar grows it requires high lethal dose to kill 50% of the test populations. The results concludes that the larval mortality increases with an increase in doses of microbial pesticides and time interval.

Table 3: Cumulative dose mortality response of second and third instar *Spodoptera frugiperda* larvae against *Metarhizium anisopliae* 1.15% WP after 6, 8 and 10 days of treatment

Sr. No	Days after treatment	LD ₅₀	Chi-square (X)	Regression	Fiducial Limits	
					Lower	Upper
Second Instar						
1	6	6.77	0.875	1.60+3.66	3.82	11.99
2	8	3.76	0.975	1.60+3.66	2.30	6.15
3	10	2.36	0.928	1.60+3.66	1.31	4.26
Third Instar						
1	6	9.79	0.933	1.60+3.66	4.57	20.99
2	8	4.90	0.989	1.60+3.66	2.65	9.05
3	10	2.69	0.934	1.60+3.66	1.33	5.44
Cumulative Mortality second Instar						
1	-	3.80	0.708	1.60+3.66	2.16	6.68
Cumulative Mortality third Instar						
1	-	4.10	0.862	1.60+3.66	2.33	7.23



Eggs of fall armyworm



Larvae of fall armyworm



FAW larvae infected by *Metarhizium anisopliae* infected



Mycosis on FAW larvae

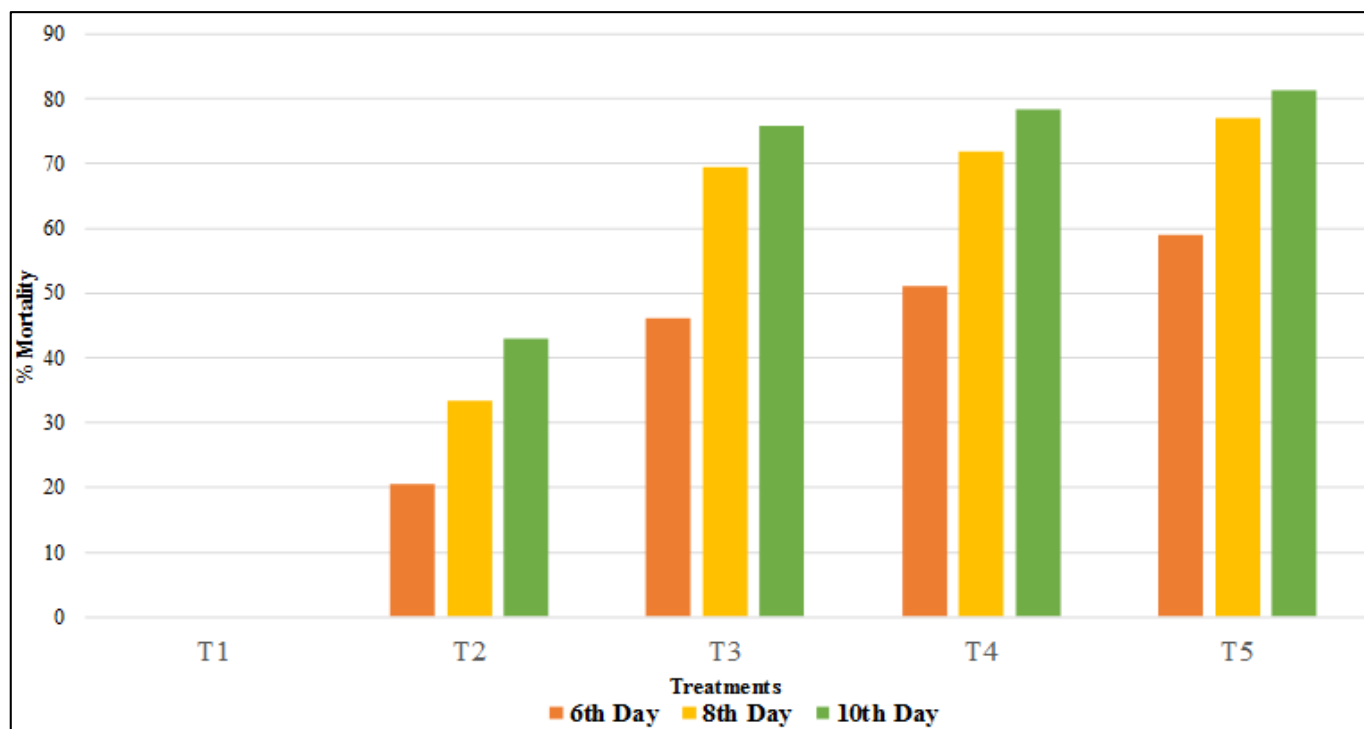


Fig 1: Corrected larval mortality of second instar *Spodoptera frugiperda* larvae at 6, 8 and 10 days after treatment

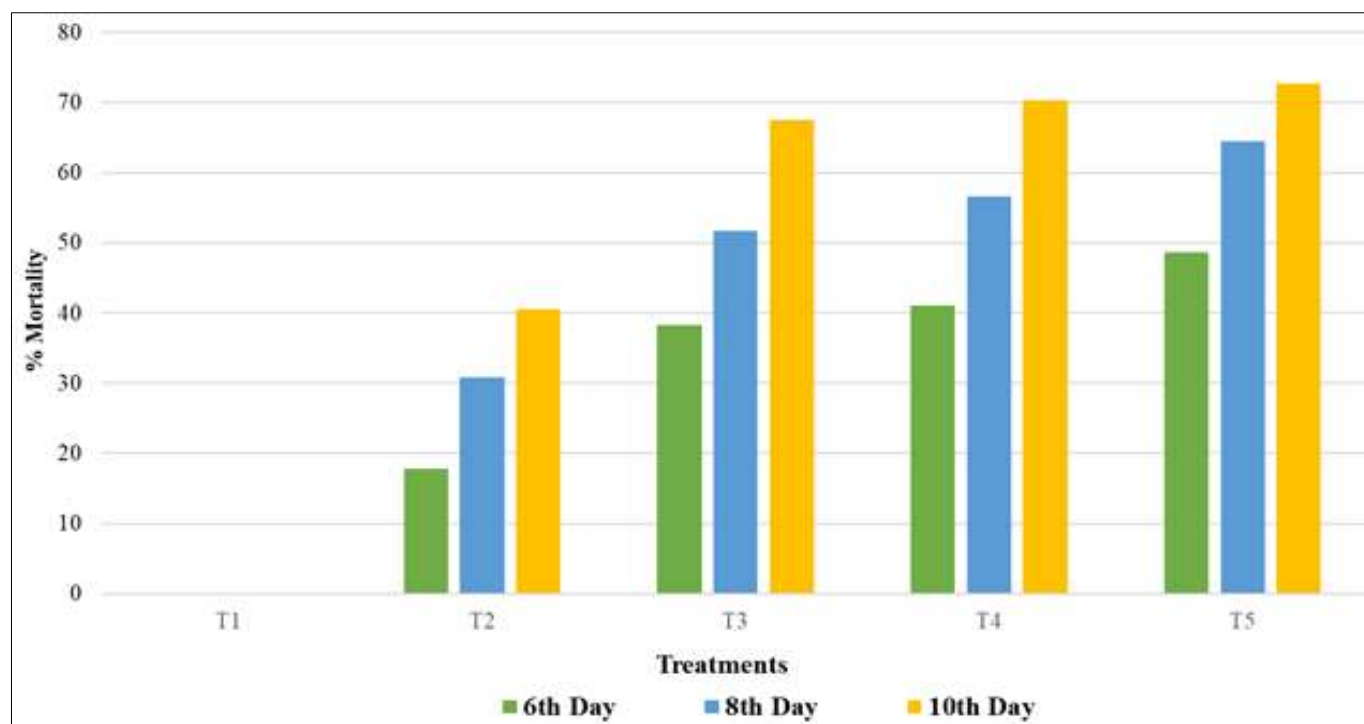


Fig 2: Corrected larval mortality of third instar *Spodoptera frugiperda* larvae at 6, 8 and 10 days after treatment.

The chi square test revealed the homogeneity of the test populations in all the bioassays which reveals the good fit of observed and expected responses.

Different researchers reported different mortalities of *Spodoptera frugiperda* by *Metarhizium anisopliae* and the variation in results could be due to variation in the strain utilized in the investigation as well as differences in the concentrations and doses employed in the study. Lezama-Gutierrez *et al.* (2001) [18] reported 100% mortality rates. According to current studies, mortality rates ranged from 40.55 to 81.38 percent. Sanchez-Pena *et al.* (2007) [25] found

90% FAW mortality. Sisay *et al.* (2018) [28] performed a laboratory bioassay and reported mortality rates ranging from 80 to 100 percent. Apirajakamol *et al.* (2022) [3] observed 53% mortality in 2nd instar larvae by *Metarhizium anisopliae*.

The current study results are in line with those of Ramanujam *et al.* (2020) [23] found that all strains of *M. anisopliae* were effective against *S. frugiperda* larvae with larval mortality ranging from 10.70 to 67.80 percent with treatment of *M. anisopliae* ICAR-NBAIR Ma-35 (67.80%) causing the highest larval mortality.

The current findings corroborate with those of Kiruthiga *et al.* (2022) ^[17] who examined the pathogenicity of *Metarhizium anisopliae* against *S. frugiperda* 2nd instar larvae raised in a laboratory. They reported mortality ranging from 18.87 to 86.67 percent. In the current investigation, mortality at various doses ranged from 43.05 to 81.38 percent.

The current research supports the findings of Barros *et al.* (2020) ^[4] who found that *Spodoptera frugiperda* mortality rates ranged from 74 to 84 percent as a result of *Metarhizium anisopliae*. The mortality rate for 2nd instar larvae in the current study caused by *Metarhizium anisopliae* 1.15% WP was 81.38 percent after 10 days of treatment.

The current findings are slightly different from results of those obtained by Patel *et al.* (2020) ^[21] who found that the AAU strain of *M. anisopliae* caused the highest larval mortality rates (57.56 and 50.63%) against 2nd and 3rd instar larvae respectively. In present study *M. anisopliae* at a dose of 5g/l caused mortality of 81.38 and 72.77 percent in 2nd and 3rd instar larvae respectively.

Present findings are in collaboration with the finding of Montecalvo *et al.* (2021) ^[19] who recorded lethal infestation to the larval instars (1st to 6th) ranged from 23.13 to 61.33 percent in *M. anisopliae* at 10 days after treatment under laboratory conditions. In present study mortality of *Spodoptera frugiperda* 2nd instar ranged from 46.11 to 75.83 percent with a dose of 5 g/l.

The current outcomes are in line with Romero-Arenas *et al.* (2014) findings that a native strain of *M. anisopliae* resulted in a mortality rate of 72.5 percent and a lowest of 32.5 percent. In the current investigation mortality rates for second instar larvae at a dose of 5 g/litre were 75.83 percent and for third instar larvae 67.50 percent.

The current findings are in accordance with those of Bosa *et al.* (2004) who found that *M. rileyi* isolates were effective and caused 73 - 100 percent mortality in *S. frugiperda* second instar. In the current investigation the maximum mortality rate of 81.38 percent was found in the second instar of *S. frugiperda* at a dose of 10 g/litre.

The current findings support those of Akutse *et al.* (2019) ^[11] who reported that *M. anisopliae* strain IC1PE 41 causes the maximum mortality of 96.5 percent in FAW. In the current research the second instar reported the highest mortality rate (81.38%).

The current findings are consistent with those of Gutierrez-Cardenas *et al.* (2019) ^[14] who reported that the *M. anisopliae* CP-MA 1 strain causes 72.50 percent mortality. At a dose of 10 gram/ litre in the current investigation *Metarhizium anisopliae* resulted in a 72.77 percent mortality rate.

The current findings are in accordance with those of Garcia *et al.* (2011) ^[13] who stated that *M. anisopliae* strains showed 96.6 percent larval mortality. Mortality for *M. anisopliae* in the current investigation ranged from 40.55 to 81.38 percent at various dosages.

The current findings are in line with those of Montecalvo and Navosero (2021) ^[19]. At 10 days following treatment the lethal infestation of the larval instars ranged from 23.13 to 61.33 percent in *M. anisopliae*. In the current investigation 3rd instar mortality ranged from 40.55 to 72.77 percent at a dosage of 5 g/litre.

The current findings are slightly different from the findings of Shahzad *et al.* (2021) ^[26] who found 59 percent mortality in 2nd instar larvae whereas the current data showed 75.83 percent mortality in 2nd instar larvae at a dose of 5 g/litre.

Conclusion

The recent study on Pathogenicity of *Metarhizium anisopliae* against *Spodoptera frugiperda* larvae under laboratory conditions conclude that 2nd instar larvae with treatment of *M. anisopliae* with different doses showed mortality ranging from 43.05% to 81.38%. The treatment with highest mortality in descending order was observed in treatment with a dose of 10 g/liter (81.38%) > treatment with a dose of 7.5 g/liter (78.33%) > treatment with a dose of 5 g/liter (75.83%) and treatment with a dose of 2.5 g/liter (43.05%).

In 3rd instar larvae *M. anisopliae* with different doses showed mortality of FAW ranges from 40.55% to 72.77%. The treatment with highest mortality in descending order was observed in treatment with a dose of 10 g/liter (72.77%) > treatment with a dose of 7.5 g/liter (70.27%) > treatment with a dose of 5 g/liter (67.50%) and treatment with a dose of 2.5 g/liter (40.55%).

The results are suggestive that the earlier instar larvae of *S. frugiperda* are more susceptible than later instar larvae. Similarly in laboratory conditions higher doses of *M. anisopliae* gave higher mortality rates than lower doses.

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