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Impact of *Metarhizium rileyi* infected larvae on cannibalism behavior in *Spodoptera frugiperda*

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ABSTRACT

A laboratory experiment was undertaken during 2021-22 and 2022-23 to assess the impact of entomopathogen, Metarhizium rileyi infected larvae on cannibalism behavior in Spodoptera frugiperda. It helps to study how this behavior will affect cannibalism. M. rileyi is a significant contributor to natural epizootics, causing mortality among S. frugiperda larvae. Upon analyzing the combined data, it became evident that varying degrees of cannibalism were observed among different instar healthy larvae of S. frugiperda. Furthermore, when interactions occurred between healthy and diseased larvae of different instars, the instances of cannibalism escalated. Notably, a pronounced increase in cannibalism was noted in smaller healthy larvae when exposed to larger diseased larvae, in contrast to cannibalism rates between larvae of the same age. This phenomenon also had an impact on the biological characteristics and pupal weight of the cannibalistic individuals.

Keywords: *Cannibalism, Entomopathogen, Epizootics, Metarhizium rileyi, Mortality, Puapl weight and Spodoptera frugiperda*

INTRODUCTION

Cannibalism is the process of killing and consumption of conspecifics which is a taxonomically widespread behavior in phytophagous insects, mostly in lepidopteran species [2]. Cannibalism often accounts for substantial mortality that may influence population dynamics and community structure [3]. Cannibalism may confer direct fitness benefits, in the form of increased survival, developmental rate, or fecundity. Cannibals may also benefit indirectly from the removal of potential competitors [6]. It is often favored under high population densities and when resources are limited but can occur even when food is not limited [6]. This behavior is costly in terms of energy, time, risk of injury, and death for competing individuals that may have repercussions for an individual's fitness. Cannibalistic insects are most likely to prey on smaller conspecifics. However, they may also attack larger conspecifics. Cannibals can obtain nutrients such as salt, protein, and amino acids by consuming conspecifics [8]. Cannibalism is a frequent behavior of *Spodoptera frugiperda* accounting for 40–60 percent mortality in laboratory culture [1]. On maize, the larvae usually feed on the wrapped leaves of the developing whorl. The density of larvae in wrapped leaves may lead to food competition and then attack individuals weaker than themselves.

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MATERIAL AND METHODS

The study was conducted under laboratory conditions during 2021-22 and 2022-23. Later instar larvae of fall armyworm were collected from the field and reared on maize leaves in cavity trays to avoid cannibalism in order to get nucleus culture. The larvae were reared up to pupal stage. Pupae were placed in petri dishes containing sand to simulate natural conditions and were kept in wooden cage (36 x 36 x 36 cm size). When adults emerged, they were provided with 10 percent honey solution as food for adults. Fresh tender maize leaves were kept in a glass conical flask inside the cage for oviposition. The cut end of the leaf whorl was covered with a wet cotton wad for maintaining turgidity and freshness. Freshly laid egg masses were kept in rearing plastic boxes provided with wet blotting paper at the bottom to protect the eggs from desiccation. After two days when eggs turned to black color, they were provided with fresh maize leaves as food for neonate larvae. Egg masses laid at different days were kept separately. The neonate larvae were released on leaves with the help of a soft hair brush in cavity trays. For the study, the larvae of each instar were taken and placed in a rearing box whose top was covered with muslin cloth in order to facilitate aeration. The food was changed after every 24 hours. One day-old larvae of each instar, based on molting date and the presence of shredded head capsules were taken for the study.

Larvae of *S. frugiperda* were placed individually in cavity trays and were fed with maize leaves treated with *M. rileyi*. Five healthy larvae of *S. frugiperda* were placed into a transparent plastic box containing a wet blotting paper to maintain the moisture and enclosed with a plastic lid having holes to allow ventilation. Each box was considered as one replicate with three replicates per treatment in a completely randomized design. To these boxes, five *S. frugiperda* larvae after infection with *M. rileyi*

were placed. One day-old *M. rileyi* treated larvae were used for the experiment. Larval feeding preference and number of larvae cannibalized were observed. Survived larvae were observed till pupation and adult emergence to study the impact of cannibalism on the biology of the insect. Number of normal and abnormal pupae, percent pupation, number of normal and abnormal adults, and percent adult emergence were calculated. Observations were calculated using following formulae:

$$\text{Per cent cannibalism} = \frac{\text{Number of larvae cannibalized}}{\text{Total number of larvae}} \times 100$$

$$\text{Per cent pupation} = \frac{\text{Number of pupae formed}}{\text{Total number of larvae}} \times 100$$

$$\text{Percent adult emergence} = \frac{\text{Number of adults emerged}}{\text{Total number of pupae}} \times 100$$

RESULTS

Percent cannibalism

When compared to control, cannibalism of 2nd instar larvae of *S. frugiperda* was found to be 30.00, 20.00, 13.33, 13.33, 10.00, 6.67, 0.00 and 0.00 percent at 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively. This contributed to a total of 93.33 percent in the presence of *M. rileyi* infected 3rd instar larvae. The percentage of cannibalism among 2nd instar larvae of *S. frugiperda* displayed the following pattern over time: 33.33, 20.00, 13.33, 10.00, 10.00, 6.67, 0.00 and 0.00 percent at 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively. These cumulative rates amounted to 93.33 per cent in the presence of *M. rileyi* infected 4th instar larvae, in comparison to the control. When compared to control, cannibalism of 2nd instar *S. frugiperda* larvae was found to be 33.33, 16.67, 13.33, 13.33, 10.00, 6.67 and 0.00 percent at 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively in the presence of *M. rileyi* infected 5th instar larvae. This contributed to a total of 93.33 percent. The percentage of 3rd instar larvae of *S. frugiperda* that were cannibalized was 23.33, 16.67, 13.33, 10.00, 10.00, 6.67, 0.00 and 0.00 per cent at 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively. This contributed to a total of 80.00 per cent when *M. rileyi* infected 4th instar larvae were present compared to control. The percentage of cannibalism among 3rd instar larvae of *S. frugiperda* followed a pattern over time: 30.00, 23.33, 13.33, 10.00, 6.67, 3.33, 3.33 and 0.00 percent at 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively. These cumulative values added up to 90.00 percent in the presence of *M. rileyi* infected 5th instar larvae, as compared to the control. The cannibalism percentages among 4th instar larvae of *S. frugiperda* over time was 13.33, 13.33, 13.33, 10.00, 13.33, 3.33, 0.00 and 0.00 percent at 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively. These cumulative values amounted to 66.67 percent in the presence of *M. rileyi* infected 5th instar larvae, in comparison to the control.

The percentage of cannibalism among 2nd instar larvae of *S. frugiperda* was found to be 26.67, 16.67, 13.33, 13.33, 6.67, 6.67, 3.33 and 0.00 percent at 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively, adding up to a total of 86.67 percent in the presence of *M. rileyi* infected 2nd instar larvae compared to control. There was a varying cannibalism percentages among 3rd instar larvae of *S. frugiperda* across time intervals: 20.00, 16.67, 13.33, 13.33, 6.67, 3.33, 3.33 and 0.00 percent at 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively. These cumulative percentages summed up to 76.67 percent in the presence of *M. rileyi* infected 3rd instar larvae, compared to the control. The percentage of 4th instar larvae of *S. frugiperda* that were cannibalized was 13.33, 13.33, 6.67, 10.00, 6.67, 3.33, 10.00 and 0.00 percent at 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively. This contributed to a total of 63.33 percent in the presence of *M. rileyi* infected 4th instar larvae compared to control. The results of the data analysis using the two samples t-test showed that there was a significant difference between treatment and control. Cannibalism percentages among 5th instar larvae of *S. frugiperda* across different time intervals was 10.00, 10.00, 6.67, 10.00, 13.33, 0.00, 0.00 and 0.00 per cent at 12, 24, 36, 48, 60, 72, 84 and 96 hrs, respectively. These cumulative percentages amounted to 50.00 per cent in the presence of *M. rileyi* infected 5th instar larvae, compared to the control. Employing a two-sample t-test for data analysis indicates no statistically significant difference between the conditions being compared (Table 1).

Impact of cannibalism on the biology of *S. frugiperda*

According to pooled data, when 2nd instar larvae were released alongside *M. rileyi* infected 3rd instar larvae, their larval lifespan was extended by 22.38 percent as opposed to when they were raised separately (without cannibalism). When released together with *M. rileyi* infected 2nd instar larvae, it increased in 2nd instar larvae by 18.88 per cent over control (Table 2). There were no pupae formed when 2nd instar larvae were released with *M. rileyi* infected 4th or 5th instar larvae and 3rd instar larvae were released with *M. rileyi* infected 5th instar larvae compared to control. Percent normal pupae formed was 0.00 per cent in early instar larvae (Fig. 1). When released with *M. rileyi* infected 3rd instar larvae, male pupal duration of 3rd instar larvae increased by 9.30 per cent over control. When 4th instar larvae were released with *M. rileyi* infected 5th instar larvae, it rose by 7.04 per cent over control. When *M. rileyi* infected 3rd and 5th instar larvae were released with healthy 3rd and 4th instar larvae, respectively, female pupal duration increased by 10.00 and 7.50 per cent over control (Table 2). There were no adults emerged in 2nd instar larvae when released along with *M. rileyi* infected 2nd or 3rd or 4th or 5th instar larvae and 3rd instar larvae were released with *M. rileyi* infected 5th instar larvae compared to control (Fig. 2). The adult male's longevity demonstrated an elevation of 13.79 and 10.34 per cent over control during the 3rd and 4th instar larval phases, respectively, when introduced in the company of *M. rileyi* infected 3rd and 5th instar larvae. Likewise, the adult female's longevity exhibited a rise of 10.81 per cent over control during the 4th instar larval stage upon release alongside *M. rileyi* infected 5th instar larvae. Furthermore, it exhibited a rise of 5.41 percent over control during the 4th instar larval stage when released alongside *M. rileyi* infected 4th instar larvae (Table 2).

Fitness cost analysis

Combined data analysis revealed that the most significant reduction percentage (51.59 %) in pupal weight (111.96 mg) occurred in 2nd instar larvae when introduced alongside *M. rileyi* infected 3rd instar larvae. This was succeeded by a reduction of 47.64 per cent in 3rd instar larvae (122.03 mg) when released in the presence of *M. rileyi* infected 5th instar larvae, as compared to the control (243.50 mg). On the other hand, the least notable reduction percentage (35.41 %) in pupal weight was observed in 5th instar larvae (153.21 mg) when released alongside *M. rileyi* infected 5th instar larvae. This was followed by a reduction of 38.94 per cent in 4th instar larvae (144.20 mg) when introduced alongside *M. rileyi* infected 4th instar larvae (Table 3).

DISCUSSION

Interestingly, our findings suggest that, no larvae consumed the disease-infected larvae in the presence of food. They did not

even step near to the disease-infected larvae. In some cases, *i.e*., when there was no food, larvae had gone near disease infected larvae and tried to feed on them even though they were covered with fungal spores (Unpublished data). Our results suggest that healthy larvae avoided cannibalizing on disease-infected larvae, rather they cannibalized the healthy larvae at a higher rate. This may be because disease-infected larvae became less active until their death. After their death, fungal spores start coming out of the larvae. So, the healthy larvae avoided coming near to the larvae which were infected because of the fungal toxins released by the disease-infected larvae. In the majority of the cases, larvae cannibalise on healthy larvae which are actively moving and, behaviorally attacking and defending, posing threat of encounter. Rarely they will feed on conspecifics that are already injured, partially fed or disease-infected. Sometimes they even feed on disease-infected individuals when the pathogen is virus. Varied level of cannibalism was noticed among different instar larvae of *S. frugiperda*. Cannibalism was increased when different instars of healthy and diseased larvae were interacted. Cannibalism was at a greater extent among smaller healthy larvae in the presence of larger diseased larvae, compared to cannibalism among equal aged larvae. This might be because when the healthy larvae are smaller than disease-infected larvae, fungal toxins released from the bigger ones may be at a greater extent due to larger surface area. It was also observed that cannibalism was highest in early instar larvae rather than late instar larvae. Deaths due to cannibalism occurred gradually and regularly so that there was a decreasing number of insects in a situation becoming decreasingly crowded and less encountered by the conspecifics. Results are in line with the findings of [4,5,7] who documented that cannibalism was prevalent among larvae in a group when the pathogen was present compared to when it was absent.

CONCLUSION

Cannibalism had a more pronounced impact on early instar larvae in contrast to their later instar counterparts in the presence of entomopathogen. The findings of our study carry significant implications for the management of *S. frugiperda*. Given that *S. frugiperda* larval populations are influenced by the entomopathogenic fungus *M. rileyi*, the application of EPF spray could potentially lead to an increase in cannibalistic behavior among larvae that remain unaffected by *M. rileyi*.

FUTURE SCOPE

Investigating the population dynamics of *S. frugiperda* larvae in field conditions while coexisting with *M. rileyi* infected larvae stands as a significant objective for upcoming research endeavors.

CONLICT OF INTEREST

The authors declare no conlict of interest.

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Table 1: Intraspecific interaction of Spodoptera frugiperda with Metarhizium rileyi infected larvae (Pooled data of 2021-22 and 2022-23)

Tr. No.	Treatment details	Per cent Cannibalism									
		12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	Total	t statistic
T1	2nd instar (H) vs 3rd instar larvae (D)	30.00	20.00	13.33	13.33	10.00	6.67	0.00	0.00	93.33	4.91**
T2	2nd instar (H) vs 4th instar larvae (D)	33.33	20.00	13.33	10.00	10.00	6.67	0.00	0.00	93.33	4.91**
T3	2nd instar (H) vs 5th instar larvae (D)	33.33	16.67	13.33	13.33	10.00	6.67	0.00	0.00	93.33	6.50**
T4	3rd instar (H) vs 4th instar larvae (D)	23.33	16.67	13.33	10.00	10.00	6.67	0.00	0.00	80.00	4.16*
T5	3rd instar (H) vs 5th instar larvae (D)	30.00	23.33	13.33	10.00	6.67	3.33	3.33	0.00	90.00	4.43*
T6	4th instar (H) vs 5th instar larvae (D)	13.33	13.33	13.33	10.00	13.33	3.33	0.00	0.00	66.67	3.54*
T7	2nd instar (H) vs 2nd instar larvae (D)	26.67	16.67	13.33	13.33	6.67	6.67	3.33	0.00	86.67	5.50**
T8	3rd instar (H) vs 3rd instar larvae (D)	20.00	16.67	13.33	13.33	6.67	3.33	3.33	0.00	76.67	3.54*
T9	4th instar (H) vs 4th instar larvae (D)	13.33	13.33	6.67	10.00	6.67	3.33	10.00	0.00	63.33	4.00*
T10	5th instar (H) vs 5th instar larvae (D)	10.00	10.00	6.67	10.00	13.33	0.00	0.00	0.00	50.00	2.12 NS
T11	Five 2nd instar larvae (H) (Control 1)	0.00	6.67	13.33	6.67	6.67	6.67	10.00	0.00	50.00	
T12	Five 3rd instar larvae (H) (Control 2)	0.00	10.00	3.33	10.00	3.33	10.00	6.67	0.00	43.33	
T13	Five 4th instar larvae (H) (Control 3)	0.00	3.33	10.00	3.33	3.33	13.33	3.33	0.00	36.67	
T14	Five 5th instar larvae (H) (Control 4)	0.00	6.67	6.67	6.67	6.67	3.33	0.00	0.00	30.00	

H- Healthy larvae D- Diseased larvae
*t table (0.05): 2.776 t table (0.01): 4.604 *Significant at 5 % level of significance ** Significant at 1 % level of significance [NS]Non-significant*

Table 2: Impact of cannibalism on biology of Spodoptera frugiperda in intraspecific interaction with Metarhizium rileyi infected larvae (Pooled)

Tr. No.	Treatment details	Larval duration (days)	% increase over control	Pupal duration (days)				Adult longevity (days)			
				Male	% increase over control	Female	% increase over control	Male	% increase over control	Female	% increase over control
T1	2nd instar (H) vs 3rd instar larvae (D)	17.50	22.38	--	--	--	--	--	--	--	--
T2	2nd instar (H) vs 4th instar larvae (D)	--	--	--	--	--	--	--	--	--	--
T3	2nd instar (H) vs 5th instar larvae (D)	--	--	--	--	--	--	--	--	--	--
T4	3rd instar (H) vs 4th instar larvae (D)	14.00	16.18	9.50	6.50	--	--	--	--	--	--
T5	3rd instar (H) vs 5th instar larvae (D)	--	--	--	--	--	--	--	--	--	--
T6	4th instar (H) vs 5th instar larvae (D)	11.08	13.68	9.50	7.04	10.75	7.50	8.00	10.34	10.25	10.81
T7	2nd instar (H) vs 2nd instar larvae (D)	17.00	18.88	--	--	--	--	--	--	--	--
T8	3rd instar (H) vs 3rd instar larvae (D)	13.75	14.11	9.75	9.30	11.00	10.00	8.25	13.79		
T9	4th instar (H) vs 4th instar larvae (D)	10.92	11.97	9.13	2.82	10.50	5.00	7.88	8.62	9.75	5.41
T10	5th instar (H) vs 5th instar larvae (D)	8.25	9.27	9.13	2.82	10.25	4.27	7.75	3.33	9.63	4.96
T11	Single 2nd instar larvae (H) (Control 1)	14.30		9.00		9.63		7.58		9.38	
T12	Single 3rd instar larvae (H) (Control 2)	12.05		8.92		10.00		7.25		9.00	
T13	Single 4th instar larvae (H) (Control 3)	9.75		8.88		10.00		7.25		9.25	
T14	Single 5th instar larvae (H) (Control 4)	7.55		8.88		9.83		7.50		9.17	

H- Healthy larvae D- Diseased larvae

Table 3: Pupal weight of Spodoptera frugiperda in intraspecific interaction with Metarhizium rileyi infected larvae

Tr. No.	Treatment details	Pupal weight (mg)					
		2021	% Reduction over control	2022	% Reduction over control	Pooled	% Reduction over control
T1	2nd instar (H) vs 3rd instar larvae (D)	111.06	52.69	112.85	50.46	111.96	51.59
T2	2nd instar (H) vs 4th instar larvae (D)	--	--	--	--	--	--
T3	2nd instar (H) vs 5th instar larvae (D)	--	--	--	--	--	--

T4	3rd instar (H) vs 4th instar larvae (D)	122.11	48.41	121.95	46.85	122.03	47.64
T5	3rd instar (H) vs 5th instar larvae (D)	--	--	--	--	--	--
T6	4th instar (H) vs 5th instar larvae (D)	134.79	43.49	133.82	42.13	134.31	42.82
T7	2nd instar (H) vs 2nd instar larvae (D)	123.47	47.88	129.40	43.89	126.44	45.91
T8	3rd instar (H) vs 3rd instar larvae (D)	133.68	43.92	135.99	41.27	134.84	42.61
T9	4th instar (H) vs 4th instar larvae (D)	145.09	39.50	143.31	38.37	144.20	38.94
T10	5th instar (H) vs 5th instar larvae (D)	152.20	36.75	154.22	34.04	153.21	35.41
T11	Control*	247.00		240.00		243.50	

H- Healthy larvae D- Diseased larvae
*- *Mean pupal wight of single 2nd, 3rd, 4th and 5th instar larvae of Spodoptera frugiperda*

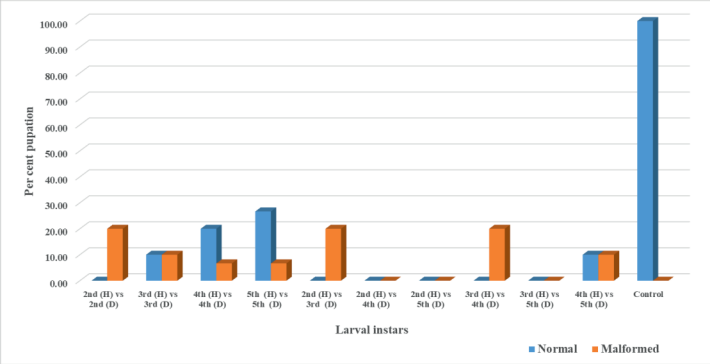

Figure 1: Impact of cannibalism on pupation of Spodoptera frugiperda in presence of Metarhizium infected larvae
H- Healthy larvae D- Diseased larvae

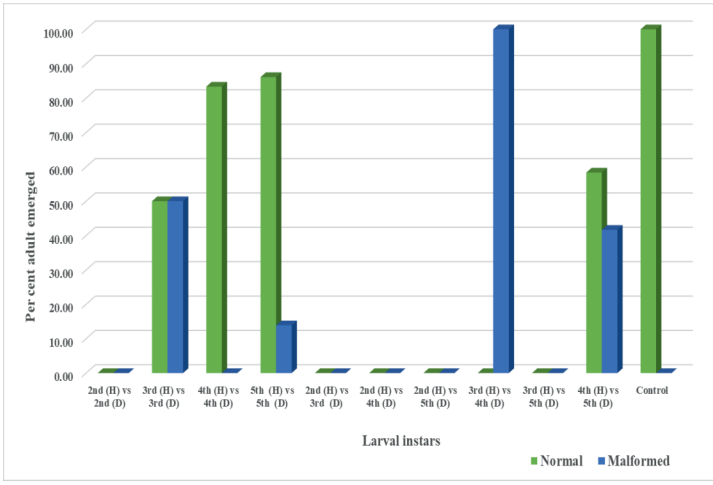

Figure 2: Impact of cannibalism on adult formation of Spodoptera frugiperda in presence of Metarhizium infected larvae
H- Healthy larvae D- Diseased larvae

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