EFFECTS OF BACTERIA ISOLATED FROM SALIBAT BOGS ON THE BIOLOGY OF LESSER GRAIN BORER *RHYZOPERTHA DOMINICA* (F)

Ahmed Ayad Al-Nuaimy[1]*, Sofia Jabbar Jassim[1], Meeri Kadhim Al-Fatlaw[1] and Alyaa Adnan Makki[2]

[1]Department of Desert studies center and Sawa lake, Al-Muthanna University, Iraq
[2]Agricultural Extension and Training Office, Al-Muthanna, Iraq
*Email: ahmed.ayad@mu.edu.iq (corresponding author): ORCID ID 0000-0002-4164-5708

ABSTRACT

In the 2022-2023 season, this study done at the Desert and Sawa Lake Research Center Laboratory of Al-Muthanna University explored the effects of bacterial isolates *Raoultella planticola* and *Providencia rettgeri* on insects. Different concentrations and periods of exposure were evaluated against life stages of *Rhyzopertha dominica* (F). The results showed that after treatment with *P. rettgeri*, the mortality rate in second-instar larvae of male and female significantly increased (13.14- 40.26%). The period of exposure of 96 hr was significantly superior (18.44- 47.18%); the triple interference treatment (*P. rettgeri* + 96 hr + 106) was significantly superior than others in giving mortality of all life stages to an extent of 23.33 - 84.96%.

Key words: Salibat bogs, *Rhyzopertha dominica*, biological control, *Raoultella planticola*, *Providencia rettgeri,* toxicity, mortality, concentration, dose, exposure, life stages

The lesser grain borer *Rhyzopertha dominica* (F) belonging to the order: Coleoptera and family: Bostrichidae, is a widespread storage pest. It causes significant damage to wood, and dried fruits (Ziaee,.2016). Its larvae enter the grain and feed on its contents, leaving only the peel behind. Besides their ability to pierce, they consume more food than they need, even in the driest grains that other insects cannot pierce (Rajabpour et al., 2019). Many insects cause significant damage tin storage, thereby negatively affecting their marketing value (Chen, 2013). This prompts use of farmers to overuse pesticides resulting in contamination and residues (Ahmed et al., 2021). Hence, insect control using biological controls, which include the use of predators, insect parasites and pathogens such as fungi, viruses, bacteria, and insect growth promoters is emerging as an ecofriendly alternative (Zhang et al., 2019). Bacteria have a wide distribution in the environment and interact with insects in various ways, including basic symbiosis (Feldhaar, 2011). While many bacteria inhabit insects and establish mutualistic symbioses to varying degrees, only a limited number play a role as insect pathogens. The latter have developed multiple strategies to invade the host, overcome its immune response, and infect and kill the host. It is thinking that the mechanisms leading to these types of interactions have ancient origins and developed during a long process of coevolution (Vilcinskas, 2010). After decades of research on microbial pest management, mainly *Bacillus thuringiensis*, new bacterial species with innovative modes of action are being discovered and developed into new products. Important examples include the symbiosis of the pathogenic nematode *Photobacterium* spp. and *Xenorhabdus* spp., *Serratia* spp., *Yersinia entomophagus*, *Pseudomonas entomophaga*, and the recently discovered betaproteobacteria genus Burkholderia. As well as the type of bacteria *Streptomyces* spp. and *Saccharopolyspora* and *Providencia* species (Vallet-Gely et al., 2008). The goal of this study was to evaluate how effective a biocide containing *Raoultella planticola* and *Providencia rettgeri* (either alone or combined) is against *Rhyzopertha dominica*.

MATERIALS AND METHODS

Local bacterial isolates *R. planticola* and *P. rettgeri* were obtained from the laboratories of Badia Research Center and Sawa Lake/Muthanna University. Add 5 ml of 0.9% saline solution to the bacterial culture to prepare a bacterial suspension with a concentration of (102, 104, and 106 ml/ CFU), use a sterile L-shaped harvester to separate the bacterial growth, and use a wine medical syringe to remove the metal part, After adding 5 ml of sterile saline solution, filter the suspension through two layers of gauze fixed on the glass funnel to ensure the removal of all bacteria and remove residues from the food environment. Collect the bacterial suspension in a 100 ml conical glass flask. A basic bacterial stock suspension is obtained from this. The bacterial concentration is prepared in 8 sterile test tubes with a capacity of 10 ml. We pipette 1-8 and put 9 ml of saline solution with a concentration of 0.9% into each tube, and then we pre-add 1 ml of basic bacterial suspension, which we will prepare with a micropipette. To achieve the concentration, I removed the solution and added it to test tube No. 2. Add 108 ml from test tube No. (2) to test tube No. (3) to gain a concentration of 107. Continue concentrating on the remaining tubes until you reach concentration No. 101. In this way, the concentrations required for the experiment (102, 104, 106) were obtained (Lacey, 1997).

After preparing the desired concentration, prepare the nutrient medium Nutrient Agar according to the manufacturer's recommendations by dissolving 28 g of the medium in 1 liter of distilled water and sterilizing it in an autoclave at a temperature and pressure of 121°C. 1.5 bar for 15 min, then pour into a Petri dish and allow to solidify, transfer 2 µl of the desired concentration using a (micropipette) and spread using a sterile L-shaped glass rod and incubate at 30°C± 2. After 24 hr, after confirming the growth of the isolate, count the number of colonies growing in the petri dish according to (Lacey, 1997) to prove that two isolates are growing on the same medium, and the dual vaccine can be obtained. There is no antagonism between the two isolates (Jassem, 2017). Bacterial commentator: After the counting of the bacterial colonies for the R. After counting the bacterial colonies for *R. planticola* and *P. rettgeri*, we placed the dishes at a temperature of 30 ± 2 C° and then added 5 ml of the phosphate salt solution (PBS) with a pH of 7.2 to the nutritional agar culture containing the bacterial colonies. We collected the bacteria and transferred them to sterile tubes, which were then stored in the refrigerator until needed (Thiery, 1997).

Effects of *Raoultella planticola* and *Providenccia rettgeri* bacteria on R. Dominica insects' various to test the three concentrations of *R. planticola* (6.1 x 102, 6.1 x 104, 6.1 x 106) ML /CFU, and P. Rettgeri (4.39 x 102, 4.39 x 104, 4.39 x 106) ML/ CFU, While the double inoculum vaccine R. The experiment tested three concentrations of *R. planticola* (6.1 x 102, 6.1 x 104, 6.1 x 106) ML/ CFU, and P. Rettgeri (4.39 x 102, 4.39 x 104, 4.39 x 106) ML/ CFU. Additionally, a double inoculum vaccine of *R. planticola* and *P. rettgeri* (5.52 x 102, 5.52 x 104, 5.52 x 106) ML/ CFU was sprayed using a small sterile sprayer to ensure even distribution of the entire Petri dish. The control treatment involved spraying sterile distilled water and then culturing the bacterial suspension. Treat in an incubator with a temperature of 30± 2°C and a humidity of 70± 5%. Then the killing rate is calculated after 7 days of treatment (Ahmedani et al., 2008). The results are based on the Orell and Schneider equation (Shaaban and Al-Mallah, 1993). For statistical analysis, R C B D design was used with treatments replicated thrice. Upon designing several ratios and converting them to arcsine transformed values, mean values were compared (LSD, $p \leq 0.01$).

RESULTS AND DISCUSSION

Table 1 shows that there are significant differences in the mortality of the second instar larvae of the insect among different treatments, If the results overall show that biological factors at different concentrations are significantly better than the control treatment, concentration 106 records the highest mortality rate of 59.29%, while the mortality rate of the control treatment is 0%. Significant differences were observed between the resistance factors used in the studies. The study showed that the second-instar larvae of *R. dominica* treated with isolate of *P. rettgeri* showed the highest mortality rate of 40.26%, while the mortality rate of the two-bacteria treatment was the lowest at 30.26%. Mortality rate of the second-instar larvae was significantly different with exposure periods; maximum was 47.18% at 96 hr of exposure; and the least of 19.49% was at 24 hr. The interaction between concentration and exposure was significant- as 96 hr exposure of *P. rettgeri* gave maximum mortality (81.81%). Triple intervention treatment has obvious significant differences (*P. rettgeri* + 96 hr + 106) with mortality reaching 84.96%. As regards concentration factor, single and double biological resistance factors, and exposure time have significant effects on the mortality of fourth-instar larvae; Concentrations (102, 104, 106) are better than the contrast treatment; rate of concentration 106 is 41.36%; *P. rettgeri* isolate was significantly better than the biological resistance agent, with the highest mortality rate of fourth-instar larvae at 39.79%, while the double treatment (*R. planticola* and *P. rettgeri*) had the lowest rate at 17.07%, There was a significant difference in the impact of exposure time. The fourth-instar larvae at exposure time of 96 hr showed the highest mortality (42.38%), while the exposure time of 24 hr showed only 12.78%.

The significant effect of the interaction between the biological resistance factor added singly or double with the duration of exposure to it, as the treatment of the

Table 1. Effects of *R. planticola* and *P. rettgeri* bacteria on *R. dominica*

Bacteria	Concentration	Time (hr)				R×C	R
		T1	T2	T3	T4		
Second-instar larvae							
R1	C0	0	0	0	0	0	
	C1	26.51	40.08	45.31	51.49	40.85	
	C2	33.44	56.22	63.23	71.51	56.10	
	C3	36.65	63.31	77.26	84.96	65.52	
R1×T		24.12	39.90	46.45	51.99		40.26
R2	C0	0	0	0	0	0	
	C1	16.91	35.96	34.04	37.18	31.02	
	C2	30.08	50.40	60.02	69.79	52.57	
	C3	32.19	60.90	73.31	82.00	62.10	
R2×T		19.79	36.81	41.84	47.24		36.42
R3	C0	0	0	0	0	0	
	C1	16.20	26.24	29.59	32.81	26.21	
	C2	18.60	39.71	53.25	57.97	42.38	
	C3	23.37	44.59	63.40	78.49	52.46	
R3×T		14.54	27.63	36.56	42.31		30.26
T		19.49	34.73	41.62	47.18	average c	
C×T	C0	0	0	0	0	0	
	C1	19.87	34.09	36.31	40.49	32.69	
	C2	27.37	48.78	58.83	66.42	50.35	
	C3	27.76	56.27	71.33	81.81	59.29	
LSD	R×C×T	C×T	R×T	R×C	T	C	R
	0.560	0.323	0.280	0.280	0.161	0.161	0.140
Fourth-instar larvae							
R1	C0	0	0	0	0	0	
	C1	13.57	25.97	30.03	35.94	26.38	
	C2	19.95	43.00	48.97	55.94	41.96	
	C3	23.21	51.15	62.64	66.79	51.02	
R1×T		18.91	40.04	47.31	52.89		39.79
R2	C0	0	0	0	0	0	
	C1	6.86	13.98	22.01	24.89	16.93	
	C2	16.11	29.91	42.02	48.97	34.28	
	C3	20.02	34.74	46.64	63.18	41.15	
R2×T		14.33	26.21	36.93	45.68		30.79
R3	C0	0	0	0	0	0	
	C1	3.13	8.28	17.85	18.56	11.96	
	C2	6.19	12.17	36.12	43.12	24.40	
	C3	11.04	22.32	41.65	52.61	31.91	
R3×T		5.09	10.69	23.90	28.57		17.07
T		12.78	25.65	36.05	42.38	average c	
C×T	C0	0	0	0	0	0	
	C1	7.85	16.07	23.30	26.47	18.42	
	C2	14.09	28.36	42.40	49.34	33.55	
	C3	18.09	36.07	50.41	60.86	41.36	
L.S.D	R×C×T	C×T	R×T	R×C	T	C	R
	0.329	0.164	0.190	0.164	0.095	0.095	0.082

(contd.)

(contd. Table 1)

Adult females							
R1							
	C0	0	0	0	0	0	
	C1	3.09	7.98	12.28	17.37	10.18	
	C2	5.47	16.49	23.25	30.99	19.05	
	C3	8.02	19.16	27.06	39.08	23.33	
R1×T		4.14	10.90	15.64	21.86		13.14
R2	C0	0	0	0	0	0	
	C1	1.91	4.19	8.83	11.89	6.70	
	C2	4.13	11.80	20.13	27.06	15.78	
	C3	6.93	15.89	25.05	37.01	21.22	
R2×T		3.24	7.97	13.50	18.99		10.92
R3	C0	0	0	0	0	0	
	C1	0.59	2.18	5.56	8.19	4.13	
	C2	4.04	4.12	12.87	21.74	10.7	
	C3	10.35	7.11	17.52	27.97	15.73	
R3×T		3.75	3.35	8.98	14.47		7.64
T		3.71	7.41	12.71	18.44	average c	
C×T	C0	0	0	0	0	0	
	C1	1.86	4.78	8.89	12.48	7.00	
	C2	4.55	10.80	18.75	26.59	15.17	
	C3	8.43	14.08	23.21	34.68	20.09	
L.S.D	R×C×T	C×T	R×T	R×C	T	C	R
	0.485	2.485	2.152	2.152	1.243	1.243	1.076
Adult males							
R1	C0	0	0.22	1.17	1.47	0.71	
	C1	3.98	9.92	14.75	21.28	12.48	
	C2	5.87	17.69	26.06	34.92	21.14	
	C3	8.34	23.27	34.04	43.58	27.31	
R1×T		4.55	12.77	19.01	25.31		15.41
R2	C0	0.60	0.36	1.04	1.30	3.74	
	C1	2.12	5.12	10.81	14.99	6.77	
	C2	5.59	13.00	22.95	29.96	15.95	
	C3	7.73	17.72	27.99	41.18	22.50	
R2×T		4.01	9.05	15.70	21.86		12.24
R3	C0	0.24	0.10	0.97	1.19	0.62	
	C1	0.91	4.40	9.71	11.88	6.73	
	C2	2.78	6.02	18.38	26.05	13.31	
	C3	4.96	10.40	23.77	33.01	18.04	
R3×T		2.22	5.23	13.21	18.03		9.67
T		4.86	12.52	20.98	27.82	average c	
C×T	C0	0.28	0.23	1.06	1.32	0.72	
	C1	2.34	6.48	11.76	16.05	9.16	
	C2	4.75	12.23	22.47	30.31	17.44	
	C3	7.01	17.13	28.60	29.25	23.00	
L.S.D	R×C×T	C×T	R×T	R×C	T	C	R
	0.347	0.200	0.173	0.173	0.100	0.100	0.086

R1: First bacterial vaccine (*P. rettgeri*), R2: Second bacterial vaccine (*R. planticola*), R3: Double bacterial vaccine (*R. planticola + P. rettgeri*); C0:first concentration (0), C1: second concentration (10^2), C2: third concentration (10^4), C3: fourth concentration (10^6); T1- 24 hr; T2- 48 hr; T3- 72 hr; T4- 96 hr.

bacterial isolate *P. rettgeri* showed maximum mortality of 52.89% at an exposure period of 96 hr, compared to the double bacterial treatment with an exposure period of 24 hr. The results also showed that the interaction coefficient between concentration and exposure time was better than the comparison treatments if the concentration of 106 at 96 hr (60.86%). There were significant differences in the triple interference coefficients. There are significant differences in the mortality of adult females with treatments. There were also significant differences between the biological resistance factors, with *P. rettgeri* giving maximum mortality rate of 13.14%, while interactions between bacterial isolates recorded the lowest mortality rate of 7.64%. There were significant differences in the mortality rate during exposure periods; maximum was 18.44% after 96 hr of treatment, while it was only 3.71% after 24 hr; also significant differences between disturbance treatments was observed- maximum being with disturbance treatments viz., *P. rettgeri* + 96 hr, *R. planticola* + 96 hr and *R. planticola* and *P. rettgeri* + 96 hr). As regards for the effect of the bilateral interaction between resistance and concentration factors, the results showed that the interaction treatment (*P. rettgeri* + 106) was dominant at 23.33%, while the interaction rate among the contrasting treatments was the lowest at 0%. Effect of triple intervention was found to be significant, as the results showed the superiority of the two triple intervention treatments (*P. rettgeri* + 96 hr + concentration 106) and (*R. rettgeri* + 96 hr + concentration 106 (mortality sequentially reached (39.08, 37.01%). There are significant differences due to treatment on the mortality of (Table 1); mortality at concentration 106 is maximum of 23% as against 23% in control; and it is significantly better than biologically resistant pathogens; as regards exposure time maximum mortality was at 27.82% at 96 hr; interaction between biological resistance factors and exposure time revealed that *P. rettgeri* +96 hr gave mortality of 25.31% while with *R. planticola* and *P. rettgeri* + 24 hr was only 2.22%; while *P. rettgeri* + concentration 106 outperformed all treatments by 27.31%; triple intervention has a significant effect on the treatment i.e., *P. rettgeri* + 96 hr + concentration 106 gave maximum mortality of 43.58%.

Thus, the results showed that the use of this biological agent resulted in the death of second and fourth instar larvae. The bacteria disappear within 3-6 hr causing the larvae to become immobile, dry and unviable within 24 hr; this is approximately one hour, depending on the larval stage (Louis et al., 2020); there was lower mortality in the fourth larval stage. These results corroborate those of Fillinger et al. (2003); late stages have immunity as shown by Jactel et al. (2014). It may be due to bacteria entering insect's body, or because of a wound that allows bacteria to enter (Al-Hasnawi, 2014); and adults have more immunity (Harris, 2006; Ruiu, 2015). The current results show that the killing rate is low immediately after treatment and increases over time after a few days. This is because the bacteria need enough time to reach the stomach and release the toxin. At high doses, midgastric epithelial cells undergo lysis, leading to rapid death, In less sensitive insects, damage to gastric cells at low doses is enough to prevent normal secretion in the stomach, thereby reducing the acidity, allowing bacterial cells to penetrate and multiply in the haemolymph, causing damage to blood cells and death (Hanford, 2020). Dahi et al. (2021) pointed out that midget epithelial cells completely get detached from the basement membrane, severing some epithelial cells and the surrounding nutrient membrane, and causing most of the epithelial cells to shrink.

AUTHOR CONTRIBUTION STATEMENT

AAA, SJJ, MKA, and AAM conceived of the original idea. AAA, SJJ, MKA and AAM developed the theoretical and performed the statistical analysis for experimental data. SJJ, MKA and AAM verified the analytical methods. AAA, MKA and AAM worked on lab analysis and supervises the project. AAA, SJJ, MKA, and AAM discussed the results and contributed to the wrote the manuscript .

CONFLICT OF INTEREST

No conflict of interest

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