FALL ARMYWORM STRAINS (LEPIDOPTERA: NOCTUIDAE) IN ARGENTINA, THEIR ASSOCIATE HOST PLANTS AND RESPONSE TO DIFFERENT MORTALITY FACTORS IN LABORATORY

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ABSTRACT

The aim of this research was to identify the existence of different *Spodoptera frugiperda* (J.E. Smith) (FAW) host strains in Argentina, and to determine their behaviors in the presence of different mortality factors. Populations belonging to these strains were tested with the pesticides chlorpyriphos and cypermethrin, transgenic corn germplasms expressing Cry proteins 1Ab or 1F, and an indigenous *Bacillus thuringiensis* strain. The relation of these strains with the host plant species and crop utilization, subsistence or commercial ones, is discussed. The response to the assayed insecticides, Bt transgenic corn and Bt suspension was diverse, showing wide variability in mortality rates. This research validates the need of intensive studies relating resistance phenomena with the differential behavior of the host strains inhabiting Argentina. Host plant and crop utility is not a determinant for the identity of the colonizing strain, so molecular identification of the strains is highly recommended before study of any aspect of the FAW in Argentina.

Key Words: *Spodoptera frugiperda*, mitochondrial DNA, transgenic corn, *Bacillus thuringiensis*, pesticides

RESUMEN

El objetivo de esta investigación fue establecer la existencia de diferentes líneas o cepas del cogollero del maíz, *Spodoptera frugiperda* (J. E. Smith), en Argentina, y determinar su comportamiento frente a diversos factores de mortalidad. Poblaciones correspondientes a diferentes cepas fueron testadas con los insecticidas clorpirifos y cipermetrina, maíces transgénicos que expresan las proteínas Cry 1Ab y 1F, y una cepa nativa de *Bacillus thuringiensis*. Se discute la relación entre las cepas, las plantas desde donde fueron colectadas y el objetivo agrícola del cultivo, subsistencia o comercial. La respuesta a los insecticidas, maíces Bt transgénicos y a la suspensión de Bt fue diversa, mostrando una amplia variabilidad en las tasas de mortalidad. Esta investigación exhorta la necesidad de intensificar estudios relacionados a los fenómenos de resistencia, y a determinar el comportamiento diferencial de las cepas del cogollero que afectan cultivos en Argentina. La planta hospedadora y el tipo de cultivo no es determinante de la identidad de la cepa que lo coloniza, por lo que es altamente recomendable realizar la identificación de las mismas mediante técnicas moleculares antes de estudiar cualquier aspecto relacionado a esta especie plaga en Argentina.

Translation provided by the authors.

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a polyphagous pest that causes important damage in different regions of America (Sparks 1979). The significance of this pest in Argentina was discussed by Virla et al. (1999).

There are at least 2 morphologically identical host strains of FAW. The rice strain is associated with rice and bermudagrass, while the corn strain predominates on corn, sorghum, and cotton, although this host specificity is not completely exclusive (Pashley 1986; McMichael & Prowell 1999). Additional behavioral and physiological distinctions between these strains have been reported, including differences in pesticide resistance, susceptibility to transgenic plants, and nutritional adaptation (Pashley 1988a, b; Pashley et al. 1995; Veenstra et al. 1995; Adamczyk et al. 1997).

The rice and corn strains can be distinguished by several restriction fragment-length polymorphisms in mitochondrial DNA (mtDNA). Lu & Adang (1996) reported the *Msp*I mtDNA restriction pattern as a diagnostic marker for corn and rice strains. Later, a polymorphic *Msp*I restriction site located in the mitochondrial cytochrome oxidase subunit I (COI) gene was used for strain identity (Levy et al. 2002).

For many years, control of FAW has been exclusively dependent on insecticides; as a result, this pest has developed resistance to major classes of insecticides in many areas (Yu et al. 2003). Resistance of FAW to various carbamate,

organophophate, and pyrethroid insecticides were observed in field strains collected from corn in north, central, and south Florida (Yu 1991, 1992). In Argentina, the organophosphate chlorpyriphos has been extensively used. Berta et al. (2000) demonstrated that the application of this pesticide did not reduce FAW populations but drastically diminished the establishment of the parasitoid *Campoletis grioti* (Blanchard) (Hymenoptera, Ichneumonidae).

Genetically modified or transgenic maize (*Zea mays* L.) may improve agricultural productivity in controlling FAW in Argentina (Frizzas 2003). For example, MON810 (Monsanto Company, St. Louis, MO) expressing *Bacillus thuringiensis* var. *kurstaki* (Bt) Cry1Ab endotoxins, provided high dose and season-long control of another lepidopteran pest of maize, the European corn borer *Ostrinia nubilalis* (Hübner) (Koziel et al. 1993; Armstrong et al. 1995). Another viable alternative for insect control in agriculture is the use of entomopathogens. Among these, Bt is the most widely employed. The use of this microorganism is compatible with sustainable and environmentally friendly agricultural practices.

The purpose of this work was to identify FAW host strains in Argentinean territory and to determine their behaviors in the presence of pesticides, transgenic corn plants or Bt water suspension.

Material and Methods

Origin and Maintenance of Fall Armyworm Colonies

Larvae of FAW were collected as 3rd to 6th instars from different localities in northern Argentina (Table 1). Larvae were manually collected from the whorl of the plants and placed individually in glass tubes (12-cm length × 1.5-cm diameter) with host leaves and carried to the laboratory.

Adults were maintained in polyethyleneterephthalate cylindrical cages (30-cm high × 10-cm diameter). For aeration, the top was closed with a nylon mesh cloth. These cages contained pieces of paper that allowed the females to oviposit. Food was provided via a cotton plug saturated with a mixture of honey and water (1:1 vol/vol). The cages were checked daily for oviposition and adult mortality; egg masses were collected and deposited in glass tubes as mentioned above.

Table 1. Origin of FAW populations.

FAW	Locality (Province)	Altitude (m)	Host plant (purpose)	Date of collection
1	La Morocha (La Rioja) S 29°35'03.9–W 66°49'46.9)	914	Corn (Subsistence)	11/XI/05
3	Campo Largo (Chaco) S 26°46'57.3–W 60°49'17.6	110	Corn (Commercial)	02/I/06
4	Gral. Capdevila (Chaco) S 27°25'39.8–W 61°29'50.4	115	Sorghum (Subsistence)	02/I/06
5	Quimilí (S. del Estero) S 27°38'39.0–W 62°21'18.1	144	Sorghum (Subsistence)	02/I/06
6	La Virginia (Tucumán) S 26°44'43.3–W 64°47'41.6	397	Sorghum (Commercial)	19/XII/05
7	San José (Catamarca) S 26°48'40.6–W 66°04'30.4	1978	Corn (Subsistence)	18/XII/05
8	Purmamarca (Jujuy) S 23°44'47.8–W 65°28'50.5	2275	Corn (Subsistence)	12/I/06
9	Guemes (Salta) S 24°47'23.2–W 65°02'10.8	790	Corn (Commercial)	12/I/06
10	Rosario de la Frontera (Salta) S 25°58'25.9–W 65°05'08.6	965	Corn (Commercial)	12/I/06
11	El Talar (Jujuy) S 23°42'57.8–W 64°31'49.6	365	Corn (Subsistence)	12/I/06
12	Metán (Salta) S 25°38'31.5–W 64°56'50.8	807	Corn (Commercial)	12/I/06
14	Barcena (Jujuy) S 23°59'10.5–W 65°27'15.3	1856	Corn (Subsistence)	12/I/06
16	Vicuña Makena (Córdoba) S 33°55'51.2–W 64°21'12.1	224	Corn (Commercial)	31/I/06
17	Castelli (Chaco) S 25°57'19.4–W 60°35'49.7	110	Corn (Commercial)	12/III/06
18	El Manantial (Tucumán) S 26°49'50.2–W 65°16'59.4	445	Corn (Subsistence)	12/III/06

Once emerged, the neonate larvae were placed in 250-cc plastic pots covered with nylon mesh cloth until they reached the 3rd instar. These older larvae were isolated in glass tubes to prevent cannibalism.

All cultures were maintained separately in the laboratory on an artificial diet (Osores et al. 1982), in rooms at 25 ± 0.5°C, 14:10 (L:D) artificial photoperiod, and 70 ± 15% RH. Data were recorded by a HOBO® data logger every hour. All biological assays were done with individuals of the second laboratory generation and under the same conditions used for FAW population maintenance. Mortality was calculated for both treatments and controls. Larvae that could not crawl after being touching with a brush were considered dead.

DNA Preparation and PCR Analysis

Total DNA was isolated from individual FAW larvae with a commercial kit (GE Healthcare genomic prep™ Cells and Tissue DNA). All genomic DNAs used in this study were tested by PCR for the mitochondrial COI gene restriction fragment-length polymorphism to confirm strain identity. PCR amplification of genomic DNA was performed in a 25-µL reaction mix containing 2.5 µL 10 × STR reaction buffer (Promega), 20 ng total DNA, 20 pmol L^{-1} of JM76 and JM77 primers and 2 units of Taq DNA polymerase (Promega). Amplification was performed on a DNA thermocycler (Perkin-Elmer) with the following program: an initial incubation at 94°C (5 min), followed by 35 cycles of 94°C (1 min), 58°C (1 min), 72°C (2 min), and a final segment of 72°C for 7 min. Samples of 8-10 µL were electrophoresed in 7% PAGE gel, followed by ethidium bromide staining and photography under UV light. The PCR amplified DNA products (0.5 µL) were digested in separated reactions with either SacI or MspI restriction endonuclease. Samples were incubated at 37°C for 1 h. The restriction enzyme profiles were also visualized with ethidium bromide on an 8.0% PAGE gel.

Primers were synthesized by Tecnolab S.A. They included JM 76 (5'-GAGCTGAATTAGG(G/A)ACTCCAGG-3') and JM 77 (5'-ATCACCTCC(A/T)CCTGCAGGATC-3') (Levy et al. 2002).

Bioassays with Pesticides

Two insecticides that are widely used in corn production, chlorpyriphos (3.33 cc/L H_2O) and cypermethrin (0.53 cc/L H_2O), were tested. Both insecticides were obtained commercially. Single corn leaves from a non-transgenic hybrid known as "precomercial 22" were submerged in insecticide solutions for 15-20 s and then were left to dry for 2 h before the bioassay was started. Single control leaves were placed in distilled water.

For both insecticides and the control, 3rd instars (Stadler 1996) were held in separate glass tubes (10 cm length × 1 cm diameter) and were supplied with treated pieces of leaf (about 2 cm^2 each). Cotton plugs closed the vials. Each experiment was replicated 6 times with 10 larvae (total n = 60). Mortality data were collected after 6, 24, 30, 48, 54, 72, and 78 h after exposure.

Bioassays with Transgenic Corn Plants

Two newly developed transgenic corn germplasms, NK 120 TDmax® (Syngenta Seeds) expressing the Cry 1Ab protein, and Herculex® I (Dow AgroSciences) expressing the Cry 1F protein, were tested. The former germplasm reportedly provides partial control of FAW, while the latter germplasm reportedly provides complete protection against FAW. The non-transgenic hybrid "precomercial 22" was used as a control.

For each germplasm, 6 replicates of 10 newly-emerged 1st instars were held in separate 2.0-mL Eppendorf vials. Each larva was supply with a piece of leaf (about 2 cm^2) that was replaced when necessary. Mortality data were collected after 6, 24, 30, 48, 54, 72, and 78 h after exposure.

Bioassays with *Bacillus thuringiensis* (Bt)

A native *B. thuringiensis* strain RT from our own culture collection was used throughout this study. The partial nucleotide sequence of the 16S rRNA from Bt RT was deposited in Genebank database under accession number EF638795. Bacteria were grown in LB-agar. The presence of crystal proteins was checked with Coomasie blue reagent (Sharif & Alaeddinoğlu 1988).

Six replicates of 10 newly-emerged 1st instars were held in separate 2.0-mL Eppendorf vials. Each larva was supply with a piece of artificial diet (approximately 0.25 cm^2) immersed in a suspension of Bt in water (DO_{600} = 1.7; 8.9×10^6 CFU). Controls were maintained with a similar piece of artificial diet but immersed in distilled water. Mortality data were collected 6, 24, 30, 48, 54, 72, 78, 92, 98, 116 h after exposure.

Data Analysis

Mean survivorship was analyzed by analysis of variance (ANOVA), and means were compared with the Tukey test ($P \leq 0.05$). Lethal times for 50% of the population (LT_{50}) were computed by Probit analysis (Minitab 2004). Mean survival time data were organized into a 1-0 matrix and the similarity degree among the FAW populations was estimated with the simple matching coefficient. Clusters were then constructed by the unweighted pair group method with arithmetic average (UPGMA) algorithm with the NTSYS program (Rohlf 1998).

Results and Discussion

The existence of 2 FAW host strains is well established in the USA (Pashley 1986, 1988a, b), and Brazil (Busato et al. 2004), and its possible presence was mentioned in Mexico (Lopez-Edwards et al. 1999). No report has been given for the presence of these host-strains or their behaviors in Argentina.

Strain specificity of FAW samples from several locations in Argentina was determined by the presence of diagnostic mitochondrial markers recently reported by Nagoshi et al. (2006). In that work, the fragment produced by PCR amplification with the JM-76/JM-77 primers was separately digested with SacI and MspI restriction enzymes. The amplified product from the rice strain was cut once by SacI but not by MspI, whereas the corn strain DNA showed a reciprocal pattern. Thus, according to these molecular markers, populations of 4 rice strains and 11 corn strains were found in Argentina (Figs. 1 and 2). The results also showed that a small fraction of rice strain individuals readily use corn as a host. This overlap in host usage also was established by Pashley (1989) and McMichael & Prowell (1999).

An important consideration for the management of this pest is that differences were found in the response of the rice and corn strains to different mortality factors such as insecticides and/or pathogens (Nagoshi & Meagher 2004). The degree of similarity among the FAW populations was studied based on their mean survival time obtained in the presence of pesticides, transgenic corn plants, or Bt water suspensions. As shown in the dendrogram (Fig. 2), the numerical analysis clearly revealed 2 major clusters at a similarity level of 54%. Cluster A comprised seven FAW populations, all of these were identified as corn strain,

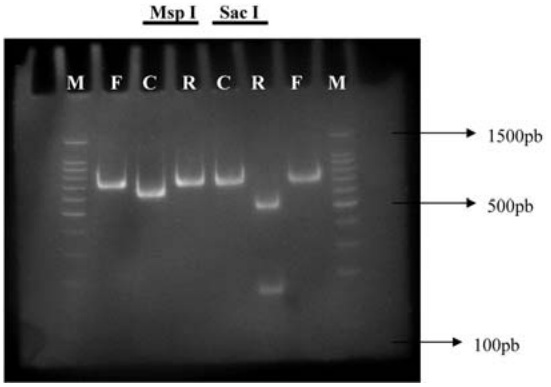

Fig. 1. PAGE gel displaying PCR-amplified fragment produced by the JM-76/JM-77 primers (F) and cut with the specified restriction enzymes. Respectively, M, C, and R denote molecular marker, corn strain, and rice strain.

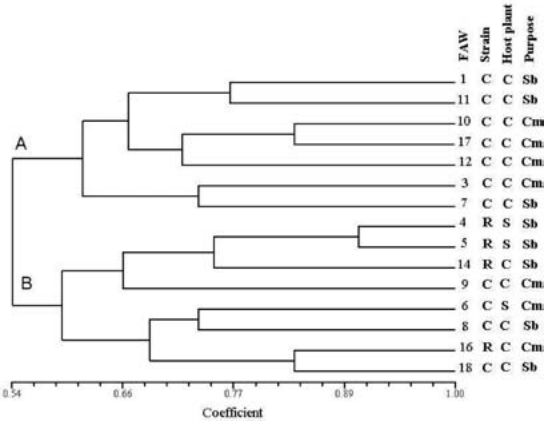

Fig. 2. Dendrogram showing clustering (groups A and B), and relationships of FAW populations based on their survival time obtained in the presence of insecticides, transgenic corns or Bt RT water suspension. Associations were produced by using UPGMA clustering method. Strain: corn (C), rice (R). Host plant: corn (C) and sorghum (S). Purpose: subsistence (SB), commercial (CM). for FAW population references see Table 1.

while cluster B included both rice (4) and corn (4) FAW strains. In addition, rice strain 16 showed a level of similarity of 59% with the other rice strain populations (4, 5, and 14), which displayed a higher level of similarity between them (75%). Interestingly, rice strain 16 was collected from a completely different geographic location (Table 1). Rice strain populations 4 and 5 shared the same host usage and had a similarity level of 90%.

Five of the 8 FAW populations collected from subsistence crops were found in group B (Fig. 2). Only 1 of the 11 corn strain populations was from sorghum, whereas 2 of the 4 rice strain populations were collected from corn.

Pesticides are a critical component of insect pest management in corn production. In general, the application of chlorpyriphos or cypermethrin resulted in more rapid death of FAW populations than the use of transgenic corn or Bt water suspension; with the LT_{50} values ranged from 3.01 to 20.7 h (Table 2). FAW population 12 displayed a completely different result. After 78 h in the assay, 50% mortality with LT_{50} of 76.34 h was obtained. This mortality level was the same as that found in the presence of Bt water suspension (Table 4). However, assays conducting with corn plants expressing either the Cry 1F or Cry 1Ab gene resulted in 86.67 and 85.00% mortality, respectively (Table 3).

Since the first genetically engineered corn was commercialized, there have been numerous advancements for insect control with transgenic technology. In this work, the impact of transgenic corn expressing Cry 1F or Cry 1Ab against FAW

TABLE 2. MEAN SURVIVAL TIME*, LETHAL TIME (LT_{50}), AND MORTALITY OF FAW POPULATIONS EXPOSED TO PESTICIDES (CHL: CHLORPYRIPHOS; CYP: CYPERMETHRIN; CONTROL: DISTILLATED WATER).

		Mean survival time (h)			LT_{50} (h)		Mortality (%)		
FAW	Strain	CHL	CYP	Control	CHL	CYP	CHL	CYP	Control
1	C	2.95 ab	6.10 abc	74.40 b	6.63	5.53	100.00	95.00	5.0
3	C	4.73 bc	16.35 d	74.30 b	7.99	20.70	100.00	100.00	6.7
4	R	1.42 ab	3.15 ab	74.70 b	4.80	6.50	100.00	100.00	5.0
5	R	1.33 ab	2.05 ab	75.70 b	6.63	4.49	100.00	100.00	8.3
6	C	1.33 ab	1.80 ab	76.60 b	4.72	4.12	100.00	100.00	5.0
7	C	1.63 ab	11.40 bcd	76.20 b	3.89	12.32	100.00	95.00	5.0
8	C	1.75 ab	4.47 abc	74.40 b	5.05	8.64	100.00	100.00	5.0
9	C	7.07 c	10.47 abcd	72.60 ab	12.86	14.06	100.00	95.00	11.7
10	C	4.58 ab	13.47 cd	65.00 a	4.94	14.58	100.00	93.33	21.7
11	C	7.15 c	8.67 abcd	76.60 b	11.60	12.41	98.33	98.33	5.0
12	C	1.50 ab	55.17 e	75.20 b	4.87	76.34	100.00	50.00	5.0
14	R	2.42 ab	10.12 abcd	76.60 b	5.44	13.57	100.00	100.00	5.0
16	R	2.13 ab	3.13 ab	76.10 b	4.62	4.61	100.00	100.00	6.7
17	C	1.92 ab	13.90 cd	76.70 b	5.16	15.15	100.00	90.00	3.3
18	C	1.00 a	1.00 a	76.80 b	3.10	3.01	100.00	100.00	3.3

*To facilitate the comparison, we arbitrarily assign 1 h survival for those larvae dead before the first observation (6 h). In each column, means followed by different letters are significantly different ($P \leq 0.05$) (ANOVA; Tukey analysis).

populations range from slight to highly toxic. However, some considerations must be made. First, as expected, the Cry 1F transgene provided higher mortality than corn producing Cry 1Ab (83.5 ± 6.2% vs. 68.8 ± 6.8%, respectively). As shown in Table 3 and during the 78-h assays, 73% of the FAW populations exposed to corn with Cry1F resulted in mortality values higher than 75%, while this percentage was achieved by only a 40% of the colonies tested with corn expressing Cry 1Ab protein. Luo et al. (1999) also reported high toxicity of Cry 1F protein against FAW larvae. Second, transgenic corn displayed differential mortality with respect to the rice strain populations. Rice strain populations 4 and 5 from subsistence sorghum sustained markedly less mortality than all other populations. It is not known why these populations were more resistant than the others.

TABLE 3. MEAN SURVIVAL TIME, LETHAL TIME (LT_{50}), AND MORTALITY OF FAW POPULATIONS FEEDING ON EITHER GENETICALLY MODIFIED OR NON-TRANSGENIC CORNS.

		Mean survival time (h)			LT_{50} (h)		Mortality (%)		
FAW	Strain	Cry 1F	Cry 1Ab	Control	Cry 1F	Cry 1Ab	Cry 1F	Cry 1Ab	Control
1	C	55.00 defg	61.40 efgh	76.20 bc	62.70	75.28	85.00	53.33	16.67
3	C	45.50 cd	57.60 cdef	76.70 bc	52.26	67.06	98.33	70.00	6.67
4	R	72.10 h	74.20 i	73.70 bc	120.16	97.70	21.67	20.00	21.67
5	R	62.80 gh	68.90 hi	73.40 bc	92.64	206.26	36.67	20.00	8.33
6	C	41.80 c	45.70 b	74.10 bc	48.32	53.09	100.00	90.00	16.67
7	C	46.10 cde	58.00 defg	75.20 bc	53.09	66.54	96.67	71.67	13.33
8	C	48.40 cdef	56.40 bcde	75.50 bc	55.77	64.57	93.33	81.67	8.33
9	C	28.10 b	46.90 bc	76.80 bc	34.91	54.53	100.00	96.67	1.67
10	C	56.50 fg	59.20 defgh	74.90 bc	24.90	72.87	66.67	56.67	18.33
11	C	53.60 defg	68.40 fghi	78.00 c	58.47	90.90	88.33	45.00	0.00
12	C	55.50 efg	58.60 defgh	76.80 bc	62.37	66.31	86.67	85.00	10.00
14	R	15.50 a	28.10 a	63.90 a	24.27	36.72	100.00	93.33	30.00
16	R	55.30 efg	54.20 bcde	73.40 bc	62.52	61.20	93.33	98.33	16.67
17	C	51.90 def	62.80 efgh	78.00 c	59.43	73.31	98.33	55.00	0.00
18	C	53.10 defg	50.30 bcd	70.40 ab	59.44	54.51	88.33	95.00	28.33

In each column, means followed by different letters are significantly different ($P \leq 0.05$) (ANOVA; Tukey analysis).

TABLE 4. MEAN SURVIVAL TIME, LETHAL TIME (LT_{50}), AND MORTALITY OF FAW POPULATIONS EXPOSED TO EITHER *Bt*-TREATED OR UNTREATED ARTIFICIAL DIET.

FAW	Strain	Mean survival time (h)		LT_{50} (h)	Mortality (%)	
		Bt RT	Control	*Bt* RT	*Bt* RT	Control
1	C	99.50 cd	114.60 c	149.27	26.67	1.7
3	C	94.80 cd	114.90 c	124.52	38.33	3.3
4	R	106.80 d	113.30 bc	253.79	10.00	3.3
5	R	91.80 cd	108.40 bc	191.44	26.00	8.3
6	C	67.10 ab	102.20 ab	78.35	71.67	25.0
7	C	89.10 bcd	116.00 c	121.24	35.00	0.0
8	C	56.60 a	115.00 c	64.33	75.00	1.7
9	C	93.90 cd	112.60 bc	136.23	38.33	8.3
10	C	66.90 ab	110.90 bc	77.21	65.00	11.7
11	C	83.90 bcd	110.10 bc	110.08	53.33	10.0
12	C	86.30 bcd	111.90 bc	107.14	50.00	8.3
14	R	97.70 cd	113.50 bc	171.02	21.67	5.0
16	R	88.50 bcd	116.00 c	125.32	33.33	0.0
17	C	56.90 a	115.60 c	65.43	91.67	1.7
18	C	82.00 bc	114.60 c	104.52	45.00	1.7

In each column, means followed by different letters are significantly different ($P \leq 0.05$) (ANOVA; Tukey analysis).

The use of Bt products as an alternative to chemical insecticides has encouraged many research centers to focus their efforts in the isolation of native strains that provide mortality to FAW. As shown in Table 4, although the assays were conduced with artificial diet, the native strain Bt RT was in general active against FAW populations. Particularly interesting was the response of rice strain colonies to this Bt water suspension; all of these populations presented low mortality values with LT_{50} values ranging from 125.32 to 253.79 h. Therefore, strain identity must be taken into consideration when evaluating the effectiveness of new biological agents.

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