International Journal of Agriculture and Biosciences 2025 14(2): 244-251.
https://doi.org/10.47278/journal.ijab/2024.215

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Characterization of the Entomopathogenic Bacteria of the Fall Armyworm (*Spodoptera frugiperda* J.E. Smith,) in Western Burkina Faso

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ABSTRACT

Utilizing entomopathogenic microorganisms as a means of management is a compelling technique to control *Spodoptera frugiperda*. This polyphagous insect poses a significant threat to maize growing given the limits of chemical pesticides control. Surveys were carried out in maize fields to collect dead larvae, live larvae and chrysalids of *Spodotera frugiperda*. Dead larvae were utilized to isolate bacteria, whereas live larvae were cultivated in order to carry out pathogenicity assessments of the isolated bacteria. The pathogenicity tests involved introducing the bacterial strains into the larvae's diet, which consisted of maize leaves, to observe and evaluate the larvae's growth and developmental stages across different phases. A total of 240 live and 10 dead larvae were collected from maize fields, and 259 strains were isolated. Comparative analysis of the 24 representative strains, 16 gram-positive and 8 gram-negative, revealed that gram-positive strain 5 and gram-negative strain 20 induced 100% and 56% mortality, respectively, in the larvicidal test. Gram-positive strains 1, 4, 5, 6, 11, 13, and 15 and gram-negative strains 18, 19, 23, and 24 completely inhibited the laying of eggs. In addition, gram-positive strains 5 and 11 produced 100% and 90% rate of organic essences that are out of harm's way insect limits. For gram-negative strains 18 and 20, these rates were 80% and 88% respectively. Thus, gram-positive strains 5, 11, and 20 and gram-negative strains 18 and 20 can be considered entomopathogenic bacteria of *Spodoptera frugiperda*. In addition, it is important to carry out molecular identification of these strains to facilitate testing under real-life conditions.

Keywords: Entomopathogenic bacteria, *Spodoptera frugiperda*, Pathogenicity, Characterization, Biological control, Burkina Faso

Article History
Article # 24-740
Received: 03-Aug-24
Revised: 21-Aug-24
Accepted: 19-Sep-24
Online First: 01-Jan-25

INTRODUCTION

Spodoptera frugiperda (J.E. Smith, 1797) is an polyphagous insect that originated to tropical and subtropical regions of the Americas (Ayala et al., 2013; Sharanabasappa et al., 2018; Yainna et al., 2022; Aleem et al., 2023; Mukanga et al., 2024). The presence of *S. frugiperda* was detected and confirmed in Burkina Faso during the agropastoral campaign of 2017/2018 (DPVC, 2018). The phytosanitary survey revealed that all of the areas infected by this pest in the 2019 rainy season were found in cereal fields, with maize fields alone representing 89.9% of the total infected areas (MAAH, 2020; Yaméogo et al., 2024). Due to the significant damage and economic losses caused by *S. frugiperda*, as well as the importance of cereals, particularly maize, in ensuring food and nutritional security for the population, the use of synthetic pesticides for chemical control against this pest has been extensively employed. In Burkina Faso, aside from the intentional use of pesticides by producers, a total of 14,000 liters and 220

Cite this Article as: Zerbo KBF, Yameogo F, Damiba P, Kafando A, Sawadogo MW, Somda I and Wonni I, 2025. Characterization of the entomopathogenic bacteria of the fall armyworm (*Spodoptera frugiperda* J.E. Smith,) in western Burkina Faso. International Journal of Agriculture and Biosciences 14(2): 244-251. https://doi.org/10.47278/journal.ijab/2024.215

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kg of insecticides were provided to farmers during the 2017/2018 agricultural season to control this pest (DPVC, 2018). Despite the application of these insecticides, the pest persists at a notable level of harm, which includes the adverse impacts of these pesticides on the environment, human health, and non-target organisms such as natural predators (Barzman et al., 2015; Son, 2018; Gutiérrez-Moreno et al., 2019). Given these discoveries, it is imperative to seek more efficient options within the framework of sustainable development. Therefore, the increasing utilization of biological control is evident. In recent decades, there has been a significant increase in the utilization of natural enemies such as predators, parasitoids and pathogens to control pests (Kenis et al., 2019; Sree & Varma, 2015). Various entomopathogenic microorganisms have been used to control *S. frugiperda*. The entomopathogenic fungi, include *Beauveria bassiana*, *Metarhizium anisopliae* and *Nomuraea rileyi* (Thomazoni et al., 2014; Khan & Ahmad, 2015; Rivero-Borja et al., 2018), as well as the entomopathogenic bacteria *Bacillus thuringiensis* (Polanczyk et al., 2000). The entomopathogenic nematode species of the Steinernema and Heterorhabditisgenera have been used in conjunction with specific insecticides to control *S. frugiperda* (Negrisoli et al., 2010). The aim of this study is to contribute to the efficient management of *Spodoptera frugiperda* in an agroecological context. Specifically, the aim is to isolate bacteria from *Spodoptera frugierda* larvae and assess their pathogenicity on the insect. This will make it possible to reduce the use of synthetic chemical pesticides.

MATERIALS & METHODS

Collecting Sites

The survey and collection of live and dead larvae and chrysalids (pupae) of *S. frugiperda* were carried out in the Hauts-Bassins and Cascades regions. The localities surveyed include Bama and Kodeni in Hauts-Bassins region, Toussiana, Bérégadougou, Tingrela and Banfora in the Cascades region (Fig. 1). These areas are located in the agro-climatic zone of South Sudan, receiving an annual rainfall ranging from 900 to 1200mm. The study utilized a total of eighteen (18) fields. Each prospecting patch had a minimum area of one hectare and had not undergone any chemical treatment.

Collecting Larvae

The scale model promoted by (FAO, 2018) (Fig. 2) was used as the method for surveying and collecting *S. frugiperda* organic essences (dead larvae, live larvae and chrysalids) in the fields. The field diagnostics consisted in making observations on the actual presence of *S. frugiperda* larvae on maize plants.

The organic essences (larvae andchrysalids) collected in the fields were stored in 1.5mL Eppendorf tubes. Live larvae from each individual plot were placed in tubes containing a few maize leaves before being transported to the insectarium for breeding. The dead larvae and chrysalids were transported directly in the laboratory for isolation Also, all larvae that died during rearing were transported to the laboratory for isolation.

Site Infestation Rate

The infestation rate was evaluated for each plot (Tip) using the following formula:

$$\textbf{Tip} = \frac{\sum \text{of infested plants per plot}}{\text{Total number of plants}} \text{ X } 100$$

The overall infection rate of the fields (Tigs) under study was determined by calculating the sum of the infestation rates according to the following formula:

$$\textbf{Tigs} = \frac{\sum \text{Tip of site}}{\text{Number of plots prospected}} \text{ X } 100$$

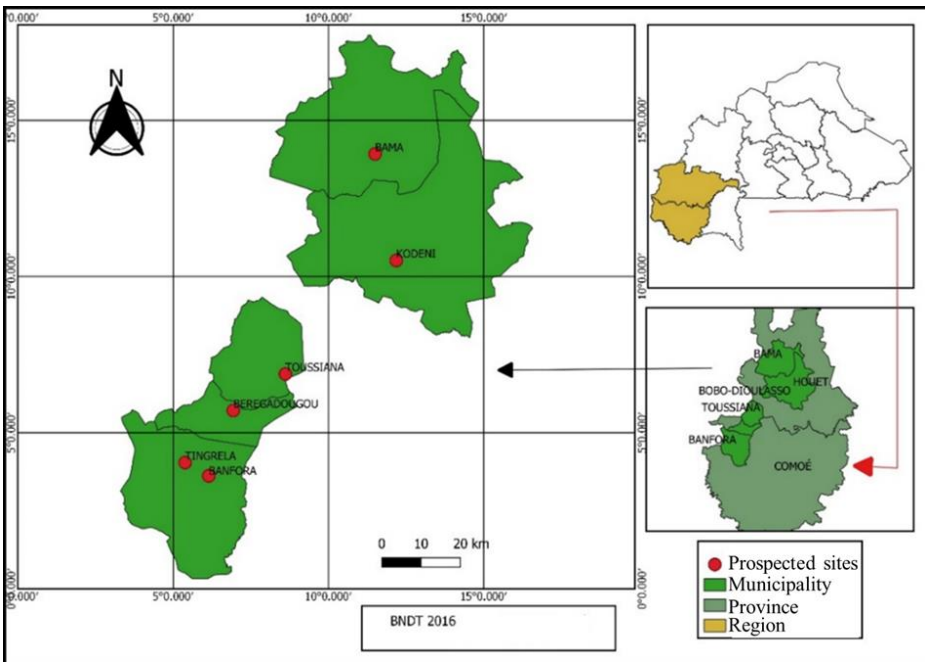

Fig. 1: Sites for the prospecting and collection of larvae and chrysalids of *S. frugiperda*

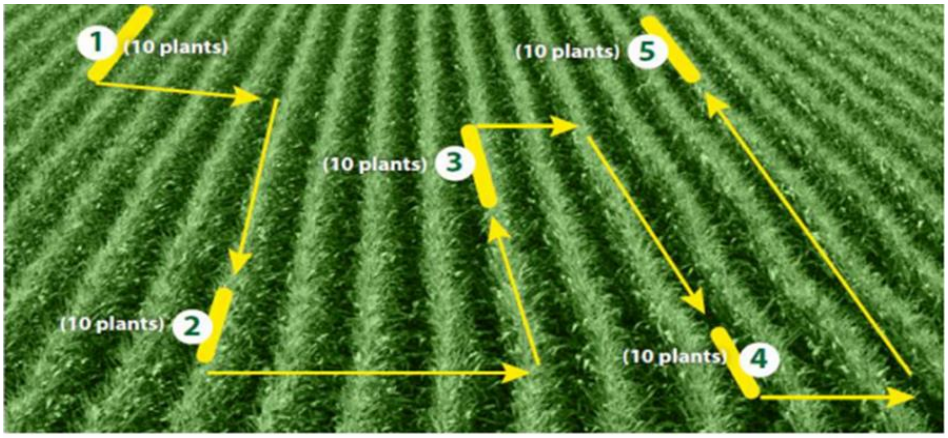

Fig. 2: Prospection and collection model for *S. frugiperda* larvae and chrysalides. **Legend:** 1, 2, 3, 4 and 5 represent the collection points within a plot

Experimental Sites

The experimental activities were carried out in two laboratories. This is the Phytopathology/Bacteriology Laboratory of the National Centre for Specialization in Fruits and Vegetables (CNS-FL) of the Institute of Environment and Agricultural Research (INERA) at the Farako-Bâ isolation station. The Laboratory of Natural Systems, Agro-Systems and Environmental Engineering (SyNAIE), section of the Plant Clinic of Nazi BONI University (UNB), served for the breeding of *S. frugiperda* and the pathogenicity testing of bacterial strains on larvae.

Isolation through Dead Larvae and Chrysalids

Isolations were carried out on dead larvae and chrysalids that had not completed their biological cycle. The samples were rinsed in sterile distilled water, crushede in BIOREBA brand paper and suspended in 1mL of sodium chloride solution (NaOH = 90%). The suspension was left at room temperature for at least 30 minutes and shaken periodically to promote bacterial diffusion (Zerbo et al., 2024).

The dilution method was used. Two dilutions (10^{-2} and 10^{-3}) were prepared based on the initial suspension (10^{-1}). Twenty (20) µL of the suspension was spread over pre-solidified Nutrient Agar (NA) media plates (for 1000mL of distilled water at pH=7.2 we used Beef extract 7g, peptone 7g glucose, 7g and Agar 18g) to which are added antibiotics including kasugamycin (20mg/L), cephalexin (40mg/L) and propiconazole (fungicide at 40mg/L). (Zerbo et al., 2024). Petri dishes are incubated for 48 to 72hours between 25 and 28°C.

Bacterial colonies obtained after incubation are subject to successive purification until a pure colony is obtaining.

Identification of Isolated Strains

The identification of pure bacterial colonies was made on the basis of the morphological characteristics on the Petri dishes and biochemical through the gram test.

- ***Morphological identification***

The morphological characterization of the isolates was based specifically on the observation of pure bacterial colonies. The colony morphology of the isolates was studied under a light microscope (Rohomania et al., 2015). This included shape, diameter, color, opacity, elevation, surface, consistency and smell. All these morphological aspects and strains originate localities are used to group the strains that have the same characteristics.

- ***Biochemical identification: gram test***

The biochemical gram staining test was then carried out on bacterial colonies to classify them according to their belonging to the gram-positive or gram-negative. To do this, a 3% KOH solution was prepared and the viscosity of the colony was assessed using a toothpick. The formation of a sticky and thread-like appearance within 45s when the toothpick lifted indicates that the test bacterium is gram-negative and the formation of an insoluble white precipitate indicates that it is a gram-positive strain. (Dimri et al., 2020; Hossain et al., 2021).

Breeding of Larvae of *S. frugiperda*

Living larvae from prospections are placed individually in Petri dishes containing moistened blotting paper. The photoperiod of the breeding laboratory has been set to 12: 12 h (light: dark), at a temperature of 28±2°C and 60±15 % relative humidity, which are ideal conditions for thegrowth and development of *S. frugiperda*. The different stages of biological cycle of *S. frugiperda* is represented by Fig. 3 (Cokola & Tech, 2019). The larvae were fed with tender leaves of variety KEJ of maize due to its high infestation by *S. frugiperda* (Yaméogo et al., 2023). The chrysalids from larvae metamorphosis were placed in a cage (60x40x40cm) and kept until the emergence of adults (butterflies). The emerging butterflies were sexed and coupled into Petri dishes covered with a 120 x 2.3mm white fabric with small nets allowing the necessary ventilation for the butterfly's breathing. A 5% honey water prepared from honey and sterile distilled water is used for feeding butterflies. The eggs laid after coupling the adults were harvested and incubated.

Hatching occurred within 48 to 72 hours post-incubation. The larvicide assay was performed using healthy L1 stage larvae and the identified bacterial strains. Cattle monitoring was conducted daily, with observations recorded at 24-hour intervals.

Evaluation of Pathogenicity of Isolated Strains

The larvicidal effect of bacterial strains was assessed by feeding larvae with tender maize leaves previously soaked in a bacterial inoculum with titers of 10^8 and 10^{12} CFU/mL (colony forming unit) forgram-positive and gram-negative, respectively. Each titrated inoculum contained 10 drops of

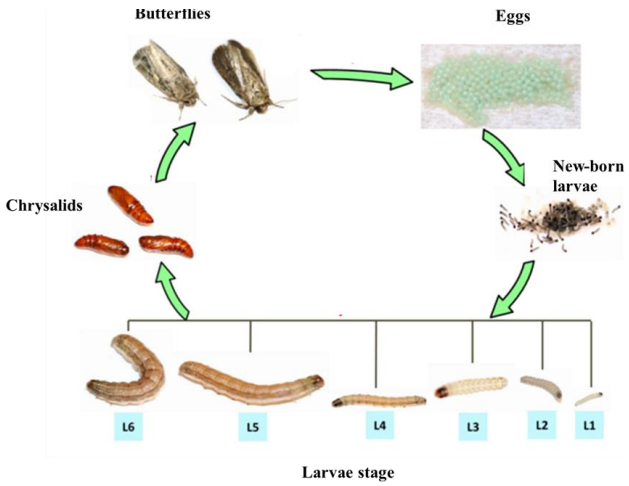

Fig. 3: Biological cycle of *Spodoptera frugiperda*

tween 20 and one drop of triton. The test was conducted on 25 larvae, with one larva per Petri dish. As for the negative control, it consisted of sterile distilled water (EDS) + 10 drops of tween 20 and one drop of triton. After inoculations, the Petri dishes were placed at a temperature of 28°C and observed daily. The surviving larvae after the larvicide test were followed through the over stages, namely the chrysalis and adult stages, as well as through ovular oviposition. This allowed for obtaining information on the fate of the insect after inoculation, provided that it did not die immediately after the larvicidal test. The experimental design is a completely randomized block.

The degree of pathogenicity of the *S. frugiperda* bacterial strains tested was assessed on the following parameters.

- Average lifespan of larvae in hours (DVLH)

$$\textbf{DVLH} = \frac{\sum \text{lifespan of larvae surviving treatment}}{\text{Number of larvae surviving treatment}}$$

- Average lifespan of chrysalids in hours (DVCH)

$$\textbf{DVCH} = \frac{\sum \text{of the lifespan of chrysalises from treated larvae that have emerged as butterflies}}{\text{Number of chrysalids resulting by larvae}} \text{ X } 100$$

- Average life of butterflies in hours (DVPH)

$$\textbf{DVPH} = \frac{\sum \text{of the lifespan of butterflies from treated chrysalids that have emerged as butterlies}}{\text{Number of butterflies resulting from treatment}} \text{ X } 100$$

- Larval Mortality Rate (TML) of a Treatment

$$\textbf{TML} = \frac{\text{Number of dead larvae}}{25}$$

- Rate of unemerged chrysalids (TCNE)

$$\textbf{TCNE} = \frac{\text{Number of unemerged chrysalises}}{\text{Number of chrysalids}} \text{ X } 100$$

- Rate of malformed butterflies (TPM)

$$\textbf{TPM} = \frac{\text{Number of malformed butterflies}}{\text{Number of butterflies}} \text{ X } 100$$

- Number of eggs laid (NOP)

$$\textbf{NOP} = \frac{\sum \text{of eggs laid by pairs from the treatment}}{\text{Number of pairs}}$$

- Rate of organic essences that are out of harm's way insect limits (TEBHN)

$$\textbf{NOP} = \frac{\sum \text{of dead larvae} + \sum \text{non} - \text{emerged chrysalises} + \sum \text{of malformed butterflies}}{25} \text{ X } 100$$

Assessing the Virulence of Isolated Strains on Maize Plants

The phyto-pathogenicity of the strains was verified by inoculating 21-day-old maize leaves with a bacterial inoculum containing 10^{12} UCF/mL, which was induced from strains that had a larval mortality rate of at least 50%. The seedlings were monitored for a duration of three (03) weeks in order to identify any signs of disease through the appearance of symptoms.

Data Processing and Analysis

Data entry and organization were completed using Excel 2016 spreadsheet software, while statistical analyses were performed using SPSS version 22.0 and XLSTAT version 2016. An analysis of variance (ANOVA) was conducted using the Student-Newman-Keuls (SNK) test at a 5% significance level. Additionally, the Kruskal-Wallis test, accompanied by multiple comparisons based on Dunn's test at a 5% significance level, was employed for non-parametric data.

RESULTS

Rate of Infestation in the Field

The prevalence of *S. frugiperda* varied according to localities. As an illustration, Bama and Bérégadougou are the most infested, with infestation rates of 46% and 41% respectively (Table 1). However, the locality with the lowest infestation rate (11%) is Tingrela

Table 1: Average rate of site infestations by *S. frugiperda*

Sites	Infestation rate (%)
Bama	46
Kodéni	38
Toussiana	29
Bérégadougou	41
Banfora	27
Tingréla	11

Isolated Bacterial Strains

During surveys, 240 live larvae and 10 dead larvae were collected from maize fields. During larval breeding, 55 larvae died, making a total of 65 larvae isolated. Of the 65 larvae isolated, 41gram-negative and 218gram-positive strains were obtained. Based on morphological characteristics, 24 bacterial strains were obtained, of which 16gram-positive and 8gram-negative strains were used for pathogenicity tests (Table 2).

Pathogenicity of Gram-positive Bacterial Strains

- ***On the mortality of larvae***

Evaluation of the pathogenicity of gram-positive strains on larval mortality is shown in Table 3. It reveals that the strains caused variable rates of larval mortality. Strain 5 induced 100% larval mortality. In contrast, with strain 12 and the control, 0% larval mortality was recorded.

- ***On the lifespan of organic essences***

Table 4 shows the pathogenic effect of strains on the life span of larvae, chrysalis and moths, expressed in hours.

Table 2: Characteristics of isolated strains

Sites prospected	Live larvae collected in the field	Dead larvae collected in the field	Dead larvae collected in the laboratory	Gram+ strains identified	Gram-identified bacterial strains	Number of gram+ strains for the test	Number of gram- strains for the test
Bama	94	4	21	27	136	4	2
Kodéni	63	0	11	1	25	3	1
Toussiana	30	0	5	0	13	0	1
Bérégadougou	20	2	4	3	5	3	1
Banfora	12	2	6	6	10	3	1
Tingréla	21	2	8	4	29	3	1
Total	240	10	55	41	218	16	8

Legend*:* gram+: gram-positive and gram-:gram-negative

Table 3: Mortality rate of inoculated L1 larvae

Bacterial strain code	Larve mortality rate (%)
1	40±07.35abc
2	12±05.6bc
3	12±06.33bc
4	60±13.40ab
5	100±00a
6	40±00abc
7	12±06.33bc
8	20±07.11abc
9	20±02.83abc
10	16±04.95abc
11	12±02.83bc
12	00±00c
13	60±07.07ab
14	40±06.48abc
15	40±06.21abc
16	20±09.06abc
Control	00±00c
Probability	< 0.0001
Meaning	HS

The groups affected by the same letter are not significantly different at the 5% threshold according to the Kruskal-Wallis and Dunn tests.

Table 4: Effect of gram-positive strains on larvae, chrysalids and butterflies

	Lifetime (in hour)		
Stem Codes	Larvae	Chrysalids	Butterflies
1	312±16.97abcd	226±12.81abc	156±16.97abc
2	235±19.72bcde	216±16.97abc	144±24abc
3	355±10.63a	197±19.72bc	150±10.39abc
4	254±12.81abcde	192±16.97bc	100±11.31ab
5	115±10.63e	-	-
6	312±16.97abcd	226±31.69abc	156±16,97abc
7	339±23.43ab	213±12.37abc	144±16.97abc
8	327±20.13abc	219±28.30abc	100±8.94ab
9	305±22.14abcde	219±28.30abc	144±24abc
10	302±12.81abcde	245±19.72ab	168±24abc
11	295±16.40abcde	240±24ab	96±00ab
12	290±13.71abcde	230±13.71abc	266±25.38c
13	197±19.72de	288±00a	192±16.97c
14	254±27.20abcde	216±33.94abc	144±16.97abc
15	216±29.39cde	204±33.94abc	144±16.97abc
16	235±20.03abcde	216±24abc	288±24c
Control	256±11.31abcde	182±13.86bc	185±21bc
Probability	<0.0001	< 0.0001	< 0.0001
Meaning	HS	HS	HS

Legend: **HS =** Highly significant. The groups affected by the same letter in the same column are not significantly different at the 5% threshold according to the Kruskal-Wallis and Dunn tests.
- Absence of chrysalids and butterflies

The effect of strains on larval lifespan is variable. In the presence of strain 5, larval lifespan was reduced to 115±10.63 hours, compared with strains 1, 3, 6, 7, 8 and 9, which reduced larval life span to between 312±12.81 and 355±10.63 hours. However, with the control, larval lifespan was estimated at 256±11.31 hours and statistical analysis revealed no difference with strains.

With strain 5, no larvae have reached the chrysalis stage. Statistically, strain 13 with 288 hours differs from the control with 182 hours.

Again, with strain 5, no larvae reached the butterfly stage. In the presence of the other strains, the butterfly lifespan was statistically identical to that induced in the presence of the control, with 185±21 hours of lifespan.

- ***Effect on organic essences***

The following Fig.s represent the effect of bacterial strains on non-emerged chrysalid levels (Fig. 4A), on malformed butterflies (Fig. 4B), on butterfly capacity to lay eggs (Fig. 4C), and on the non-harmful organic essence rate (Fig. 4D). As a reminder, with strain 5 no larvae have passed the larval stage.

The rate of non-emerged chrysalises in the presence of the water control (sample 17) and strains 3, 4, 7, 8, 9, 13, 14 and 16 was 0%. Strain 11 produced the highest rate of non-emerged chrysalids at around 45%, followed by strain 15 at around 38%.

The rate of malformed moths varied according to strain (Fig. 1B). Strains 1, 2, 3, 6, 7, 10, 11 and 12 produced malformed moths. Strain 11 induced a rate of around 80% of malformed moths, followed statistically by strains 7 and 12.

The egg-laying capacity of the butterflies varied according to strain (Fig. 1C). Thus, in the presence of strains 1; 4; 6; 11, 13 and 15, no egg-laying was observed in the butterflies.

Compared with the control (sample 17), all bacterial strains induced a non-harmful rate of organic essences, but at variable levels (Fig. 1D). This rate was 100% in the presence of strain 5 and around 90% in the presence of strain 11 strain 5 and around 90% in the presence of the strain 11.

Pathogenicity of Gram- bacterial Strains

- ***Larval mortality***

The highest larval mortality rates were observed with strains 20, 23, and 24, with values of 56±9.06, 48±7.87, and 28±12.08% respectively, compared to the control where no mortality was observed (00±00%) (Table 5).

- ***On the lifespan of organic essences***

Table 6 shows the pathogenicity data of gram-negative strains on the lifespan of larvae, chrysalids, and butterflies.

In the case of larvae, all strains showed a tendency to increase larval life except strains 20 and 23. However, statistically, the larval lifetimes induced by strains 20 and 23, 261±18.23 hours and 228±29.39hrs, respectively, were no different from those induced by the water control.

The life span of chrysalids treated with strain 18 (240±29.39hrs) and strain 20 (238±33.53hrs) was statistically higher than that of the other strains.

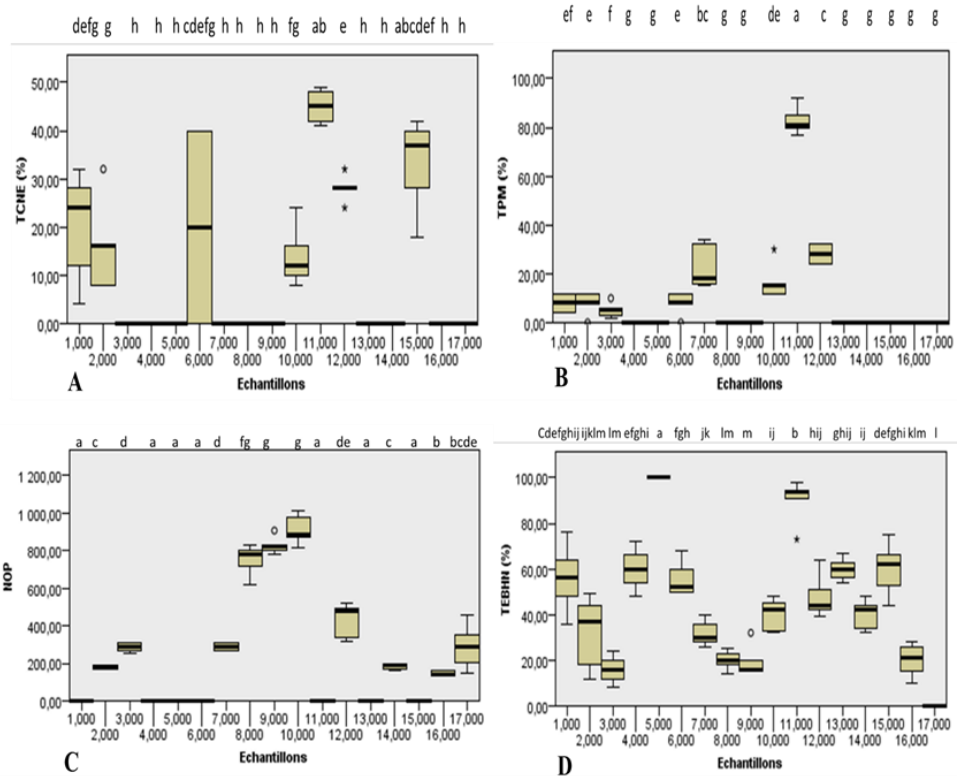

Fig. 4: Effect of strains on the development and fate of organic essences
TCNE = Rate of unmerged chrysalids, **TPM** = rate of malformed butterflies, **NOP** = Number of eggs laid, **TEBHN** = Rates of non-harmful biological substances. Groups affected by the same letter are not significantly different at the 5% threshold according to the Kruskal-Wallis and Dunn tests.

Table 5: Mortality rate of inoculated L1 larvae

Bacterial Isolate Code	Rate of larval mortality (%)
18	16±5.66ab
19	16±4.69ab
20	56±9.06a
21	16±9.38ab
22	24±7.35ab
23	48±7.87a
24	28±12.08a
25	16±7.35ab
Control	00±00b
Probability	< 0.0001
Meaning	HS

Legend: **HS** = Highly significant; groups affected by the same letter are not significantly different at the 5% threshold according to the Kruskal-Wallis and Dunn tests.

Table 6: Effect of strains on the lifespan of organic essences

Bacterial strains codes	Lifetime (in hour)		
	Larvae	Chrysalids	Butterflies
18	381±18.25a	240±29.39c	192±16.97abc
19	300±23.50b	178±21.26a	240±24.00d
20	261±18.23c	238±33.53c	192±33.94abc
21	296±29.93b	186±19.90a	192±24.00abc
22	228±29.39c	216±29.39ab	216±24.00cd
23	304±12.96b	200±23.15ab	152±11.31a
24	326±12.81b	202±21.82ab	160±21.73ab
25	291±29.33b	211±20.03ab	200±11.31bc
Control	256±11.31c	182±13.86a	185±21.84abc
Pr>F	0.000	0.001	0.000
Meaning	HS	HS	HS

Legend: ***HS*** *= Highly significant; groups that are assigned the same letter in the same column are not substantially different at the 5% threshold, as determined by the Kruskal-Wallis and Dunn tests. The correspondence of the isolate codes may be found in annex 1.*

In terms of butterfly lifespan, none of the strains reduced the duration compared with the control (185±21.84hrs).

- ***On the development of organic essences***

Table 7 shows the relative pathogenicity of gram-negative strains on the development of organic essences. On chrysalises, strain 18 produced the highest rate of non-emerged chrysalises (52±13.04%) compared with the control (0%). The rate of malformed butterflies obtained in the presence of strains 18, 19 and 20 were 70±10.39, 48±11.92 and 3838±8.78% respectively. Compared with the control, these rates are higher and statistically different. With regard to the rate of organic essences out of harm's way, strain 18 and strain 20 recorded 88±7.75% and 80±15.94% respectively. Butterflies treated with strains 18, 19, 23 and 24 showed no egg-laying.

DISCUSSION

The dynamics of *S. frugiperda* infestations in maize fields in western Burkina Faso exhibit spatial variability. Infestation rates range from 11% to 46% depending on the location. This diversity is associated with cultural behaviors and climatic factors. Regarding agricultural practices, Baudron et al. (2019) and Yaméogo et al. (2023) have demonstrated that the selection of corn varieties significantly affects the occurrence of *S. frugiperda*, as different types exhibit varying levels of susceptibility. For instance, the infection rate of the most commonly cultivated Komsaya variety in western Burkina Faso was expected to be 39.92±12.26% in 2021 and 44.44±11.08% in 2022 (Yaméogo et al., 2023). These findings validate our result. Furthermore, (FAO, 2018; Prasanna et al., 2018) demonstrated that the late or staggered seedlings, the presence of weeds, favorable climatic conditions exacerbate the development of *Spodoptera frugiperda* populations.

Pathogenicity tests showed that mortality rates varied according to the stage of development of *S. frugiperda* and the nature of the strain. On larvae, gram-positive strain 5 and gram-negative strains 20, 23 and 24 induced the highest mortality rates. These results show that these

Table 7: The impact ofgram-negative bacterial strains on the biological characteristics of *S. frugiperda* extracts.

Bacterial strains codes	Rate of unmerged chrysalids	Malformed butterflies rate	Rate of organic essences out of harm's way	Number of eggs laid
18	52±13.04a	70±10.39a	88±7.75a	00±00a
19	00±00b	48±11.92a	56±6.33ab	00±00a
20	27±10.34ab	38±8.78a	80±15.94a	268±20.40b
21	19±8.12ab	24±9.06ab	48±11.66ab	4.80±10.73ab
22	00±00b	32±7.67ab	48±10.95ab	13.20±29.52ab
23	00±00b	00±00b	48±7.07ab	00±00a
24	00±00b	22±8.37ab	44±10.95ab	00±00a
25	19±7.52ab	18±3.54ab	44±12.65ab	245±40.88ab
Control	00±00b	00±00b	00±00b	290±131.17b
Probability	<0.0001	<0.0001	<0.0001	<0.0001
Meaning	HS	HS	HS	HS

Legend: HS = Highly significant. Groups that are labeled with the same letter in the same column are not statistically different at the 5% threshold, as determined by the Kruskal-Wallis and Dunn tests.

strains are larvicidal on *S. frugiperda* larvae. Similar results were obtained by (Polanczyk et al., 2000), who tested 4 strains of *Bacillus thuringensis* to control *S. frugiperda* larvae, with efficiencies reaching 100%. In terms of butterflies' egg-laying capacity, gram-positive strains 1; 4; 5; 6; 11, 13 and 15 and gram-negative strains 18, 19, 23 and 24 failed to oviposit in *S. frugiperda* butterflies. These results show that these bacterial strains disrupt the reproductive life cycle of *S. frugiperda*. Studies by Polanczyk & Alves (2005) showed that *Bacillus thuringensis* affected biological parameters such as oviposition and female fecundity. Furthermore; in the presence of gram-positive strains 5 and 11, respectively, 100% and 90% of organic essences of *S. frugiperda* were kept out of harm's way, compared with 88% and 80% forgram-negative strains 20 and 18. This result shows that these strains can keep *S. frugiperda* populations below the harmful threshold. Over one hundred bacteria, classified into three families (Bacillaceae, Enterobacteriaceae, and Pseudomonadaceae) (Tchao et al., 2022), have been identified as entomopathogenic bacteria (Starnes et al., 1993). Entomopathogenic bacteria are widely distributed around the globe and can be found in diverse settings (Lacey, 2012; Thais, 2011). The majority of bacteria found in insects are primarily located within the digestive tract (Priest, 2000). Bacteria within the digestive system secrete enzymes such as lecithinase, proteinase, and chitinase. These enzymes specifically target the cells of the middle intestine, facilitating the bacteria's entry into the hemocoel (Tanada & Kaya, 2012; Zafar et al., 2020; Zafar et al., 2022).

Moreover, these results show that despite the difference in concentration of gram-negative higher than gram-positive used, gram-positive present the best aptitudes on *S. frugiperda*. This confirms the findings of several authors (Lacey, 2012; Gichuhi et al., 2019; Ren et al., 2019) that gram-positive strain bacteria are more entomopathogenic than gram-negative strain bacteria.

Conclusion

The search for effective entomopathogenic bacterial strains on *S. frugiperda* essences led to the testing of 24 strains of bacteria, including 16 of gram-positive and 8 of gram-negative isolated from insect larvae collected in maize plots in western Burkina Faso. The pathogenicity tests showed the larvicidal capacity of isolated bacterial strains, in particular strain gram-positive strain 5 with 100% larval mortality and strain 20 of gram-negative with 56% mortality. Furthermore, on the fertility of *S. frugiperda* and the laying of eggs it appears that strains 1; 4; 5; 6; 11, 13 and 15 of gram-positive and strains 18, 19, 23 and 24 of gram-negative prevented laying eggs completely. As for the rate of non-harmful organic essences, an efficacy of 100% and 90% was obtained with gram-positive strains 5 and 11 respectively and 80% and 88% was achieved with gram-negative strains 20 and 18, respectively. This study confirms that these strains have an entomopathogenic effect against *S. frugiperda*. These results represent an interesting breakthrough and remain a real prospect in the fight against this insect. It is therefore important to deepen research into these bacterial strains. In the light of these results, it is imperative and essential to proceed with the molecular identification of gram-positive strains 5 and 11, as well as gram-negative stems 20 and 18 and to carry out an on-station test in order to evaluate the effectiveness of these strains.

Author Contributions: Conceptualization, IW and KBFZ; Methodology, KBFZ, FY, PD and AK; Software, KBFZ and FY; Formal analysis, KBFZ, MS and AK; Writing and original draft preparation, KBFZ, FY and PD., Supervision, IW and IS. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: The authors would like to thank the phytopathology/bacteriology laboratory of INERA Farako-Bâ, Bobo Dioulasso (Burkina Faso), LMI PathoBios, France, and the plant clinic of Nazi BONI University, Bobo Dioulasso (Burkina Faso).

Conflicts of Interest: The authors of this article declare that they have no conflicts of interest.

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