第 48 卷 第 6 期
2021 年 6 月

湖南大学学报（自然科学版）
Journal of Hunan University（Natural Sciences）

Vol. 48. No. 6.
June 2021

Open Access Article

The Cytotoxic Activity of Crude Aqueous *Annona* Plant-Derived Extracts on *Spodoptera frugiperda*, Cell Lines

Jesús A. Polo Olivella[1,2], Marlinda Lobo de Souza[3], Ericsson Coy-Barrera[4], Jhon F. Betancur Pérez[2], Jorge W. Arboleda Valencia [2,5]

[1] Maestría de Biología, Facultad de Ciencias Básicas, Universidad del Atlántico, Barranquilla, Colombia

[2] Centro de Investigaciones en Medio Ambiente y Desarrollo -CIMAD, Facultad de Ciencias Económicas, Contables y Administrativas, Universidad de Manizales, Manizales, Colombia

[3] Brazilian Agricultural Research Corporation -EMBRAPA, Embrapa Genetic Resources and Biotechnology (CENARGEN), Brasília, Brazil

[4] Laboratorio de Química Bioorgánica, Universidad Militar Nueva Granada, km 2 Cajicá – Zipaquirá, Nueva Granada Campus, Cajicá, Colombia

[5] Instituto de Biología, Facultad de Ciencias Exactas y Naturales, Universidad de Antioquia, Medellín, Colombia

Abstract: The excessive use of agrochemicals in agriculture to control insect pests has generated several environmental problems related to pollution and health risks for farmers. Therefore, chemical control has been proposed, including using products with plant origin, as an alternative to problematic, chemically-synthetized agents. These natural sources produce great numbers of bioactive molecules, and many have been reported to exhibit insecticidal effects. Recent studies have described the presence of bioactive compounds in tissue extracts from *Annona* plants, which are now considered useful in support of certain agricultural production processes. The cytotoxic effect of *A. squamosa* and *A. muricata* leaf extracts was tested on SF-21 and SF-9 insect cell lines obtained from *Spodoptera frugiperda*. Results showed that extracts of *A. squamosa* were more effective, as they gradually reduce cellular viability at 72 hours following exposure. Treatments with *A. squamosa* were significantly different from the negative control, whereas treatment with *A. muricata* behaved similarly to the control (i.e., no cytotoxic effect). Morphological changes in cells exposed to *A. squamosa* extracts were also observed. These included modifications in the structure and the shape of cell nuclei, generalized vacuolization, and membrane lyses. The effect observed by *A. squamosa* extracts on the tested cell lines represents relevant information regarding the potential use of plant-derived compounds from these species as alternatives for insect-specific pest control.

Keywords: bioprospecting, *Annona sp.*, biological activity.

粗制番荔枝植物提取物对草地贪夜蛾细胞系的细胞毒活性

摘要：在农业中过度使用农用化学品来控制害虫已经产生了一些与污染和农民健康风险相关的环境问题。因此，已经提出化学控制，包括使用植物来源的产品，作为有问题的化学合成剂的替代品。这些天然来源产生大量的生物活性分子，据报道，许多具有杀虫作用。最近的研究描述了番荔枝植物组织提取物中存在生物活性化合物，这些化合物现在被认为可用于支持某些农业生产过程。在从草地贪夜蛾获得的顺丰-21 和顺丰-9 昆虫细胞系上测试了鳞

Received: March 3, 2021 / Revised: April 6, 2021 / Accepted: May 2, 2021 / Published: June 28, 2021

About the authors: Jesús A. Polo Olivella, Maestría de Biología, Facultad de Ciencias Básicas, Universidad del Atlántico, Barranquilla, Colombia; Centro de Investigaciones en Medio Ambiente y Desarrollo -CIMAD, Facultad de Ciencias Económicas, Contables y Administrativas, Universidad de Manizales, Manizales, Colombia; Marlinda Lobo de Souza, Brazilian Agricultural Research Corporation -EMBRAPA, Embrapa Genetic Resources and Biotechnology (CENARGEN), Brasília, Brazil; Ericsson Coy-Barrera, Laboratorio de Química Bioorgánica, Universidad Militar Nueva Granada, Cajicá, Colombia; Jhon F. Betancur Pérez, Centro de Investigaciones en Medio Ambiente y Desarrollo -CIMAD, Facultad de Ciencias Económicas, Contables y Administrativas, Universidad de Manizales, Manizales, Colombia; Jorge W. Arboleda Valencia, Centro de Investigaciones en Medio Ambiente y Desarrollo -CIMAD, Facultad de Ciencias Económicas, Contables y Administrativas, Universidad de Manizales, Manizales, Colombia; Instituto de Biología, Facultad de Ciencias Exactas y Naturales, Universidad de Antioquia, Medellín, Colombia

茎和一种. 穆里卡塔叶提取物的细胞毒性作用。结果表明，鳞茎提取物更有效，因为它们在暴露后 72 小时逐渐降低细胞活力。用鳞茎处理与阴性对照显着不同，而用一种. 穆里卡塔处理的行为与对照相似（即，无细胞毒性作用）。还观察到暴露于鳞茎提取物的细胞的形态学变化。这些包括细胞核结构和形状的改变、普遍的空泡化和膜裂解。鳞茎提取物对测试细胞系观察到的影响代表了有关可能使用这些物种的植物衍生化合物作为昆虫特异性害虫控制替代品的相关信息。

关键词：生物勘探，番荔枝，生物活性。

1. Introduction

For years, cell lines have been used as a tool in cell physiology studies to obtain valuable information about the mechanisms related to insect pathological processes. In addition, assays on cell lines have been demonstrated to be useful for studying vectors of mammal diseases and the characterization of new products [1, 2]. SF-21 is a cell line obtained from *Spodoptera frugiperda* pupae ovarian tissue (Lepidoptera: Noctuidae) [3], This cell line currently used in many studies related to baculovirus infections and the production of recombinant proteins, as its cell clone, SF-9 [4]. *S. frugiperda*, commonly referred to as the fall armyworm, is a cosmopolitan tropical insect that is widely distributed throughout America [5–7] and is recognized as one of the most economically important pests for crops such as corn, cotton, rice, and specific vegetables [8, 9]. In addition, the fall armyworm was recently reported for the first time in Africa and has since spread rapidly across more than 30 countries in the continent [10].

Studies regarding *S. frugiperda* control have been primarily focused on biological control and have used entomopathogenic bacteria, such as *Bacillusthuringiensis* [11]. However, chemical control is still required, so searching for novel insecticidal compounds from natural sources has become a challenging focus for researchers [12, 13]. It is well-known that the excessive use of synthetic pesticides has serious environmental and toxicological implications and increasing problems related to pest resistance [14]. For this reason, it is necessary to conduct major studies of novel, non-toxic compounds that exhibit broader action spectrums [13]. In this sense, plant tissues constitute a valuable source of novel chemical entities with insecticidal potential. These tissues synthesize and accumulate various pesticide compounds in response to various stimuli or environmental conditions [13, 15, 16].

Colombia boasts an exceptional geographical position and possesses the greatest biodiversity in Latin America, with 56,343 species reported. This is distributed in 30,776 plants, 7,500 of which are endemic [17]. As part of this biodiversity, various species belong to the Annonaceae family and are widely distributed throughout the country, with the *Annona* genus being the most representative. Of the various plants which belong to this genus, the presence of several phytoconstituents that exhibit cytotoxic activity is common [15, 18–20], anti-tumor [20–22], and anti-parasitic effects [23, 24], among other properties [16, 25–27] has been reported. The present study evaluated the cytotoxic effect of aqueous leaf extracts from *Annona* plants on *S. frugiperda* cell lines as the first step to screening alternatives for insect-specific pest control with this kind of plant material.

2. Materials and Methods

2.1. Study Area

The plant material was collected near the Sabanalarga municipality, in the Atlántico department of Colombia, located at 10º35'43.7" North and 74º59'81.5" West, at 87 meters of altitude. The annual average temperature in the area is 29.2 °C, annual rainfall totals 1.021 mm, and relative humidity remains between 62 and 92% monthly.

2.2. Crude Extract Preparation

Fresh leaves were comminuted, weighed (20 g), and then soaked in distilled water with a mass/volume ratio of 1:1. The obtained extract was subsequently filtered through No. 1 Whatman filter paper and centrifuged at 10,000 rpm for five minutes in a microcentrifuge at room temperature. Once the material had precipitated, the supernatant was collected and stored at 4 °C ± 1 in 50 ml falcon tubes for use in subsequent analyses and bioassays.

2.3. Insect Cell Cultures and Treatments

The SF-21 and SF-9 Lepidoptera (*Spodoptera frugiperda*) cell lines, obtained from an envelope of pupal ovaries and stably transformed cell clones, respectively [3], were maintained at 27 °C ± 1 in a TNM-FH medium supplemented with 10% FCS and 1% penicillin-streptomycin–amphotericin solution.

Cultures in the early stationary phase (typical cell density with cell viability of approximately 85%) were split every five days, with a starting density of 2 x 10^5 cells/ml. Different concentrations of crude aqueous extracts of *A. muricata* and *A. squamosa* leaves were evaluated; later, total phenols and flavonoids in the crude extracts were quantified. Afterward, the insect cells were seeded in 12-well cell culture plates at the early stationary phase (1 x 10^6 cells/ml), and test solutions were added at a ratio of 1:100 (v/v). Plates were finally incubated for 24, 48, and 72 hours at 27 °C ± 1.

2.4. Bioassays: Cell Viability and Ultrastructural Analysis

Trypan blue dye (0.4%) was added to insect cell cultures in a 1:1 ratio for further observation and counting of viable and non-viable cells with the 10X objective of a Nikon Eclipse TS100 inverted microscope. The cells were kept at 27 °C ± 1 °C [28]. The evaluation was performed at 24, 48, and 72 hours after treatment, using a Neubauer chamber. All tests were evaluated in triplicate.

For ultrastructural analysis, the SF-21 cells exposed to the *A. squamosa* extract during 72 hours were initially pelleted at 3,500 rpm for five min. Cells were fixed using 2.5% glutaraldehyde and a 0.1 M (pH 6.8) sodium cacodylate buffer and were kept at 4 °C ± 1 °C overnight. The fixed material was centrifuged at 4,500 g for five minutes, and the supernatant was discarded. This process was repeated three times. Subsequently, the pellet was post-fixed in 2% osmium tetroxide for two hours, following centrifugation and five subsequent washes with Milli-Q water. The pellet was suspended in 0.5% uranyl acetate and stored overnight at 4°C, in the dark. Additionally, scaled, progressive dehydration with ethanol (10%, 30%, 50%, 70%, 90%, and 100%) was implemented, with a 10-minute wash in each solution, followed by centrifugation. Following the manufacturer's protocol, the infiltration was performed by removing ethanol; afterward, the spur resin was added in a tissue rotator. Samples were placed in molds and polymerized for 48 hours, in an oven, at 70 °C ± 1 °C. Then, they were cut into semi-thin 500 nm, and ultrathin 50 nm sections were prepared in an ultramicrotome (LKBUltrotome III). These were subsequently stained with uranyl acetate 2% for one hour in the dark. The analysis was performed with an EM 109 transmission electron microscope (Zeiss) [29].

2.5. Chemical Analysis of Total Phenols and Flavonoids

Supernatants from S-L aqueous extraction, after centrifugation, were lyophilized. Each crude extract was used to prepare stock solutions at 2 mg/mL to quantify total phenols through the spectrophotometric Folin-Ciocalteu procedure, using gallic acid as a reference [30]. Standard work solutions were prepared from a 200-ppm gallic acid stock solution via dilutions with distilled water to attain different concentrations (0.9, 4.7, 9.5, 14.2, and 19.4 ppm) for the calibration curve at 765 nm. Test extracts and reference solutions were placed into 96-well plates, 20 µL of test solution was added, as was 40 µL of 10% Folin-Ciocalteau reagent, and after three minutes, 150 µL of 7.35% Na2C03 was added. Absorbance was monitored with a microplate reader at 765 nm [31]. Results are expressed as gallic acid equivalents (ppm) after three replicates.

2.6. HPLC-ESI-MS Analysis

Crude extracts were diluted in a 0.1% formic acid and acetonitrile solution (1:1 ratio). Chromatographic analysis of these samples was performed with the Shimadzu Prominence HPLC system (Shimadzu Corp., Japan), equipped with an SPD-M20A detector and MS2020 mass spectrometry detector with Electrospray Ionization (ESI) that operated simultaneously in negative and positive ion mode. Analysis was performed with a Shimadzu C-18 Premier column (5 µ, 150 x 4.6 mm). A 0.1% formic acid (phase a) and acetonitrile (phase b) mixture was employed as mobile phase for the chromatographic method, in gradient elution (0-30% B 0-25 minutes, 30-50% B 25-32 minutes, 50-58% B 32-25 minutes, 58-100% B 35-39 minutes, 100-100% B 39-42 minutes, 100-0% B 42-50 minutes), using a flow of 0.7 mL/min. Separation was carried out for 52 minutes, and UV detection was performed at 270 nm. Compounds were tentatively identified by analyzing their UV-vis and mass spectra compared to those in the Mass Bank, Knapsack databases, and data reported in the literature.

2.7. Statistical Analysis

Results were defined as the mortality variable and were subjected to descriptive statistics to determine the mean and standard deviation for all cases. Mortality correction was performed using the formula proposed by Schneider-Orelli [32, 33]. They were then subjected to the Shapiro-Wilk normality test. Depending on the assumption of obtained normality, either the Kruskal Wallis or ANOVA variance analysis was applied. Tukey comparisons were used, with a significance level of 95%. Simple bivariate correlations were performed, using the Pearson, Spearman, and Kendall coefficients, with the R software, version 3.4.2.

3. Results

3.1. Chemical Characterization

LC-MS-based analysis of aqueous *A. squamosa*-derived extracts showed the presence of two flavonoids as main constituents, identified as quercetin 3.7-diglucoside and kaempferol 3-glucoside (Fig. 1). Total

phenols and flavonoids were corroborated through quantification, using the Folin-Ciocalteau method. *A. squamosa* extracts showed higher concentrations (0.242 ppm ± 0.002) than *A. muricata* extracts (0.027 ppm ± 0.004). In both extracts, additional compounds were detected, mostly alkaloids and acetogenins, typical Annonaceae compounds [34].

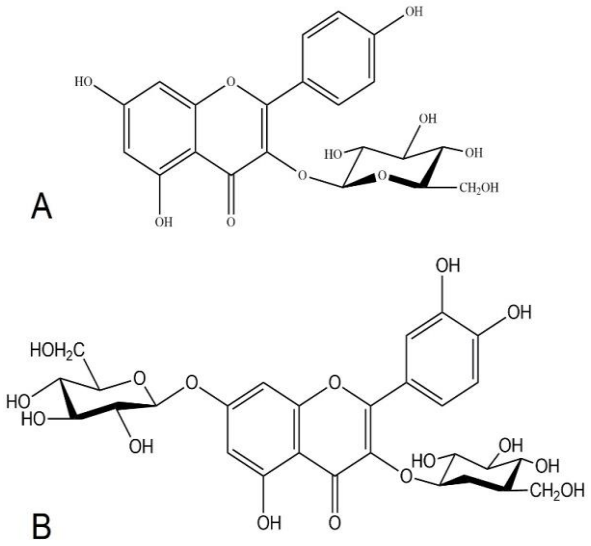

Fig. 1 Quercetin 3,7- diglucoside, C27H30O17, Mw: 626.148, b. Kaempferol 3-O-beta-D-glucoside, C21H20O11, Mw: 448.100

3.2. Bioassays: Cell Viability

A. squamosa extract showed a greater cytotoxic effect on SF-21 compared to *A. muricata* extracts during observations at 24, 48, and 72 hours. A direct decrease in cell viability when exposed to the *A. squamosa* extract was observed, as shown in Fig. 2. On the other hand, exposure to *A. muricata* extracts did not show any cytotoxic effect. Further, cell viability was approximately 90%, which is very similar to that of the initial control. On the evaluation of the correlation between viability and exposure time, no relationship was found for *A. muricata* extracts (Table 1), but rather an inverse relationship of dependence was revealed between these variables for *A. squamosa* extracts, with $p < 0.05$ for all coefficients (Table 1). This association was confirmed by the correlation coefficients of Pearson, Spearman, and Kendall (Table 1). On correlating the total phenol content of crude extracts (*A. squamosa* 0.242 ppm ± 0.002; *A. muricata* 0.027 ppm ± 0.004) with cell viability, it was observed that, in all cases, the hypothesis of independence was rejected, as $p \leq 0.05$. Therefore, in all exposure times, and considering the three coefficients, it was concluded that there was an inverse relationship of dependence between said variables (Table 2). This association seems to be stronger at 72 hours when observing Pearson's correlation coefficient, followed by that of 48 hours and 24 hours (Table 2). Spearman and Kendall's coefficients show a significant but equal relationship at the three exposure times (Table 2).

Table 1 Analysis of simple bivariate correlations, contrasting the exposure times and cell viabilities observed for each extract

	Time vs. viability					
	Pearson´s coefficient		**Spearman´s coefficient**		**Kendall´s coefficient**	
	p	**Correlation**	***p***	**Correlation**	***p***	**Correlation**
A. muricata	0.285	-0.401	0.197	-0.474	0.222	-0.353
A. squamosa	0.003	-0.896	0.005	-0.869	0.012	-0.784

Table 2 Simple bivariate correlation analysis contrasting the concentration of crude extracts (*A. squamosa* and *A. muricata*) and cell viability observed for each extract

	Concentration vs. viability					
	Pearson´s coefficient		**Spearman´s coefficient**		**Kendall´s coefficient**	
	p	**Correlation**	***p***	**Correlation**	***p***	**Correlation**
24 hours	0.050	0.812	0.21	0.878	0.050	0.775
48 hours	<0.001	0.982	0.021	0.878	0.050	0.775
72 hours	<0.001	0.998	0.021	0.878	0.050	0.775

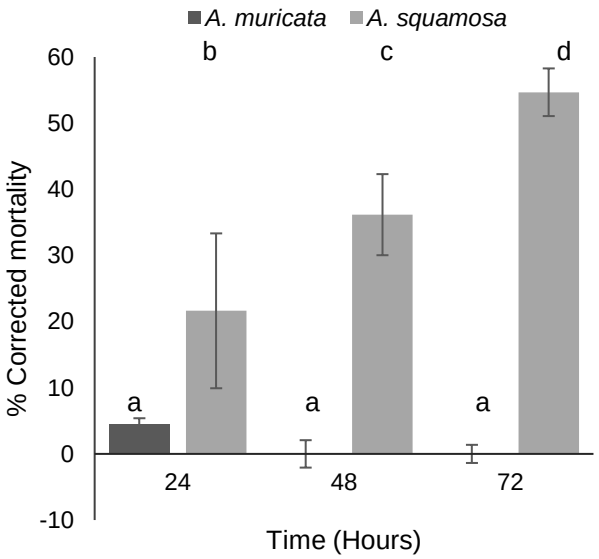

Fig. 2 Corrected SF-21 mortality when exposed to *A. squamosa* and *A. muricata* extracts (Schneider - Orelli's formula). The error bars represent the standard deviation. Each trial was performed in triplicate. Statistically significant differences were indicated with different letters, Tukey ($p \leq 0.05$)

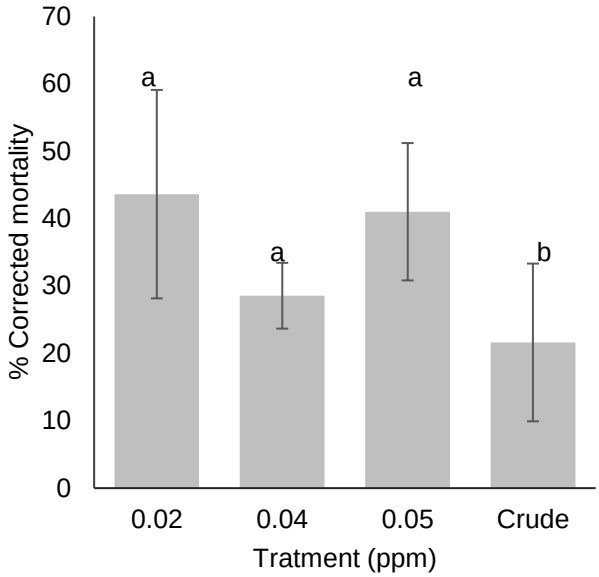

Fig. 3 Corrected SF-21 mortality when exposed to different concentrations of *A. squamosa* extract (Schneider - Orelli's formula). The error bars represent the standard deviation. Each trial was performed in triplicate. Statistically significant differences are indicated with different letters, Tukey ($p \leq 0.05$) (Shapiro-Wilks: $p = 0.5871$)

Normality tests showed that the data behaved non-parametrically (Shapiro-Wilk: $p = 0.00982$). A Kruskal-Wallis (K-W) variance analysis was performed, which reflected the differences between each treatment (K-W: $X2= 21.231$, $p = 0.0065$). However, when comparisons were drawn separately, *A. muricata* crude extracts were statistically similar to the controls. *A. squamosa* crude extracts showed statistical differences from other treatments and between the evaluation times (Fig. 2 and Table 3).

Table 3 Multiple comparisons of means: Tukey contrasts. Comparisons are shown between the control at each exposure time and the respective treatment with *A. squamosa* Pr (>|t|) crude extract

		A. squamosa		
		24 hours	**48 hours**	**72 hours**
Control	24 hours	0.005	< 0.001	< 0.001
	48 hours	-	0.041	< 0.001
	72 hours	-	-	0.005

The *A. squamosa* extract dilution effect on SF-21 was evaluated, with concentrations like that found in the crude extracts of *A. muricata* (0.02 ppm, 0.04 ppm, 0.05 ppm). Statistically significant differences were not found among these concentrations ($p \leq 0.05$) (Fig. 3). However should be highlighted that the cell viability of the SF-21 exposed to *A. squamosa* extracts at 24 hours decreases in proportion to the concentration, as compared to the crude extract (0.242 ppm ± 0.002), which indicated statistically significant differences between treatments ($p = 0.0266$) (Fig. 3).

The evaluation of the effect of *A. squamosa* crude extract on SF-9 showed a similar result to that observed in SF-21. The effect of exposure time was revealed, considering that, at 24 hours, viability was greater than 80%, at 48 hours, it was greater than 55%, and at 72 hours, at its time of greatest effect, it showed the viability of no more than 40%. Compared to the negative control, statistical differences were shown at each exposure time, as evaluated by variance analysis (ANOVA) and the Tukey contrast of multiple comparisons of means (Fig. 4).

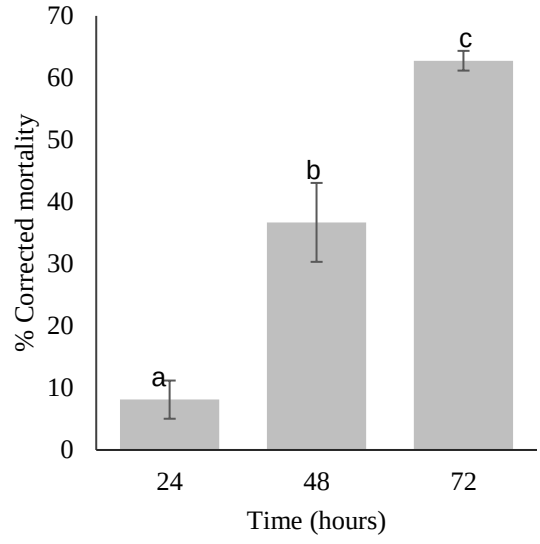

Fig. 4 Corrected SF-9 mortality following exposure to *A. squamosa* extracts (Schneider - Orelli's formula). The error bars represent the standard deviation. Each trial was performed in triplicate. Statistically significant differences are indicated with different letters, Tukey ($p \leq 0.05$)

Negative controls were established to monitor the effect of the treatments. All controls showed approximate viability of 90%, which suggests treatment correction in the control function, with Schneider-Orelli's formula.

The effect observed on SF-21 cells, when exposed to the *A. squamosa* extract, is due to morphological changes in cell structure and shape, such as nuclear condensation, generalized vacuolization, and cell membrane lysis (Fig. 5 and 6).

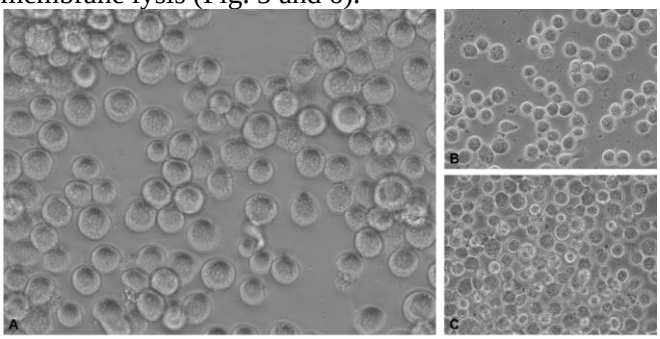

Fig. 5 Optical micrographs of the in-vitro effect produced by the *A. squamosa* extract on SF-21 cells. A: Control cells without treatment. B, C: Cells treated with the *A. squamosa* extract. Images were obtained using 40X objectives at 24 hours of exposure

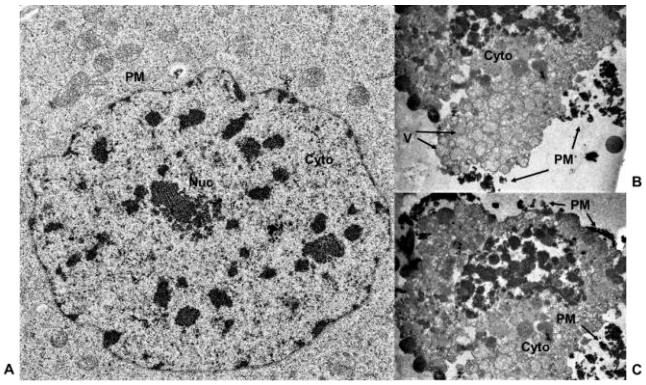

Fig. 6 Transmission Electron Microscopy (TEM) images of 3000x SF-21 cells at 72 hours. A: Cell control. B, C: Cells treated with the A. squamosa extract. Labels indicate the presence of cellular structures such as nuclei (Nuc), plasma membranes (PM), cytoplasm (Cyto), and vacuoles (V)

4. Discussion

There is a link between pesticide overuse, pollution-related environmental problems, and human medical disorders [35, 36]. This has been the starting point in searching for controlling alternatives that are friendly to the environment and contribute to sustainable agriculture. It is necessary to provide new methods for farmers to control pests. So the search for these alternatives has spurred increased use of compounds from natural origin, which are both accessible and economically sustainable [37]. However, exploring these compounds and evaluating their potential is only the first step in the prospection of compounds with biotechnological utilities in the agroindustrial sector [25].

Methods to assess organism susceptibility to extracts or pure compounds are complex to carry out due to the economic and infrastructure implications necessaries for their realization. Cell lines used for in-vitro susceptibility assessments, in this case for insects, are viable alternatives, as with these, relevant information may be obtained regarding the behavior of cells following exposure to extracts or compounds of diverse origin; these may be extrapolated to the behavior of the organism, using similar methodologies to those applied in medicine, as is the case with feasibility counts [38, 39]. This research job represents just the first report on the cytotoxicity of extracts obtained from *Annona* plants on *S. frugiperda* insect cell lines.

The cytotoxicity evaluation of crude *A. squamosa* and *A. muricata* leaf aqueous extracts was initially carried out on the SF-21 cell line. This showed maximum effectiveness of 21.629 % in the first 24 hours of exposure, whereas the *A. muricata* extract was shown to have no effect (Fig. 3); thus, Yamthe et al. [24] findings demonstrating the limited cytotoxic effect of *A. muricata* extracts, as tested on human erythrocytes. However, the antineoplastic potential of the n-butanol leaf extract from *A. muricata* was reported [27]. It may thus be inferred that the type of extract evaluated in this study is inadequate to obtain interesting results regarding the cytotoxic potential of *A. muricata*. However, when evaluating the correlation between the concentration of polyphenols and viability (Table 2), a high correlation was found at all exposure times, at 48 and 72 hours of more than 90% and at 24 hours of 81.2%. This permits the inference that low polyphenol concentration in the *A. muricata* extract could be responsible for the poor effect observed compared to *A. squamosa*extract. This pattern indicates that the higher the concentration of polyphenols (Fig. 2), the lower the cell viability of SF-21. Additionally, the effect observed in the SF-21 cell viability decrease when exposed to *A. squamosa* extracts follows a temporal pattern, supported by the high correlation shown between *A. squamosa* extracts when cell viability was compared to the exposure time. This indicates that *A. squamosa* crude extract (0.242 ppm ± 0.002) is more cytotoxic after 72 hours of exposure to SF-21 cells than other hours evaluated. When the concentration of *A. squamosa* extracts decreased, an increase in the cytotoxic effect was observed (Fig. 4). It is thus possible that the cytotoxic effect of the compounds present in *A. squamosa* extracts increases in concentrations near 0.02 ppm. These polyphenolic compounds are attributed properties related to the inactivation of cellular enzymes due to hydroxyl groups [40, 41]. These interact with the cell membrane, causing fatty acid and phospholipid modifications, which produce damage to the cellular components and deteriorate energy metabolism [42]. The effect of the *A. squamosa* extract on the SF-9 cell line was evaluated, considering that they were slightly more sensitive than

the first, as shown in Fig. 4. Similar results were obtained for cell viability, with values of 88.09%, 59.55%, and 35.93% at 24, 48, and 72 hours, respectively.

Recently, studies have attributed the cytotoxic characteristics of *A. squamosa*, mainly evaluated on human tumor cell lines, to different compounds, within which flavonoids play a significant part. However, the wide variety of chemical compounds representing this plant family makes its form of action variable. In many cases, it acts as a cell-proliferation inhibitor [43]. In the present study, it was possible to observe that the *A. squamosa* species presents significant cytotoxic activity on SF-21 and SF-9 cell lines. This can be corroborated by studies in which the presence of cytotoxic compounds and insecticides in the *Annona* genus leaves can inhibit the feeding and growth of *S. frugiperda* larvae [16]. In addition, the cytotoxic activity of these extracts on an insect line only suggests their potential for final pest control, given that the insecticidal effect on whole larvae involves different factors, including concentrations, synergistic effects, environmental variables, genotypes, and individual chemical responses. It has been shown that compounds in plant extracts with insecticidal activity may act as feed inhibitors in insects and may disrupt the organism's growth, development, reproduction, and behavior [16]. Furthermore, the synergistic effect of quercetin and kaempferol on human tumor cell lines has been reported to be due to cell-cycle arrest, proliferation inhibition, and apoptosis induction [44].

We observed effects on SF-21 cells when exposed to the *A. squamosa* extract, as registered in Fig. 5 and 6. Similar studies reported results regarding apoptosis induction by extracts from plants in the *Annona* genus on SMMC-7721 cancer cells [14]. More specifically, *A. squamosa* seeds extracts have been reported to have the same effect on BC-8 cells [22].

5. Conclusions

The observed effect of the *A. squamosa* extracts on evaluated cell lines (SF-21 and SF-9) represents relevant information regarding the potential use of plant-based compounds from the Annonaceae family, or more specifically from the *Annona* genus, as pest-control alternatives. It should be noted that the cytotoxic effect is attributed to flavonoids, and specific extraction forms, which allow for the obtention of these compounds in their purest form, should be evaluated to evaluate the biotechnological potential of these compounds as pest control agents. Considering that the addition of *A. squamosa* extracts to SF-21 cells leads to intense cell lysis, analyzing this effect on the molecular level is worthwhile. In terms of the effects of apoptotic processes, the action of the endonucleases responsible for digesting DNA in small fragments must be verified. Finally, these results may permit the future obtention of alternatives to control several insect pests based on biotechnological strategies.

Funders

We thank the Universidad del Atlántico and Universidad de Manizales-Colombia (Grant number B0601X0209) for supporting the present study. We also thank Embrapa Recursos Genéticos e Biotecnología (CENARGEN) for all assistance provided with insect cell culture and TEM analyses, by William Sihler and Rosana Falcão from Insect Virology Laboratory and Bioimage Laboratory, respectively.

References

[1] AHMED T., SHAHID M., NOMAN M., NIAZI M. B. K., ZUBAIR M., ALMATROUDI A., KHURSHID M., TARIQ F., MUMTAZ R., and LI B. Bioprospecting a native silver-resistant Bacillus safensis strain for green synthesis and subsequent antibacterial and anticancer activities of silver nanoparticles. *Journal of Advanced Research*, 2020, 24: 475-483. https://doi.org/10.1016/j.jare.2020.05.011

[2] FERNANDES B., VIDIGAL J., CORREIA R., CARRONDO M. J., ALVES P. M., TEIXEIRA A. P., and ROLDÃO A. Adaptive laboratory evolution of stable insect cell lines for improved HIV-Gag VLPs production. *Journal of Biotechnology*, 2020, 307: 139-147. https://doi.org/10.1016/j.jbiotec.2019.10.004

[3] VAUGHN J. L., GOODWIN R. H., TOMPKINS G. J., and MCCAWLEY P. The establishment of two cell lines from the insect spodoptera frugiperda (lepidoptera; noctuidae). *In Vitro*, 1977, 13: 213–217. https://doi.org/10.1007/BF02615077

[4] MISHRA V. A Comprehensive Guide to the Commercial Baculovirus Expression Vector Systems for Recombinant Protein Production. *Protein and Peptide Letters*, 2020, 27(6): 529-537. https://doi.org/10.2174/0929866526666191112152646

[5] KHATRI S., PAKUWAL P., and KHANAL S. Integrated pest management of fall armyworm infestations in maize fields in Nepal: A review. *Archives of Agriculture and Environmental Science*, 2020, 5(4): 583-591. https://doi.org/10.26832/24566632.2020.0504023

[6] NAGOSHI R. N., NAGOSHI B. Y., CAÑARTE E., NAVARRETE B., SOLÓRZANO R., and GARCÉS-CARRERA S. Genetic characterization of fall armyworm (Spodoptera frugiperda) in Ecuador and comparisons with regional populations identify likely migratory relationships. *Plos One*, 2019, 14(9): e0222332. https://doi.org/10.1371/journal.pone.0222332

[7] MARTINELLI S., CLARK P. L., ZUCCHI M. I., SILVA-FILHO M., FOSTER J., and OMOTO C. Genetic structure and molecular variability of Spodoptera frugiperda (Lepidoptera: Noctuidae) collected in maize and cotton fields in Brazil. *Bulletin of Entomological Research*, 2007, 97: 225–231. https://doi.org/10.1017/S0007485307004944

[8] THUMAR R. K., ZALA M. B., VARMA H. S., DHOBI C. B., PATEL B. N., PATEL M. B., and BORAD P. K. *Evaluation of insecticides against fall armyworm, Spodoptera frugiperda (JE Smith) infesting maize.* 2020.

[9] KALLESHWARASWAMY C. M., MARUTHI M. S., and PAVITHRA H. B. Biology of invasive fall army worm

Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) on maize. *Indian Journal of Entomology*, 2018, 80(3): 540-543. https://doi.org/10.5958/0974-8172.2018.00238.9
[10] BATEMAN M. L., DAY R. K., LUKE B., EDGINGTON S., KUHLMANN U., and COCK M. J. Assessment of potential biopesticide options for managing fall armyworm (Spodoptera frugiperda) in Africa. *Journal of Applied Entomology*, 2018, 142(9): 805-819. https://doi.org/10.1111/jen.12565
[11] LEMES A. R., MARUCCI S. C., COSTA JR. V., et al. Selection of strains from Bacillus thuringiensis genes containing effective in the control of Spodoptera frugiperda. *Bt Research*, 2015, 6: 8.
[12] DOS SANTOS C. A. M., DO NASCIMENTO J., GONÇALVES K. C., SMANIOTTO G., DE FREITAS ZECHIN L., DA COSTA FERREIRA M., and POLANCZYK R. A. Compatibility of Bt biopesticides and adjuvants for Spodoptera frugiperda control. *Scientific Reports*, 2021, 11(1): 1-8. https://doi.org/10.1038/s41598-021-84871-w
[13] ALVES D. S., MACHADO A. R. T., CAMPOS V. A. C., OLIVEIRA D. F., and CARVALHO G. A. Selection of Annonaceae Species for the Control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and Metabolic Profiling of *Duguetia lanceolata* Using Nuclear Magnetic Resonance Spectroscopy. *Journal of Economic Entomology*, 2016, 109: 649–659. https://doi.org/10.1093/jee/tov396
[14] KEDIA A., PRAKASH B., MISHRA P. K., SINGH P., and DUBEY N. K. Botanicals as eco friendly biorational alternatives of synthetic pesticides against Callosobruchus spp. (Coleoptera: Bruchidae)—a review. *Journal of Food Science and Technology*, 2015, 52: 1239–1257. https://doi.org/10.1007/s13197-013-1167-8
[15] SHANIBA V. S., AZIZ A. A., JOSEPH J., JAYASREE P. R., and KUMAR P. M. Synthesis, Characterization and Evaluation of Antioxidant and Cytotoxic Potential of Annona muricata Root Extract-derived Biogenic Silver Nanoparticles. *Journal of Cluster Science*, 2021. https://doi.org/10.1007/s10876-021-01981-1
[16] FREITAS A. F., PEREIRA F. F., FORMAGIO A. S. N., LUCCHETTA J. T., VIEIRA M. C., and MUSSURY R. M. Effects of Methanolic Extracts of Annona Species on the Development and Reproduction of Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae). *Neotropical Entomology*, 2014, 43: 446–452. https://doi.org/10.1007/s13744-014-0225-x
[17] SIB COLOMBIA ¿Cuántas especies registradas hay en Colombia? 2021. https://cifras.biodiversidad.co/
[18] FERRAZ R. P. C., BOMFIM D. S., CARVALHO N. C., SOARES M. B. P., PINHEIRO M. L. B., COSTA E. V., and BEZERRA D. P. Cytotoxic properties of the leaf essential oils of *Guatteria blepharophylla* and *Guatteria hispida* (Annonaceae). *Flavour and Fragrance Journal*, 2014, 29: 228–232. https://doi.org/10.1002/ffj.3199
[19] OWOLABI M. S., OGUNDAJO A. L., DOSOKY N. S., and SETZER W. N. The Cytotoxic Activity of Annona muricata Leaf Oil from Badagary, Nigeria. *American Journal of Essential Oils and Natural Products*, 2013, 1(1): 1–3. https://www.essencejournal.com/archives/2013/1/1/A/1
[20] WANG D. S., RIZWANI G. H., GUO H., AHMED M., AHMED M., HASSAN S. Z., HASSAN A., CHEN Z. S., and XU R. H. Annona squamosa Linn: cytotoxic activity found in leaf extract against human tumor cell lines. *Pakistan Journal of Pharmaceutical Sciences*, 2014, 27: 1559–1563.
[21] NAIK A. V., DESSAI S. N., and SELLAPPAN K. Antitumour activity of Annona muricata L. leaf methanol extracts against Ehrlich Ascites Carcinoma and Dalton's Lymphoma Ascites mediated tumours in Swiss albino mice. *Libyan Journal of Medicine*, 2021, 16(1): 1846862. https://doi.org/10.1080/19932820.2020.1846862
[22] HAYKAL T., NASR P., HODROJ M. H., TALEB R. I., SARKIS R., MOUJABBER M. N. E., and RIZK S. Annona cherimola seed extract activates extrinsic and intrinsic apoptotic pathways in leukemic cells. *Toxins*, 2019, 11(9): 506. https://doi.org/10.3390/toxins11090506
[23] BRÍGIDO H. P. C., CORREA-BARBOSA J., DA SILVA-SILVA J. V., COSTA E. V. S., PERCÁRIO S., and DOLABELA M. F. Antileishmanial activity of Annona species (Annonaceae). *SN Applied Sciences*, 2020, 2(9): 1524. https://doi.org/10.1007/s42452-020-03340-7
[24] YAMTHE L. R. T., FOKOU P. V. T., MBOUNA C. D. J., KEUMOE R., NDJAKOU B. L., DJOUONZO P. T., MFOPA A. N., LEGAC J., TSABANG N., GUT J., ROSENTHAL P. J., and BOYOM F. F. Extracts from Annona Muricata L. and Annona Reticulata L. (Annonaceae) Potently and Selectively Inhibit Plasmodium Falciparum. *Medicines*, 2015, 2(2): 55–66. https://doi.org/10.3390/medicines2020055
[25] ALI S. S., AHMAD S., AHMED S. S., RIZWANA H., SIDDIQUI S., ALI S. S., RATTAR I. A., and SHAH M. A. Effect of Biopesticides Against Sucking Insect Pests of Brinjal Crop Under Field Conditions. *Journal of Basic & Applied Sciences*, 2016, 12: 41–49. http://dx.doi.org/10.6000/1927-5129.2016.12.06
[26] CHEN Y. Y., MA C. Y., WANG M. L., LU J. H., HU P., CHEN J. W., LI X., and CHEN Y. Five new ent-kaurane diterpenes from Annona squamosa L. pericarps. *Natural Product Research*, 2020, 34(15): 2243-2247. https://doi.org/10.1080/14786419.2019.1582048
[27] GEORGE V. C., KUMAR D. R. N., RAJKUMAR V., SURESH P. K., and KUMAR R. A. Quantitative assessment of the relative antineoplastic potential of the n-butanolic leaf extract of Annona Muricata Linn. in normal and immortalized human cell lines. *Asian Pacific Journal of Cancer Prevention*, 2012, 13: 699–704. https://doi.org/10.7314/APJCP.2012.13.2.699
[28] STROBER W. Trypan Blue Exclusion Test of Cell Viability. *Current Protocols in Immunology*, 2015, 111: 1–3. https://doi.org/10.1002/0471142735.ima03bs111
[29] PINTO M. F. S., FENSTERSEIFER I. C. M., MIGLIOLO L., SOUSA D. A., DE CAPDVILLE G., ARBOLEDA-VALENCIA J. W., COLGRAVE M. L., CRAIK D. J., MAGALHÃES B. S., DIAS S. C., and FRANCO O. L. Identification and structural characterization of novel cyclotide with activity against an insect pest of sugar cane. *Journal of Biological Chemistry*, 2012, 287: 134–147. https://doi.org/10.1074/jbc.M111.294009
[30] SINGLETON V. L., ORTHOFER R., and LAMUELA-RAVENTÓS R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 1998, 299: 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1
[31] BAJČAN D., HARANGOZO Ľ., HRABOVSKÁ D., and BONČÍKOVÁ D. Optimizing conditions for spectrophotometric determination of total polyphenols in

wines using Folin-Ciocalteu reagent. *Journal of Microbiology, Biotechnology and Food Sciences*, 2013, 2: 1271-1280. https://www.jmbfs.org/18_jmbs_bajcan2_fbp_f/?issue_id=1716&article_id=17

[32] PÜNTENER W. *Manual for Field Trials in Plant Protection*. Google Books. 1992. https://books.google.com.co/books/about/Manual_for_Field_Trials_in_Plant_Protect.html?id=hEWxnAEACAAJ&redir_esc=y

[33] BAKR E. *Abbot and Henderson-Tilton calculations.* http://www.ehabsoft.com/ldpline/onlinecontrol.htm

[34] EGYDIO-BRANDÃO A. P. M., NOVAES P., and SANTOS D. Y. A. C. Alkaloids from Annona: Review from 2005 to 2016. *JSM Biochemistry & Molecular Biology*, 2017, 4(3): 1031. https://www.researchgate.net/profile/Anary-Egydio/publication/322102874_Central_Bringing_Excellence_in_Open_Access_Alkaloids_from_Annona_Review_from_2005_to_2016/links/5a452acf0f7e9ba868a93161/Central-Bringing-Excellence-in-Open-Access-Alkaloids-from-Annona-Review-from-2005-to-2016.pdf

[35] CASSOU E., TRAN D. N., NGUYEN T. H., DINH T. X., NGUYEN C. V., CAO B. T., JAFFEE S., and RU J. *An overview of agricultural pollution in Vietnam: summary report 2017*. World Bank, Washington, District of Columbia, 2017. https://openknowledge.worldbank.org/handle/10986/29242

[36] KHALID M. A., & SHAHID S. M. A. Environmental Pollution and Climate Change Impacts on Human Health with Particular Reference to Brain: A Review. In: KHALID M. A., & SHAHID S. M. A. *Phytopharmaceuticals for Brain Health*. CRC Press, New York, 2017: 39-68. https://www.taylorfrancis.com/chapters/edit/10.1201/9781315152998-3/environmental-pollution-climate-change-impacts-human-health-particular-reference-brain-monowar-alam-khalid-syed-monowar-alam-shahid?context=ubx&refId=da23a50c-8997-4fa4-a745-27a38c90e327

[37] HAJEK A. E., & EILENBERG J. *Natural enemies: an introduction to biological control*. Cambridge University Press, 2018. https://doi.org/10.1017/9781107280267

[38] ZHOU K., GOODMAN C. L., RINGBAUER J., SONG Q., BEERNTSEN B., and STANLEY D. Establishment of two midgut cell lines from the fall armyworm, Spodoptera frugiperda (Lepidoptera: Noctuidae). *In Vitro Cellular & Developmental Biology-Animal*, 2020, 56(1): 10-14. https://doi.org/10.1007/s11626-019-00420-w

[39] VALENCIA J. W. A., BUSTAMANTE A. L. G., JIMÉNEZ A. V., and GROSSI-DE-SÁ M. F. Cytotoxic activity of fungal metabolites from the pathogenic fungus Beauveria bassiana: An intraspecific evaluation of beauvericin production. *Current Microbiology*, 2011, 63: 306–312. https://doi.org/10.1007/s00284-011-9977-2

[40] MINATEL I. O., BORGES C. V., FERREIRA M. I., GOMEZ H. A. G., CHEN C. Y. O., and LIMA G. P. P. Phenolic compounds: Functional properties, impact of processing and bioavailability. In: SOTO-HERNÁNDEZ M., PALMA-TENANGO M., and GARCÍA-MATEOS R. (eds.) *Phenolic Compounds - Biological Activity*. IntechOpen, London, 2017: 1-24. https://doi.org/10.5772/66368

[41] CHEN L., TENG H., XIE Z., CAO H., CHEANG W. S., SKALICKA-WONIAK K., GEORGIEV M. I., and XIAO J. Modifications of dietary flavonoids towards improved bioactivity: An update on structure–activity relationship. *Critical Reviews in Food Science and Nutrition*, 2018, 58(4): 513-527. https://doi.org/10.1080/10408398.2016.1196334

[42] KUNDU N., ROY S., MUKHERJEE D., MAITI T. K., and SARKAR N. Unveiling the interaction between fatty-acid-modified membrane and hydrophilic imidazolium-based ionic liquid: understanding the mechanism of ionic liquid cytotoxicity. *The Journal of Physical Chemistry B*, 2017, 121(34): 8162-8170. https://doi.org/10.1021/acs.jpcb.7b06231

[43] CORIA-TÉLLEZ A. V., MONTALVO-GÓNZALEZ E., YAHIA E. M., and OBLEDO-VÁZQUEZ E. N. Annona muricata: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arabian Journal of Chemistry*, 2018, 11(5): 662-691. https://doi.org/10.1016/j.arabjc.2016.01.004

[44] MUTLU ALTUNDAĞ E., YILMAZ A. M., KOÇTÜRK S., TAGA Y., and YALÇIN A. S. Synergistic induction of apoptosis by quercetin and curcumin in chronic myeloid leukemia (K562) cells. *Nutrition and Cancer*, 2018, 70(1): 97-108. https://doi.org/10.1080/01635581.2018.1380208

参考文:

[1] AHMED T.、SHAHID M.、NOMAN M.、NIAZI MBK、ZUBAIR M.、ALMATROUDI A.、KHURSHID M.、TARIQ F.、MUMTAZ R. 和 LI B. 对天然抗银芽孢杆菌进行生物勘探用于银纳米粒子的绿色合成和随后的抗菌和抗癌活性的菌株。高级研究杂志，2020，24：475-483。 https://doi.org/10.1016/j.jare.2020.05.011

[2] FERNANDES B.、VIDIGAL J.、CORREIA R.、CARRONDO M. J.、ALVES P. M.、TEIXEIRA A. P. 和 ROLDÃO A. 用于改进艾滋病病毒-堵嘴 VLP 生产的稳定昆虫细胞系的适应性实验室进化。生物技术杂志，2020，307：139-147。 https://doi.org/10.1016/j.jbiotec.2019.10.004

[3] VAUGHN J. L.、GOODWIN R. H.、TOMPKINS G. J. 和 MCCAWLEY P. 从昆虫草地贪夜蛾（鳞翅目；夜蛾科）建立两种细胞系。体外，1977，13：213-217。 https://doi.org/10.1007/BF02615077

[4] MISHRA V. 用于重组蛋白生产的商业杆状病毒表达载体系统综合指南。蛋白质和肽快报，2020，27(6)：529-537。 https://doi.org/10.2174/0929866526666191112152646

[5] KHATRI S.、PAKUWAL P. 和 KHANAL S. 尼泊尔玉米田秋粘虫虫害的综合虫害管理：综述。农业与环境科学档案，2020, 5(4): 583-591. https://doi.org/10.26832/24566632.2020.0504023

[6] NAGOSHI R. N.、NAGOSHI B. Y.、CAÑARTE E.、NAVARRETE B.、SOLÓRZANO R. 和 GARCÉS-CARRERA S. 厄瓜多尔秋粘虫（草地夜蛾）的遗传特征以及与区域种群的比较确定了可能的迁徙关系。普洛斯一号，2019, 14(9): e0222332。https://doi.org/10.1371/journal.pone.0222332

[7] MARTINELLI S.、CLARK P. L.、ZUCCHI M. I.、SILVA-FILHO M.、FOSTER J. 和 OMOTO C. 在巴西玉米和棉花田中收集的草地贪夜蛾（鳞翅目：夜蛾科）的遗传结构和分子变异性。昆虫学研究公报，2007，97：225-231。https://doi.org/10.1017/S0007485307004944

[8] THUMAR R. K., ZALA M. B., VARMA H. S., DHOBI C. B., PATEL B. N., PATEL M. B., 和 BORAD P. K. 对侵染玉米的秋粘虫、草地贪夜蛾 (杰·史密斯) 的杀虫剂评价。2020。

[9] KALLESHWARASWAMY C. M.、MARUTHI M. S. 和 PAVITHRA H. B. 玉米上入侵性秋粘虫草地贪夜蛾（草地夜蛾）(J. 乙.史密斯)（鳞翅目：夜蛾科）的生物学。印度昆虫学杂志，2018，80（3）：540-543。https://doi.org/10.5958/0974-8172.2018.00238.9

[10] BATEMAN M. L.、DAY R. K.、LUKE B.、EDGINGTON S.、KUHLMANN U. 和 COCK M. J. 评估用于管理非洲秋粘虫（草地夜蛾）的潜在生物农药选择。应用昆虫学杂志，2018, 142(9): 805-819。https://doi.org/10.1111/jen.12565

[11] LEMES A. R., MARUCCI S. C., COSTA JR. V.，等。从含有有效防治草地贪夜蛾的基因的苏云金芽孢杆菌中筛选菌株。Bt 研究，2015，6：8。

[12] DOS SANTOS C. A. M.、DO NASCIMENTO J.、GONÇALVES K. C.、SMANIOTTO G.、DE FREITAS ZECHIN L.、DA COSTA FERREIRA M. 和 POLANCZYK R. A. Bt 生物杀虫剂和索普达佐剂的兼容性。科学报告，2021, 11(1): 1-8。https://doi.org/10.1038/s41598-021-84871-w

[13] ALVES D. S.、MACHADO A. R. T.、CAMPOS V. A. C.、OLIVEIRA D. F. 和 CARVALHO G. A. 选择番荔枝科物种控制草地贪夜蛾（鳞翅目：夜蛾科）和杜盖蒂亚 Specticola 磁性刺血针的代谢分析经济昆虫学杂志，2016，109：649-659。https://doi.org/10.1093/jee/tov396

[14] KEDIA A.、PRAKASH B.、MISHRA P. K.、SINGH P. 和 DUBEY N. K. 植物药作为对菖蒲的合成杀虫剂的生态友好生物理性替代品。(鞘翅目: 布鲁奇科)——综述。食品科学与技术杂志，2015，52：1239-1257。https://doi.org/10.1007/s13197-013-1167-8

[15] SHANIBA V. S.、AZIZ A. A.、JOSEPH J.、JAYASREE P. R. 和 KUMAR P. M. 番荔枝根提取物衍生的生物银纳米颗粒的抗氧化和细胞毒性潜力的合成、表征和评估。集群科学杂志，2021. https://doi.org/10.1007/s10876-021-01981-1

[16] FREITAS A. F.、PEREIRA F. F.、FORMAGIO A. S. N.、LUCCHETTA J. T.、VIEIRA M. C. 和 MUSSURY R. M. 番荔枝属物种的甲醇提取物对草地贪夜蛾 (J.E.新热带昆虫学，2014，43：446-452。https://doi.org/10.1007/s13744-014-0225-x

[17] 国际商业银行哥伦比亚哥伦比亚有多少注册物种？2021. https://cifras.biodiversidad.co/

[18] FERRAZ R. P. C.、BOMFIM D. S.、CARVALHO N. C.、SOARES M. B. P.、PINHEIRO M. L. B.、COSTA E. V. 和 BEZERRA D. P. 绦虫(绦虫) 的叶子精油的细胞毒性特性。香精香料杂志，2014 年，29：228-232。https://doi.org/10.1002/ffj.3199

[19] OWOLABI M. S.、OGUNDAJO A. L.、DOSOKY N. S. 和 SETZER W. N. 来自尼日利亚巴达加里的番荔枝叶油的细胞毒性活性。美国精油和天然产品杂志，2013，1(1)：1-3。https://www.essencejournal.com/archives/2013/1/1/A/1

[20] WANG D. S.、RIZWANI G. H.、GUO H.、AHMED M.、AHMED M.、HASSAN S. Z.、HASSAN A.、CHEN Z. S. 和 XU R. H. 番荔枝：叶提取物对人类肿瘤细胞系的细胞毒性活性。巴基斯坦药物科学杂志，2014，27：1559-1563。

[21] NAIK A. V.、DESSAI S. N. 和 SELLAPPAN K. 番荔枝叶甲醇提取物对瑞士白化小鼠的艾氏腹水癌和道尔顿淋巴瘤腹水介导的肿瘤的抗肿瘤活性。利比亚医学杂志，2021, 16(1): 1846862. https://doi.org/10.1080/19932820.2020.1846862

[22] HAYKAL T.、NASR P.、HODROJ M. H.、TALEB R. I.、SARKIS R.、MOUJABBER M. N. E. 和 RIZK S. 番荔枝种子提取物激活白血病细胞中的外在和内在凋亡途径。毒素，2019，11(9)：506。https://doi.org/10.3390/toxins11090506

[23] BRÍGIDO H. P. C.、CORREA-BARBOSA J.、DA SILVA-SILVA J. V.、COSTA E. V. S.、PERCÁRIO S. 和 DOLABELA M. F. 番荔枝属（番荔枝科）的抗利什曼原虫活性。SN 应用科学，2020, 2(9): 1524. https://doi.org/10.1007/s42452-020-03340-7

[24] YAMTHE L. R. T., FOKOU P. V. T., MBOUNA C. D. J., KEUMOE R., NDJAKOU B. L., DJOUONZO P. T.,

MFOPA A. N., LEGAC J., TSABANG N., GUT J., ROSENTHAL P. J., 和 BOYOM F. F. 从安娜娜·穆里卡塔升. 和番荔枝.（番荔枝科）有效且选择性地抑制恶性疟原虫。药物，2015，2(2)：55-66。https://doi.org/10.3390/medicines2020055

[25] ALI S. S.、AHMAD S.、AHMED S. S.、RIZWANA H.、SIDDIQUI S.、ALI S. S.、RATTAR I. A. 和 SHAH M. A. 生物农药在田间条件下对茄子作物吸食害虫的效果。基础与应用科学杂志，2016，12：41-49。http://dx.doi.org/10.6000/1927-5129.2016.12.06

[26] CHEN Y. Y., MAC C. Y., WANG M. L., LU J. H., HU P., CHEN J. W., LI X., 和 CHEN Y. 来自番荔枝果皮的五种新的进入-考拉内二萜。天然产物研究，2020，34(15)：2243-2247。https://doi.org/10.1080/14786419.2019.1582048

[27] GEORGE V. C.、KUMAR D. R. N.、RAJKUMAR V.、SURESH P. K. 和 KUMAR R. A. 安娜·穆里卡塔·林恩正丁醇叶提取物相对抗肿瘤潜力的定量评估。在正常和永生化的人类细胞系中。亚太癌症预防杂志，2012，13：699-704。https://doi.org/10.7314/APJCP.2012.13.2.699

[28] STROBER W. 细胞活力的台盼蓝排除试验。当前免疫学协议，2015，111：1-3。https://doi.org/10.1002/0471142735.ima03bs111

[29] PINTO M. F. S., FENSTERSEIFER I. C. M., MIGLIOLO L., SOUSA D. A., DE CAPDVILLE G., ARBOLEDA-VALENCIA J. W., COLGRAVE M. L., CRAIK D. J., MAGALHÃES B. S., DIAS S. C., 和 FRANCO O. L. 鉴定和结构表征与活性新环浪潮防治甘蔗害虫。生物化学杂志，2012，287：134-147。https://doi.org/10.1074/jbc.M111.294009

[30] SINGLETON V. L.、ORTHOFER R. 和 LAMUELA-RAVENTÓS R. M. 通过福林 - 乔卡尔特试剂分析总酚和其他氧化底物和抗氧化剂。酶学方法，1998，299：152-178。https://doi.org/10.1016/S0076-6879(99)99017-1

[31] BAJČAN D.、HARANGOZO Ľ.、HRABOVSKÁ D. 和 BONČÍKOVÁ D. 使用福林-乔卡尔特试剂优化分光光度法测定葡萄酒中总多酚的条件。微生物学、生物技术与食品科学杂志，2013，2：1271-1280。https://www.jmbfs.org/18_jmbs_bajcan2_fbp_f/?issue_id=1716&article_id=17

[32] PÜNTENER W. 植物保护现场试验手册。谷歌图书。1992. https://books.google.com.co/books/about/Manual_for_Field_Trials_in_Plant_Protect.html?id=hEWxnAEACAAJ&redir_esc=y

[33] BAKR E. 方丈和亨德森 - 蒂尔顿计算。http://www.ehabsoft.com/ldpline/onlinecontrol.htm

[34] 来自安诺娜的 EGYDIO-BRANDÃO A. P. M.、NOVAES P. 和 SANTOS D. Y. A. C. 生物碱：2005 年至 2016 年的回顾。JSM 生物化学与分子生物学，2017，4(3)：1031。https://www.researchgate.net/个人资料/Anary-Egydio/publication/322102874_Central_Bringing_Excellence_in_Open_Access_Alkaloids_from_Annona_Review_from_2005_to_2016/links/5a452acf0f7e9ba868a93161-fromAlkaloids-from_Annona_Review_from_2005_to_2016/links/5a452acf0f7e9ba868a93161-fromAlkaloids-fromAlkaloids-from_Centralo1/A93161-fromAlkaloids-from_Central-0-Central-fromAlkaloids-from_Centralo

[35] CASSOU E.、TRAN D. N.、NGUYEN T. H.、DINH T. X.、NGUYEN C. V.、CAO B. T.、JAFFEE S. 和 RU J. 越南农业污染概览：2017 年总结报告。世界银行，华盛顿哥伦比亚特区，2017. https://openknowledge.worldbank.org/handle/10986/29242

[36] KHALID M. A. 和 SHAHID S. M. A. 环境污染和气候变化对人类健康的影响，特别是对大脑的影响：综述。在：KHALID M. A. 和 SHAHID S. M. A. 用于大脑健康的植物药物。CRC 出版社，纽约，2017：39-68。https://www.taylorfrancis.com/chapters/edit/10.1201/9781315152998-3/environmental-pollution-climate-change-impacts-human-health-particular-reference-brain-monowar-alam-khalid-syed-monowar-alam-shahid?context=ubx&refId=da23a50c-8997-4fa4-a745-27a38c90e327

[37] HAJEK A. E. 和 EILENBERG J. 天敌：生物防治简介。剑桥大学出版社，2018。https://doi.org/10.1017/9781107280267

[38] ZHOU K.、GOODMAN C. L.、RINGBAUER J.、SONG Q.、BEERNTSEN B. 和 STANLEY D. 从秋粘虫草地夜蛾（鳞翅目：夜蛾科）中建立两种中肠细胞系。体外细胞与发育生物学 - 动物，2020，56(1)：10-14。https://doi.org/10.1007/s11626-019-00420-w

[39] VALENCIA J. W. A.、BUSTAMANTE A. L. G.、JIMÉNEZ A. V. 和 GROSSI-DE-SÁ M. F. 病原真菌白僵菌的真菌代谢物的细胞毒活性：白僵菌素生产的种内评价。当前微生物学，2011，63：306-312。https://doi.org/10.1007/s00284-011-9977-2

[40] MINATEL I. O.、BORGES C. V.、FERREIRA M. I.、GOMEZ H. A. G.、CHEN C. Y. O. 和 LIMA G. P. P. 酚类化合物：功能特性、加工影响和生物利用度。在：SOTO-HERNÁNDEZ M.、PALMA-TENANGO M. 和 GARCÍA-MATEOS R.（编辑）酚类化合物 - 生物活性。英泰开放，伦敦，2017：1-24。https://doi.org/10.5772/66368

[41] CHEN L.、TENG H.、XIE Z.、CAO H.、CHEANG W. S.、SKALICKA-WONIAK K.、GEORGIEV M. I. 和 XIAO J. 改善膳食黄酮类化合物以提高生物活性：结构-活性关系的更新.食品科学与营养评论，2018，58(4)：513-527。 https://doi.org/10.1080/10408398.2016.1196334

[42] KUNDU N.、ROY S.、MUKHERJEE D.、MAITI T. K. 和 SARKAR N. 揭示脂肪酸改性膜与亲水性咪唑基离子液体之间的相互作用：了解离子液体细胞毒性的机制。物理化学杂志乙，2017，121(34)：8162-8170。 https://doi.org/10.1021/acs.jpcb.7b06231

[43] CORIA-TÉLLEZ A. V.、MONTALVO-GÓNZALEZ E.、YAHIA E. M. 和 OBLEDO-VÁZQUEZ E. N. 番荔枝：对其传统药用、植物化学物质、药理活性、作用机制和毒性的全面回顾。阿拉伯化学杂志，2018，11(5)：662-691。 https://doi.org/10.1016/j.arabjc.2016.01.004

[44] MUTLU ALTUNDAĞ E.、YILMAZ A. M.、KOÇTÜRK S.、TAGA Y. 和 YALÇIN A. S. 槲皮素和姜黄素在慢性粒细胞白血病 (钾 562) 细胞中协同诱导细胞凋亡。营养与癌症，2018，70（1）：97-108。 https://doi.org/10.1080/01635581.2018.1380208