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Tree Tobacco Extract (Nicotiana glauca) as a Plithogenic Bioinsecticide Alternative for Controlling Fruit Fly (Drosophila immigrans) using n-SuperHyperGraphs

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Abstract. Concern regarding the negative effects of synthetic pesticides has driven the development of bioinsecticides as sustainable alternatives. Consequently, *Nicotiana glauca* has gained prominence for its insecticidal properties due to phytochemical compounds effective against pests such as *Drosophila immigrans*. This study, therefore, evaluated the efficacy of *Nicotiana glauca* extract as a bioinsecticide by optimizing extraction parameters and analyzing its insecticidal action under laboratory conditions across eight combined treatments. For this purpose, multiple experimental combinations were analyzed through Plithogenic n-SuperHyperGraphs, allowing a multidimensional evaluation of the attributes and properties of each treatment. Results indicated that treatment with 96% ethanol, 48 hours of maceration, and fresh leaves maximized insecticidal metabolites and pest mortality. It is concluded that *Nicotiana glauca* represents a promising bioinsecticide alternative for sustainable agriculture, with optimal treatments for pest control.

Keywords: treatment efficacy, tree tobacco, bioinsecticide, plithogenic n-SuperHyperGraphs.

1 Introduction

In recent years, concern has grown regarding the effects of synthetic insecticides on the health of farmers and the safety of both human and animal food consumers [1]. This has driven the development of naturally-derived toxins for pest control, offering effective botanical alternatives for pests that have developed resistance to synthetic compounds [2]. The repeated use of synthetic agrochemicals not only increases producers' costs but also poses a public health risk. Consequently, the search for more sustainable and affordable alternatives for producers has intensified, promoting environmentally friendly options.

Among new alternatives, natural compounds have proven effective in reducing contamination and improving the health, productivity, and economic and social sustainability of farmers [3]. Plants synthesize chemical compounds as a defense mechanism against pests, and over 2,000 plant species with insecticidal properties have been identified. In the Americas, wild tobacco (*Nicotiana glauca*) stands out for its tolerance to drought and soil salinity and contains anabasine, an alkaloid toxic to insects, presenting a viable option for the production of natural insecticides [4][5]. This compound, with an action similar to organophosphate and carbamate insecticides, is highly toxic due to rapid dermal absorption, thus its use is recommended in open spaces [6].

Generally, plant extracts are obtained using polar solvents such as water and ethanol, which are suitable for extracting secondary metabolites and ensure a low toxicity level in bioinsecticide bioassays [7]. Extracting these compounds is particularly relevant in fruit farming, where pest control—such as for fruit flies of the *Anastrepha* genus—is one of the primary productivity challenges [8]. This pest causes crop losses that can reach between 30% and 50% in Latin America and up to 100% in some regions of Ecuador.

The fruit fly represents a global threat due to its adaptability and prolific life cycle, which can reach up to 12 generations per year under favorable conditions [9]. The larvae of this pest cause physical damage to the pulp, allowing microorganisms to enter, affecting post-harvest quality, and impeding fruit export due to phytosanitary restrictions [10]. These losses impact both local and international markets due to fruit decomposition [11], as fruits become colonized by fungi and bacteria after infestation.

Therefore, the present study focuses on evaluating the effectiveness of tree tobacco (*Nicotiana glauca*) extract as a bioinsecticide by optimizing extraction parameters and analyzing its composition and insecticidal action against *Drosophila immigrans*. To this purpose, eight treatments derived from combining the extract characteristics under laboratory conditions are proposed, along with the selection of the best treatment using Plithogenic n-SuperHyperGraphs to facilitate multidimensional evaluation based on the attributes and sub-attributes defined at each proposed vertex.

2 Materials and Methods

2.1 Plithogenic n-SuperHyperGraphs

Plithogenic n-SuperHyperGraphs were defined by Smarandache in the field of decision-making in [12] [13]

First, an n-SuperHyperGraph is defined as follows:

Given $V = \{V_1, V_2, \cdots, V_m\}$, where $1 \leq m \leq \infty$ is a set of vertices, containing *Single Vertices* that are classical, *Indeterminate Vertices* that are unclear, vague, partially known, and the *Null Vertices* that are empty or completely unknown [14].

$P(V)$ is the power set of V including $\emptyset$. $P^n(V)$ is the n-power set of V, which is defined recursively as follows:

$P^1(V) = P(V)$, $P^2(V) = P(P(V))$, $P^3(V) = P(P^2(V))$, … , $P^n(V) = P(P^{n-1}(V))$, for $1 \leq n \leq \infty$.
Where it is also defined as $P^0(V) = V$.

An n-SuperHyperGraph (*n-SHG*) is an ordered pair $n - SHG = (G_n, E_n)$, where $G_n \subseteq P^n(V)$ and $E_n \subseteq P^n(V)$, for $1 \leq n \leq \infty$. Such that, G_n is the set of vertices and E_n is the set of edges.

G_n contains all possible types of vertices in the real world:

- *Single Vertices* (the classics),
- *Indeterminate Vertices* (unclear, vague, partially known),
- *Null Vertices* (empty, totally unknown),
- *SuperVertex* (or *SubsetVertex*) contains two or more vertices of the above types put together as a group (organization).
- *n- SuperVertex* which is a collection of vertices, where at least one of them is a *(n-1) - SuperVertex,* and the others can be *r- SuperVertex* for $r \leq n - 1$.

E_n contains the following types of edges:

- *Single Edges* (the classics),
- *Indeterminate Edges* (unclear, vague, partially known),
- *Null Edges* (totally unknown, empty),
- *HyperEdge* (connecting three or more single vertices),
- *SuperEdge* (connecting two vertices, at least one of them is a SuperVertex),
- *n- SuperEdge* (connecting two vertices, at least one of them is an n- SuperVertex and may contain another that is an r-SuperVertex with $r \leq n$).
- *SuperHyperEdge* (connects three or more vertices, where at least one of them is a SuperVertex),
- *n-SuperHyperEdge* (contains three or more vertices, at least one of which is an n-SuperVertex and may contain an r-SuperVertex with $r \leq n$),
- *MultiEdge* (two or more edges connecting the same two vertices),

- *Loop* (an edge that connects an element to itself),

Graphs are classified as follows:

- Directed Graph (the classic one),
- Undirected Graph (the classic one),
- Neutrosophic Directed Graph (partially directed, partially undirected, partially indeterminate directed).

Within the framework of the theory of Plithogenic n-SuperHyperGraphs, there are the following concepts:

Enveloping vertex: A vertex representing an object comprising attributes and sub-attributes in the graphical representation of a multi-attribute decision-making environment.

SuperEnveloping vertex: An enveloping vertex comprises of SuperHyperEdges.

Dominant Enveloping Vertex: An enveloping vertex that is with dominant attribute values.

Dominant Super Enveloping Vertex: A super enveloping vertex with dominant attribute values.

Dominant Enveloping Vertex is classified into *input*, *intervene,* and *output* based on the nature of the object's representation.

Plithogenic Connectors: The connectors associate the input enveloping vertex with the output enveloping vertex. These connectors associate the effects of input attributes with output attributes and these connectors are weighted by plithogenic weights.

3 The Study

Case study: This study evaluated the effectiveness of *Nicotiana glauca* (false tobacco) extracts for pest control by using fresh and dried leaves macerated in 96% ethanol and distilled water for 24 and 48 hours. The analysis of treatments was conducted using the plithogenic n-SuperHyperGraphs method, identifying the optimal combination to achieve the highest concentration of insecticidal metabolites. For this purpose, fly infestation was monitored in specific areas using traps and fruit sampling. Larvae were collected from infested guavas, and their development was managed under suitable conditions until reaching the adult stage.

Weighting and consensus of the results were achieved through the application of the Plithogenic n-SuperHyperGraphs method, which facilitates the multidimensional evaluation of treatments in complex studies. This method allowed for the integration and weighting of evaluations of multiple parameters, considering both treatment variability and the relative importance of each variable in the insecticidal effect (see Table 1).

Table 1: Plithogenic evaluation criteria. Source: Own elaboration.

Vertex Sub Sub-Attributes	Description	Plithogenic criterion (PC)
NO	The state is deficient, not meeting the basic requirements established.	Non-optimal
LO	State slightly below an acceptable standard, with evident issues.	Low
ST	Meets minimum quality standards, though still susceptible to variations.	Stable
GO	State above minimum, with stable and acceptable quality.	Good
OP	The highest level of quality, achieving ideal parameters without variation.	Optimal

Additionally, component analyses were conducted to identify and weigh the most significant variables, enabling greater precision in selecting the optimal treatment. Feedback from participating experts was incorporated through a consensus aggregation process to ensure the applicability of results in bioinsecticide applications.

In this study, the Plithogenic n-SuperHyperGraphs methodology allows for a detailed analysis of the combinations of *Nicotiana glauca* extract characteristics to optimize its effectiveness as a bioinsecticide (see Table 2). The n-SuperVertices organize each main attribute (type of solvent, maceration time, and leaf condition) into SuperVertices and Vertices that specify experimental values (such as distilled water or 96% ethanol). The SuperVertex defines the treatments (T1 to T8) by representing specific interactions among these values; for example, T1 includes distilled water, 24 hours of maceration, and fresh leaves (see Table 3). This structure enables an analysis of variable interactions and facilitates the selection of treatment configurations, demonstrating potential for bioinsecticide use.

Table 2: Extract characteristics. Source: Own elaboration.

n-SuperVertex (NSV)	SuperVertex (SV)	Vertex (V)	Vertex Attributes (V)
Extract characteristics (V_1)	Solvent type ($SV_{1,2}$)	Distilled water (V_1)	Purity, pH, conductivity, temperature, and volume.
		Ethanol 96% (V_2)	pH, polarity, temperature, and volume
	Maceration time ($SV_{3,4}$)	24 hours (V_3)	Standard or non-standard conditions
		48 hours (V_4)	Standard or non-standard conditions
	Leaf status ($SV_{5,6}$)	Fresh (V_5)	Leaf moisture and integrity
		Dry (V_6)	Leaf moisture and integrity

Table 3: Proposed treatments derived from the grouping of Vertex into SuperVertex. Source: Own elaboration.

Treatments	HyperEdge (HE)	SuperVertex (SV)	Vertices
T1	$(HE_{1,3,5})$	$(SV_{1,3,5})$	$\{V_1, V_3, V_5\}$
T2	$(HE_{1,3,6})$	$(SV_{1,3,6})$	$\{V_1, V_3, V_6\}$
T3	$(HE_{1,4,5})$	$(SV_{1,4,5})$	$\{V_1, V_4, V_5\}$
T4	$(HE_{1,4,6})$	$(SV_{1,4,6})$	$\{V_1, V_4, V_6\}$
T5	$(HE_{2,3,5})$	$(SV_{2,3,5})$	$\{V_2, V_3, V_5\}$
T6	$(HE_{2,3,6})$	$(SV_{2,3,6})$	$\{V_2, V_3, V_6\}$
T7	$(HE_{2,4,5})$	$(SV_{2,4,5})$	$\{V_2, V_4, V_5\}$
T8	$(HE_{2,4,6})$	$(SV_{2,4,6})$	$\{V_2, V_4, V_6\}$

Interaction Analysis: The statistical results in Table 4 indicate a significant difference in the SuperVertex ($SV_{1,2}$) and in the interaction of the SuperVertices ($SV_{1,2} \leftrightarrow SV_{5,6}$). Meanwhile, the following SuperVertices (SV_{34}, $SV_{5,6}$) do not show significant differences, nor do the interactions between ($SV_{1,2} \leftrightarrow SV_{3,4}$) and ($SV_{34} \leftrightarrow SV_{5,6}$). Additionally, the interaction among the SuperVertices comprising the treatments ($SV_{1,2} \leftrightarrow SV_{3,4} \leftrightarrow SV_{5,6} \leftrightarrow SV_{1,2}$) shows no significant differences. Thus, a 2.40% coefficient of variation is obtained, indicating a low error rate in the study and demonstrating effective handling of the experimental data.

Table 4: ADEVA from the tobacco extracting tree (Nicotiana glauca). Source: Own elaboration.

VF	SC	GL	CENTIMETER	F	p Value	
$SV_{1,2}$	6905.08	1	6905.08	95703.84	<0.0001	**
SV_{34}	0.39	1	0.39	5.41	0.0233	ns
$SV_{5,6}$	0.01	1	0.01	0.09	0.7598	ns
$SV_{1,2} \leftrightarrow SV_{3,4}$	0.13	1	0.13	1.85	0.1787	ns
$SV_{1,2} \leftrightarrow SV_{5,6}$	4.55	1	4.55	63.06	<0.0001	**
$SV_{34} \leftrightarrow SV_{5,6}$	0.01	1	0.01	0.16	0.6943	ns
$SV_{1,2} \leftrightarrow SV_{3,4} \leftrightarrow SV_{5,6} \leftrightarrow SV_{1,2}$	0.39	1	0.39	5.41	0.0233	ns
REPLICA	1.72	2	0.86	11.95	<0.0001	**
ERROR	4.47	62	0.07			
TOTAL	6916.76	71				
% CV		2.40				

VF	SC	GL	CENTIMETER	F	p Value
** significant with p≤0.001, ns: not significant, CV: Coefficient of variation.					

Once the treatments have been defined, the most effective bioinsecticide is determined by measuring the new properties obtained and the connection through HyperEdges (HE). The following table shows the vertices, attributes, and sub-attributes to measure the effectiveness of the treatments (see Table 5). In addition, it can be observed that the sub-attributes V_{721}, V_{811}, and V_{821} are input envelope vertices, while V_{723}, V_{812}, and V_{822} are the output envelope vertices. As for the rest of the envelope vertices, they are considered as intermediate.

Table 5: Vertices, attributes, and sub-attributes to measure the efficacy and resistance of the bioinsecticide. Source: Own elaboration.

Vertex	Vertex Attributes	Vertex Sub Attributes	Vertex Sub Sub-Attributes
Dosage and duration of treatment (V_7)	Extract concentration (dilutions in bioassays) (V_{71})	5% (V_{711})	• Plithogenic criterion (PC) (V_{7111})
		10% (V_{712})	• CP (V_{7121})
		20% (V_{713})	• CP (V_{7131})
	Application time (duration in minutes) (V_{72})	Initial phase: 3 to 10 minutes (V_{721})	• CP (V_{7211})
		Intermediate phase: 10 to 20 minutes (V_{722})	• CP (V_{7221})
		Advanced phase: 20 to 30 minutes (V_{723})	• CP (V_{7231})
Performance and resistance to bioinsecticide (V_8)	Pest mortality (V_{81})	Initial Phase (V_{811})	• Low (V_{8111}) • Moderate (V_{8112}) • High (V_{8113})
		Final Phase (V_{812})	• Low (V_{8121}) • Moderate (V_{8122}) • High (V_{8123})
	Tolerance levels (V_{82})	Initial Phase (V_{821})	• Low tolerance (V_{8211}) • Moderate tolerance (V_{8212}) • High tolerance (V_{8213})
		Final Phase (V_{822})	• Low tolerance (V_{8221}) • Moderate tolerance (V_{8222}) • High tolerance (V_{8223})

3.1 Analysis between treatments and n-SuperVertex V_7 and V_8.

The results in Table 6 indicate that treatment T8 presents the highest Brix concentration, making it the most suitable for application. The extract consists of 96% ethanol with a 48-hour maceration. Meanwhile, using fresh leaves, treatment T7, which also contains 96% ethanol with a 48-hour maceration, follows closely. The organic solvent ethanol is polar and shows a greater interaction with polar organic compounds compared to water.

Table 6: Tukey test at 5% extract. Source: Own elaboration.

Treatments	Mean	Ranks
T1	0.98	A
T2	1.33	AB
T3	1.62	BC
T4	1.73	C
T5	20.64	D

Treatments	Mean	Ranks
T6	20.88	DE
T7	21.19	EF
T8	21.30	F

Therefore, the particularity of the two mentioned treatments lies in the use of fresh leaves, which prove to be more effective than dried ones. The progressive drying of the plant removes the bitter taste and, in many cases, also the secondary metabolites. The analysis of the variance of dead flies is presented next in Table 7.

Table 7: ADEVA dead flies. Source: Own elaboration.

VF	SC	GL	CENTIMETER	F	p Value	
DOSE	135.85	2	67.92	28.89	0.0002	**
TIME	424.28	9	47.14	20.05	0.0001	**
DOSE*TIME	46.39	11	4.22	1.79	0.2082	ns
REPETITION	6.19	2	3.10	1.32	0.3202	ns
ERROR	18.81	8	2.35			
TOTAL	631.52	32				
% CV		19.46				
**significant with p≤0.001, ns: not significant, CV: Coefficient of variation.						

According to Table 8, there is a significant difference in "dose" and "time," while the interaction of "dose-time" is not significant. This demonstrates that the different doses of the tree tobacco extract do not affect the fly mortality time variable (Drosophila immigrans). The coefficient of variation is 19.46%, confirming that the development and process of the bioinsecticide regarding the "dose" and "time" parameters were correctly adapted to the study. A Tukey test at 5% was then performed for the dose variable, as shown below in Table 8.

Table 8: Tukey test at 5% for the dose. Source: Own elaboration.

Dose	Mean	Ranks
5%	4.67	C
10%	8.50	BC
20%	9.67	A

Table 8 shows three ranges of significance, where the 20% concentration yields the best results in controlling the attack of the fly (Drosophila immigrans), followed by the 10% dose, and the statistically lowest range corresponds to the 5% concentration. The study determined that higher concentration results in greater effectiveness of the bioinsecticide. Previous studies mention that, after 24 hours of aqueous application of tobacco (N. tabacum) at a 10% concentration, 50% of the flies are killed. This indicates that the increase in concentration is directly proportional to fly mortality.

Within the results of Table 9, 10 different "application times" of the tree tobacco bioinsecticide extract are presented, showing 4 ranges of significance, with the most significant being 20 minutes (A). The times of 2, 9, and 15 minutes show no difference among them, and shorter times exhibit a low probability of mortality.

Table 9: Tukey test at 5% for time. Source: Own elaboration.

Time	Mean	Ranks
3	3.50	D
4	5.00	D

Time	Mean	Ranks
5	5.20	D
6	5.50	D
8	5.60	D
12	6.75	CD
9	9.00	ABC
15	11.67	BC
19	13.00	AB
20	17.50	A

Once the relationships and interactions of each vertex represented in Table 5 have been defined, the evaluations for each treatment are represented. These treatments are conceptualized as the union of vertices that form SuperVertices and n-SuperVertices within the Plithogenic n-SuperHyperGraphs, allowing each treatment to be represented as a complex system. This system groups attributes and sub-attributes, such as extract concentration, application time, pest mortality, and tolerance levels, facilitating the analysis of the interactions between these elements and their contribution to overall performance. The treatments are configured as composite entities, whose performance depends on the interaction of their specific qualities. Through the union of several SuperVertices, n-SuperVertices are formed, adding complexity to the structure and allowing exploration of how different treatment configurations complement or compete according to the evaluated criteria (see Table 10).

Table 10: Treatment evaluation based on the connection between vertices, attributes, and sub-attributes. Source: Own elaboration.

Vertex Attributes	Vertex Sub Attributes	Vertex Sub Sub-Attributes	T1	T2	T3	T4	T5	T6	T7	T8
Extract concentration (V_{71})	5% (V_{711})	CP (V_{7111})	NO	NO	NO	NO	NO	BA	BA	BA
	10% (V_{712})	CP (V_{7121})	ES	ES	ES	ES	ES	ES	ES	ES
	20% (V_{713})	CP (V_{7131})	BU	BU	BU	ES	ES	ES	BU	OP
Application time (V_{72})	Initial phase: 3 to 10 minutes (V_{721})	CP (V_{7211})	BA	BA	ES	ES	ES	ES	OP	BU
	Intermediate phase: 10 to 20 minutes (V_{722})	CP (V_{7221})	ES	ES	ES	BU	BU	BU	OP	OP
	Advanced phase: 20 to 30 minutes (V_{723})	CP (V_{7231})	OP	OP	OP	OP	OP	OP	OP	OP
Pest mortality (V_{81})	Initial Phase (V_{811})	Low (V_{8111})	BA	BA	BA	BA	BA	BA	ES	ES
		Moderate (V_{8112})	ES	ES	ES	ES	ES	ES	ES	BU
		High (V_{8113})	BU	BU	BU	BU	BU	BU	OP	OP
	Final Phase (V_{812})	Low (V_{8121})	BA	BA	BA	BA	BA	BA	OP	BU
		Moderate (V_{8122})	ES	ES	ES	ES	ES	ES	OP	BU
		High (V_{8123})	BU	BU	BU	BU	OP	OP	OP	OP
Tolerance levels (V_{82})	Initial Phase (V_{821})	Low tolerance (V_{8211})	B	B	B	B				
		Moderate tolerance (V_{8212})					M	M	M	M
		High tolerance (V_{8213})								
	Final Phase (V_{822})	Low tolerance (V_{8221})								
		Moderate tolerance (V_{8222})	M	M		M				
		High tolerance (V_{8223})			A		A		A	A

The connections between SuperVertices and n-SuperVertices are established based on the affinity of their attributes, revealing patterns of efficacy or compatibility between treatments. This approach facilitates the identification of treatment groupings with high effectiveness under specific conditions. Meanwhile, the plithogenic connections, highlighting the extract concentration, application time, pest mortality, and tolerance, emphasize that T7 is the optimal treatment, followed by T8. Both treatments stand out due to the optimization of quality and effectiveness criteria, supporting their selection as the most suitable based on their specific interactions and overall performance.

Plant extracts, such as the ethanolic extract of Nicotiana glauca (T7), present key physicochemical characteristics for evaluating their quality. In this case, the total solids reach 20.25%, the pH is 5.92, the acidity is 0.05%, and the density is 0.8467 g/mL, meeting the established parameters. The phytochemical profile highlights the predominant presence of alkaloids and phenolic compounds, with pharmacological and antioxidant properties, suggesting their potential as a natural insecticide and for health purposes. Factors such as genetics, environmental conditions, and the extraction process influence the quality of bioactive compounds. Thus, the need to control these aspects to obtain high-quality extracts, essential for various industrial applications, is emphasized. Regarding maceration time, this should be considered in future research, as appropriate maceration for a bioinsecticide is generally done over a period of six days, during which the plant's secondary metabolites remain unchanged. Furthermore, although maceration is an efficient process for extracting active principles from plants, its effectiveness decreases significantly over time.

4. Conclusion

This study demonstrated the effectiveness of Nicotiana glauca extracts as bioinsecticides, highlighting that the combination of 96% ethanol, 48 hours of maceration, and fresh leaves optimized the concentration of insecticidal metabolites, thereby maximizing pest mortality. Similarly, the use of the Plithogenic n-SuperHyperGraphs method allowed for precise analysis of the interactions between experimental variables and the selection of optimal configurations, confirming that the solvent and maceration time are dominant enveloping vertices in the efficacy of the extracts. These results emphasize the potential of Nicotiana glauca as a sustainable and effective alternative in biological pest control. Furthermore, it is suggested that future research evaluate its applicability under field conditions and for other pest species, promoting its integration into sustainable agricultural strategies.

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