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Biocidal Effect of the Ethanolic Extract of Nerium Oleander, in the Control of Larvae of Prodiplosis Longifila Infested in Asparagus

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Abstract

The objective of the study was to evaluate the biocidal effect of the ethanolic extract of Nerium oleander leaves in the control of larvae of Prodiplosis longifila infested in asparagus shoots. Phytochemical screening was carried out using the Miranda & Cuellar method. The cardiotonic glycosides were obtained using the partition coefficient method. The evaluation of the biocidal effect was performed every 4, 12, 24 and 48 hours following the experimental design by randomized complete blocks with a factorial arrangement of 5x4x1, facing concentrations that were 100%, 60% and 40% volume/volume. The ethanolic extract, reported the presence of lactones, steroids, catechins, reducing sugars, resins, saponins, polyphenols, flavonoids, cardiotonic glycosides and leucoanthocyanidins. The concentration of the extract at 60% volume/volume showed a mortality rate of 40.5% of larvae of Prodiplosis longifila

Keywords: Efecto Biocida, Nerium Oleander, Prodiplosis Longifila, Valor Letal, Control Biológico

Introduction

The control of noctuids in current asparagus production in Peru is based mainly on chemical control and monitoring through some tools, which do not lead to sustainable management of populations and solving the export problem. This at the level of small producers conditions a situation of productive inefficiency. In asparagus fields throughout the country, methodical and sustained biological information on the populations has not been defined, so control is not efficient or sustainable either. The management components in use have not been adequately validated, including the inefficient evaluation and monitoring system, except for some isolated and specific investigations, focused on control rather than management.

However, with the recovery of the international position of Peruvian asparagus, the need to eliminate fumigation with methyl bromide has been resumed in recent years through concerted work between the National Agrarian Health Service (Senasa), the companies agro-exporters and the inclusion of small producers, for the development of a national program for integrated pest management in this crop, standardized and efficient. This involves constant contact and monitoring of the process by the phytosanitary entities of the destination countries such as APHIS (the US Animal and Plant Health Inspection Service) [1].

Recently, P. longifila has been included in a list of organisms harmful to tomatoes, with risks of being introduced to Europe through fruit import (Grousset et al. 2015), which could become a phytosanitary alert, potentially restrictive of imports of that and other agricultural products. An important aspect raised by these abrupt changes in the status of a species as a pest, due to marked and atypical population increases, has to do with the factors that may have produced them. This imposes the need to review the changes that have occurred in agronomic management and especially with the use of insecticides since the entry of organosynthetics into the market (1947), which, from very early on, caused serious problems in pest management in

that region, due to its "indiscriminate" use (Herrera 2010). The temporal continuity between the previous agronomic events and the beginning of the problems caused by P. longifila, suggests that this was a case of a "secondary or potential pest" of high status due to imbalances in the control by its natural enemies, as a result of said use of insecticides to control other species considered "pests" [2].

With the purpose of controlling pests in agriculture, the use of pesticides has increased indiscriminately, which causes a problem of biological imbalance in the environment by destroying natural controllers [3, 4]. Despite the damage, the

Agrochemicals continue to be used, harming not only the environment but also the safety of products obtained with chemical residues [5].

Asparagus, Asparagus officinalis, is a perennial vegetable that is highly appreciated in the diet, with Peruvian asparagus being the favorite, due to its color, aroma and texture [6]. The first producer of asparagus in the world is China with 70,000 hectares, followed by Peru, which has a cultivated area of 25,000 hectares, with production throughout the year, of which 65% is white asparagus and 35% is green asparagus [7].

The asparagus crop is attacked by pests and diseases. Prodiplosis longifila, a small, yellowish leaden fly approximately 1.7 mm in length, is a polyphagous pest that causes great loss in daily harvest volume, which results in an economic deficit for farmers; and in the case of asparagus at harvest time [8]. Its small larvae are found inside the bracts of asparagus spears, which it twists and causes loss in yield, quality and competitive price and state that P. longifila. was first reported in Florida. USA, by Raine W., 1934, feeding on wild cotton and were described and identified taxonomically by Gagné, 1986 [9, 10]. The key pest that affects asparagus cultivation is P. longifila, called "bud fly", whose management and control It is difficult given its short biological cycle and acquired resistance to insecticides [11].

To control P. longifila in asparagus cultivars, chemical control is used almost exclusively, such as chlorpyrifos and methiomyl, two chemical insecticides that contaminate the soil, the environment with toxic substances and They can even cause damage to the health of workers [12-14]. An alternative with less agricultural practice to control the pest is the use of traps such as: water, color, pheromone traps, baits and plant extracts [15]. When it comes to biodegradable and environmentally friendly plant extracts, it is necessary to look for new alternatives, such as Nerium oleander extract.

In Trujillo, Peru; Pink laurel plants are grown as ornamental shrubs in parks and on the city's main avenues. N. oleander contains secondary metabolites with a chemical structure similar to digoxin, a recognized toxic digitalis glycoside; They are generally low molecular weight compounds [16]. Secondary metabolites are not important for the primary metabolism of the plant, but in many cases they are of great importance for the survival of the plant in its environment [17]. N. oleander contains a mixture of cardiotonic glycosides such as neriantin, neriantogenin, neriin, oleandrin, pseudocuranin and strophantin; among them oleandrin, which turns out to be one of the most poisonous components, being considered an extremely toxic plant high [18, 9]. Of the isolated cardiac glycosides, oleandrin turns out to be of greater toxicity [19]. Oleandrin rotates the plane of light to the left and has a molecular weight of 576.7, represented by the formula C32H48O9 [20, 21].

In humans and animals, the pharmacological and toxic effect of Nerium oleander leaves has been proven, but not The toxic effect as an extract has been proven in the control of Prodiplosis longifila larvae infested in asparagus shoots [22-25].

With this information, the objective of this work was to verify the lethal effect of the ethanolic extract obtained by maceration of N. oleander leaves on P. longifila larvae using infested asparagus spears, to control the insect Pest.

Material and Methods

Biological Material

N. oleander leaves were collected from the University City of the National University of Trujillo, La Libertad region. Peru, located at an altitude of 34 meters above sea level. P. longifila larvae obtained from infested asparagus spears from the CESCAM experimental area of the National University of Trujillo and the "Libertad" Farm of the Virú Province, La Libertad Region.

Figure 1: Larva de P. longifila en turiones de espárragos.

Processes to Obtain the Biocide

Pharmacognostic Study

With the help of sterile gloves, leaves were collected from the central part of pink laurel branches in the morning. The plant drug was selected to separate damaged and growing leaves. A complete specimen was taken to the Truxillense Herbarium of the National University of Trujillo and deposited with the code 58167. The selected leaves were spread on kraft paper placed on a table to be dried under shade at room temperature for 60 days. The leaves were removed once a week to achieve uniform desiccation; After time, craft paper bags were assembled and placed in the SD 343 oven at a temperature of 40 °C for 24 hours. Once the leaves were dried, they were ground in a mechanical mill to the appropriate particle size. The pulverized material was sieved (particle size 5 mm), and

It was properly stored in amber bottles in a place without humidity and direct light, until use. In a two-liter amber bottle, 250 g of pulverized drug material and 1.2 L. of 96° GL ethanol were added. The extract was macerated for 15 days, shaking it once a day. Then extract was filtered through a Thomas scientific Mod 5KH33DN68 vacuum pump and concentrated in a rotary evaporator Heidolph hei vap advantage 240 L up to 2/4 of the total volume. With the concentrated extract, the method of Miranda & Cuellar was used to prepare the dichloromethane, methanolic and aqueous extracts; which were then identified through different coloring and/or precipitation reactions in each extract [26].

Separation of Cardiotonic Glycosides by the Partition Coefficient Method

Five settling bulbs were placed on tripods and 100 mL of hexane was added to each settling bulb. 50 mL of ethanolic extract was taken and diluted with 50 mL of distilled water, transferring the mixture to the first decanting bulb, then shaking gently and letting it rest for 3 minutes. The mixture formed two phases: an immiscible phase made up of cardiotonic glycosides and the other phase with the other metabolites, chlorophyll and hexane.

Subsequently, the lower part with the cardiotonic glycosides was decanted into a 250 mL flask, listing them respectively. The upper phase, which contained the emulsifying mixture, was transferred to the second bulb containing 100 mL of hexane, stirred and allowed to decant. The lower phase of the second settling bulb was separated into a second numbered flask. The process continued only until the third decanting pear in which very little amount of cardiotonic glycosides was appreciated. The greatest amount of cardiotonic glycosides were extracted in the first decantation bulb.

Evaluation of the Biocidal Effect of Nerium Oleander Extract

The experimental design used in the research to determine the toxic effect of the ethanolic extract obtained from dry leaves of N. oleander on asparagus shoots infested with P. longifila, was carried out using a complete randomized block design, with a 5x4x1 factorial arrangement, where there were 5 treatments, 4 were the experimental repetitions for each treatment and 1 was the biological cycle of P. longifila due to the need to exercise mortality control, given the existence of larval heterogeneity in the instars of the biological unit in each asparagus shoot. The experimental treatments consisted of three concentrations of the bioinsecticide given in volume/volume percentages of 40%, 60% and 100%, as well as distilled water representing the absolute control and 96° GL ethanol. as a commercial witness. The evaluation of the biocidal lethal effect of the ethanolic extract on P. longifila larvae was carried out using Model Nz 1930 stereoscope. Each batch was evaluated at 4, 12, 24, and 48 hours, after spraying the extract with a manual atomizer at the Asparagus spears infested with P. longifila. The evaluation of the optimal concentration was performed statistically by averaging the sum averages of the live and dead units of the biological sample in each block. The verification of the lethal effect of the ethanolic extract by ingestion or contact was carried out using an experimental block to

each concentration of 40%, 60% and 100% of the extract, with 20 biological units (P. longifila larvae) which were sprayed with the biocidal extract, evaluating the effect at 15 and 30 min. at each concentration.

Results and Discussion

The external macromorphological study of the leaves of Nerium oleander was carried out using the perception method [27].

Table 1: Macromorphological characteristics of Nerium oleander leaves

Shape	**Limbo**	Falcada
	Smooth	Edge
	Acute	Apex
	Base	Cuneated
	Petiole	Short
	innervation	Pignated
	Rough	
Surface	Flexible	
Consistency	3.5 cm wide	
Average leaf measurements	21cm long	

The perception method allows the management of all the sensations that bring together the sense of observation for the study of research and its significance in perception in the face of information.

Table 2: Physicochemical characteristics of the bioinsecticide obtained

Characteristics	Most common uses
Líquido color verde	Bioinsecticida
Densidad: 0.85 gr/cm3	Extracto biodegradable
Punto Ebull: 87.3 (20 °C)	Insecticida ecológico
Indice de Refrac: ND20 = 1.732	

The physical-chemical characteristics are intrinsic properties of the biocide obtained from the dried hours of Nerium Oleander.

The identification of the phytoconstituents was determined through chemical reactions of coloration and/or precipitation, following the method described by Miranda and Cuellar for increasing polarity, using as solvents: dichloromethane, methanol and distilled water. The phytochemical screening of the ethanol extract, obtained by maceration of dry Nerium oleander leaves, followed an order of increasing polarity; because the cells from which the phytoconstituents are extracted constitute internal hydrophilic systems, immersed within a vesicle, which consists of a lipophilic membrane. When the leaves are sprayed, cell walls and membranes are broken, leaving the vesicles that store the active ingredients or metabolites free.

secondary that the plant produces for its protection [28]. From the ethanolic extract, three extracts were prepared: methanolic, dichloromethane and aqueous, identifying phytoconstituents such as lactones, steroids, catechins, reducing sugars, resins, saponins, polyphenols, flavonoids, cardiac glycosides and leucoanthocyanidins.

Table 3: Preliminary phytochemical march of Nerium oleander leaves

Metabolito	Ensayo	Extracto Diclorometano	Extracto metanólico	Extracto Ácuoso
Alcaloides	Dragendorf			
	Mayer			
	Wagner			
	Hager			
Lactonas (Cumarinas)	Baljet	+	++++	+
Triterpenos/esteroides	Liebermann-Burchard	+	+	
Catequinas			+	
Resinas			+	
Azucares reductores	Fehling		+	
Saponinas	Espuma			+
Polifenoles	Cloruro férrico		+	+
Aminoácidos	Ninhidrina			+
Quinonas	Bornträger	+		
Flavonoides	Shinoda		+	
Glicósidos cardiotónico	Kedde		++++	
Antocianidinas	Rosenhein			
Taninos	Gelatina			
Leucoantocianinas				+

Leyenda: + = Intensidad baja, ++++ = Intensidad alta

The preparation of the dichloromethane, methanolic, and aqueous portions of the ethanolic extract allowed us to know, through polar affinity, whether the oleandrin that is part of the cardiotonic glycosides was present in the leaves of Nerium oleander. From the evaluation of the phytoconstituents, it was observed that pink bay leaves have a greater amount of lactones and total cardiotonic glycosides, corroborating the presence of oleandrin, which has these metabolites as its main components. The evaluation of the best lethal concentration of the ethanolic extract applied to P. longifila larvae allowed us to know that 100% is the optimal concentration with 41.86% mortality, but for economic reasons, 60% is the best concentration, having results of 40.38% mortality, very close to 100% concentration. The results of the toxic effect are shown in the following table.

Table 4: Deadly toxic effect of the extract against Prodiplosis longifila larvae

The determination of the biocidal effect by ingestion or by contact was carried out using 20 larvae of P. longifila for each concentration of 40%, 60% and 100% of the extract, resulting in: by contact, analyzed in a time of 15 min , where the ethanolic extract was sprayed directly on larvae that were in the bracts of the asparagus shoots, the result showed that the larvae had movement, with which it was concluded that P. longifila does not die by contact. Regarding the ingestion analysis, carried out with 20 P. lonfigila larvae, where the extract was sprayed directly on the bracts of the turions with larvae, after 30 min. They begin to lose mobility, so it was concluded that P. longifila dies from ingestion by sucking the sap mixed with biocide in the asparagus. The spears of asparagus are covered by bracts on the upper part; Those closest to the tip of the turion are more closed and make it difficult for the biocide to enter, which did not allow for a better lethal effect against P. longifila larvae found in that area. The use of the extract with an optimal concentration of 100% can be contrasted with the best treatment at 100% concentration found by Alarcon & Machado in their research with extracts of poisonous plants for pest control [28]. The percentage of mortality of Prodiplosis longifila larvae with the Nerium oleander extract, represented by 41.86%, is greater than the 40.30% determined by Preciado, using a neem extract [29]. In the statistical analysis, the analysis of variance and Duncan were used in which the three doses 40%, 60%, 100% and the controls water and ethanol were evaluated on the abscissa axis.

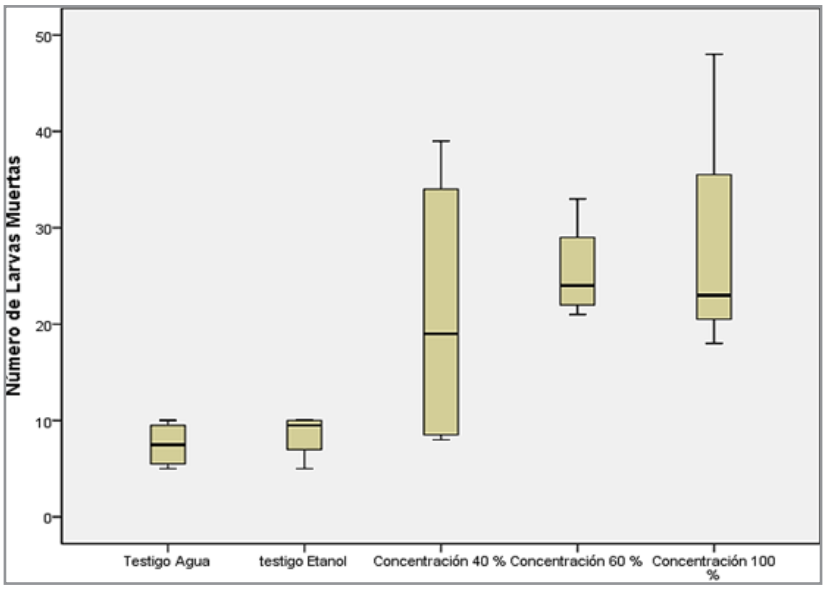

Figure 2: Concentración del Extracto

The logit transformation was considered in the response, considering the proportion of dead larvae found in each experimental unit. The dependent variables were used: dead and live larvae, found in each experimental unit, which have been evaluated at different times: 4, 12, 24 and 48 h. respectively. Each experimental batch had a different number of P. longifila larvae. At the end of the evaluation time established for each experimental unit, the number of dead and live larvae was determined [30]. In the four experimental lots, which received water treatment, the number of dead larvae were: 6,10, 5, 9 and the number of live larvae were: 23, 37, 20 and 28; respectively. Likewise, in the four experimental lots, which received the 96° GL ethanol treatment, the number of dead larvae were: 10,10, 5, 9 and the number of live larvae were: 23, 37, 20 and 28; respectively. With the statistical analysis, it is concluded that there is a linear relationship between the five sets of values, with a degree of adjustment of R^2 equal to 47.15%; This allows us to statistically choose 50% concentration of the extract as the best alternative with a lethal effect [31-38].

Conclusion

The bioinsecticide obtained from the leaves of N. oleander "pink laurel" had a lethal effect on the different instars of P. longifila larvae. The optimal lethal dose of the ethanolic extract evaluated was 60% v/v with 40.48% mortality. The mortality assessment carried out after of 4, 12, 24 and 48 hours, report that the mortality efficiency is not subject to the exposure time of the P. longifila larvae to the bioinsecticide.

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