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Research Article

Influence of Bioactives on the Germination of Different Varieties of *Phaseolus vulgaris* L.

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Abstract

The present investigation was carried out in areas of the farm of an individual producer from the Cumanayagua Municipality, Province of Cienfuegos, Cuba, with an average annual temperature that ranges between 28 and 30 celsius, with an average annual rainfall of 700 mm, a brown soil with carbonate. With the objective of determining the influence of some bioactives on the germination process of specific varieties of *Phaseolus vulgaris* L., in order to contribute to improving the integrated management carried out in this crop. It was found that the extracts of garlic, tabaquina and Neem, in doses of 50 and 100%, respectively, cause an inhibitory effect on the germination of the seeds of *Phaseolus vulgaris* L,, both on the Variety Cuba Cueto 25-9N and on the Variety Velazco Also as another result, it was obtained that the varieties of *Phaseolus vulgaris* L., Cuba Cueto 25-9 N and Velazco Largo show a similar behavior, in relation to the contrast between the extracts and their doses between them, without showing significant differences between the extracts values of their means; however, there are significant differences between the mean values of the extracts and their doses compared to their controls.

Keywords

Germination, Inhibition, Varieties, Beans, Extracts and Dosage

1. Introduction

The production and consumption of *Phaseolus vulgaris* L (bean) on a global scale is becoming increasingly important, beans are very valuable species for human consumption because this along with rice, is part of the daily diet of thousands of people, for its richness in protein (22.0%), essential amino acids, iron and excellent taste qualities that improve appetite, and a caloric intake of 340 cal/100g, so it is of vital importance, especially for the vast majority of the population of less economic resources, Valdez et al, (1997), in addition to the fact that it is a product that can provide food for a long period of time due to the possibility of being stored without many difficulties. It is stated with propriety that it is practically cultivated all over the world [1].

The most suitable planting time for beans is the one that, in addition to offering the climatic conditions for good crop development, allows the harvest to coincide with the period of low or no rainfall to avoid damage to the grain due to excess moisture. The range of bean planting in Cuba is from September 1 to January 30, with optimal date October 15 to November 30 and areas without irrigation from September 1 to October 15; however, it is proposed that the month of September is for those who do not have irrigation and December

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for those who have irrigation.

September is a month in which temperatures are still somewhat high (27 ℃), and temperatures for beans should be around 23 ℃, but the rains are taken advantage of, in Frank País municipality, Holguín province, considered one of the largest producers of this grain in Cuba, two fundamental planting seasons are well differentiated, which fall between September-November, because at this stage it precipitates 80% of the annual historical rainfall in the municipality and the other suitable time is in the month of January the latter is where the best agricultural yields have been obtained, bearing in mind the possible occurrence of the White Fly and the damage it can cause [2].

The seed originates from a complotropic ovule, it is exalbuminous, that is, it has no albumen; at maturity it lacks endosperm, the nutritional reserves are concentrated in the cotyledons. The reserves in the cotyledons supply the needs of the seedling until about 12 days after sowing. It has a wide variation in color, shape and size [3].

When the viable seed is provided with humidity, good aeration and a certain temperature, it germinates and the embryo that was resting resumes its growth. The first thing that emerges from the testa is the radicle [4].

The germination of any seed is a complex process and at the same time determinant for the future stability and yields of any crop. In Cuba and the rest of the world, studies of the germination process of *Phaseolus vulgaris* L. seeds have been aimed at measuring the influence of certain factors that influence germination, both from the intrinsic and extrinsic point of view, Maqueiras *et al*., [5], when studying the influence of temperature on the germination process of *Phaseolus vulgaris* L, found that there is sensitivity of different bean cultivars to a variation in temperature in the germination process; therefore, the effect of temperature is closely related to the genetic material being worked with, and they also stated that temperatures above 30 ℃ decrease the speed of germination in the cultivars under study.

Little or almost nothing is known about the influence of bioactives in the germination process of *Phaseolus vulgaris* L. seeds, however, the influence of bioactives in the germination process of other cultivated plants has been found in the literature review. Dago *et al.* found that the biostimulants used in their research favored the germination dynamics of Vignasesquipedalis (Bean) by 98% [6].

Bean is attacked by insects at all stages of its life [7]. Selection of bean genotypes for horizontal resistance to the conchid Epilachna varivesti Mulsant. Coleoptera Coccinellidae.

Effect of biostimulants on germination and seedling growth of Vigna unguiculata Subsp. Sesquipedalis l [6].

The most critical phenological stages are: seedling, flowering and pod filling [7]. This period consists mainly of germination until 30 days after germination [8].

The ability of microorganisms to increase the rate of germination, growth and development of plants, especially their root system, has been known for many years. However, these mechanisms are not known with certainty even though it can be assured that many of them are linked to indirect biological control mechanisms, as they allow a better nutritional status in the plant and a better response to different types of stress [9].

Biostimulants are organic compounds that differ from nutrients. In small amounts they promote, inhibit or modify plant physiological processes [10].

Fungi of the genus Trichoderma have been found to produce substances stimulating plant growth and development through the establishment of symbiotic relationships with plants. These substances act as catalysts or accelerators of the primary meristematic tissues in the young parts of those, so they induce their cellular reproduction, and thus reach a faster development than those that have not been treated with that microorganism [11].

Hernández *et al*., obtained that at concentrations 1:200 as 1:400, the strains of T. harzianum A29 and A20 stimulated the germination of bean and maize seeds with respect to the control. At the same concentrations, the T. viride T4 and T5 strains had a lower germination stimulating effect than *T. harzianum* A29 and A20 but, in the case of the aforementioned seeds, it was superior to that obtained with the control [12].

The application of plant growth stimulators with the aim of increasing crop quality and yields is an aspect of agricultural research of great importance for agriculture because of the social and economic implications [13].

In the early 1960s some researchers hypothesized that accelerated germination and growth of pollen grains might be associated with the presence of growth promoters. In 1970, it was reported that some extracts of rapeseed (*Brassica napus* L.) pollen produced a powerful elongation (lengthening) effect on the bean stem. This response was distinct from that produced by other hormones called gibberellins. The most active growth-promoting substances were isolated from Brassica napus, hence they were called brassinos. These authors prophetically attributed plant hormone status to brassinos because they were specific organic compounds, isolated from plants, that had induced growth when applied in minute amounts to other plants [14].

According to Vuelta *et al*., the application of the foliar biostimulant Biobras-16 at a dose of 37.5 mg/ha produced the highest yield in the crop of *Phaseolus vulgaris* L. In addition, these authors also obtained that the application of Biobras-16 positively influenced all the indicators evaluated in each of the treated plants. The highest percent of pods and first-category grains was achieved in treatment three, which also had the highest values of diameter and height [15].

The application of bioactives in agriculture is a fairly novel element, up to this moment the tendency has been to employ plant extracts fundamentally in pest control and to inhibit germination of weeds, an example of this is: Cano [16], which evaluated the influence of three plant extracts (Aji-Garlic, Ruda, Neem) as bio-controllers of the main pests of the Bean crop; highlighting the importance of starting to reduce the use of agrochemicals in the management of the crop, which, has become a product of daily consumption in the department of Antioquia and therefore of economic importance, both for consumers, as well as for producers. This author obtained that: the extracts of Ruda and Neem corresponding to treatments two (Rutinal 10 cc/liter), five (Bioneen 3 cc/liter) and six (Bioneen 5 cc/liter) reached the lowest average values in terms of the total number of insects and homoptera in relation to the other vegetal extracts evaluated, during the two crop cycles, exerting the greatest control of the population.

In view of the above, the following Scientific Research Problem is posed:

The use of certain bioactives in a systematic way for the control of pests and diseases, in certain varieties, of the crop *Phaseolus vulgaris* L, may be influencing its germination process and be performing an inadequate management of the crop.

Hypothesis:

If the influence of certain bioactives on the germination process is established, in certain varieties of the *Phaseolus vulgaris* L. crop, then it will be possible to have information in this sense, which will allow a better management of this crop.

General Objective.

To determine the influence of some bioactives on the germination process of specific varieties of *Phaseolus vulgaris* L., in order to contribute to improve the integrated management of this crop.

Specific Objectives.

To measure the influence of specific doses of specific bioactives on the germination process of some *Phaseolus vulgaris* L. varieties.

2. Materials and Methods

2.1. Characterization of the Experimental Area

The present experiment was established in the productive form of agriculture belonging to an individual producer, in the Municipality of Cumanayagua, Cienfuegos Province, adjacent to the East with the Municipality of Trinidad, Sancti Spiritus Province, to the South with the Caribbean Sea, to the North with the Guamuhaya Mountain Massif and to the West with the Municipality of Cienfuegos, Cienfuegos Province, the average annual temperature oscillates over 30 C0, with an average annual rainfall of 700 mm, a brown soil with carbonate, the farm occupies a total of 2 ha, distributed in the production of various crops, with a predominance in the planting of beans, but also produces bananas and a small level of vegetables. There is a source of water supply from an artesian well, as well as fruit and forest trees.

2.2. Experimental Stage

For the fulfillment of this important stage several phases were established:

Obtaining, Collection and Conservation of the Plants for the Preparation of Their Extracts

Procedure

There are different factors that determine the quality of plants to be used in the process of preparing their extracts, such as humidity, impurities, where it is fundamental that the plants should be free of toxic compounds, free of microbial load and the obtaining of biological properties depends on the phases in which they are: vegetative state, fruiting and flowering, etc. The collection and use of the bioactive properties of these plants depend on the collection of the plant organs such as leaves, flowers, stems, among others. On the other hand, to generate a quality product, the drying of the plants, either by air drying or shade drying, is essential for the conservation of the active principles and essential oils of the plants.

In the case of the present experiment, we opted for drying in the shade for a period of 72 hours; sun drying was discarded because several authors have reported variations in the composition of the active compounds present in the biopreparations due to the incidence of the sun, which causes an increase in temperatures.

The following plants were collected in the present experiment:

Allium sativum L. (Garlic).

Garlic is a plant that belongs to the Liliaceae family, like onions and leeks, is cultivated as an annual, is grown for human consumption but can also be used in plant protection as an insecticide, fungicide and antibacterial. Both bulbs and leaves contain active substances that can be applied to crops.

Garlic according to Gimeno [17], can act as a repellent, it also has the property of acting by ingestion, causing certain digestive disorders, since it prevents the insect from feeding, it also works as a high spectrum systemic, since it can be absorbed by the vascular system of the plant, it can also generate a natural odor change of the plant, which prevents the attack of pests, it is based on a masker of the odor of food, pheromones, preventing the reproduction of pests.

Garlic extract is completely biodegradable, does not change the odor and flavor of crops and owes this characteristic odor to allicin, it is also rich in sulfur compounds, in studies the basic active agent of garlic was isolated, allin, which when released interacts with an enzyme called allinase and in this way Alicina is generated [17].

The efficacy of garlic extract (A. sativum) as a natural pesticide has been demonstrated with certain pests, such as lepidopteran larvae, aphids, small bugs and several fungal diseases, its mechanism of action is by ingestion, causing an excitation of the nervous system, which causes repellency [18].

Cano, when evaluating the influence of chili garlic extracts on pests acting on the crop of *Phaseolus vulgaris* L. determined that they did not have effective control over the total population of insects and homopterans with respect to the other extracts evaluated, presenting the highest averages [16].

In the case of this plant, it was feasible to take its heads and shelling the teeth that compose it to submit to treatment and then carry out the process of preparation of the extracts.

Azadirachta indica A. Juss. (Neem).

According to Pérez, one of the most studied plant extracts in recent years is that obtained from the Neem tree, whose effectiveness in the control of insects, mites and nematodes has been widely demonstrated. The importance of Neem extracts for sustainable agriculture lies in the fact that they have only a slight contact action, the substance has to be ingested for it to act, so its effect on natural enemies is limited, and the diversity of bioactive substances it contains means that the risk of resistance developing is minimal and it is not toxic to humans or other mammals. Its mechanisms of action are varied from repellency, anti-feeding, sterilizing, oviposition repellent, insecticide and growth regulator [19].

Azadirachta indica A. Juss.(Neem).

According to Pérez [19], one of the most studied plant extracts in recent years is that obtained from the Neem tree; its effectiveness in the control of insects, mites and nematodes has been widely demonstrated. The importance of Neem extracts for sustainable agriculture lies in the fact that they have only a slight contact action, the substance has to be ingested for it to act, so its effect on natural enemies is limited, and the diversity of bioactive substances it contains means that the risk of resistance developing is minimal and it is not toxic to humans or other mammals. Its mechanisms of action are varied, ranging from repellency, anti-feeding, sterilizing, oviposition repellent, insecticide and growth regulator.

Perez himself, proposes azadirachtin, which appears to be effective on more than 90% of pests, and research carried out in recent years has shown it to be the most potent regulator and deterrent, as it will repel or reduce the feeding of many species of insect pests, as well as some nematodes. Also note the other active ingredients in Neem such as meliantriol and salanin which act as powerful inhibiting agents to the feeding process. Nimbin, like nimbidin (another component of Neem) has antiviral properties [19].

Cano, in his study conducted to determine the influence of plant extracts on pests that acted on the crop of *Phaseolus vulgaris* L., found that the combination of Ruda and Neem extracts corresponding to treatments two (Rutinal 10 cc/liter), five (Bioneen 3 cc/liter) and six (Bioneen 5 cc/liter) obtained the lowest average values in terms of the total number of insects and homopterans in relation to the other plant extracts evaluated, during the two crop cycles, exerting the greatest control of the population [16].

In the case of Neem, and following the recommendation of several authors in the bibliographic review carried out for the present work, it was decided to use the leaves, which were collected to be subjected to treatment and subsequent preparation of the extracts.

Nicotiana tabacum L.

It can be argued that Tabaquina, due to its gassing and adult asphyxiation properties, together with its low residuality, conserves natural enemies better. The general mode of action of Tabaquina is based on intervening in the agroecosystem only at those points where the behavior of the harmful organisms deviates from the levels that do not cause damage to agronomic yield or crop quality, following conservative measures. Cuba contributed tobacco cultivation to the world. The insecticide properties of this plant have been known since pre-Columbian times. Its broths (tabaquina) have been used by farmers for pest control because of its alkaloid content, availability and less aggressive to the environment or environment of the farms.

2.3. Preparation of Extracts and Doses of Collected Palntas

In obtaining extracts, thanks to the long contact time that the solvent must have with the plant material, which must be pulverized or ground to achieve a greater contact surface with the solvent, this process is carried out at room temperature. It is convenient to carry out frequent agitations for the homogenization of the procedure and thus try to influence the extraction yield, the extraction power of the solvent decreases as the contact time with the plant material passes; for the realization of this type of extracts it is convenient to protect the extraction container from sunlight, since this can decompose photo labile substances. After the realization of the extract by filtering it is necessary to wash the remaining plant material with more solvent for the obtention of the total extract [20].

Once the collected material was ready, it was proceeded to crush the material, this step has the objective of seeking to increase the contact surface of the same so that when applying the solvent to be used, a greater dissolution is produced for this, the Maceration Method was chosen: which consisted of extracting the active principle at room temperature, in a closed container, using water as extracting solvent; the mixture of the macerated material obtained from each plant with the extracting liquid is carried out. The mixture is then left to stand for three days. This method has the advantage that the extraction of natural products is achieved without any modification. In some cases the maceration can be done several times (four or five days) [21].

There are several established methodologies for obtaining the doses, depending on the objectives to be achieved and the nature of the plant material collected. For example, for 100 ml of extract, 10 g of the plant to be used will be weighed and placed in 90 ml of hot water (it must be distilled water or weakly mineralized mineral water). Logically the resulting quantity will not be 100 ml, since the plant, being dry, will absorb a good part of the water. This must be taken into account when calculating the amounts depending on the formula in which the extract is to be incorporated [22].

The previous methodology has the disadvantage that the use of hot water can alter the nature of the active substances that the biopreparation may contain and mask its effects, which is why several authors prefer not to use it.

To obtain the doses in the present work, the Gizasa Methodology [23] was used, for which the different parts of the plants that would be used for the preparation of the extracts were taken, and from 1000g of these macerated parts were diluted in 10 liters of water, This dose would represent the dose at 100% concentration, then 500g of each part of the plant were taken and diluted in 10 liters of water, achieving a concentration of 50%, these would be the two doses to be applied to the bean seeds of the different varieties to be sown in the bags used in the experiment.

With these doses the different treatments to be used in the experiment were established which were:

Treatment 1: dose at 50% concentration of Garlic.
Treatment 1: dose at 100% garlic concentration.
Treatment 2: dose at 50% concentration of Tabaquina.
Treatment 2: dose at 100% concentration of Tabaquina.
Treatment 3: dose at 50% concentration of Neem.
Treatment 3: dose at 100% Neem concentration.
Treatment 4: Control.

In summary, the experimental design is a Latin Square, with four replicates of each treatment per block and two replicates of each block.

A	B	C
C	A	B
B	C	A
A	B	C

Caption:
A: Control
B: Treatment 1 with 50 % dose.
C: Treatment 1 with 100% dose.

E	F	G
G	E	F
F	G	E
E	F	G

Caption:
E: Control
F: Treatment 2 with 50 % dose.
G: Treatment 2 with 100% dose.

I	J	K
K	I	J
J	K	I
I	J	K

Caption:
E: Control
F: Treatment 3 with doses at 50 %.
G: Treatment 3 with doses at 100 %.

Figure 1. *Latin Square design for the variety Cuba Cueto 25-9 N and Velazco Largo variety.*

This design was followed in the same way as previously proposed for the Velazco Largo variety, see Figure 1.

2.4. Statistical Processing of the Results

The statistical processing of the results was carried out using the statistical package Statgraphics Centurion Version 19.5.01. Assuming germination as the dependent variable and treatments, doses, and type of extracts as independent variables, an ANOVA test and a correlation test of the means were carried out.

3. Results and Discussion

As can be observed in Table 1, until day three of sowing the seeds of *Phaseolus vulgaris* L., Variety Cuba Cueto 25-9 N in the bags, there is no germination, it is from the fourth day of sowing the seeds that the germination process begins, this is valid for all the seeds of *Phaseolus vulgaris* L. var. Cuba Cueto 25-9 N, sown in the bags, under all the treatments and doses applied, resulting logical if it is kept in mind that in different germination tests carried out with *Phaseolus vulgaris* L, by different authors, independently of the variety used, it is common that germination begins on the fourth day of sowing the seeds of this plant and extends up to seven days.

These results agree with those obtained by the Delegation of Agriculture of the Trinidad municipality [24], where in an investigation carried out with the objective of evaluating the behavior of 15 varieties of beans, which were sown in the late season: seven black on December 31, six red on January 2 and two white on the same day with the objective of determining those that adapt to the climatic and edaphic conditions of the Trinidad municipality, to introduce new varieties and contribute to the increase of genetic biodiversity and the improvement of soil fertility, research where the two bean varieties under study in this work were tested, one of the results of this research was that the black varieties showed uniform germination four days after sowing.

Table 1. *Germination performance of Phaseolus vulgaris L. var. Cuba Cueto 25-9 N, under the influence of different treatments and doses.*

Days	% of germination Control	% of germination Garlic (50%)	% of germination Garlic (100%)	% of germination Control	% of germination Tabaquina (50%)	% of germination Tabaquina (100%)	% of germination Control	% of germination Neem (50%)	% of germination Neem (100%).
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	31,2	6,2	0	31,2	3,1	0	31,2	0	0
5	59,4	13,2	6,0	59,4	5,3	0	50,0	0	0
6	78,1	21,3	13,7	65,6	7,7	3,1	81,2	0	0
7	93,8	33,1	23,1	93,8	11,3	5,7	93,8	0	0

Own elaboration.

In the above table, it can be observed that from the fourth day of sowing the seeds in the bags, the germination process begins, reaching in all the witnesses (seeds sown in the bags watered with water) a uniform average value of 31.2% of germination of the total seeds sown in the witness condition, it should be noted that following the pattern of the experimental design established for this research, which is a Latin Square, in which the influence of three treatments (Garlic extracts, Tabaquina extracts and Neem extracts) is being evaluated in three doses (control in water, 50% dose of each of the extracts, and 100% dose of each of the extracts) and that four replicates were made for each treatment and dose, respectively, that in each bag two seeds of *Phaseolus vulgaris* L. were deposited, subjected to the influence of the different treatments. subjected to the influence of the different treatments and doses were placed in each bag.

Therefore, based on the above, it is assumed that the number of bags used for the control in water was a total of 24, and that by sowing two P. vulgaris. seeds per bag, this would give a total of 24, by bag that would give a total of 48 seeds in control condition for the whole experiment, when applying a simple Rule of Three, where the 48 seeds would represent 100% and 32.1% of the germinated seeds under the control condition would represent the unknown (x) sought, when producing a clearance and the corresponding calculation, it will be obtained as a result that on the fourth day of sown seeds of *P. vulgaris* in the control condition, 15% of the seeds sown under this condition were already germinated, out of the total of 48, that is, less than half of the total.

If this value is compared with those obtained in other studies where the germination response to *P. vulgaris*, an example of this are the results obtained by Araya and Hernandez [25], where in the protocol established by them for the production of bean seeds in Costa Rica, obtained that on the fourth day of sown bean seeds, there was more than 50% germination, although in this research, and in this specific case, only 31.2% germination is reached, a value that compared with 50% obtained by the aforementioned authors, has differences.

In the case of Cuba, in this sense it is more rigorous and it is stated by different authors that the minimum economic profitability for sowing and germination of bean seeds should be at 85%, and the optimum above 90% [26].

The effect of plant extracts from certain plants on physiological processes in other plants is well established, as decomposing plant residues release a large amount of allelopathic agents. Factors influencing this process include the nature of the residue, the type of soil, and the conditions of decomposition [27].

When creating a contrast test between the different treatments and doses used to establish their behavior on the germination process of *Phaseolus vulgaris* L., Variety Cuba Cueto 25-9 N, in the case of the garlic extracts, both in doses of 50 and 100%, respectively, show significant differences with the germination means of the controls, the germination means of the *P. vulgaris* seeds under the influence of the doses of 50 and 100% respectively, of the garlic extracts being lower than those of the controls, this makes it clear that there is a marked inhibitory influence of these doses on the germination of the Phaseolus variety under study.

This same behavior is repeated for the 50 and 100% doses, respectively, of the Tabaquina and Neem extracts; However, when a comparison is made of the results of the means of the different doses (50 and 100%, respectively), with the different extracts (Garlic, Tabaquina and Neem) it can be seen that there are no significant differences between them, so it is not possible to establish a level of greater or lesser influence of each treatment on the germination of the seeds of this variety of bean, Therefore, in order to decide which extract would cause a greater inhibitory effect on the germination of the seeds of the variety in question, it would be necessary to analyze the behavior of the means of the different doses and treatments on the controls.

In the bibliographic review carried out for the present investigation, no evidence was found of similar works in which the influence of the extracts and doses tested in the present investigation on the germination of these varieties in another place, or at a different time of the year, or of other varieties, so that there are no possible patterns of comparison with the behavior obtained in the present investigation.

In order to determine the influence of the extracts used and their doses in this research on the germination process of the seeds of *Phaseolus vulgaris* L., Velazco Largo Variety and Cuba Cueto Variety 25-9 N, a comparison of their means was made, applying a Multifactor ANOVA for their processing, where germination was used as a dependent variable, the combination of extracts as a factor and the variety as covariates, Table 2, it can be observed that for both varieties Velazco Largo and Cuba Cueto Variety 25-9 N, it is fulfilled that the Neem extract, both in its doses at 50 and 100%, respectively, when applied on the bean seeds, shows differences in the germination process, when applied on the bean seeds, shows significant differences between the means of germination that reach the same and the means that shows the germination of bean seeds under the application of extracts of Tabaquina and Garlic, also with doses of 50 and 100%, respectively, also show significant differences the means of germination of bean seeds under the influence of extracts of Garlic in the doses of 50 and 100%, respectively, and between the means of the seeds that are under the influence of the doses of Garlic at 100% and the means of the controls and finally show significant differences between the means of the bean seeds under the influence of Tabaquina extracts at doses of 50% and the means of the controls, Following the analysis, and referring to the values of the means of germination that show the bean seeds in their two varieties under study, where it appears that the value of the germination of these seeds under the influence of Neem extracts, both in doses of 50 and 100%, both in doses of 50% and 100%, respectively, reach a value of zero, there being no significant differences between these means, so it can be inferred that the degree of inhibition reached by the Neem extracts on the germination of the bean, in its two varieties under study, is the highest with respect to the doses and 100%, respectively, and that the inhibition of the Neem extracts on the germination of the bean, in its two varieties under study, is the highest with respect to the doses and 100%, is the highest with respect to the doses and of the other two extracts (Tabaquina and Garlic), inferring also that the level of efficiency reached by the doses at 50 and 100%, respectively, of the Neem extracts on the inhibition of the germination of the seeds of Bean, in its two varieties under study is the same.

Table 2. *ANOVA analysis of the means of germination of Phaseolus vulgaris L., in its varieties Cuba Cueto 25-9N and Velazco Largo under the influence of the different doses and treatments under study.*

Contrast	Sig.	Difference	+/- Limits
Garlic at 100% - Garlic at 100%		222874,	816606,
Ajo al 100% - Ajo al 50%	*	750437,	677094,
Ajo al 100% - Neem al 100%	*	885723,	677094,
Ajo al 100% - Neem al 50%	*	885723,	677094,
Ajo al 100% - Tabaquina al 100%		496437,	677094,
Ajo al 100% - Tabaquina al 50%		246437,	677094,
Ajo al 100% - Testigo		522714,	763865,
Ajo al 100% - Testigo	*	885329,	677094,

Contrast	Sig.	Difference	+/- Limits
Ajo al 100% - Testigo		477160,	816606,
Ajo al 100% - Testigo	*	703944,	677094,
Ajo al 100% - Ajo al 50%		527563,	677094,
Ajo al 100% - Neem al 100%		662849,	677094,
Ajo al 100% - Neem al 50%		662849,	677094,
Ajo al 100% - Tabaquina al 100%		273564,	677094,
Ajo al 100% - Tabaquina al 50%		23563,0	677094,
Ajo al 100% - Testigo		299840,	816606,
Ajo al 100% - Testigo		662456,	677094,
Ajo al 100% - Testigo		254286,	763865,
Garlic at 100% - Control		481070,	677094,
Garlic at 50% - Neem ay 100%		135286,	540134,
Garlic at 50% - Neem at 50%		135286,	540134,
Garlic at 50% - Tabaquina at 100%		-253999,	540134,
Garlic at 50% - Tabaquina at 50%		-504000,	540134,
Garlic at 50% - Control		-227723,	677094,
Garlic at 50% - Control		134893,	540134,
Garlic at 50% - Control		-273277,	677094,
Garlic at 50% - Control		-46493,0	540134,
Neem at 100% - Neem at 50%		0	540134,
Neem at 100% - Tabaquina at 100%		-389285,	540134,
Neem at 100% - Tabaquina at 50%	*	-639286,	540134,
Neem at 100% - Control		-363009,	677094,
Neem at 100% -Control		-393,5	540134,
Neem at 100% - Control		-408563,	677094,
Neem at 100% - Control		-181779,	540134,
Neem at 50% - Tabaquina at 100%		-389285,	540134,
Neem at 50% - Tabaquina at 50%	*	-639286,	540134,
Neem at 50% - Control		-363009,	677094,
Neem at 50% - Control		-393,5	540134,
Neem at 50% - Control		-408563,	677094,
Neem at 50% - Control		-181779,	540134,
Tabaquina at 100% - Tabaquina at 50%		-250000,	540134,
Tabaquina at100% - Control		26276,5	677094,
Tabaquina at 100% - Control		388892,	540134,
Tabaquina at 100% - Control		-19277,5	677094,
Tabaquina at 100% - Control		207507,	540134,
Tabaquina at 50% - Control		276277,	677094,

Contrast	Sig.	Difference	+/- Limits
Tabaquina at 50% - Control	*	638892,	540134,
Tabaquina at 50% - Control		230723,	677094,
Tabaquina at 50% - Control		457507,	540134,
Control - Control		362615,	677094,
Control - Control		-45554,0	816606,
Control - Control		181230,	677094,
Control - Control		-408169,	677094,
Control - Control		-181385,	540134,
Control- Control		226784,	677094,
E.E= ±0.133413 C.V= 3.89			

* denotes significant differences. P-values ≤0.05.

Brief economic evaluation of the results obtained in this research.

If it is assumed that for flat land, in the sowing of *P. vulgaris*, a distance of 1 m between furrows is used; on sloping land, the distance between furrows is greater, between 1.10 and 1.50 m. The recommended distance between plants is 20 centimeters, placing one seed per site, this would give about 100 000 plants planted per hectare, if it is kept in mind that in the specific case of Neem extract obtained in this work, both in doses of 50 and 100%, respectively, cause a complete inhibition, or zero value in germination, to be applied both on the varieties Cuba Cueto 25-9 N, this would mean a loss of all the seeds that can be planted in a hectare of beans of these varieties, if in addition it is borne in mind that the cost of production of one hectare is $ 3,800.00 (Three thousand five hundred pesos 00/100 M.N). 25 jul 2023, this would be equivalent to a total loss of that amount of money if we apply the above extract as a preemergete for the control of any pest that is in the soil, or at any stage of the crop close to harvest, is also valid for if applied in the period between day one and seven of sown the seeds of these varieties.

Seen from another angle, if we bear in mind that the price of a kilogram of certified bean seed, today in Cuba, is around $317.86 and that it is recommended to use 20 to 25 kilograms of seed per hectare, with these amounts of seed we will have a population of 100,000 to 150,000 plants per hectare; then if Neem extracts are applied, in doses of 50 and 100%, respectively, on seeds of bean varieties Cuba Cueto 25-9 N and Velazco Largo, and assuming that 20 kg / ha are needed for planting, as the effect caused by the application of this specific extract, in those doses, on the germination of these varieties is complete inhibition of germination, this would mean a loss of $6357.20, since the 20 kg of seeds would be lost.

4. Conclusions

Garlic, Tabaquina and Neem extracts, in doses of 50 and 100%, respectively, cause an inhibitory effect on the germination of *Phaseolus vulgaris* L. seeds, both on the variety Cuba Cueto 25-9N and on the Velazco Largo variety.

The varieties of *Phaseolus vulgaris* L., Cuba Cueto 25-9 N and Velazco Largo show similar behavior in terms of their response to germination under the influence of the extracts and doses applied, showing no significant differences between the values of their means; however, there are significant differences between the values of the means of the extracts and their doses with respect to their controls.

The doses of 50 and 100%, respectively, of the Neem extracts do not show significant differences between the values of their means, reaching equal efficiency in the inhibition of germination of the two varieties of *Phaseolus vulgaris* L., and at the same time they are the ones that show the highest levels of inhibition on germination with respect to the other doses and extracts applied.

5. Recommendations

It is recommended to continue the present study to verify if the behavior shown by the extracts of Garlic, Tabaquina and Neem, in doses of 50 and 100%, respectively, cause inhibitory effects on other *Phaseolus vulgaris* L. varieties.

Do not apply extracts of Garlic, Tabaquina or Neem on Cuba Cueto 25-N and Velazco Largo varieties when they are sown, that is, between the first and seventh day of their germination process, since they cause inhibitory effects on this process.

Conflicts of Interest

The authors declare no conflicts of interest.

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