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by

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CANAVALIA AND DOLICHOS EXTRACTS FOR SUSTAINABLE PEST BIOCONTROL AND PLANT NUTRITION IMPROVEMENT IN EL SALVADOR

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Botanical repellents and pesticides are now being rediscovered as new tools for integrated pest management in order to reduce the use of toxic chemicals in crop production. Canavalia gladiata and Dolichos lablab are two Fabaceae very well adapted to farmlands of El Salvador, effective as living barriers and mostly as cover crops, however, they are not yet very well disseminated. This document describes the potential for using the liquid extracts and the dry flour of raw seeds of those plants for economic benefit and practical convenience for pest management in Salvadorian agriculture under field conditions. Seed extracts were useful when applied to the foliage to repel white flies (Aleyrodidae spp.) and cucumber beetle (Diabrotica spp.), at the dose of 10% v/v; the repellent effect lasted approximately up to 8 days. Thrips, in contrast, were not affected by any dose. The flour produced from the ground seeds were effective for preventing infections of Meloidogyne spp. and Phyllophaga spp., when mixed with soil prior to transplant; the beneficial effect lasted for about one month. Another added value of those flours was the contribution to plant nutrition in the short term, yet applying this treatment must be delayed until after crop germination and emergence because it can cause growth disorder in young seedlings. These leguminous crop seed extracts and flours appear to have promise for commercial application, especially by limited resource farmers.

1. Introduction

During the dry season of recent years, western Salvadorian farmers have experienced severe pest problems in their plantations. For example, entire watermelon and cucumber fields have succumbed to the damage caused by *Thrips palmi* Karney; this insect is usually controlled by the use of very toxic insecticides which are applied repeatedly, even a few days before harvesting. Desperate farmers tend to use mixtures of pyrethroids and other insecticides, often exceeding the recommended doses. Those treatments are not only dangerous for farmers, consumers and the environment, but they have also proven to be too expensive and sometimes ineffective. Home made solutions composed of detergents, cinnamon oil and other ingredients exhibit only limited pest control on the field. The situation is worsened because small farmers execute their own improvised management activities and when one of them they fails, insects are abundant and later can easily spread across fields.

With the intention of reducing pest populations in soils and foliage, this research explored the use of natural extracts of ***Canavalia gladiata*** and ***Dolichos lablab*** to test their repellent effects and other possible contributions to plant nutrition.

Farmers in a subsistence agriculture environment could benefit from an improved production model of these legumes, planting them in impoverished soils to regenerate soil fertility while they can produce organic substances at a low cost for pest control and plant nutrition contribution.

Botanical insecticides were widely used by ancient farmers. In the past century, those substances were carelessly replaced by new chemical products. However, there have been consequences due to their residues that produce negative effects on human health, livestock and many other kind of organisms, while pests rapidly adapt to those compounds making them innefective. While returning to some old agricultural practices, it is possible to speculate that *"a number of plant substances have been considered for use as insect antifeedants or repellents. [...] In the context of agricultural pest management, botanical insecticides are best suited for use in organic food production in industrialized countries but can play a much greater role in the production and post-harvest protection of food in developing countries"* (Isman, 2006). Several authors agree with Sofia et al., (2006) who suggested that *"it would not be wrong in saying that we are reinventing traditional methods [...] coming close to nature again"*; however, this idea does not intending a drastic rejection of chemical products but their prudential use only until needed. There is recent interest in rediscovering natural sources of pest prevention/control; for this use, legume grains particularly contain a relatively high amount of such natural defenses, as indicated by Enneking & Wink (2000): *"Anti-nutritional factors (ANFs) in grain legumes can be divided into several groups based on their chemical and physical properties such as non protein amino acids, quinolizidine alkaloids, cyanogenic glycosides, pyrimidine glycosides, isoflavones, tannins, oligosaccharides, saponins, phytates, lectins or protease inhibitors"*.

Arim et al. (2006) conducted a study of the effects of using *Canavalia ensiformis* (very similar to *Canavalia gladiata*) as green manure, in the context of subsistence agriculture. A nematode reduction of *Pratylenchus* spp. up to 70% was shown in maize fields, also increasing yield because of nitrogen fixed by the cover crop. Follmer et al. (2001) had already described the active ingredient as "canatoxin", a compound 86% similar to urease, which has insecticidal effects and it is lethal to mice at 2 mg/Kg. Carlini et al. (1997) demonstrated that it is lethal for insects displaying cathepsin-based digestion at 0.25% wt:wt, affecting harmful insects like *Phyllophaga* spp. and also thrips, according to Kuipers et al. (2004).

Canavalia gladiata is very similar to *C. ensiformis* not only in their appearance but also in its toxicity potential. Ekanayake et al. (2007) discovered more than one chemical with insecticidal effects on mature *Canavalia* beans, previously soaked for a few hours and subsequently squeezed to obtain a natural substance rich in *"antinutritional factors such as haemagglutinins (concanavalin A), protease inhibitors (Laurena et al., 1994), hydrocyanic acid, tannins, phytates and canavanine (Kay, 1979)."*

Dolichos lablab contains a chitinase-like protein that inhibits fungus activity, especially for *Fusarium oxysporum, Rhizoctonia solani*, and *Coprinus comatus* (Ye et al., 2000). Among many indigenous legumes investigated for their toxicity, *Dolichos* bean had the highest trypsin inhibitor activity ranging from 14 to 27 units/mg (Laurena et al., 1994), which consists of a powerful agent that helps to avoid the attack of many kind of insects. Barbehenn et al. (2011) described that in the first 5 hours when moisture starts the germination process in *Dolichos*, the highest concentrations of tannins are reached, increasing toxicity for hervibore pests (Osman, 2007). The reason those tannins are so useful for bio-protecting the crop is that "*tannins are especially prone to oxidize in insects with high pH guts, forming semiquinone radicals and quinones, as well as other reactive oxygen species. Tannin toxicity in insects is thought to result from the production of high levels of reactive oxygen species"* (Barbehenn et al., 2011). According to Janarthanan et al. (2008) extracts of raw seeds of *Dolichos* contain high amounts of proteins similar to isoforms of arcelins 3 and 4 and pathogenesis-related protein 1 (PvPR1), causing the complete death of Coleoptera: *Callosobruchus maculatus,* at doses of 5% of its regular diet. Those experiments prove that *Dolichos* raw beans contain more than one kind of toxic substance, all of them capable of controling different types of pests and with possible synergistic effect between them.

2. Methods

2.1. Location. This study was conducted on a farmland located in Canton San Cristobal, municipality of El Porvenir, department of Santa Ana, Republic of El Salvador, Central America. Its coordinates were 14°02'22.5" N, 89°37'49.9" W, 693 masl. All the tests were performed in an area of a few acres on the same farm. Trial places were adjacent even though they did not occur at the same time. There was no apparent soil spatial variability or other important measurable differences about soil pH and E.C.

2.2. Production of raw material. For a first phase, half hectare for each legume, *Canavalia* and *Dolichos,* was planted in a well-drained silty loam soil with some stoniness and low fertility. The plantations were stablished at open sky and without the use of agrochemicals. Seeds proceeded from self-owned cultivars and received no prior treatment.

2.3. Natural extracts obtaining. As literature suggests, seeds are the part of the plant where most of the toxins are contained and their concentration is increased a few hours after exposing to moisture (Barbehenn et al., 2011; Ekanayake et al., 2007), reason for which they were immersed in water until swelling point and then compressed to separate solids from liquids. The extrusion process is efficient, for example it can obtain up to 90% of the essential oils of soybeans (Bargale et al., 1999), whose structure is similar to the legume seeds used in this study. Extrusion offers a convenient method for tissue disruption due to strong compression forces and slight heating in a fraction of time, both in a single-step operation. This treatment contributes greatly to the retention of the natural characteristics of the natural substances while avoiding activation of damaging enzymes before they adversely affect essential oil quality (Nelson et al. 1987). Soetaredjo et al., (2008) conducted a similar experiment using dry neem seeds in order to obtain oil for pest bio control, arriving at two main conclusions: pressure should be at least of 3000 psi (5000 was optimum for neem), and no pre heating of seeds was required. For the present study, seeds were collected and cleaned with an air flow at atmospheric temperature, oil was extracted with a 20 Ton hydraulic jack, which extruded seeds trough a recycled engine cylinder of 4 inches diameter, previously perforated with 12 holes of 4 milimeters diameter, creating a presion of 10,000 psi which exceeded the minimum required. This simple machine was a low cost adaptation of the very well known principle of a hydraulic press.

Additionally, flours of these seeds were used to conduct additional tests including plant nutrition effect. Seeds of each legume were water soaked during a period of 24 hours, after which were grinded to obtain a natural paste, then it was sun dried and finally grinded again to produce fine flour.

2.4. Expected products. In case of *Canavalia*, the compound of major interest is canatoxin, with a concentration of 3.4% of the raw essential oil (Stanisçuaski et al., 2005; Oliveira et al., 1999; Carlini et al., 1997); additionally, each gram of raw seed contains: 4.8 mg of phytic acid, 2.4 mg of tannic acid and 22 µg of extremely poisonous Hydrogen cyanide (Laurena et al. 1994). The extract of the second legume, *Dolichos*, has an average tannin content of 2% (Deka, 1990) and different amounts of anti-nutritional factors: dolichin (Ye et al., 2000), trypsin inhibitor (Osman, 2007), polyphenols and phytic acid (Ramakrishna et al., 2006). These last kinds of chemicals are enzyme inhibitors which are difficult to be measured in absolute terms (e.g. grams), because their purity tends to be low and a proportion of them could be inactive, a reason for which they are often designated as Inhibitory Units per gram (IU/g). Ramakrishna et al. (2006) concluded that raw seeds of *Dolichos* (after 24 hours of soaking) have an average of 1916 trypsin IU/g, 82 phytic acid IU/g, and 3.5 total polyphenols IU/g.

2.5. Treatment considerations. Several studies of botanical pesticides were compared to determine the experiment design. Sarwar, M. (2015) recommends using 50 grams of plant parts like peeled garlic cloves or hot pepper, diluted in a liter of water for foliar spraying in a sufficient amount for uniform coverage; in the case of neem, 10 grams of ground seeds are used in 1 litre of water. Oparaeke et al. (2006) used a concentration of 10% w/v of extract mixtures composed by eucalyptus, neem, starch and soap. Nas (2004) tested some bacteriostatic effects using extracts of black tea, green tea, cocoa and coffee at the concentrations of 0.95% to 3.8% w/v. However, as it is logical, low doses are ineffective; for example, Kamatenesi-Mugisha et al. (2013) used essential oils from 5 different plant extracts to test different doses between 2.3 to 15 mg/Kg, with no significant results. However, high concentrations of extracts also were not a good choice because of their probable counter effects; Sinzogan et al. (2006) reported abnormal development of plants after applying extracts of neem seeds, Hyptis leaves and Khaya bark, with doses ranging from 40% to 80%. More similar to the experiment plans for the present study, was the case of Adeyemo et al. (2014), who reported the use of Zingiberoideae extracts, using doses from 1 to 5% in order to test effects on a Lepidoptera pest and obtaining significant results.

2.6. Experimental design. For the present study all tests were performed with young plants in order to determine beneficial and adverse effects during the period in which crops are most susceptible. Biological compounds extracted from ***Canavalia gladiata*** and ***Dolichos lablab*** were simply named as ***Canavalia*** and ***Dolichos*** in the thesis**.** Their extracts were administered at different concentrations to be applied over the foliage of tomato, red beans and cucumber plants in order to test their repellent effects on thrips, *Diabrotica* and white fly. Other treatments were drenched on rice, tomato and cucumber plants, in order to evaluate their contribution to plant nutrition and the potential for repelling of *Phyllophaga*. The goal was to measure whether one dose for each treatment would be beneficial enough to contribute to the control of each specific pest problem. Tests were applied to two families of crops in one repetition for getting exploratory results, except for the Meloidogyne and plant nutrition experiments, in which a single crop was chosen to be tested in two repetitions. Several measurements like pest populations and plant growth were recorded periodically according to the particular conditions of each experiment, subsequently explained in this document. Bidimensional matrices were built for comparing results of treatment by plant families, and later confirmed with a post-hoc Tukey test to determine the significance of differences among the treatments and also comparing them to the controls. Considering that there were no repetitions for those experiments, a specialized statistical software was used to bootstrap the input data for increasing reliability of the results. Using this analysis, the dose with "more difference" compared to the control treatments was considered as the more adequate for practical uses, also including the criteria of selecting the lowest concentration to recommend for those cases with similar results.

To test repellence against *Meloidogyne* and beneficial effects on plant nutrition, treatments were directly applied to soil in the root zone of tomato plants during transplant. Two repetitions were included for a better statistical analysis of both experiments. Also, as organic matter from treatments was expected to decompose and release nutrients in soil, another experiment with tomato at the same doses of treatment was run in two repetitions to observe the effects on plant development. Each dose of all treatments was tabulated as an individual data series for regression analyses and obtaining of the correlation index between repetitions.

In detail, each of the six mentioned experiments were performed as follows:

2.6.a) Repellence of thrips. Seven hundred cucumber plants were planted close to a previously established commercial watermelon plantation that was already damaged by a thrips attack. Fifteen days after germination, the foliage of one hundred plants was sprayed with the treatment of liquid extracts of **Canavalia** and **Dolichos**, each of them at the doses of 5%, 10% and 20% $^{V}/_{V}$, diluted in distilled water, plus a Control treatment containing no extract. The same treatments were applied to a similar planting of watermelon. The number of thrips was determined by selecting a square centimeter of two different leaves per plant and then counting the adults; the average of those two numbers was considered as representative for each plant. This procedure was used at three different times for comparison purposes: before treatment, two days after treatment, and seven days after treatment.

2.6.b) Repellence of white flies. Seven hundred red bean plants were planted during the dry season when the incidence of this pest tends to be highest. Fifteen days after germination, the foliage of every hundred plants were treated as described in the previous experiment (3.6.a). Tomato was the selected crop due to its susceptibility to white fly. The number of insects was determined using the following procedure: a leaf was gently held between two pieces of transparent acrylic sheet and then cut off the plant, a square centimeter was selected as a sample and the adults found inside were counted. This procedure was repeated with one another plant. Data was obtained at two different times for comparison purposes: two days after treatment and seven days after treatment.

2.6.c) Repellence of *Diabrotica*. This experiment followed the same procedure described in 3.6.b. However, in this case the crops used for testing were maize and red beans, and the count of *Diabrotica* individuals was carefully done by the observation of adults in the whole plant.

2.6.d) Repellence of *Phyllophaga*. Seven hundred *Phyllophaga* insects were collected from an infected rice field, their identification and collection were easy because of the symptoms of the plants that were being parasitized. Larvae were deposited in plastic bags filled with half kilogram of ground. Each bag contained one *Phyllophaga* and was located at open field, previously perforating 4 small holes for drainage. The reason for using plastic bags is that the pest is able to move underground to avoid unfavorable conditions, making it difficult to determine if individuals would die after treatment or just be repelled. The bags were classified in 14 groups of 50 units, to test six different treatments of **Canavalia,** six of **Dolichos** and two controls. Half of each bag group was transplanted with rice and the other with cucumber plants. The doses for each treatment were 3 grams, 6 grams and 12 grams of **Canavalia** and **Dolichos**. One of the control treatments contained no flour and the other consisted on 6 grams of corn starch. After 8 days, bags were opened in order to observe the death or repellence of the insect. In the second phase of the experiment, the whole experiment was repeated but with the difference that bags were not opened until the activity of the insects reappeared, to evaluate the possible loss of the repellent effects.

2.6.e) Repellence of *Meloidogyne*. A *Meloidogyne* culture was developed in a local laboratory in order to obtain a reliable pathogenic inoculum. The experiment was conducted with the same number of plastic bags and the same treatment schema as in the first phase of the experiment described in section 3.6.d. However, the differences were: the crop used for the tests was tomato and the whole experiment had two repetitions. Five nematode nodules of a previously infected host were situated aside the root zone, and the plants were fertilized with 4 grams of urea to increase the possibility of nematode infection by nitrogen stimulation. Plastic bags were opened for root examination after 6 weeks from transplant. Nodulation was visually recognized using the method of Weighted Nematode Rating (UC-IPM, 2004; Flint, 2012) in which index 0 is assigned when 0% of root system exhibits galling, index 1 is for damage ranging from 1% to 25%, 3 for 26% to 50%, 5 for 51% to 75 % and 7 for 76% to 100%.

2.6.f) Effects of plant nutrition. The experiment was conducted with the same treatment schema as the first phase of the experiment described in section 3.6.d. However, in this experiment tomato seedlings were transplant to natural soil (instead of plastic bags) inside of a macro tunnel at day number 30 after germination. There were two repetitions. Treatments were applied at two moments: during transplant and two weeks after transplanting. Plant height was measured weekly every five days for statistical analysis.

2.7. Data analysis. Statistical analyses were based on the quantification of survival or repellency of insects in every treatment. ANOVA and Tukey tests were based on the simple averages of the results per every treatment, determining the significance of differences among them.

2.8. Climate and weather during the experiment. El Salvador has only two defined seasons: dry (from November to April) and rainy (from May to October). The described methodology gives the impression that all treatments were possible to be executed at once. However, in fact we know that pathogens had different virulence peaks during the year. To make these experiments most rigorous and appropriate, those related to foliage pests were conducted during the dry season, while those concerning soil pests were conducted during the rainy season.

3) RESULTS

3.1) Thrips repellence

Both cucumber and watermelon crops were severely infected by *Thrips palmi* Karny. The average population of this insect per square centimeters of leaf area was reduced only slightly by the second and seventh day after treatment, compared to the counts on initial days and control plants (Table 3.1). Even the plants treated with the highest concentration of ***Canavalia*** and ***Dolichos*** extracts, which was 20%, exhibited a very small change with respect to their initial conditions and the control (Figure 3.1) By comparing all the ***Canavalia*** treatments through a Tukey test (Table 3.4), it was possible to observe that the mean difference among treatments and control was very small, their respective standard errors were too large, and the significance index did not support a clear difference among treatments. The same results were obtained by comparing ***Dolichos*** treatments (Table 3.4). Therefore, it is possible to infer Thrips were not affected for the applied treatments.

Table 3.1: Repellence of thrips on foliage of mature cucumber and watermelon plants, two days after treatment. Measurements were the count of individuals per square centimeter of leaf area.

Crop tested		***Canavalia*** extract (concentration)			***Dolichos*** extract (concentration)			Control
		5%	10%	20%	5%	10%	20%	
Cucumber	0 days	18.9	18.4	19.1	17.1	18.3	17.4	17.5
	2 days	17.1	18.0	15.3	18.6	17.7	18.5	17.6
	7 days	19.1	18.6	17.2	16.4	17.1	18.8	18.3
Watermelon	0 days	13.7	14.7	14.3	14.4	13.8	12.1	14.7
	2 days	13.9	11.3	10.1	13.5	14.2	12.9	13.4
	7 days	14.3	12.7	12.8	15.5	14.8	16.1	14.5

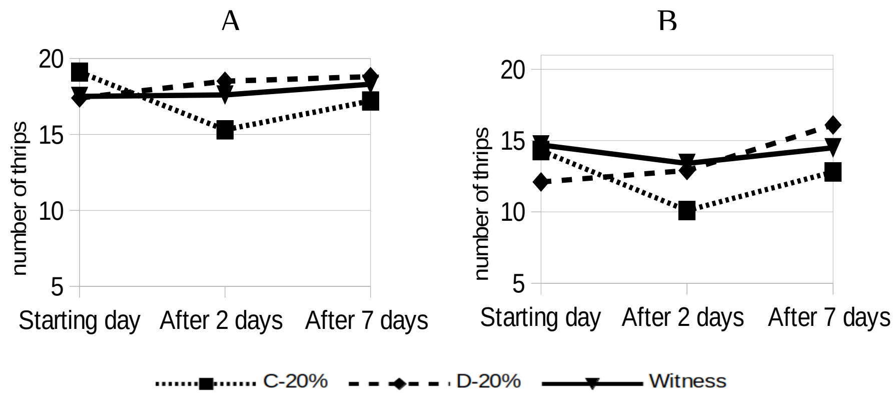

Figure 3.1: Average number of thrips in cucumber (A) and watermelon (B) plants treated with the most concentrated treatment of Canavalia and Dolichos, compared to Control; differences were not significant [p=0.05]

Table 3.2: Tukey HSD test for multiple comparisons of ***Canavalia*** treatments used to test thrips repellence in watermelon and cucumber crops.

Crop tested	(I) extract concen-tration	(J) extract concen-tration	Mean Difference (I-J)	Std. Error	Signifi-cance
Watermelon	10%	20%	0.50	1.16	0.97
		5%	-1.07	1.16	0.79
		Control	-1.30	1.16	0.69
	20%	10%	-0.50	1.16	0.97
		5%	-1.57	1.16	0.56
		Control	-1.80	1.16	0.45
	5%	10%	1.07	1.16	0.79
		20%	1.57	1.16	0.56
		Control	-0.23	1.16	1.00
	Control	10%	1.30	1.16	0.69
		20%	1.80	1.16	0.45
		5%	0.23	1.16	0.997
Cucumber	10%	20%	1.13	0.92	0.63

		5%	-0.03	0.92	1.00
		Control	0.53	0.92	0.94
	20%	10%	-1.13	0.92	0.63
		5%	-1.17	0.92	0.61
		Control	-0.60	0.92	0.91
	5%	10%	0.03	0.92	1.00
		20%	1.17	0.92	0.61
		Control	0.57	0.92	0.93
	Control	10%	-0.53	0.92	0.94
		20%	0.60	0.92	0.91
		5%	-0.57	0.92	0.93

Table 3.3: Tukey HSD test for multiple comparisons of ***Dolichos*** treatments used to test thrips repellence in watermelon and cucumber crops.

Crop tested	(I) extract concentration	(J) extract concentration	Mean Difference (I-J)	Std. Error	Significance
Watermelon	10%	20%	0.57	1.02	0.94
		5%	-0.20	1.02	1.00
		Control	0.07	1.02	1.00
	20%	10%	-0.57	1.02	0.94
		5%	-0.77	1.02	0.87
		Control	-0.50	1.02	0.96
	5%	10%	0.20	1.02	1.00
		20%	0.77	1.02	0.87
		Control	0.27	1.02	0.99
	Control	10%	-0.07	1.02	1.00
		20%	0.50	1.02	0.96
		5%	-0.27	1.02	0.99
Cucumber	10%	20%	-0.53	0.63	0.83

	5%	0.33	0.63	0.95
	Control	-0.10	0.63	1.00
20%	10%	0.53	0.63	0.83
	5%	0.87	0.63	0.54
	Control	0.43	0.63	0.90
5%	10%	-0.33	0.63	0.95
	20%	-0.87	0.63	0.54
	Control	-0.43	0.63	0.90
Control	10%	0.10	0.63	1.00
	20%	-0.43	0.63	0.90
	5%	0.43	0.63	0.90

3.2) White flies repellence

White flies could be temporary controlled by the application of tested extracts on red beans and tomato crops (Table 3.4). A 10% dose of ***Canavalia*** extract showed a good repellent effect on white flies within a week after application. The dose of 20% did not show a significant increase of effectiveness but did cause a counter effect by an apparent biochemical unbalance in foliage which was noticeable because the plants in those treatments stopped growing during that period of time. Such a side effect was less pronounced in ***Dolichos*** treatments, which showed an acceptable repellent effect at the dose of 20%. Figure 3.2 compares the effectiveness of every treatment against the Control.

Table 3.4: Numbers of white flies present in foliage of young plants.

Crop		***Canavalia*** extract (concentration)			***Dolichos*** extract (concentration)			Control
		5%	10%	20%	5%	10%	20%	
Red beans	After 2 days	12.9	3.4	2.4	23.5	20.1	11.2	34.0
	After 7 days	23.8	19.4	15.0	33.3	29.9	17.3	59.1
Tomato	After 2 days	5.3	1.1	2.2	11.7	10.8	5.7	22.0
	After 7 days	14.5	7.7	8.4	15.2	13.6	10.6	52.9

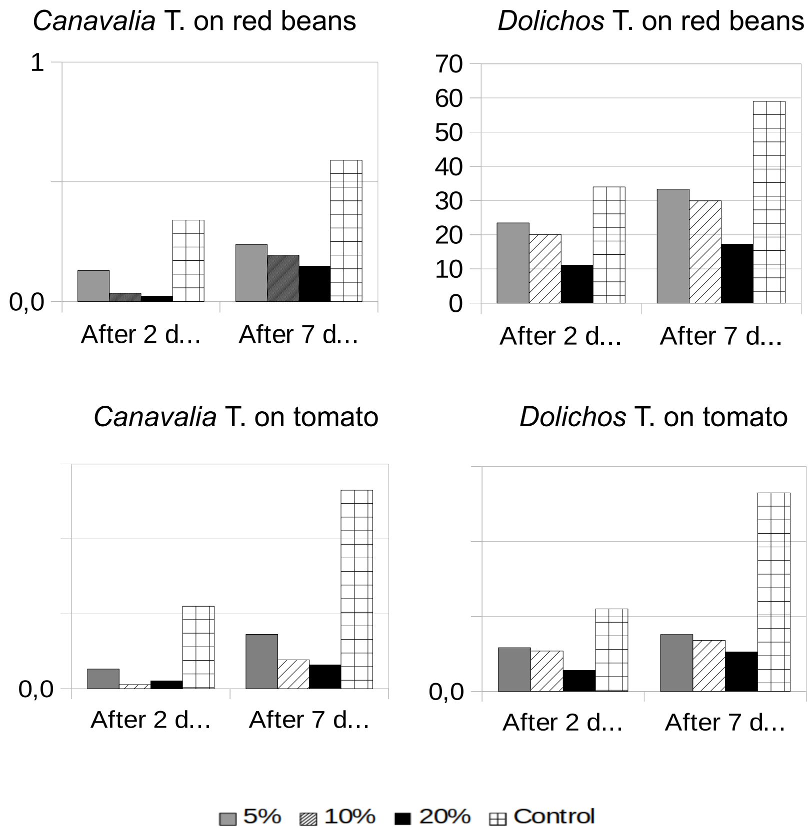

Figure 3.2: Average number of white flies observed in red beans and tomato crops, for each **Canavalia** (Can.) and **Dolichos** (Dol.)

Tukey tests (see Table 3.5 and 4.6) demonstrated that there is a significant difference between the insect populations at the 10% and 20% concentration with respect to the control for both extracts tested. The mean difference is big enough to support that assertion and the significance index indicates quantitative proof of this difference. The Pearson correlation obtained by comparing the data results of ***Canavalia*** extracts on red beans vs tomato was $r^2 = 0.976$, significant at the 0.01 level with a 95% confidence interval, run on a model of 1000 bootstrap samples. The high level of correlation indicate that the results of the treatments can be considered equivalent among the two different crops, although they were in adjacent fields and not truly replicated. In the case of ***Dolichos*** treatments, correlation results were similar, with a value of $r^2 = 0.950$.

Table 3.5: Tukey HSD test for multiple comparisons of ***Canavalia*** treatments used to test white fly repellence in watermelon and cucumber crops.

Crop tested	(I) extract concentra -tion	(J) extract concentra -tion	Mean Differenc e (I-J)	Std. Error	Signifi-cance
Red beans	10%	20%	2.70	12.06	1.00
		5%	-6.95	12.06	0.93
		Control	-35.1	12.06	0.14
	20%	10%	-2.70	12.06	1.00
		5%	-9.65	12.06	0.85
		Control	-37.9	12.06	0.11
	5%	10%	6.95	12.06	0.93
		20%	9.65	12.06	0.85
		Control	-28.2	12.06	0.23
	Control	10%	35.2	12.06	0.14
		20%	37.9	12.06	0.11
		5%	28.2	12.06	0.23
Tomato	10%	20%	-0.90	11.84	1.00
		5%	-5.50	11.84	0.96
		Control	-33.1	11.84	0.15
	20%	10%	0.90	11.84	1.00
		5%	-4.60	11.84	0.98
		Control	-32.2	11.84	0.16
	5%	10%	5.50	11.84	0.96
		20%	4.60	11.84	0.98
		Control	-27.6	11.84	0.24
	Control	10%	33.1	11.84	0.15
		20%	32.5	11.84	0.16
		5%	27.6	11.84	0.24

Table 3.6: Tukey HSD test for multiple comparisons of ***Dolichos*** treatments used to test white fly repellence in watermelon and cucumber crops.

Crop tested	(I) extract concentra -tion	(J) extract concentra -tion	Mean Differenc e (I-J)	Std. Error	Signifi- cance
Red beans	10%	20%	10.8	10.4	0.74
		5%	-3.40	10.4	0.99
		Control	-21.6	10.4	0.30
	20%	10%	-10.8	10.4	0.74
		5%	-14.2	10.4	0.58
		Control	-32.3	10.4	0.11
	5%	10%	3.40	10.4	0.99
		20%	14.2	10.4	0.58
		Control	-18.1	10.4	0.41
	Control	10%	21.5	10.4	0.30
		20%	32.3	10.4	0.11
		5%	18.1	10.4	0.41
Tomato	10%	20%	4.05	11.2	0.98
		5%	-1.25	11.2	1.00
		Control	-25.2	11.2	0.25
	20%	10%	-4.05	11.2	0.98
		5%	-5.30	11.2	0.96
		Control	-29.3	11.2	0.18
	5%	10%	1.25	11.2	1.00
		20%	5.30	11.2	0.96
		Control	-24.0	11.2	0.28
	Control	10%	25.2	11.2	0.25
		20%	29.3	11.2	0.18
		5%	24.0	11.2	0.28

3.3) *Diabrotica* spp. repellence

Diabrotica balteata could be temporarily repelled by the application of tested extracts on red beans and maize crops (Table 3.7). As in the last test, a 10% dose of the two extracts showed a good repellent effect on white flies within a week after application.The mean difference is big enough to support that assertion and the significance indicates quantitative proof. The Pearson correlation obtained by comparing the data results of Canavalia extracts on red beans vs tomato, was r2 = 0.971, significant at the 0.01 level with a 99% confidence interval, run on a model of 1000 bootstrap samples. The high level of correlation indicates that the results of the treatments can be considered equivalent in the two crops. In the case of Dolichos treatments, correlation result was similar, with a value of r2 = 0.976 (significant at the 0.01 level)

Table 3.7: Amount of *Diabrotica balteata* individuals present per plant.

Crop		***Canavalia*** extract (concentration)			***Dolichos*** extract (concentration)			Control
		5%	10%	20%	5%	10%	20%	
Red beans	After 2 days	0.2	0	0	0.4	0.3	0.5	1.7
	After 7 days	1.1	0.5	0.6	1.1	0.8	0.7	2.1
Corn	After 2 days	0	0	0	0.2	0.4	0.3	2.4
	After 7 days	0.6	0.3	0.2	0.7	0.2	0.4	2.6

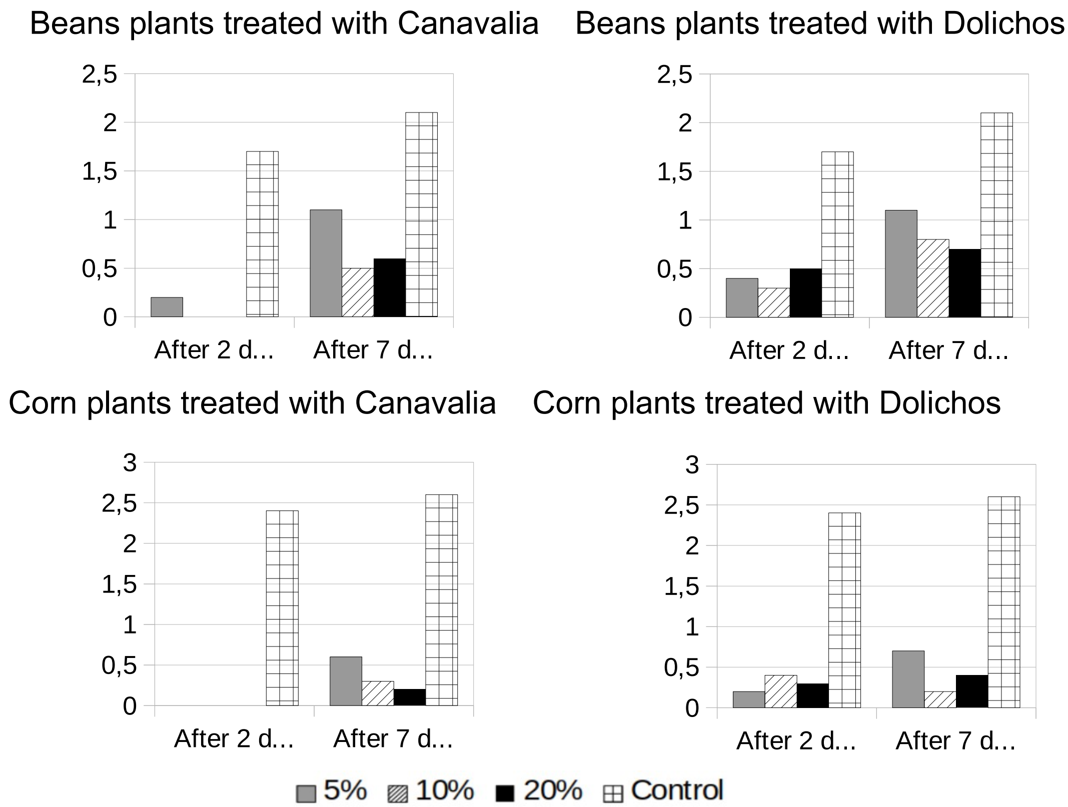

Figure 3.3: Average number of *Diabrotica* observerd in red beans and maize crops, for each ***Canavalia*** (Can.) and ***Dolichos*** (Dol.) treatment (T.)

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Table 3.8: Tukey HSD test for multiple comparisons of *Canavalia* treatments used to test *Diabrotica* balteata repellence in watermelon and cucumber crops.

Crop tested	(I) extract concentra -tion	(J) extract concentra -tion	Mean Differenc e (I-J)	Std. Error	Signifi-cance
Red beans	10%	20%	-0.05	0.44	1.00

		5%	-0.40	0.44	0.81
		Control	-1.65	0.44	0.07
	20%	10%	0.05	0.44	1.00
		5%	-0.35	0.44	0.86
		Control	-1.60	0.44	0.07
	5%	10%	0.40	0.44	0.81
		20%	0.35	0.44	0.86
		Control	-1.25	0.44	0.15
	Control	10%	1.65	0.44	0.07
		20%	1.60	0.44	0.07
		5%	1.25	0.44	0.15
Corn	10%	20%	0.05	0.29	1.00
		5%	-0.15	0.29	0.95
		Control	-1.75	0.29	0.01
	20%	10%	-0.05	0.29	1.00
		5%	-0.20	0.29	0.89
		Control	-1.80	0.29	0.01
	5%	10%	0.15	0.29	0.95
		20%	0.20	0.29	0.89
		Control	-1.60	0.29	0.02
	Control	10%	1.75	0.29	0.01
		20%	1.80	0.29	0.01
		5%	1.60	0.29	0.02

Table 3.9: Tukey HSD test for multiple comparisons of *Dolichos* treatments used to test *Diabrotica* balteata repellence in watermelon and cucumber crops.

Crop tested	(I) extract concentra-tion	(J) extract concentra-tion	Mean Difference (I-J)	Std. Error	Significance

Red beans	10%	20%	-0.05	0.32	1.00
		5%	-0.20	0.32	0.92
		Control	-1.95	0.32	0.01
	20%	10%	0.05	0.32	1.00
		5%	-0.15	0.32	0.96
		Control	-1.90	0.32	0.01
	5%	10%	0.20	0.32	0.92
		20%	0.15	0.32	0.96
		Control	-1.75	0.32	0.02
	Control	10%	1.95	0.32	0.01
		20%	1.90	0.32	0.01
		5%	1.75	0.32	0.018
Corn	10%	20%	-0.05	0.21	0.99
		5%	-0.15	0.21	0.88
		Control	-2.20	0.21	0.00
	20%	10%	0.05	0.21	0.99
		5%	-.10	0.21	0.96
		Control	-2.15	0.21	0.00
	5%	10%	0.15	0.21	0.88
		20%	0.10	0.21	0.96
		Control	-2.05	0.21	0.00
	Control	10%	2.20	0.21	0.00
		20%	2.15	0.21	0.00
		5%	2.05	0.21	0.00

3.4) *Phyllophaga* repellence

Canavalia flour had a strong repellent effect on *Phyllophaga* spp. While both control treatments were seriously affected by this pest, most of the plants provided with this treatment could be kept safe for about a month (Table 3.10). This effect was similar for rice and cucumber crops, along with the observation of improved plant vigor as the dose was increased. About half of the pest individuals died after the application. Survival was measured in a separate experiment a month after treatment; it was found that about half of the surviving pests were able to reinfest crop plants. In the case of ***Dolichos*** treatments, results were proportional but less intense. Graphs in Figure 3.4 show that in the case of ***Canavalia*** treatment, almost all pest individuals could be controlled with either dose. ***Dolichos*** treatment proved to be relatively less effective and its control power was proportional to the dose provided.

Table 3.10: *Phyllophaga* repellence test over rice and cucumber.

Crop	Effect [a]	***Canavalia*** flour			***Dolichos*** flour			Controls	
		3g	6g	12g	3g	6g	12g	No treatment	Corn starch
Rice	Repelled	27 [b]	25	24	12	16	27	0	0
	Dead	22	25	26	0	1	7	0	0
	No effect	1	0	0	38	33	16	50	50
Cucumb.	Repelled	34	24	23	19	13	26	0	0
	Dead	16	26	27	0	4	9	0	0
	No effect	0	0	0	31	33	15	50	50

(a) Measured 8 days after treatment.

(b) Each amount represent one sample containing one individual.

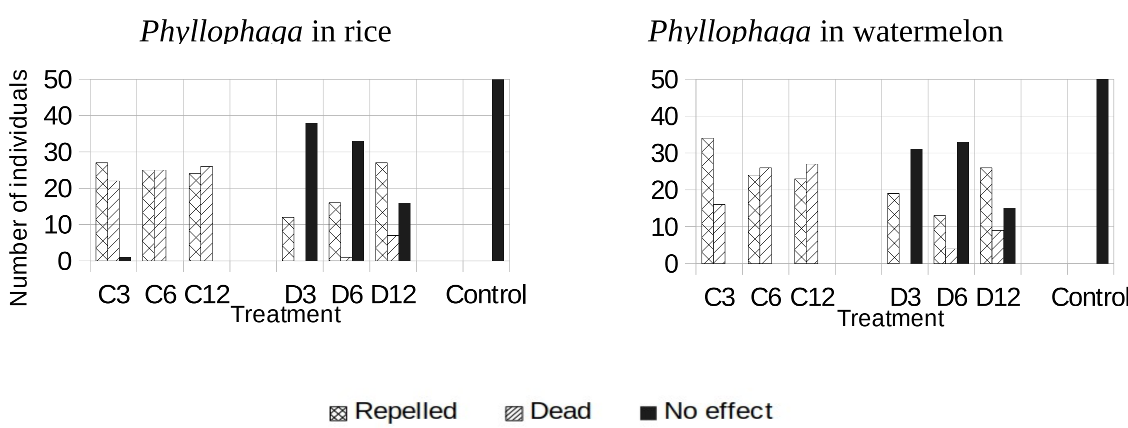

Figure 3.4 Count of Phyllophaga individuals in rice (A) and watermelon (B) plants,

*treated with three different concentrations of **Canavalia** and **Dolichos** flours and then compared with two "Control" treatments of identical results: corn starch and no bio chemical added. In the labels of X axis, letter "C" stands for **Canavalia** treatment and "D" for **Dolichos**; the contiguous numbers of 3, 6 and 12, indicate the amount of grams of flour per treatment.*

Tukey tests (see Table 3.11 and 4.12) demonstrated that there is a significant difference between the *Phyllophaga* populations at every concentration of ***Canavalia*** flour. The mean difference is big enough to support that assertion and the significance index indicates quantitative proof. The Pearson correlation obtained by comparing the data results of ***Canavalia*** extracts on red beans vs tomato, was $r^2 = 0.987$, significant at the 0.01 level with a 95% confidence interval, run on a model of 1000 bootstrap samples. The high level of correlation indicate that the results of the treatments can be considered equivalent between the two crops. In the case of ***Dolichos*** treatments, correlation result was similar, with a value of $r^2 = 0.984$ (significant at the 0.01 level).

Table 3.11: Tukey HSD test for multiple comparisons of Canavalia treatments used to test *Phyllophaga* repellence in watermelon and rice crops.

Crop tested	(I) grams of flour	(J) grams of flour	Mean Difference (I-J)	Std. Error	Signifi-cance
Watermelon	12	3	-6.25	13.9	0.97
		6	0.50	13.9	1.00
		Control	-7.00	13.9	0.96
	3	12	6.25	13.9	0.97
		6	6.75	13.9	0.96
		Control	-0.75	13.9	1.00
	6	12	-0.50	13.9	1.00
		3	-6.75	13.9	0.96

		Control	-7.50	13.9	0.95
	Control	12	7.00	13.9	0.96
		3	0.75	13.9	1.00
		6	7.50	13.9	0.95
Rice	12	3	-7.75	13.8	0.94
		6	-1.50	13.8	1.00
		Control	-8.00	13.8	0.94
	3	12	7.75	13.8	0.94
		6	6.25	13.8	0.97
		Control	-0.25	13.8	1.00
	6	12	1.50	13.8	1.00
		3	-6.25	13.8	0.97
		Control	-6.50	13.8	0.96
	Control	12	8.00	13.8	0.94
		3	0.25	13.8	1.00
		6	6.50	13.8	0.96

Table 3.12: Tukey HSD test for multiple comparisons of Dolichos treatments used to test phyllophaga repellence in watermelon and rice crops.

Crop tested	(I) grams of flour	(J) grams of flour	Mean Difference (I-J)	Std. Error	Significance
Watermelon	12	3	-7.75	13.6	0.94
		6	-1.00	13.6	1.00
		Control	-7.75	13.6	0.94
	3	12	7.75	13.6	0.94
		6	6.75	13.6	0.96
		Control	0.00	13.6	1.00

	6	12	1.00	13.6	1.00
		3	-6.75	13.6	0.96
		Control	-6.75	13.6	0.96
	Control	12	7.75	13.6	0.94
		3	0.00	13.6	1.00
		6	6.75	13.6	0.958
Cucumber	12	3	-9.50	14.2	0.91
		6	-2.50	14.2	1.00
		Control	-9.50	14.2	0.91
	3	12	9.50	14.2	0.91
		6	7.00	14.2	0.96
		Control	0.00	14.2	1.00
	6	12	2.50	14.2	1.00
		3	-7.00	14.2	0.96
		Control	-7.00	14.2	0.96
	Control	12	9.50	14.2	0.91
		3	0.00	14.2	1.00
		6	7.00	14.2	0.96

3.5) Nematode repellence

Disease caused by *Meloidogyne* was significantly reduced by the addition of 6 grams of ***Canavalia*** flour in tomato root zones at the time of transplant (Table 3.13). The dose of 12 grams did not exhibit a great change on disease prevention but it provided an apparent increased foliage vigor to the plant.

For ***Dolichos*** treatments, 12 g dose was the more effective but still exhibited less advantages compared to ***Canavalia*** (Figure 3.5). The inoculated control plants proved that the infection method was successful and the non inoculated control eliminates other variables as the cause of differences between treatments. The coefficient of correlation of the data obtained during the first and second repetition, were 0.99 for ***Canavalia*** treatments and 0.98 for ***Dolichos*** *treatments,* leading to the assumption that the resulting averages were in concordance.

Table 3.13: Degree of visual injury in tomato root systems by *Meloidogyne.*

Test	***Canavalia* flour**			***Dolichos* flour**			Inoculated Control	Non inoculated Control
	3 g	6 g	12 g	3 g	6 g	12 g		
Tomato, first repetition	3.2	1.5	1.1	5.3	4.2	3.1	7.0	0.0
Tomato, second repetition	5.2	2.4	1.2	5.5	4.8	3.3	7.0	0.0

* Numbers represent the average of WNR index values.

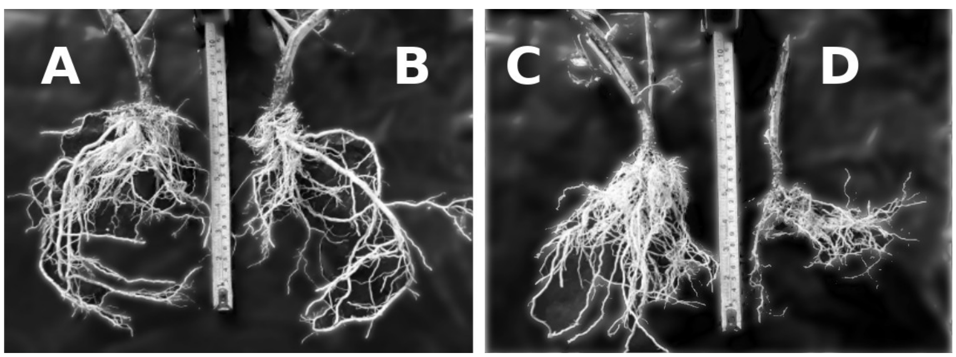

Figure 3.5: Tomato roots, 30 days after infestation with *Meloidogyne*. Central measurement tape in both pictures is indicating 10.5 inches, for visual reference. Roots treated with (A) X mg of ***Canavalia*** flour manifested a mild level disease, (B) same treatment with ***Dolichos*** exhibited a moderate level disease, (C) a non inoculated healthy root system served for control and (D) another successfully infected plant with no treatment showed a severe disease damage. *Photos: Carlos Martínez.*

3.6) Effects on plant nutrition

Table 3.14 describes the conditions of the soil in where the experiments of plant nutrition took place. According to the composition of ***Canavalia*** and ***Dolichos*** flours (see Table 3.15), it was estimated that their major contributions to plants were nitrogen, boron, phosphorus, potassium and sulfur.

Doses of 3 grams of each flour per plant showed less effective results than the dose of 6 grams. However, the application of 12 grams did not show any undesired side effect, but also showed an important advantage over its predecessor because the extra amount of matter had a slower decomposition rate; its nutrients would be beneficial in a longer period of time than the lapse of 50 days considered in this study, meaning that 6 grams per plant would be a more suitable dose for results in the short term.

Figure 3.6 compares the results of treatment after first repetition and Figure 3.7, the second repetition. There were differences on the growth pattern of the subject plants because environmental conditions were hotter and dryer during the first test than in the second. However, correlation indexes between repetitions were 0.992 for ***Canavalia*** treatments, 0.997 for ***Dolichos***, 0.938 for Control 1 (corn starch) and 0.948 for Control 2 (no extra treatment). Both treatments proved to have beneficial effects on plant nutrition; the reason because ***Dolichos*** exhibited more beneficial effects could be related to the fact that its decomposition seemed to be faster.

Table 3.14: Soil test results

Texture	Clay loam
O.M.	3.5%
pH	4.6
High	Mg, Fe
Good	K, Ca, S, Mo, Cu, Mn
Low	P, B, Zn
Very low	N

Table 3.15: Mineral nutrient content of flours			
Nutrient	Canavalia flour	Dolichos flour	Method
N	2.83 %	2.48 %	Kjeldahl
P	0.07 %	0.07 %	Colorimetry
K	0.89 %	0.65 %	Emission spectrophotometry
Ca	0.08 %	0.06 %	AA spectrophotometry
Mg	0.10 %	0.11 %	AA spectrophotometry
S	0.06 %	0.37 %	Colorimetry
Fe	0.02 %	0.02 %	AA spectrophotometry
Mn	0.00 %	0.00 %	AA spectrophotometry
Zn	0.00 %	0.00 %	AA spectrophotometry
B	0.23 %	0.30 %	Colorimetry
Cu	0.00 %	0.00%	AA spectrophotometry

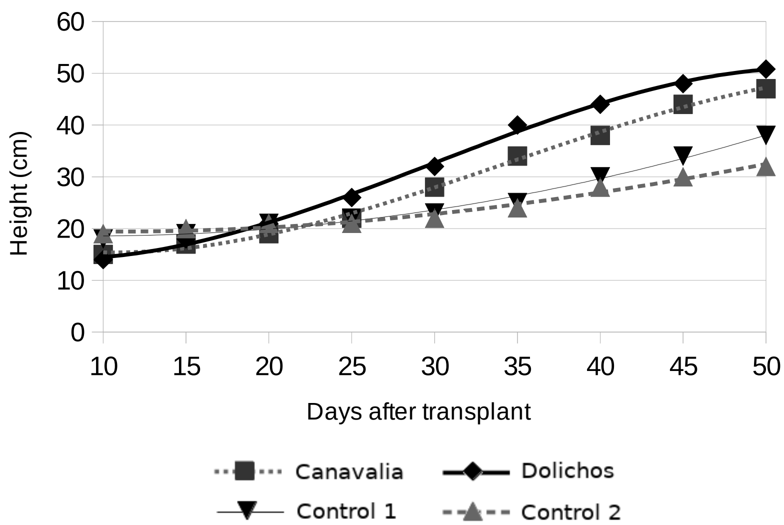

Figure 3.6: Comparison of the growth of tomato plants in four treatments, during the first repetition of the experiment. The dose of 6 grams of ***Canavalia***, ***Dolichos*** and corn starch (Control 1) were 6 grams of dry flour. Control 2 was provided with no flour. Points represent the average of each group and the regression line for ***Canavalia*** was $f(x) = -0.081x^3 + 1.77x^2 - 5.99x +$

21.3; for ***Dolichos,*** $f(x) = -0.101x^3 + 1.81x^2 - 4.68x + 17.4$; for Control 1, $f(x) = 0.026x^3 + 0.175x^2 - 1.41x + 15.8$ and for Control 2: $f(x) = -0.005x^3 + 0.287x^2 - 1.19x + 20.7$.

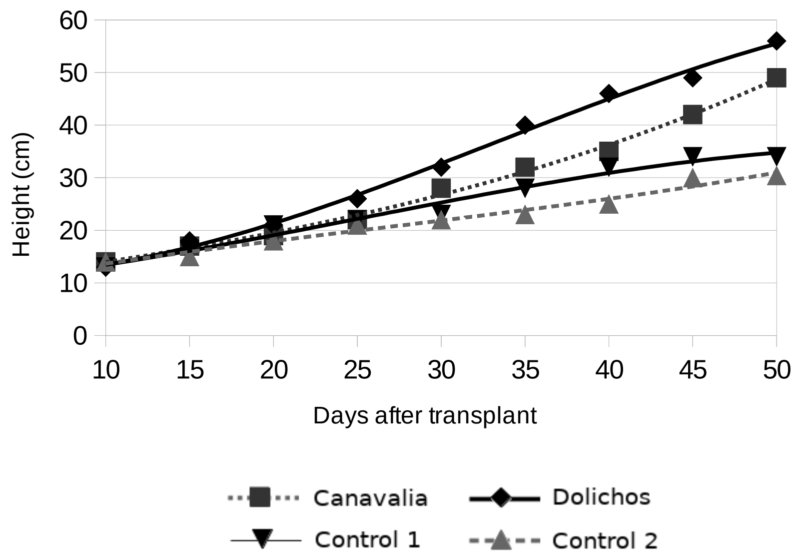

Figure 3.7: Comparison of the growth of tomato plants in four treatments, during the second repetition of the experiment. The dose of 6 grams of ***Canavalia, Dolichos*** and corn starch (Control 1) were 6 grams of dry flour. Control 2 was provided with no flour. Points represent the average of each group and the regression line for ***Canavalia*** is $f(x) = -0.014x^3 + 0.74x^2 - 2.30x + 11.6$; for ***Dolichos***, $f(x) = -0.055x^3 + 0.945x^2 - 0.853x + 11.7$; for Control 1, $f(x) = 0.024x^3 + 0.293x^2 - 1.96x + 11.2$ and for Control 2, $f(x) = -0.013x^3 + 0.169x^2 - 2.66x + 11.1$

3.7) Other non-measured observations

There were other phenomena that occurred unexpectedly and were not properly measured because they were not part of the experiment design. However, these ‘emergent properties’ could build interest in the topic for future research, the reason for which they are briefly mentioned in this section. The first was the observation of a certain repellent effect on lepidoptera in both treatments, ***Canavalia*** and ***Dolichos***. A second situation was that several tests on soil had to be repeated due to the development of ecto mycorhizae (under field conditions), especially in those treatments with 6 and 12 grams of Canavalia flour, suggesting that the substance was not antagonist to this symbiotic microorganism and yet favored the infection. One last finding was that flours applied over the soil surface seemed to repel defoliation by ants and also tended to benefit inflorescence development.

4. Discussion

Trials to test thrips control in cucumber and water melon plantations, exhibited no important repellence effect at any dose of ***Canavalia*** and ***Dolichos*** treatments. In fact, Tukey tests demonstrated that there were no significant differences between Control and other treatments. This results are in concordance the laboratory tests performed by Shan et al. (2012).

An important number of white flies (tested on cucumber and tomato) could be repelled for a period of seven days with ***Canavalia*** treatment at the dose of 10%. In contrast, 20% concentration showed only little improvement and caused a dark green leaf coloration with plant stunting for an approximate period of one week; development was later resumed and plants could eventually reach the same size as Control plants. ***Dolichos*** showed a more limited repellence effect with the same temporal plant stunting. However, this results may not be beneficial enough since white flies are vectors of virus and the reduction on the population of this pest can not completely guarantee the absence of infestation. For this reason, preventive treatmentss with ***Canavalia*** at the concentration of 5% are preferable than corrective treatments.

Diabrotica could be repelled for an approximate time of 8 days in red beans and tomato. Any dose of 5%, 10% and 20% was effective, but the highest dose exhibited the same mentioned counter effect. Repellence was more satisfactory with ***Canavalia*** than ***Dolichos*** treatment.

Phyllophaga, also a coleoptera as *Diabrotica*, could be effectively repelled by ***Canavalia*** treatment. However, ***Dolichos*** exhibited a slightly weaker effect. About half of the insects died and the survivors could resume their parasitic activity after a month. The presence of increased organic matter in soil must not be considered the cause, because the corn starch control treatment exhibited a zero repellence activity. In other terms, the ***Canavalia*** and ***Dolichos*** flours applied to soil in the previous moment to transplant did not exhibit any kind of counterproductive effect. In contrast, they appeared to have a beneficial influence on plant nutrition. These results are in concordance with Pino et al. (2013) and Meseguer et al. (2008) who described that the powder of a species of *Canavalia* possesses an effective insecticidal and repellent effect over *Sitophilus zeamais* Motschulsky, which is a Coleptera similar to the Diabrotica and Phyllophaga tested in this study. Other control effects could be found in literature in order to compare the results of this study: a insecticidal peptide derived from a *Canavalia* species, which was convenient for control of *Spodoptera frugiperda* and *Dysdercus peruvianus* (Mulinari et al., 2007), toxicity for *Oncopeltus fasciatus* (Defferrari et al., 2011)*,* Atta sexdens (Hebling et al., 2000) and Acyrthosiphon pisum (Sauvion et al., 2004)

Results from some experiments indicate that ***Dolichos*** have a real but limited insecticidal/repellent effect. For example, ***Dolichos*** has been reported as adulticidal for many *Diptera* (Kamaraj et al., 2010; Hazarika et al., 2012; Mavundza et al., 2014), and toxic to some *Adisura atkinsoni* at the dose of 10% v/v (Chakravarthy et al., 1985), ***Canavalia gladiata*** flour demonstrated a reduced infestation of *Meloidogyne*, as it has been previously demostrated for *Canavalia ensiformis* (Lopes et al., 2009; Crozzoli et al., 2001), which is capable to produce *"oxidation of the cuticle with periodate under mild conditions"* (Spiegel et al., 2011). ***Dolichos lablab*** flour showed a certain suppression level for *Meloidogyne* damage, similarly as proven in laboratory conditions (Araya, et al., 1994), or in open field when the foliar biomass is incorporated as cover crop (Brandenburg et al., 2010).

In all experiments, corn starch had a non-relevant beneficial effect, indicating that the positive results of ***Canavalia*** and ***Dolichos*** flours cannot be achieved by every kind of organic substances. The ground powders of the these seeds possess a useful effect for integrated pest management programs and can significantly contribute to plant nutrition in the short term, which is comparable to the evidence of those cases in which soil fertility has been restored by the incorporation of cover crops of a similar species, *Canavalia ensiformis* (Pohlan et al., 2008; Fageria et al., 2005; Buckles et al., 1998) and also *Dolichos lablab* (Karuma et al., 2011; Carsky et al., 2001; Schaaffhausen, 1963).

5. Conclusions

Canavalia gladiata and *Dolichos lablab* are two legumes that can be used to improve soil fertility. In subsistence agriculture, this practice is particularly recommended because of its low cost and the possibility of using raw seeds to produce botanical insecticides or repellents that contribute to the organic production of vegetables and cereals. Their extracts can repel some foliage insects and the flour of their ground seeds applied to soil can contribute to preventing some Coleptera pests, reducing the need of dangerous agrochemicals. *Canavalia* exhibited more benefits than *Dolichos* in the case of Salvadorian agriculture. Integrated nematode management plans can also be improved by the addition of these types of flour in the root zones during the transplant, also contributing to plant nutrition as the organic matter is decomposed. All the mentioned treatments are recommended for plants that have already past the emergence phase, in order to avoid negative side effects of the applied flour from these two legumes.

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