

Effects of bean seed treatment to the imidacloprid-gaucho on the Bean Stem Maggot, the Black Bean Aphids attacks and the Bean Common Mosaic Virus transmission

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ABSTRACT

The common bean (*Phaseolus vulgaris L.*) is one of the principal food crops of Rwanda. It is cultivated by 97% of the farms and constitutes the principal source of proteins for the majority of the Rwandan population. One observes since 2000 a considerable reduction in the bean outputs; among the principal causes, one can quote the transmitted diseases and damage caused by insects.

In order to contribute to the production of healthy seeds and plant of bean, a test was carried out (February-May 2009) to the station of Rubilizi-ISAE Busogo, from which the objective was to protect the plants of bean against the attacks of insects during the first 8 weeks by coating the seeds with the imidacloprid-gaucho in order to avoid the viral diseases on the plants which result from these seeds and to also thwart the attacks of the principal devastating insects of the bean among which there were the black bean aphid (BBA), *Aphis fabae*, vector of bean common mosaic virus and the bean stem maggot (BSM), *Ophiomyia spp.* Three doses of imidacloprid-gaucho were compared to the control, namely 2, 4 and 6g of active matter per kilo of seeds.

The results made possible to draw the following conclusions:

- the imidacloprid expressed its effects up to eight weeks after sowing; indeed one recorded few close Bean Common Mosaic Virus-attacked plants (less than 3%) until 56 days after sowing; the percentage of virus attacked plant increased beyond to reach 42% for the dose of 2g of imidacloprid per kilo of seeds and only 25% maximum for 4 and 6g of the insecticide product per kilo of seeds;
- the imidacloprid allowed also to control the Bean Stem Maggot in the proportion of 58%, 44%, 22% and 18% respectively for the control (0 g), 2, 4, and 6 g of the product per kg of seeds.

Within the sight of these results, we can recommend the producers, the pelleting of bean seeds to the dose 4g of imidacloprid-gaucho per kilo of seeds within the framework of the integrated pest management (IPM) against the common mosaic and the damage of Black Bean Aphids and Bean Stem Maggots.

Key words: *Imidacloprid-Gaucho, Black bean aphid, Bean stem maggot, Bean common mosaic virus, Integrated pest management.*

2- INTRODUCTION

The common bean (*Phaseolus vulgaris L.*) is the most important grain legume crop in Eastern and Southern Africa. Since common beans provide an inexpensive

source of proteins compared to animal sources, they are particularly important to low-income families. More than ninety percent of the beans in Eastern Africa are grown by small holder farmers with few

inputs. The source of seed for these farmers is either saved from their previous harvests or bought from local markets (HART and SAETTLER, 1981).

The common bean (*Phaseolus vulgaris* L.) currently constitutes one of the principal food crops in Rwanda. It covers itself 29% of the entire cultivated surface for all the food crops, according to MINAGRI (2008). On mean, a rwandan citizen would annually consume 51 kg of leguminous grain whose $\frac{3}{4}$ consist of bean (CIAT, 2000). The bean constitutes a significant source of proteins because it brings, with him only 48.6% of protein provided by the food crops (NDAMAGE, 2010).

According to Minagri (2010), the bean is cultivated in the areas ranging between 800 and 2300 m of altitude while the producing bean areas are: Imbo, Mayaga, Impala and Plates of the East.

Several eco-climatic, edaphic, socio-economic and biological constraints weigh on the bean cropping and inflict to him considerable fall of output (ISAR, 1987). The bean yield varies from 2500 to 3000 kg/ha and 900 to 1500 kg/ha respectively in station and rural areas (MINAGRI, 2006).

The bean yield regresses if not stagnates and several factors among which the weak fertility, the devastating insects and diseases contribute to this reduction. According to ISAR (2008), the diseases and devastating insects of bean recorded in Rwanda are mainly dominated by the common mosaic virus transmitted by the infected seed and the Black Bean Aphids (*Aphid fabae*), the Bean Stem Maggot (*Ophiomyia spp.*), the beetles (*Acanthoscelidae detectus*), Gray Worms (*Agrotis segetum*) and the anthracnose (*Colletotrichum lindemuthianum*).

Among the diseases and devastating insects of bean, the Black Bean Aphids (BBA) and the mosaic virus which it conveys as well as the Bean Stem Maggot (BSM) currently occupy a significant place especially on the currently popularized climbing varieties.

The virus of the common mosaic is transmitted mainly by the infected seed

(principal source of primary inoculums) and by various types of aphids (AUTRIQUE and PERREAUX, 1991) like by the mechanical way (HOWARD and GUILLERMO, 2000; NIENHAUS, 2002).

The damage caused by the Black Bean Aphids (BBA) and the Bean Stem Maggots (BSM) varies according to the sensitivity of the cultivated variety, of the stock of the infecting virus, the moment and the origin of the infection as well as the environmental conditions (NIENHAUS, 2002).

The means of fight against the bean common mosaic remain preventive because there does not exist until today any plant health for the treatment of the plants already attacked by virus.

Current insecticide pulverizations having given very unsatisfactory results on the one hand and being very expensive and requiring a regular monitoring of the aphids 'attacks on the other hand, new alternatives of protection such as varietal resistance and integrated control must be required.

At the time the first option concerns a long-term research with a long-term result, the second with the recent appearance on the market of the very powerful insecticidal products for the seeds' treatment seems more promising.

The treatment of the healthy bean seeds would seem to be an effective, economic and integrated means of fight against the populations of Black Bean Aphids vectors of the mosaic virus and other devastating insects whose Bean Stem Maggot which threatens the bean crop at the beginning of its cycle. Such is the objective of this study.

3. MATERIALS AND METHODS

3.1. Experimental design

Within Rubilizi Station of the Higher Institute of Agriculture and Animal Husbandry, a trial was led out in a Completely Randomized Block Design (CRBD), where three different

treatments/doses of imidacloprid were compared to the control with five replications (blocs). The trial was carried out during the 2007B rainfall season in February-Mai 2007.

In each bloc, four 3m x 3m plots were established and in each plot beans were planted after the pelleting of seeds during 6 hours.

The treatments were: T0 = control; T1 = 2g of active matter of imidacloprid-gaucho/kg of seeds; T2 = 4g of active matter of imidacloprid-gaucho/kg of seeds; T3 = 6g of active matter of imidacloprid-gaucho/kg of seeds.

3.2. Observations and data collection

Field survey of bean insects' attacks was conducted four weeks after bean emergence, to determine the incidence and prevalence of black bean aphids and bean stem maggot as well as of Bean Common Mosaic Virus symptoms.

In each plot, which the number of bean plants attacked by the black aphids and bean stem maggot or showing common mosaic symptoms was counted and expressed in percentage of the emerged bean plants.

For the bean stem maggot, the following symptoms were appreciated: poor plant growth, leaf chlorosis, premature

defoliation and death; stems thicker than normal with crack above soil.

For the incidence the black bean aphid on common beans, the parameter assessed included stems, leaves and flowers and the percent numbers of plants having the black bean aphids were expressed as the percentage incidence for that particular plot.

After the angular transformation, the data obtained was subjected to ANOVA using EXCEL. Comparisons were made between treated and untreated plots for the common mosaic and black bean aphid and bean stem maggot incidence. Differences were tested using the Least Significant Difference (LSD) test at various significance levels ($p \leq 0.05$; $p \leq 0.01$ and $p \leq 0.001$).

4. RESULTS

4.1 Effect of imidacloprid on the development of black bean aphids population

1. Data presentation

In each block and for all the treatments under study, the number of plants colonized by the black bean aphids is counted at the end of each week. The table 1 below presents the cumulated number of the plants attacked by treatment and by replication at the end of the experiment.

Table 1- Cumulated number and percentage of plants colonized by the black bean aphids

Treatments	Bloc 1		Bloc 2		Bloc 3		Bloc 4		Bloc 5		Mean	
	N	%	N	%	N	%	N	%	N	%	N	%
T0	18	42.6	20	48.8	19	51.3	22		48.9	57.5	20.4	50.2
T1	2	3.8	2	3.8	3	5.7	0		0.0	2.0	1.6	3.8
T2	1	1.9	2	3.8	1	1.9	1		1.9	2.0	1.2	2.3
T3	0	0.0	1	1.9	0	0.0	1		1.9	1.8	0.6	1.1

Table 2- The angular transformation of the cumulated percentages of plants colonized by the black bean aphids*

Treatments	Bloc 1	Bloc 2	Bloc 3	Bloc 4	Bloc 5	Averages
T0	1.49	1.55	1.60	1.55	1.72	1.58
T1	0.39	0.39	0.48	0.00	0.28	0.31
T2	0.28	0.39	0.28	0.28	0.28	0.30
T3	0.00	0.28	0.00	0.28	0.27	0.16

*The angular transformation ($y = 2 \arcsin \sqrt{\frac{y}{n}}$) for having a normal distribution of data before the statistical analysis.

2- Data analysis

Indicate: v, the number of treatments r, the number of replications yij, the number of plants colonised by black bean aphids of the i^{th} treatment and j^{th} replication R_j , the total relating to the j th replication for the v treatments V_i , the total relating to the i th treatment for the r replications G, the grand total

(i) Calculation of the sums of the squares and the degrees of freedom
 The factor of correction, FC, is obtained by the following law:

$$FC = \frac{G^2}{vr} = \frac{(\sum_{i=1}^v \sum_{j=1}^r y_{ij})^2}{vr} = 6.95$$

The total sum of squares (TSS) is calculated as follows :

$$TSS = \sum_{i=1}^v \sum_{j=1}^r (y_{ij})^2 - FC = \sum_{i=1}^v \sum_{j=1}^r (y_{ij})^2 - \frac{(\sum_{i=1}^v \sum_{j=1}^r y_{ij})^2}{vr} = 6.902$$

with (vr - 1)=12 df

The sum of squares relating to the replications (SSR)

$$SSR = \frac{\sum_{j=1}^r (R_j)^2}{v} - FC = \frac{\sum_{j=1}^r (R_j)^2}{v} - \frac{(\sum_{i=1}^v \sum_{j=1}^r y_{ij})^2}{vr} = 0.050$$

; with (r-1)=4 df

The sum of squares relating to the treatments (SST)

$$SST = \frac{\sum_{i=1}^v (V_i)^2}{r} - FC = \frac{\sum_{i=1}^v (V_i)^2}{r} - \frac{(\sum_{i=1}^v \sum_{j=1}^r y_{ij})^2}{vr} = 6.632$$

with (v-1)=3 df

The residual sum of the squares (error)

This one is obtained by the following difference:

$$\text{Error} = TSS - SSR - SST; \text{ with } (v-1)(r-1)=12 \text{ df}$$

$$\text{Error} = \sum_{i=1}^v \sum_{j=1}^r (y_{ij})^2 - \frac{\sum_{i=1}^v (V_i)^2}{r} - \frac{\sum_{j=1}^r (R_j)^2}{v} - \frac{(\sum_{i=1}^v \sum_{j=1}^r y_{ij})^2}{vr} = 0.22$$

Into final, one has:

$$\text{Error} = \sum_{i=1}^v \sum_{j=1}^r (y_{ij})^2 - \frac{\sum_{i=1}^v (R_j)^2}{v} - \frac{\sum_{i=1}^v (V_i)^2}{r} + \frac{(\sum_{i=1}^v \sum_{j=1}^r y_{ij})^2}{vr} = 0.22$$

with (r-1)(v-1)=12 df

(ii) Analysis of the variance and interpretation of the results

From calculations carried out above, the results of the analysis of the variance are summarized in the table hereafter:

Table 3- Summary of the variance analysis of the plants attacked by the black bean aphids

Source of variation	df	SS	MS	F _{obs}	F _{th(0.05)}	F _{th(0.01)}	F _{th(0.001)}	Conclusion
Blocs	4	0.050	0.01	0.69	3.26	5.41		NS
Treatments	3	6.632	2.21	120.63	3.49	5.95	10.8	***
Error	12	0.22	0.02					
Total	19	6.902						

NS: Differences statistically Non significant;
 ***Differences statistically very highly significant

The results of the analysis of the variance summarized in the table 3 reveal that there is any statistically significant difference between the blocks at the lowest significance level $p=0,05$ [$F_{obs}=0.69 \leq F_{th(0.05)}=3.26$]; this proves that the experimentation was started from a relatively homogeneous material and was led under homogeneous conditions in all plots. Conversely, the analysis shows very highly significant differences between the averages of the treatments even at the significance level $p=0.001$ [$F_{obs}=120.63 \leq F_{th(0.01)}=10.8$]. These

results lead us to conclude that the imidacloprid is very highly effective against the black bean attacks.

Being given these statistically very highly significant differences between the treatments, i.e. between the various doses of imidacloprid, it is significant to calculate the Least Significant Difference (LSD) at the quoted levels of significance; and to carry out the separation of the averages in homogeneous groups (table 4). The LSD is given according to DAGNELIE P. (2003), by the following relation from the law of Student:

$$PPDS = t_{\alpha} ES_m \sqrt{2} = t_{\alpha} \frac{ET}{\sqrt{r}} \sqrt{2} = t_{\alpha} \frac{\sqrt{CMR}}{\sqrt{r}} \sqrt{2} = t_{\alpha} \frac{\sqrt{2CMR}}{\sqrt{r}}$$

Table 4- Results of averages' separation in homogeneous groups

Treatments	Means	Homogeneous groups		
		LSD _(0.05) =0.19	LSD _(0.01) =0.26	LSD _(0.001) =0.34
T0	1.58	a	A	a
T1	0.31	b	B	b
T2	0.30	b	B	b
T3	0.17	b	D	b

It arises from table 4 that on all the levels of significance, only two homogeneous groups are dissociated. The three treatments of imidacloprid (T1, T2 and T3) gather in the same class and are distinguished very clearly from the control (T0) who, remains to him only in its group. These results once again show the effect of the imidacloprid-gaucho in seeds' treatment on the attacks of the

black bean aphids even to the least dose of 2g per kg of seed.

3. Effect of imidacloprid-gaucho on the epidemiologic evolution of the black aphids on the bean plants

The averages' comparison carried out for the various dates of observation (table 5) and the epidemiologic curve of BBA (figure1) show in a general way the effectiveness of the imidacloprid in seeds' treatment against the black aphids.

Table 5- Averages' comparison of the black bean aphids attacked plants at various dates of observation (March- April 2007)

Treatments	Dates of observation						
	19/03	26/03	02/04	09/04	16/04	23/04	30/04
T0	7.2 a	14.6 a	31.1 a	33.7 a	47.5 a	48.9 a	50.5 a
T1	0.0 b	0.0 b	0.4 b	0.4 b	2.3 b	3.0 b	3.1 b
T2	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c	1.1 bc	2.3 b
T3	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c	0.7 c	1.1 b

In the same column, the averages followed by the same letter are not significantly different according to the test of Newman & Keuls at the significance level $p = 0.001$.

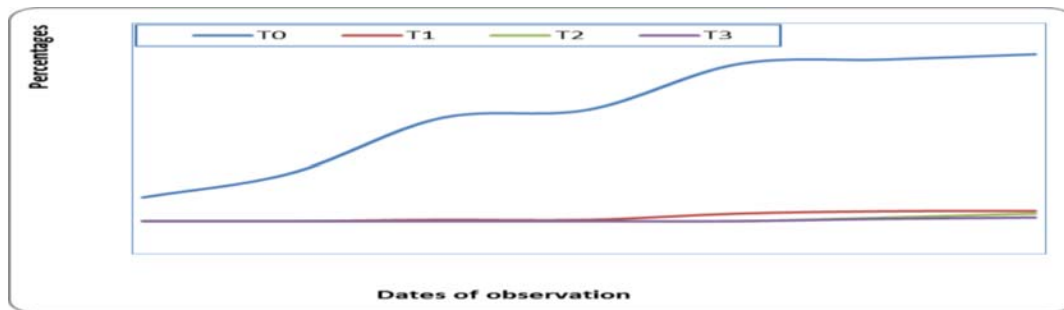


Figure 1: Epidemiologic curve of black bean aphids' evolution on plants by treatment

By comparing the observations on the evolution of the attacks of the black bean aphids with those on the evolution of the virus disease plants, one notes that the virus disease preceded the installation of

the black aphids on the bean plants; as well on the control as on the treated plants. The early appearance of the virus disease would thus not be due to the black aphids, they could be other winged species which

would have fed on these plants and would have thus transmitted the disease.

Indeed, MESSIAEN (1980) informs that there are three species of plant louses which attack bean and transmit the virus of the common mosaic; black aphids being most significant. Also the black aphids that one often meets on bean are those which are in the apterous phase. With the appearance of the black bean aphids, the virus disease is higher in the control plots than in the treated plots.

4.2- Evolution of the bean common mosaic disease in time

Table 6- ANOVA summary of data relating to the last date of observation on bean common mosaic evolution

Source of variation	Df	SS	MS	F _{obs}	F _{th(0.05)}	F _{th(0.01)}	F _{th(0.001)}	Conclusion
Blocs	4	0.0356	0.0089	3.07	3.26	5.41		NS
Treatments	3	5.5435	1.8478	637.38	3.49	5.95	10.8	***
Error	12	0.0348	0.0029					
Total	19	5.6139						

NS: Differences statistically Non significant; *** Differences statistically very highly significant

The comparison and separation of the averages of the mosaic virus attacked plants for the various dates of observation

The evolution of the bean common mosaic plants (number and percentage) on various dates of observation, the angular transformation of these percentages as well as the analysis of the variance of these data were carried out.

The data analysis on the common mosaic virus disease evolution (for various observation' dates) always revealed differences statistically very highly significant between the treatments and of the nonsignificant differences between the blocks as shown in the summary of the ANOVA of the cumulated data relating to the last date of observation (Table 6).

by the test of Newman & Keuls (DAGNELIE P, 1975) give the decision summarized in the table7.

Table 7- Averages' comparison of the mosaic virus attacked plants at various dates of observation (March- April 2007)

Treatments	Dates of observation						
	19/03	26/03	02/04	09/04	16/04	23/04	30/04
T0	0.0 a	8.8 a	29.7 a	40.1 a	66.7 a	79.8 a	82.7 a
T1	0.0 a	0.7 b	1.8 b	2.6 b	21.4 b	38.2 b	41.6 b
T2	0.0 a	0.4 b	0.7 b	0.7 b	8.7 c	23.3 c	24.3 b
T3	0.0 a	0.0 b	0.8 b	0.8 b	6.7 c	19.7 c	21.6 b

In the same column, the averages followed by the same letter are not significantly different according to the test of Newman & Keuls at the significance level $p = 0.001$. With the first observation, neither the control, nor the treated seedlings expressed the symptoms of the mosaic virus.

That wants to say that all the seeds were healthy or that the primary inoculum was null. From the second date of observation, the symptoms of the virus disease appeared very early on the control to reach the 30/04/2007 a very high percentage from approximately 83%. On the treated plants, the average percentage of mosaic virus attacked plants is very low and remains the same whatever the dose is until the 9/04/2007, or one month and half after sowing.

From the 16/04/2007 the plants of T1 express more symptoms of virus disease than those of T2 and T3; this is explained again by the quantity of product still in the plant, more significant quantity than that of T1.

If thus the literature informs that the imidacloprid has an eight weeks activity after sowing (KANSAS STATE UNIVERSITY, 1997 and LECLERCQ, 1992), under our conditions this activity was maintained for six weeks. This is explained by the faster degradation of the product in bean under our experimental conditions. The results of table 6 also show that beginning at the end of the observations treatments T2 and T3 behaved in the same way. All this is well illustrated by the epidemiologic curve of the virus disease of the figure 2.

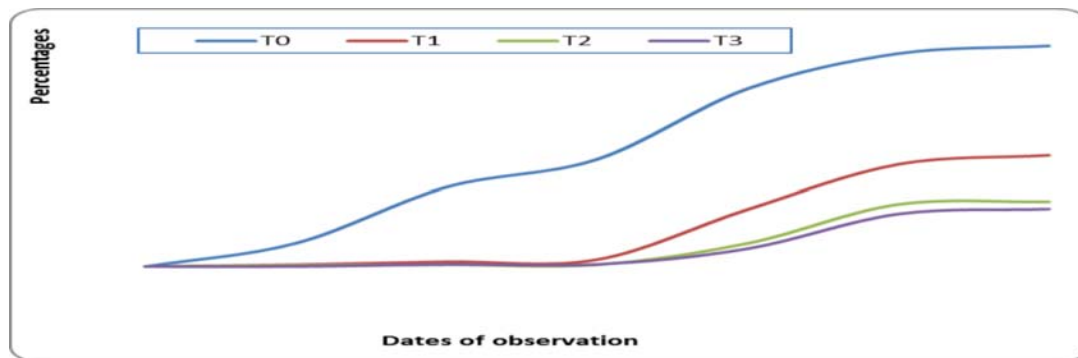


Figure 2: Epidemiologic curve of the bean common mosaic virus evolution by treatment

4.3 Effect of imidacloprid on the bean stem maggot attacks

The evolution of the number and the percentage of plants destroyed by the bean stem maggot at the various dates of observation, the angular transformation of these percentages as well as the analysis of the variance of these data were carried out. On each date of observation, the analysis of the variance of the data revealed

differences statistically very highly significant between the treatments ($p = 0.001$) and nonsignificant between the blocks ($p = 0.05$).

The comparison and the separation of the averages of the treatments in homogeneous groups according to the test of Newman & Keuls ($p = 0.001$) provide the decision summarized in table 8.

Table 8- Averages' comparison of the bean stem maggot attacked plants at various dates of observation (March- April 2007)

Treatments	Dates of observation						
	19/03	26/03	02/04	09/04	16/04	23/04	30/04
T0	4.6 a	6.0 a	9.7 a	12.1 a	16.9 a	21.3 a	24.2 a
T1	0.0 b	0.0 b	0.0 b	0.7 b	2.2 b	2.2 b	2.6 b
T2	0.0 b	0.0 b	0.0 b	0.7 b	2.6 c	2.6 b	2.6 b
T3	0.0 b	0.0 b	0.0 b	0.4 b	1.8 c	2.2 b	2.2 b

In the same column, the averages followed by the same letter are not significantly different according to the test of Newman & Keuls at the significance level $p = 0.001$. The examination of the table 8 results shows that the imidacloprid expressed a very effective effect against the bean stem maggot. Indeed, the percentage of

destruction of the plants by the bean stem maggot was higher (24%) on the control and very low on the treated plants (approximately 3%). Compared to the doses of the product, all the doses have a comparable effectiveness; this is still better illustrated on figure 3.

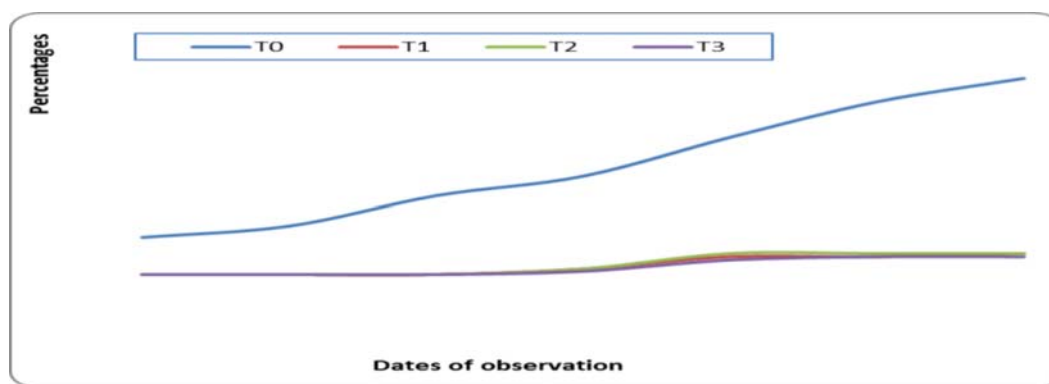


Figure 3- Evolution epidemiologic curve of the bean stem maggot by treatment

5. CONCLUSION AND RECOMMENDATIONS

The purpose of this study was to evaluate the effect of the imidacloprid in seeds' treatment on the black bean aphids (vector of the bean common mosaic virus) and the bean stem maggot in order to fight against these diseases. This fight would allow a good protection of the plants and would be the least polluting of the environment by reducing insecticide pulverizations in the fields.

With this intention, 3 doses of products: 2, 4, and 6 g of active matter per kg of seeds were compared with untreated seeds in an experimental design of completely randomized blocks with 5 replications. The observations related to the evolution of the virus disease and of the plants colonized by the black bean aphids as well as the evolution of the bean stem maggot attacked plants.

For one eight weeks period after sowing (period in which young plants are very sensitive to diseases and insects' attacks), the systemic insecticide imidacloprid gaucho expressed a great effectiveness against the black bean aphids' populations and also as consequence against the bean mosaic virus which they convey. Indeed, on all the observation dates, the data analysis always revealed differences statistically very highly significant between the treated and untreated plots. As results of imidacloprid action, one recorded close few bean common mosaic virus-attacked plants (less than 3%) until 56 days after sowing; the percentage of virus attacked plant increased beyond to reach 42% for the dose of 2g of imidacloprid per kilo of seeds and only 25% maximum for 4 and 6g of the insecticide product per kilo of seeds.

For the same period, the imidacloprid gaucho was also shown very effective against the bean stem maggot. The statistical analysis of the collected weekly data expressed differences statistically very highly significant between the treated and

untreated pieces. The product allowed to control the Bean Stem Maggot in the proportion of 58%, 44%, 22% and 18% respectively for the control (0 g), 2, 4, and 6 g of the product per kg of seeds.

Within the sight of these results, we can recommend the producers, the pelleting of bean seeds with the dose of 4 g of imidacloprid-gaucho per kilo of seeds within the framework of the integrated pest management (IPM) against the populations of black bean aphids and BCMV transmission as well as the damage caused by the bean stem maggots.

This crop protection method by seeds' treatment, once effective, has enormous advantages in the sense that it is less expensive and less polluting of the environment and that the insecticidal product has sufficient time to disintegrate before the harvest and the human consumption of the agricultural produce.

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