

Effect of Imidacloprid Gaucho-Seed Coating on Mosaic Virus Transmission and the Dynamics of the Main Insect Pests of Common Bean (*Phaseolus vulgaris* L.) Cultivation in Rwanda

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Abstract

The common bean (*Phaseolus vulgaris* L.) is one of the principal food crops of Rwanda and Central Africa. It is cultivated by 92% of the family farms and constitutes the principal source of proteins for the majority of the Rwandan population. Since 2000, there has been a considerable decrease of the common bean yields and among the main causes are diseases and insect pests in particular the black aphid (*Aphis fabae*) vector of the Bean Common Mosaic Virus (BCMV), the bean stem maggots (*Ophiomyia* spp.), Cutworm (*Agrotis segetum*) and Bean leaf beetle (*Cerotoma trifurcata*). For contributing to the search of affordable methods and less polluting for the protection of common bean against insect pests, three doses of imidacloprid for seed coating (2g, 4g and 6g of active ingredient per kg of seeds) were tested and compared to the control (untreated) in a Completely Randomized Block Design (CRBD) with 5 replications in 2016B agricultural season. The overall objective of the trial was to protect the bean plants from insect attacks for the first eight (8) week period of young age when the plants are vulnerable to disease and insect attack. The imidacloprid seed coating has shown positive effect up to 8 weeks after the seed emergence; very few virus-infected plants (less than 3%) were recorded until 8 weeks after plant emergence; the percentage of infected plants increased up to 42% for the dose of 2 g of active ingredient per kilo of seeds and 25% and 23.5% maximum, respectively for the doses of 4 and 6 g of active ingredient per kilo. However, the symptoms of common mosaic appeared before the presence of wingless aphids on common bean plants. This suggests that winged forms of the black aphid stung the plants before the wingless forms of the black aphid settled. The used product has also controlled other insect pests, namely the bean stem maggots (*Ophiomyia* spp.), Cutworm (*Agrotis segetum*) and bean leaf beetle (*Cerotoma trifurcata*). In view of these results, we can recommend to bean producers, the coating of bean seeds at the dose of 6g of active ingredient per kilo of seeds to protect common bean plants against the mosaic virus and other insect pests.

Key words: Imidacloprid, Bean Common Mosaic Virus, Black aphids, Cutworm, Bean Stem Maggots, Bean leaf beetle, Insect pests, Rwanda.

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Introduction

The development of food crops and cash crops is accompanied by a proliferation of pests which find favorable conditions in the tropics to their development and cause significant losses whatever the type of crop. The establishment of plant protection programs in the field becomes a priority objective. This explains the interest shown by universities and agricultural research centers in Rwanda, Africa and around the world. The common bean crop should be protected as its protein-rich grains enter the diets of the majority of Africans (Jerry and Smith, 1989).

It is currently recognized that 30 to 40% of production losses are caused by pests, parasites, pathogens and weeds (Umubyeyi and Rukazambuga, 2017). To ensure production, phytosanitary protection is still largely dominated by the use of synthetic pesticides. These products are very much accessible despite their cost.

According to Huignard *et al.* (2011), the predominance of chemical control has made it possible to limit losses, but can have negative repercussions on the environment, the health of farmers and consumers and encourage the development of resistance in pathogens. The concept of integrated pest management which highlights the management of pest populations by combining various approaches (cultivation techniques, plant resistance, biological antagonists, etc.) can help keep the levels of chemical pesticides below harmful thresholds. It is then necessary to put in place alternative strategies, easily usable by African farmers, thus allowing the appropriation of a real integrated pest management. This change of approach is not so new in itself, but is widely shared these days.

For facts and figures according to CIAT and PABRA (2016), African smallholder farmers cultivate more than four million hectares of beans each year, a source of food for more than 100 million Africans. East Africa holds the world record for per capita bean consumption at around 50 to 60 kg per year. With a rate of 22%, the bean is rich in protein; it also contains iron, zinc, fiber and slow carbohydrates.

The common bean (*Phaseolus vulgaris L.*) is the most important legume plant in East and South Africa. Compared to meat and fish, common bean is a cheaper source of protein; which makes it particularly important in feeding poor and low-income families. Over 90% of the beans consumed in East Africa are produced by small holder farmers with very little use of agricultural inputs (Huignard, 2011).

It is currently one of the main food crops in Rwanda and covers 27.2% of the total area sown for all food crops, according to NISR (2018). On average, the Rwandan consumes 51 kg of legume seeds annually, 75% of which are made up of beans (CIAT, 2000). Beans are an important source of protein because they alone contribute to 48.6% of the proteins provided by food crops (Ndamage, 2010).

Several eco-climatic, edaphic, socio-economic and biological constraints weigh on the bean crop and inflict a very considerable drop in yield (MINAGRI, 2008). Bean yields vary from 2,500 to 3,000 kg/ha and 900 to 1,500 kg/ha respectively in stations and in rural areas (MINAGRI, 2006). The yield of the bean decreases if not stagnates and several factors among which low soil fertility, pests and diseases contribute to this decrease. According to ISAR (2008), the bean crop diseases and pests recorded in Rwanda are mainly dominated by the Bean Common Mosaic

Virus transmitted by infected seed and the black aphid (*Aphis fabae*), the Bean Stem Maggots (*Ophiomyia spp.*), the Weevils (*Acanthoscelidae detectus*), the Cutworms (*Agrotis segetum*) and the Anthracnose (*Colletotrichum lindemuthianum*). The common mosaic virus is mainly transmitted by infected seeds (main source of primary inoculum) and by different types of aphids (Autrique and Perreaux, 1991) as well as by mechanical means (Howard and Guillermo, 2000; Nienhaus, 2002).

Among the common bean diseases and insects, the black aphid and the mosaic virus it carries, as well as the bean stem maggots currently occupy an important place, especially on the voluble varieties currently widely recommended for their productivity in Rwanda (MINAGRI, 2008).

The damage caused by aphids and bean stem maggots varies according to the sensitivity of the cultivated variety, the strain of the infecting virus, the time and origin of the infection as well as environmental conditions (Nienhaus, 2002 and Abate et al., 1996). The means to fight the common mosaic remain preventive because there is as of today no phytosanitary product for the treatment of viral diseases.

The current spraying of insecticides against insect pests having given unsatisfactory results and highly polluting the environment, new protection alternatives should be sought such as varietal resistance and integrated control. While the first option is long-term research with long-term results, the second with the recent appearance on the market of high-performance insecticide products for seed treatment seems more promising. The healthy bean seeds coating would appear to be an effective, cost-effective and integrated means of controlling populations of insect pests of bean cultivation.

Methodology

Study Area and Period

This experimental research was conducted during the agricultural season 2016B in Rubilizi Agricultural Station of the College of Agriculture, Animal Sciences and Veterinary Medicine, University of Rwanda. The station is located in Kanombe Sector of Kicukiro District at 1° 58' 37" South Latitude, 30° 6' 42" East Longitude, 1,530 m above sea level and is characterized by an annual average temperature of 13 to 27°C and a rainfall regime varying around 970 mm (European Union and Government of Rwanda, 2006, and MINITERE, 2005).

The previous crop on all the trial plots was eggplant fertilized with NPK while the surrounding crops were maize, soybeans and pineapple.

Plant Material

The plant material used was the NM 82 variety. It is currently recommended in Rwanda for its productivity and is very sensitive to several diseases including common virus. However, it manifests resistance genes against rust and angular spot diseases.

Experimental Setting

The test was conducted in Completely Randomized Bloc Design (CRBD) comprising 5 replications where 3 different treatments (doses of imidacloprid-gaicho) were compared to the control and thus leading to the setting of 20 plot units of 9 m² each. The treatments under study are as follow:

T0 = untreated control;

T1 = 2g of active ingredient/kg of seeds;

T2 = 4g of active ingredient/kg of seed

T3 = 6g of active ingredient /kg of seed.

Sowing was carried out at same time on all the plots six hours after the seeds have been coated with the product.

Data Collection and Analysis

The data collection focused on the seed emergence rates 10 days after sowing, the effect and development of insects on bean plants through meticulous observations on the insect attacks (black aphids, bean stem maggots, cutworm and bean leaf beetle) as well as the evolution of bean common mosaic in various plot units while the percentages of plants attacked were calculated compared to the total number of plants raised per plot and per treatment. For each date of observation the data obtained was subjected to the statistical analysis using Analysis of Variance (ANOVA). The comparison of the means and their separation into homogeneous groups were performed by calculating the Least Significant Difference (LSD) at different significance levels (p=0.05%) according to the Newman & Keuls test (Dagnelie, 2003).

Results and discussion

Effect of Imidacloprid on Black Aphids Attacks and Development on Common Bean Plants

The percentage evolution of plants colonized by aphids at different observation dates and the analysis of the variance of these data were carried out. For each date of observation, the analysis of variance has shown very highly significant differences between the treatments (p = 0.001) and non-significant differences between the blocks (p = 0.05). The separation of means into homogeneous groups according to the Newman & Keuls test (Dagnelie, 1975) gives the decision summarized in the Table 1 below.

Table 1. Means' comparison and separation into homogeneous groups of bean plants (%) colonized by aphids at the various dates of observation

Treatments	Number of weeks after sowing (WAS)													
	3 WAS		4 WAS		5 WAS		6 WAS		7 WAS		8 WAS		9 WAS	
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	4.7	b	5.1	b
T2	0.0	b	0.0	b	0.0	b	0.0	b	0.0	c	1.1	c	1.9	c
T3	0.0	b	0.0	b	0.0	b	0.0	b	0.0	c	0.7	c	1.1	c

In the same column, the means followed by the same letter are not significantly different according to the Newman & Keuls test at the significance level p = 0.5.

For the first four observations from 3 WAS to 6 WAS, the attack of aphids is almost zero for the treated plants, while it is higher on the control whose the cumulative

percentage reaches around 34%. This is explained by the product activity in the plant which negatively affects the development and therefore prevents the

establishment of colonies of black aphids on bean common plants. From 7 WAS to 9 WAS, the T2 and T3 treatments differ from the T1 treatment, by presenting an average percentage of colonized plants significantly lower than that of T1 who's the cumulative amount of attacked plants was 5.1% over

1.9% and 1.1% respectively for T2 and T3. This is due to the amount of product in the plant explained by a larger dose for T2 and T3 treatments. This is best illustrated in the Figure 1 of the plant-epidemiological evolution by aphid attack (50.5% for T0 against 1.1% for T3).

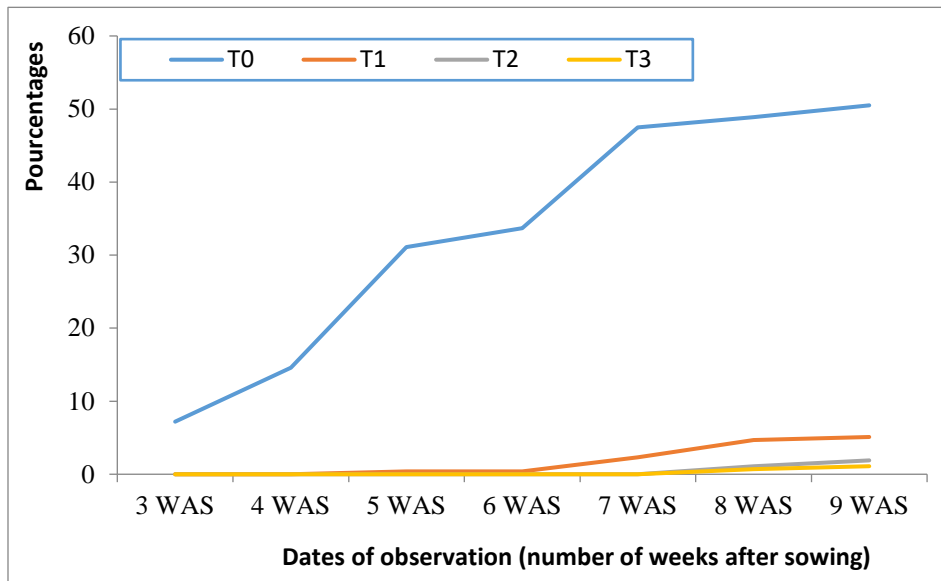


Figure 1. Extend (%) of the plants colonized by black aphids at various dates of observation

BCMV transmission by Aphids Influenced by Imidacloprid

The study then looked at the transmission of mosaic virus-attacked plants (%) at different observation dates. The analysis of variance at each date revealed also statistically very

highly significant differences between the treatments ($p = 0.001$) and non-significant differences between the blocks ($p = 0.05$). The means' comparison and separation into homogeneous groups by the Newman & Keuls test (Dagnelie, 1975) gives the decision summarized in the Table 2.

Table 2. Means' comparison and separation into homogeneous groups of mosaic virus attacked plants (%) at various dates of observation

Treatments	Number of weeks after sowing (WAS)												
	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS	8 WAS	9 WAS	3 WAS	4 WAS	5 WAS	6 WAS	9 WAS	
T0	7.2	14.6	31.1	33.7	47.5	48.9	50.5	a	a	a	a	a	a
T1	0.0	0.0	0.4	0.4	2.3	4.7	5.1	b	b	b	b	b	b
T2	0.0	0.0	0.0	0.0	0.0	1.1	1.9	b	b	b	c	c	c
T3	0.0	0.0	0.0	0.0	0.0	0.7	1.1	b	b	b	c	c	c

In the same column, the means followed by the same letter are not significantly different according to the Newman & Keuls test at the significance level $p = 0.5$.

On the first date of observation (3 WAS), neither the control nor the treated plants showed symptoms of the common mosaic. This means that all seeds were healthy or that the primary inoculum was zero. From the second date of observation (4 WAS), the symptoms of the common mosaic appeared very early on the control to reach at 9 WAS a very high percentage of approximately 83%. However, for the treated plots, the average of virus-attacked plants was very low (3%) and remained statistically and significantly the same regardless the dose until the fourth date of observation (6 WAS) or one and a half months after sowing. From 7WAS, plants from T1 treatment were severely attacked than those of T2 and T3 (Table 2);

what could be explained by the amount of product still in the plant, greater than that of T1.

If therefore the literature indicates that imidacloprid in the plant remains active for a period of eight weeks after sowing (Kansas State University, 1997 and Leclercq, 1992), under our conditions this activity was maintained for six weeks. This is explained by the quick degradation of the product in the bean under our experimental conditions. The results in Table 2 also show that from the start to the end of the observations, the T2 and T3 treatments behaved in the same way as illustrated by the epidemiological curve of the Figure 2.

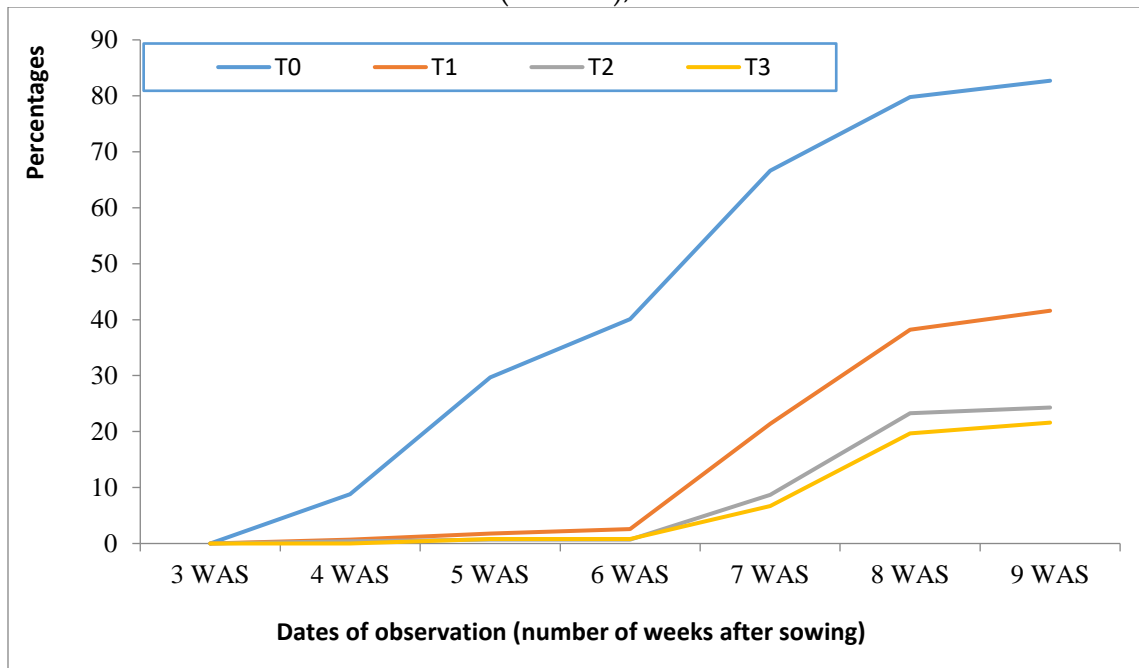


Figure 2. Transmission extends (%) of the viral mosaic attacked plants per treatment

While comparing the observations on the evolution of the black aphid attacks with those on the evolution of viral mosaic attacked plants, we notice that the viral mosaic preceded the installation of black aphids on bean plants; both on the control and treated plants.

The early appearance of the virus is therefore not due to black aphids, it could be other winged species which would feed on these plants and thus transmit the disease. Indeed, Messiaen (1980) informs that there are three species of aphids which attack the common bean and transmit the common mosaic virus; black aphids being the most

important. Also the black aphids that are often encountered on beans are those that are in the flightless phase. With the installation and development of black aphids, the number of virus was higher in the control than in the treated plots. Common bean seedlings damaged by Cutworms negatively affected by Imidacloprid

The evolution (%) of the plants sheared by cutworms at different dates of observation and the analysis of the variance were carried

out. The summary of the analysis and the means separation into homogeneous groups revealed also very highly significant differences between the averages ($p = 0.001$) and led to the situation summarized in the Table 3 hereafter.

Table 3. Means comparison and separation into homogeneous groups for bean plants (%) sheared by cutworms at various dates of observation

Treatments	Number of weeks after sowing (WAS)							
	3WAS		4 WAS		5 WAS		6 WAS	
T0	4.76	a	7.1	a	8.72	a	8.72	a
T1	0.64	b	1.46	b	1.82	b	1.82	b
T2	0.36	b	0.72	b	1.06	b	1.06	b
T3	0.36	b	0.72	b	0.72	b	0.72	b

In the same column, the means followed by the same letter are not significantly different according to the Newman & Keuls test at the significance level $p = 0.5$.

The Table 3 shows that the product had a negative effect on cutworms. In fact, on the untreated plants, about 8.72% of plants sheared by cutworms were recorded against only about 1% for the treated plants. On the other hand, there is no significant difference between the different treatments (doses).

Effect of Imidacloprid on Bean Leaf Beetle

Observations on the number and percentage of plants with leaves damaged by bean leaf beetle on different observation dates and analysis of variance on each date was carried out. The statistical analysis has always shown statistically very highly significant differences between the treatments ($p = 0.001$) and non-significant differences between the blocks ($p = 0.05$). The separation of the means by the Newman and Keuls test led to the situation summarized in Table 4.

Table 4. Means comparison and separation into homogeneous groups for bean plants (%) pierced by bean leaf beetle at various numbers of weeks after emergence of bean plants

Traitements	Number of weeks after sowing (WAS)					
	3 WAS		4 WAS		5 WAS	
T0	77.8	a	88.1	a	97.3	a
T1	9.4	b	13.8	b	16.4	b
T2	1.8	c	2.2	c	2.6	c
T3	1.1	c	1.4	c	2.2	c

In the same column, the means followed by the same letter are not significantly different according to the Newman & Keuls test at the significance level $p = 0.5$.

From the data in Table 4, it can be seen that the product had a negative effect on bean leaf beetle which usually attack bean leaves from emergence, causing a large number of holes in the leaves when fed on them. The holes thus created on the leaves decrease the photosynthetic surface and hence the yield. By comparing the different doses of product, it can be seen that the doses T2 and T3 are of comparable effectiveness and have been shown to be more effective than the dose T1.

Effet of Imidacloprid on the Bean Stem Maggots

This study also looked at the evolution (%) of bean plants with damage symptoms resulting from bean stem maggots feeding at various dates of observation. The ANOVA always performed did no cease to reveal statistically very highly differences between the treatments ($p = 0.001$) and no significant differences between blocs (0.05). The means separation into homogeneous groups by the Newman and Keuls test gave the decision summarized in Table 5.

Table 5. Means comparison and separation into homogeneous groups for bean plants (%) damaged by the bean stem maggots at various dates of observation.

Treatments	Number of weeks after sowing (WAS)													
	3 WAS		4 WAS		5 WAS		6 WAS		7 WAS		8 WAS		9 WAS	
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	b	2.6	b	2.6	b
T3	0.0	b	0.0	b	0.0	b	0.4	b	1.8	b	2.2	b	2.2	b

In the same column, the means followed by the same letter are not significantly different according to the Newman & Keuls test at the significance level $p = 0.5$.

The analysis of the results in Table 5 shows that the product had a very negative effect against the bean stem maggots. In fact for the control, the percentage of damaged common bean plants by the stem maggots

was higher (24%) and very low on the treated plants (approximately 3%) and it has not been noticed statistically significant differences between the doses of imidacloprid.

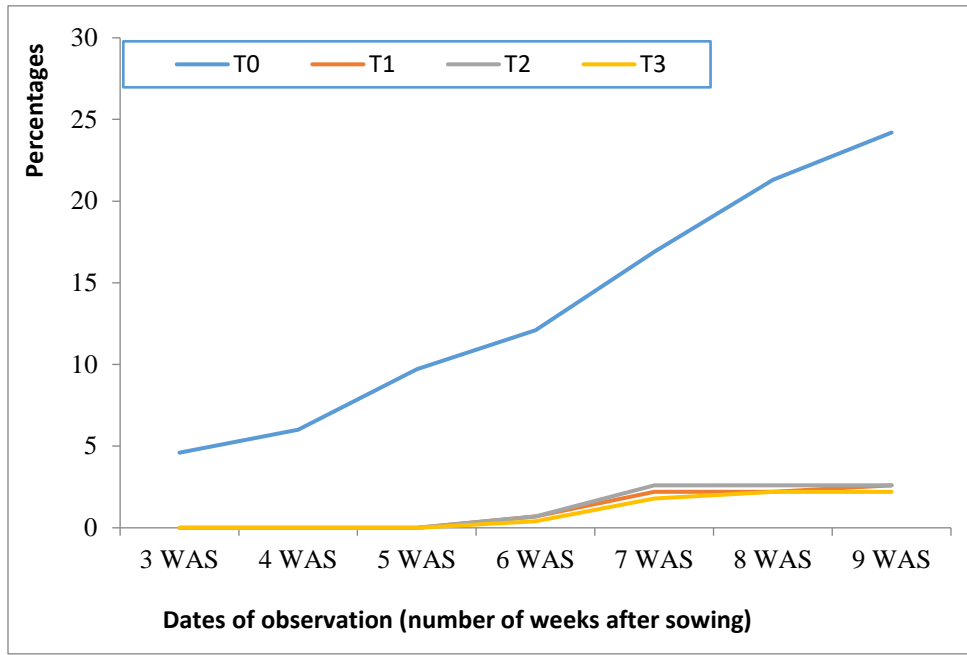


Figure 4. Extent of damage caused by the bean stem maggot over the growing period of bean plants

Conclusion and recommendation

The purpose of this work was to assess the effect of the imidacloprid-seed coating on the main insects and the diseases they transmit. The proposed technique under study would allow good protection of plants and would be the least polluting of the environment by reducing the spraying of insecticides in the fields.

To do this, 3 doses of products, namely 2, 4, and 6 g of active ingredient per kg of seed were compared with untreated seed in a Completely Randomized Bloc Design with 5 replications. Observations focused on the extend of common mosaic attacked plants, installation and development of black aphids on common bean plants as well as the

damage of other insects (bean stem maggot, cutworms and bean leaf beetle).

The imidacloprid has been shown to be very effective in protecting bean plants up to eight weeks after sowing. Through the control of populations of vector insects (*Aphis fabae*) in fact very few virus-infected plants were recorded (less than 3%) until 56 days after sowing; the percentage of these infected plants has increased beyond this to reach 42% at the dose of 2 g of active ingredient per kilo of seeds and 25% maximum for 4 and 6 g of active matter per kilogram of seeds. The symptoms of common mosaic appeared before the development of black aphids colonies on common bean plants; this suggests that black aphid winged forms stung the plants

before the black aphid flightless forms settled. The imidacloprid has also made it possible to effectively control other insects, namely cutworms, bean leaf beetle and bean stem maggots. In view of these results, we could recommend to bean grower, the coating of bean seeds at a dose of 4g of active matter per kilogram of seeds as part of the integrated control of common mosaic and other bean pest insects.

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