



# ECONOMIC FEASIBILITY OF APPLYING MICROBIAL INOCULANTS TO DARK TOBACCO CROP

## Factibilidad económica de la aplicación de inoculantes microbianos en el cultivo del tabaco negro

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**ABSTRACT.** During 2010/2011 and 2011/2012 in tobacco crop seasons, a research was carried out at the Tobacco Experimental Station of San Juan y Martínez, Pinar del Río, Cuba, with the objective of determining the economic feasibility of two microbial inoculant methods applied to dark tobacco crop in the sun. A random block was designed with 19 treatments and four repetitions. Different mineral fertilizer rates were studied combined with two microbial inoculants (*Azotobacter chroococcum* and *Bacillus megatherium* var. *phosphaticum*). Two variants were employed: an inoculation at sowing time of the seedbeds and another which included the initial inoculation plus a reinoculation at transplanting time. Better results were achieved by reinoculation and mineral fertilizer reduction than by inoculation method. The economic feasibility of *A. chroococcum* + *B. megatherium* var. *phosphaticum* reinoculation plus 75 % of total nitrogen and phosphorus rates was proved in dark tobacco cultivated at the sun.

**RESUMEN.** La investigación se realizó durante las campañas tabacaleras 2010/2011 y 2011/2012 en la Estación Experimental del Tabaco de San Juan y Martínez, Pinar del Río, Cuba. El objetivo fue determinar la factibilidad económica de dos métodos de aplicación de inoculantes microbianos en el cultivo del tabaco negro al sol. Se utilizó un diseño experimental de bloques al azar con 19 tratamientos y cuatro repeticiones. Se estudiaron diferentes dosis de fertilizante mineral en combinación con dos inoculantes microbianos (*Azotobacter chroococcum* y *Bacillus megatherium* var. *phosphaticum*). Se utilizaron dos variantes: una inoculación en el momento de la siembra del semillero y otra variante que incluye la inoculación inicial y una reinoculación en el trasplante. Con la reinoculación y disminución del fertilizante mineral se lograron mejores resultados que con el método de inoculación. Se demostró la factibilidad económica de la reinoculación de *A. chroococcum* + *B. megatherium* var. *phosphaticum* y el 75 % de la dosis total de nitrógeno y fósforo en el cultivo del tabaco negro al sol.

**Key words:** *Azotobacter*, *Bacillus megatherium*,  
production costs

**Palabras clave:** *Azotobacter*, *Bacillus megatherium*,  
costos de producción

## INTRODUCTION

In Cuba, the cultivation of black tobacco is a crucial line for the economy, as the leaves produced are exported raw or used directly in the production of pure Habano (1).

The tobacco is a very sensitive crop to the nutrient availability and suffers considerable variation in performance and quality as a result of inadequate

fertilization. Nitrogen (N) is the element that causes the greatest effects on growth and crop development and the quality of the leaves; however, the production of nitrogen fertilizers is limited by the large consumption of fuel required for the establishment of high temperatures and pressures for industrial production. This makes that nitrogen fertilizer production reaches high costs (2).

Improper use of mineral fertilizers, on the other hand, can adversely affect the environment, acidifying the soil, polluting the groundwater with nitrates washing and the nitrogen atmosphere by releasing gases (3). Therefore, it is essential, the search and evaluation of alternatives that fulfill the

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nutritional needs of crops and allow obtaining adequate levels of performance and product quality enable partial or total savings of fertilizers and permit to increase the biological processes soil as a sustainability index of the agricultural process (4).

The urgent need to find ways to improve the efficiency of mineral fertilizer utilization and acquired boom in technology implementation, increasingly more respectful of the ecosystem and natural resources have given new life and significant boost to the use of bio-fertilizers in agriculture (5). In recent decades, biotechnology has come to become a major field of scientific knowledge and agricultural technologies (6).

Biofertilization is one of the most valuable elements available to organic farming, which includes the use of micro-organisms living in the soil (7). Biofertilizers help to improve the quality and crop productivity by the partial or total elimination of chemical fertilizer addition (8).

In Cuba, different bioproducts have been tried that have proved their effectiveness as biofertilizers, biostimulants and biocontrol, all with a definition in their action mechanisms. Stimulator plant growth rhizobacteria are able to fix nitrogen, solubilize phosphates, and produce hormones, antibiotics and other compounds for crop development (9).

With all this, the importance of the joint implementation of bio-fertilizers and stimulants to bring about positive effects on crops (10) and part of the alternatives to be considered in sustainable agricultural systems is demonstrated, as they constitute an economically attractive means and ecologically acceptable to reduce external inputs, improve the quantity and quality of internal resources and ensure greater efficiency in the use of mineral fertilizers, achieving partial replacements of these (11).

The objective of this research was to determine the economic feasibility of two methods of application of biofertilizers in sun-grown black tobacco.

## MATERIALS AND METHODS

The research was conducted at the Experimental Station of tobacco in San Juan y Martínez, Pinar del Río, during the 2010/2011 and 2011/2012 tobacco campaigns. An experimental design of randomized blocks with 19 treatments and four replications was used. Microbial inoculants as *Azotobacter chroococcum* and *Bacillus Megatherium* var *phosphaticum* were applied. They were sprinkled on the seedbed immediately after planting at a dose of 2 L ha<sup>-1</sup>. At 45 days after sowing the transplant was carried out where two variants were used:

- ♦ Seedlings were reinoculated by dipping the root in a 50 % solution of the corresponding inoculum in each case.
- ♦ Seedlings were transplanted without reinoculating only with the initial inoculation.

The treatments were as follows:

- 1- 100 % of the mineral fertilizer (control)
- 2- 100 % of the mineral fertilizer + *A. chroococcum*
- 3- 100 % mineral fertilizer + reinoculation of *A. chroococcum*
- 4- 100 % of the mineral fertilizer + *B. Megatherium* var. *phosphaticum*
- 5- 100 % mineral fertilizer + reinoculation of *B. Megatherium* var. *phosphaticum*
- 6- 100 % of the mineral fertilizer + *A. chroococcum* + *B. Megatherium* var. *phosphaticum*
- 7- 100 % mineral fertilizer + reinoculation of *B. Megatherium*, *A. chroococcum* + var. *phosphaticum*
- 8- 75 % 100 % N + P + *A. chroococcum*
- 9- 75 % 100 % N + P + re-inoculation of *A. chroococcum*
- 10- 75 % 100 % P + N + *B. Megatherium* var. *phosphaticum*
- 11- 75 % 100 % P + N + reinoculation of *B. Megatherium* var. *phosphaticum*
- 12- 75 % N + 75 % + P + *A. chroococcum*, *B. Megatherium* var. *phosphaticum*
- 13- 75 % N + 75 % P + re-inoculation of *A. chroococcum* + *B. Megatherium* var. *phosphaticum*
- 14- 50 % 100 % N + P + *A. chroococcum*
- 15- 50 % 100 % N + P + reinoculation of *A. chroococcum*
- 16- 50 % 100 % P + N + *B. Megatherium* var. *phosphaticum*
- 17- 50 % 100 % P + N + reinoculation of *B. Megatherium* var. *phosphaticum*
- 18- 50 % 50 % N + P + + *B. Megatherium*, *A. chroococcum* var. *phosphaticum*
- 19- 50 % N + 50 % P + reinoculation of *A. chroococcum* + *B. Megatherium* var. *phosphaticum*

The selection was made from 10 strains of bacteria from both collections from INIFAT, according to the established procedure<sup>A</sup>.

Cultural activities are performed according to the Technical Manual for the Cultivation of Tobacco (12), except mineral fertilization, which was made using ammonium nitrate as carriers, Super Simple Phosphate, Potassium sulfate and Magnesium sulfate. In all cases, the potassium and magnesium fixed background were applied as recommended in the manual dose, while nitrogen and phosphorus were applied according to the limits specified for each treatment.

<sup>A</sup>Martínez, R.; López, M.; Dibut, B.; Parra, C. y Rodríguez, J. *La fijación biológica del nitrógeno atmosférico en condiciones tropicales*. edit. MPPAT, Caracas, Venezuela, 2007, 172 p.

After the curing stage, the leaves were selected to determine the overall performance and upper classes used for cigar twisted (13). The data obtained were subjected to an analysis of variance of simple classification and the difference between means were compared by the multiple range test of Duncan, with a probability of less than 0,05 and equal error. The economic analysis of the results was performed from the cost and value of the output produced on a hectare of tobacco for each of the treatments. The production cost was calculated from the date for tobacco semi-mechanized sun, Tobacco enterprise "Hermanos Saiz" (14), which was adjusted for the different variants, depending on the costs for data-cost mineral and organic fertilization, which were those with the most significant variations depending on the treatments analyzed. Biofertilizers used are marketed by LABIOFAM at a price of 50 \$ L<sup>-1</sup>.

The production value was calculated according to the official prices (15) and from these values the profits, profitability and cost economic indices were calculated by weight, as described below:

Utilities = value of total production-cost of production (\$).  
Productivity = profit / total cost of production 100 (%).  
Cost/weight = total cost of production/production value (\$).

## RESULTS AND DISCUSSION

In analyzing the effect of microbial inoculants (*A. chroococcum* and *B. Megatherium* var. *Phosphaticum*), its combination with different levels of mineral fertilization and the two methods of inoculation (Table I) on yields in upper types, it was observed a positive and superior effect on production quality with the reinoculated variant 75 % N+ 75 % P. With this variant the yield was increased to higher types to 238,75 kg ha<sup>-1</sup> in relation to the control treatment and decreased nitrogen and phosphoric mineral fertilizers by 25 %. Other authors have obtained satisfactory results in crops of the *Solanaceae* (16) family, such as the production of potato (*Solanum tuberosum* L), where the combination was used 75% of mineral fertilizer + Azomeg (*Azotobacter* and *Bacillus Megatherium*) and an increase was achieved in 2,88 % yield.

**Table I. Effect of the treatments in the total yield and in higher types**

Treatments	Yields in higher types (kg ha <sup>-1</sup> )	Total yield (kg ha <sup>-1</sup> )
1- 100 % of mineral fertilizer (control)	1474,05 c	1833,75 b
2- 100 % of mineral fertilizer + <i>A. chroococcum</i>	1320,02 ef	1738,60 c
3- 100 % of mineral fertilizer + reinoculation of <i>A. chroococcum</i>	1441,50 cd	1749,25 c
4- 100 % of mineral fertilizer + <i>B. megatherium</i> var. <i>phosphaticum</i>	1561,72 b	1899,62 b
5- 100 % of mineral fertilizer + reinoculation of <i>B. megatherium</i> var. <i>phosphaticum</i>	1304,10 ef	1721,35 c
6- 100 % of mineral fertilizer + <i>A. chroococcum</i> + <i>B. megatherium</i> var. <i>phosphaticum</i>	1378,57 de	1705,17 cd
7- 100 % of mineral fertilizer + reinoculation of <i>A. chroococcum</i> + <i>B. megatherium</i> var. <i>phosphaticum</i>	1229,87 gh	1571,00 efg
8- 75 % N + 100 % P + <i>A. chroococcum</i>	1068,82 k	1474,55 ij
9- 75 % N + 100 % P + reinoculation of <i>A. chroococcum</i>	1128,22 ijk	1491,15 hij
10- 75 % P + 100 % N + <i>B. megatherium</i> var. <i>phosphaticum</i>	1165,70 hij	1483,30 ij
11- 75 % P + 100 % N + reinoculation of <i>B. megatherium</i> var. <i>phosphaticum</i>	1321,65 ef	1624,20 ef
12- 75 % N + 75 % P + <i>A. chroococcum</i> + <i>B. megatherium</i> var. <i>phosphaticum</i>	1099,12 jk	1498,47 ghi
13- 75 % N + 75 % P + reinoculation of <i>A. chroococcum</i> + <i>B. megatherium</i> var. <i>phosphaticum</i>	1712,80 a	2228,20 a
14- 50 % N + 100 % P + <i>A. chroococcum</i>	1103,77 jk	1492,62 hij
15- 50 % N + 100 % P + reinoculation of <i>A. chroococcum</i>	1310,37 ef	1638,50 def
16- 50 % P + 100 % N + <i>B. megatherium</i> var. <i>phosphaticum</i>	1071,70 k	1416,97 j
17- 50 % P + 100 % N + reinoculation of <i>B. megatherium</i> var. <i>phosphaticum</i>	1279,02 fg	1643,82 de
18- 50 % N + 50 % P + <i>A. chroococcum</i> + <i>B. megatherium</i> var. <i>phosphaticum</i>	953,30 l	1475,35 ij
19- 50 % N + 50 % P + reinoculation of <i>A. chroococcum</i> + <i>B. megatherium</i> var. <i>phosphaticum</i>	1186,97 hi	1564,47 fgh
CV (%)	15,01	11,92
ES (x)	23,785	24,499

Similar results to those obtained in performance in upper types were achieved when evaluating total yields. In general, with the use of variants reinoculated in combination with reducing higher total mineral fertilizer, yields were achieved with the inoculated variants and reduction of mineral fertilizer. The best result was obtained and used to reinoculated the variant 75 N % +75 % P + *A. Megatherium* + *B. chroococcum* var. *phosphaticum*, with statistical differences for other treatments. The control treatment was overcome by this variant in 394,45 kg ha<sup>-1</sup> of total yield.

Several authors argue that the application of both biofertilizers in other crops of agricultural interest has been compelling and demonstrate the possibility of

reducing from 30 to 50 % applications of nitrogen fertilizer, achieving increases in yields between 10 and 20 % by the possibility of fixing nitrogen, phosphorus solubilize while favoring the absorption of other nutrients and produce active substances involved in growth and plant development (17, 18, 19).

In conducting the economic assessment (Table II), it was observed the increase in total costs by the application of microbial inoculants in treatments where 100 % of the mineral fertilizer is applied, which were more pronounced in treatments where it was used the double inoculation. Decreasing the 25 % and 50 % of mineral fertilizer costs were lower compared to the control treatment.

**Table II. Economic valoration**

Treatments/Factors	Total costs (\$ ha <sup>-1</sup> )	Value of production (\$ ha <sup>-1</sup> )	Utilies (\$ ha <sup>-1</sup> )	Profitability (%)	Cost/weight
1- 100 % of mineral fertilizer (control)	18 179,66	47 952,25	29 772,59	163,77	0,38
2- 100 % of mineral fertilizer + <i>A. chroococcum</i>	18 279,66	43 370,79	25 091,13	137,26	0,42
3- 100 % of mineral fertilizer + reinoculation of <i>A. chroococcum</i>	18 329,66	44 291,44	25 961,78	141,64	0,41
4- 100 % of mineral fertilizer + <i>B. megatherium</i> var. <i>phosphaticum</i>	18 279,66	49 147,38	30 867,72	168,86	0,37
5- 100 % of mineral fertilizer + reinoculation of <i>B. megatherium</i> var. <i>phosphaticum</i>	18 329,66	42 504,80	24 175,14	131,89	0,43
6- 100 % of mineral fertilizer + <i>A. chroococcum</i> + <i>B. megatherium</i> var. <i>phosphaticum</i>	18 379,66	42 468,36	24 088,7	131,06	0,43
7- 100 % of mineral fertilizer + reinoculation of <i>A. chroococcum</i> + <i>B. megatherium</i> var. <i>phosphaticum</i>	18 479,66	41 097,81	22 618,15	122,39	0,45
8- 75 % N + 100 % P + <i>A. chroococcum</i>	17 422,07	37 569,60	20 147,53	115,64	0,46
9- 75 % N + 100 % P + reinoculation of <i>A. chroococcum</i> reinoculated	17 472,07	39 029,50	21 557,43	123,38	0,45
10- 75 % P + 100 % N + <i>B. megatherium</i> var. <i>phosphaticum</i>	17 422,07	38 866,04	21 443,97	123,09	0,45
11- 75 % P + 100 % N + reinoculation of <i>B. megatherium</i> var. <i>phosphaticum</i>	17 472,07	41 671,63	24 199,56	138,50	0,42
12- 75 % N + 75 % P + <i>A. chroococcum</i> + <i>B. megatherium</i> var. <i>phosphaticum</i>	17 522,07	40 230,74	22 708,67	129,60	0,44
13- 75 % N + 75 % P + reinoculation of <i>A. chroococcum</i> + <i>B. megatherium</i> var. <i>phosphaticum</i>	17 622,07	49 342,03	31 719,96	180,00	0,36
14- 50 % N + 100 % P + <i>A. chroococcum</i>	16 564,48	39 342,43	22 777,95	137,51	0,42
15- 50 % N + 100 % P + reinoculation of <i>A. chroococcum</i>	16 614,48	42 067,23	25 452,75	153,20	0,39
16- 50 % P + 100 % N + <i>B. megatherium</i> var. <i>phosphaticum</i>	16 564,48	37 367,53	20 803,05	125,59	0,44
17- 50 % P + 100 % N + reinoculation of <i>B. megatherium</i> var. <i>phosphaticum</i>	16 614,48	42 181,04	25 566,56	153,88	0,39
18- 50 % N + 50 % P + <i>A. chroococcum</i> + <i>B. megatherium</i> var. <i>phosphaticum</i>	16 664,48	38 300,59	21 636,11	129,83	0,44
19- 50 % N + 50 % P + reinoculation of <i>A. chroococcum</i> + <i>B. megatherium</i> var. <i>phosphaticum</i>	16 764,48	40 470,84	23 706,36	141,41	0,41



The value of production was influenced by the yields achieved with each treatment classes, which maintained a direct relationship. The control treatment was only surpassed by 100 % variants of mineral fertilizer + *B. Megatherium* var. *phosphaticum* inoculated and 75% N + 75% P + *A. Megatherium* + *B. chroococcum* var. *phosphaticum* reinoculated. In all cases where the reduction of mineral fertilizer was studied, the variants reinoculated had greater economic effect than the inoculated variants. Treatment of better economic result was when we used 75 % N + 75 % P + *A. Megatherium* + *B. chroococcum* var. *phosphaticum* reinoculated, which provided higher performance classes and higher throughput, so the production value was higher with gains of 1 \$ 947,37 ha<sup>-1</sup> and a yield of 16,23 % over the control treatment, respectively and with a lower cost/weight.

It is important to note that this treatment can achieve a 25 % reduction of nitrogen and phosphate mineral fertilization; therefore there is also a positive influence on the environmental and economic ambient, since the application of products to enrich the soil microbial population and reduce the consumption of mineral fertilizers (20).

These results are related to others obtained in the cultivation of chickpea, where the biggest gains were achieved with the application of *Rhizobium*, followed by the genus *Azotobacter* strain INIFAT12 that influenced positively on physiological parameters and yield, they produced gains in production and lower costs of inputs, such as chemical fertilizers (21).

The use of these biofertilizers is an alternative for obtaining increasingly ecological products and less harmful to the environment which contributes to increase yields and reduce the use of mineral fertilizers (22, 23).

## CONCLUSIONS

- ◆ The use of the reinoculation method, with mineral fertilizer decreased by 25 %, permits to achieved better results than a single inoculation.
- ◆ The reinoculation of microorganisms such as *Azotobacter chroococcum* + *Bacillus Megatherium* in the cultivation of tobacco, saving 25 % of N and P fertilization, with increases in yields and profits in the production process.

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