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Insecticide Thiamethoxam: A Bioactive Action on Carrot Seeds (*Daucus carota* L.)

Andréia da Silva Almeida¹, Francisco Amaral Villela²,
Maria Ângela André Tillmann² and Geri Eduardo Meneghelo²

¹*Ciência e Tecnologia de Sementes;*

²*Ciência e Tecnologia de Sementes;*

University Federal de Pelotas

Brazil

1. Introduction

Explorations of the substances potential that can act as promoters of growth-inducing activities are in substantial contribution of research to agriculture. Many of these activities are related to the activation capacity of the plants resistance mechanisms, thus enabling seek control through integrated management.

With the modernization of agriculture, marked advances in farming techniques have been obtained, allowing mitigate the limiting factors with weather conditions such diseases, pests, among others. The plant physiology has promoted great advances in recent years with the advent of modern techniques such as the production of plants by tissue culture, genetic engineering and biotechnology. Among these modern techniques, the use of bioactive, capable of increasing the productive potential of plants, is an increasing use in the practice of modern agriculture and widespread in countries highly technical.

In Brazil, the use of bioactive beginning to be explored and the results of several studies have shown that these substances provide significant increases in productivity and, in quality, as observed, for example, significant increases in the amount of larger fruits. Bioactivators are natural substances of plant origin that have actions similar to the main plant growth regulators, aimed at growth and development of the plant. Provide better physiological balance, favoring closer ties to the genetic potential of culture.

Moreover, they are complex organic substances, not bioregulators, growth modifiers, capable of working in the plant transcription factors and gene expression in membrane proteins by altering the ion transport. They also act in metabolic enzymes could affect the secondary metabolism and may alter the mineral nutrition, induce the production of precursors of plant hormones, leading to hormone synthesis and more intense response to nutrients and plant hormones.

Applied to plants, bioactivators cause modification or alteration of specific metabolic and physiological processes, such as increasing the division and cell elongation, stimulation of chlorophyll synthesis and photosynthesis, flower bud differentiation, increasing the life of plants, softening the effects of adverse weather conditions and increasing the absorption of nutrients and setting their roots.

The bioactivator acts in the expression of genes responsible for synthesis and activation of metabolic enzymes related to plant growth by altering the production of amino acid precursors of plant hormones. With the increased production of hormones, plant expressed greater vigor, germination and root development. With a greater number of roots, increases the absorption and resistance of plant stomata to water loss, which benefits the metabolism and increases resistance to stress.

Thiamethoxam, 3-(2 - chloro - thiazole - 5 - ylmethyl) - 5 - methyl (1,3,5) oxadiazinan - 4 - ylidene-N- nitroamine, whose chemical structure is shown in Figure 1, is a systemic insecticide neonicotinoid group, family nitroguanidine, which acts on acetylcholine nicotinic receptor in membrane of insects, damaging the nervous system and causing them to death. It is used successfully in pest control in beginning cycle from different cultures. Due to numerous reports of field observations describing increases in vigor development and productivity, even in the absence of pests, has been considered a product that has phytotonic effect.

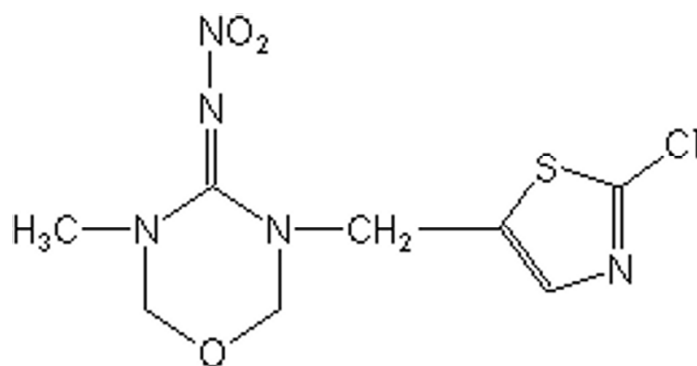


Fig. 1. Chemical structure of thiamethoxam. Source: Oliveira, et al., (2009).

With reference to the mechanism of action of thiamethoxam, the molecule has the ability to induce physiological changes in plants. Due to results obtained, it is concluded that this insecticide acts as bioactive two ways. The first, activating carrier proteins of cell membranes allowing greater ion transport by enhancing plant mineral nutrition. This increase in the availability of minerals promotes positive responses in the development and plant productivity. The second is related to increased enzyme activation caused by thiamethoxam at both the seed and the plant, thereby increasing both primary and secondary metabolism. It also increases the synthesis of amino acids, precursors of new proteins and endogenous synthesis of plant hormones. The responses of plants to these proteins and hormone biosynthesis may be related to significant increases in production.

Results of research related with soybean (*Glycine max*) (Castro et al., 2008; Cataneo, 2008), rice (*Oryza sativa*) (Clavijo, 2008; Almeida et al.; 2010), cotton (*Gossypium hirsutum*) (Lauxen et al., 2010), bean (*Phaseolus vulgaris*) (Almeida et al., 2010) e vegetables like, lettuce (*Lactuca sativa*), tomato (*Lycopersicon esculentum*), pumpkin (*Cucurbita pepo* L.) and carrot (*Daucus carota*) indicate benefit effect of thiamethoxam uses.

2. Bioactivator in physiological performance of carrot seeds

Carrot (*Daucus carota* L.) is the most economical expression of vegetables among those whose edible portion is the root, and to highlight the nutritional value, as a major vegetable

sources of provitamin A (Spinola et al, 1998). Careful selection of cultivars allows sowing of carrots over the years in many regions (Filgueira, 2000).

The success of horticulture generally depends on the establishment of suitable stand for each crop; otherwise reductions may occur in the quantity and quality of final product (Silva and Vieira, 2006).

Under field conditions, carrot seed germination may have low, slow and irregular, resulting in uneven emergence and a heterogeneous population of plants (Corbineau et al., 1994).

With increasing mechanization in vegetable production, establish rapid and uniform culture becomes increasingly important, and it is desirable that the evaluation of seed quality to provide information on their performance on the field.

Establishment of appropriate stand depends on the use of seeds with high physiological potential, able to germinate rapidly and uniformly under a large variation of the environment. Speed and timing are very important because they allow reducing the degree of exposure of seeds and plants to adverse factors (Marcos Filho, 2005).

Reduced or uneven emergence can lead to developmental delays, problems with weed control, non-uniformity of culture in different phenological stages, interference on product quality and characteristics related to the efficiency of harvesting (Marcos Filho, 2005).

In vegetables, backwardness and uneven development may be reflected in product quality and reducing the commercial value, such as lettuce, cabbage, carrots, cauliflower, eggplant and onion (Kikuta and Marcos Filho, 2007).

A survey on physiological quality of seeds of different kinds of vegetables such as carrots, peas, beets, tomato and watermelon industry, marketed and / or used by farmers, it was observed that germination of these seeds do not always fit the minimum standard of marketing required for each species (Nascimento, 1994).

Therefore, failure to stand and seedling vigor at low field level are frequent, with the need for appropriate and sensitive methods to detect these differences in seed quality.

In this context, considering the lack of information concerning the effect of thiamethoxam and the potential benefits that treatment can provide, the present study was to evaluate the influence of thiamethoxam in physiological performance of carrot seeds.

3. Methodology

This work was conducted at the Laboratory of Seed Analysis Textbook and greenhouse, Faculty of Agronomy Eliseu Maciel, Universidade Federal de Pelotas (UFPel), Pelotas / Brazil.

Seed lots of carrot cultivar Brasília represented by four lots.

To establishment concentrations of thiamethoxam, it was used the following concentrations: 0.0, 0.05, 0.1, 0.2, 0.4, 0.8 mL / L, based on germination test three were selected. Tests conducted to evaluate the quality of seeds were performed with and without water stress.

The seeds were treated in a plastic bag containing distilled water at 0.1 mL of water to 0.05 mL and 0.4 mL of product thiamethoxam to 3g of carrot seeds. The product was applied directly to the bottom of the plastic bag before putting the seeds. Then the seeds were placed in plastic bag and mixed until uniform coating of the seeds. It was used a volume of mixture (product + water) sufficient to promote a more even distribution of product on the seeds. For measurement of product and distilled water were used micropipettes.

Water stress was achieved by the water potential of -0.4 MPa, using aqueous solutions of polyethylene glycol (PEG 6000). The calculation of the solute quantities was performed

according to Villela et al. (1991). Polyethylene glycol solutions, thus obtained were applied to the paper substrate, in an amount equivalent to 2.5 times its dry weight in all parameters evaluated in the laboratory involving the germination test.

To evaluate the physiological quality of seeds were conducted the following tests:

Germination: it was used four replications of 50 seeds of each batch distributed in transparent plastic boxes (gerbox) on two sheets of white blotter paper, moistened 2.5 times the weight of paper, placed in a germination chamber set to maintain the temperature constant 25 ° C. Counts were made in the seventh and fourteenth days after sowing, and assessments, carried out according to ISTA (2010) by computing the percentages of normal seedlings.

Accelerated aging: conducted with 4.0 g of seeds distributed in wire screen suspended and placed inside plastic boxes, type gerbox (mini-camera). Inside the germination boxes were placed 40 mL of water and then the boxes were taken to an incubator set at a constant temperature of 41° C for 48 hours and subsequently subjected to germination tests, as described above. The evaluation was performed seven days after sowing, by computing the percentage of normal seedlings.

Root length: four replicates of 50 seeds were sown on a line drawn in the upper third part of paper substrate. The rolls containing the seeds remained at 25 ° C for seven days, after being evaluated, the root length of normal seedlings, with the aid of a millimeter ruler. The root length was obtained by adding the measurements of each replicate and dividing by the number of seedlings, with results expressed in centimeters.

Speed of germination: performed according to the methodology of the germination test, determined by daily counts to stabilize the number of seedlings in the test and the speed calculation made according to Maguire (1962).

Emergence of seedlings in the greenhouse: four replications of 50 seeds were distributed in individual cells of polystyrene trays (Styrofoam), containing commercial substrate Plantimax ®. The trays were kept in the greenhouse and evaluations were performed at 16 days after sowing, counting seedlings in length and more than 1.0 cm. The results were expressed as a percentage of emergence.

Statistical procedure: completely randomized factorial 4x3 (four lots and three concentrations of the product) separately in the evaluation with and without water stress, with three replications. For comparison of means between control and concentrations, it was used Dunnet test, probability level of 5%.

4. Results establishment of concentrations

The concentrations selected based on the result of germination test of treated seeds with different concentrations of the product thiamethoxam, beyond control were 0.05 and 0.4 mL.

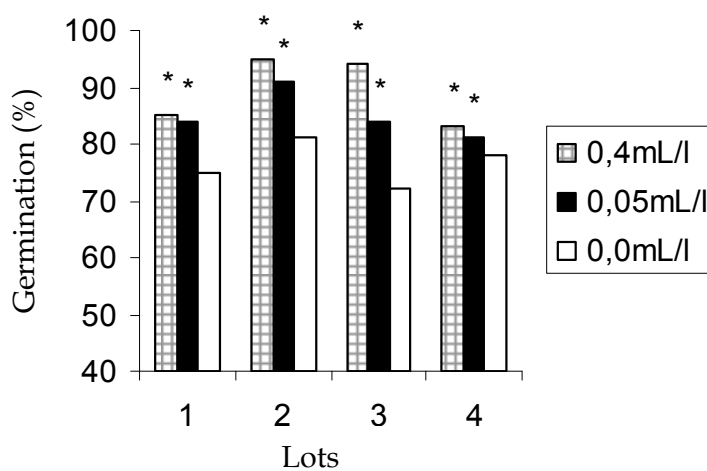
Germination of treated seeds in accordance with product concentrations were 70% (zero), 75% (0.05 mL / L), 72% (0.1 mL / L), 72% (0.2 mL / L), 75% (0.4 mL / L) and 70% (0.8 mL / L) without stress. The choice of the concentration of 0.05 mL / L was based on the fact that seeds showed germination similar to other concentrations and spend less of the product. On the other hand, the concentration of 0.4 mL / L was selected because germination test were seedlings showed well developed, open cotyledons and normal roots. At the concentration 0.8 mL / L was found that the seedlings were developed, but their roots had necrosis.

Statistical analysis performed by Dunnet's test showed significant results for comparison of means between control and concentrations of all parameters.

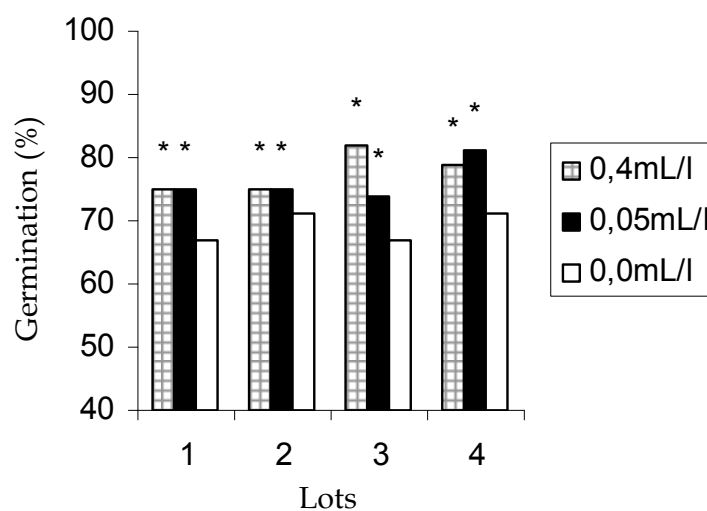
It is noted in Figure 1 that germination of four seed lots, without (Figure 1A) and with (Figure 1B) water stress, treated with thiamethoxam showed significant difference compared to control. Increases in germination were marked and varied according to the lots from 5 to 23 percentage points if the seeds have not been subjected to water stress and 4 to 15 to be subjected to stress.

In Figure 1B it appears that water stress reduced the percentage germination of seed lots. Lots 1 and 3 not treated, after the water stress reached below the standard of marketing, however, treatment stimulated germination of seeds and lots reached the minimum germination (70%) of the standard marketing. In soybean seeds was also observed that thiamethoxam accelerates germination, induces more growth of the embryonic axis and minimize the negative effects in situations of presence of aluminum, salinity and water deficiency (Cataneo et al., 2006).

There is a trend of germination of treated lots with different concentrations of the product showed similar results, with the exception of Lot 3, in which the concentration of 0.4 mL / L was more efficient.



(A)

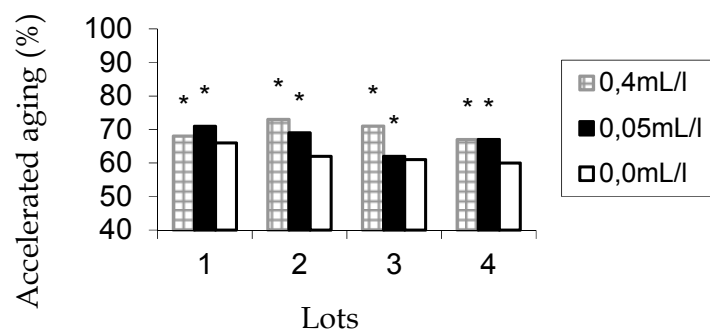


(B)

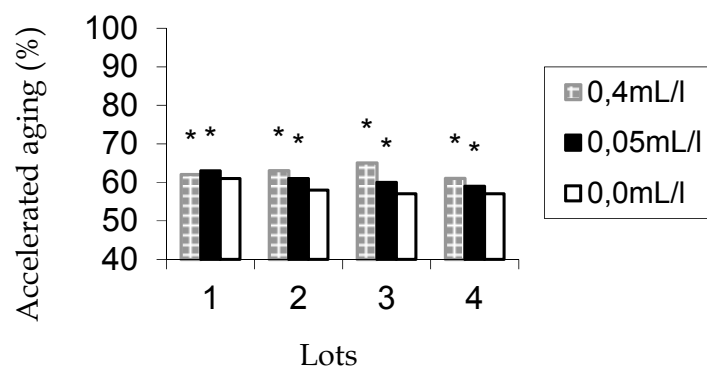
Fig. 1. Germination (%) of four seed lots of carrot, cultivar Brasilia, submitted (A) or not (B) to water stress. * Different from the control by Dunnet test at probability level of 5%.

According to Figure 2, germination after accelerated aging of treated seeds without (Figure 2A) and with (Figure 2B) water stress showed significant differences related to control. Positive difference varied according of lots, 2 to 11 percentage points in seed not submitted to stress and 2 to 9 in submitted to water stress. This superiority resistance occurs because thiamethoxam move through plant cells and activates several physiological reactions, such as functional protein expression related with plant defense mechanism avoid stress factors like drought, high temperatures, toxic effects, among others, improving productivity, leaf and radicular area, as found in soybean seed (Tavares e Castro, 2005).

Concentrations showed positive results in situations with and without water stress, but the concentration of 0.4 mL / L performed better for lots 2 and 3, without stress and 2, 3 and 4 with stress.



(A)



(B)

Fig. 2. Accelerated aging (%) of four seed lots of carrot, cultivar Brasilia, without (A) and with (B) water stress.* It differs from the control by Dunnet test at probability level of 5%.

According to data presented in Figures 3 and 4, treated seeds showed marked differences in root length compared to untreated, on average 4 cm, in both cases without (Figure 3 and 4 A) and with (Figures 3 and 4 B) water stress. This effect of thiamethoxam of supporting the growth of the root system, confirming the effect of rooting observed by Pereira et al. (2007) in sugar cane and potatoes; and also by Tavares et al. (2007) in soybean. It is believed that the thiamethoxam increase water uptake and stomatal resistance, improving the water

balance of the plant, tolerating water deficit better (Castro, 2006). As observed in soybean root development increases the absorption of nutrients, increases the expression of leaf area and plant vigor (Tavares and Castro, 2005).

The data speed of germination, without (Figure 5A) and with (Figure 5B) stress show that the treated seeds had a higher rate compared to control. The concentrations used had similar results. Treated seeds germinated on average one day soon if they have not been subjected to water stress and two days are subject to stress. This effect is very promising because carrot seeds in field conditions have poor germination, slow and irregular resulting in uneven emergence (Corbineau et al., 1994). This increased speed of germination is caused by physiological changes that occur in the plant indirectly stimulating the production of hormones, resulting in increased vigor, root growth, water absorption and primary and secondary metabolism, as observed in the sugarcane crop (Castro, 2007).

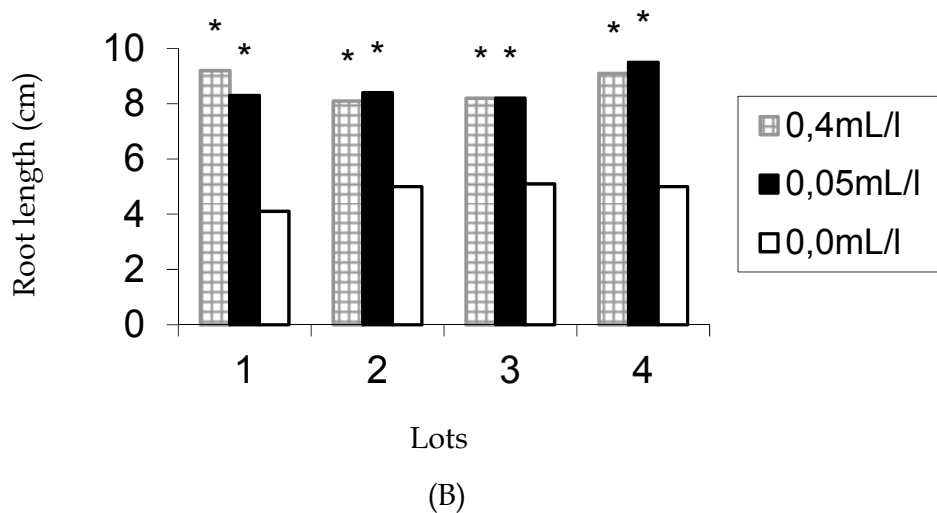
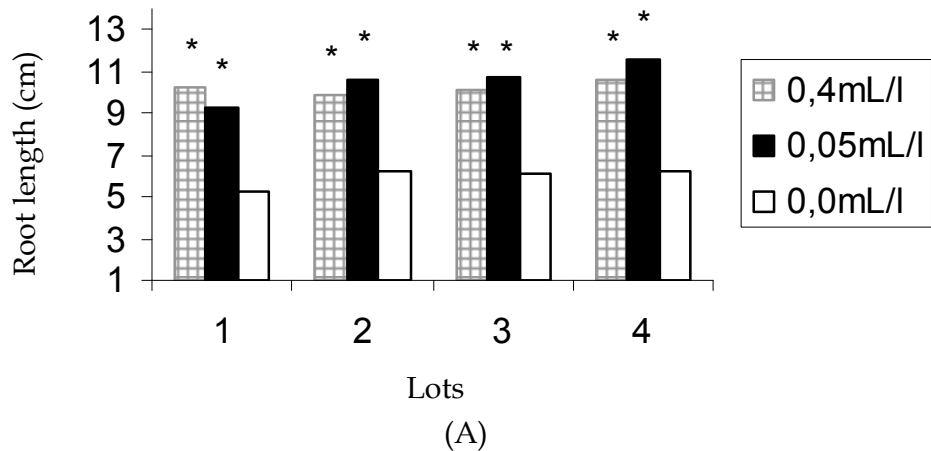


Fig. 3. Root length (cm) of seedlings of four seed lots of carrot, cultivar Brasilia, without (A) and with (B) water stress. * Different from the control by Dunnet test at probability level of 5%.

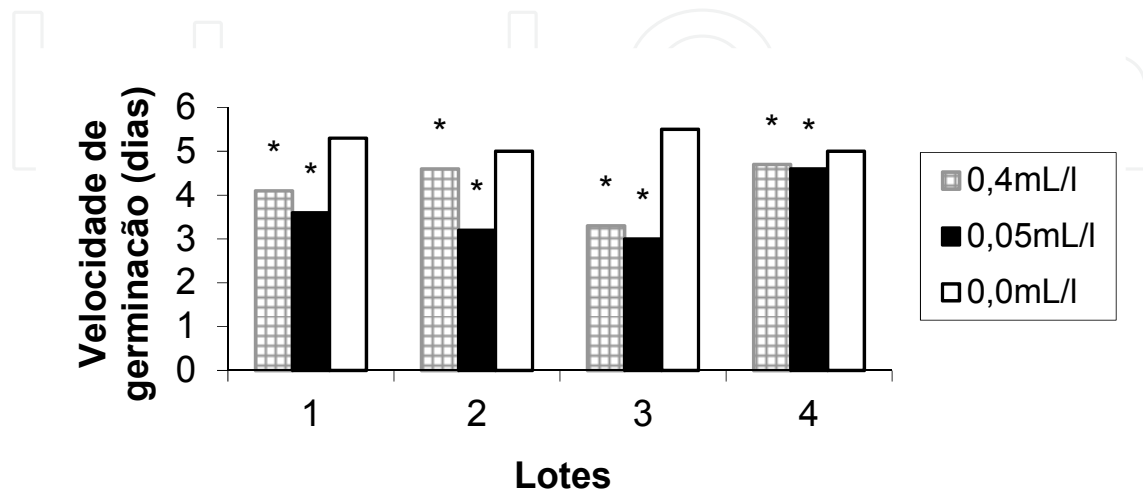


(A)

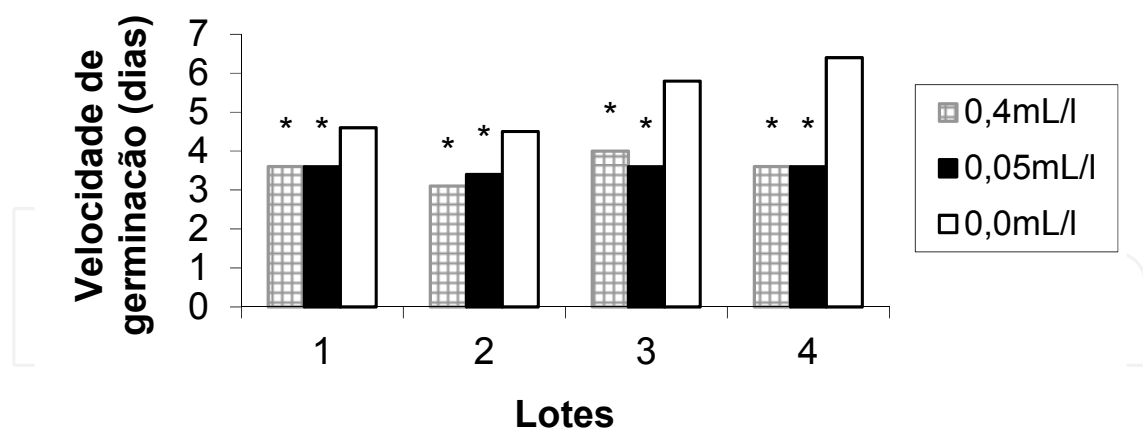
mL of product/ 3g of seed



Fig. 4. Root length (cm) of seedlings of four seed lots of carrot, cultivar Brasilia, without (A) and with (B) water stress.

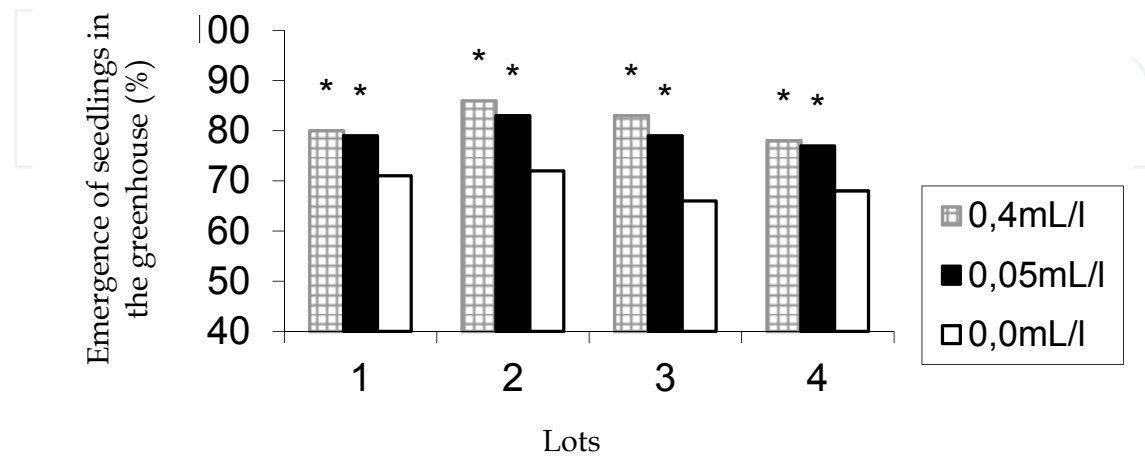


(A)



(B)

Fig. 5. Speed of germination (days) of four seed lots of carrot cultivar Brasilia, without (A) and with (B) water stress.* It differs from the control by Dunnet test at probability level of 5%.



(A)

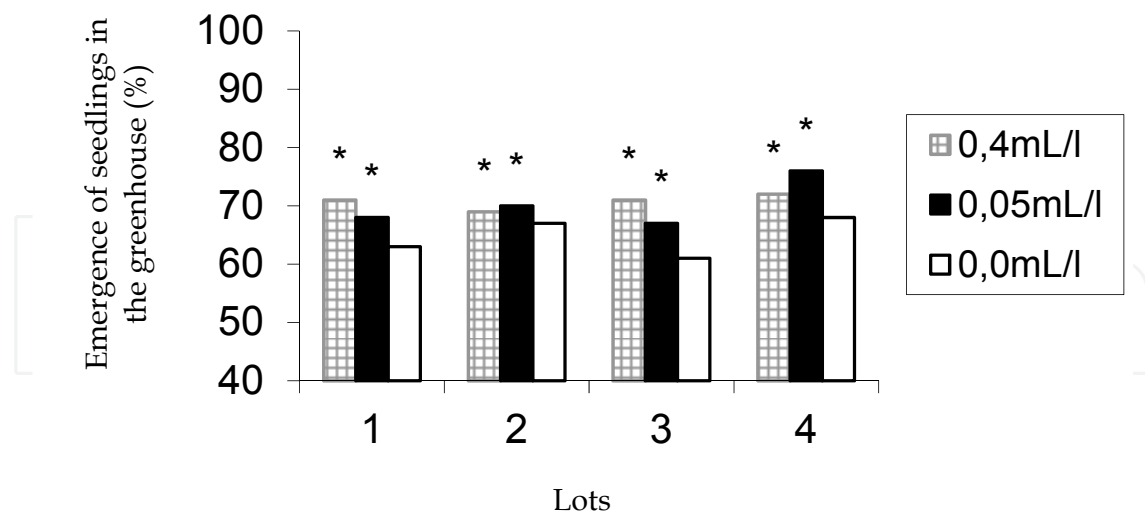


Fig. 6. Emergence of seedlings in the greenhouse for four seed lots of carrot, cultivar Brasilia without (A) and with (B) water stress. * Different from the control by Dunnet test at probability level of 5%.

In Figure 6, without (Figure 6A) and with (Figure 6B) water stress, it was observed that the emergence of seedlings in the greenhouse was stimulated, and the seeds treated with thiamethoxam showed significant differences compared to control. The positive differences compared to control vary according to lots, 9 to 17 percentage points if the seeds have not been subjected to water stress and 20 to 10 percentage points when subjected to stress. The two concentrations showed similar responses. These results confirm those found in soybean, to be seen increase in the root system and the percentage of seedling emergence also in water deficit conditions (Castro et al., 2006). According to the literature, soybean seeds treated with thiamethoxam have higher levels of amino acids, enzyme activity and synthesis of plant hormones that increase the plant responses to these proteins and these events provide significant increases in production and reducing the time of establishment of culture in the field, making it more tolerant to stress factors (Castro, 2006).

The results obtained can be described that the product stimulated the performance of carrot seeds in all parameters evaluated, both in seeds subjected to water stress or not. Carrot seeds treated with the product thiamethoxam showed significant increases in germination and vigor for all lots. Among the aspects of vigor, the product stimulated the growth of the root length, which is of great importance to the culture of carrots and this result was obtained in the laboratory confirmed in the greenhouse.

The product was more effective in stimulating the quality of seeds not subjected to water stress, with the exception of root length which positive change was similar for seeds subjected to stress or not. In all parameters evaluated, increases in the quality varied according to the lot. Concentrations of the product for most tests evaluated did not differ, however there was a trend of higher concentration to the higher values.

The application of thiamethoxam has strong interest for the culture of carrot, whose edible portion is the root and, moreover, by presenting, in field conditions, poor germination, slow, irregular with uneven emergence, the product acts as an enhancer, by allowing the expression of seed germination potential, accelerate the growth of roots and increase the absorption of nutrients by the plant. These features of thiamethoxam combined with the use of genetics and physiological high-quality seed powers the productive capacity of the culture.

5. Conclusions

Thiamethoxam product stimulates the physiological performance of carrot seeds subjected to water stress or not, with variable intensity according to lot.

Concentrations of 0.05 and 0.4 mL of the product is effective, however there is a tendency of higher concentration to the higher increases in quality.

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It is our hope that this book will be of interest and use not only to scientists, but also to the food-producing industry, governments, politicians and consumers as well. If we are able to stimulate this interest, albeit in a small way, we have achieved our goal.

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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