

MATHEMATICAL MODELING IN THE APPLICATION OF BIOFERTILIZERS IN BELL PEPPER CROPS

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Abstract

*Mathematical modeling is a useful tool for assessing the potential risks in pepper crops. It has been demonstrated that biofertilizers can improve plant growth and development by mobilizing nutrients from the soil. The objective of this study is to analyze a mathematical model to assess the stimulatory capacity of a biofertilizer on the germination of bell pepper seeds following their wetting in solutions of varying concentrations of the product. Furthermore, the study employs collembola (*Folsomia candida* L.) to assess the ecotoxicity of the utilized product, thereby providing a multifaceted examination of soil health in relation to plant development and evolution under the influence of diverse substances. The population dynamics of *F. candida* serve as a barometer for this relationship, offering insights into the interactive dynamics between soil health, plant growth, and the impact of chemical substances.*

Key words: mathematical modeling; seed germination; biofertilizers; *Folsomia candida*; *Capsicum annuum* L.

INTRODUCTION

In the context of Romanian agriculture, the cultivation of bell peppers (*Capsicum annuum* L.) holds particular significance, particularly within the southern regions of the country. This prominence can be attributed to the optimal soil and climatic conditions that prevail in these areas, resulting in a high demand from consumers and food processors (Barcanu-Tudor & Drăghici, 2018).

Peppers are a highly suitable crop for sunny, fertile, and irrigated vegetable land in our country, where they are predominantly cultivated in the field (Barcanu-Tudor & Drăghici, 2018). In response to the high demand and interest in year-round consumption, the area under peppers has expanded in recent years to include protected areas (greenhouses), as well as intensive and industrial systems.

Pepper crops demonstrate a reduced susceptibility to diseases and pests; however,

they exhibit an increased vulnerability to soil contamination, which can impede germination (Iosob et al., 2019). The impact of this inhibition is amplified in soils that are already contaminated. The risk factors for pepper crops include the following: seedling depreciation (inappropriate age and density), the presence of diseases and/or pests, delayed cultivation due to the implementation of inappropriate technological measures, and climatic factors (Iosob et al., 2023).

The objective of this study is to assess the stimulatory capacity of a biofertilizer on the germination of pepper seeds, whilst concomitantly utilising collembola (*Folsomia candida*) to detect the ecotoxicity of the product. Germination rate and seed vigour are pivotal factors in determining the efficiency of seedling production, which is crucial for successful crop establishment (Larson et al., 2020). The time required for germination can vary significantly between different *C. annuum* L. seeds, with

some completing the process within a few days, while others requiring up to 21 days (Samarah et al., 2016). The seeds of bell pepper exhibit sensitivity to elevated temperatures and salt stress, which result in diminished seed physiological potential at temperatures of 20°C and 35°C and augmented NaCl concentrations (de Assis et al., 2025). Pepper seeds contain substantial amounts of both free and bound polyphenols, which undergo changes during the germination process. These compounds are utilised by the seeds to scavenge free radicals. These compounds may also function as signal compounds or be bound to plant defence, participating in induced systemic resistance (Ureche et al., 2021).

Biofertilizers are defined as substances containing naturally occurring biocompounds and micro-organisms. When applied to the seed or plant surface or soil, biofertilizers promote plant growth and development by increasing the supply or availability of primary nutrients to the host plant. The utilization of biofertilizers exerts a positive impact on both the environment and the crop by increasing plant productivity (Dobrin et al., 2019; Avasiloaiei et al., 2024). These biofertilizers may comprise micro- or macro-organisms, plant residues, enzymes, or substances that promote plant growth by fixing atmospheric nitrogen, solubilizing phosphorus, or producing growth-promoting substances. They function as an environmentally friendly alternative to chemical fertilizers and play an important role in sustainable agriculture (Avasiloaiei et al., 2023).

Groundfix® (Figure 1) is a root biofertilizer that has been demonstrated to mobilize phosphorus and potassium from insoluble compounds, fix nitrogen, and increase the efficiency of mineral fertilizer use.



Figure 1. Groundfix® biofertilizer

The compound contains cells of the bacteria *Bacillus subtilis*, *Bacillus megaterium* var.

phosphaticum, *Azotobacter chroococcum*, *Enterobacter* spp., *Paenibacillus polymyxa*, as well as beneficial bacteria (lactic acid bacteria, enzyme producers), vitamins, amino acids, and other physiologically active substances.

The OECD has issued a recommendation that all products utilized in agricultural settings, including biofertilizers, be subjected to a rigorous evaluation of their toxicity. This evaluation aims to ascertain their impact on the environment, particularly with regard to their effect on soil, plants, and even groundwater. In the context of organic agriculture, it has become imperative to undertake an ecotoxicological risk assessment of these substances on soil.

The utilization of bioindicators, such as the *F. candida*, as outlined in OECD 232, serves as a crucial element in the assessment of the ecotoxicity of products employed in pepper cultivation. This approach ensures the safety and efficacy of biofertilizers, while also offering a cost-effective alternative to more traditional methods. The benefits of this approach are evident, as it leads to a marked enhancement in the health of the soil, which, in turn, positively impacts the vitality of the plants and the effectiveness of the substances used.

MATERIALS AND METHODS

In this study, we utilized seeds of the bell pepper variety Dariana Bac, obtained from Vegetable Research and Development Station Bacau, as the biological material. This variety is characterized by an early maturation period, with a vegetation period of 120 days leading to the initial harvest. The plant exhibits average vigor and a 50% erect and 50% horizontal growth pattern.

The application of biofertilizers to seeds prior to planting has been demonstrated to facilitate the establishment of beneficial microorganisms during the early stages of plant development (Kuts et al., 2024, brochure Groundfix®)

The Groundfix® biofertilizer was applied to the pepper seeds in accordance with the mathematical model of type 2³.

Factorial design

In this paper, the influence of two parameters for the germination of Dariana Bac bell pepper (*Capsicum annuum* L.) was investigated, which are expressed in terms of amount of fertilizer

(X₁) and wetting duration (X₂) upon the five response functions. The response functions investigated were: initial germination (Y₁), final germination (Y₂), mean germination time (Y₃), mean germination rate (Y₄) and germination speed (Y₅). The variation ranges are summarized in Table 1.

Table1. Parameters that influence the germination of bell pepper and their variation domain

Paramet ers (x _i)	Reduced variable	Minimal level (X _{i,min})	Median level (X _{i,med})	Maximal level (X _{i,max})	ΔX _i
Amount of fertilizer [mL]	x ₁	2.5	3.75	5	1.25
Wetting duration [h]	x ₂	1	2.5	4	1.5

The ecotoxicity of Groundfix® biofertiliser was the subject of an evaluation which employed the collembola, *F. candida*, as the test organism. This evaluation was conducted in accordance with the OECD 232 protocol.

F. candida is a common soil-dwelling organism found across the globe, playing a pivotal role in the decomposition process and nutrient cycling within terrestrial ecosystems (Calara et al., 2020; Martins et al., 2023).

Adult individuals of *F. candida*, maintained in the laboratory on activated carbon medium and fed yeast, were exposed to soil samples containing biofertilizer at the established concentrations in plastic containers (Figure 2) with lids and kept in the Sanyo chamber at constant temperature and humidity for the duration of the experiment.

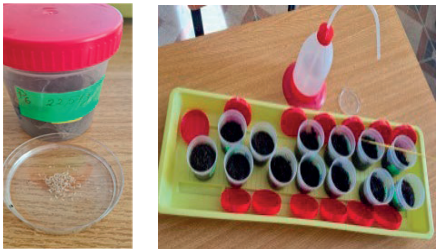


Figure 2. Preparation of soil samples

In preparation for the experiment, soil samples were collected in plastic containers and stored in the Sanyo chamber set at 20-22°C. Prior to the introduction of the collembola, the soil samples

were sterilized and saturated with distilled water. Following the addition of the biofertilizer, the samples were aerated every 48 hours and subjected to foliar feeding. The biofertilizer solutions employed in the experiment adhered to the maximum degradation time of the compound, not exceeding 4 hours until the moment of use, wetting the samples at a frequency of 24-48 hours, each 5 ml. The duration of the experiment was 28 days, during which the survival and reproduction of the collembola were evaluated.

RESULTS AND DISCUSSIONS

Design of experiments

The five response functions investigated were: initial and final germinations, mean germination time, mean germination rate and germination speed, which values are presented in Table 1. The reduced values of the variables are presented in parenthesis. Three other tests were also realized in the central point of the domain (0, 0) to calculate the significance of the program. The obtained values are shown in Table 2.

Table 2. Various germination parameters

Sample	Amount of fertilizer [mL]	Wetting duratio n [h]	Initial germina tion [mm]	Final germina tion [mm]	Mean germina tion time [h]	Mean germina tion rate [mm]	Germina tion speed [%]
	x ₁	x ₂	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅
1	2.5 (-1)	1 (-1)	64	90	5.52	0.18	18.25
2	2.5 (-1)	2.5 (0)	24	73.33	6.76	0.15	15.2
3	2.5 (-1)	4 (+1)	44.67	56	5.16	0.19	19.38
4	3.75 (0)	1 (-1)	36	56.67	5.42	0.18	18.44
5	3.75 (0)	2.5 (0)	33.33 (34.00; 34.56; 34.33)	76.00 (75; 77; 78)	5.89 (5.75; 5.92; 5.89)	0.17(0.17; 0.17; 0.18)	17.01 (17.05; 17.03; 17.01)
6	3.75 (0)	4 (+1)	20	49.33	6.57	0.16	15.53
7	5 (+1)	1 (-1)	72	95.33	5.25	0.19	19.05
8	5 (+1)	2.5 (0)	26.7	61.33	7.71	0.13	13.05
9	5 (+1)	4 (+1)	30.67	82.67	6.29	0.16	16.13

Elaboration of the mathematical model

The particular form of response function for factorial program of 3² types is:

$$Y = a_0 + a_1 \cdot x_1 + a_2 \cdot x_2 + a_{12} \cdot x_1 \cdot x_2 + a_{11} \cdot x_1^2 + a_{22} \cdot x_2^2 \quad (1)$$

In Table 3 are presented the coefficients values of polynomial mathematical models, which were calculated according to the literature specifications (Lazic, 2004).

Table 3. Values of the polynomial coefficients

Coeffi cients	Coeffi cient value for Y_1	Coeffi cient value for Y_2	Coeffi cient value for Y_3	Coeffi cient value for Y_4	Coeffi cient value for Y_5
a_0	18.75	59.71	6.68	0.152	15.18
a_1	-0.55	3.33	0.30	-0.007	-0.76
a_2	-12.78	-9	0.31	-0.008	-0.78
a_{12}	-3.67	3.56	0.23	-0.007	-0.67
a_{11}	13.90	15.78	0.15	-0.002	-0.15
a_{22}	16.55	1.44	-1.08	0.027	2.71

According to the polynomial coefficients, the mathematical models which describe the response functions of the optimizing criterion are:

$$Y_1 = 18.75 - 0.55 \cdot x_1 - 12.78 \cdot x_2 - 3.67 \cdot x_1 \cdot x_2 + 13.90 \cdot x_1^2 + 16.55 \cdot x_2^2 \quad (2)$$

$$Y_2 = 59.71 + 3.33 \cdot x_1 - 9 \cdot x_2 + 3.56 \cdot x_1 \cdot x_2 + 15.78 \cdot x_1^2 + 1.44 \cdot x_2^2 \quad (3)$$

$$Y_3 = 6.68 + 0.3 \cdot x_1 + 0.31 \cdot x_2 + 0.23 \cdot x_1 \cdot x_2 + 0.15 \cdot x_1^2 - 1.08 \cdot x_2^2 \quad (4)$$

$$Y_4 = 0.152 - 0.007 \cdot x_1 - 0.008 \cdot x_2 - 0.007 \cdot x_1 \cdot x_2 - 0.002 \cdot x_1^2 + 0.027 \cdot x_2^2 \quad (5)$$

$$Y_5 = 15.18 - 0.76 \cdot x_1 - 0.78 \cdot x_2 - 0.67 \cdot x_1 \cdot x_2 - 0.15 \cdot x_1^2 + 2.71 \cdot x_2^2 \quad (6)$$

Determination of the coefficients' significance

In order to determine the significance of the polynomial coefficients of the five response functions, the *t* - student test was used. The average values of the response function and the average values of the measurement errors were calculated according to the algorithm presented in the literature (Georgescu et al., 2013; Georgescu et al., 2017; Georgescu et al., 2023). The *t*-student test results for the five response functions are presented in Table 4.

Table 4. T-student test results

t_j	t_0	t_1	t_2	t_{12}	t_{11}	t_{22}
Values for Y_1	104.04	-3.05	-70.91	-20.35	77.11	91.81
Values for Y_2	918.52	51.28	-138.46	54.70	242.73	22.22
Values for Y_3	102.82	4.62	4.69	3.58	2.37	-16.68
Values for Y_4	2.34	-0.11	-0.12	-0.1	-0.02	0.41
Values for Y_5	233.63	-11.78	-12.06	-10.37	-2.31	41.75

According to *t*-student test results, the individual coefficient (a_1) has to be eliminated in the case of mathematical model which describes Y_1 response function and in the case of mathematical model of Y_5 , the quadratic term coefficient (a_{22}) has to be eliminated.

The effects simulation in the optimization study involves analyzing both the absolute value and the sign of each coefficient individually.

In the case of the first response function, the a_0 value indicates that the optimal initial germination is close to 18.75 mm. Although this value is not the maximum value obtained in the experimental program, it can be concluded that the optimal initial germination is not the one with the highest value. The individual coefficients (a_1 and a_2) are negative, so the x_1 and x_2 variables have an unfavorable individual effect on the germination process. The individual effect of the x_{12} term is negative, so the x_1 and x_2 variables have an unfavorable effect on the germination process. Analyzing the quadratic term coefficients (a_{11} and a_{22}) which values are positive, it can be said that the response function (initial germination) is characterized by a minimum in relation to variables x_1 and x_2 , having approximately the same curvature in relation to variable x_1 and variable x_2 , because they have close values.

In the case of the second response function, the value of a_0 (59.71) indicates that we have an optimal final germination at a value close to this value. The coefficient for simple effects a_1 is positive, so that variable x_1 has an individual favorable action to the process. On the other hand, the coefficient for simple effects a_2 being negative, variable x_2 has an unfavorable influence on the process. Since the interaction coefficient a_{12} is positive (3.56), it can be concluded that the variables x_1 and x_2 , through their interaction, have a favorable effect on the process. This effect has approximately the same intensity as the individual action of the variable x_1 . Since the quadratic coefficients a_{11} and a_{22} have the same sign, it results that the response function is characterized by a minimum, having greater curvature in relation to the variable x_1 , compared to the variable x_2 .

Regarding the simulation of the effects in the case of the response function Y_3 (mean germination time), the value of a_0 indicates that we have an optimal mean germination time at a value close to 6.68 h. The coefficients for simple effects (a_1 and a_2) being positive, it can be said that the amount of fertilizer and the wetting duration have individual favorable actions to the process. The interaction coefficient a_{12} is positive (0.23), so the variables x_1 and x_2 ,

through their interaction, have a favorable effect on the process. This effect has approximately the same intensity as the individual action of the variable x_1 or of variable x_2 , since the values are close. Analyzing the quadratic term coefficients (a_{11} and a_{22}) which values are positive, respectively negative, it can be said that the response function (mean germination time) is characterized by a minimum in relation to variable x_1 and by a maximum in relation to variable x_2 . These coefficients having opposite signs, it results that the response function is characterized by an inflection point, the graphic representation being in the form of a saddle.

In the case of the fourth response function, the value of a_0 indicates that we have an optimal mean germination rate at a value close to 0.152 h. The coefficients for simple effects (a_1 and a_2) being negative, it can be said that the amount of fertilizer and the wetting duration have individual unfavorable actions to the process. The interaction coefficient a_{12} is negative (-0.007), so the variables x_1 and x_2 , through their interaction, have an unfavorable effect on the process. This effect has approximately the same intensity as the individual action of the variable x_1 or of variable x_2 , since the values are approximately the same. Analyzing the quadratic term coefficients (a_{11} and a_{22}) which values are negative, respectively positive, it can be said that the response function (mean germination rate) is characterized by a maximum in relation to variable x_1 and by a minimum in relation to variable x_2 . These coefficients having opposite signs, it results that the response function is characterized by an inflection point, the graphic representation being in the form of a saddle.

In the case of the last response function (germination speed), the value of a_0 indicates that we have an optimal mean germination rate at a value close to 0.152 h. The coefficients for simple effects (a_1 and a_2) being negative, it can be said that the amount of fertilizer and the wetting duration have individual unfavorable actions to the process. The interaction coefficient a_{12} is negative (-0.007), so the variables x_1 and x_2 , through their interaction, have an unfavorable effect on the process. This effect has approximately the same intensity as the individual action of the variable x_1 or of variable x_2 , since the values are approximately

the same. Analyzing the quadratic term coefficients (a_{11} and a_{22}) which values are negative, respectively positive, it can be said that the response function (mean germination rate) is characterized by a maximum in relation to variable x_1 and by a minimum in relation to variable x_2 . These coefficients having opposite signs, it results that the response function is characterized by an inflection point, the graphic representation being in the form of a saddle. For response functions obtained, the partial derivatives of first order were calculated, in rapport with each variable.

In the case of the first response function (Y_1), by equating the partial derivatives of first order with 0, the linear system resulted was resolved. The optimal point searched was (0.052; 0.392), represented in dimensionless coordinates. It can be seen, that the optimal values for x_1 and x_2 are within the limits of the domain (-1, 1) which initially were supposed. The real values of the optimal conditions for bell pepper germination process were obtained for an amount of fertilizer of 3.82 mL and a wetting duration of 3.09 h.

In the case of the second response function (final germination), the optimal point searched was (0.532; 3.78), represented in dimensionless coordinates. It can be seen, that the optimal value for x_1 is within the limits of the domain (-1, 1) which initially were supposed, but in the case of optimal value of x_2 it isn't. The real values of the optimal conditions for bell pepper germination process were obtained for an amount of fertilizer of 4.42 mL and a wetting duration of 8.17 h. The results obtained in mathematical modeling are in accordance with literature (Ciofu et al., 2019) because the seeds can be damaged during a longer soaking time. In the case of the third response function (Y_3), the optimal point searched was (-1.02; 0.034), represented in dimensionless coordinates. It can be seen, that the optimal values for x_1 and x_2 reflect the gentle action of the Groundfix® biofertilizer. The real values of the optimal conditions for bell pepper germination process were: 2.48 mL and 2.55 h.

In the case of mean germination time response function (Y_4), the optimal point searched was (-1.63; -0.06), represented in dimensionless coordinates. It can be seen, that the optimal values for x_1 and x_2 reflect the gentle action of the Groundfix® biofertiliser. The real values of

the optimal conditions for bell pepper germination process were: 1.71 mL and 2.41 h. In the case of germination speed, the optimal point searched was (-1.16; -0.61), represented in dimensionless coordinates. It can be seen, that the optimal values for x_1 and x_2 reflect again the gentle action of the Groundfix® biofertiliser. The real values of the optimal conditions for bell pepper germination process were: 2.3 mL and 1.59 h.

In this study, the initial and final germinations, mean germination time, average germination rate and germination speed, were established according to the two factors, amount of fertilizer and wetting duration for the germination of Dariana Bac bell pepper, dependence that can be illustrated using the curves presented in Figures 3-7.

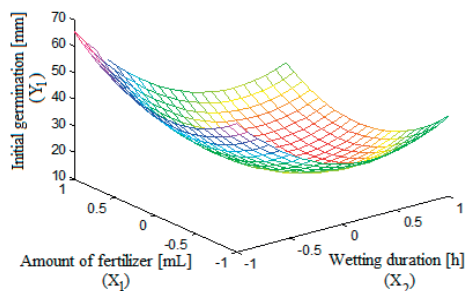


Figure 3. Influence of amount of fertilizer (X_1) and wetting duration (X_2) on initial germination (Y_1)

$$Y_1 = 18.75 - 12.78 \cdot x_2 - 3.67 \cdot x_1 \cdot x_2 + 13.9 \cdot x_1^2 + 16.55 \cdot x_2^2$$

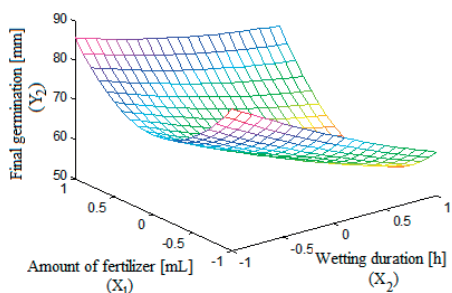


Figure 4. Influence of amount of fertilizer (X_1) and wetting duration (X_2) on final germination (Y_2)

$$Y_2 = 59.71 + 3.33 \cdot x_1 - 9 \cdot x_2 + 3.56 \cdot x_1 \cdot x_2 + 15.78 \cdot x_1^2 + 1.44 \cdot x_2^2$$

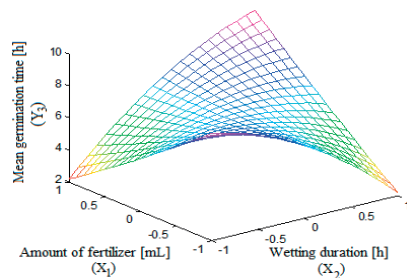


Figure 5. Influence of amount of fertilizer (X_1) and wetting duration (X_2) on mean germination time (Y_3)

$$Y_3 = 6.68 + 0.3 \cdot x_1 + 0.31 \cdot x_2 + 0.23 \cdot x_1 \cdot x_2 + 0.15 \cdot x_1^2 - 1.08 \cdot x_2^2$$

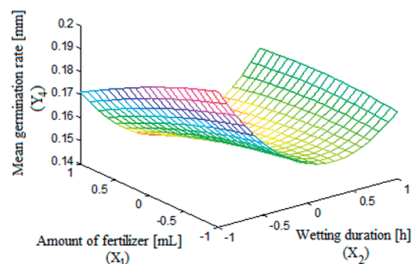


Figure 6. Influence of amount of fertilizer (X_1) and wetting duration (X_2) on mean germination rate (Y_4)

$$Y_4 = 0.152 - 0.007 \cdot x_1 - 0.008 \cdot x_2 - 0.007 \cdot x_1 \cdot x_2 - 0.002 \cdot x_1^2 + 0.027 \cdot x_2^2$$

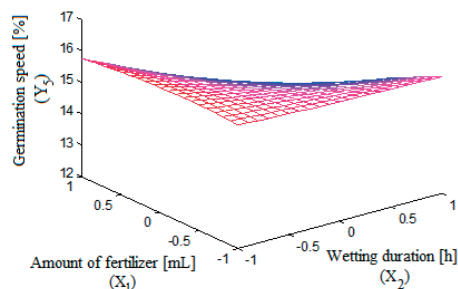


Figure 7. Influence of amount of fertilizer (X_1) and wetting duration (X_2) on germination speed (Y_5)

$$Y_5 = 15.18 - 0.76 \cdot x_1 - 0.78 \cdot x_2 - 0.67 \cdot x_1 \cdot x_2 - 0.15 \cdot x_1^2$$

Mathematical modeling highlighted the optimal results of the parameters analyzed in the relationship between bell pepper and the Groundfix® biofertiliser.

The utilization of *F. candida* to evaluate the ecotoxicity of Groundfix® biofertiliser in pepper crops is indicative of the health status of the soil in relation to the plant and the fertilizers employed. The alterations in the collembola population substantiate this assertion. The findings of our study demonstrated a favourable survival rate of colembolla following the incorporation of 3.8 mL/L of biofertilizer. From the number of experimental variants analysed (Figure 8), the number of adults of *F. candida* suffered a significant decrease at 5 mL/L Groundfix® concentration (P7, P8, P9).

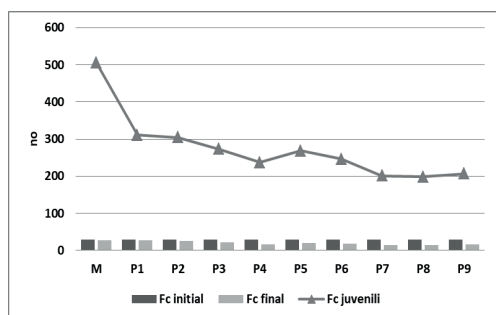


Figure 8. Dynamics of the number of *Folsomia candida* to biofertilizer action

The number of juveniles reflects a low level of toxicity of the product compared to the control: 41.40% at a concentration of 2.5 mL/L (P1, P2, P3), 50.43% at a concentration of 3.8 mL/L (P4, P5, P6) and 60% at a concentration of 5 mL/L (P7, P8, P9) biofertilizer.

CONCLUSIONS

The optimized values obtained from the experimental design are in agreement with the values of parameters initially explored, with the exception of final germination of bell pepper. Following mathematical calculations, a germination time value of approximately 8 h was obtained for a quantity of almost 5 mL of biofertilizer (Y_2 response function), which is in accordance with the maximum quantity recommended by the manufacturer for use.

The fact that the final germination and the average germination rate are within the limits imposed by the factorial program (2.5-5 mL biofertilizer), shows that the bell pepper seeds used in this research have good germination capacity.

From the analysis of the data obtained from the modeling and those observed from the behavior of *Folsomia* in relation to the biofertilizer Groundfix®, its efficiency in bell pepper cultivation since germination is justified.

As a perspective, we recommend the using of mathematical modeling also for other categories of substances allowed in organic agriculture.

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