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Application of Design of Experiments Box-Behnken to improve the growth of the Argan tree in Morocco

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Abstract
This work is devoted to the modelling and optimization of the growth tracking of the Argan tree from southwestern planted in Rabat, in the presence of phosphorus and heavy metals (Cr, Cd). The determination of optimum condition of this growth was realized by the methodology of experimental design: Box-Behnken. The studied factors that have a major influence on this growth are: the phosphorus concentration (M), the cadmium concentration (ppm) and chromium concentration (ppm). The optimum is reached at a quantity of phosphoric acid equal to 3M, an amount of 28 ppm of Cr and 0,775 ppm of Cd, comparing with the permissible limit for human consumption.

Keywords: Argan tree, phosphoric acid, Heavy metals, Design of experiments, Modelling and Optimization.

1. Introduction
The Argan is a multipurpose tree forest (forest, fruit and fodder). It is important biologically, economically and socially [1-3]. It has great medicinal interest [4] and therapeutic [5], thanks to the oil extracted from the fruit. Despite the interest of this ecosystem which is under strong pressure from various anthropogenic factors more the climate. The degradation threat of the Argan tree is a major concern for both the population as well as for scientists [6]. Thus the services of Water and Forests attempted to regenerate this species by planting repeatedly but without success [7]. However, watering of the plant by phosphoric acid during growth gave promising results showing a positive and noticeable effect on the size of the tree [8, 9]. For tracking the Argan trees originating from Moroccan southwestern and planted in Rabat. A study of the effects of phosphorus in different concentrations was done, based on Argan trees planted since 2008 [8, 9]. For the manufacture of a specific fertilizer designed for the Argan trees, the target is to find the favorable conditions for the phosphorus content. It is in this perspective that the present work is aiming to study the influence of certain chemical elements on the size, determining a mathematical model describing the evolution of the size depending on the contents of some heavy metals (Cr, Cd) and phosphoric acid which allow finding optimal conditions.

The objective of this work is the modeling and optimization of the growth of the Argan tree with presence of phosphorus and heavy metals (Cr, Cd) using design of experiments: Box-Behnken. The factors studied which may influence the size of Argan at four sites A, B, C and D are the concentration of phosphoric acid solutions (M), the cadmium concentration (ppm) and the concentration of chromium (ppm).

2. Materials and methods
This study was carried out on Argan trees from Moroccan southwestern planted in Rabat since 2008. The remaining four trees (A,B,C,D) were watered weekly with 20 ml of phosphoric acid (H_3PO_4) solution, each tree with different concentration (0.38, 0.75, 1.5 and 3M). The length of the rods was measured every six months. The sizes are reported in Table 1.

2.1. *Sampling*

The samples of soil, wood and leaves were collected from different trees growing in the garden of Faculty of Science in Rabat. Samples were either manually collected or harvested and analyzed for their metal content.

2.2. *Analysis*

For mineral analysis, 0.5 g samples of Argan were washed with distilled water, dried in an oven at 550°C, and then crushed. Samples were incinerated prior to analysis. For this, each sample was placed in a 30 ml Teflon flask to which 15 ml of HNO_3 65% was added. The suspension was heated at 110°C close to dryness. Then 5 ml of HF 40% was added, the flask sealed and the suspension heated at 120°C for 5 h. Hydrogen peroxide 35% (1 ml) was carefully added to the solution at 80°C together with 10 ml of HNO_3 65%. The solution was heated at 140°C to dryness. After cooling, the residue was suspended in deionized water, filtered and the volume adjusted to 100 ml [10].

2.3. *Apparatus*

Heavy metals content (Cd, Cr) were determined using an ICP-AES spectrometer (Jobin Yvon, Ultima 2) with axial viewed plasma. The plasma was operated with a concentric nebulizer coupled to a cyclone type nebulization chamber. The operating conditions were set as follows: power 1150-1200 W; plasma flow gas 10.5-15 l/min; auxiliary gas flow 1.5 l/min; nebulizer gas flow 0.2 l/min. Wavelengths used for quantification were: cadmium 226.5 nm, chromium 205.5 nm. Table: 2

Table: 1.The size of each tree after every six months watering

		size(cm)									
trees	H_3PO_4 (M)	0 (month)	6 (months)	12 (months)	18 (months)	24 (months)	30 (months)	36 (months)	42 (months)	48 (months)	54 (months)
A	0 .38	19	33.4	55	63.9	79.8	86.9	100	115	126.4	134.3
B	0.75	16	35	60	75.4	100	110.5	130.8	144.3	152.2	159.5
C	1.5	18	40.3	70	87.9	115	127	154.2	167.2	172	179.2
D	3	19	44.7	80	100.6	130.6	144.9	177.2	190	197.4	205.3

Table: 2. Results obtained by the analysis of heavy metals (Cd,Cr) in three parts of the Argan

		1month		6months		14months		48months		54months	
trees	Sample	Cd(ppm)	Cr(ppm)	Cd(ppm)	Cr(ppm)	Cd(ppm)	Cr(ppm)	Cd(ppm)	Cr(ppm)	Cd(ppm)	Cr(ppm)
	Leaves	0.275	32.229	0.21	34.01	0.4	42.22	0,414	41.465	0.472	43.211
A	Wood	0.306	20.076	0.32	20.12	0.48	23.79	0.513	25.146	0.55	27.3
	Soil	0.215	9.5266	0.27	9.5266	0.67	15.54	0.60	14.86	0.731	17.864
	Leaves	0.284	35.242	0.28	35.242	0.284	38.46	0.781	40.07	0.90	42.238
B	Wood	0.346	16.242	0.37	16.242	0.41	18.98	0.52	17.32	0.654	19.21
	Soil	0.298	14.011	02.37	14.36	0.91	15.23	0.87	15.30	1.161	16.76
	Leaves	0.981	33.092	0.98	34.12	1.83	56.1	1.824	38,59	1.98	42.04
C	Wood	0.488	16.046	0.5	16.91	0.69	21.03	0.751	20.89	0.937	23.84
	Soil	0.836	18.541	0.88	19.11	0.91	20.61	0.92	21.876	1.24	24.143
	Leaves	0.994	37.354	0.99	37.51	1.05	43.76	1.022	41.63	1.072	45.8
D	Wood	0.57	14.344	0.61	14.48	0.96	11.35	0.838	11.01	0.98	14.5
	Soil	0.923	35.638	0.92	35.4	1.67	35.638	1.515	34.453	1.827	35.761

3. Results and discussion

3.1. *Experimental design and optimization by response surface methodology (RSM)*

Our research identifies the factors that influence the size of Argan (Y (cm) response) at four trees A, B, C and D. These parameters are the concentration of phosphoric acid (M), the cadmium concentration (ppm) and the chromium concentration (ppm). In the case of design of experiments 2 (k-p), Plackett and Burman (1946) developed highly split planes to obtain the maximum number of main effects with minimum of trials [11]. The equivalent for a plan 3 (k-p) is a plan Box-Behnken [12, 13]. These plans do not have a simple plan of generators, they are built by combining two-level factorial designs with incomplete blocks plans and they have complex interactions confused. However, these plans have the advantage of being economical when the experimental tests are expensive. The modelling is to determine an empirical model that describes the variations of the Y response as a function of various factors which may influence on these changes, using the Box-Behnken plans [14-19]. The quadratic equation for the independent variables was expressed as follows:

$$Y = a_0 + a_1 X_1 + a_2 X_2 + a_3 X_3 + a_{12} X_1X_2 + a_{13} X_1X_3 + a_{23} X_2X_3 + a_{11} X_{12} + a_{22} X_{22} + a_{33} X_{32} \pm \varepsilon$$

Where Y represents the predicted response, a_0 is the interception coefficient, a_1 a_2 and a_3 are the linear coefficients,a_{11},a_{22} and a_{33} are the quadratic coefficients,a_{12}, a_{13} and a_{23} is the cross-product coefficients, X_1, X_2 and X_3 represent the independent variables studied and ε. is the random error.

The various factors that influence the growth of the Argan tree in Morocco is shown in table 3.

Table 3: Experimental range and levels of the independent process variables to study the growth of the Argan tree in Morocco

Coded variables	X_1, X_2, X_3	-1	0	1
Natural variables x_i	x_1= Molarities H_3PO_4(M)	1	2	3
	x_2= Cd Concentration (ppm)	0.5	0.75	1
	x_3= Cr Concentration (ppm)	20	30	40

Table 4 presents the various experiments based on the Box-Behnken plans for various factors that influence the size of Argan irrigate with the phosphorus solution.

Table 4: Matrix of experiments plans Box- Behnken of the size of Argan watered with the phosphorus solution in four sites A, B, C and D

N°	X_1	X_2	X_3	Response (size (cm))			
				Site			
				A	B	C	D
1	-1	-1	0	134.3	159.5	179.2	205.3
2	1	-1	0	126.4	152.2	172	197.4
3	-1	1	0	115	144.3	167.2	190
4	1	1	0	100	130.8	154.2	177.2
5	-1	0	-1	86.9	110.5	127	144.9
6	1	0	-1	79.8	100	115	130.6
7	-1	0	1	63.9	75.4	87.9	100.6
8	1	0	1	55	60	70	80
9	0	-1	-1	33.4	35	40.3	44.7
10	0	1	-1	26.2	25.7	30	33
11	0	-1	1	19	17.1	19.9	21.4
12	0	1	1	19	17.1	19.3	21.5
13	0	0	0	19	16.8	19	21
14	0	0	0	19	16.5	18.9	20.7
15	0	0	0	19	16	18	19

Calculation results of the model coefficients of the Argan growth according to the different trees are shown in Table 5.

Table 5: Model factors for the growth of the Argan tree in the presence of P, Cd and Cr

Coefficients	The estimated coefficients	The standard errors	t_{exp}	Confidence level
$a_{0\,A}$	19.0000	4.2976	0.0442	0.0069
$a_{0\,B}$	16.4333	5.1119	0.0321	0.0236
$a_{0\,C}$	18.6333	5.2163	0.0357	0.0160
$a_{0\,D}$	20.2333	6.0577	0.0334	0.0206
a_{1A}	-4.8625	2.6317	-0.0185	0.1239
$a_{1\,B}$	-5.8375	3.1304	-0.0186	0.1212
$a_{1\,C}$	-6.2625	3.1943	-0.0196	0.1072
$a_{1\,D}$	-6.9500	3.7096	-0.0187	0.1199
$a_{2\,A}$	-6.6125	2.6317	-0.0251	0.0537
$a_{2\,B}$	-5.7375	3.1304	-0.0183	0.1263
$a_{2\,C}$	-5.0875	3.1943	-0.0159	0.1721
$a_{2\,D}$	-5.8875	3.7096	-0.0159	0.1733
a_{3A}	-8.6750	2.6317	-0.0330	0.0216
$a_{3\,B}$	-12.7000	3.1304	-0.0406	0.0098
$a_{3\,C}$	-14.4000	3.1943	-0.0451	0.0064
$a_{3\,D}$	-16.2125	3.7096	-0.0437	0.0072
a_{12A}	-1.7750	3.7218	-0.0048	0.6535
$a_{12\,B}$	-1.5500	4.4271	-0.0035	0.7405
$a_{12\,C}$	-1.4500	4.5174	-0.0032	0.7612
$a_{12\,D}$	-1.2250	5.2461	-0.0023	0.8246
$a_{13\,A}$	-0.4500	3.7218	-0.0012	0.9085
$a_{13\,B}$	-1.225	4.4271	-0.0028	0.7931
$a_{13\,C}$	-1.4750	4.5174	-0.0033	0.7573
$a_{13\,D}$	-1.5750	5.2461	0.0030	0.7761
$a_{23\,A}$	1.8000	3.7218	0.0048	0.6491
$a_{23\,B}$	2.325	4.4271	0.0053	0.6219
$a_{23\,C}$	2.4250	4.5174	0.0054	0.6144
$a_{23\,D}$	2.9500	5.2461	0.0056	0.5982
$a_{11\,A}$	73.4625	3.8738	0.1896	<0.0001
$a_{11\,B}$	96.5083	4.6879	0.2094	<0.0001
$a_{11\,C}$	111.0583	4.7019	0.2362	<0.0001
$a_{11\,D}$	128.0588	5.4603	0.2345	<0.0001
$a_{22\,A}$	26.4625	3.8738	0.0683	0.0010
$a_{22\,B}$	33.7583	4.6879	0.0733	0.0007
$a_{22\,C}$	38.4583	4.7019	0.0818	0.0004
$a_{22\,D}$	44.1833	5.4603	0.0809	0.0005
$a_{33\,A}$	-21.0625	3.8738	-0.0544	0.0029
$a_{33\,B}$	-26.4667	4.6879	-0.0574	0.0022
$a_{33\,C}$	-29.7167	4.7019	-0.0632	0.0015
$a_{33\,D}$	-34.2667	5. 4603	-0.0628	0.0015

The model equations of growth of the Argan tree in the presence of phosphorus and heavy metals (Cr, Cd) are:

$$Y_A = 19.0000 - 4.8625.X_1 - 6.6125.X_2 - 8.6750.X_3 - 1.7750.X_1X_2 - 0.4500.X_1X_3 + 1.8000.X_2X_3 + 73.4625.X_1^2 + 26.4625.X_2^2 - 21.0625X_3^2$$

$$Y_B = 16.4333 - 5.8375.X_1 - 5.7375.X_2 - 12.7000.X_3 - 1.5500.X_1X_2 - 1.2250.X_1X_3 + 2.325.X_2X_3 + 96.5083.X_1^2 + 33.7583.X_2^2 - 26.4667.X_3^2$$

$$Y_C = 18.6333 - 6.2625.X_1 - 5.0875.\ X_2 - 14.4000.X_3 - 1.4500.\ X_1X_2 - 1.4750.X_1X_3 + 2.425.X_2X_3 + 111.0583.X_1^2 + 38.4583.X_2^2 - 29.7167.X_3^2$$

$$Y_D = 20.2333 - 6.9500.X_1 - 5.8875.X_2 - 16.2125.X_3 - 1.2250.X_1X_2 - 1.5750.X_1X_3 + 2.9500.X_2X_3 + 128.0583.X_1^2 + 44.1833.X_2^2 - 34.2667.X_3^2$$

At 64% confidence level (A significance level of 36%), only the coefficients a_0, a_{12}, a_{13} and a_{23} can be considered significant for the Site A. The equation of the model estimated is:

$$\hat{Y}_A = 19.0000 - 1.7750.X_1X_2 - 0.4500.X_1X_3 + 1.8000.X_2X_3$$
$$(\pm 0.04) \quad (\pm 0.005) \quad (\pm 0.001) \quad (\pm 0.005)$$

At a 62% confidence level (a 38% significance level) for the Site B, the model equation is:

$$\hat{Y}_B = 16.4333 - 1.5500.X_1X_2 - 1.2250.X_1X_3 + 2.3250.X_2X_3$$
$$(\pm 0.03) \quad (\pm 0.004) \quad (\pm 0.003) \quad (\pm 0.005)$$

For the site C, at a 61% confidence level (A significance level of 39%), the model equation is:

$$\hat{Y}_C = 18.6333 - 1.4500.X_1X_2 - 1.4750.X_1X_3 + 2.4250.X_2X_3$$
$$(\pm 0.04) \quad (\pm 0.003) \quad (\pm 0.003) \quad (\pm 0.005)$$

The equation of the model estimated the growth of the Argan tree in the site D to 59% confidence level which is a significance level 41%, is:

$$\hat{Y}_D = 20.2333 - 1.2250.X_1X_2 - 1.5750.X_1X_3 + 2.9500.X_2X_3$$
$$(\pm 0.03) \quad (\pm 0.002) \quad (\pm 0.003) \quad (\pm 0.005)$$

3.2. *The optimization of the growth of the Argan tree*

The goal of this optimization is to meet a specific objective to find the optimum phosphorus quantity for the growth of the Argan tree and the minimum contents of the heavy metals Cr and Cd. So, the optimization is to find the set of values of the factors causing the desired response which is in our case the size of the Argan tree for four sites A, B, C and D based on economic constraints. After the modeling of the response as a function of various factors, the optimum can be located by plotting curves isoréponse [20-21]. The response surface plots for the Argan growth are shown in the figures 1, 2, 3 and 4.

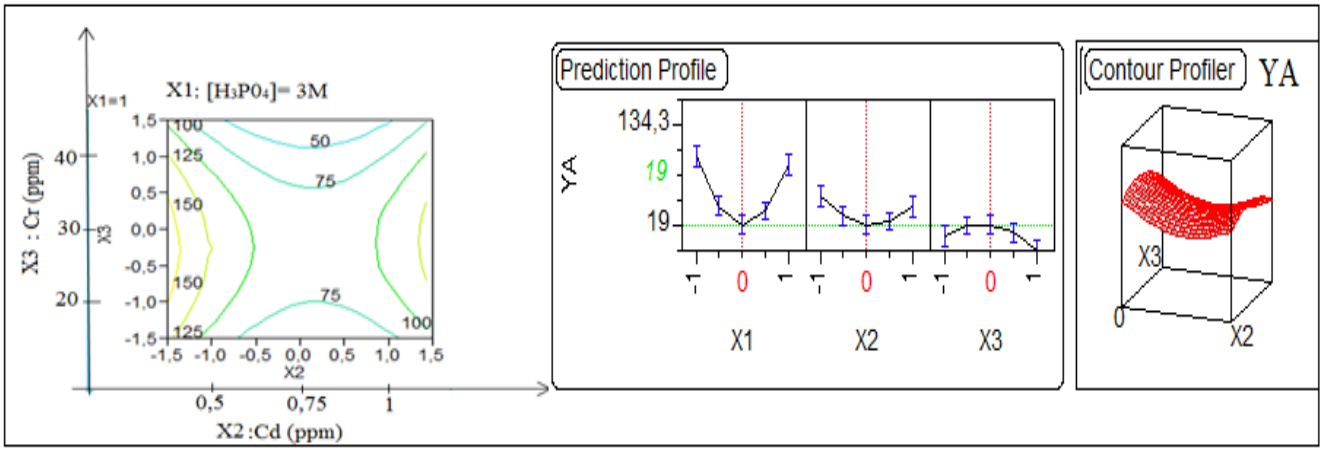

Figure 1: The Response surface plots for the growth of the Argan tree in site A.

The optimum is reached in the site A to an amount of phosphoric acid equal to 3M, an amount of 30 ppm in Cr and 0.825 ppm in Cd.

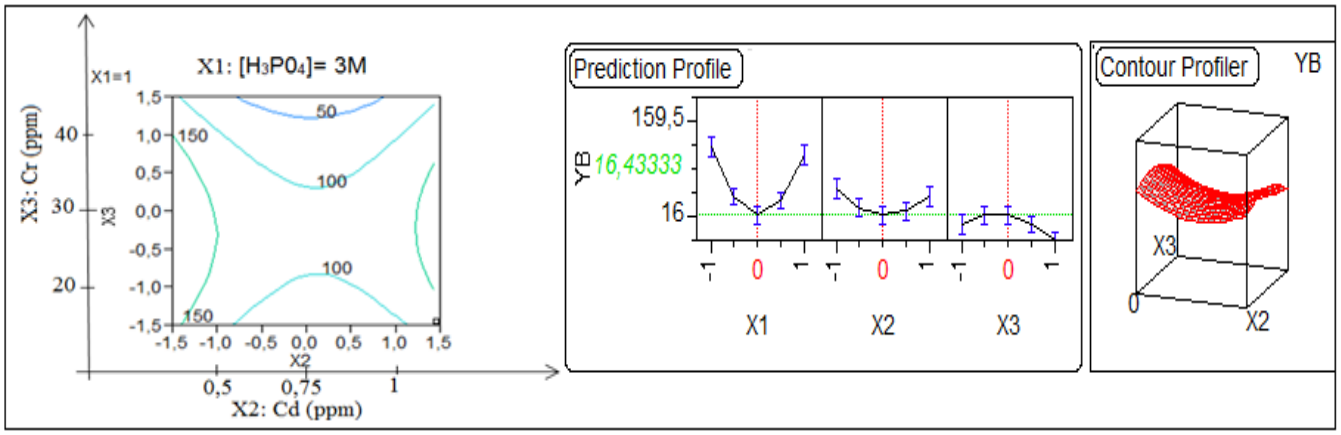

Figure 2: The Response surface plots for the growth of the Argan tree in site B.

For the site B, the optimum is reached at an amount of phosphoric acid equal to 3M, a quantity of 28 ppm of Cr and 0.775 ppm of Cd.

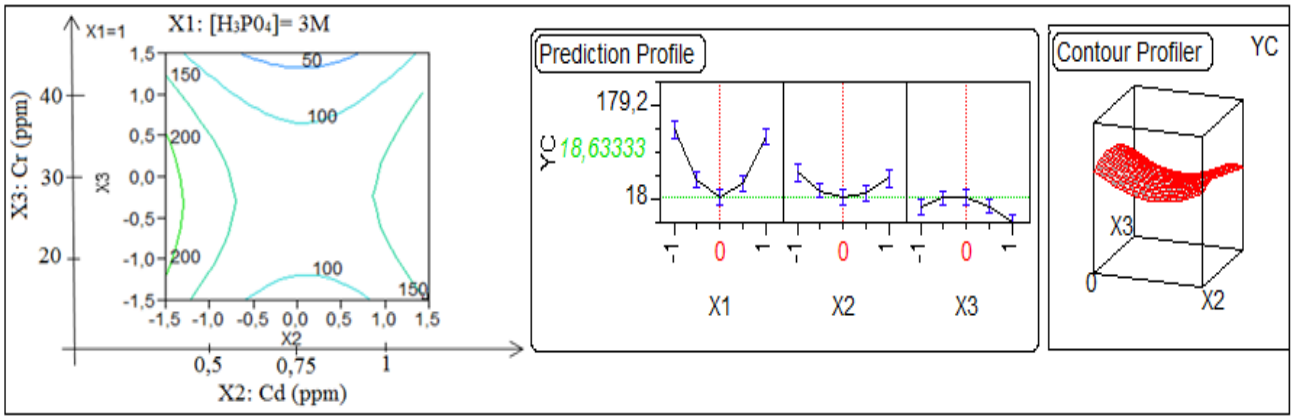

Figure 3: The Response surface plots for the growth of the Argan tree in site C.

For the site C, the good sized for the Argan is obtained at the phosphorus concentration equal to 3M, a quantity of 28 ppm in Cr and 0.775 ppm in Cd.

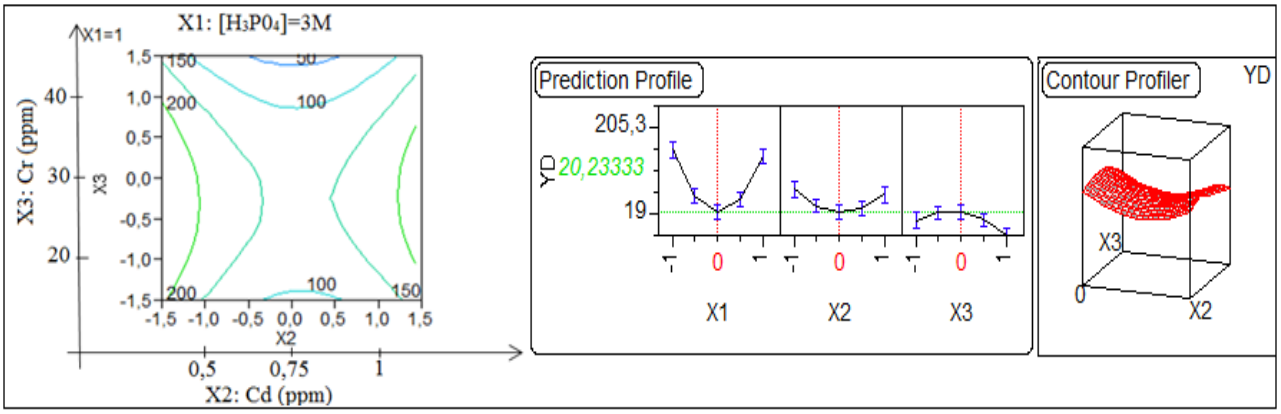

Figure 4: The Response surface plots for the growth of the Argan tree in site D.

For the last tree D, we found that the optimum concentration is 3M for the phosphoric acid, 28.5 ppm in Chromium and 0.775 ppm in cadmium.

Conclusion

In this article we used the methodology of design of experiments Box-Behnken. The factors that influence the Argan growth are phosphoric acid concentrations, cadmium concentrations and chromium concentrations. Models equations in the four sites are:

$$\hat{Y}_A = 19.0000 - 1.7750.X_1X_2 - 0.4500.X_1X_3 + 1.8000.X_2X_3$$
$$(\pm 0.04) \quad (\pm 0.005) \quad (\pm 0.001) \quad (\pm 0.005)$$

$$\hat{Y}_B = 16.4333 - 1.5500.X_1X_2 - 1.2250.X_1X_3 + 2.3250.X_2X_3$$
$$(\pm 0.03) \quad (\pm 0.004) \quad (\pm 0.003) \quad (\pm 0.005)$$

$$\hat{Y}_C = 18.6333 - 1.4500.X_1X_2 - 1.4750.X_1X_3 + 2.4250.X_2X_3$$
$$(\pm 0.04) \quad (\pm 0.003) \quad (\pm 0.003) \quad (\pm 0.005)$$

$$\hat{Y}_D = 20.2333 - 1.2250.X_1X_2 - 1.5750.X_1X_3 + 2.9500.X_2X_3$$
$$(\pm 0.03) \quad (\pm 0.002) \quad (\pm 0.003) \quad (\pm 0.005)$$

The optimum is obtained at a high concentration of the phosphoric acid (3M) which represents the commercial concentration; the values of the chromium and the cadmium are 28 ppm and 0.775 ppm respectively.
These results show that the phosphoric acid concentrations have a positive effect on the argan growth studied, against, the presence of heavy metals (Cr, Cd) limits the growth for some trees and slowed down for some others.

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