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SEASONAL CHANGES OF SEED GERMINATION OF *Conium maculatum* L. - REGRESSION MODELS FOR ESTIMATION

SUMMARY

Conium maculatum L. is distributed in all floristic regions in Bulgaria. In the recent years field infestation with this species has increased especially in cereal crops. Thus, the knowledge of the seasonal changes in seed germination is of great importance. The investigations were carried out during the period 2001-2004. The seeds were buried outside at the different depths (5, 10, 20, 30 and 40 cm) and different duration (2, 6, 8, 9, 11, 12, 13, and 18 months). The effects of soil depth, time of burial, air temperature and humidity as well as the hydrothermal coefficient on the seed germination were estimated using the tools of mathematical modeling. The correlation analysis showed burial duration and hydrothermal coefficient as a main factors of influence. The soil depth impact increases with increase of the burial duration and is most significant if the seeds have been buried during 11-12 months. Different models were developed to describe the single or combined impact of the observed factors. A fractional-polynomial relationship was obtained between the dynamic of the seed germination and burial duration. The fitted regression models showed that the maximum seed germination appears in the 12th month.

Keywords: *Conium maculatum* L., seeds, germination, seasonal changes, regression models.

INTRODUCTION

Conium maculatum L. (C.mL), Family Apiaceae is troublesome annual or biennual weed species. It is native to the area of Europe, Northern Tropical Africa, Asia, the Azores and South Atlantic Islands. (Weber, 2005). This species is very dangerous because all plant parts contain toxic alkaloids and may be fatal to both livestock and wildlife (Lopez et al., 1999; Weber, 2005; Barlow, 2006). C.mL is distributed in all floristic regions in Bulgaria up to 1200 m above sea level. In the recent years field infection by this species has increased, especially in cereal crops. It is spread quickly from margin to the field (Milanova et al. 2003; Milanova et al. 2007). It occurs as a summer annual weed with flushes of emergence occurring after September (Nikolova and Milanova, 2001). At the beginning of winter the plants form a rosette of 8-15 leaves and the vegetation continues in the spring of next year. The seeds of C.mL are easily dispersed by human activities, agricultural produce, winds and birds. Under the conditions in

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Bulgaria mature seeds germinate in the autumn of the same year or spring and summer in the following year. Plants flower from the end of April to September and seeds are mature in June to October. The seeds germination (SG) are 1.75% in darkness, 2.75% in 12-h photoperiod at 26oC, 34.25% in 12-h photoperiod at 25/30oC and 45.25% in 24-h photoperiod at 25/30oC (Milanova et al., 2003). The bioecological characteristics of C.mL are nearly the same in the other parts of the world (Parsons, 1973; Geoden and Ricker, 1982). Aliloo et al., (2014) investigated integrated effects of salicylic acid priming, seed moisture content and temperature during storage on germination and seedling performance of fennel seeds.

One way to understand, predict and manage a system that is hidden beneath the soil and involves a large number of complex, long-term interactions is to create a computational model of that system (Renton et al., 2006a). Bouwmeester and Karsen (1993) have developed a regression model that estimates the changes in SG of *Chenopodium album* L. in fields and found out that, despite of absence of clear seasonal changes in the temperatures suitable for germination (computed with the model), SG showed well expressed seasonal periodicity. Many authors have developed mathematical models to estimate the different aspects of the changes of dormancy, survival and germination of weed seeds: SG for *Polygonum persicaria* L. at field temperature was accurately described with a model (Bouwmeester and Karssen, 1991); a logistic regression for five weed species - *Stellaria media*, *Chenopodium album*, *Capsella bursa-pastoris*, *Matricaria perforata*, and *Veronica hederifolia* (Grundy and Mead, 2000); non-linear model for *Beta vulgaris* L (Sester et al., 2006). Alvarado and Brandford (2002) reported that the weed SG responses across a range of sub-optimal temperature and water potential could be described by a general hydrothermal time (HTT) model. Grundy et al. (2003) use a generic model to quantify seed response to burial depth and to improve the wider application of existing seedling germination models.

Many authors use the HTT model for predicting of SG depending on the temperature and water potential (Bradford, 1995; Bradford, 2002; Larsen et al., 2004; Gundel et al., 2006). Renton et al. (2006b) use a model to simulate the effect of crop choice, sowing date, seeding rate, herbicide application on SG and reported that modelling framework can be used for more theoretical investigation of the complex mix of factors affecting seed bank and SG. Batlla and Benech-Arnold (2007) have developed a model that allow the prediction of timing and extent of weed emergence that, on their opinion, is essential for planning more effective weed control strategies in agricultural systems.

The objective of this study was to investigate the seasonal changes in seed germination of *Conium maculatum* L and, by use of mathematical modelling tools, to estimate the range of single or combined impact of different environmental factors affecting SG.

MATERIAL AND METHODS

Seed germination test: Seasonal changes in seed germination of *Conium maculatum* L. (*C. maculatum*) were examined over a 4 year period in two separate trails (2001-2003 and 2002-2004). The seeds were buried in the different depths outdoors and compared with germination of dry stored seeds at room temperature. Matured seeds were collected from parental plants in the middle of August 2001 and 2002. Samples of 100 seeds were placed in fine mesh nylon bags. Each was buried to a depth of 5, 10, 20, 30 or 40 cm in sandy clay loam soil in 6.5 cm diameter plastic pots (volume 65 ml) with drainage holes. The mesh was small enough to retain the seeds but still allow for passage of water, gases and microorganisms through the screen.

Pots were in turn buried outdoors on 5 October 2001 and 2002 in split-plot design with four replications. Subplots consisted of pots corresponding to the five duration of burial: 2, 6, 9, 12, 18 and 6, 8, 11, 13, 18 months in 2001 and 2002, respectively. At each time of recovery, four pots of each depth were removed from the field and bags were exhumed and washed under running tap water in the laboratory and dried before opening. The seeds from each bag were placed on two sheets of filter paper in 5.5 cm Petri dishes. Distilled water (3ml) was added to each Petri dish and replaced regularly.

These were set to germinate in the laboratory under incubation conditions (24 hour photo-period at 30/25oC) that had been found to be favorable in the preliminary studies (Milanova et al., 2003). Seeds showing radicle emergence were counted and removed from the Petri dishes. The final germination percentage was scored after 35-day incubation period. Control seeds were stored at room temperature and were set to germinate under the same conditions as recovered seeds (soil depth (SD) - 0 cm).

Statistical analysis and modelling: Statistical procedures and calculations were performed with the computer software in FORTRAN developed on the base of standard statistical algorithms for small biological data sets (Sokal et al., 1981; Maneva, 2007). The data were processed by analysis of variance with F for test significance and LSD for significance of mean differences between variants and the control at $P< 0.05$, 0.01 or 0.001, depending on the dispersion in the variants. There were two sources of variation – the burial duration and the soil depth. Correlation analysis was also performed. The hydrothermal coefficients (HTC) according Selyaninov (Radomsky, 1973) were calculated. To develop the regression models and to fit them to the data we used the Statistica5.0 testing standard model design or models designed by the author. The models that gave highest regression coefficients (R) and lowest estimated variance were chosen for functional description of seed germination of *C. maculatum* and respective observed factors, which showed well expressed impact.

RESULTS AND DISCUSSION

Statistical analysis: For both sources of dispersion (soil depth (SD) and burial duration (BD)) the analysis of variance showed statistically significant differences between the means of variants and the respective control. The differences were strongly statistical significant when the BD was the source of variance (Table 1).

Table 1. Statistical significance of the differences between variants and respective control at source of variation the burial duration. Comparative analysis at the different soil depths

BD	Soil depth - cm					
	0	5	10	20	30	40
2	22.8 C	32.0 C	15.3 C	16.3 C	18.7 C	14.8 C
6	21.3 NS	20.9 ***	17.3 NS	17.0 NS	19.4 NS	17.0 NS
8	21.5 NS	11.0 ***	22.3 **	24.5 ***	31.8 ***	24.0 ***
9	23.0 NS	29.5 NS	22.8 **	23.8 ***	26.8 *	24.5 ***
11	23.3 NS	9.0 ***	43.0 ***	49.3 ***	52.3 ***	68.8 ***
12	23.3 NS	53.8 ***	58.8 ***	72.3 ***	68.5 ***	23.3 ***
13	21.5 NS	4.0 ***	4.0 ***	8.5 ***	8.0 ***	8.5 **
18	24.5 NS	5.2 ***	8.4 ***	10.9 ***	12.6 **	19.7 NS
LSD F	1.40 187.95	1.81 187.54	2.17 141.8	1.77 307.93	2.17 181.06	1.41 138.79

C – control, NS – P>0.05, * - P<0.05, ** - P<0.01, ** - P<0.001

Table 2 Dependence of the seed germination on the observed factors of influence presented as correlation coefficients $r_{i/j}$ – i – factor of influence, j – seed germination (SG).

	Correlation coefficient $r_{i/j}$				
Factors	**A** : Soil depth (SD)				
	5 cm	10 cm	20 cm	30 cm	40 cm
DB/SG	0.714 **	0.754 **	0.771 **	0.883 ***	0.866 ***
HTC/SG	0.864 ***	0.827 ***	0.729 **	0.742 **	0.720 **
Tmin/SG	0.182	0.192	0.170	0.207	0.364
Tmax/SG	0.362	0.212	0.172	0.230	0.317
Taver/SG	0.190	0.126	0.088	0.162	0.308
Precipitation	0.319	0.512 *	0.570 *	0.570 *	0.522 *
% air humid.	0.372	0.338	0.423	0.368	0.368

B: Burial duration (BD) in months

	2	6	8	9	11	12	13	18
SD/SG	0.441	0.691 *	0.712 **	0.882 **	0.967 ***	0.967 ***	0.857 **	0.435

Only marked by stars coefficients are statistically significant at level: * - P<0.05, ** - P<0.01, *** - P<0.001

Correlation coefficients rij, (i - one of the observed impact factors – burial duration (BD), HTC, daily temperatures (minimal (Tmin), maximal (Tmax), average (Taver)), precipitation or percentage of air humidity, and j - seed germination (SG), were calculated for every soil depth (Table 2,A). Increase of SD did not change significantly the values of the correlation coefficients. This showed that SD is not the main factor affected the SG. Best correlation was found out between BD/SG ($0.714<r<0.883$, $P<0.01$ or $P<0.001$) and HTC/SG ($0.720<r<0.864$, $P<0.01$ and $P<0.00$). Increase of the SD enhanced the correlation between BD and SG but decreased it for the HTC. The precipitation and percentage of air humidity showed higher impact on the SG than the temperature. The increase of BD increases the impact of the SD. This determined the BD as a main factor affecting the SG. The best correlation between SD and SG appeared if the seeds have been 11-12 months buried (Table 2,B). Calculated linear r were stronger than the non linear ($0.800<nonlinear<0.872$, $P<0.001$), and kept the same trends described by the linear ones. They showed again a significant influence of the BD ($0.820<r<0.872$, $P<0.001$). The initial regression analysis showed that the relationship between SG and the climatic factors –daily temperature (Tmin, Tmax or Taver), precipitation and percentage of air humidity as well as BD, SD and HTC were not first degree linear.

Models: The regression analysis was performed to be described the influence of he observed climatic factors (daily temperatures (Tmin, Tmax or Taver), average precipitation (AP), hydrothermal coefficient HTC and relative air humidity (RAH)) as well as burial duration (BD) on the seed germination (SG) under different soil depths (SD). The initial regression analysis showed that the relationships between SG and the climatic factors were not first degree linear. The impacts of observed factors on SG, separately for every SD, were described by least squares regression models. The best fit to the data was found when the impact factors were AP, HTC and RAH (Fig. 1, Fig 2 and Fig. 3). The obtained models showed that HTC decreases the SG for $HTC<6$ and increased it slightly for $HTC > 6$ (Fig 2). The trends for all SD were same as at the deepest SD (40 cm) the SG is the best. After $HTC > 6$ the SG increased faster at 30 cm SD. Models for influence of average precipitation and relative soil humidity showed same trends – SG has a pick at the respective values of the impact factor. The pick of SG appeared at $60 \text{ mm} < AP < 65\text{mm}$ (Fig. 1). RAH caused the maximum of SG when RAH is near 72-73% (Fig. 3). In both cases best seed germination showed the seeds buried deepest (40 cm) and the SG decreased with decrease of SD.

Several regression models describing the impact of burial duration BD on the soil germination (SG) were tested to be finding out the best fit to the experimental data. The relation SG = F(BD) for every one of the observed SD was best described by the fractional-polynomial equation (1).

$$Y=X/(Ao+A_1X+A_2X^2) \qquad (1)$$

Where: Y – is the percentage of seed germination, X – burial duration, Ai, i = 0,1,2 – the model parameters.

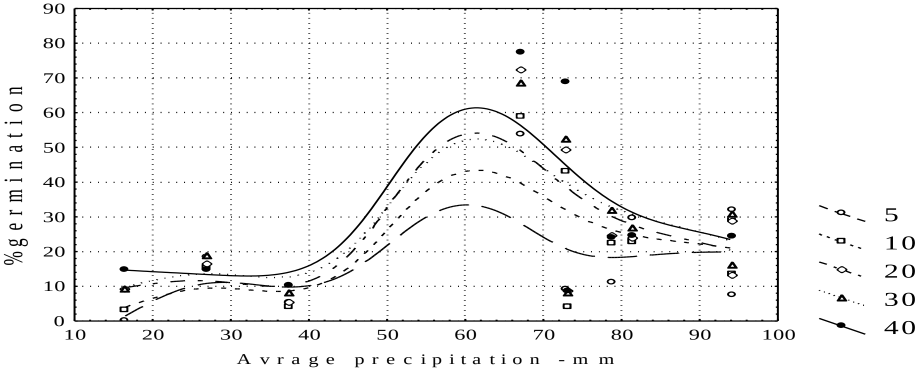

Fig 1. Least squares regression models described the influence of average precipitation (mm) on seed germination (SG) under different soil depths (cm).

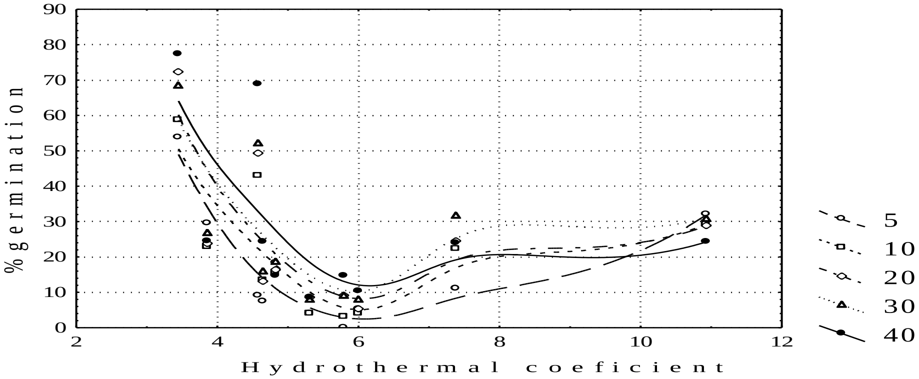

Fig 2. Least squares regression models described the influence of HTC on seed germination (SG) under different soil depths (cm).

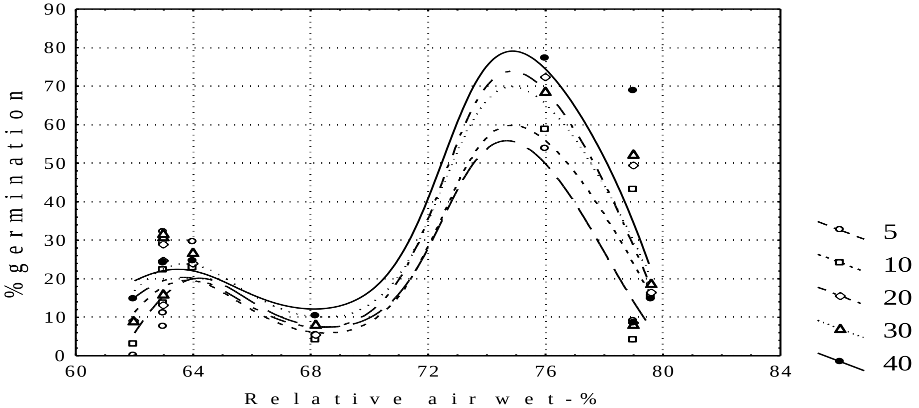

Fig 3. Least squares regression models described the influence of relative air humidity (RAH in %) on seed germination (SG) under different soil depths (cm).

It is clear that the soil depth did not change the respective trends. In SD of 10, 20 or 30 cm SD impacts the SG if the seeds were buried longer then 11-12 months (Fig. 4, B, C and D). In the smaller SD (5 cm) the pick of SG appeared at BD of 4 days 9Fig.4, A). In the deepest SD (40 cm) we found out the highest SG after 12 days burial duration (Fig. 4,E).

The model was fitted to the data from all SD with good statistical significance (0.686<R< 0.912 at P<0.05 0 or 0.001).The obtained models described a stable trend of the investigated process. All SD fitted regression models showed a peak of the SG depending on the BD. The SD only changed the period of the appearing of the peak. The significance of the fit is presented on Table 3. The regression coefficients are in ranger of 0.686<R<0.918 at P<0.01 or 0.001. Errors of the regression parameters (Ai, i = 1,2 or 3) are small with enough good t values and P<0.001 for the any of SD.

Table 3. Coefficients Ai, (*i* = 0, 1, 2, 3) of equation (1) and their statistical estimations

	Soil depth														
	5 cm			10 cm			20 cm			30cm			40 cm		
	Parameters of equation (1) fitted to the data of respective soil depth														
	A_0	A_1	A_2	A_0	A_1	A_2	A_0	A_1	A_2	A_0	A_1	A_2	A_0	A_1	A_2
Value	0.23	-0.9	0.01	4.4	-0.8	0.4	3.4	0.61	0.03	1.96	0.34	0.02	2.57	0.41	0.02
Err.	0.05	0.02	0.003	0.01	0.01	0.02	0.1	0.01	0.01	0.09	0.01	0.01	0.09	0.01	0.01
T	4.82	-4.0	5.46	23.0	-82	20.6	26	-97	25.6	23.0	-57	19.4	27.6	-69	30.4

The combined effect of two observed factors on the SG was estimated by the two-factor regression model (2)

$$Z= A0 +A1X+A2Y+A3X2+A4XY+A5Y2 \qquad (2)$$

Where Z is SG, and (X; Y) – the respective couples of investigated factors of influence of SG.

Plotting the SG versus (BD;SD) by model (2) indicated that BD was stronger factor than SD, as well as that the peak of SG appeared at BD of 12 months (Fig.5,A). Increase of SD slightly increased the percentage of SG. Both fit with the results found out by the one factor models.. Relation SG=F(HTC<BD) showed that increase of HTC decreased the SG in the shorter BD and increased it when seeds were longer in the soil (Fig.5,B). SG = F(SD;*Taver)* showed a well expressed but not very strong influence on the SG (Fig. 5,C). The higher T*aver* depresed SG in the near soil surface since the increasing of both Taver and SD increased SG (Fig. 5,C). The combination (SD,HTC) had significant effect on SG (Fig. 5,D). All 4 models fit to the data with good statistical significance (R>0.9, P<0.001)

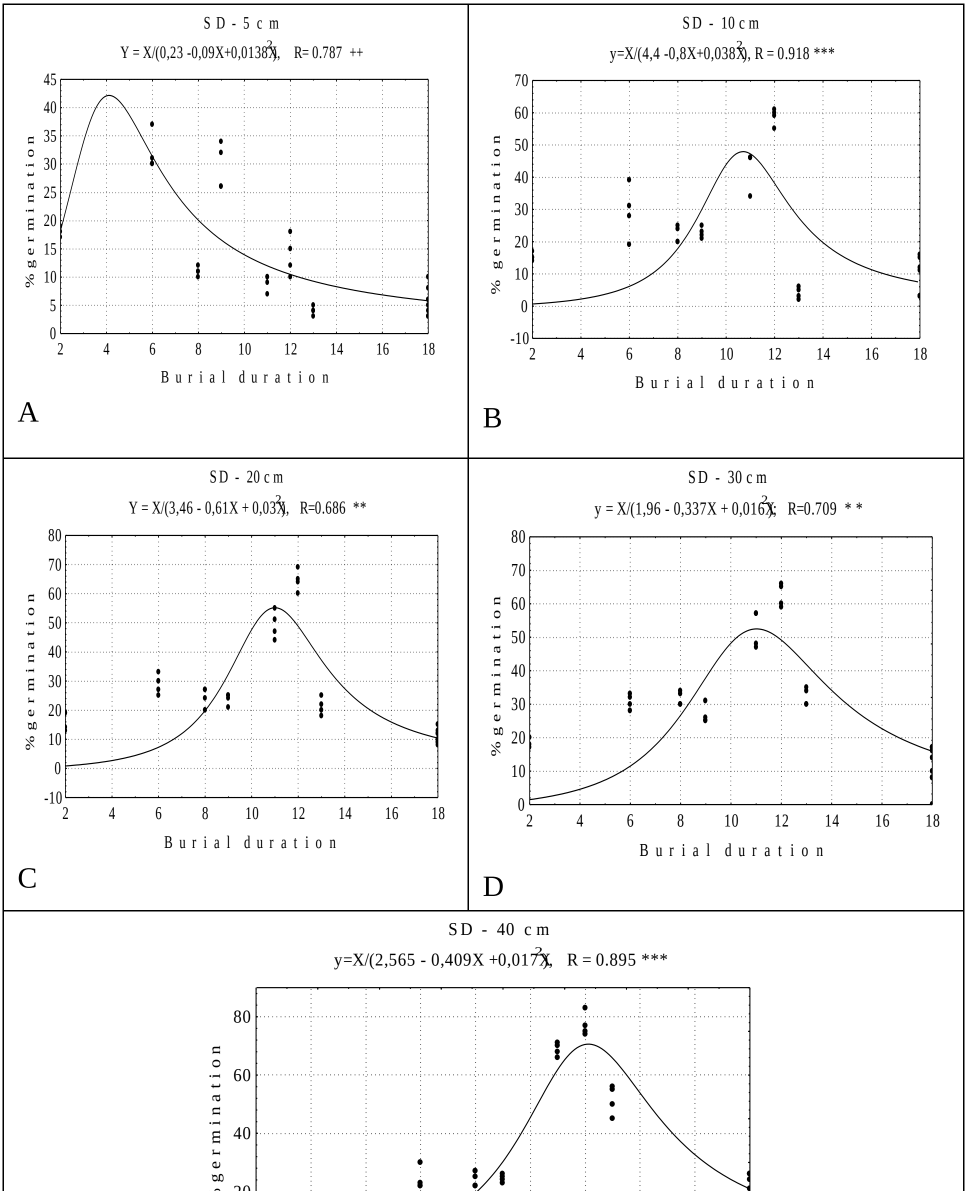

Figure 4. Functional dependence of seed germination (SG) on the burial duration (BD) in soil depths (SD) of 5, 10, 20, 30 and 40 cm

Table 4. Coefficients Ai, (*i* = 0, 1, 2, 3, 1, of equation (3) and their statistical estimations

	Parameters of the regression model					
	A_0	A_1	A_2	A_3	A_4	A_5
Value	36.583	14.16	0.155	-4.635	-0.658	0.038
Err	1.81	0.68	0.042	0.548	0.044	0.0005
t	8.03	20.74	3.74	-8.463	-14.94	7.34
P	0.001	0.001	0.001	0.001	0.001	0.001

The stronger effect on the SG showed the combination of (BD, SD, HTC). Their effect on SG was best described by equation (3):

$$Y = A0 + A_1X_1 + A_2X^2 + A_3X^3 + A_4X1^2 + A5X3^2 \quad (3)$$

Where Y – % germination, X1 – burial duration, X2 – soil depth, X3 – hydrothermal coefficient, Ai, i=0÷5 -the model parameters.

When the equation was applied including the squared SD (X_2^2), the fitting to experimental data was worse (R=0.714 **) than if expel it (R=0.942 ***), as well as the model parameters had better statistical estimations (Table 4). This once again confirmed that the SD is not the main factor affecting SG

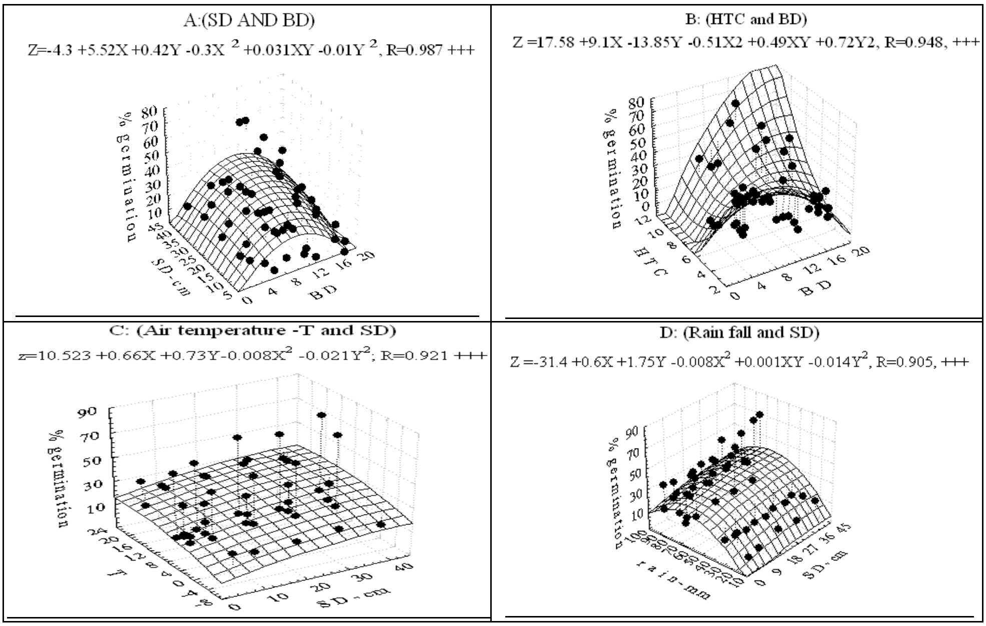

Figure 5. Seed germination (SG) as function of two factors: A – Burial duration (BD) and soil depth (SD), B – Burial duration (BD) and Hydrothermal coefficient (THC), C – Soil depth (SD) and average air temperature, D - Soil depth (SD) and Hydrothermal

CONCLUSIONS

The results showed that there were well expressed non-linear relationships in the observed processes that made them good subjects for mathematical modelling. On the basis of the statistical analysis and obtained models BD was determined to be the main factor that affects the SG changes. It was identified that fraction-polynomial regression was the best at describing SG as a function of BD. The model showed that SG increased with increasing the time of burial and reached its peak with in BD of 10-12 months. In this way the model estimation confirmed the conclusion, reported by Milanova et al. (2003) on the base of one year previous experiment, that exhumed seeds of *C. maculatum* L. after 12 months (in October) had higher SG.

Two-factor regression models described well the combined effect of the factors on SG changes. By them was determined the influence degree of every pair of factors. The combinations of (BD;SD), (BD;HTC), (BD; precipitation), (SD;HTC) and (SD; percentage of air humidity) had a stronger effect than the other combinations. The impact of (BD;SD;HTC) was described by polynomial regression of the third degree expelling the square of SD, that could be explained with the lower effect of SD. Boumeester et al. (1993) established that, despite the absent of clear air temperature changes, SG in the field showed seasonal periodicity. Our models confirmed the conclusion that air temperature is not main factor when relate with the BD and add the knowledge that it was a stronger factor when related with SD. Irregularly SG in the growing season was attributed to the seasonal changes in the biological dormancy of seed due to the complex of factors. The mathematical modelling is a good way to understand, predict and manage a system that depends on complex mix of factors affecting on the seasonal changes of seed germination.

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