FINAL REPORT

Covering period from: December 2005-December 2008

Submitted to the U.S. Agency for International Development; Bureau for Economic Growth, Agriculture, and Trade

TITLE OF PROJECT: Irrigation of Megacarpaeae and Crambe plants with brackish water and its effect on seed lipids and other lipophylic components

Project Number: CA22-020 and Award Number: TA-MOU-02

Principal Investigator: Bruria Heuer
Grantee Institution: ARO, Volcani Center
Address: Institute of Soil, Water and Environmental Sciences, POB 6, Bet Dagan 50250, Israel
Telephone Number: 972 3 9683704
FAX Number: 972 3 9604017
E-mail address: bruriah@volcani.agri.gov.il

Co-Principal Investigators: Tibor Markovitz
Address: as above

Principal Investigators: A. I. Glushenkova
Grantee Institution: Uzbek Academy of Sciences
Address: Institute of Plant Substances Chemistry, 77 Kh. Abdullacv. Str., Tashkent 700170, Uzbekistan
Telephone Number: 998 71 1627306
FAX Number: 998 71 1206475
E-mail address: cnc@icps.org.uz

I. Akhmedzanov
Institution: Uzbek Academy of Sciences
Address: Institute of Physiology and Biophysics, 1 A. Niyazov Str., Tashkent 700095
Telephone Number: 998 71 2 469412
FAX Number: 998 71 1917404
E-mail address: iskan_bioph@mail.ru

Co-Principal Investigators: M. Ionov, N. T. Ulchenko, N. Yuldasheva, F. Mustaev, A. Halilov
Institution: Uzbek Academy of Sciences
Address: Institute of Plant Substances Chemistry, 77 Kh. Abdullacv. Str., Tashkent 700170, Uzbekistan
Telephone Number: 998 71 1627306
FAX Number: 998 71 1206475
E-mail address: cnc@icps.org.uz

Administration Official: D. Pruski
Institutional Affiliation: ARO, Volcani Center

Administration Official: A. Abdulaev
Institutional Affiliation: Uzbek Academy

Address: POB 6, Bet Dagan 50250

Telephone Number: 972 3 9683226
FAX Number: 972 3 9604017
E-mail address: vkmichal@agri.gov.il

of Sciences
Address: Institute of Plant Substances Chemistry, Tashkent 700170

Telephone Number: 998 71 1625913
FAX Number: 998 71 1206475
E-mail address: cnc@icps.org.uz

Executive Summary:

The Aral Sea crisis and other ecological disasters responsible for the salinization of a significant part of the soils and water in Uzbekistan and other countries of Central Asia, lead to the deterioration of the vegetative resources in this region. More than 1.4 million ha of seabed in the Republic of Uzbekistan were exposed following a catastrophic drop in the level of the Aral Sea, resulting in a new desert.

The possibility to cultivate new crops under saline conditions will permit a further development of the affected areas. The purpose of this study was to introduce new crops of great potential from agricultural, nutritional and industrial aspects, which, after adaptation to the local growth conditions, might have higher economical benefit than the present agricultural crops. *Megacarpaea gigantean* could not be grown as a cultivated crop, thus no research was carried out on this species. Wild *Crambe* species are growing naturally in the Aral Sea region, with a soil salinity level ranging from 3 to 10 %; therefore we decided to determine its potential as a cultivated crop irrigated with saline water. The effect of irrigation with saline water on oil content and the composition of lipids and lipophilic components of seeds of *Crambe amabilis, Crambe abyssinica*, *Crambe kotchayana* and safflower (*Carthanus tinctoris)* was studied in greenhouse experiments in Israel and in the field in Uzbekistan. This included plant growth, yield and plant analysis. The results showed that all crops accomplished full life cycle and produced seeds at all salinity levels, including the very high salinity of 9.0 dS m^{-1} suggesting tolerance to moderate salinity levels and feasibility of their culture in areas of the Aral Sea with adequate salinity levels. The results obtained are encouraging and reproducible. Moreover, seeds from high salinity obtained in the 1st year and used in the 2nd greenhouse experiment showed better performance with minimal salinity effect.

Research Objectives:

The Aral Sea crisis and other ecological disasters were responsible for the salinization of a significant part of the soils and water in Uzbekistan and other countries of Central Asia, leading to the deterioration of the vegetative resources in this region. Deforestation and an excessive use of the remaining pastures have caused serious soil erosion in vast areas. These processes led to an aggravation of the catastrophic changes in the natural conditions of the region in respect to environmental and socio-economic aspects. In order to improve the socio-economic situation in this region, it was extremely important to develop a scientific basis and technologies to prevent the negative outcomes of the Aral Sea drying. More than 1.4 million ha of seabed in the Republic of Uzbekistan were exposed following a catastrophic drop in the level of the Aral Sea, resulting in a new desert. It was essential to find different ways and methods to stop further salinization of the exposed seabed. One possibility was to cultivate drought- and/or salt-resistant plants, which are also capable to stop the movement of sands and the enlargement of saline soils. The possibility to cultivate new crops under saline conditions could permit a further development of the affected areas, as well as other similar regions in many developing countries. The region of Central Asia is inhabited by two wild species of *Crambe*, *C. kotschyana* and *Crambe edentula* F. and by *Megacarpaea gigantean,* used as forage for cattle. Natural growth of wild *Crambe* species in the Aral Sea region, with a soil salinity level ranging from 3 to 10 %, suggested its relatively high resistance as compared to other plants. The necessity of *Crambe* cultivation at the dried Aral Sea bottom was proved by the institute of Botany of the Uzbek Academy of Sciences. *Crambe* plants are characterized by a high tolerance to salinity and by the ability to prevent movement of sand and dust resulting from storms and which are toxic for all alive creatures thousands of kilometers around. Cruciferae seeds are relatively rich in oil and biologically active lipophylic components. *Crambe* seeds contain 30 to 35 percent oil, which is one of the richest known sources of erucic acid, which makes up 55% to 65% of the oil glycerides. The hull makes up about 30% of the harvested product. Dehulled *Crambe* seed has an oil content of 33 to 54% and a protein content of 30 to 50%. Refined *Crambe* seed oil may be used as it is or erucic acid may be extracted from the oil. *Crambe* oil is useful in the textile and steel industries for spinning lubricants and sheet steel fabrication. The lipids obtained from *Crambe* can be used in a food-processing industry, soap, lacquer-paint industry and other technical purpose. Preliminary research of the Uzbek team devoted aimed to study the chemical composition of a number of *Crambe* species and of *Megacarpaea* gigantean growing under natural conditions, showed that the oil content in the seeds of *C. kotschyan*a is 38.2% and 25.0% in *M. gigantean* and about 60% of it is erucic acid.

At the beginning of our research we did not have enough information on the effect of salinity on oil yield and its components as well as on the long-term response of these plants to high salinity, but we believed that *Crambe* plants and *Megacarpaea* could be successfully cultivated on a much larger scale under the conditions of the soil and water salinity in the Aral Sea region. Consequently, this was the main objective of the research proposal. A successful solution of this problem was crucial from both, the environmental and socio-economic aspects, taking into consideration the valuable productive features of *Cramb*e and *Megacarpaea* (the use of the emergent part as forage for cattle and the use of the seed isolated lipids for food, paint and other technical industries).

The specific objectives of the research were:

1. To study the feasibility of irrigating *Crambe* and *Megacarpaea* with brackish water.
2. To investigate the influence of irrigation management on oil yield and its quality.
3. To study the effect of salinity on seed lipids and other lipophylic components.

The urgent necessity, from the ecological and economical aspects, to cultivate species of *Crambe* and *Megacarpaea* rich in oil and lipophylic components in the regions affected by salinity, emphasized the importance and actuality of the research not only for Central Asia, but also for other regions in the world with similar problems. The novelty of this research was the introduction of new crops with high economic value.

The economic development of the five states of the Aral Sea basin, namely, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan, is closely connected with the solution of complicated environmental problems caused by the deterioration of the Aral and the Caspian seas. These states have enormous water and land resources, vast irrigation systems, significant hydro energetic potential, critically important mineral resources, considerable labor resources and reserves of oil, gas and coal. However, in order to use these natural reserves, it is first necessary to solve the environmental problems.

<u>Methods and Results</u>:

This report summarizes the greenhouse experiments carried out in Israel and the field experiment carried out by the Uzbek team on the same plant species: safflower (*Carthamus tinctorius*) from Uzbekistan, *Crambe kotchayana* and *Crambe amabilis* from Kazakhstan and *Crambe abyssinica* from USA. It is important to mention that this is the first trial ever to grow *Crambe kotchayana* and *Crambe amabilis* under controlled conditions.

Irrigation system

General view of the greenhouse

Treatment initiation started about 3 weeks after planting. They consist of 4 salinity levels: $EC_i = 1.5, 3.0, 6.0$ and 9.0 dS m^{-1}.

In Israel and in the 1st year in Uzbekistan, both *Crambe kotchayana* and *Crambe amabilis* could not flower, therefore no seeds were obtained. However, in the 2nd year, all the plants in the field completed a full vegetative cycle including flowering, which consequently, produced fruits and seeds.

Crambe kotchayana grown in the field experiment in Uzbekistan at blooming

We assume that the main reason for not flowering was the much higher temperatures in Israel (Fig. 1) than in Uzbekistan where minimal temperatures drop below zero (Fig. 2). All field plants survived, although the high salinity level significantly reduced growth and yield.

A summary of the EC of leachates is presented in Figs. 3 and 4 for all plant species. In general, in both experiments the EC was maintained at the pre-established values, although in the greenhouse values were somehow higher. The EC of the leachates from the field does not represent fluctuations between soils washing in order to keep a more or less constant value, therefore we are not aware how much time the plants have seen higher EC values.

Crambe amabilis grown in the field experiment in Uzbekistan at blooming

Crambe kotchayana (right) and *Crambe abyssinica* (left) at blooming in the greenhouse

Growth of *Crambe abyssinica* in the greenhouse was very little affected by salinity (Fig. 7) while in the field, growth decreased gradually with the increase in salinity yielding 14-42% inhibition (Fig.8). Interesting results were obtained with *Crambe kotchayana*, the Uzbek species of Crambe; moderate salinity values between 3 to 6 dS m^{-1} enhanced growth by 13% and the high level of 9 dS m^{-1} resulted the same growth as the controls (Fig. 9), suggesting that this crop is mostly suitable for growth in the Aral Sea area with similar salinities values.
Safflower plants grown in the greenhouse were much taller than those in the field (Fig. 5). Its growth in the greenhouse was only slightly affected by high salinity while in the field it was reduced by 70% (Fig. 6). We assume that the reason for this is the fact that the experiments were carried out with an Uzbek variety which probably is less tolerant than other genotypes

reported in the literature as well as the low temperature in Uzbekistan during winter time.

Safflower in the field experiment in Uzbekistan

Safflower in the greenhouse at blooming

We could not measure *Crambe kotchayana*'s height in the greenhouse as the plants did not develop above soil level.

Highest chlorophyll content was measured in plants exposed to high salinity probably because of early senescence following exposure to high salinity (Fig. 10). In the field, chlorophyll content decreased slightly in safflower and by 50% in all Crambe species (Table 1).

In the first year, fruit number of *Crambe abyssinica* in the greenhouse was decreased at high salinities, but at the second year this effect almost disappeared resulting in a very similar fruit number at all treatments (Fig. 11). However, in the field, a gradual decrease in fruit number was observed in the first year (Fig. 12). There are no data for the second year as many fruits were aborted for an unknown reason. No significant effect in safflower heads was observed in the greenhouse (Fig. 13).

We assume that the main reasons for the decrease in growth and yield obtained in the field are low temperatures and exposure to high EC levels between soil washings.

Table 1: Chlorophyll content in field grown plants

	14.5.07			1.6.07		
safflower	chl a	chl b	chl a+b	chl a	chl b	chl a+b
1.5	4.29	2.20	6.57	4.37	1.94	6.39
3.0	4.26	2.10	6.27	4.30	1.89	6.28
6.0	3.92	1.95	6.12	4.16	1.65	5.90
9.0	3.87	1.83	5.79	4.11	1.66	5.97
C. kotchayana						
1.5	2.23	1.50	3.78	2.05	1.69	3.78
3.0	2.17	1.46	3.68	2.07	1.57	3.69
6.0	2.10	1.24	3.39	2.00	1.60	3.64
9.0	2.10	1.20	3.34	1.94	1.44	3.42
C. abyssinica						
1.5	4.06	2.90	7.05	3.87	2.96	6.91
3.0	2.17	1.46	3.68	2.07	1.57	3.69
6.0	2.10	1.24	3.39	2.00	1.60	3.64
9.0	2.10	1.20	3.34	1.94	1.44	3.42

As both, safflower and the Crambe species are oil plants, seed yield and quality is important. Total seed weight in the greenhouse as well as seed number was increased by irrigation with saline water up to 6 dS m^{-1} (Fig. 14, 16). Weight of 100 seeds was even slightly increased at 9 dS m^{-1} (Fig. 15). Contrariwise, total seed weight of *Crambe abyssinica* was remarkably decreased by irrigation with saline water (Fig. 17) emphasizing the difference between a local genotype to another. As weight of 100 seeds was almost not changed (Fig. 18), the reason for the decrease in total weight is a remarkable decrease in seed number, especially in the first year (Fig. 19). Total seed yield was significantly decreased as well as weight of an individual seed but not seed number (at 9 dS m^{-1}). The main reason is that many pods were empty without any seeds.

Table 2: Seed yield in the field on 2007

	Crambe abyssinica			Safflower		
EC_i	Seed weight	Weight of 100 seeds	Seed number	Seed weight	Weight of 100 seeds	Seed number
1.5	4.67	1.12	417	28.69	4.24	677
3.0	3.14	0.97	324	15.30	4.04	379
6.0	5.26	0.8	658	4.12	2.72	151
9.0	2.88	0.72	400	0.47	-	-

Oil and carotenoids content in seeds of *Crambe abyssinica* from both, greenhouse and field, was almost not affected by salinity (Table 3). Oil and carotenoids content in seeds of safflower from the greenhouse experiment was also not significantly changed, while in field plants, their content was reduced by approximately 85% at 9 dS m^{-1} (Table 4).

Table 3: Oil and carotenoids content in *Crambe abyssinica* seeds from year 2007

EC_i (dS m^{-1})	Greenhouse		Field	
	Oil (%)	Carotenoids (%)	Oil (%)	Carotenoids (%)
1.5	21.31	10.44	10.27	20.39
3.0	18.83	10.94	9.53	10.85
6.0	18.60	11.18	8.31	16.48
9.0	18.85	10.03	9.98	21.52

Tables 5 and 6 show the distribution of the fatty acids in safflower seeds grown in the greenhouse and in the field during 2006-2007. Significant changes can be observed between the two contents; at high salinity palmitic acid (16:0) and stearic acid (18:0) were doubled while oleic (18:1) and linoleic acid (18:2) were reduced by 30% when grown in the field. There are also changes in the content of the fatty acids in *Crambe abyssinica* with the most significant one a 20% reduction in erucic acid (22:1) in the seeds from the field plants exposed to very high salinity level (Tables 7 and 8). In both experiments the changes as a result of exposure to different salinity levels are minor.

Table 4: Oil and carotenoids content in safflower seeds from year 2007

EC_i (dS m^{-1})	Greenhouse		Field	
	Oil (%)	Carotenoids (%)	Oil (%)	Carotenoids (%)
1.5	22.52	1.56	26.17	3.90
3.0	22.79	1.37	19.99	4.04
6.0	26.30	1.16	5.32	3.66
9.0	20.75	1.02	3.31	4.18

Table 5: Fatty acid content in safflower seeds from year 2007 in the greenhouse

dS m^{-1}	Fatty acids (%)					
	14:00	16:00	16:01	18:00	18:01	18:02
1.5	0.29	6.6	0.57	2.06	11.67	78.81
3.0	0.29	7.6	0.54	1.92	12.78	76.87
6.0	0.21	7.68	0.48	2.22	12.61	76.8
9.0	0.27	8.15	0.55	3.25	14.01	73.77

Table 6: Fatty acid content in safflower seeds from year 2007 in the field

dS m^{-1}	Fatty acids (%)					
	14:00	16:00	16:01	18:00	18:01	18:02
1.5	0.19	6.78	0.34	1.82	14.74	76.13
3.0	0.22	7.52	0.33	1.9	15.69	74.34
6.0	0.36	13.21	0.59	1.7	31.99	52.15
9.0	0.41	16.22	0.44	1.52	31.23	50.18

Table 7: Fatty acid content in *Crambe abyssinica* seeds from year 2007 in the greenhouse

	Fatty acids (%)									
dS m^{-1}	14:00	16:00	16:01	18:00	18:01	18:02	18:03	20:01	22:00	22:01
1.5	0.44	3.41	0.59	0.53	38.17	14.06	3.93	10.44	0.6	27.83
3.0	0.17	4.55	0.88	1.48	29.95	16.42	6.01	10.8	0.86	28.88
6.0	0.18	4.31	0.81	1.18	33.27	16.49	5.91	10.79	0.77	26.28
9.0	0.21	3.84	0.46	1.15	35.82	14.63	4.17	8.01	1.04	30.66

The results of the second year field experiment are still under evaluation and could not be included in this report. It is important to mention that *Crambe abyssinica* is not the local genotype of Crambe which probably may explain the differences observed in their behavior. *Crambe kotchayana* flowered only on the second year therefore we still don't have the results of its chemical analysis of the seeds.

Table 8: Fatty acid content in *Crambe abyssinica* seeds from year 2007 in the field

	Fatty acids (%)									
dS m^{-1}	14:00	16:00	16:01	18:00	18:01	18:02	18:03	20:01	22:00	22:01
1.5	0.21	4.46	0.67	1.21	31.48	17.49	6.82	8.9	1.62	27.14
3.0	0.31	5.43	0.58	1.76	37.41	15.17	4.87	8.34	1.18	24.95
6.0	0.21	4.6	0.76	1.8	34.96	14.39	5.92	8.87	2.08	26.41
9.0	0.35	5.48	0.78	1.27	33.17	16.75	6.26	9.75	1.95	24.24

In conclusion, both greenhouse experiments show that irrigation of all 3 species with moderate salinities is feasible. As far as the field experiment is concerned, in spite of a serious effect on final yield, irrigation with moderate saline water is also feasible. These results are very encouraging for large area around the Aral Sea where salinity level is moderate transforming our project in a successful one .

Impact, Relevance, and Technology Transfer:

Due to the Aral Sea crisis and other ecological disasters, a significant part of the soils and water in Uzbekistan is salt contaminated, leading to the deterioration of the vegetative resources. Nevertheless, the area has a broad range of salinity levels which permit partial use of the land for agriculture. The same is true for saline water that might be diluted with fresh water according to crop salt tolerance. The results obtained in this study allow the use of these lands for essential crop cultivation for the benefit of local farmers and from similar regions in many other developing countries. The selection of field crops to be irrigated with marginal water is improved and farmers' income might be increased. Although the crops tested showed different sensitivity to salinity and as a result, seed yield was reduced, this might be partially overcome by increasing plant density in the field and improving the irrigation management. Both crops, Crambe and safflower, are of a great economic importance in all the Central Asian region with a population of more than 50 millions which may immediately benefit from the results of this research.

The Uzbek team was acquainted with a new irrigation method which might be implemented to other crops such as cotton which is the main crop in Uzbekistan. A modern HPLC was purchased for the laboratory of Prof. Glushenkova facilitating the separation of methyl ethers of fatty acids, triacylglycerols, fatty and cyclic alcohols and carbohydrates.

The leaders of the project from both countries coherently exchanged information by E-mail or by international calls. Reports, pictures and results are periodically sent from the Uzbek team. New cooperation relations between several institutes of the Academy of Science were established through our intermediary efforts. Thus, teams from 3 different institutes worked and contributed to the success of the field. Two young scientists were in Israel for different periods of time for training. An Uzbek student did her candidate thesis on the theme of this project. No doubt, very good and firm relations between the two teams were created with a reciprocal interest to further cooperation.

Project Activities/Outputs:

Dr. Maksim Ionov was trained in Israel for 12 months, performing the greenhouse experiments, and on his return, he transferred all the information obtained to a local team of soil researchers and plant physiologists. Dr. Zarif Adilov was trained for one month in order to achieve seed germination. Nigora Yuldasheva carried out her candidate thesis while working on the project.

No meetings or publications came out yet as the project was only recently terminated.

Project Productivity:

Results are still not implemented although it is clear that irrigation with saline water is feasible. All the plants may be cultivated at the Aral Sea region. The project ended only last December and the chemical analysis of the second year will be finished by the end of the month. We really hope that the leaders of this country which are the one to decide on the crops to be cultivated and their related policies will choose to make use of our results and implement them in the near future. The project accomplished all of the proposed goals.

Future Work: Unfortunately, the CDR program has stopped its activity. We would very much like to continue this research in order to improve crop tolerance and find out the best irrigation regime for maximal yield. Moreover, we would like to carry out experiments at the Aral Sea region and test there our crops. We will look for any possibility in the future to renew our fruitful collaboration.

Fig. 1: Average temperatures in the greenhouse during 2006-2007

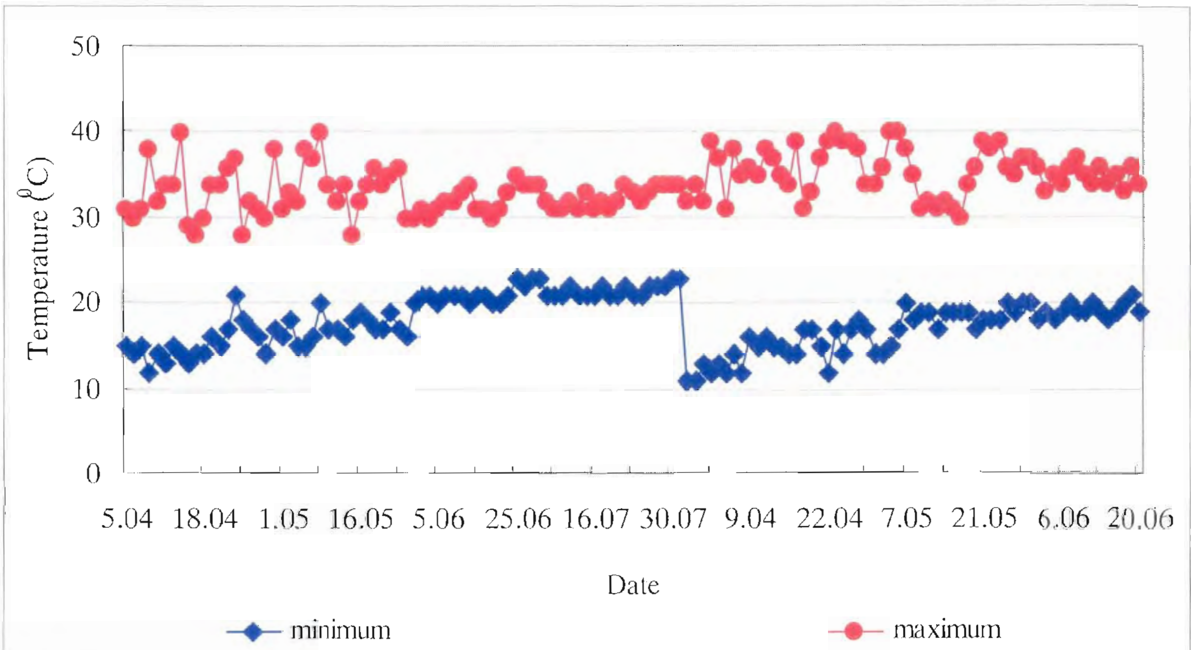

Fig. 2: Average temperatures in the field during 2007-2008

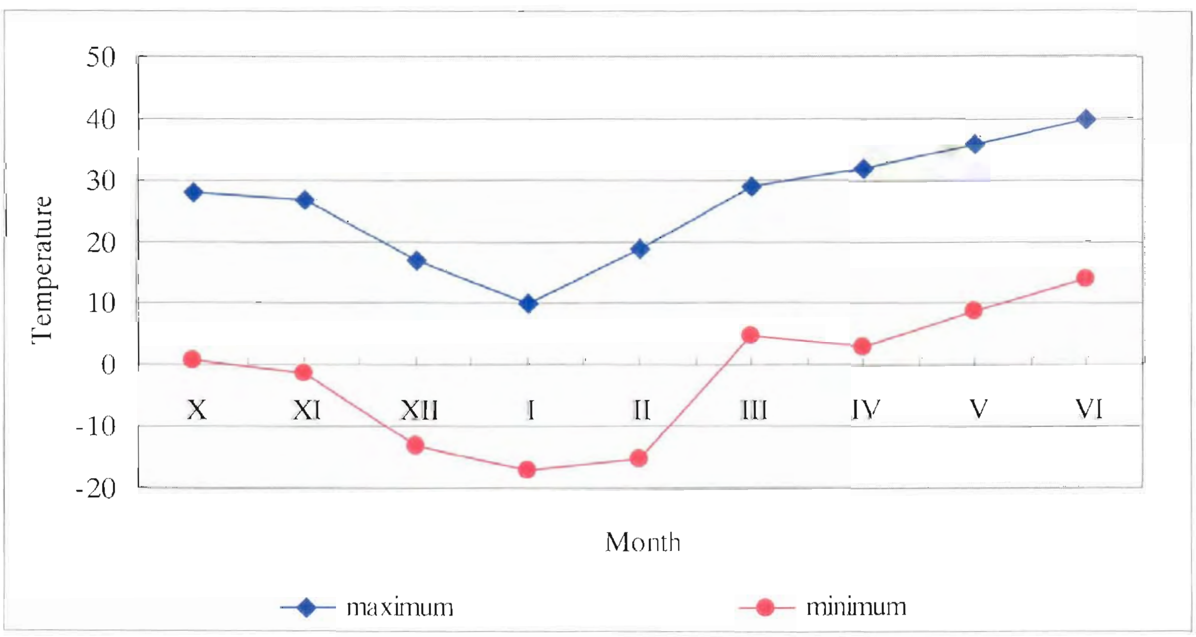

Fig. 3: Average electrical conductivity of all greenhouse plant species

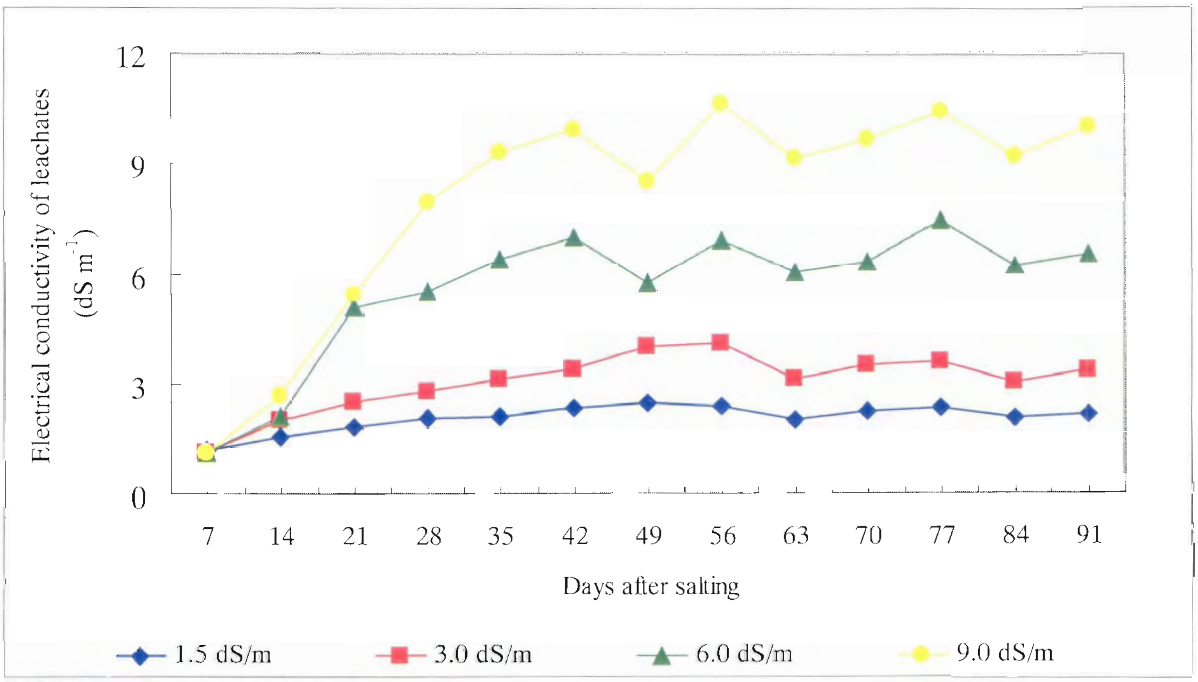

Fig. 4: Average electrical conductivity of all field plant species

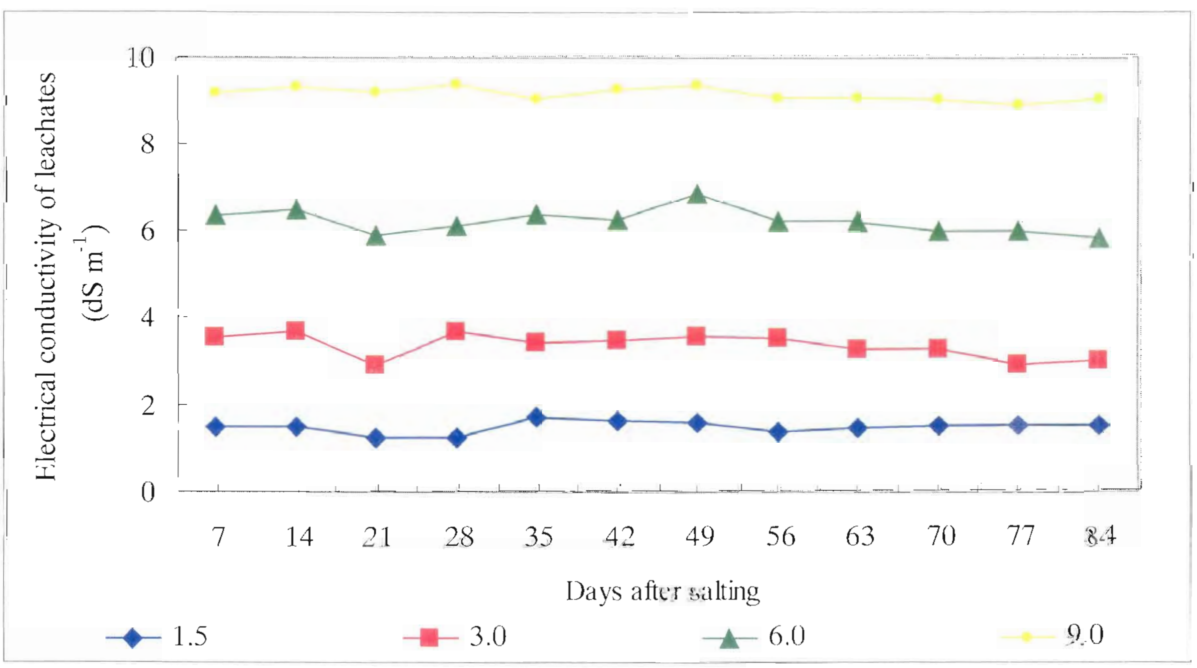

Fig. 5: Plant height: safflower (greenhouse experiment)

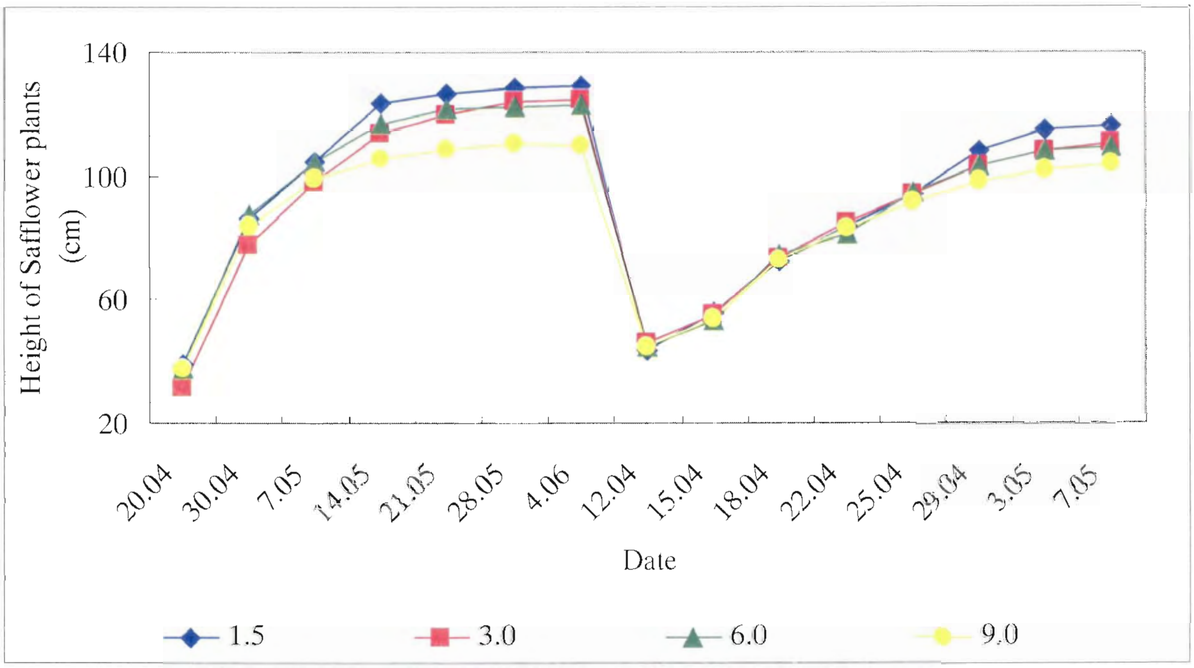

Fig. 6: Plant height: safflower (field experiment)

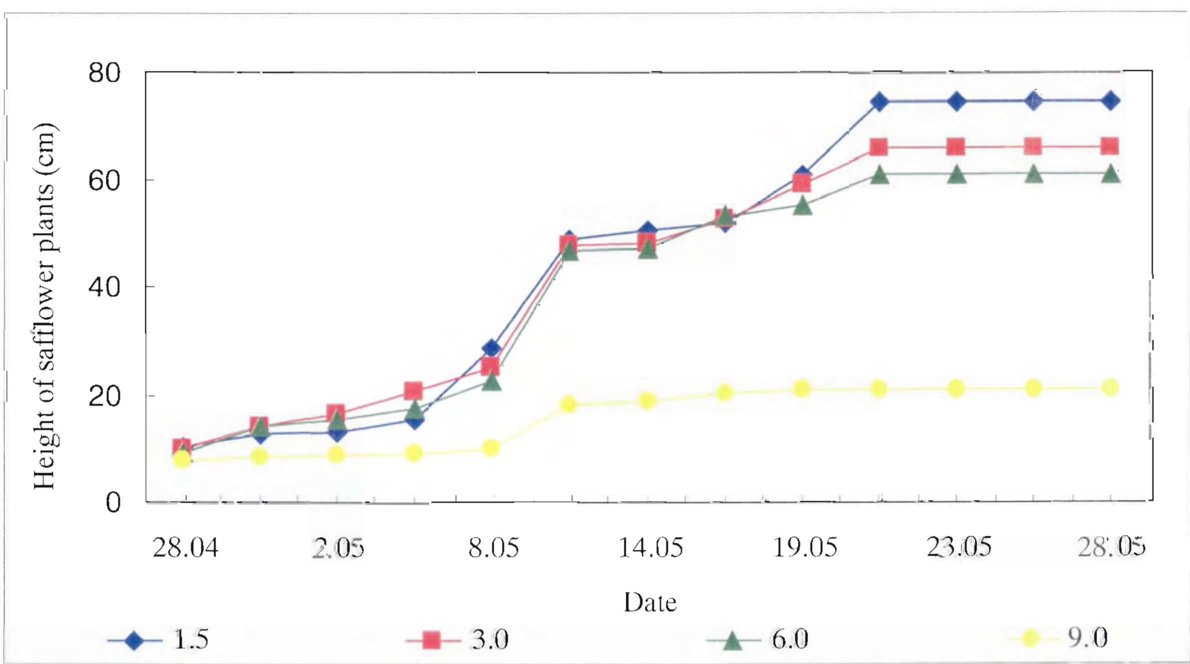

Fig. 7: Plant height: *Crambe abyssinica* (greenhouse experiment)

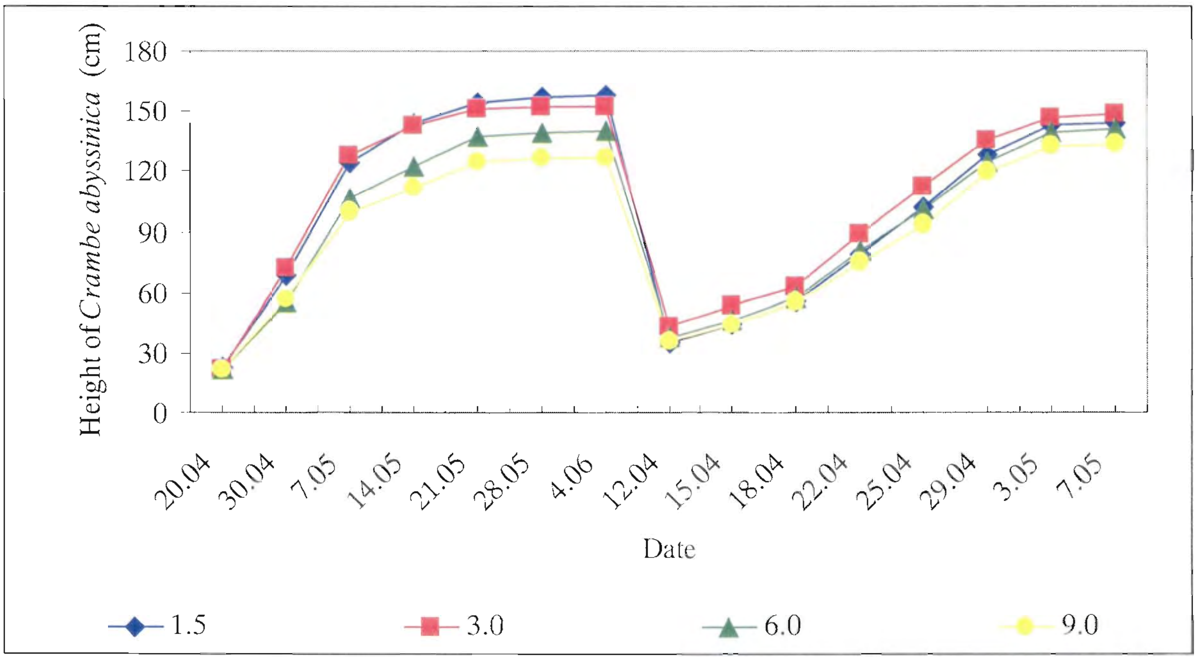

Fig. 8: Plant height: *Crambe abyssinica* (field experiment)

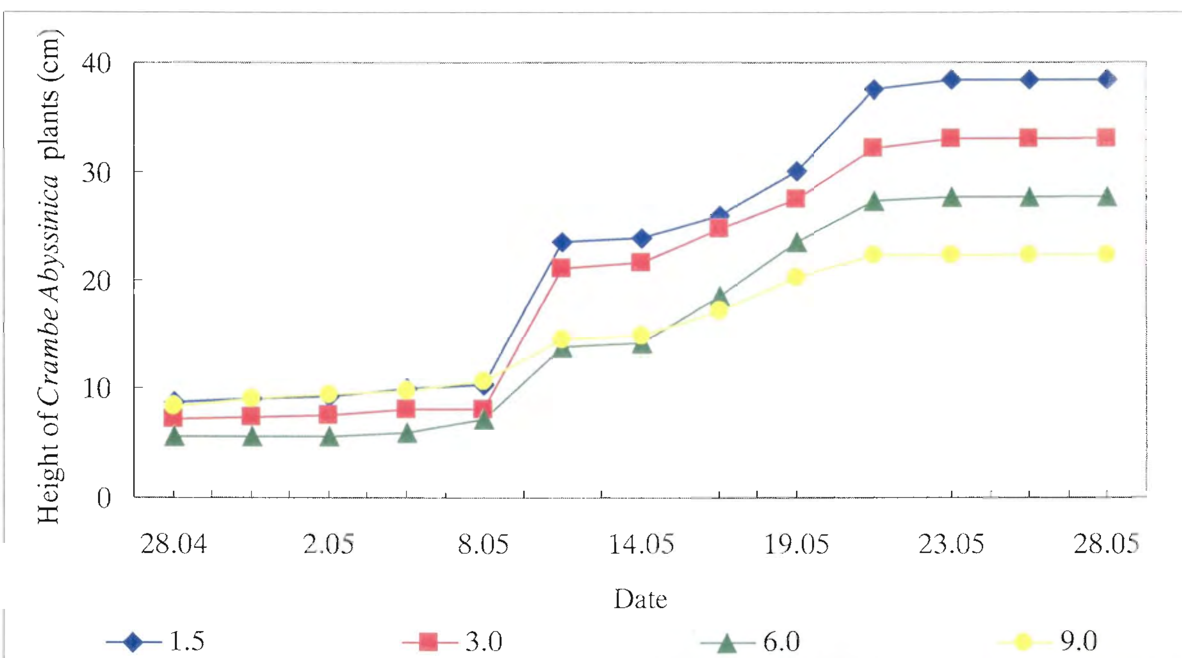

Fig. 9: Plant height: *Crambe kotchayana* (field experiment)

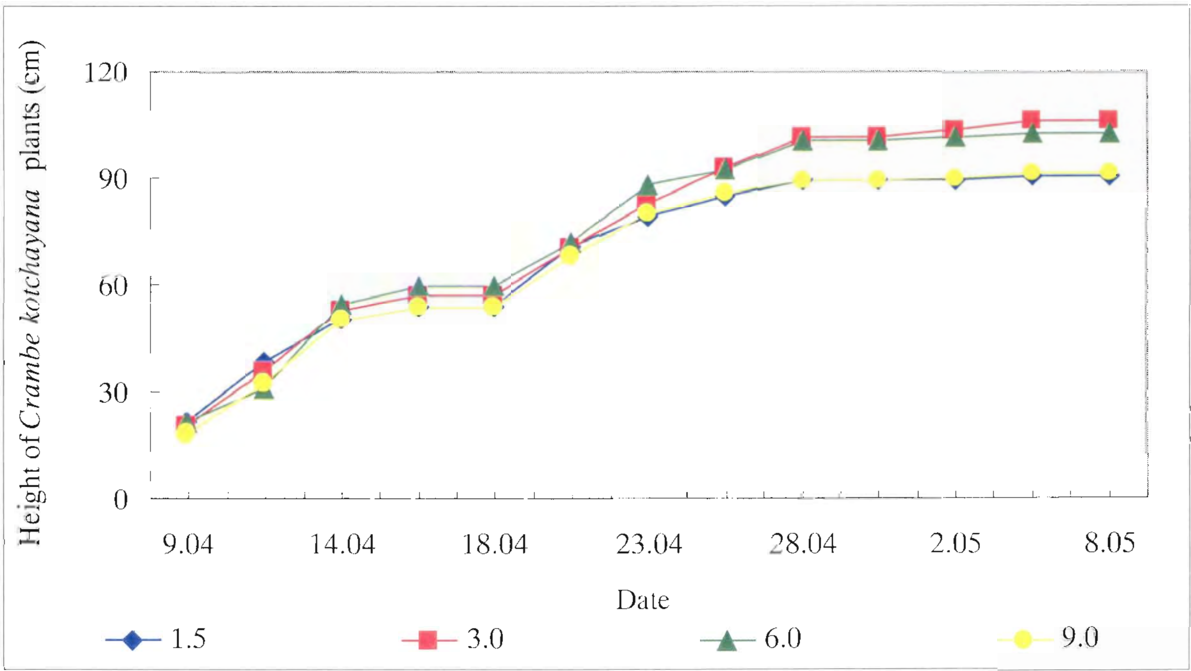

Fig. 10: Chlorophyll content in *Crambe Kotchayana* leaves (greenhouse experiment)

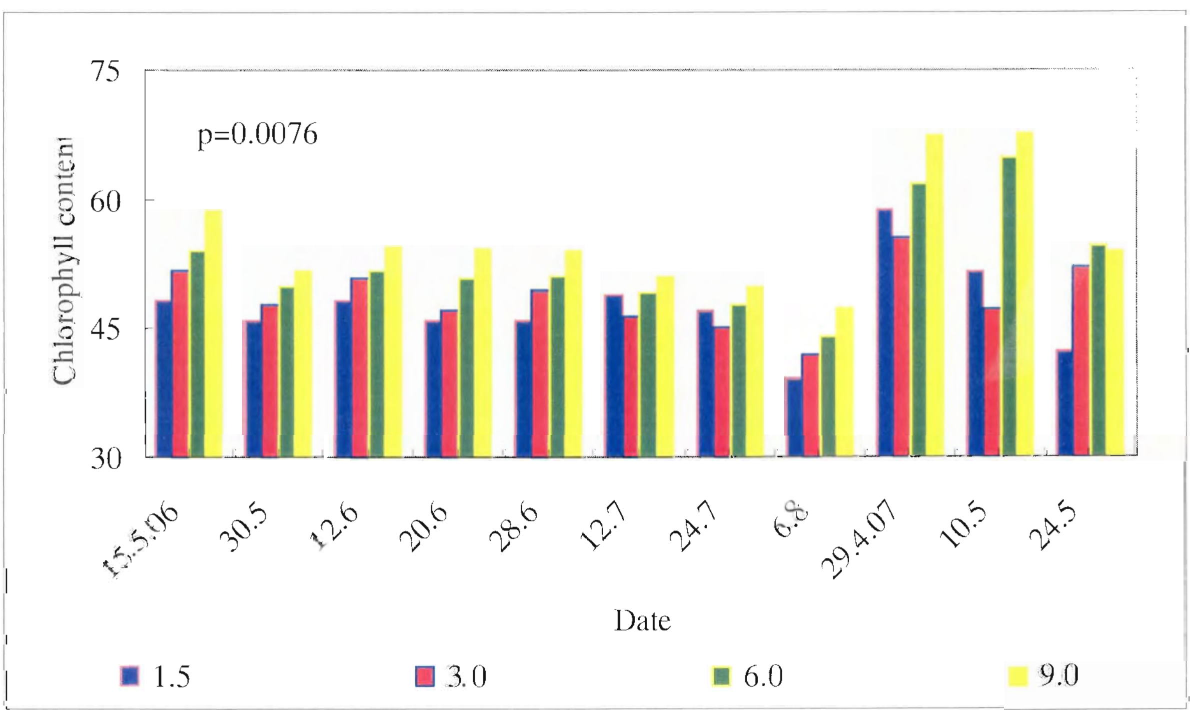

Fig 11: Fruit number of *Crambe abyssinica* in the greenhouse

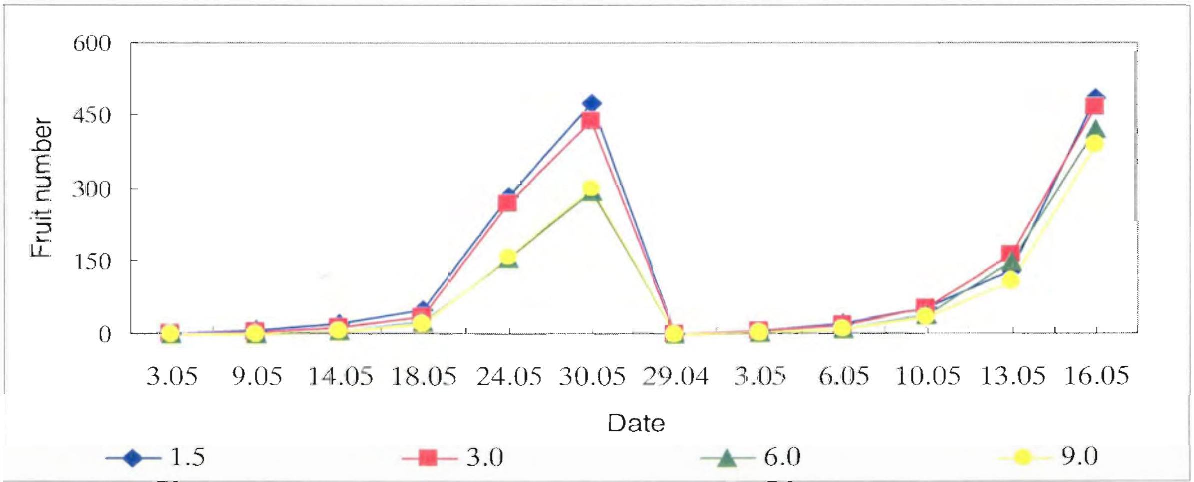

Fig 12: Fruit number of *Crambe abyssinica* in the field

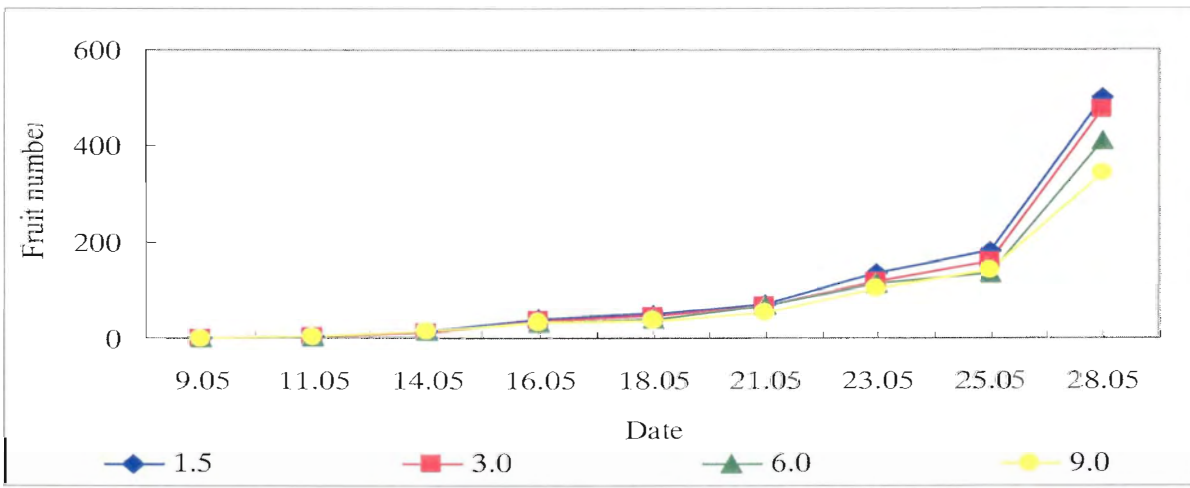

Fig 13: Fruit number of safflower in the greenhouse

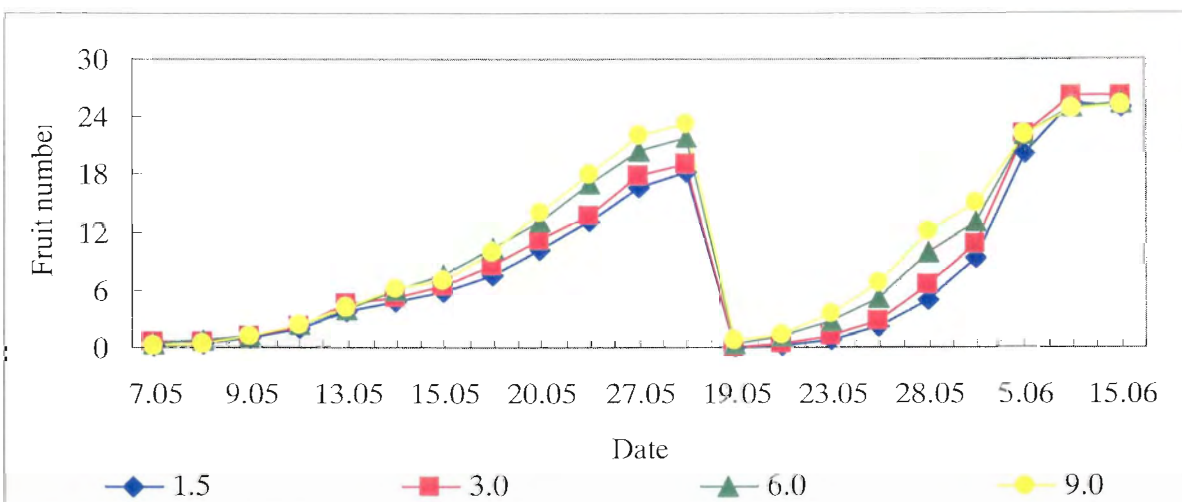

Fig 14: Total seed weight of safflower in the greenhouse

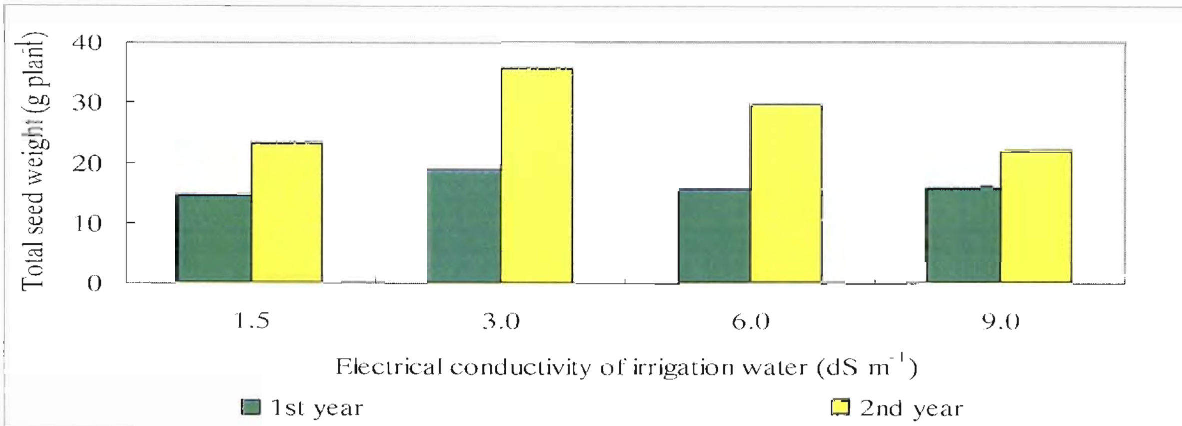

Fig 15: Weight of 100 seeds of safflower in the greenhouse

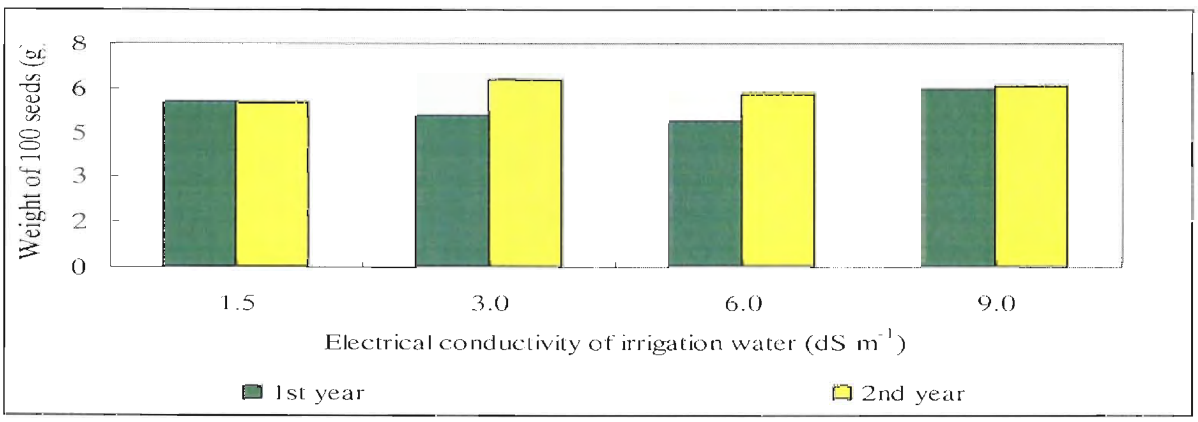

Fig 16: Seed number of safflower in the greenhouse

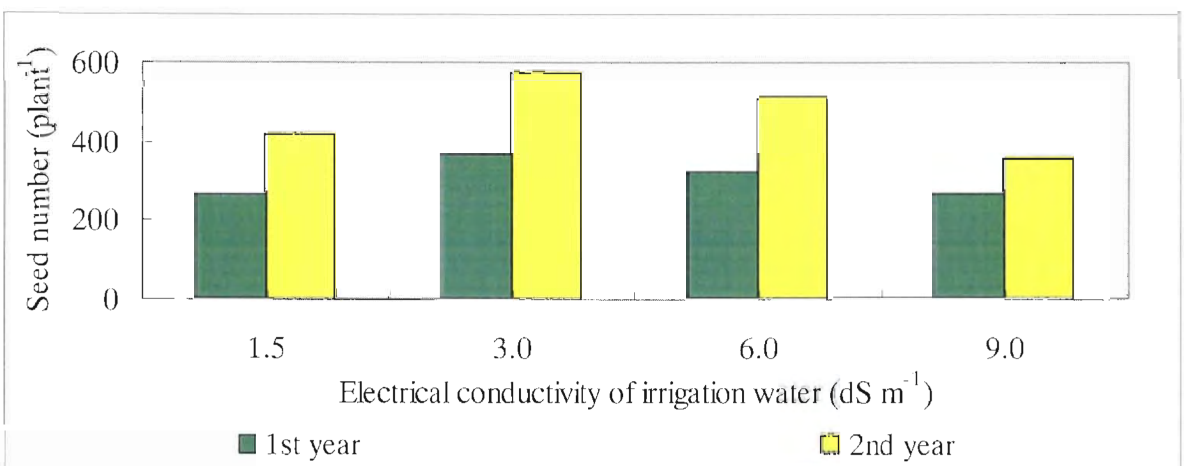

Fig 17: Total seed weight of *Crambe abyssinica* in the greenhouse

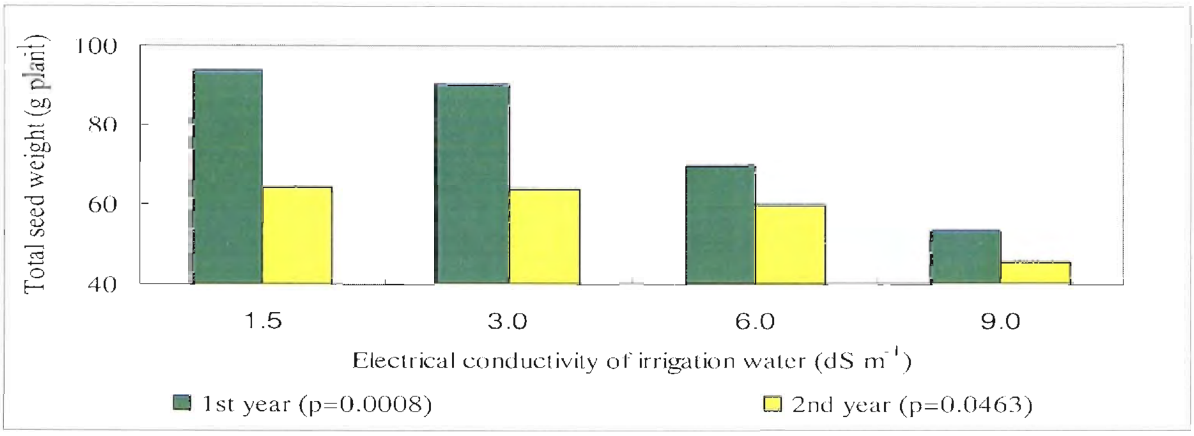

Fig 18: Weight of 100 seeds of *Crambe abyssinica* in the greenhouse

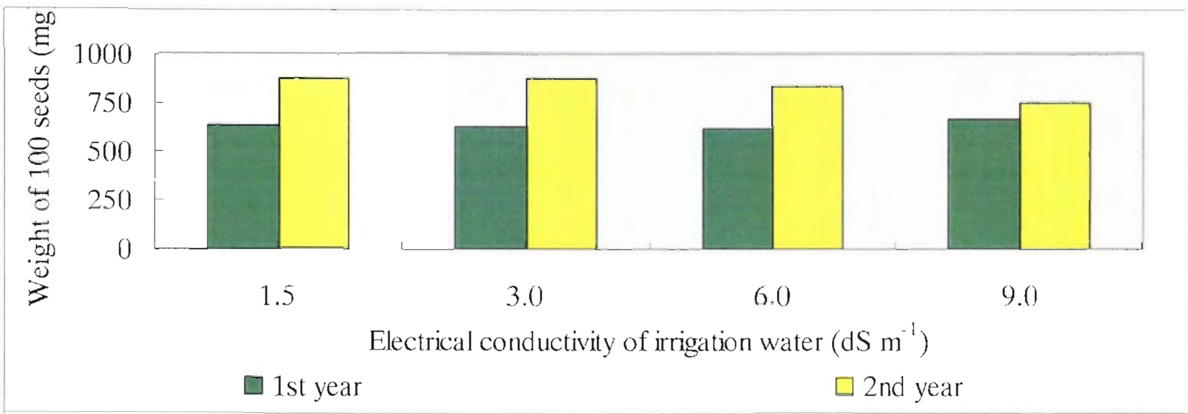

Fig 19: Seed number of *Crambe abyssinica* in the greenhouse