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FEASIBILITY FOR THE DISINFESTATION OF
PULSES AND CEREAL GRAINS BY IRRADIATION

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FEASIBILITY FOR THE DISINFESTATION OF PULSES AND CEREAL GRAINS BY IRRADIATION

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ABSTRACT

The faba bean seed beetle, *Bruchus dentipes* Baudi, the lentil seed beetle, *B. ervi* Froel, and the European lentil seed beetle, *B. lentis* Froel are the most important insect pests of lentil and faba bean in Syria. Adults lay their eggs on the green pods in the field and immature stages develop inside the seeds. Infestation rate differs from year to year and from one location to another. In 1991, it ranged between 9.6 and 13.90 for lentil seeds and 31.00 to 57.39% for faba bean seeds depending on the region. This reduced the economic value by about 50% for faba bean seeds and 20-30% for lentil seeds. Current disinfestation methods are unsafe and not adequately effective. Ionizing radiation could be an alternative approach. An investigation was initiated to determine the possibility of applying the irradiation disinfestation technique against these pests. The dose of gamma radiation needed for disinfesting faba bean seeds infested with *B. dentipes* was found to be 90 Gy for the last two larval instars. Immature stages of *B. ervi* and *B. lentis* develop very rapidly in the field and reach the pupal or adult stage by harvest. This makes the application of this technique for disinfesting lentil seeds of little or no value. However, the results indicate that the irradiation disinfestation method could be an advantageous approach for disinfesting faba bean seeds.

1. INTRODUCTION

Pulses and cereal grains form a large part of the diet of the world population. These products are stored as dry seeds and form an enormous reserve of the human food supply. Unfortunately, all of them are subject to attack during storage by a number of pests that cause an intolerable amount of damage and great economic losses. Subrahmanyam (1) indicated that the world food supply could be increased by 25 - 30 % if post-harvest food losses are avoided. Bakal (2) estimated the annual post-harvest food losses to be 33 million tones which is enough to feed about 250 million people for one year. In general, it is estimated that 5 - 10 % of the annual world production of pulses and cereal grains is lost in storage and that insect pests take the blame for most of the damage. The situation is even more critical in the developing countries where this percentage is most

likely to double or even triple and more food is needed to feed the ever increasing population. Because of that, it is very essential to reduce post-harvest food losses by protecting stored food products from attack by insect pests.

Although excellent control measures are some times available, the search for new, more effective, and economically acceptable ones is necessary. Fumigation, though by far the most effective method for disinfecting stored products, has serious limitations. For instance, it is difficult for fumigants to penetrate some commodities in sufficient concentrations. In addition, it gives rise to concern from the stand point of public health and occupational safety. Chemical treatment may also create problems by leaving undesirable residues or having deleterious effects on grain quality. The development of resistance to these chemicals by insects is another incentive for developing an alternative technique.

The possibility of disinfecting stored products by ionizing radiation has long been attractive to scientists. The practical approach to radiation disinfection began early this century. Murgan and Runner (3) examined the possibility of disinfecting packed cigars infested with the cigarette beetle, *Lasioderma sericorne* , by means of X-radiation. Although, it was later proved to be possible (4), the use of that technique was foreshadowed for some time by their early unsuccessful attempt (3). In addition, their work was of little practical value at that time due to the absence of sufficiently powerful sources of ionizing radiation. However, the conduction of much more powerful ones after the second world war revived the interest in this technique.

Over the last eighty years, a considerable amount of research has been done on the effects of ionizing radiation on stored product insects. The results show that ionizing radiation is as effective as or even more effective than other insect disinfection methods. In fact, it has some advantages over chemicals or fumigants. For instance, it takes less time than fumigation and leaves no undesirable residues in treated commodities. In addition, it does not cause any significant changes in the nutritive value of the treated product and there is no fear of the pest developing resistance to ionizing radiation. The information has been summarized by several authors (5-9). Current research activities are devoted primarily to the practical application of ionizing radiation to reduce or prevent post-harvest food losses. However, gaps still exist in the basic information needed for the application of this technique for some insect species. Three of these species are of a particular economic importance in Syria. These are the faba bean seed beetle, *B. dentipes* Baudi, the lentil seed beetle, *B. ervi* Froel and the European lentil seed beetle, *B. lentis* Froel (10, 11, 12). All of them are univoltine species, attack the crop in the field and lay their eggs on the green pods. The larvae penetrate the seed coat and develop inside the seeds to adults. The developmental process takes about 5 months in the case of *B. dentipes* (11) and 1.5 to 5 months in the other two species (12-13). Adults stay inside the seeds until they are planted when they leave them and

hibernate in the field. Hibernating insects start their activity again next spring and form the basic source of infestation for the new crop.

This report presents data on the economic losses caused by these three species to lentil and faba bean in Syria. In addition, it studies the radiosensitivity of two larval instars of *B. dentipes* Baudi. It also discusses the possibility of radiation disinfestation of faba bean seeds infested with this species.

2. MATERIALS AND METHODS

To estimate the economic losses caused by these pests, samples of faba bean and lentil seeds (about 2 Kg of faba bean and 1 Kg of lentil seeds) were obtained at harvest. The samples were collected from 10 locations in each one of the main faba bean and lentil producing regions in the country. Seeds from each location were mixed well in the lab and smaller samples of 1000 seeds each were obtained. The new samples were placed in paper bags and labeled properly. Faba bean samples were examined for the presence of black dots on the seeds as an indication of *B. dentipes* infestation (14-15) and percentage infestation of each sample was recorded. Because it is difficult to distinguish infested lentil seeds from their appearance, the seeds were soaked in water for 12 hours, dissected and examined for the presence of *B. ervi* and *B. lentis*. This experiment was done immediately after harvest and repeated three months later.

As a rule, disinfestation of legumes infested with univoltine bruchids using ionizing radiation, or any other means, should be done as early as possible after harvest. At that time, insects are most likely to be in their early developmental stages and damage is minimal. However, in some species, insects develop very rapidly and reach the pupal or adult stage by harvest. When this happens, disinfestation will be of little or no practical value. This means that the developmental stage of the insects inside the seeds should be determined and the amount of the damage to the seeds should be assessed before any decision could be made. To do that, one hundred infested faba bean seeds from each sample in the previous experiment were examined. Seeds were soaked in water for 12 hours, insects were dissected out of the seeds, the stage of development and movement of the insects inside the seeds were recorded. The stages of development for lentil seed beetles were also determined from the previous experiment.

To investigate the possibility of using the irradiation disinfestation technique for a particular insect species, its radiosensitivity has to be determined. However, because radiosensitivity changes with age, larval instar, and the developmental stage of the insect, disinfesting doses have to be determined for each one of those or at least for the most resistant one. The stage of development for insects is relatively easy to tell and larval

instars are commonly determined by counting the number of molts the larvae go through. In seed feeders and wood borers, however, molts are difficult to determine, and the frequency distribution of head-capsule width can be used if the distribution shows a clear separation of peaks (16). Reichart (17,18) used three methods to determine the larval instars of *B. pisorum*. He measured the maximum width of the head-capsule, width between eyes, and width between the antennal bases. All three methods showed that the insect had four larval instars. Tahhan and Emden (11) measured the head-capsule width of *B. dentipes* to determine the number of larval instars. They concluded that this species, similar to *B. Pisorum*, has four larval instars. They also found that the first and the second larval instars are relatively short and end by harvest. The third larval instar, however, is the longest one and persists from harvest (late May) to early September. The fourth instar, by contrast, takes about a month and so for the pupal stage.

To determine the radiosensitivity of *B. dentipes*, a series of doses and radiation disinfestation experiments were performed. The first experiment was done in the first week of June immediately after harvest and repeated in the first week of July, the first week of August and the first week of September. In each experiment, faba bean seeds infested with only one larvae/seed of *B. dentipes* were irradiated with seven levels of gamma radiation ranged between 0 and 90 Gy with 15 Gy intervals. One hundred seeds were irradiated for each dose level and each dose was replicated five times. Irradiation was done using a Cs 137 source and the dose rate ranged during the irradiation process from 521 Gy/h to 517 Gy/h at the end of the experiment. All faba bean seeds used in this experiment were from the local cultivar and obtained from the International Center for Agricultural Research in Dry Areas (ICARDA). Irradiated seeds were placed in paper bags, labeled, and left under lab temperature (25±2 C) until early October when they were examined. Before examination, seeds were soaked in water for 12 hours, dissected and the number of living insects and their developmental stages were recorded.

The larval instar of the treated insects at the time of irradiation of each experiment and the amount of damage to the seeds were determined. Fifty larvae were dissected out of the infested seeds, preserved in 70% alcohol and the head-capsule width across the widest point of the head was measured. A stage micrometer and a microscope were calibrated and used for this purpose. The movement of the larvae inside the seeds at the time of irradiation and in October for the seeds treated with 90 Gy was recorded as an indication of seed damage.

3. RESULTS

Table 1 presents the results of examining faba bean and lentil seeds obtained from the main faba bean and lentil producing regions in Syria for

infestation with Bruchids. The data show that percentage lentil infestation during the year 1991 ranged from 9.6 to 13.90%. Although, differences were not very high, percentage infestation among districts was significantly different ($F = 5.43$, 3 and 9 df, $P < .001$). The lowest infestation was recorded in Idleb and the highest in Hama. The results also show that percentage infestation did not change significantly within three months of examination ($P > 0.1$). This indicates that there were no undetected insects at harvest. These results are different from those obtained by Kalkan (19). He found that percentage infestation reaches about 80% in Turkey. Percentage infestation of faba bean seeds in the same year ranged from 31.00 to 57.39%. The statistical analysis showed that infestation was significantly different between districts with the highest infestation rate in Aleppo and the lowest in Homs ($F = 7.58$, 3 and 9 df, $P < 0.001$). These results are similar to those reported by Tahhan and Emden (11). Differences in percentage infestation are probably due to different environmental factors among locations. Planting dates and the availability of a source of infestation may have contributed to the different results.

Heavily infested faba bean seeds are not suitable for human consumption. Instead, they are sold as an animal feed and this reduces their economic value by about 50%. Infested lentil seeds are often rejected by foreign markets and lose about 20-30% of their economic value in the local market.

Table I. Percent infestation of faba bean and lentil seeds with *B. dentipes*, *B. ervi* and *B. lentis* in the main producing regions (1991).

District	Faba bean		Lentil	
	% Infes. Seeds at Harvest \pm SD	% Infes. Seeds at Harvest \pm SD	% Infes. Seeds at Harvest \pm SD	% Infes. Seeds 3 Months Later \pm SD
Aleppo	57.39 \pm 15.89	11.57 \pm 2.01	11.75 \pm 1.99	
Hamma	32.83 \pm 13.17	13.48 \pm 2.73	13.51 \pm 2.52	
Homs	31.02 \pm 10.85	X	X	
Damascus	46.21 \pm 15.36	X	X	
Dar'a	X	13.70 \pm 2.47	13.91 \pm 2.27	
Idleb	X	9.06 \pm 3.43	9.12 \pm 3.17	

X = No samples were taken.

The results of examining the stage of development of faba bean and lentil seed beetles immediately after harvest (early June) are presented in Table 2. The results indicate that irradiation disinfestation of lentil seeds is of little or no practical value. This is because *B. ervi* and *B. lentis*

immature stages develop very quickly in the seeds and reach the pupal or adult stage by harvest. At these stages, most of the damage would be done and since reinfestation with these species does not occur in storage, any control measures may not be necessary. Although both species are believed to exist in Syria (10, 20, 21), their rapid development is similar to that reported by Dortbudak for *B. ervi* in Turkey (12). However, these results are different from those obtained by Pajni and Mittal (13) in India for *B. Lentis*. They found that the development of immature stages of this species takes about 5 months. This might be due to differences between geographical strains and environmental factors may have played an important part. It is also possible that *B. ervi* is the dominant species in Syria. The same table (Table 2) shows that all *B. dentipes* examined specimens were in the larval stage. Damage was also restricted to one cotyledon. This indicates that the irradiation disinfestation technique for *B. dentipes* could be an advantageous approach.

Table II. The stage of development of *B. dentipes*, *B. ervi*, and *B. lentis* in faba bean and lentil seeds in Syria (June, 1991).

District	<i>B. dentipes</i> (Faba bean)			<i>B. lentis</i> & <i>B. Ervi</i> (Lentil)		
	No. Exam. Insects	(%)Stage L. P. A.	No. Exam. Insects	(%)Stage L. P. A.		
Aleppo	396	100	115	46.08 38.92		
Hamma	299	100	135	71.11 28.89		
Homs	331	100	X	X X		
Damascus	370	100	X	X X		
Dar'a	X	X	137	56.93 43.07		
Idleb	X	X	96	60.41 39.59		

L = larvae, P = pupae, & A = adult

X = No samples were taken

In selecting the optimal dose of ionizing radiation for grain disinfestation, it is necessary to analyze the effect of greater and smaller doses. High doses greatly increase labor costs and may also cause unfavorable changes in the quality of the grain. Radiation doses which do not guarantee death or sterility of the treated insects, on the other hand, may create problems of reinfestation. Table 3 presents data on the effects of a series of doses of gamma radiation on different ages and larval instars of *B. dentipes*. It is clear from the table that irradiation reduced significantly the number of larvae that were able to pupate ($P < 0.001$). Percentage of larvae that were able to develop to the pupal stage decreased

with increasing radiation dose from about 80% for the controls to zero percent for the 90 Gy dose. However, the increase in mortality was not proportional to the dose. For instance, in the first experiment, 15 Gy killed about 32% of the larvae (mortality was corrected for the controls) and increasing the dose by one hundred percent (30Gy), increased death by about 30% too. However, when the dose was increased again by 15 Gy (45 Gy), the death rate increased by about 20% only. The increase in mortality was reduced to 15% for the fourth dose (60 Gy) and less than that (about 3%) for the 75 Gy dose. The proportional decrease in mortality with increasing radiation dose can be explained on the bases of "One-hit versus multi-hit" theory for inducing dominant lethal mutations (22, 23).

Ninety Gy is most likely to be the optimal disinfestation dose. This dose killed all irradiated larvae regardless of the age or larval instar irradiated. This may seem a little confusing because the sensitivity of insects to ionizing radiation varies with age, instar and stage of development. To clarify that, it should be mentioned that ionizing radiation kills living organisms mainly by injuring the cell nucleus. Thus radiation is more effective against cells that are dividing. At high doses, however, both dividing and non dividing cells are damaged (24). Once the larva hatches from the egg, most of its growth is accomplished by cell enlargement. At each molt, however, there is a brief period of mitotic activity during which a new and larger cuticle is produced by the epiderm. If this process is disturbed, molting will not occur. Irradiation of the developmental stages of insects may have short and/or long term effects on development. Short term effects cause the failure of the insect to pass properly to the next stage. This may occur as death in the process of molting or complete failure to molt (25). Long term effects, however, appear as lethal effects occurring in a stage later than the irradiated one (26). Apparently, irradiation disturbs the molting process by inhibiting mitosis or causing hormonal disturbances. It is still not clear which one of them causes the death, however, a series of studies by Baldwin and Salthouse (27-29) indicated that interruption of the mitotic process is most likely to be the cause. A study of stage sensitivity with all four larval instars of the grainary weevil also showed that the increase in radioresistance was minimal during the larval period (30).

Comparing these results with similar ones obtained for some other Bruchids shows the high sensitivity of this species to gamma radiation. For instance, 470 Gy was the dose required to kill all immature stages of *Callosobruchus chinensis* (31) and 400 Gy needed for *B. rufimanus* and *B. incranatus* (32).

Table III. Effects of gamma radiation on the larvae of the faba bean seed beetle *B. dentipes* Baudi.

Exp. No.	Dose (Gy)	% Pupation±SD
1	0	82.4±2.30
	15	56.4±12.74
	30	32.2±6.06
	45	15.0±5.52
	60	3.6±1.95
	75	1.2±1.30
	90	0.0
2	0	78.8±3.11
	15	59.4±4.16
	30	31.0±7.97
	45	17.8±4.87
	60	4.0±2.55
	75	1.2±1.30
	90	0.0
3	0	78.8±6.98
	15	55.2±7.36
	30	27.4±2.51
	45	16.0±5.35
	60	3.0±1.22
	75	1.8±0.84
	90	0.0
4	0	83.2±3.35
	15	67.4±3.78
	30	33.0±7.45
	45	18.0±2.35
	60	3.4±1.25
	75	1.8±0.84
	90	0.0

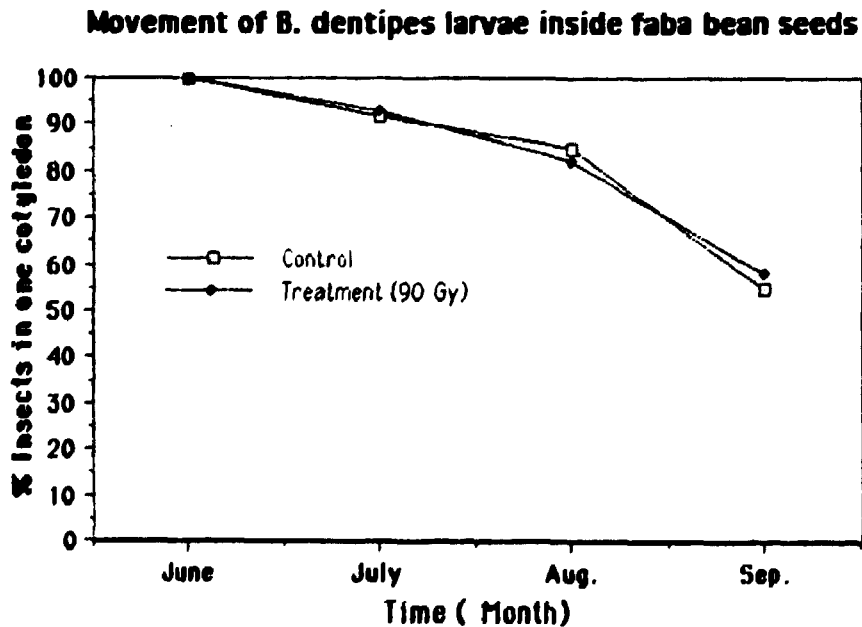
The results of examining the larval head-width at the time of irradiation for each experiment (Table 4) indicates that experiments 1, 2, and 3 were done on the third larval instar. Experiment 4, however, was done on the fourth larval instar. This is indicated by a highly significant difference between the head width measurements for the larvae in the fourth experiment ($F = 311.75, 3$ and 196 df, $P < 0.001$).

Table IV. Head-capsule width (mm) of different larval instars of *B. dentipes* Baudi.

Exp. No.	Mean (mm) Head-Width	F Value	Probability value	Larval Instar
1	0.57±0.05	311.75	>0.001	3
2	0.59±0.06			3
3	0.60±0.06			3
4	0.93±0.09			4

Examining the damage caused by the larvae showed that it was restricted to one cotyledon at the time of the first experiment. In July, however, only 92% of the larvae were still in the first cotyledon and this percentage decreased to 85% in August and only 55% in September. Comparing these results with the amount of damage for the treated seeds with 90 Gy (Fig. 1) showed that percentage damage did not significantly increase after irradiation. This indicates that the larvae died within a short period of time after irradiation. However, it has been reported that irradiated larvae live longer than unirradiated ones (33). This is probably not the case here, and if it is, irradiated larvae should have stopped feeding shortly after irradiation.

Fig. 1



4. DISCUSSION

The purpose of this study was to estimate the economic losses caused by three univoltine Bruchids attacking faba bean and lentil seeds in Syria. It also discussed the feasibility of radiation disinfestation of the seeds of these two crops as an alternative technique to the currently used one. In addition, it studied the radiosensitivity of the third and fourth larval instars of *B. dentipes* Baudi.

The results showed that these pests cause enormous economic losses. They also indicated that disinfesting lentil seeds, by irradiation or any other means, is of little or no practical value. This is because, by the time the seeds are ready for disinfestation, most of the damage would be done. However, examining faba bean seeds after harvest showed that all examined insects were still in the third larval instar and damage was very limited. This indicates that radiation disinfestation of this crop immediately after harvest can be useful.

Studying the relationship between radiation dose and mortality response of *B. dentipes* larvae showed that 90 Gy applied to the third and fourth larval instars caused 100% mortality. The study also showed that the radiosensitivity of the larvae did not change with age or even with larval instars. This means that irradiation can be carried out within at least three months of harvest without the need for increasing the dose. It also indicated that the larvae died soon after irradiation or at least stopped feeding as showed by the size of the chamber the insect left behind.

In general, the results of the study indicate that it is possible to use ionizing radiation to disinfest faba bean seeds infested with *B. dentipes* Baudi. It is also clear that the dose required for that is relatively a very low one. This means that irradiated seeds do not require any analysis to prove that they are suitable for human consumption.

As for the practical application of this technique, it should be pointed out that any new pest control agent has to be assessed in relation to the existing practices. This means that numerous questions have to be answered before any recommendations could be made. Some of the main questions to be asked are whether there are deficiencies in the currently used technique? Is it safer than the presently used one? Is it more reliable? Is it more economical? Can we integrate it in the existing agricultural production system? and does it affect consumer acceptance? Only by providing reliable answers to these questions, can the potential of the new technique be assessed.

The currently used technique for faba bean seed disinfestation depends on fumigation with hydrogen phosphide (PH₃). This method has several problems. The chemical is not adequately effective and it is very dangerous from the point view of occupational safety. Further more,

treatment takes a long time and there is the potential of the pest developing resistance.

The irradiation disinfestation method could be safer than fumigation from the stand point of occupational safety and public health. The required dose is a very low one and by all standards does not cause any possibly undesirable changes in the treated product. Extensive studies on the wholesomeness of irradiated food in the USA (34) and England (35) showed that the nutritional quality was not impaired. The studies also showed that irradiation did not produce any toxic or harmful material in the irradiated food at the recommended dose. Further more, the joint FAO/IAEA/WHO expert committee on the wholesomeness of irradiated food concluded that irradiation of any food commodity to an over all dose of 10 KGy presents no toxicological hazards (36). This conclusion was later (1983) incorporated by the Codex Alimentarius Commission in its Existing Recommended International General Standard for Irradiated Food. It is obvious that this dose is more than one hundred times higher than the dose needed for disinfesting faba beans. From the stand point of occupational safety, the process could be fully automatic, no danger to the workers and not subject to human error.

The technique is very reliable, provides homogeneous and instant treatment, cause a 100% disinfestation and no fear of the pest developing resistance to ionizing radiation. In fact, it preserves its nutritive value, stops degradation caused by insect infestation and leaves no undesirable residues.

The cost of radiation disinfestation depends on the efficiency in operating the facility and the kind of source being used. Cornwell (7) compared the cost of disinfestation of wheat by fumigation with hydrogen phosphide (PH₃) and irradiation using electron accelerators or gamma source. He found that the cost of irradiation using accelerated electrons costs the same as fumigation. However, because of the high costs of shielding, disinfestation using gamma irradiators costs double the amount. In another comparison (37), the cost estimate of irradiation treatment of grain was compared with fumigation. It was found that fumigation is fairly constant. The cost of irradiation, however, decreases with increasing the amount of treated material. Irradiation of faba bean seeds should be done within a short period of time after harvest. This means that operation costs will be very high. However, if such facility was used for some other purposes, the cost can be reduced significantly.

Various systems for irradiating grains have been proposed (38). Some workers advocate the use of electron accelerators with high grain flow rate and low penetrating power. Others, however, favor radioisotopic source such as Co-60 or Cs-137 for their very high penetrating power. Suggestions have been made for mobile irradiation facilities installed on trucks, railroad cars and ships. Irradiators of this type might be suitable for

faba bean seed disinfection. By moving such facilities to locations where they are needed, maximum use can be made of the irradiators.

Consumer acceptance of the irradiated product depends on several factors such as the price, the quality and consumer confidence in the irradiated product. Although this issue is still probably the most important factor in the development and practical application of this technique, public opinion is changing in favor of the new technology

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تقرير نهائي عن بحث علي



امكانية تطهير بذور البقوليات
والمحاصيل الحبية بالتشميع

الدكتور محمد منصور

الدكتور محفوظ البشير

قسم الزراعة الاشماعية

الجزء ١٩٩٣

خط درس - زراعت - ن ١٩

سورية - دمشق - ص ١٩٩١

مكتبة الامم المتحدة

الجمهورية العربية السورية
هيئة الطاقة الذرية

قسم الزراعة الاشعاعية

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أيار ١٩٩٢

هـ ط نس - ز/ت ن بع ٦٩

حقوق النشر

يسمح بالنسخ والنقل عن هذه المادة العلمية للاستخدام الشخصي بشرط الاشارة الى
المرجع ، أما النسخ والنقل لأهداف تجارية فغير مسموح بهما الا بموافقة خطية مسبقة
من ادارة الهيئة .

التقرير النهائي

الاجتماع التنسيقي الثالث

لبرنامج تشجيع الأغذية

لدول أوروبا والشرق الأوسط

فرنسا ، كاداراش ، ٨ - ١٢ آذار ١٩٩٢

عنوان مشروع البحث

إمكانية تطهير بذور البقوليات

والحاصلات الحبية بالتشجيع

قدم التقرير

د. محمد منصور ، الباحث الرئيسي لهذا البحث

المعهد

هيئة الطاقة الذرية السورية

ص.ب ٦٠٩١ ، دمشق ، سورية

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