J. Agric. Sci. Mansoura Univ., 29 (3): 1495 - 1503, 2004

EFFECT OF LASER IRRADIATION ON SEED BORNE FUNGI OF RICE

Moustafa, M. S. H., M. R. Helal and H. A. M. El- Dakar,
Plant Pathology Research Institute, Agricultural Research Center, Giza , Egypt.

ABSTRACT

In this work, an apparatus for irradiation of the rice seeds with LASER beam. was developed .This apparatus design is described.
Seeds of rice (Giza 159 cv.) were irradiated with LASER beam for different periods (0, 15, 30, 45 and 60 s.). Irradiation of rice seeds resulted in increasing of the seed germination percent; however, prolonging the irradiation period to 60 s. had lethal effect on the seeds. Isolation of different seed-borne fungi from irradiated and non-irradiated rice seeds showed obvious differences either in total number or number of each fungus isolated from the different treatments. Increasing the irradiation period was correlated with decreasing of the seed-borne fungi. While 41 isolates were obtained from untreated seeds, only 26 isolates were obtained from seeds treated with LASER beam for 15 s. compared with 3 isolates obtained from seeds treated for 30 s. Irradiation of the seeds for 45 s. resulted in complete disinfections of the seeds.

Trichoderma sp. and *Penicillium* sp. were isolated from the surface of the seeds. *Trichoderma* isolates were more sensitive to the irradiation than the *Penicillium* isolates.

Isolation from seed coat showed that the dominant fungi were *Pyricularia oryzae* and *Helminthosporium* sp. while *Fusarium* sp. was found in the endosperm. Irradiation period of 30 or 45 s. resulted in complete elimination of these fungi.

Under greenhouse conditions the plants, emerged from irradiated and non-irradiated seeds, showed different disease severity, which was correlated negatively with prolonging of the irradiation period; in contrary, the height of plants and fresh weight of the foliage and roots were correlated positively with prolonging of the irradiation period

INTRODUCTION

Environmental pollution is an extremely harassed problem which facing whole the world. Pesticides are considered one of the most important environmental pollutants. Soil and ground water and subsequently lacks and rivers water are subjects to be polluted; either indirectly with the pesticides used to control foliar pests or directly with the pesticides used as seed- or soil treatment.

Rice is a subject for infection with many diseases, which can be transmitted by seeds i.e. *Helminthosporium oryzae, Alternaria tenuis, Curvularia lunata, Nigrospora oryzae, Epicoccum, Fusarium* and *Pyricularia oryzae* (Benoit and Mathur, 1970; Vaidehi and Ramarao, 1972; Majumdar and Chattopadhyay, 1976; Potlaichuk *et al.* 1976 and Chung and Lee 1983). In order to save the environment it is important to reduce the pesticides amount introduced in the environment; therefore other procedures have to be applied to control the pests. LASER beam (light amplification by stimulated emission of radiation) is a promising candidate to be used for such purposes. The effect of LASER radiation on reparative and immune processes to plant diseases on barley seeds, and another number of fruit crops and berries were tested (Bel'-skii and Mazulenko, 1984 and Borodin *et al.* 1996)

This work is aiming to study the effect of LASER irradiation on the rice seed-borne fungi and seed viability.

MATERIALS AND METHODS

Apparatus used for seed irradiation:

Seed irradiation was carried out using a self-made apparatus; the design of this apparatus based on the same design of rotary evaporator with some modifications. These modifications can be summarized as follows: Smaller flask (100 ml) was used. This flask was supplied with a van, which was inclined attached to the internal wall of the flask to perform continues seeds reeling; in addition, the flask spin was raised to reach about 400 rpm. Instead of the water path a low power LASER sours (semiconductor LASER diode, wave length 630-660 nm with max output < 1mW), was mounted 5 cm under the flask bottom. The power supply was controlled using a photographic laboratory electronic timer.

Seed irradiation:

Small amounts of rice seeds (Giza 159 cv.), enough to form only one layer of the seed (25 seeds) on the bottom, were inserted in the flask. The flask was allowed to spin, and then the LASER source was switched on for the desired irradiation period (0.0, 15, 30, 45 or 60 seconds). For each irradiation period 300 seeds were used.

Seed germination and seed-borne fungi isolation:

The irradiated seeds as well as the untreated seeds were subjected to germination test on sterilized wet filter papers in humidity chamber. In addition, superficial, external (in the coat) and internal (in endosperm) seed-borne fungi were isolated. The seed wash water technique described by Neergaard (1983) was modified; and performed as follows: Ten seeds of each treatment were immersed in 10 ml sterilized distilled water in test tube; shacked thoroughly for two minutes. With sterilized micropipette 500 µl of the wash water were transferred to the surface of PDA medium in 9 cm. Petri-dish and spread evenly on the surface of the medium then incubated for one week at 25^{o}C. Each treatment was replicated five times. The immerged colonies were counted periodically and identified. Mean number of each immerged fungus was calculated. In another step the seed coat was separated from the endosperm. The separated seed coat and endosperm were surface sterilized; the seed-borne fungi in both parts were isolated using the Agar Plate method (Neergaard, 1983) on banana slant agar and PDA media.

Another part of the, irradiated and non- irradiated seeds were sown in sterilized soil in pots under green house conditions. The pots were placed in trays full of water for about 2 cm height and each pot was covered tightly with fine pored plastic page. Number of diseased plants, disease severity on the leaves and panicles, plant height and fresh weight of foliage and roots of the plants were estimated. Any abnormal symptoms on the treated plates were recorded.

The leave infection grades were estimated according to the following numerical scale.

Numerical value	leaf infection grad
0	No lesion
1	1-2 lesions
2	3-5 lesions
3	More than 5 lesions but less than ¼ of the leaf is destructed.
4	Forth but less than half of the leaf is destructed.
5	Half but less than 3/4 of the leaf is destructed.
6	¾ or more of the leaf is destructed.

Infection grades on the panicles were determined using the following numerical scale used by Sayed (1986)

Numerical value	Panicles infection grad
0	No infection
1	One infected spikelet / panicle
2	Two infected spikelets / panicle
3	Three infected spikelets / panicle
4	Four infected spikelets / panicle
5	Five infected spikelets / panicle
6	Six infected spikelets / panicle
7	Seven infected spikelets / panicle
8	Eight infected spikelets / panicle
9	Nine infected spikelets / panicle
10	More than nine infected spikelets / panicle

The severity of leaf and panicle infection was calculated using the following equation developed by Townsend and Heuberger (1943).

$$P = \frac{\Sigma(n \times v)}{k N} \times 100$$

where :

P = infection severity)
n = number of leaves or panicles within infection grade.
v = numerical value of each grade.
k = highest numerical value
N = total number of samples

RESULTS AND DISCUSSION

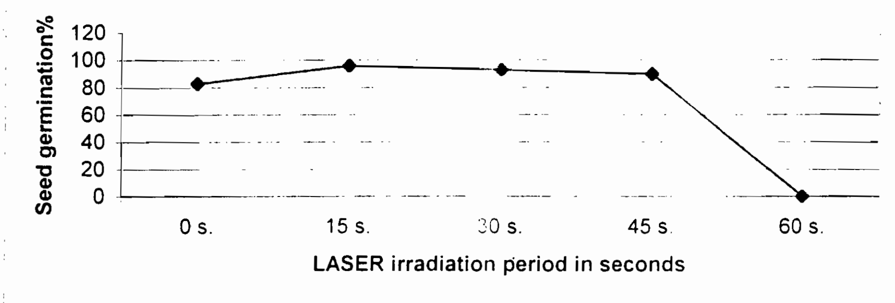
Effect of LASER irradiation on the viability of irradiated seeds:

Irradiation of rice seeds with LASER for different periods resulted in different effect on the viability of the seeds (table 1 and figure 1). Irradiation of rice seeds resulted in obvious increasing of seeds germination. These increase differed as the irradiation period differed. The shortest irradiation period (15 s.) resulted in the highest germination percentage and increased the germination from 83% to 96%. Prolonging the irradiation period to 30 s. resulted in less increase of the seed germination, since the germination rose from 83 to 93%. Compared with only 90% when the irradiation period prolonged to 45 s. More prolong in the irradiation period resulted in drastically decrease in the seed germination. Sixty-second irradiation period inhibited the seed germination completely.

Table 1: Effect of LASER irradiation on the emergence of rice seeds.

Irradiation effect	Irradiation periods in s.				
	0.0	15	30	45	60
Percentage of emerged seeds	83	96	93	90	0
Percentage of affected seeds	0.0	+ 15.6	+12.0	+8.4	-100

Figure 1: Effect of LASER irradiation on the emergence of rice seeds.



Effect of LASER seed irradiation on the number of seed-borne fungi:

Isolation of different seed-borne fungi from treated and untreated rice seed showed obvious differences either in total number or number of each isolated fungus from the different treatments (Table 2). While 41 isolates were obtained from untreated seeds, only 26 isolates were obtained from seeds treated with LASER beam for 15 s. compared with 3 isolates obtained from seeds treated for 30 s. Irradiation of the seeds for 45 s. resulted in complete disinfections of the seeds. From the same table it is clear too that the dominant fungi, which were isolated by seed wash water technique (superficial seed-borne fungi), were *Penicillium* sp. and *Trichoderma* sp. While the number of *Trichoderma* isolates drastically decreased from 10 isolate to only one isolate on seeds treaded for 15 s. and 0.0 isolates on seeds treaded for 30 and 45 s., the number of *Penicillium* isolates increased from 6 isolates on untreated seeds to 16 isolates in seeds irradiated for 15 s. Prolonging the irradiation period resulted in drastically decrease of the number of *Penicillium* isolates; it reached only 3 isolates on seeds treated for 30 s. and null isolate on seeds treated for 45 s.

Isolation from seed coat showed that the dominant fungi were *Pyricularia oryzae* and *Helminthosporium* sp. Sixteen isolates of *Pyricularia oryzae* were isolated from untreated seeds; this fungus was eliminated in all the irradiated seeds. Six isolates of *Helminthosporium* were isolated from the coat of untreated seeds. Irradiation of the seeds for 15 s. did not show any effect on this fungus. Prolonging the irradiation period to 30 or 45 s. resulted in complete elimination of this fungus.

Three isolates of *Fusarium* sp. were obtained from endosperm of the untreated seeds and seeds treated for 15 s. Longer irradiation periods (30 and 45s.) resulted in complete elimination of this fungus.

Table 2: Effect of rice seed irradiation with LASER on the seed-borne fungi

Period in second	Number of seeds carried the isolated fungus															
	Isolation from seed wash water					Isolation from seed coat					Isolation from endosperm					Total
	P.	*T.*	*H.*	*Py.*	*F.*	*P.*	*T.*	*H.*	*Py.*	*F.*	*P.*	*T.*	*H.*	*Py.*	*F.*	
0.0	6	10	0	0	0	0	0	6	16	0	0	0	0	0	3	41
15	16	1	0	0	0	0	0	6	0	0	0	0	0	0	3	26
30	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

***P* = *Penicillium* sp**
***T* = *Trichoderma* sp.**
Py* = *Pyricularia oryzae
***H* = *Helminthosporium* sp. .**
***F* = *Fusarium* sp.**

Under greenhouse conditions the plants. emerged from irradiated and non-irradiated seeds, showed different infection patterns. The diseased plants showed typical symptoms of rice blast and *Pyricularia oryzae* was isolated from all the diseased plants.

The different seeds showed different percentage of diseased plants (Table 3). Highest percentage of diseased plants was found on Plants emerged from untreated seeds (86.6; It was clear that prolonging the irradiation period was correlated negatively with the percentage of the infected plants. Treating the seeds for 15 s. resulted in 52.8% diseased plants. Prolonging irradiation period to 30 s. resulted in reduction of the percentage of diseased plants to 37.2 % ; while prolonging irradiation period to 45s. resulted in further reduction of the percentage of diseased plants to reach only 19.9 %. Exposure the seeds to LASER beam resulted in significant reduction of the severity of infection on the leaves; this reduction was correlated negatively with prolonging irradiation period. While the plants emerged from untreated seeds showed 44.7 infection severity; while only 33.6, 11.7 and 4.8% infection severity were found on leaves of plants emerged from seeds treated for 15, 30 and 45 s. On panicles no symptoms were found on the plants resulted from seeds treated for 30 or 45 s. compared with 40% on plants emerged from untreated seeds and 20.3% on plants emerged from treated seeds for 15 s.

Contrary to the last data, height of plants increased gradually as the irradiation period prolonged. While the mean height of the plants emerged from untreated seeds was 17.5 cm, mean height of the plants emerged from seeds treated for 15, 30 and 45 s. were 25.9, 28.0 and 44.7 respectively. This increasing in the plant height was correlated with increasing in the fresh weight of the foliage and roots. Irradiation of the seeds for 15, 30 and 45 s. resulted in plants foliage weight of 19.7, 24.9 and 29.2 g. respectively compared with 13.7 for plants immerged from untreated seeds. On the other hand the mean fresh weight of the roots were 8.6,10.3 and 14.3 respectively compared with 7.4 for plants from untreated seeds. No abnormal symptoms were noticed on the plants immerged from treated seeds.

Table 3: Effect of rice seeds irradiation with LASER beam on seed-borne diseases incidence, fresh weight and plant height under greenhouse condition.

Irradiation period in second	Disease incidence			Height of plant In cm.	Fresh weight in g / plant	
	Percentage of diseased plants	Severity of infection on leaves	Severity of infection on panicles		Foliage	Roots
0.0	86.6	44.7	40.0	17.5	13.7	7.4
15	52.8	33.6	20.3	25.9	19.7	8.6
30	37.2	11.7	0.0	28.0	24.9	10.3
45	19.9	4.8	0.0	44.7	29.2	14.3
LSD at 5%	9.76	5.15	5.83	7.58	6.36	2.59

LASERS have become valuable tools in industry, scientific research, communication, medicine, the military, and the arts. A number of studies were carried out in Russia to use the LASER beam in controlling seed-borne fungi, but very little information could be obtained since all these works were published in Russian language and Russian literatures. It was important to develop our apparatus by our self. It is worthy to notice that, however, we designed our apparatus to perform different irradiation periods, the periods applied (15, 30, 45 and 60 s.) can not be considered the real irradiation periods, since, the LASER beam is a very concentrated sharp beam, it can not irradiate all the seeds at the same time, in addition it can strike only certain number of the seed sites at a certain time, depending upon the probable exposure times of the seed to the beam; which in turn depends upon the reeling number of the seeds. which nearly equal to flask spin number per min. (because our apparatus flask has only one van) multiplied by applied irradiation time in min. and divided by the number of the seeds irradiated each time. Considering the last factors, the real mean seed irradiation times become 0.6 s.,1.2 s., 1.8 s. and 2.4 s. in which probably each seed will be irradiated 4, 8, 16 and 32 times respectively, in 4, 8, 16 and 32 irradiated site. for 0.15s. for each.

Irradiation of the rice seeds resulted in increasing of the seeds germination, however prolonging the irradiation period to 60s. (applied irradiation time) had lethal effect and resulted in completely inhibition of seed germination. This increasing in emergence using low doses of irradiation may due to the effect on the seed dormancy and accordingly stimulation of seed germination. Fragara (1985) pointed out that dormancy period depend upon the temperature and the humidity, which may be affected in our work by LASER irradiation.

Isolation of different seed-borne fungi from treated and untreated rice seed showed obvious differences either in total number or number of each fungus isolated from the different treatments. Increasing the irradiation period was correlated with decreasing of the seed-borne fungi; however prolonging the irradiation period to 60 s. (practical irradiation time) was lethal. In medicine, LASER beam has been used successfully to cut and cauterize certain tissues without damaging the surrounding healthy tissues (Carstanjen *et al*; 1997). That due to that the medicine instruments are supplied with high technology and the LASER beam can be directed with very high accuracy to the diseased tissues without harming the surrounding healthy tissues, contrary to our very simple apparatus which depend upon the random effect; However our data indicating that the fungi cells are more sensitive to the irradiation than the seed tissues. It indicated, too, that the *Trichoderma* isolates were more sensitive to the irradiation than the *Penicillium* isolates. It is worthy to be notice that the number of *Penicillium* isolates increased from 6 isolates on untreated seeds to 16 isolates on seeds irradiated for 15 s. (applied irradiation time) this increasing may due to the decreasing of *Trichoderma* isolates as a result of irradiation, which may antagonized *Penicillium* on untreated seeds; However prolonging the irradiation period resulted in drastically decrease of the number of *Penicillium* isolates.

Isolation from seed coat showed that the dominant fungi are *Pyricularia oryzae* and *Helminthosporium* sp. while *Fusarium* sp was found in the endosperm. These data are in accordance with that was mentioned by Agarwal *et al.* (1989), Potlaichuk *et al.* (1976). *Helminthosporium* seems to be more tolerant to the LASER irradiation than each of *Pyricularia oryzae* and *Fusarium* sp.

Under greenhouse conditions the plants, germinated from treated and untreated seeds, showed different infection patterns with *Pyricularia oryzae;* while no infection with *Helminthosporium* was noticed. That may due to that the environmental factors in greenhouse were more favorable for *Pyricularia oryzae* but not for *Helminthosporium* (Feakin, 1974). Highest percentage of diseased plants was found on plants emerged from untreated seeds. It was clear that prolonging the irradiation period was correlated negatively with the percentage of the infected plants; these data are in harmony with the data of labor tests which showed decreased seed-borne fungi; however the number of the diseased plants was higher than the number of diseased seeds in each treatment, this increasing in the number of diseased plant may due the dispersing of the fungus spores from the diseased plants to the healthy plants. Since the environmental conditions are favorable for the spread of the disease, and since all the treatments were under the same environmental conditions, the low disease severity on the plants emerged from irradiated seeds inspired to believe that LASER irradiation resulting in induced resistance in the rice plants against the disease; similar data were found by Bel'-skii and Mazulenko (1984) and Borodin *et al.* (1996). In addition LASER irradiation had positive effect on the vegetative growth; because of the rare informations available about the effect of LASER on the seed and plant physiology, more intensive studies have to be done.

It is to be concluded that, LASER irradiation is a promising tool for seed treatment, however more studies have to be done specially under field conditions; therefore cooperation between phytopathologists and electronic specialists will be very important to develop high capacity LASER seed treatment instruments, which have to be able to supply enough amounts of treated seeds for the field requirements.

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تأثير تعريض بذور الأرز لأشعة الليزر على الفطريات المحمولة عن طريق البذرة وحيوية البذور

محمد سيد حسين مصطفى - مرفت رفعت هلال و هالة على الدكر

معهد بحوث أمراض النباتات – مركز البحوث الزراعية

تم تصميم وتنفيذ جهاز لمعاملة البذور بأشعة الليزر وأستخدم فى تعريض بـــذور الأرز (صنف جيزة ١٥٩) لفترات مختلفة (١٥و ٣٠و ٤٥و ٦٠ ثانية).

أدى تعريض البذور لأشعة الليزر لفترات مختلفة (١٥ و ٣٠ و ٤٥ ثانية) إلى زيادة ة فـــي نســـبة إنبات البذور بلغت ٩٦و ٩٣ و ٩٠% على الترتيب مقارنة ب ٨٣% للبذور الغير معرضة للأشعة ، إلا أن زيادة مدة التعريض إلى ٦٠ ثانية كان له تأثير مميت على البذرة.

أدى تعريض البذور إلى خفض واضح سواء في التعداد الكلى للفطريـــات المعزولـــة أو لتعداد كل فطر على حدة. بأجراء العزل من البذور المعاملة والغير معاملـــة بالأشـــعة وجـــد أن الفطريات السائدة المحمولة خارجيا على البذور هى فطرى الترايكودرما وفطر البنيســـيليوم ولقـــد لوحظ أن فطر البنيسيليوم كان أقل تأثرا بالأشعة من فطر الترايكودرما. تم عزل كلا مـــن فطـــر البيريوكيولاريا أورايزى وفطر الهلمنثوسبوريوم من قصرة البـــذور بينمـــا تـــم عـــزل فطـــر الفيوزاريوم من الاندوسبرم. أنخفض تعداد هذه الفطريات بزيادة فترة تعريض البـــذور للأشـــعة . وقد أدت زيادة مدة التعريض ال ٣٠ أو ٤٥ ثانية الى خلو البذور من أي من هذه الفطريات.

بزراعة البذور المعاملة والغير معاملة بالأشعة فى الصوبة وتقديـــر نســـب الإصابـــة بمرض اللفحة وجد أن شدة الإصابة بالمرض تتناسب عكسيا مع مدة تعريض البذور للأشعة وفـــى نفس الوقت كان لتعريض البذور للأشعة تأثير واضح على أطوال النباتات الناتجة والوزن الرطـــب للمجموع الخضري والجذري حيث وجد تناسب طردي بين هذه الصفات ومدة التعريض للأشعة.