

Low Energy Electron Treatment - An Eco-Friendly Tool against Seed Borne Diseases

K VISHWANATH^{1*}, ANDRÉ WEIDAUER^{2*}, HM PALLAVI, FRANK-HOLM ROEGNER² AND RAMEGOWDA¹

¹University of Agricultural Sciences, GKVK, Bangalore 560 065

²Fraunhofer Institute for Electron Beam and Plasma Technology FEP,
Winterbergstrae 28, 01277 Dresden, Germany

³University of Horticultural Sciences, Bagalkot, India

*vishwakoti@gmail.com and *andre.weidauer@fep.fraunhofer.de

ABSTRACT: Healthy seedlings are of prime importance for healthy crop growth. Numerous techniques are applied to obtain disease free seedlings. This paper concentrates on chemical-free methods as electron beam radiations to eradicate seed borne pathogens. The effect of accelerated electrons on pathogens and seed quality is reviewed. Low dose of electron beam and an exactly adjusted penetration depth, that don't reach the seed embryo, is found to be effective in controlling pathogen. Such new chemical free approaches for managing seed borne diseases are promising for organic farming. However, effect of electron beam varies with infection level and status of moisture level in seeds. This needs to be addressed to increase the potentiality of the electron treatment in ecological farming.

Key Words: Electron Beam, Seed, Treatment, Pathogens

Disease-free seed is the primary means of limiting new pathogens from being introduced into new provenance or field. Quality seed is also determined by the level of seed infection with pathogens. Seed borne diseases cause not only yield reduction, but also deteriorate marketable quality of grains. Therefore, practice of treating seeds to eradicate pathogens came into existence long before chemical seed treatment. However, care is taken to maintain the germinability of seeds.

The concept of seed treatment is the application of biological, chemical, physiological and physical agents that control primary soil and seed borne infestation of diseases and insects and improve crop safety, what leads to good establishment of healthy and vigorous plants resulting in better yields.

The best recognized and the most often used methods in actual practice are chemical methods

for seed dressing, with various substances. The seeds are mainly treated with chemicals to avoid the seed borne and soil borne diseases and reduce the chemical load in the fields. The chemical seed treatment is a long lasting seed protection against seed and soil borne diseases as well as insects. There are numerous seed dressings for different kinds of seed and against different pathogens. In practice, chemicals like sodium hypochlorite and gaseous hydrogen peroxide are used for disinfecting seeds, but these are hazardous to workers' health and to environment due to non-target-effects. Besides, those chemically treated seeds that are not sold in the market due to low quality, can be processed further either as seed, food or feed, due to the masking effect of the chemicals, without being decontaminated properly. Further, depending on method of seed dressing, chemically treated seeds need to be dried to safe moisture levels, to avoid physiological deterioration. This adds an extra

cost to seed price. Thus, there were several compelling reasons to find an alternative technology without use of chemicals.

Approved seed treatment for organic agriculture

Due to the growth in organic agriculture [1] an increase in seed-borne diseases is expected in this area for which we must use organic means of seed treatment [2]. Use of electron beam technology for organic farming sector is an effective and sustainable method which guarantees to preserve the quality of the seed. Research on the use of different forms of energy for treating seeds against pathogens has been going on for quite a few years. [3, 4, 5, 6, 7, 8 & 9].

Non-chemical seed treatment

The non-chemical methods often have many drawbacks like handling and inefficient eradication of the targeted pathogen in the seeds. In this situation, the technique which is non-chemical, easy to conduct and with minor effect on the seed viability is in demand by the farmers, producers and environment. Hence, in recent years, more attention has been paid to those physical methods which favorably influence the sowing material of cultivated plants [10, 11, 12 & 13]. The prevailing opinion is that physical methods for the processing of pre-sowing seed stimulate the physiological and biochemical changes in the seeds [14, 15, 16, 17 & 18] and are also safe for the environment. There was an opinion that, no physical method could be a substitute for highly efficient chemical methods, but they could adequately supplement them. Physical treatments, such as heat (e.g. hot water treatments of 118 to 122 F for 20 to 30 min), ultraviolet light, or microwave radiation procedures can be useful for certain crops with some specific pathogens [19&20]. Treatment methods like hot water or steam treatment allow highly effective elimination of all kinds of pathogens. A disadvantage of these technologies is the necessary energy-consuming step of seed drying after disinfection. There are reports on physical methods which can be applied for seed treatment, like laser light [21, 22, 23&24] or ionizing radiation (e.g. gamma radiation, electron

beams, x-ray radiation) [4, 5, 6, 7, 8&9].

Other non-thermal approaches, including super-critical carbon dioxide (pressurized CO₂) [25] ultraviolet radiation, ultrasound treatments [26] and magnetic resonance fields, may have potential as seed treatments for reducing populations of pathogens. Exposing infected seeds for 5 sec or 10 sec in microwave irradiations has reduced seed borne pathogens in maize and paddy without affecting seed quality greatly [27]. But this strong microwave electric field irradiation, influencing the whole seed grain and therewith the seed embryo too, gave conflicting and inconsistent efficacy results on the control of spring wheat seed pathogens such as *Phaeosphaeria nodorum*, *Pyrenophora tritici-repentis* and *Fusarium spp.* [28].

Gamma rays were also used to irradiate *T. tritici*, and *T. controversa* teliospores in wheat using cobalt 60 (0-20 kGy) and this irradiation treatment did not affect the wheat seed physiological quality significantly [29], at a low dose. Gamma radiations are used successfully to disinfect products in several industrial food products. But radioactive sources for irradiation are least preferred by users because of health hazards. Similar to high energy electron beam, the gamma treatment influence the seed embryo. Radiation energy is particularly advantageous because it can penetrate seed tissues and possibly eliminate bacteria localized within protected tissues. Irradiation of seeds also has been shown to reduce *Salmonella* populations without affecting germination [30]. However, too high levels of irradiation dose or a too deep penetration depth can negatively affect the physiology of seedlings and more research is needed to assess the potential and risks of this approach.

High energy electrons from an electron accelerator do not contain any radioactive elements but instead converts electricity to ionizing energy. This type of energy treatment is being used to decontaminate variety of food products. Years ago it was reported to have effect on living organism including plant pathogens [31].

Another promising technology is the low energy electron beam technology. Several years ago it was reported that electrons have effects on living organisms including pathogens in agriculture [31, 32]. There is a report that irradiation at appropriate doses makes pathogen free sprouts of red radish, alfalfa and broccoli sprouts [6], *mucuna* seeds and Lotus seeds [7]. The effort for treating seeds with electron beam was made long back during 1995 [32] on wheat seed against *T.controversa* and *T. tritici*. The electrons at 10.2 kGy have reduced infection from 75.5 % to 0.08 %. But it has also reduced the seed germination and seedling vigor after the treatment. Hence, they concluded that the technique was found to eradicate seed pathogens but harmed the seed embryo at higher dose. However, on chickpea [33] use of medium energy electron irradiation with doses up to 1000 Gy with an acceleration voltage of approx. 500 kV found no negative effect on seed quality but disinfected the seeds. However, beyond 1000 Gy, Chickpea affected the seed quality and seed vigor, because the electrons, accelerated at a voltage of 500 kV, penetrates approx. 500 μ m deep into the material and thereby affects the whole seed grain.

Low energy electron treatment

In comparison to other irradiation processes, an electron treatment only treats the surface and near areas, like the seed coat layers. Thereby an influence on seed embryo is minimized. The basic aspects of the electron treatment of seed were studied at Fraunhofer FEP and the former Manfred von Ardenne Institute.

New areas of seed treatment application were discovered continually for the electron beam, the importance of which Manfred von Ardenne recognized early on, as being an energy intensive and flexible tool for a great number of industrial processes. The first tasks that were worked on by this Institute were predominantly those attached to the established areas of electron and ion beam technology. It is more than 25 years since the former Manfred von Ardenne Research Institute first carried out studies on the sterilizing effect of accelerated electrons on cereal seeds. However, it has taken until this year for this application to make its commercial breakthrough.

Basic principle of electron treatment

The principle is purely physical and is based on the biocidal effect of low-energy electrons. The acceleration of freely moving electrons is only possible under high vacuum conditions [34]. The technological breakthrough came with the development of band emitters (Fraunhofer FEP). This type of an electron source has a linear electron emitter which permits large linear expansion of an electron source with a compact design. The electrons are generated and accelerated in vacuum. This vacuum is separated from the process area by a so-called "Lenard Window". Such an electron generator is state of the art for many technical processes like polymer hardening, lacquer curing and the sterilization of surfaces. Hence, it became possible to treat seed in the air via a continuous process. The generation and acceleration of electrons is based on the principle of so called BRAUN tube. For Fraunhofer FEP patented seed treatment two electron generators are positioned opposite each other, each producing a beam of low-energy electrons (Fig. 1a). One can imagine this process in practice as the emergence of light from a television tube. The seed passes through this process zone and the whole surface of each seed is exposed to the electrons. The electrons hit all sides of each seed grain with a precise dose of energy and penetrate into the seed hull (Fig. 1b & 1c).

There are two basic parameters for electron treatment of products. The dose means the energy per mass that is applied on the product. The dose gives information about the number of electrons that reach a point and thereby, it indicates the disinfection capacity. The second parameter is the penetration depth. The penetration depth can be adjusted very precisely by the acceleration voltage. By optimal adjustment of the parameters, the electrons can penetrate the surface of the seed grain, but only to a certain depth - until their energy is dissipated. This means that it is possible to adjust the depth of treatment based on the thickness of the seed hull (Fig. 2).

The sensitive embryo is not touched by the electrons nor is it adversely affected in any way (Fig. 1c). The flow of electrons destroys most

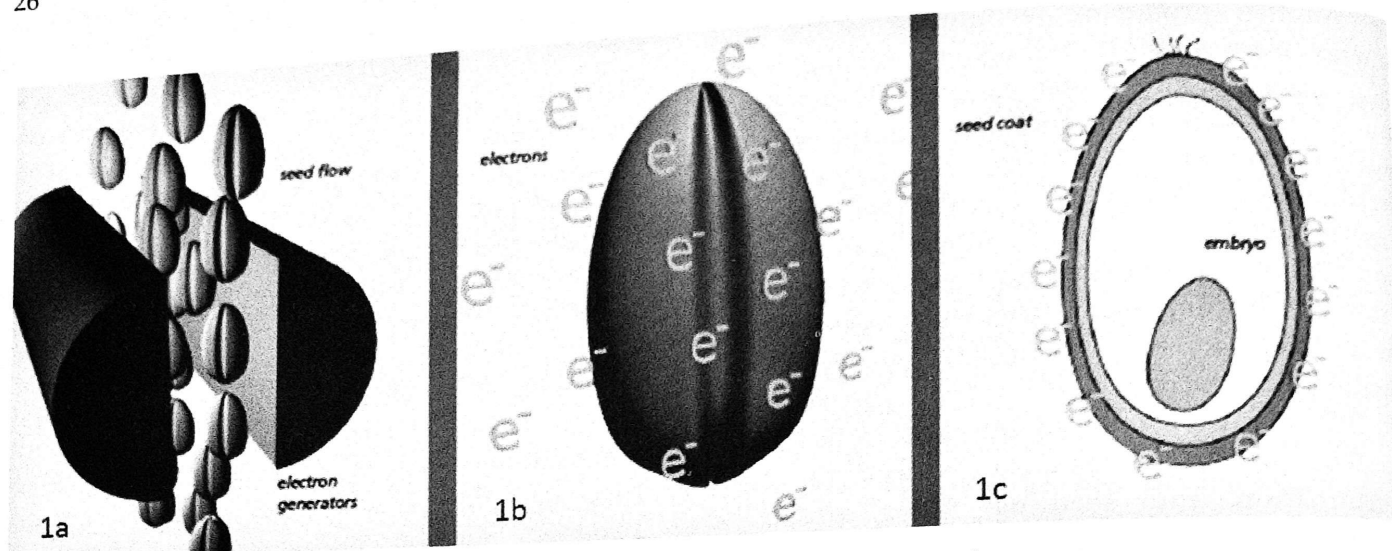


Fig. 1. Principle of electron beam treatment technology

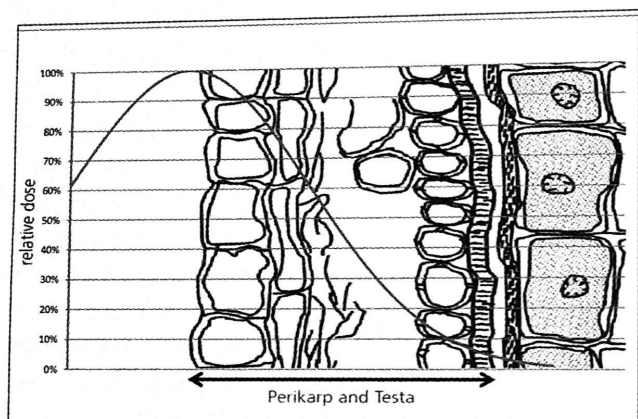


Fig. 2. Penetration depth of electrons

harmful pathogens in the seed hull, which are present within its sphere of influence, very effectively in milliseconds. Microorganisms are made harmless by the input of energy and the breaking apart of the molecule chains.

The feeding and removal of the seed occur via conveyor belts, meaning that the technology can be integrated into any existing treatment process. A special quality monitoring system permanently safeguards and sets the quality of the seed treatment, allowing the highest effective treatment and optimum plant compatibility. This is a unique seed treatment with maximum advantage and best efficacy.

Impact of electron treatment

The effectiveness of the electron treatment has

been demonstrated in extensive laboratory tests and practical trials by private industries, by federal testing institutes and by research institutes. The treatment is particularly effective against the increasingly problematic smut and bunt diseases of recent years such as common bunt in wheat and gummy stem blight in rye. Seeds infected with *Fusarium* and *Septoria* germinated better after the treatment.

In Germany, many successful trials have been carried out for treating seeds with electrons, in many crop based companies, various agricultural businesses, in particular the Nordkorn Saaten GmbH a company of Getreide AG and BayWa AG site at Hainichen/Sachsen, and collaboration with, amongst others, the Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA; now the Julius Kühn Institute) and the Saxon State Institute for Agriculture. This has enabled the gain of much practical experience and has facilitated studies on the effectiveness of the plant protection to be carried out in order to optimize the technology (Fig. 3 and 4). They have focused on treating cereal seeds; however rapeseed, seeds of leguminous crops, and vegetable seeds were also studied. An overview was published by the BBA in 2005 of some of the results of these trials [35].

The harvest yield and emergence do not differ from the yield produced from chemically dressed seeds (Fig. 3 and 4). The development of resistant

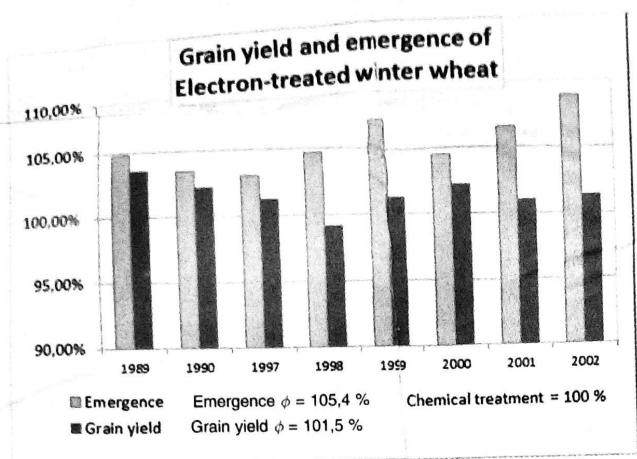


Fig. 3. Influence of electron seed treatment on yield and emergence of wheat evaluated by the federal BBA

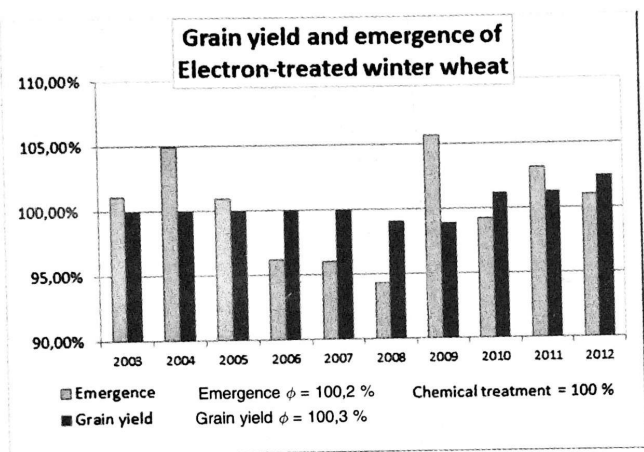


Fig. 4. Influence of electron seed treatment on yield and emergence of wheat evaluated by Fraunhofer FEP and customers

pathogens, as often occurs when using synthetic chemical agents, can be ruled out when using the electron beam treatment. The process costs are lower than that of chemical dressings.

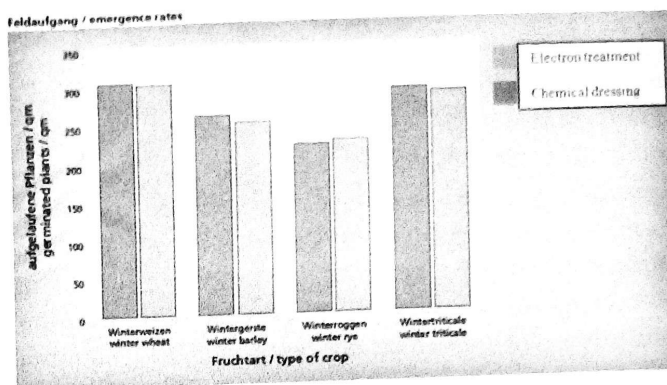


Fig. 5. Influence of electron treatment on emergence rate of several cereals

The electrons are non-chemical and non-toxic to the seeds up to certain energy levels. The energy level to be applied mainly depends upon thickness of seed coat and seed composition. The seed being a living entity, can be harmed by the electrons. Field emergence of electron treated seeds has also resulted in increased germination rate per unit area, irrespective of the kind of seed (Fig. 5).

The electron treatment is as well suited for non-cereal seeds, like vegetable seeds. Similarly electron beam at 15 kGy energy level increases seed germination in tomato (Table 1), without increasing the number of abnormal seedlings.

The chemical effects of ionizing radiation (e.g. gamma radiation, electron beams, x-ray radiation) on polymers have been known for a long time [4, 5, 6, 7, 8&9]. Accelerated electrons can be beneficially used to supply this energy, because, unlike gamma radiation sources, electron sources can be readily switched off [18] and hence plants can be managed far more easily. Similarly, complex polymer molecules in living organisms

Table 1. Influence of electron beam seed treatment on germination and seedling characters in tomato

Treatment	Germination (%)	Abnormal seedlings (%)	Seedling length (cm)	Seedling dry weight (mg)	SVI (Seedling length)	SVI (Seedling dry weight)
Untreated	92	8	13.67	1.005	1259	92
Electron @15 kGy	96	4	12.94	1.600	1249	156
Electron @ 30 kGy	88	6	10.50	1.145	920	101

such as chains of DNA are also affected by the energy input. Base loss, denaturation, and longitudinal cross linking as a result of electron bombardment lead to irreparable damage to DNA molecules and hence to cell death [31]. These effects are the key for sterilizing effect of accelerated electrons, because amongst other things harmful organisms can be killed. The sterilization of medical products with gamma radiation was established on this basis. Gamma radiation passes deeply into materials, resulting in sterilization of the whole volume [36, 37] causing minimal undesirable changes to physicochemical and sensory properties of the product. The same for seeds is totally unsatisfactory because sterile seed grains are no longer able to germinate.

In contrast to gamma radiation, the penetration of electrons into materials is much lower and can be accurately adjusted via the kinetic energy of the impinging electrons and duration of exposure is comparatively short [38]. Thus the sterilizing effect can be restricted to the surface and a defined outer layer of the seeds, without affecting the seed embryo or the endosperm inside the seed grain. Despite the recommendation of the BBA and the EPPO (European and Mediterranean Plant Protection Organization) to use this treatment method for both conventional and organic agriculture and despite its many advantages (no use of chemicals, environmentally-friendly, no dust, cost-efficient), this technology required 10 years for its commercial breakthrough. The reasons for this are varied and include:

1. The existence of an established and proven technology for the chemical dressing of seed using a functioning sales network and existing plant technology.
2. Electron treatment of seed has no effect on pathogens in the soil.
3. After the electron treatment, the seed looks the same as untreated; hence there is no simple way of proving that seeds have been successfully treated.
4. High acquisition costs for an electron treatment plant.

Recently there is a shift in opinion about seed treatment. Eco farming seeds are physically treated to remove seed infections. In United Kingdom, organic seed lots frequently do not make the grade and are often discarded because of contamination with common bunt [1]. Several chemical dressings have had their approval withdrawn or banned by several countries [39, 40]. New approvals and hence the development of new chemical dressings have decreased markedly in number. The global seed treatment market, on the basis of types of seed treatment, is further categorized as chemical and non-chemical. Bio-based seed treatments, part of non-chemical seed treatment, are made up of renewable resources and contain natural active ingredients. These seed treatments have much of a no negative impact on the environment, workforce and consumers safety when compared to chemical seed treatments. Bio-based seed treatment is expected to be one of the fastest growing seed treatment segments in the near future [41]. Many existing seed treatment methods are unsuitable for organic agriculture [1, 42]. All these factors have led to re-evaluation of alternative strategies for seed treatment worldwide.

The efforts to establish seed treatment using accelerated electrons resulted in designing of first electron treatment plant, which was developed for large scale seed treatment usage with following objectives:

- Less expensive process costs while attaining a high throughput
- Flexible adaptation to different types of seeds and throughputs
- Integration into existing seed treatment plants
- Optimal combination with biological plant protection products

The focus was made not only on traditional cereal seeds but also on corn seed, sensitive sprout seed, high-value fine seeds and invigorated seeds.

In 2011, the Fraunhofer FEP started

introducing electron beam seed treatment to the Chinese and Indian marketplace, and collaborations have been made with Indian Agricultural Universities and private seed companies to test and verify suitability of treatment plant for tropical crops seeds as well.

FUTURE PROSPECTS OF RESEARCH WORK

Seed Testing

Seed testing is a pre-requisite to confirm eradication of pathogens by any means of seed treatment. However, the ability to determine the source of microbial contamination of seed, effectively, is difficult. To improve this, statistically-sound sampling schemes must be employed for drawing samples. Inadequate sampling or small sample sizes may explain why laboratory analyses of seed samples led to the detection of pathogens in the lot even after seed treatment. It is desired that seed assays should be rapid, sensitive, specific, and easy to interpret. Many seed detection assays are available for bacteria, including semi-selective agar media, serology based techniques (ELISA and immunofluorescence staining), and culture enrichment [43].

Considering the seed being a living organism, there is a need to assess the effect of electrons on living cells of seeds, particularly on the embryo. Seeds undergo many physiological and biochemical processes before germination that ultimately leads to the emergence of plumule and radicle. The pathogens, which are present on the seed coat and inside the seed coat, will damage the embryo by utilizing stored food materials. Hence, infected seed cannot germinate very well unless pathogens are removed. The adverse effect of pathogens depends on pathogen load and position in seed. The electrons possess energy which can destroy these pathogens and make the seeds pathogen-free, without damaging the viable embryo.

Changes in the biochemical nature and structure of seeds

The seeds are the store house of many biomolecules like carbohydrates, proteins, fats, minerals and vitamins which are consumed by

humans as their lively food. Electrons may alter the chemical nature of these biomolecules. Hence, there is a need to study the biochemical changes in seeds occurring after exposing to electrons.

Following are few areas where experiments have to be conducted to obtain viable technology for seed treatment.

1. The membrane permeability study which would indicate the damage to the seed coat
2. The activity of dehydrogenase enzymes should be tested by tetrazolium test
3. Measurement of the activity of free radical scavenging enzymes SOD and catalase
4. The seeds has to be tested for the accumulation of the reactive oxygen species which is the indication of seed deterioration
5. Measuring the accumulation and content of malondialdehyde
6. Determination of protein content
7. Seed vigor should be tested using methods recommended by the ISTA

CONCLUSION

In conclusion, electron beam technology is an innovative, environmentally-friendly technology for treating seeds without using chemical agents and achieving comparable, partly higher germination rates than conventional treatment procedures. This technology has many advantages over gamma facilities and is suitable for online treatment. It is a waste-free technology and hence, it is more acceptable to the public. Research on energy levels for varied infection level and types of pathogens are under progress. Also, energy level varies with the type of infection and moisture content of seeds. There is a need to intensify research in the fields mentioned above.

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