BIO-TREATED

SEEDS

By

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PREFACE

Chemical seed treatment formulation is commonly applied during commercial pelleting or coating, both as powder and aqueous slurries or solutions. Pelleting permits applications to be made at different points as layers are built up. Active ingredients are placed either on the seed surface (e.g. to control seed borne disease), or distant from it (e.g. it minimize phytotoxicity), and to separate different additives from each other (e.g. if the formulations are incompatible). In the simpler coating process, however, treatment formulations are often mixed throughout the blend.

The chapter begins by describing the different seed treatment processes and equipment, and discusses how these processes are, or might be, utilized to inoculate seed. Additionally, new processes are considered, some still at the research and development phase. Specific microorganisms are then reviewed as case studied, with particular emphasis on Biofertilizers, Biocontrol and available inoculants formulations. All Stages of the process are considered where possible, including production of microorganisms.

This book will share in detail our knowledge on the application of microorganisms to seed, encompassing Biofertilizer inoculants, biological control agents and mycorrhizal fungi. Of these microorganisms, only rhizobial inoculants have been extensively commercialized for seed inoculation. Although the literature on the isolation and evaluation of microbial seed inoculants has grown extensively in recent years relatively little has been published on development of seed inoculation methods *per se.* Research papers often report use of the simplest inoculation techniques, Inoculation subjects an organism to a period of wetting – several hours for pelleting, during which the microbes lie in a water-saturated environment – followed by patios of rapid drying and storage. Such as wetting seed just before sowing, which are not always suitable in commercial practice. Application to seeds and their subsequent storage, and also efficacy after placement of seeds in soil. An attempt has been made to draw not only upon scientific literature, but also practerial experience, which contains much of the current expertise. One difficulty in reviewing this area, however, is that much of the work is done by commercial companies but not in India, and is not available for publication. Finally, more research needs and future prospects for application of microorganisms to seeds are summarized.

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INTRODUCTION

The Biofertilizer are microbial inoculants, which contain live cells of efficient nitrogen fixing microorganisms, which fix atmospheric nitrogen either symbiotically with host plant or free livingly, Phosphate Solubilizing/Mobilizing Microorganisms as well as potassium mobilizers. These Biofertilizer are available in markets in carrier based as well as liquid base.

Biofertilizer are inexpensive and eco-friendly etc. Many State Agricultural Universities, Govt. Agriculture/Forest Departments and a good number of commercial units in private and public sectors are producing and distributing Biofertilizer. In addition to that, Govt. of India has established a Regional Biofertilizer Development Centre in Karnataka to promote, give technical support and quality control in southern states. It is observed that, the technology of production of good quality Biofertilizer has been available for several decades but still date poor quality Biofertilizer is not uncommon in southern India. Therefore, RCOF tried to solve the different problems with Biofertilizer at every stage in production units. Although, primarily the quality of the Biofertilizer produced is the responsibility of the manufacturer, but often, the manufacturers do not follow the proper quality control protocols at various stages of production.

Application of beneficial microorganisms to seed for use in agriculture, forestry and horticulture has been under intensive investigation for many years (Scott, 1989). Microorganisms can be applied to perform specific functions, notably nitrogen fixation, phosphate solubilization, potassium mobilizing plant –growth promotion-*Pseudomonas Fluorescence, Bacillus subtilies, Aluconobacter oxydans, Alcaligenes faecalis, Bacillus coagulans, Paenibacillus, Bacillus Intermedius, Zinc Solubilizer Alcaligenes ,Lactobacillus ,Claviceps pupurea,Fraturia auerentia ,Morganella morganii , Saccharomyces Cerevisiae* , biological control like *Trichoderma Viridie, Trichomerma Harzinium, Paecilomyces fumosoroseus, Paecilomyces lilaceous, Verticillium lacanii ,Bacillus Pumilis* of plant pathogens and, more recently, biological control of pests Appling microorganisms to seed is an attractive proposition because of the combination of specific effect and limited environmental impact . Seed treatment has the potential to deliver agents " **in the right amount, at the right place, and at the right time**". With increasing public awareness of the potential environmental and health hazards of both agrochemicals and fertilizers, and the advances in biotechnology to improve the performance in microbial products, application of microorganisms to seeds (Chandra and Greep 2009; Chandra, Greep and Ramarathinam, 2006) is likely to increase in the future.

1.0 **PRESENT SCENARIO**

There are three main large-scale priming approaches, using different methods to regulate water potential are quit popular in European countries.

In osmoconditioning, seeds are incubated in an aerated solution of an osmoticum such as polyethylene glycol, or an inorganic salt such as potassium nitrate or phosphate, using high liquid: seed ratios (e.g. 10:1) in stirred bioreactors of various designs. At the end of the end of the process, seeds are rinsed before further processing. Taylor and Harman (1990) and Gray (1994) provide access to the very large literature on osmoconditioning.

In the patented solid-matrix priming technique, available commercially in the USA seed is the mixed with an approximately equivalent quantity of a friable, non-clumping, inert material, e.g. a carbonaceous, preferably ligneous shale or coal, with an osmotic component at least 90% of the equilibrium water potential, moistened sufficiently to equilibrate seeds to the correct water content . After incubation the extraneous solid material is sieved off. A related variant technique, matriconditioning, uses different inert materials in a very similar way (Khan, 1992).

The third basic priming method also incubates damp seed, but brings the seed directly to a predetermined moisture content by various means, without using an external matrix or osmotic agent to regulate seed water potential. The patented drum priming process is one version of this type of treatment (Rowse, 1991,1992).

2.0 CONVENTIAL TREATMENT

The process of applying microorganisms to seed presents a special set of technical considerations. Obviously, sufficient numbers of the inoculants must survive the process, and be able to grow in the environment of germinating seed. However, there are likely to be many crop situations where seed is to inoculated at some time and distance from the point of sowing, rather than immediately before use. In these situations, treated seed becomes in a real sense a ‘secondary formulation’ of the inoculants, and must have acceptable shelf-life qualities. In practice, since seed must be stored at low moisture levels, inoculants must be able to survive a period of low water activity. Microorganisms may also need to be applied in combination with other active ingredients, such as fungicides and insecticides. These factors raise novel issued for extended periods before application, many manufacturers stipulate that containers should not be left open. Obviously, where optimum storage conditions vary from those in the surrounding atmosphere, containers should be selected for minimum permeability to water and sometimes of oxygen.

It is well stabilized that if quality is good than symbiotic rhizobia fix atmospheric nitrogen more than the required for crop and indirectly increase soil fertility when in association with a host leguminous plant. The rhizobia infect the root hairs of the host, causing nodules to form. Within these nodules, the bacteria obtain food and energy from their host, and in turn fix nitrogen for plant growth. These are

many different types and strains of rhizobia most of which are specific to a particular legume. It is most important to note here that most soils contain a population of rhizobia but these are often ineffective strains which are unable to form active nitrogen-fixing nodules on the host, and to compete successfully with the indigenous rhizobial population. Inoculation of seed with beneficial. It is also well estabilised that co-inoculated seeds like N, P, K – bacteria (Chandra2009) perform well over individual (Chandra and Jugeshwar 1996 **;**Ramarethinam and Chandra 2007; Chandra and Greep 2009;Chandra and Greep 2010).

3.0 SEED TREATMENT PROCESSES AND MACHINERY

Seed inoculation techniques are not yet widely developed commercially, apart from the great exception of the rhizobial inoculation of legume seed. As future implementation of seed inoculation technology will need to be integrated within the existing organizational framework of the seed industry – comprising the stages of seed breeding, production, processing. Treatment with bacteria, storage, distribution and sowing – it is appropriate to note the key features of commercial seed – treatment system not only offer technical options, but also remove certain constraints.

3.1 Organization Of commercial seed treatment

Treatment of seeds before sowing to protect against pests and diseases and to enhance crop establishment and development is well established in much of the world. For many years, seed of most crops has been treated with fungicides directed against seed borne, soil borne and foliar pathogens (Maude, 1995), and for a few crop more recently with insecticides as well. The way seed treatment is organized, commercially and technically, differs considerably between crops and between markets in different regions. A variety of equipment is used, ranging greatly in terms of capacity, throughput and engineering design and sophistication. Values and volumes of seed treated also cover a tremendous range – from the so-called high-volume, low-value agricultural crops at one extreme, to low-volume, high-value horticultural and ornamental species at the other. Different types of treatment are therefore appropriate for different are crops, and cost rather then performance or biological efficacy may determine which treatments are used. Relatively expensive and slow application procedures and materials may acceptable for high-value crops such as small-seeded vegetables, where maximum performance potential is frequently required, but may not be economically reasonable for agricultural crops.

Differences also lie in where and who in the industry is responsible for carrying out he physical application of treatments. In the more developed agricultural areas of the world, much seed- treatment work is carried out at specialist facilities by, or for, seed companies or merchants. This applies particularly seed- processing technology, such as maize, cotton, sunflower, sugar beet, and many modern vegetable and flower varieties. In contrast, for other crops- particularly open-pollinated varieties of small-grin cereals and legumes – a substantial proportion of seed treatment is carried out near to the site and time of sowing, using locally installed or mobile treatment, and on seed saved by the farmers themselves. Another seed treatment situation involves outdoor crops grown from

transplants produced under cover, e.g. horticultural brassicas, onions, celery, lettuce and sugar beet (in Japan).

It is of course vital that seed processing and application of treatments do not diminish per formance potential or storability of seed. High germinability and 'vigour' are important quality parameters for seed, particularly for crop sown to a stand or produced as transplants, where as many seeds as possible should establish uniform and healthy plants.

4.0 CONVENTIONAL AND FILMCOATING TECHNIQUES

Seed-treatment techniques can be broadly divided into two basic categories:

a. Conventional treatment and thin film coating,
b. Pelleting and coating.

4.1 COMMERCIAL SEED TREATMENT

4.1.1 Pre-inoculated seed process

Equipment and processes for treatment and film coating In conventional treatment, dry powders and (increasingly) solutions, water-based wettable or water-dispersible powders, or flow able concentrates (emulsion or microencapsulated formulations) are applied directly to seed without further modification. High-volume crops such as small-grain cereals, legumes and oil seeds are usually treated in continuous- throughput machinery, but batch machines are also available. Some modern machines have very high capacities, ca 5-25 t/h or more for fixed cereal – treatment installations. In all of them, a key feature is to feed seed and formulations at controlled, metered rates, while the more sophisticated machines have failsafe controls to ensure this occurs correctly. Often, drying of treated seed is unnecessary because relatively little water is involved. Various means are employed to ensure the thorough mixing of seed and liquids, e.g. spraying formulations onto a falling curtain of seed, or stirring seed and liquid together in a trough. Mechanical damage to seed must be minimized, particularly in structurally vulnerable seed such as maize. The dwell time of seed in the machines can be less then 10 min.

By contrast, design of on-farm equipment is much simpler- at the simplest, slurring seed with formulations or dusting with powders just before sowing, in the 'planter-box' or 'hopper-box' Dry material do not adhere well to seed, giving rise to poor, uneven loading and dustiness. Alternatively, for some crop film coating is now preferred, particularly for some pelleted species, notably sugar beet. Characteristically, a uniform, dust-free, water-permeable, thin coating membrane surrounds the seed. In commercial practice the cosmetic quality standard of film coating is often high, achieving an even coating both over the seed surface and between individual seeds.

4.1.2 Liquid Inoculants surface seed coating

In the film coating process, treatment formulations are premixed with a binder, adjuvant and pigments as an aqueous suspension, and then sprayed onto the moving seed mass. The binder forms a film as the

mixture dries, so that the applied additives are stuck firmly to the seed surface. The complete film coating mixture is usually prepared only shortly before use, and may be kept just for a few hours. Typical binders are polymers of various types, such as derivatives celluloses, polyvinyl acetate, alcohol or pyrrolidone, and complex graft terpolymers, based on starch, are good to increase the number of micro-organism per seed.

Film coating formulations are applied onto seed either in modified conventional or in specialist equipment according to crop, amount of seed to be treated, treatment formulations, application rates and quality standard required. Conventional equipment is appropriate where the amount of applied liquid is relatively low, and where the seed itself can satisfactorily absorb the added moisture. If necessary, a separate drying stage can be added in the treatment process. But where the amount of additives to be applied. To the seed is relatively large, or where a higher standard of seed– to-seed distribution or of cosmetic finish is required, relatively large amounts of liquid are usually needed, and a different engineering approach towards film coating is necessary so as to increase the number of bacteria per seed. Generally, total count of all treated bacteria / Fungus must be in range of 10^3-10^5 per seed to get maximum benefits (Chandra, Mukherjee, and Karmakar 1995)**.**

In such specially engineered film coating equipment, concurrent spraying and drying takes place in an enclosed chamber, typically at up to *ca* 35^0 C, and seed is presented to the spray system many times to build up the film layer. Hence, the formulation of bacteria should be selected which can tolerate 35^0 C temperature. If, the formulation/bacteria is not able to tolerate than it is thus possible, for instance, to change the composition of the applied liquid to give a multi-layered coat. Depending on volume to be applied and spray rate, film-coating runs usually take *ca* 15 – 100 min to complete.

4.1.3 Vertical Cylindrical-seed surface treatment

In the spouted-bed system, seed is held in a vertical cylindrical or inverted cone-shaped vessel, and solutions are sprayed from the bottom into a vigorous upward-moving stream of air, which stirs and dries the seed mass. The system has relatively high drying capacity, but can damage delicate seed by mechanical attrition.

4.1.4 Rotated drum – seed treatment

In side-vented' drums, solutions are sprayed onto the surface of the seed mass, which is stirred and mixed within a horizontally inclined perforated rotating drum made with baffles or riser blades. Drying air is drawn continuously in a non-turbulent stream across the drum and through the seed. The drum is housed in a sealed chamber, which in most designs is kept under negative pressure.

In both types of machine, coating solutions are sprayed by atomization through pneumatic or, more usually, hydraulic (air-spray) guns, and extracted air is filtered to remove seed and chemical dust. It is important to minimize losses of material by spray-drying onto the vessel wall, and blemishes in the coating layer such as picking, peeling, cracking or roughness. Liquid mixture composition, inlet and outlet pressure, flow rate, relative humidity and temperature of the air, rate of stirring and spraying, and spray-gun placement are all key factors. Equipment is commercially available to accommodate a range

of batch sizes, from *ca* 200g to 50 kg seed in spouted bed coaters, and up to *ca* 200kg in drum coaters. Engineered to a high standard, with safety cut-outs and fail-safe features, the equipment is expensive and needs careful optimization for successful results with particular sizes and shapes, and so at present in practice is suited only to fixed-installation facilities. Throughputs of several t/h are achieved in semi-automated production lines, comprising machines working in series or in parallel.

4.1.5 Treatment of seeds with microbial inoculation and Chemicals

The use of conventional and film coating techniques for inoculation has several implications. Bacteria can be applied as vegetative cell cultures or spores, and fungi as mycelia fragments, conidia sclerotia or sexual spores. This variety of cultural and cellular from does not in itself present particular technical application problems, small enough to form prevent gun blockage during spray drying, and to avoid dustiness and loss of cosmetic quality where that is considered important. However, the microorganism is subjected to considerable physiological stress during structures such as spores tend to survive best.

Although powder formulations may be applied directly to seeds, as mentioned above, this approach is associated with poor adhesion. Preferably, and more likely, microbial formulation need to be dispersed in aqueous media for spraying or slurrying, which thereby exposes the inoculant organism to large and abrupt changes in water activity. In the spray mixture, at a water activity of about 1, there is additional exposure to other additives, and possibly also chemical table 1.0 (Chandra and Karmakar 1996) shows that use of chemicals like Cerbendazium,Dithane M-45,Dithane Z-78,Topsin,Ceresan had not much effect on bacteria, table 1.2 recommanded the doses – in a compete mixture that may be prepared few hours before use.It is also needed to treat the fungicide followed by bacteria , but bacteria must be competitive to the chemicals used . Then the inoculant is dried within about an hour, as water activities are reduced to levels suited to safe storage of the seed (0.5 or less). If forced drying is needed, there might also be exposure to relatively high temperatures. Finally, if seed is being treated well ahead of sowing time there may be a long dry-storage period.

On the other hand, it may not be necessary for more than a small proportion of the inoculated micro organism population to survive, providing that enough do – what may be most important is a certain minimum level of viable cells per seed. The challenge lies in devising application techniques to achieve reliable inoculation efficiencies, and to minimize loss of inoculant viability during processing and subsequent storage. Most successful was a ‘sandwich’ technique in which seeds were first coated with 5% polyvinylpyrrolidone (PVP), followed by liquid base inoculant, and them 10-15% Sepiret (Seppic, France), both as per-centage of seed weight. It is suggested that using a liquid carrier within the film coat layers provided a suitable microenvironment for the inoculant, buffering it against the stress of coating conditions.

Table 1. Survival of rhizobia for pea and soy bean into serial dilution of fungicides. Results are given as log_{10}number of rhizobia per milliliter. P, Rhizoibum for pea; NS, non-significant; LSD, least significant differences; S, Rhizobium for soybean; ng, no growth.

Fungicide	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9	10^{10}
Cerbendazium										
P	ng	ng	ng	ng	ng	4.36	6.83	7.20	7.42	6.52
S	ng	ng	ng	ng	ng	3.10	5.63	6.63	6.81	6.80
Dithane M-45										
P	ng	ng	ng	ng	5.36	6.56	7.36	7.30	7.56	7.63
S	ng	ng	ng	4.36	6.20	6.10	6.36	6.42	6.63	6.63
Dithane Z-78										
P	ng	ng	ng	5.10	6.36	6.26	6.42	6.52	6.63	6.60
S	ng	ng	2.00	41.0	5.36	6.30	6.42	6.48	6.48	6.52
Topsin										
P	ng	ng	ng	ng	ng	6.36	6.42	6.52	6.42	6.52
S	ng	ng	ng	ng	ng	ng	6.42	6.10	6.10	6.10
Ceresan										
P	ng	ng	ng	ng	ng	ng	5.36	6.63	6.59	6.63
S	ng	ng	ng	ng	ng	ng	4.56	5.52	6.63	5.63
LSD (0.05)	ng	ng	NS	0.14	0.075	0.083	0.092	0.088	0.10	0.084

Table 1.1-Effect of Rhizobium growth fungicides yeast mannitol broth RG FYMB) on nodulation of pea and soyabean crops. X, not tested; P, Rhizobium for pea, S, Rhizobium for soybean

Fungicide	Cerbandazium		Dithane M-45	Dithane Z-78	Topsin	Ceresan
Dilution	P	S	P S	P S	P S	P S
10^3	X	X	X X	X 18.10	X X	X X
10^4	X	X	X 42.66	88.66 32.33	X X	X X
10^5	X	X	90.56 65.33	X X	X X	X X
10^6	28.66 18.66		X X	X X	75.00 X	X X
10^7	X	X	X X	X X	X 80.00	45.00 23.66

Table 1.2- Minimum toleran ce doses (MTD) of Rhizobium for pea and soybean. P, Rhizobium for pea; S, Rhizobium for soybean.

Fungicide	Dose (ppm)
Cerbendazium	
P	1.0
S	1.0
Dithane M-45	
P	10.0
S	100.0
Dithane Z-78	
P	100.0
S	1,000.0
Topsin	
P	1.0
S	0.1
Ceresan	
P	0.1
S	0.1

5.0 PELLETING AND COATING TECHINIQUES

The main purpose of pelleting and coating is to change the handling characteristics of seed by altering the shape, weight or size. Both processes are commonly used for applying chemical seed treatment formulations, and both are, or could potentially be, used to inoculate seeds with living microorganisms.

5.1 Crops pelleted or coated Pelleting

By far the largest commercial use of pelleting is for monogerm sugarbeet – almost all the crop in Europe and Japan is grown from pelleted seed. Other species pelleted in commercial quantity include carrot, carrot, onion, pepper, and endive, leek, lettuce, onion, pepper, tomato and, to a lesser extent, some brassicas and super-sweet corn varieties, as well as certain flower species, particularly those with tiny seeds.

Makes irregularly shaped seed round and smooth, usually specifically for use with precision drills, to mechanically singulate (prevent clustering without obtaining standard spacing) or space-plant in glasshouse or field sowings. Pellet size tolerances are therefore normally strictly specified, but differ for a given seed species between markets, depending on the particular drill settings used. By contrast, seed coating – sometimes called encrusting – applies relatively little material, still more or less revealing the original seed shape. Structurally, a coated seed can thus be regarded as a 'partial' pellet. Seed coating is used for a variety of purposes, for example to upgrade the size range of a seed batch, or to increase seed weight (e.g. range grasses for air-seeding).

5.2 Use of Inert material for pelleting and coating Pelleting

This processing steps, comprising wet, drying and size-grading stage. Both processes are usually on a batch or batch-continuous basis. Typically, pellet batches are *ca* 250 g to 100kg seed, though some commercial systems operate by true continuous throughput. In practice, pelleting has a slower throughput than coating: more material is added and addition is more gradual to control final shape and size. Success depends on culture and equipment can serve as nucleus during the process, it is important beforehand to remove extraneous matter of comparable size to seed. Both processes of carried out commercially at specialist facilities, usually run on a secret basis. For some seed species, notably sugar beet, pelleting is done semi-automatically in production lines comprising several machines, but for others skilled operatives must perform it manually. Although much information on the technology is proprietary, and indeed involves a great deal of 'skill and art', the general types of equipment, blend and binder materials used have been reviewed (e.g. Halmer, 1988; Taylor and Harman, 1990).

Pelleting involves addition of materials in a rotating horizontal or inclined pan or drum to achieve the required control of size. Seed is wetted, and then the powdered blend is progressively added along with more water by layering until the desired weight or size increase is reached. Examples of materials used include chalk, clays, diatomaceous earths, perlite, sand, vermiculite, peat, and vermiculite, peat, and wood fibers or other organic materials ;the water phase also may contain dissolved or suspended binders with inoculants N,P,K bacteria and disease control fungus or other scurried materials . Powder-size grades are important: larger particles of some materials, e.g. above *ca* 100 *u*m, may not be

incorporated into the growing pellet but remain as free dust. Throughout the size build-up stage, which typically takes *ca* 20-200 min to complete, the seed mass is continuously stirred mechanically to prevent formation of clumps, and the agglomeration of 'empty' pellets. When large quantities and longer times are involved, frictional heating can slowly warm the mass by up to ca10^0 C above ambient. Finally, the wet pellets seed is removed from the coating pan, and dried at *ca* 30-50^0C depending on the physiological tolerance of the species, by forced air convection in static or fluidized beds or rotating perforated drums. Depending on the amount of wet pellets and their moisture content which can be at least 50% on a fresh weight basis – drying usually takes *ca* 30-300 min. After size grading, any undersized pellets can be returned to the process for rebuilding. The seeds can be packed presence of Nitrogen gas to avoid further grow of contamination.

6.0 SEED INOCULATION BY ENCAPSULATION

Gel-encapsulation technology has been devised for delivery both of somatic embryos (Redenbsugh *et. al.,* 1987) and microbe granules (Fravel *et al.,* 1985; Bashan, 1986,) and could perhaps be used to deliver inoculated seed to soil.

One gel-encapsulation system, developed for the formulation of biocontrol fungi, hard alginate prill, (Lumsden and Lewis, 1989) could conceivably be adapted to coat or pellet seeds. Digat (1991)has patented a process to produce granules of up to about 8mm diameter, with a core containing liquid-cultured bacteria and an outer protective coating layer. The process was capable of producing *ca* 2000 granules per hour per injector. A variation on the technique was devised to encapsulate seeds (soybean, pea, maize, pelleted lettuce, sugarbeet and tomato-a fairly round shape was a prerequisite) with *Pseudomonas* or rhizobia inoculants. Seeds were dropped one at a time onto a having double meniscus formed from two immiscible liquids, so as to from around the seed an inner coating layer containing bacterial culture mixed with algal polysaccharide as a thickener, and an outer envelope layer of kaolin. Capsules were immediately gelled by dropping into calcium gluconate solution, the dried and treated with a layer of bentonite clay to aid handing. Freshly encapsulated seeds had 10^7 - 10^8 living bacteria per seed, but after only partial drying (1 h at 40 ^{0}C) most recoverable titters fell *ca* 10-fold. There was evidence of more bacterial death after further drying to normal seed storage moisture contents: recoverable titters of bacteria in inoculated lettuce pellets fell from *ca* 10^4 to 10^5 c.f.u. Per seed after drying to a residual water content of 8-10%.

The effect on seed encapsulation with bacterial inoculant was done with following

Inoculant of *Pseudomonas fluorescence, Bradyrhizobium japonicum, PSM, KSM, T.V* in liquid medium used

Adjust P_H was adjusted to 7.0

Sterile aqueous 10% kaolin (w/v), was used to maintain viscosity *ca* 350 c.p.s.

Seeds of Soybean, pea, sunflower were used

Injector assembly prepared Inoculant layer is fed though a downward-pointing injector, and envelope material is simultaneously fed through an outer concentric injector. Diameter of the central injection orifice depends on size of the seed to be coated

Methods

1 Pump liquids simultaneously under through the injector assembly to form a lower,

denser (envelope) meniscus, overlayed with an upper, lighter (inoculant)

Meniscus layer.

2 A single seed was released over the double meniscus, so that as it falls under its own weight the two liquid layers surround it

3 Allowed the liquid-coated seed to fall into 0.1 M calcium gluconate, remove after

1 min and immediately dry

4 Coat with bentonite clay

It is important to evaluate in this type of approach what effect the inoculant coating has on germination and handing properties of the seed

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7.0 IMMERSION TECHNIQUES

7.1 Priming Seed

Priming techniques for the physiological enhancement of germination have been extensively researched and developed over the past 25 years. Priming is now in widespread commercial use as a tool to help crop establishment over a range of environmental conditions, especially for horticultural crops, e.g. carrot, celery, leek, lettuce, onion, pepper, tomato and some flowers, and recently in some markets for higher volume crop such as grasses and sugar beet. Some priming techniques also have potential for the inoculation of seeds.

All priming techniques incubate a batch of hydrated seed at a predetermined water activity for a time at a controlled temperature, followed usually by drying (and treatment and handling in the normal ways).Incubation allows some of the germinative physiological processes to occur, but germination itself is not completed in any individual seeds – i.e. embryo structures do not begin to emerge from the seed coat. As a result, the subsequent germination of the seed population is faster and more synchronized. This response increases the speed, uniformity and final level of field emergence, particularly under stress – such as cold wet or a combination of the two, supra-optimal germination temperatures, or unstable soil structure prone to crusting soon after sowing. Typically, enhanced germination

performance of dried, primed seed is retained in storage for at least several months, providing that suitable priming and storage conditions are used.

Depending on species, priming may take *ca* 1 – 14 days or more, at 15-25^{0}C, with water potentials between – 0.5 and – 2.0 MPa. After priming, seed is either pelleted or directly dried, e.g. using fluidized-bed techniques. Basic protocols are established for a species, but often individual seed batches are pre-checked to assess suitability for priming and establish optimal conditions: there extra steps take several days to complete.

In all priming-treatment approaches seed must be regularly mixed and aerated to

Table 2.0 Procedure for solid-matrix priming of seed (Taylor,1990)

Optimal ratio of seed:solid water for effective priming at 150C

Parts by weight

Crop	*Matrix material*	*Seed*	*Matrix*	*H2O*(%)*	*Duration (days)*
Tomato	*Agro-lig+*	*1.0*	*1.5*	*95*	*6*
	Soft coal+	*1.0*	*1.5*	*95*	*6*
	Sphagnum moss	*1.0*	*1.5*	*90*	*6*
Carrot	*Agro-Lig*	*1.0*	*1.5*	*90*	*6*
Onion	*Agro-Lig*	*1.0*	*2.0*	*80*	*6*
Lettuce	*Agro-Lig*	*1.0*	*2.0*	*85*	*1*
Cucumber	*Agro-Lig*	*1.0*	*1.5*	*60*	*6*

CalibrationPer-test small Sample of the seed at a range water amount and temperature, according to experience (guide condition are shown in Table). Establish that just prevent radicle emergence of seed

Methods (small-scale)

1 Wet seed with about 25% of its weight of water

2 Coat wet seeds with predetermine amounts of dry, flowable, particulate solid matrix material with thiram at 0.2% w/v

3 Add reminder of predetermined amount of water. Mix. Incubated conditions, but so as to minimize evaporation, e.g. by standing in closed metal container with a ventilation hole

4 After incubation, mechanically separate matrix material from seed. Rinse seed, blot dry.

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* Based on dry weight of matrix component.

\+ Leonardite ligneous shawl. Total percentage organic 84%, <1% nitrogen. Typically 90% < 200 mesh.

\+ Total percentage organic 90% < 1% nitrogen.

The matrix and damp-seed priming approaches are particularly suited to inoculation of seeds. Both are akin to solid-substrate fermentation, and allow the populations of microbes to colonize the seed coat itself. Several such biopriming studies have been reported. Indeed, solid-matrix priming was originally conceived partly to encourage colonization of seeds after inoculation with bioprotectant slurry. The technique has been used experimentally with *Enterobacter cloacae and Trichoderma spp.* (Harman and Taylor, 1988; Harman, 1991).Callan *et al.* (1990, 1991) also used this approach to inoculate super-sweet corn varieties with *Pseudomonas*, and used a commercial biopriming technique to inoculate tomato seed with *Trichoderma*, both to control *Pythium*.

7.2 Hydration Seed humidification

Is a physiological enhancement technique particularly suited to large-seeded species such as beans and soybean that are highly susceptible to imbibitional damage? The purpose is to raise seed moisture contents by a relatively small degree, and hydration levels reached are much lower than in priming. Recently, Szafirowska and Khan (1995) effectively treated snap bean seeds with *Bacillus subtillis* applied before humidification.

7.3 Pregerminated/seedling-inoculation technique

Fluid drilling is a preconditioning technique related to osmoconditioning, which delivers Pregerminated seedlings to the soil, and which can also be used to introduce inoculants like N, P, K and biocontrol to germinated seeds. The technique allows a seed batch to complete germination in an aerated liquid medium (but without high concentrations of osmotic agents) then, after removal of any ungerminated seeds by density separation, directly sows the seedlings by extrusion in a viscous nutrient gel which may also contain fungicides which does not effect the count (Pill, 1991,Chandra1996). The technique is particularly applicable to small-seeded vegetables, since success depends on timely production of the germinated seed suspension; it is effectively an on-farm technique. It requires reliable sowing equipment for precisions to ensure development of the young seedlings, which are very vulnerable to desiccation damage at this stage. Although fluid drilling has been used by some specialist horticultural growers, e.g. for Celery and tomato establishment, it has not met with widespread commercial success, for both practical and logistical reasons.

The fluid-drilling suspension can be used to apply microbial inoculants to the germinated seed. This has the potential advantage that the microbes do not have to be dried, and may have the opportunity directly to colonize the growing seedling root before delivery to the soil. Conway (1986) successfully used a modified hydroxyethyl cellulose gel to deliver sclerotia of *Laetisaria arvalis* to control *Rhizoctonia* damping-off of pepper seedling and Southern blight *(Sclerotium rilfsii)* in apple seedlings. Also, gel solutions have been investigated to inoculate soybeans with rhizobia to inoculate pea and radish seeds with *Trichoderma* spp.

7.4 Steeping and heat several physical treatment techniques

Could theoretically be combined with seed inoculation. Prolonged seed steeps with water-dispersed fungicides to penetrate seed tissue and control deep-seated seed borne pathogens. Most notably, thiram soaking is commercially used in the UK to treat vegetable and sugarbeet seed. The thiram recommended quantity does not affect the screened bacteria (Chandra and Karmakar 1996)

7.5 Soaking with inoculants

Such as streptomycin to control seed borne bacteria. However, these can, be phytotoxic; also commercial use is proscribed in many countries because of the importance of antibiotics in medicine.

7.6 Hot water or aerated steam treatments,

30 min at 50-60 ^{0}C, are used commercially for some small-seeded species, e.g. flowers and celery. Success depends on finding conditions lethal to the pathogen with minimal damage to seed quality. Further details of these techniques are given by Maude (1995). Though it is possible to envisage such treatment techniques being used as a preliminary sterilization step before the application of beneficial microorganisms, no research seems to have been reported on these lines.

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