Review

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Seed treatments to control seedborne fungal pathogens of vegetable crops

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Abstract

Vegetable crops are frequently infected by fungal pathogens, which can include seedborne fungi. In such cases, the pathogen is already present within or on the seed surface, and can thus cause seed rot and seedling damping-off. Treatment of vegetable seeds has been shown to prevent plant disease epidemics caused by seedborne fungal pathogens. Furthermore, seed treatments can be useful in reducing the amounts of pesticides required to manage a disease, because effective seed treatments can eliminate the need for foliar application of fungicides later in the season. Although the application of fungicides is almost always effective, their non-target environmental impact and the development of pathogen resistance have led to the search for alternative methods, especially in the past few years. Physical treatments that have already been used in the past and treatments with biopesticides, such as plant extracts, natural compounds and biocontrol agents, have proved to be effective in controlling seedborne pathogens. These have been applied alone or in combination, and they are widely used owing to their broad spectrum in terms of disease control and production yield. In this review, the effectiveness of different seed treatments against the main seedborne pathogens of some important vegetable crops is critically discussed.

Keywords: *Alternaria* spp; biocontrol agents; essential oils; *Leptosphaeria maculans*; physical treatment; seedborne pathogens

1 INTRODUCTION

Management of plant diseases is important for most crops, and it is particularly critical for the production of high-quality seed. Plant pathogens can reduce the quantity and quality of the seed harvested, and in addition they can be preserved in seed lots in the case of seedborne pathogens. In this way, seeds can inadvertently provide an efficient means of plant pathogenx dissemination.[1]

Although the treatment of seeds does not replace the availability and use of healthy seeds, it can be an effective means to increase seedling emergence when used on seeds of low vigour, and when the seed coat has been damaged. Similar benefits can also be obtained when germination is delayed because of unfavourable soil or weather conditions, such as early planting in cool or cold soil, planting in dry soil or planting in a poor seedbed. Indeed, treatment of seeds can become an extremely important means of eradicating or reducing seedborne pathogens, especially when seeds are grown for seed production, or where good-quality seed with a lower percentage of fungal infection is required.

In the past, seed treatments were carried out mainly by applying fungicides, and even now this remains the most effective means. However, new methods that exclude the use of fungicides are increasingly required, especially in organic farming. A prerequisite for organic farming is that seeds or other propagation materials should be produced under organic farming conditions (in the EU, according to EEC Regulation 2092/91). For several vegetable crops it is very difficult to produce organic seeds using the same quality standards as for conventional farming. This is especially the case for biennial vegetable crops, such as cabbage, carrot and onion, where difficulties are encountered in the production of high-quality organic seeds. Several non-chemical methods of seed treatment are being developed or are under study, and these include physical treatments and seed coating using plant extracts and biocontrol agents (BCAs).[2,3]

The successful outcome of a seed treatment depends not only on the intrinsic effectiveness of the compound applied but also on the degree of internal infection of the seed, the amount of inoculum in a seed lot and the specificity and potential phytotoxicity of the treatment.[1] Different kinds of treatment can be used, which will depend on precisely where the pathogen is localised on or in the seed, and these can include seed disinfestation, disinfection and/or protection.[4] Seed disinfestation is the control of spores and other forms of disease organism on the seed surface. Seed disinfection is the elimination of a pathogen that has penetrated into the living cells of the seed, infected it and become established. Seed protection is the application of a treatment to protect the seed from seedborne and soilborne disease organisms, such as *Pythium*, *Fusarium* and *Rhizoctonia* in particular, which can cause seed rot, pre-emergence damping-off and seedling blight of many crops. With systemic fungicides used as seed protectants, these can often provide post-emergence protection of the crop against foliage diseases for several weeks after plant emergence.

2 SEED TREATMENTS

2.1 Fungicide treatments

Historically, fungicides were developed from sulfur, copper and mercury compounds. The toxicity of these compounds for seeds

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and the development of newer and more specific molecules contributed to the decline in the use of such inorganic compounds. In the case of mercury compounds, their toxicity to warm-blooded animals and the accumulation of mercury in the environment have resulted in their being banned.[5] The newer systemic fungicides have largely replaced inorganic compounds, and they can be extremely efficient. Furthermore, systemic fungicides can pose less of a risk to crops, animals and the environment because they may be readily degraded by soil microorganisms, which prevents their accumulation in the soil. Before the mercurials were banned, fungicides for treating seed were classified as volatile and non-volatile. With the removal of the volatile mercurials, most fungicides now approved for use on seed are classified as non-volatile.[6]

Fungicides applied to seeds can be broad spectrum, i.e. toxic to all or many kinds of fungus, or narrow spectrum, i.e. effective only against a few species. Contact fungicides are only effective against fungal spores on the surface of a seed, and consequently they have no effects on internal fungal seed infections, such as loose smuts infection. Translaminar or cytotropic fungicides can penetrate into the superficial layers of seeds to counter shallow fungal infections. Other fungicides are characterised by systemic activity, and these are effective against fungal diseases deep within the seed, and can also give protection against early infection from airborne and soilborne diseases. However, such agents are more effective at later stages of seedling development, when seed treatment is supplemented with foliar sprays.

2.2 Physical treatments

Physical treatments consist of heat treatments of seeds, with the most common being hot water, hot air and electron treatments. Thermotherapy inactivates or kills the pathogen, while it leaves the host tissue viable.[7] Among these physical treatments, hot water treatment is a long-known technique that consists in the immersion of plant material in agitated water at a predetermined temperature and time. In the past, hot water treatment was frequently used for sanitisation of contaminated cereal seeds,[8] and this is now receiving new attention.[9,10] Aerated steam[11] and electron seed treatments are two of the more modern physical seed treatments, and these are under intensive investigation, particularly as they have proved to be highly effective in several host–pathogen systems.[3,12] Seed treatments with carefully regulated aerated steam at a correct intensity make it possible to kill the pathogens while leaving the seeds unharmed. During electron seed treatment, the electrons act within milliseconds on the surface and in the seed coat, destroying the DNA of present harmful organisms and keeping intact the interior of the seed. In recent years, numerous tests have been carried out to determine whether these treatments can be used to eradicate pathogens that affect vegetable seed crops.[13] For the success of these treatments, pretests with germination assays are necessary to determine the optimum treatment for a given seed batch. In experimental trials, the performance of physical treatments under controlled conditions and in the field appears to be largely comparable.

2.3 Treatments with biopesticides

Increasing use of chemical inputs can have several negative effects, which include the development of pathogen resistance to the applied agents and the non-target environmental impact. There is a growing awareness that integrated pest management strategies can provide more environmentally sound and economically feasible alternatives for seedborne and soilborne disease management.[14] Furthermore, the growing cost of pesticides, particularly in the less affluent regions of the world, and consumer demand for pesticide-free food have led to the search for substitutes for such chemical compounds. There are also certain diseases for which chemical solutions are few, ineffective or non-existent.[15] All of these factors have led to an increasing demand for alternatives to the use of synthetic fungicides. Biological control is thus being considered as an alternative or a supplement to reduce the use of synthetic chemicals in agriculture.[16] Seed treatments that include plant extracts and biocontrol agents offer an attractive way to replace the use of synthetic fungicides.

2.3.1 Plant extracts and natural compounds

Plants extracts can contain natural antimicrobial compounds, and these can be used for seed disinfection as an alternative to fungicide treatments, or in combination with physical treatments.[17] These extracts include essential oils, of which there are several kinds that have shown good antifungal activities in *in vitro* trials, including tea tree, clove, peppermint, rosemary, laurel, oregano and thyme oils. Such oils have been reported to be active against pathogens like *Ascochyta* spp., which are responsible for Ascochyta blight on *Fabaceae*, and *Alternaria* spp., which affect carrot seeds.[18] Among the essential oils, thyme oil has most frequently shown the best effectivity in *in vitro* and field tests. Thyme oil contains thymol and other antifungal compounds,[19] which provide general antimicrobial activity against seedborne bacteria and fungi.[20] Other effective natural compounds have been extracted from plants that belong to the genus *Allium*. These plants produce various sulfur-containing compounds, and some of these have been shown to have antimicrobial effects.[21] Onion seed exudates include sugars, amino acids, organic acids and phenolic compounds, which can be released during seed imbibitions, and these can have an inhibitory effect on pathogenic fungi,[22] which are not able to colonise seeds.[23]

Chitosan is derived from crab-shell chitin, and it is a biopolymer with antifungal properties that have been shown to be effective against several fungi. Chitosan acts by chelating nutrients and minerals, which prevents pathogens from accessing them or enhances plant innate defences following induction of the host defence responses.[24,25] The resistance that is induced by this abiotic agent is broad spectrum and long lasting, although it will rarely provide complete control of an infection. This plant resistance is influenced in the field by environment, genotype and crop nutrition.[26] When chitosan is applied to seeds, the most frequently produced effects are an increase in germination index, a reduction in mean germination time and greater shoot height, root length and root and shoot weights. The efficacy of chitosan as a seed protectant against several pathogens has been reported in numerous studies carried out on cereal crops, such as for wheat,[27] maize,[28] pearl millet[29] and oil-bearing crops,[30] and also on several horticultural crops.

2.3.2 Biocontrol agents

Biological control of fungal plant pathogens appears to be an attractive and realistic approach, and numerous microorganisms have been identified as biological control agents (BCAs) in terms of both fungi and bacteria. BCAs can be used in combinations or as replacements for fungicides, and they have gained wide acceptance. This is especially so for fungal-based BCAs, primarily because of their broader spectrum in terms of disease control and production yield.[31] These microorganisms can be used as seed treatments, and in some cases might be applied during seed priming. Seed priming is a hydration treatment that includes application of osmotic stress to the seeds prior to drying-back. It allows controlled imbibition and induction of the pregerminative metabolism, but radicle emergence is prevented: in this way the emergence of directly seeded crops is improved, particularly under wet or cold conditions, and quick and uniform emergence is provided.[32–34] With the use of this technique, BCAs can be applied and three main stages are provided: hydration, incubation and drying of the seeds. BCAs are added as suspension in water during the first phase. On onion and carrot, fungal-based BCAs, applied during seed priming, seem to survive better than bacterial ones: *Clonostachys rosea* (Link:Fr.) Schroers, Samuels, Siefert and W. Gams and *Trichoderma harzianum* Rifai showed good survival in the following weeks, and in particular the former increased significantly in number.[32]

For effective protection against plant pathogens, an antagonist must be able successfully to colonise the rhizosphere of a plant[35] and to compete with other microorganisms in the root system of the plant, to inhibit the attack of pathogens.[36–38] It has been shown that colonisation patterns differ, depending on the type of antagonist microorganism used.[39]

Another aspect to take into consideration for the success of colonisation by BCAs is the physical conformation of the seed coat (texture and ornamentation). This determines the different spatial colonisation patterns of microorganisms, because some sites, such as grooves or cracks on the surface of a seed, are more favourable for pathogen growth; for example, the roughness of carrot and tomato seeds might provide more niches for pathogen survival than smooth onion seeds.[40,41] Therefore, it is important to examine seed type and microorganism combinations for their compatibility, as not all microorganisms will become established on every seed type.

The inoculation of seeds with BCAs does not lead to changes in the ecophysiological structure and physiological profiles of the rhizosphere bacterial community. This is unlike fungicide treatments, which can alter the metabolic profiles of the culturable rhizosphere bacterial communities.[42] The subsequent survival and establishment of the beneficial microorganisms in the rhizosphere once the seed is planted is of fundamental importance for continued promotion of plant growth and disease control. Although microorganisms behave differently from each other, bacteria such as *Pseudomonas chlororaphis* (Guignard & Sauvageau 1894; Bergey *et al.*, 1930) and *Pseudomonas fluorescens* (Trevisan 1889) Migula 1895 tend to decrease in number in rhizosphere soil and on roots over time, whereas fungi such as *C. rosea* and *T. harzianum* remain constant or increase in number.[32] Another important aspect to consider is that many BCAs are difficult to formulate as products, and, in spite of their demonstrated effectiveness against phytopathogenic microorganisms, they do not reach the marketplace.[43]

To control different diseases that affect the same crop, the association of several microorganisms is needed, although most BCAs are specific only for a given type of target pathogen. The combination of two or more BCAs means multiple registration processes, with increased costs and difficulties in providing all of the studies required according to strict legislation. However, a solution might be the labelling of already registered biofungicides, based on different antagonistic strains, as compatible with each other and proposed for joint use.[14]

Among BCAs, species belonging to the genus *Trichoderma*, which is a free-living fungus that is common in soil and root ecosystems, have been widely used as antagonistic agents against several pests, in addition to their use as plant-growth enhancers. Their biocontrol actions include mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites and induction of plant defences. Experimental trials conducted with several crops have shown the effectiveness of *Trichoderma* spp. as seed treatments against soil- and seedborne pathogens such as *Pythium*, *Phytophthora*, *Rhizoctonia* and *Fusarium* spp.[44–46]

Other BCAs used for seed coating are the plant-growth-promoting rhizobacteria (PGPR), a group of free-living bacteria that colonise the rhizosphere while producing hormones,[47,48] vitamins and growth factors that improve plant growth and increase plant yield.[49] PGPR promote a reduction in the populations of a broad spectrum of root and foliar pathogens that are found in the rhizosphere, and they show different mechanisms of action: antibiosis,[50] competition for space and nutrients, parasitism and induction of systemic resistance in plants.[51–53]

The success of PGPR is influenced by a number of biotic and abiotic components that represent limiting factors for root colonisation. The quality of PGPR formulations, in terms of viability and efficacy, determines their large-scale adoption at the end-user level.[54,55] Among PGPR, the gram-negative bacteria *Pseudomonas* spp. are widely used because they have a number of positive features, such as: the ability to grow rapidly *in vitro* and to be mass produced, rapidity in the use of seed and root exudates, colonisation and multiplication in the rhizosphere and within the plant, production of a wide spectrum of bioactive metabolites (i.e. antibiotics, siderophores, volatiles and growth-promoting substances), aggressive competition towards other microorganisms and adaptability to environmental stress.[56]

In addition, pseudomonads are responsible for the natural suppression of some soils towards soilborne pathogens.[57] Several species of *Pseudomonas* have been shown to be effective against plant diseases caused by soilborne and seedborne pathogens and the agents of canker. The major weakness of pseudomonads as biocontrol agents is their inability to produce resting spores (as do many *Bacillus* spp.), which complicates the formulation of these bacteria for commercial use; indeed, they are formulated as frozen cell pellets that must be kept on dry ice until application.[58] Fluorescent pseudomonads are the most applied as biocontrol agents for horticultural seed production.

The gram-positive *Bacillus* spp. are also PGPR, and their principal mechanisms of growth promotion include the production of growth-stimulating phytohormones, solubilisation and mobilisation of phosphate, siderophore production, antibiosis, inhibition of plant ethylene synthesis and induction of plant systemic resistance to pathogens.[59–63] The species that can promote significant reductions in the incidence or severity of many diseases include *Bacillus amyloliquefaciens* (Priest *et al.*, 1987), *Bacillus subtilis* (Cohn, 1872), *Bacillus pasteurii* (currently known as *Sporosarcina pasteurii* Bergey 2004), *Bacillus cereus* Frankland & Frankland, 1887, *Bacillus pumilus*, *Bacillus mycoides* and *Bacillus sphaericus*.[64,65] Other such gram-positive bacteria include the streptomycetes, which are active in the rhizosphere and effective in the biocontrol of plant pathogens through different modes of action, including antibiosis, lysis of the fungal cell wall, competition and hyperparasitism.[66,67] In particular, a strain of *Streptomyces griseoviridis*, the K61 strain, was isolated from light-coloured *Sphagnum* peat and has been developed as a biofungicide. This is due to its ability to control or suppress some root rot and wilt diseases caused by *Pythium*, *Fusarium*, *Rhizoctonia* and *Phytophthora* spp. by colonising the rhizosphere prior to these pathogens.[68,69] Unlike gram-negative microorganisms, sporulating gram-positive microorganisms can be formulated readily into stable products such as a dry powder through their heat-resistant and desiccation-resistant spores. However, in spite of the greater ease of use, gram-positive bacteria have received less attention in the literature on biocontrol than the fluorescent pseudomonads, in part because gram-positive organisms have been less tractable for genetic studies, and in part because less is known about the mechanisms by which they suppress disease.[58] A recently discovered BCA, but not yet applied as seed treatment, is *Piriformospora indica* Sav. Verma, Aj. Varma, Rexer, G. Kost & P. Franken (1998), an arbuscular mycorrhizal fungus belonging to Basidiomycota. This BCA, besides having a positive influence on growth and development of many different plants, induces tolerance against salt stress and resistance against root and shoot pathogens, e.g. wheat and tomato pathogens,[70] and therefore its future development may be of considerable interest in the context of seed treatments.

Of note, according to the experimental trials carried out to date, is the fact that the efficacy of biological treatments on vegetable seeds is often better in a greenhouse than in the field.

3 TREATMENT OF DIFFERENT PATHOSYSTEMS

In the following sections, fungicide, physical and biopesticide treatments against the main seed-transmissible fungal pathogens of some important vegetable crops will be defined and discussed (Table 1).

3.1 *Daucus carota/Alternaria dauci, A. radicina*

Alternaria leaf blight and *Alternaria* black rot are the most destructive diseases of carrot, and they are caused by *Alternaria dauci* (Kühn) Groves & Skolko and *Alternaria radicina* Meier Drechsler & Eddy respectively. These diseases have spread to all carrot production areas in the world, and they commonly occur when carrots are cultivated under conditions of moderate temperatures, where the leaves are exposed to prolonged periods of wetness due to rainfall, dew or sprinkler irrigation.[71–79]

Fungicide applications to seeds can minimise seedborne infections of both *A. dauci* and *A. radicina*, which results in fewer infections in the early season in production crops. Fungicides commonly applied for the control of both of these *Alternaria* species include chlorothalonil,[80] iprodione,[81,82] pyraclostrobin and azoxystrobin,[80] which have been shown to provide excellent disease control in field evaluations. These fungicides are used both as foliar sprays and as seed treatments to control seedborne *A. radicina* and *A. dauci*. Other fungicides that are applied as seed treatments are also effective in the control of both of these pathogens, including thiram,[10] boscalid,[80] maneb, macozeb, benomyl and thiofanate methyl.[83] *In vitro* trials of other active ingredients, such as trifloxystrobin and tebuconazole, have shown sufficient inhibitory activity on the growth of *Alternaria* spp. colonies, including for *A. alternata* and *A. dauci*.[84] Infected seeds treated with fungicides can reduce, but not eliminate, contamination,[85–87] which highlights the fact that the contamination of carrot seed by *Alternaria* spp. is a continuing problem, and new methods of seed decontamination are required.

Table 1. Main seed-transmissible fungal pathogen species on some vegetable crops

Vegetable host crops		Seed-transmissible fungal pathogen species
Apiaceae	*Daucus carota* L.	*Alternaria dauci* (Kühn) Groves & Skolko
		Alternaria radicina Meier Drechsler & Eddy
Brassicaceae	*Brassica* spp.	*Alternaria brassicicola* (Schwein.) Wiltshire
		Leptosphaeria maculans (Desmaz.) Ces. & De Not.
Solanaceae	*Lycopersicon esculentum* Mill.	*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hans.
		Alternaria solani Sorauer
Fabaceae	*Pisum sativum* L.	*Ascochyta pisi* Lib.
		Ascochyta pinodes L.K. Jones
		Ascochyta pinodella L.K. Jones
	Cicer arietinum L.	*Ascochyta rabiei* (Passerini) Labrousse
	Lens culinaris Medik.	*Ascochyta lentis* Bond. & Vassil.
	Vicia faba L.	*Ascochyta fabae* Speg.

Good and consistent disease control has generally been achieved by physical methods. Hot water treatment of carrot seed at 50–53 °C for 10–30 min has been shown to be >95% effective against *A. dauci* and *A. radicina* for the reduction of disease symptoms in carrot, following blotter and greenhouse tests.[88] Similar results were reported in another study, where water treatment of carrot seeds at 44 °C and 49 °C reduced the incidence of *A. dauci* and eradicated it following treatment at 54 °C for 20 min.[89] However, in a blotter test, where a highly infested carrot seed lot was used, the same treatment did not completely eradicate the pathogens.[10] On carrot seeds in the field, hot air treatments have the best eradicating effects against *A. dauci* and *A. radicina*, which are as effective as chemical treatments, while electron treatments have shown low efficiency.[10] However, the results obtained in different studies regarding the efficiency of physical treatments have not always been in agreement, which might be due to the application of fixed treatments that are not specifically adapted to specific seed lots.

Thyme oil *in vitro* tests have shown antimicrobial activity against *A. dauci*.[20] For carrot seeds emulsified in water, thyme oil percentages from 0.1 to 1% provide good efficacy for the reduction of *A. dauci* and *A. radicina*.[10] Another essential oil that has shown good antimicrobial activity against *A. dauci* in *in vitro* assays is manuka oil.[20] Also allicin, which is a volatile antimicrobial substance that is produced in garlic when the tissues are damaged and the substrate alliin (*S*-allyl-L-cysteine sulfoxide) mixes with the enzyme alliin lyase, has shown antimicrobial action against several pathogens, including seedborne *Alternaria* spp. in carrot. Soaking carrot seeds with allicin-containing preparations, followed by subsequent drying-down, resulted in a high rate of germination of *Alternaria*-infested carrot seeds, comparable with results obtained with the industrial seed dressing with thiram.[90]

Among the BCAs, strains of *Pseudomonas*, including *P. fluorescens*, applied to carrot seeds led to significant protection of carrot plants against *A. dauci* and *A. radicina*, promoting seed emergence and controlling disease. This efficacy was generally lower than that of the chemical standards and the best physical treatments,[10] but in the case of *Burkholderia cepacia* (Palleroni and Holmes 1981) Yabuuchi *et al.* 1993, its efficacy against carrot black rot was as good as that of chemical treatment with iprodione.[81] Another microorganism that is effective in controlling *Alternaria* spp. on carrot is *C. rosea*, which when applied as seed treatments produces a significant increase in plant stands, both in seed tray tests and in field experiments.[10] Furthermore, the biopriming of highly infested carrot seeds with this BCA reduced incidence of *A. radicina* from 29 to <2.3%, and incidence of *A. dauci* from 11 to <4.8%.[82]

3.2 *Brassica* spp./*Alternaria brassicicola, Leptosphaeria maculans*

Alternaria brassicicola (Schwein.) Wiltshire and *Leptosphaeria maculans* (Desmaz.) Ces. & De Not., the causal agents of black spot disease and of blackleg disease respectively, are important seedborne pathogens affecting every important cultivated *Brassica* species.[91–96]

The most common fungicides that are effective in controlling *A. brassicicola* infection of *Brassica oleracea* L. seeds are those based on thiram and iprodione, which can reduce disease both in the greenhouse and under field conditions.[97,98] Against *L. maculans*, the use of fungicides based on flutriafol and fluquinconozole has provided reliable economic responses. These are applied as a seed dressing to the canola before sowing, and they reduce the damage caused by the fungus also with high disease severity and with cultivars with lower blackleg resistance.[99,100] Flutriafol decreases the severity of blackleg disease on the canola with remarkable effectiveness at the cotyledon stage, and also when applied to the seeds at a low dose (6 g kg^{-1} seed) so as not to be phytotoxic. Seed treatments with acibenzolar-*S*-methyl alone as the active ingredient, which is an activator of systemic acquired resistance, do not reduce disease severity; however, in combination with flutriafol, a synergistic reduction in disease severity on the cotyledons is obtained when this is applied to seeds.[101]

Hot water treatments of cabbage seeds have been shown to be effective in controlling *A. brassicicola* and *L. maculans*. Specific water treatments at 50 °C for 25–30 min and at 53 °C for 10 min were shown to reduce *L. maculans* infections by 87–92%, and *A. brassicicola* infections by 92–99% respectively.[88] As cabbage seed treatments at too high a temperature, i.e. at 53–55 °C, for periods longer than 20 min reduce seedling emergence, temperatures lower than 53 °C or shorter treatment times are recommended, to avoid delay in germination and emergence, as cabbage is a sensitive crop.[88,102] Another important parameter to consider is seed maturity: the less mature the *B. oleracea* seeds, the more susceptible they are to hot water and aerated steam treatments.[2] In another study, aerated steam and electron treatment appeared to be more reliable against *A. brassicicola* than hot water treatment, with the latter providing more variable results.[98] Negative results are probably due to the limited pretesting carried out for hot water treatments.[10] These results can be improved with better adaptation of the chosen treatment parameters to the seed lot used.

Good control of *A. brassicicola* on cabbage has also been reported following seed treatment with thyme oil. However, for thyme oil the choice of concentrations that do not impair seed germination is important.[98]

Among the BCAs, several products based on *B. subtilis* have been applied as seed treatments and have increased the numbers of healthy cabbage plants while reducing infection by *A. brassicicola*;[98] similarly, a strain of *S. griseoviridis* has been shown to reduce the inoculum of the same pathogen on cabbage[98] and on Chinese cabbage [*Brassica pekinensis* (Lour.) Rupr. (Valkonen & Koponen 1990)].

Seed treatments of oilseed rape (*Brassica napus* L.) directed against the blackleg disease caused by *L. maculans* have shown that the rhizobacteria *Serratia plymuthica* Breed *et al.* 1948 and *P. chlororaphis* can reduce disease severity by 71.6 and 54% respectively. However, with the application of these two rhizobacteria in combination, using the biopriming technique, the effectiveness of the combined treatment was not superior to that with *S. plymuthica* alone.[103] In another study, a greater reduction in disease infestation under field conditions was provided by the combined treatments of *S. plymuthica* and *Clonostachys rosea* f. *catenulata* (J.C. Gilman & E.V. Abbott) Schroers with metconazole.[104]

3.3 *Lycopersicon esculentum*/*Fusarium oxysporum* f. sp. *lycopersici*, *Alternaria solani*

Tomatoes are parasitised by several pathogens, and the main seed-transmissible ones are *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hans., the causal agent of *Fusarium* wilt of tomato,[105–107] and *Alternaria solani* Sorauer, which is responsible for early blight.[108–110]

The fungicides that are most commonly applied to tomato seeds to limit the onset of *Fusarium* wilt symptoms are chlorothalonil, mancozeb, mefenoxam, thiram and azoxystrobin.[111]

In terms of plant extracts, *in vitro* tests have demonstrated that neem leaf extracts added to growth media prior to inoculation effectively suppress mycelial growth of *A. solani* and *F. oxysporum* f. sp. *Lycopersici*. These effects are seen at different concentrations (5, 10, 15 and 20%) in aqueous, ethanol and ethyl acetate solutions, and they are gradually enhanced with increasing concentration. When assayed at a concentration of 20%, both ethanol and ethyl acetate extracts of neem leaves completely suppressed the growth of *F. oxysporum* f. sp. *lycopersici* and inhibited *A. solani* by 52–63%.[112] Leaf extracts of zimmu (*Allium cepa* L. × *Allium sativum* L.) demonstrated very high inhibition of mycelia growth of *A. solani* (87%) *in vitro* and good reduction of symptoms under greenhouse conditions.[113]

Pretreatment of tomato by soaking the seeds in 0.1 mM of methyl jasmonate, a volatile organic compound and plant signalling substance, efficiently reduced the development of early blight caused by *A. solani*, while also increasing the levels of defence markers, such as total phenolics, anthocyanins and phenylalanine ammonia-lyase activity.[114] Several biocontrol agents have been shown to be effective in controlling tomato wilt. This includes *P. fluorescens*, which after seed biopriming increases seedling emergence and reduces *Fusarium* wilt incidence on tomato.[115] Similarly, in addition to a clear boost in plant growth, *Brevibacillus brevis* (Migula 1900) Shida *et al.* 1996 considerably reduced tomato wilt.[116] Also, talc-based formulations of *S. griseus* Waksman and Henrici 1948 AL applied to tomato seeds are effective in controlling *F. oxysporum* f. sp. *lycopersici* incidence under greenhouse conditions, especially if applied with chitin, which shows their ability to interfere with the wilt disease cycle.[117] The combined use of more than one biocontrol agent, or their combination with other alternative methods, has often ensured better effectiveness of seed treatments. The combination of fluorescent *Pseudomonas*,

Table 2. Effectiveness of the different treatments against the pathogens considered

	Type of treatment									
	Fungicides		Physical means		Plant extracts or essential oils		BCAs		BCAs + plant extracts or essential oils	
	Rate	Cases (*n*)	Rate	Cases (*n*)	Rate	Cases (*n*)	Rate	Cases (*n*)	Rate	Cases (*n*)
Alternaria dauci, *Alternaria radicina*	+++[a]	9	+/+++	3	++	3	+/++	3	/	
Alternaria brassicicola, *Leptosphaeria maculans*	+++	5	+/+++	5	++	1	++	3	++	2
Fusarium oxysporum f. sp. *lycopersici*, *Alternaria solani*	+++	1	/		++/+++	3	++	4	++	1
Ascochyta spp.	+++	9	−	3	++	1	+	1	/	

[a] +++ = effectiveness >80% compared with the control; ++ = effectiveness between 50 and 80%; + = effectiveness between 20 and 50%; − = effectiveness <20%.

T. harzianum and *Glomus intraradices* N.C. Schenck & G.S. Sm., which is an arbuscular mycorrhizal fungus, was more effective than the single isolated treatments and reduced *Fusarium* wilt incidence and severity by 74 and 67% in pots and fields respectively, and also increased the yield by 20%.[118] In another study, a talc-based formulation of two strains of *P. fluorescens*, a strain of *B. subtilis* and zimmu leaf extract was applied as seed treatment and as foliar spray. This produced the greatest reductions in early blight disease and the greatest seed germination when compared with other treatments, including mancozeb treatment. A previous study suggested that the application of BCAs along with plant extracts might help to overcome pathogen infections by also increasing the levels of defence-related enzymes and phenolic substances.[113]

3.4 *Fabaceae/Ascochyta* spp.

Species belonging to the genus *Ascochyta* are the main seedborne fungi that affect several vegetable crops of *Fabaceae*, thus causing *Ascochyta* blight.[119] These include *Ascochyta pisi* Lib. and *Ascochyta pinodes* L.K. Jones on pea, *Ascochyta rabiei* (Passerini) Labrousse on chickpea, *Ascochyta lentis* Bond. & Vassil. on lentil and *Ascochyta fabae* Speg. on faba bean. Another closely related species found on pea is *Ascochyta pinodella* L.K. Jones, which frequently occurs on pea and causes symptoms on internodes and leaves that are similar to those induced by *A. pinodes*. This is one of the three species in the *Ascochyta* blight complex of pea,[120,121] which differentiates *Ascochyta* blight of pea from that of lentil, faba bean and chickpea, all of which are caused by a single fungal species.

Fungicide seed coatings have been shown to prevent spore germination and to reduce mycelial growth on the Fabaceae seed surface,[122] which increases seedling emergence. Fungicides against *A. rabiei* applied to chickpea seeds with high levels of natural or artificial infection can reduce mycelial growth and spore germination to a minimum; these include treatments based on maneb, on benomyl plus thiram or plus captan, on tridemorph plus maneb and on thiabendazole.[122–126] Also, active ingredients, such as metalaxyl, thiabendazole, ipconazole and azoxystrobin, can increase the percentages of healthy plants, with greater effectiveness if they are applied as a mixture.[127] Fungicide seed treatments with thiabendazole and benomyl, which were tested under field conditions on *A. lentis*-infected seeds, can induce considerable seedling emergence and significant yield benefits.[128] Thiram and thiabendazole applied as seed treatments are also recommended against *Ascochyta* spp. in faba bean and for other grain legumes.[129] Under certain conditions, some fungicides show phytotoxic actions when coated onto Fabaceae seeds, where treatments with imazalil, thiram and tridemorph/maneb can cause stunting, chlorosis and loss of seed vigour,[130] and treatments with benomyl plus thiabendazole and with tridemorph and chlorothalonil can cause reduction of chickpea seed germination.[125,126] Similarly, when triadimefon, triadimenol, etaconazole and thiram were applied as a soak treatment for lentil seeds, these adversely affected plant growth, vigour and yield.[128]

Among the alternative treatments, seeds treated with a strain of *C. rosea* and thyme oil at 40 °C have been shown to increase the numbers of healthy plants and reduce *Ascochyta* spp. on pea. This good control with thyme oil might be due to the elevated temperature, as this can facilitate the penetration of the active components into the seed coat, or it might be due to the elevated temperature alone.[12] Hot water, hot carbon tetrachloride and steam/air mixtures all failed to provide control of *Ascochyta* infection on legumes.[123,125,128] The difficulty of controlling seedborne *Ascochyta* spp. with alternative treatments might at least partly be explained by the position of these pathogens in the seed.[131] In most seeds infected with *A. pisi* the fungus is situated beneath the testa, and in about 40% of the seeds the embryo is also attacked,[12] making it also particularly difficult to control *Ascochyta* spp. with fungicide seed treatments.

4 CONCLUDING REMARKS

The present review has covered seed treatments that are effective in the reduction or eradication of the main seedborne pathogens that affect some important vegetable crops. As well as reducing the quantity and quality of the seed harvested, seedborne pathogens can be preserved in seed lots, which provides a massive boost to the spread of plant pathogens.[1] Besides traditional seed treatment with fungicides, further support can be provided by alternative, environmentally friendly seed treatments. Some of these have already been in use in the past, like hot water treatments, while others have only been applied in more recent years, like treatments with BCAs. Recourse to natural products with antimicrobial properties that can reduce inoculum levels in seeds and disease incidence in seedlings is increasingly frequent, even given the limitations in the use of conventional chemical products through the implementation of integrated pest management techniques that are to become obligatory practice in the EU (Directive 2009/128/EC) by 2014. In general, in all of the vegetable/pathogen systems considered, fungicide treatments can reduce, if not eradicate, seedborne pathogens, showing an effectiveness always greater than 80% compared with the control, thus remaining more reliable than alternative treatments (Table 2). In spite of this, alternative treatments in some cases have proved to be as effective as chemical treatments, especially for physical treatments, which provide the best seed protection, in particular against *A. dauci*, *A. radicina*, *A. brassicicola* and *L. maculans*. For this type of treatment, the adaptation of its duration and temperature to the specific seed lot is fundamental; otherwise there may be a lack of effectiveness. Plant extracts and essential oils showed a good effectiveness against all the pathogens, ranging from 50 to 80% compared with the control. Among the plant extracts, thyme oil has been shown to be more frequently effective than other natural compounds against various pathogens.

BCAs have in some cases proved to be less effective than the previous alternative treatments, e.g. against *A. dauci*, *A. radicina* and *Ascochyta* spp. The lack of effectiveness of BCAs against some pathogens is probably due to several extrinsic factors that influence the seed-coat colonisation, particularly involving the physical conformation of the seed coat, and the BCA formulation as a commercial product. Indeed, the latter is of paramount importance for the viability and efficacy of any antagonistic microorganisms. The combination of essential oils or plant extracts with BCAs, compared with the use of these individually, has ensured a greater protection against pathogens such as *A. brassicicola*, *L. maculans*, *F. oxysporum* f. sp. *lycopersici* and *A. solani*.

In conclusion, the treatments of seeds with fungicides or alternative compounds represent good methods for their protection, disinfestation or disinfection from seedborne pathogens. The success of such treatments depends on the pathogen localisation at the seed level, but they can provide improved stand quality and increased yields.

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