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Review of the detection tools for seed-borne pathogens and the seed treatments that are applicable in organic seed production

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1. Introduction

With changing climatic conditions and a rapidly growing world population estimated to reach 9 billion by 2050, humankind faces the serious challenge of increasing food production by at least 70 %. The vision of BRESOV is to tackle this challenge by exploring the genetic diversity of three of the economically most significant vegetable crops (broccoli, snap bean, and tomato) and to improve the competitiveness of these three crops in an organic and sustainable environment. The consortium's overall aim is to increase the plants' tolerance to biotic and abiotic stresses and adapt the varieties to the specific requirements of organic and low-input production processes.
In this frame we have pointed our attention to microbes actively involved in vegetable production generally called plant growth promoting bacteria (PGPBs) and plant growth promoting rhizobacteria (PGPRs) which improve the performance and health of the crops playing a positive role on supplying nutrients to crops, producing phytohormones, biocontrol of pathogens, improving soil structure, bioaccumulation of inorganic compounds and bioremediation of metal contaminated soils. In addition, the natural compounds, as such as glucosinolates (GLSs) or propolis, are widely utilized for pathogens control in organic agrosystems and the list of natural compounds (NCs) useful for this task is long.
Sustainable agriculture needs to implement the interactions among beneficial soil microbiome and organic matter, NCs and the plant, improving plant health and soil fertility and reducing the conventional agricultural inputs through combining beneficial microorganisms.

It is known that the two main factors affecting the development of organic farming in Europe are the limited quantity and the poor quality of organic seed available on markets (bad germination, pest contamination, and contamination with weed seed). Therefore, WP4 (High quality organic seed production) aims to develop the protocols and tools which suit to the specific conditions of organic farming to maximize yield (T4.1) and ensure high quality (T4.2 and T4.3) of organic seeds in broccoli, snap bean and tomato.

Specific objectives of WP4:
- O4.1: Develop protocols adapted to the specific conditions of organic farming to improve organic seed yield.
- O4.2: Determine products and tools to control the sanitary and genetic quality of organic seed lots.
Task 4.2 foresees the evaluation of alternative seed treatments to the use of chemical treatments to control sanitary quality of seed lots.

In fact the organic farming prohibits the use of conventional chemicals to control pests and diseases, so alternative Biocontrol agents (BCAs) and NCs, as well as mechanical treatments, will be evaluated on seed for its protection against seed-borne pathogens and for seed vigor enhancement.

2. Description of Activities

The review was prepared by the partners involved in the T4.2 in order to support BRESOV stakeholders to adopt the new detection tools for the target major pathogens under organic cultivation and the seed treatments for diseases control under these conditions. The crops/pathogens under study are: tomato/ *Clavibacter michiganensis*, *Pseudomonas syringae* pv. *tomato, Xanthomonas* spp., tomato mosaic virus (ToMV), *Fusarium oxysporum f.sp. radicis lycopersici*; broccoli/, *Xanthomonas campestris* pv. *campestris*, *Alternaria* spp., *Phoma lingam* (*Leptosphaeria maculans*); bean/ *Pseudomonas savastanoi* pv. *phaseolicola*, *Fusarium solani* f.sp. *phaseoli*, *Colletotrichum lindemuthianum*. For these diseases each partner involved in

T4.2 collected relevant literature references which provided information on the detection tools for the diseases and the BCAs, NCs and physical treatments till now evaluated and/or validated for their control in seeds.
Particular attention was paid not only to individuate BCAs and NCs that are useful for controlling the above cited seed-borne diseases but also to the methodologies of their use in order to facilitate the inoculation of the microorganisms for increasing their adaptation to sub-optimal environmental conditions or to use NCs avoiding phytotoxicity to the crop and maximizing their effects.

Each partner shared, in relation to the own expertise, their knowledge and reference related to:
i) Pathogen on-line resources and seed detection (official protocols);
ii) Bibliography detection methods;
iii) Biocontrol agents (BCA) treatments,
iv) Plant extracts or compounds treatments;
v) Physical treatments;
vi) Registered bio-active substances mainly for seed treatments.

With regards to the pathogen detection we elaborated a table that could be available on line as public resource that summarize all information that regards the pathogens under study in the different research groups and updated official seed detection methods when available or laboratory consolidated methods. The resources were extracted from the European and Mediterranean Plant Protection Organization (EPPO), International Seed testing Association (ISTA) protocols, International Seed Federation (IFS) protocols, European Food Safety Authority (EFSA).
Although official protocols are available and most of them have been updated recently a table was prepared to be filled during the project to scout new methods that could have a further exploitation for pathogen detection in seeds. The table, in which the revised pathogen nomenclature was updated has space available for new methods based on molecular detection (PCR, Real-time PCR, isothermal amplification, other methods) as well for serological methods.

For the BCA, natural phytoextracts and physical treatments we listed the BCA/natural phytoextracts/physical treatments, *in vivo* and *in vitro* tests, bibliography and their DOI or link. Finally for the registered substances we listed: the registered active substances for seed coating, the registered active substances (Italy), Company, products and active substances.

3. Results

For the target diseases, each partner involved in T4.2 has collected several relevant references which dealt with both the detection tools for the disease and the BCA, NCs and physical treatments evaluated up to now and validated for their control. Many of the microorganisms and natural compounds are not registered for seed treatments.

On the basis of our research we found only two commercial products registered in EU for seed-borne diseases which are Cerall (Pseudomonas chlororaphis) commercialized by Serbios company and Mycostop (Streptomyces griseoviridis K61) commercialized by Bioplanet. On the other hand, we have found many other microrganisms and natural compounds where any interaction on seed borne pathogens will be evaluated as well as PGPR and biostimulant activities.

The registrations of microorganisms and natural produces (phytoextracts, oils, etc.) will be implemented by the recent revision proposal of the Regulation of the European Parliament and of Council laying down rules on the making available on the market of CE marked fertilising products and amending Regulation (EC) No 1069/2009 and (EC) No 1107/2009, which are aimed at ensuring an internal market in fertilisers. This regulation mainly addresses mineral fertilisers and deters the introduction of new types of fertilisers, as such as biostimulants, mainly represented by microorganisms mixtures and natural phytoextracts. The negotiation of the proposal among the stakeholders, EU representative and experts will open new perspectives for the use of biostimulants in the next future.

In this frame WP4 aims to test the beneficial effect of the BCAs, NCs and physical seed/plant treatments in order to provide high quality organic seed production to the growers.

In Annex no. 1 we listed the actual nomenclature for each disease and the revised one for *Xanthomonas spp.* pathogenic to tomato crop, and for *Alternaria spp.* and for *Leptosphaeria maculans* of broccoli crop.
In addition we listed for all the eleven diseases studied. For each of them we listed the availabes EPPO diagnostic standard number and the online link of protocols and documents, providing the more recent information about.
In Annex no. 2 we listed the most recent detection methods for seed by PCR, real-time PCR, isothermal amplification and other methods.
The BCA seed treatments are listed in Annex no. 3 for each of the eleven diseases studied indicating for each of them the microorganism utilised and the related bibliography and their DOIs or web links.
The natural compounds and the physical agents for seed treatments evaluated and validated for controlling the studied diseases are listed in the Annexes no. 4 and 5. Finally are listed in annex no. 6 the only two substances registered for seed coating only for cereals and the active substances registered in Italy for organic agriculture use utilized for disease control. Annex no. 6 also lists the products allowed for seed treatment in organic agriculture in Switzerland.

This resources will be also implemented during the project.

Among the above-cited treatments the more difficult to use are related to the BCAs which aim to inoculate the microorganism supporting their symbiosis with the plant (intercellular bacteria, iPGPRs) or the colonization of surface of the root hairs (extracellular, free-living bacteria). For the PGPRs their benefit leads to the improvement of germination of the seeds, increase branches in root hairs, enhance a fast nodule performance, increase leaf surface, plant vigor and carbohydrates accumulation, release of phytohormones, increase the plant nutrients and water uptakes.
The explored and/or investigated PGPRs mechanisms of the recent literature include:
solubilization and mineralization of phosphorus;
ii) nitrogen fixation by symbiosis and/or asymbiosis;
iii) release phytohormones as such as gibberellins, cytokinins, IAA (indole acetic acid), ABA (abscisic acid), AAC-deaminase (1-aminocyclopropane-1-carboxylate deaminase) reduce ethylene level in roots increasing length and vigour of the roots system;
iv) disease antagonism by producing cyanides and antibiotics;
v) implement the availability of nutrients, as such as iron by chelating and siderophores;

vi) increase resistance to abiotic oxidative stresses;
vii) production of water soluble vitamins as such as biotin, niacin, thiamine and riboflavin;
viii) detoxification of heavy metals;
ix) plant tolerance of salinity;
x) biological control of pests and diseases.

PGPRs inoculation implemented the stress resistance and production of tomato, lettuce, wheat, rice, soybean, groundnut, maize, chickpea, barley, sugar beet, strawberry, grapes and raspberry, increasing yield from 25% to 65%. The microorganism inoculation is the critical step and different methods have been described, by several Authors, for increasing the microorganism colonization of the several matrices (plant, soil, etc.), as such as seed coating, pelleting, foliar application, direct soil application by inoculation which represent the practise utilized since the advent of BCAs use.

For implement PGPRs inoculation is worldwide utilised to soak the seeds for a variable time in liquid suspension of BCAs in order to stimulate the physiological processes support the germination one preventing radicle and plumule emergence until the seed sown. Following this method the proliferation of the PGPRs inside the seed is 10-folds than for the other pathogens enabling the plant to survive and show good productive performances. The application methods of PGPRs contributes their survival and proliferation efficiency into the soil and on the seed. Some of the inoculation methods include seed treatment, soil amendment and roots dipping in PGPRs suspensions before transplanting; the latter suspensions could be utilized by foliar spray or drip irrigation.

Several PGPRs carrier materials have been tried in order to keep the microorganisms viable for longer times, for reducing PGPRs desiccation and for improving their adhesiveness to the plant tissue. Broth and agar cultures and powder carriers have been used but the widely utilised are the peat based inoculants which are sensible to high temperatures, water scarcity if not well irrigated, and peat quality often contaminated by pollutants like Pb, Ni, As, Me, etc., or by NaCl increases the peat EC, etc. Peat soil is the better PGPRs carrier for plant inoculation but its critical points are its quality and its availability limited for environmental restrictions of the traditional areas of extraction. Rice husk is utilised as carrier in Asia whereas was utilised bentonite clay for increasing PGPRs survival in fine textured soils or barley straw improved the root colonization of the roots by the several strains.

Inoculation techniques are not well standardized and there is scarce information about their detailed protocols but is quite well known the PGPRs population into the soil is positively correlated with the initial stalk of its inoculum on the seed. In some case some other variables of the soil, as such as the texture, structure, temperature, water amount, nutrient presence and pH, affecting the PGPRs survival. *Pseudomonad* strains survived 10-fold better in sandy loam than in clay one, whereas mineral bentonite amendment of the soil improve the PGPRs survival in loam sand soil through their protection against protozoa.

The real bottleneck of the diffusion and of the efficiency of PGPRs are represented by the several inoculation methods and techniques, as such as seed coating and covering, root dipping, foliar spray, direct soil application and seed inoculation which showed controversial aspect to take in consideration. Seed coating/covering method consist in suspending the seeds in the PGPRs liquid suspension in order to cover homogeneously its surface. Some constraints of this method are the use of adhesive for well cover the seed

surface, micronutrients ,presence as such as molibden, in the carrier, permeability to the seed gaseous exchange in *Fabaceae* seeds reducing nitrogen fixation, strain desiccation.
The root dipping in PGPRs suspension has been largely adopted for inoculating PGPRs for controlling *Fusarium, Meloidogyne incognita* in tomato crops increasinging significantly the yield for strawberry. Foliar application not desired results of increase bacteria to the plants but this method is utilised for biocontrol of fungus and for increase the yield, and its parameters, of strawberry, apricot, sweet cherry and apple. Mulberry crops react well with foliar spray of *Azotobacter, Azospirillum* and *Beijerinckia* liquid suspensions.

Soil inoculation of the PGPRs inoculum can be effective to control antagonistic microbes or pesticides in plant tissues. Inhibitory substances on the plant tissue can partially inhibit inoculation of some organs. Solid inoculum could be easily managed but difficulties are registered for the liquid ones because they need particular care for their transportation and application into the soil.

Seed inoculation is implemented by carriers for improving transportation and application of the inoculum, its adhesivity on the seed surface ensuring its sticking activity and avoiding its desiccation. Since the discovery of the *Rhizobium* for the *Fabaceae* crops the peat-based inoculum. seed inoculation could be favoured by adhesive agents on the seed surface followed inoculum spreading under shade conditions. The most adhesive agents utilized for seed inoculation are arabic gum, caseinate salt and polyvinylacetate, sugar solutions, polyvinylpyrollidone, methylcellulose.
Efficient PGPRs inoculation and colonization lead to improve the performance of the plant and of the crops. Some PGPRs, like endophytic bacteria and fungi, spent part of their life in symbiosis into plant tissues without causing any damages and similarly with the pathogens entered into the plants by several organs and mechanisms, like wounded plant organs, stomates, lenticels, radicle during germination, root cracks, facilitating the PGPBs and PGPRs inoculations. Soil inoculation has the task to reach the rhizodermis production a sting of PGPRs form biofilms or microcolonies on the surface of the rhizodermal cells colonizing them. The colonized rhizosphere is strictly related to the photosynthates translocation to the roots apparatus with its mucilages rich of exudates. Root exudates, and their concentration and composition, affect the PGPRs colonization; they are mainly represented by organic acids, amino acids and carbohydrates. Plants release malic acid for attracting PGPRs against infections which forms a protective biofilm. PGPRs compete into the soil with pathogens limiting them by secreting lytic enzymes, siderophores, secondary metabolites and antibodies. Soil nutrients affect PGPRs roots colonization. Several *Rhyzobium* species produce indol acetic acid (IAA) which is essential for nodules formation by cell proliferation and differentiation with vascular tissues; higher auxin levels are responsible in the *Fabaceae* for the nodules formation.

Host specificity in plant evolution has supported preferential interaction among plant and PGPRs and involves host recognition by root exudates variable in relation to the cultivars, the stress typologies and the plant phenophase. For organic plant breeding is very important identify soil microbiome relationship with root apparatus and PGPRs. Plant genetics goal is to identify genes involved in host specificity for increase the benefits of PGPRs for increasing plant health and performances. The new next-generation sequencing techniques can implement the studies related to host specificity of some PGPRs present in the rhizosphere. PGPRs can improve the growth and the development of the plant in relation to the strict relationship to the host exudates released in the rhizosphere and to their competitiveness to colonize the roots.

Seed priming methods are based on conventional agents which stimulate germination process, and radicle and plumule growth within the seed coat, delaying their emergences by seed redrying. Biopriming techniques

are based on the use of biological compounds for seed rehydratation in optimal conditions for PGPRs inoculation and colonization of the seed. Biopriming methods are based on the seed soaking in a PGPRs suspension for a specific time which permit the starting of the germination process preventing plumule and radicle emergences. Biopriming methods play a important role for improving the endophyte PGPRs colonization, avoiding the high temperature, and for promoting quick germination and plant growth. Seed biopriming with PGPRs improved the plant growth and the yield of carrots, sweet corn and tomato. Bio-osmopriming methods improve the uniformity of germination and the seedling establishment. The different biopriming methods differ in relation to the PGPRs mixture and concentration, to the temperature and to the soaking time; sometimes seed disinfection of their seed surface is applied before its soaking in PGPRs suspension.

Biopriming with several PGPRs, as such as *Bacillus lentus, B. subtilis, Pseudomonas fluorescens, P. putida* and *Azospirillum* increase the agro-morphological traits, dry matter accumulation and and grain yield of wheat, barley and maize.

The biopriming of *Bacillus* ssp. increase the resistance against some biotic stresses, as such as water and salinity ones, of chickpea, mungbean, potato and rice crops. Positive effects of biopriming were ascertained for controlling several diseases utilising *Serratia plymuthica* and *P. chlororaphis* for different oilseed rape cultivars as such as *Leptosphaeria maculans,* causing blackleg disease. Seed biopriming by *P. fluorescens* reduced the incidence of *Alternaria* blight was reduced and the plants to tolerated the disease efficiently. PGPRs can protect the plants to pathogens by antagonistic interaction inducing systemic resistance. Seed biopriming by *T. harzianum* reduced root rot disease caused by *Macrophomina phaseolina*, *F. solani* and *Rhizoctonia solani* in cowpea of about the 56.3%– 64% at the pre-emergence and of about 57.1%–64% at the post-emergence stages.

Seed biopriming represent a useful method for the crop biocontrol reducing the cost of pesticides enhancing plant productivity and stress resistance. The competition of action of the desired PGPRs against the local microbes permit to the formers to be already inside the seeds reducing the desiccation.
This preface about the methods utilised for biocontrol of crop diseases provide us a general frame for their use in order to improve the PGPBs and PGPRs colonization of the rhizosphere and of the plant. Several are the microorganism species and strains and well documented are their effects but the main problem is their colonization and adaptation in sub-optimal growth conditions.

In particular relating to the seed treatment of the plant species to control the plant species the methods that have been already tested for BCAs and NCs are here summarised in Annex no 7.
In the scientific literature, a range of both bacterial and fungal antagonists and natural compounds have been used experimentally to control plant diseases, but they have been used less frequently as seed treatments.
Inoculants or natural compound are applied as seed treatment by using different inoculation methods. Regarding the pathogens of the project, BCAs or natural compound were experimentally applied to seeds as liquids (sprays, drenches, root dips) or as dry formulations.
The easier and widely method used is soaking the seeds in an aqueous bacterial or conidial suspension at the concentration 10^6-10^9 cells/ ml. Several protocols have been developed and they widely varied regarding the time of incubation of the seed in the suspension ranging from 10- 60 min (Kasselakiet al., 2011; Sharma et al., 2018; Amein et al., 2011) to 5-24 h (Campbell et al., 2006; Massomo et al., 2004; Silva et al., 2004;

Umesha, 2006; Abuamsha et al., 2011; Hammoundi et al., 2011; Obes Correa et al., 2011; Mishra et al., 2012; Umesha and Roohie, 2017) until overnight incubation (Ghazalibiglar, 2014; Ghazalibiglar et al., 2015).
Adhesives or surfactants are added to the BCA suspension as wetting agents (Tween 20) or to improve their adhesion to the seeds (xanthan gum) (Boudyach et al., 2001; Umesha and Raheem, 2017). Inoculants is also applied as suspension on the seed at the time of the sowing (Hassan et al., 2017).
In slurry applications, inoculants formulated as powders are applied to the outside of seeds using a range of stickers such as carboxy methyl cellulose (Umesha,2006; Mandiriza et al., 2018). BCAs was also inoculated by soil drench method (Campbell et al., 2006).
Natural compound are usually applied to seeds as aqueous solution or are dissolved in other organic compound (Benhamou et al., 1994; Amein et al., 2011; Mbega et al., 2012; Kotana et al., 2014; Aminia et al., 2018 Mandiriza et al., 2018; Karabuyuk and Aysan, 2018).Different concentrations of natural compounds and variable time of application were tested (Kotana et al., 2014; Aminia et al., 2018).

A review of literature on the valuation of seed treatments to control seed borne disease, showed
that the effectiveness of BCAs or natural compounds is carried out using artificially infected seeds Infected seed are obtained by spraying o placing a conidial suspension on the seeds or by immersion the seeds in the inoculum (de Jensen et al., 2002; Domenech et al., 2006; Manhas et al., 2016). Generally, bacterial pathogens are inoculated on seeds by immersion in suspension for a time ranging from 5 min to 12 h (Mishra et al., 2012; Ghazalibiglar, 2014; Kotana et al., 2014; Ghazalibiglar et al., 2016; Umesha and Roohie, 2017; Karabuyuk and Aysan, 2018; Mandiriza et al., 2018; Aminia et al., 2018). To inoculate tomato seeds with Cmm or Xanthomonas spp., some researchers used a vacuum infiltration method by applying a negative pressure for 5-30 min (Kasselaki et al., 2011; Mbega et al., 2012).
Otherwise, when available, naturally infected seed are used (Umesha S., 2006; Sharma et al.,2010; Amain et al., 2011; Amin et al.,2014).
For soil-borne pathogens, such as *Fusarium* and *Alternaria* species, a conidial suspension is mixed thoroughly with soil (Thomas et al., 1998; Pereira et al., 2014; Sharma et al., 2018), or deposited in holes made in the soil near the plants (Obes Corea et al., 2014).
In other case, the pathogen is inoculated on seedling originated from treated seeds about 7-12 days after sowing or at the stage of 3 expanded leaves. A disk of actively growing mycelium or a conidial suspension was deposited close to the root system (*Fusarium* spp) (Benhamou et al., 1994; Abeysinghe,2007) or was sprayed on leaves surface (*Alternaria* spp) (Hassan et al., 2017) or used to infect previously wounded cotyledons (*Plenodomus lingam*) (Abuamsha et al., 2011; Hammoudi et al., 2012; Dawidziuk et al., 2016).
Similarly, bacterial strains are inoculated on 10-30 days seedling by spraying a bacterial suspension on the leaves (*P. syringae* tomato- *Xanthomoans* spp / tomato) (Massomo et al ., 2004; Silva et al., 2004; Campbell et al.,2006) or on the roots (Cmm/tomato)(Boudyach et al.,2001) or by applying bacterial cells on the sinus of the cotyledons (*Xanthomoans*/tomato) (Massomo et al ., 2004).

4. Deviations
No deviations to be highlighted.

5. Conclusions

Deliverable 4.1 proposes a review of the detection tools for seed-borne pathogens and the seed treatments that are applicable in organic seed production. This Deliverable was preparatory to the Deliverable 4.2 that

will describe the optimal treatments to control main seedborne pathogens affecting tomato, brassicas and snap bean. (The document is available as Annex no 8).
The analysis of available detection methods highlighted that for some pathogens a huge effort has been done to standardise methods that could be used for the seed industry and by NAPPOs. Techniques however are rapidly evolving and the more recent protocols and publications deal with molecular methods in particular PCR based methods. The sensitivity is by the fact increased as compared to serological methods.
It should be noticed that the number of published papers on the pathogens in BRESOV is wider but we focused on those that were developed or tested on seeds which is the target of the project. Seed extracts may contain inhibitors which prevent amplification. More simply official protocol on fungi often rely on agar /paper seeds grow-out tests.
The different laboratories will adopt, validate or improve the test more suitable to detect the pathogen in their pathosystem for the detection in the seeds or in the plantlets. Where applicable Real-time PCR will be chosen as it could also provide a quantitative mean to measure the pathogen presence. To date there are not validated methods that allow to count and separate death/alive cells on seeds contaminated/infected in the pathosystems in this WP.

BRESOV partners discussed deeply some problems on the availability of infected seeds, the transmission rate and other problems linked to study seed pathology and control through coating.
In particular, the main problem resides in the availability of infected seed lots to be used in the trials. This is almost impossible for regulated quarantine and non-quarantine pathogens.
At the same time, infection in naturally infected seed lots is rarely homogenous which make a standard detection and quantification of the infection rate more complex. Therefore, for these two reasons, and on advice from seed producers, it was decided to perform these experiences on artificially infected seeds, and then test the resulting most promising seed treatments methods on naturally infected seed lots, whenever available. Requests of naturally infected seed lots of these host-pathogen binomial have been already sent to a number of seed producers.
At the moment of reviewing this report probable naturally infected seed lots will be available for the binomial bean/*Colletotrichum* and Brassicas/*Alternaria*. Inoculation of seeds with bacterial cells or fungal spores is used in different protocols and for pathogenicity tests (data not shown) and already used in some BRESOV laboratories.

Regarding the application of the bioproducts in the trial the review pointed out different protocols of application on seed that in general depend on the use of commercial products or laboratory BCAs or NCs.
The most used method of application for experimental trials rely on microbiolization of the seeds in a BCA water suspension or soaking in diluted NCs. The coating technology is not suitable at the moment for the number of trials and their parcelling (products X species x pathogens) and will be later evaluated.
ITAKA made available products that are already optimised in term of formulation, therefore: they are stable, the microorganisms compatible, and the load for each strain is known; they stick to the seed in predictable quantities and remain alive.

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Annex 1

Species	revised nomenclature	EPPO List/Code	EPPO GD	EPPO diagnostic Standard n.	EPPO Standard Date	EPPO LINK	ISTA protocol	ISTA protocol date	ISF protocol	EFSA document	EFSA Link
Tomato => 5 pathogens :											
Clavibacter michiganensis subsp. michiganensis	/	A2/CORBMI	https://gd.eppo.int/taxon/CORBMI	PM7/42 (3)	2016	https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12302	https://www.worldseed.org/wp-content/uploads/2017/07/Tomato_Cmm_July2017.pdf	2017	https://www.worldseed.org/wp-content/uploads/2017/07/Tomato_Cmm_July2017.pdf	Scientific Opinion on the pest categorisation of Clavibacter michiganensis subsp. michiganensis (Smith) Davis et al. EFSA Journal 2014;12(6):3721	https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3721
Pseudomonas syringae pv. tomato		PSDMTM	https://gd.eppo.int/taxon/PSDMTM								
Xanthomonas spp. pathogenic to tomato	*X. euvesicatoria pv. euvesicatoria*	A2/XANTEU	https://gd.eppo.int/taxon/XANTEU	PM7/110	2013	https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12018			https://www.worldseed.org/wp-content/uploads/2017/07/Tomato_Xanthomonas_spp_July2017.pdf	Scientific Opinion on the pest categorisation of Xanthomonas campestris pv. vesicatoria (Doidge) Dye - EFSA Journal 2014;12(6):3720	https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2014.3856
	X. gardneri	XANTGA	https://gd.eppo.int/taxon/XANTGA								
	X. euvesicatoria pv. perforans	A2/XANTEU	https://gd.eppo.int/taxon/XANTPF								
	X. vesicatoria	A2/XANTVE	https://gd.eppo.int/taxon/XANTVE								
- ToMV	/	TOMV00	https://gd.eppo.int/taxon/TOMV00				https://www.seedtest.org/upload/cms/user/ISTARules2019SHmethods7-028.pdf	2019	https://www.worldseed.org/wp-content/uploads/2019/03/Tomato-Tobamo_CPP_2019		
- Fusarium oxysporum f.sp. radicis lycopersici		FUSARL	https://gd.eppo.int/taxon/FUSARL								
- Fusarium oxysporum subsp. lycopersici		FUSALY	https://gd.eppo.int/taxon/FUSALY								
Broccoli => 3 pathogens :											
Xanthomonas campestris pv. campestris		XANTCA	https://gd.eppo.int/taxon/XANTCA				https://www.seedtest.org/upload/cms/user/ISTARules2019SHmethods7-019a.pdf	2019	https://www.worldseed.org/wp-content/uploads/2017/08/Brassica_untreated_Xcc_Aug2017.pdf		
							https://www.seedtest.org/upload/cms/user/ISTARules2019SHmethods7-019b.pdf	2019	https://www.worldseed.org/wp-content/uploads/2017/07/Brassica_treated_Xcc_July2017.pdf		
Alternaria spp.	*Alternaria brassicicola and Alternaria brassicae*	ALTEBI and ALTEBA	https://gd.eppo.int/taxon/ALTEBI https://gd.eppo.int/taxon/ALTEBA								
Phoma lingam (Leptosphaeria maculans)	*Plenodumus lingam*	LEPTMA	https://gd.eppo.int/taxon/LEPTMA				https://www.seedtest.org/upload/cms/user/ISTARules2019SHmethods7-004.pdf	2019	https://www.worldseed.org/wp-content/uploads/2018/07/Coverpage_Brassica_Phoma_July_2018.pdf		
Snap bean => 3 pathogens :											
Colletotrichum lindemuthianum		COLLLD	https://gd.eppo.int/taxon/COLLLD				https://www.seedtest.org/upload/cms/user/ISTASHmethods20207-006.pdf	2020			

Pseudomonas savastanoi pv. phaseolicola		PSDMPH	https://gd.eppo.int/taxon/PSDMPH				https://www.seedtest.org/upload/cms/user/ISTASHmethods20207-023	2020	https://www.worldseed.org/wp-content/uploads/2017/08/Bean_Psp_July2017.pdf		
Fusarium solani f.sp. phaseoli	*Neocosmospora phaseoli* (Burkh.) L. Lombard & Crous, in Lombard, van der Merwe, Groenewald & Crous 2015	FUSAPH	https://gd.eppo.int/taxon/FUSAPH								

Sitography

https://www.eppo.int/RESOURCES/eppo_standards/pm7_diagnostics
https://www.seedtest.org/en/seed-health-methods-_content---1--1452.html
https://www.worldseed.org/our-work/phytosanitary-matters/seed-health/ishi-veg-protocols/
https://www.efsa.europa.eu/en/publications/?f%5B0%5D=im_field_subject%3A62041
https://gd.eppo.int/

Annex 2

Species	revised nomenclature	Detection in seeds (add also link or doi)					
		Serological ?	PCR	Real-time PCR	isothermal amplification	Other methods	review
Tomato => 5 pathogens :							
Clavibacter michiganensis subsp. michiganensis			Svetlana Milijašević, Biljana Todorović, Emil Rekanović, Ivana Potočnik1 and Jelica Balaž, 2007. Clavibacter michiganensis subsp. michiganensis, Bacterial Canker of Tomato: 2. Comparison of the Effectiveness of Extraction Procedures and Sensitivity of Methods for Detection in Tomato Seeds. Pestic. Phytomed. (Belgrade), 22 (2007) 121-130. http://arhiva.nara.ac.rs/handle/123456789/1272	W-J. Zhao H-Y. Chen, S-F. Zhu, M-X Xia and T-W. Tan, 2007. ONE-STEP DETECTION OF CLAVIBACTER MICHIGANENSIS SUBSP. MICHIGANENSIS IN SYMPTOMLESS TOMATO SEEDS USING A TAQMAN PROBE. Journal of Plant Pathology , 89 (3), 349-351. www.sipav.org/main/jpp/volumes/0307/030704.pdf		L.de León, F.Siverio, A.Rodríguez, 2006. Detection of Clavibacter michiganensis subsp. michiganensis in tomato seeds using immunomagnetic separation. ournal of Microbiological Methods Volume 67, Issue 1, 141-149. https://doi.org/10.1016/j.mimet.2006.03.007	
				Kameka L. Johnson Ron R. Walcott, 2011. Progress Towards a Real-time PCR Assay for the Simultaneous Detection of Clavibacter michiganensis subsp. michiganensis and Pepino mosaic virus in Tomato Seed. Journal of Phytopathology,160, Issue7-8, 353-363 https://doi.org/10.1111/j.1439-0434.2012.01911.x			
			ZHANG Y, YANG W ,LI Y , et al.2009. A multiplex PCR method for detection of Clavibacter michiganensis subsp. michiganensis with co-amplification of its host DNA[J]. Front Agric Chin, 3(2): 140-145. http://journal.hep.com.cn/fag/EN/Y2009/V3/I2/140	Han S, Jiang N, Lv Q, Kan Y, Hao J, Li J, et al. (2018) Detection of Clavibacter michiganensis subsp. michiganensis in viable but nonculturable state from tomato seed using improved qPCR. PLoS ONE 13(5): e0196525. https://doi.org/10.1371/journal.pone.0196525	Yasuhara-Bell J., Gurel F B., Miller SA, Alvarez AM., 2015 Utility of a loop-mediated amplification assay for detection of Clavibacter michiganensis subsp. michiganensis in seeds and plant tissues. Canadian Journal of Plant Pathology 37(3):260-266. DOI: 10.1080/07060661.2015.1053988		
			Mraz I, Pavel B, Kokoskov B, 2011. Detection of Clavibacter michiganensis subsp. michiganensis from tomato plants and seeds using ELISA, if and PCR with commercial and own primers. Acta horticulturae 914(914):57-60. DOI: 10.17660/ActaHortic.2011.914.7				
Pseudomonas syringae pv. tomato			Zaccardelli, M., Spasiano, A., Bazzi, C. et al.,2005. Identification and in planta detection of Pseudomonas syringae pv. tomato using PCR amplification of hrpZ Pst. Eur J Plant Pathol (2005) 111: 85. https://doi.org/10.1007/s10658-004-2734-7	Fanelli, V. , Cariddi, C. and Finetti-Sialer, M. (2007), Selective detection of Pseudomonas syringae pv. tomato using dot blot hybridization and real-time PCR. Plant Pathology, 56: 683-691. doi:10.1111/j.1365-3059.2007.01612.x			
Xanthomonas spp. pathogenic to tomato	*X. euvesicatoria pv. euvesicatoria*		Koenraadt, H., van Betteray, B., Germain, R., Hiddink, G., Jones, J.B. and Oosterhof, J. (2009). DEVELOPMENT OF SPECIFIC PRIMERS FOR THE MOLECULAR DETECTION OF				
	X. gardneri						
	X. euvesicatoria pv. perforans						
	X. vesicatoria						
- ToMV				Melo Almeida JE, dos Reis Figueir Aa, de Sousa Geraldino Duarte P, Antônio Lucas M, Edreira Alencar N (2018). Procedure for detecting tobamovirus in tomato and pepper seeds decreases the cost analysis. Bragantia. 77. 10.1590/1678-4499.2017317.			
- Fusarium oxysporum f.sp. radicis lycopersici			Hirano and Arie, 2006. PCR-based differentiation of Fusarium oxysporum ff. sp. lycopersici and radicis-lycopersici and races of F. oxysporum f. sp. lycopersici. J Gen Plant Pathol (2006) 72:273–283. 10.1007/s10327-006-0287-7 (plant)	Validov, Kamilova, Lugtenberg, 2011. Monitoring of pathogenic and non-pathogenic Fusarium oxysporum strains during tomato plant infection. Microbial Biotechnology (2011) 4(1), 82–88. 10.1111/j.1751-7915.2010.00214.x (plant)			
Broccoli => 3 pathogens :							
			Park YL, Lee BM, Ho-Hahn J., Lee GB, Park DS., 2004. Sensitive and specific detection of Xanthomonas campestris pv campestris by PCR using species-specific primers based on hrpF gene sequences. Microbiological Research 159, 419-423.https://doi.org/10.1016/j.micres.2004.09.002	Berg T, Tesoriero L, Hailstones DL., 2006.A multiplex real-time PCR assay for detection of Xanthomonas campestris from brassicas. DOI: 10.1111/j.1472-765X.2006.01887.x			
			T. Berg L. Tesoriero D. L. Hailstones, 2005. PCR-based detection of Xanthomonas campestris pathovars in Brassica seed. Plant PAthology, 54, 416-427. https://doi.org/10.1111/j.1365-3059.2005.01186.x	Laala S, Zouaoui B., Manceau C., 2010. Development of a new technique to detect living cells of Xanthomonas campestris pv. campestris in crucifers seeds: the seed-qPCR. Eur J Plant Pathol, 141,637- 646.DOI 10.1007/s10658-014-0532			

Xanthomonas campestris pv. campestris			Roohie RK and Umesha S, 2012. Development of Multiplex PCR for the Specific Detection of Xanthomonas campestris pv. campestris in Cabbage and Correlation with Disease Incidence. J Plant Pathol Microb 2012, 3:4. http://dx.doi.org/10.4172/2157-7471.1000127				
			Leu, Y. S., Deng, W. L., Yang, W. S., Wu, Y. F., Cheng, A. S., Hsu, S. T., Tzeng, K. C.,2010. Multiplex Polymerase Chain Reaction for Simultaneous Detection of Xanthomonas campestris pv. campestris and X. campestris pv. raphani. Plant Pathology Bulletin 19: 137-147.				
Alternaria spp.	*Alternaria brassicicola and Alternaria brassicae*			Iacomi-Vasilescu, B., Blancard, D., Guénard, M., Molinero-Demilly, V., Laurent, E., & Simoneau, P. (2002). Development of a PCR-based diagnostic assay for detecting pathogenic Alternaria species in cruciferous seeds. Seed Science and Technology, 30(1), 87-95. Guillemette, T., Iacomi-Vasilescu, B., & Simoneau, P. (2004). Conventional and Real-Time PCR-Based Assay for Detecting Pathogenic Alternaria brassicae in Cruciferous Seed. Plant Disease, 88(5), 490-496. https://doi.org/10.1094/PDIS.2004.88.5.490			
Phoma lingam (Leptosphaeria maculans)			Fernando, Zhang, Amarasinghe,2016. Detection of Leptosphaeria maculans and Leptosphaeria biglobosa Causing Blackleg Disease in Canola from Canadian Canola Seed Lots and Dockage. Plants (Basel). 2016 Mar; 5(1): 12. doi: 10.3390/plants5010012 (plant)				
Snap bean => 3 pathogens :							
Colletotrichum lindemuthianum			Wang, W., J. H. Tang, and Y. C. Wang. "Molecular detection of Colletotrichum lindemuthianum by duplex PCR." Journal of Phytopathology 156.7-8 (2008): 431-437. https://doi.org/10.1111/j.1439-0434.2007.01386.x	Gadaga, Stelio Jorge Castro, Carolina da Silva Siqueira, and José da Cruz Machado. "Molecular detection of Colletotrichum lindemuthianum in bean seed samples." Journal of Seed Science 40.4 (2018). DOI: 10.1590/2317-1545v40n4192761 Chen, Y. Y., et al. "A quantitative real-time PCR assay for detection of Colletotrichum lindemuthianum in navy bean seeds." Plant pathology 62.4 (2013): 900-907. Doi: 10.1111/j.1365-3059.2012.02692.x			
			CHEN, Y.Y; CONNER, R.L.; GILLARD, C.L.; BOLAND, G.J.; BABCOCK, C.; CHANG, K.F.; HWANG, S.F.;BALASUBRAMANIAN, P.M. A specific and sensitive method for the detection of Colletotrichum lindemuthianum in dry bean tissue. Plant Disease, v.91, n.10, p.271-276, 2007. link			Thomas, G. J., and K. G. Adcock. "Exposure to dry heat reduces anthracnose infection of lupin seed." Australasian plant pathology 33.4 (2004): 537-540. link	
			Gadaga, Stelio Jorge Castro, Carolina da Silva Siqueira, and José da Cruz Machado. "Molecular detection of Colletotrichum lindemuthianum in bean seed samples." Journal of Seed Science 40.4 (2018). DOI: 10.1590/2317-1545v40n4192761				
Pseudomonas savastanoi pv. phaseolicola		Wyatt, G. M., J. G. Turner, and M. R. A. Morgan. "Rapid and specific detection of Pseudomonas syringae pv. phaseolicola by immunological methods." Food and Agricultural Immunology 1.1 (1989): 53-63. link	Popovic, Tatjana, Milovanovic, Predrag, Aleksic, Goran, Gavrilovic, Veljko, Starovic, Mira, Vasic, Mirjana, & Balaž, Jelica. (2012). Application of semi-selective mediums in routine diagnostic testing of Pseudomonas savastanoi pv. phaseolicola on common bean seeds. Scientia Agricola, 69(4), 265-270. https://dx.doi.org/10.1590/S0103-90162012000400005			Kurowski, C., and P. M. Remeeus. "Proposal for a new method for detecting Pseudomonas savastanoi pv. phaseolicola on bean seeds." ISTA Method Validation Reports 4 (2007): 1-12. link	
			Molouba, F., C. Guimier, and C. Berthier. "Detection of bean seed-borne pathogens by PCR." International Symposium on Molecular Markers for Characterizing Genotypes and Identifying Cultivars in Horticulture 546. 2000. https://www.actahort.org/books/546/546_84.htm				

Fusarium solani f.sp. phaseoli						Marcenaro, Delfia, and Jari PT Valkonen. "Seedborne pathogenic fungi in common bean (Phaseolus vulgaris cv. INTA Rojo) in Nicaragua." PloS one 11.12 (2016).link	

Sitography

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https://www.efsa.europa.eu/en/publications/?f%5B0%5D=im_field_subject%3A62041

Annex 3

Species	List of Pathogen	BioControl Agents	*in vitro*	*in vivo (specify plant species)*	Bibliografy	DOI or link
Tomato	*Clavibacter michiganensis*	Bacillus spp.	+	+	Kasselaki, A.M., Goumas, D., Tamm, L., Fuchs, J., Cooper, J., Leifert, C. 2011. Effect of alternative strategies for the disinfection of tomato seed infected with bacterial canker (Clavibacter michiganensis subsp. michiganensis). NJAS - Wageningen Journal of Life Siences 58:145-147	https://www.sciencedirect.com/science/article/pii/S157352141100039X
		Pseudomonas fluoresces	+	+	Umesha S., 2006. Occurrence of bacterial canker in tomato fields of Karnataka and effect of biological seed treatment on disease incidence. Crop Protection 25: 375-381.	https://doi.org/10.1016/j.cropro.2005.06.005
		fluorescent pseudomonads		+	Boudyach, E.H., Fatmi, M., Akhayat, O., Benziri, E., Ait Ben Aoumar, A. (2001). Selection of antagonistic bacteria of Clavibacter michiganensis subsp. michiganensis and evaluation of their efficiency against bacterial canker of tomato. Biocontrol Science and Technology (11), 141-149.	https://doi.org/10.1080/09583150020029817
		Azospirillum brasiliense			Bashan Y, Luz E., 2002.Protection of Tomato Seedlings against Infection by Pseudomonas syringae pv. Tomato by Using the Plant Growth-Promoting Bacterium Azospirillum brasilense. Appl Environ Microbiol. 68(6): 2637–2643.	DOI: 10.1128/AEM.68.6.2637–2643.2002
	Pseudomonas syringae pv. tomato	BCA and PGPR (Burkholderia sp; Pseudomonas spp; Bacillus spp,Stenotrophomonas sp.)	-	+	Ji P, Campbell H.L., Kloepper J, Wilson M, Jones J, Suslow T, 2006. Integrated biological control of bacterial speck and spot of tomato under field conditions using foliar biological control agents and plant growth-promoting rhizobacteria. Biological Control 36(3):358-367	DOI 10.1016/j.biocontrol.2005.09.003
	Xanthomonas spp.	Rhizobacteria	-	+	Silva HSA, da Silva Romeiro R , Macagnan D , de Almeida Halfeld-Vieira B , Baracat Pereira MC, Mounteerd A, 2004. Rhizobacterial induction of systemic resistance in tomato plants: non-specific protection and increase in enzyme activities.Biological Control 29, 288–295	doi:10.1016/S1049-9644(03)00163-4
	ToMV					
	Fusarium oxysporum f.sp. radicis lycopersici	*Trichoderma harzianum*			Biological Control of Fusarium Crown and Root Rot of Tomato in Florida Using Trichoderma harzianum and Glomus intraradices	https://www.sciencedirect.com/science/article/pii/S1049964485710511
		A compost of vegetable waste and Posidonia oceanica mixture; Bacillus sphaericus (B12 and BS2), Pseudomonas putida PPS7 and Burkholderia gladioli BuC16.	+	+	Kouki S. , Saidi N., Ben Rajeb A., Brahmi M., Bellila A., Fumio M. , Hefiene A., `Jedidi N., Downer J., Ouzari H.,2012. Control of Fusarium Wilt of Tomato Caused by Fusarium oxysporum F. Sp. Radicis-Lycopersici Using Mixture of Vegetable and Posidonia oceanica Compost. Applied and Environmental Soil Science, Article ID 239639, 11 pages	doi:10.1155/2012/239639
		Bacillus spp.	+	+	Massomo, S.M.S., Mortensen, C.N., Mabagala, R.B., Newman, M.-A., Hockenhull, J., 2004. Biological control of black rot (Xanthomonas campestris pv. campestris) of cabbage in Tanzania with Bacillus strains. J. Phytopathol. 152, 98–105	https://doi.org/10.1111/j.1439-0434.2003.00808.x
		Bacillus subtilis strain BB Bacillus amyloliquefaciens (Priest) Priest, Bacillus pumilus, Bacillus subtilis (Ehrenberg) Cohn	roccoli; kale; cauliflower; cabbag	Broccoli; kale; cauliflower; cabbage.	Ednar G. Wulff1,, Cames M. Mguni2, Carmen N. Mortensen3, Chandroo L. Keswani2 and John Hockenhull1, Biological control of black rot (Xanthomonas campestris pv. campestris) of brassicas with an antagonistic strain of Bacillus subtilis in Zimbabwe European Journal of Plant Pathology 108: 317–325, 2002	https://link.springer.com/content/pdf/10.1023%2FA%3A1015671031906.pdf
		Paenibacillus spp.	+	+	Ghazalibiglar, H., 2014. Discovery of Paenibacillus Isolate for Control of Black Rot in Brassicas. PhD thesis. Lincoln University, Christchurch, New Zealand.	https://researcharchive.lincoln.ac.nz/handle/10182/6322

Broccoli	Xanthomonas campestris pv. campestris	*Paenibacillus polymyxa (Prazmowski) Ash*	+	Cabbage seeds of cultivar Kameron (South Pacific Seeds (NZ) Ltd)	Hoda Ghazalibiglar, John G. Hampton, Eline van Zijll de Jong & Andrew Holyoake .Evaluation of Paenibacillus spp. isolates for the biological control of black rot in Brassica oleracea var. capitata (cabbage). Biocontrol Science and Technology · December 2015	http://dx.doi.org/10.1080/09583157.2015.1129052
		Pseudomonas KA19	Brassica campestris		Shruti Mishra•Naveen K. Arora. Evaluation of rhizospheric Pseudomonas and Bacillus as biocontrol tool for Xanthomonas campestris pv campestris February 2012World Journal of Microbiology and Biotechnology (Formerly MIRCEN Journal of Applied Microbiology and Biotechnology) 28(2):693-702	DOI: 10.1007/s11274-011-0865-5
		Bacillus SE,	Brassica campestris	" "	" "	DOI: 10.1007/s11274-011-0865-6
		Pseudomonas fluorescens		cabbage (variety used Golden acre)	Y. A. KAVATHIYA, R. L. KALASARIA, J. D. TALAVIA AND M. A. VADDORIA. MANAGEMENT OF BLACK ROT CAUSED BY Xanthomonas campestris (PAMMEL) DOWSON IN CABBAGE. PESTOLOGY VOL. XLI NO. 10 OCTOBER 2017	DOI: 10.13140/RG.2.2.31993.36967
	Xanthomonas campestris pv. campestris	*Pseudomonas fluorescens*		cabbage cultivars (Pusa mukta and NBH boss)	Sharanaiah Umesha and Raheem K. Roohie. Role of Pseudomonas fluorescens and INA against Black Rot of Cabbage. J Phytopathol 165 (2017) 265–275 2017 Blackwell Verlag GmbH	doi: 10.1111/jph.12558
	Alternaria spp.	*Streptomyces hydrogenans*	+	+	Manhas, R. K. & Kaur, T. Biocontrol Potential of Streptomyces hydrogenans Strain DH16 toward Alternaria brassicicola to Control Damping Off and Black Leaf Spot of Raphanus sativus. Front. Plant Sci. 7, 1–13 (2016).	10.3389/fpls.2016.01869
		rhizobacterial isolates HMM44, HMM89, HMR25, HMR32, HMR33 and HMR70		*Indian mustard (Brassica juncea L.)*	Sharma, R., Sindhu, S. & Sindhu, S. S. Suppression of Alternaria blight disease and plant growth promotion of mustard (Brassica juncea L.) by antagonistic rhizosphere bacteria. Appl. Soil Ecol. 129, 145–150 (2018).	10.1016/j.apsoil.2018.05.013
		Streptomyces humidus-related species		*Cabbage*	Hassan, N. et al. Biocontrol Potential of an Endophytic Streptomyces sp. Strain MBCN152-1 against Alternaria brassicicola on Cabbage Plug Seedlings. Microbes Environ. 32, 133–141 (2017).	10.1264/jsme2.ME17014
		Trichoderma harzianum and Pseudomonas fluorescence		*Indian mustard [Brassica juncea (L.)]*	Meena, P. D. et al. Comparative study on the effect of chemicals on Alternaria blight in Indian mustard -A multi-location study in India. J. Environ. Biol. 32, 375–379 (2011).	https://search.proquest.com/docview/876868474?accountid=15599
		Trichoderma harzianum, Pseudomonas fluorescens and Bacillus subtilis		*Indian mustard [Brassica juncea (L.)]*	Sharma, S., Singh, J., Munshi, G. D. & Munshi, S. K. Effects of biocontrol agents on lipid and protein composition of indian mustard seeds from plants infected with Alternaria species. Arch. Phytopathol. Plant Prot. 43, 589–596 (2010).	10.1080/03235400801972350
	Alternaria spp.	*Trichoderma harzianum ISO-1, T.harzianum ISO-2 and T.piluliferum caused*	+		Shikha Thakur and N.S.K. Harsh, 2014. Phylloplane fungi as biocontrol agent against Alternaria leaf spot disease of (Akarkara) Spilanthes oleracea. Biosci. Disc., 5(2):139-144	http://jbsd.in/Vol%205%20No.%202%20July%202014/Shikaha139-144.pdf
		Gliocladium spp, B.subtilis, Pseudomonas fluorescence			effectiveness of bacterial and fungal isolated to control phoma lingam on Brassica napus	https://file.scirp.org/pdf/AJPS20120600009_79598217.pdf
		Serratia plymuthica HRO-C48 and Gliocladium catenulatum J1446		Oilseed rape (Brassica napus L.) is	Hammoudi, O., Salman, M., Abuamsha, R. & Ehlers, R.-U. Effectiveness of Bacterial and Fungal Isolates to Control Phoma lingam on Oilseed Rape Brassica napus. Am. J. Plant Sci. 03, 773–779 (2012).	10.4236/ajps.2012.36093
		Serratia plymuthica (strain HRO-C48) and Pseudomonas chlororaphis (strain MA 342)		Oilseed rape (Brassica napus L.) is	Abuamsha, R., Salman, M. & Ehlers, R. U. Effect of seed priming with Serratia plymuthica and Pseudomonas chlororaphis to control Leptosphaeria maculans in different oilseed rape cultivars. Eur. J. Plant Pathol. 130, 287–295 (2011).	10.1007/s10658-011-9753-y
		T. harzianum, T. hamatum and T. longi- brachiatum		Oilseed rape (Brassica napus L.) is	Dawidziuk, A., Popiel, D., Kaczmarek, J., Strakowska, J. & Jedryczka, M. Optimal Trichoderma strains for control of stem canker of brassicas: molecular basis of biocontrol properties and azole resistance. BioControl 61, 755–768 (2016).	10.1007/s10526-016-9743-2

	Phoma lingam(Leptosphaeria maculans)	Pseudomonas chlororaphis, Bacillus cereus and Bacillus amyloliquefaciens	canola		Ramarathnam, R., Fernando, W. G. D. & de Kievit, T. The role of antibiosis and induced systemic resistance, mediated by strains of Pseudomonas chlororaphis, Bacillus cereus and B. amyloliquefaciens, in controlling blackleg disease of canola. BioControl 56, 225–235 (2011).	10.1007/s10526-010-9324-8
	Pseudomonas savastanoi pv. phaseolicola	P. fluorescences; P. putida; bacteriophages	+	Bean seedlings	O. Hassan Eman and Z.A. El-Meneisy Afaf, 2014. Biocontrol of Halo Blight of Bean Caused by Pseudomonas phaseolicola . International Journal of Virology, 10: 235-242.	https://scialert.net/abstract/?doi=ijv.2014.235.242
			+	Beans cv. UT15 Contender seeds (Stokes Seeds Ltd., Ste-Catherine, ON, Canada)	Filion M, St-Arnaud M, Jabaji-Hare SH. Quantification of Fusarium solani f. sp. phaseoli in Mycorrhizal Bean Plants and Surrounding Mycorrhizosphere Soil Using Real-Time Polymerase Chain Reaction and Direct Isolations on Selective Media. Phytopathology. 2003 Feb;93(2):229-35	doi: 10.1094/PHYTO.2003.93.2.229.
Bean		*Trichoderma harzianum*	+	*Phaseolus vulgaris L.,*	Jackeline L. Pereira, 1 Rayner M. L. Queiroz, 1 Sébastien O. Charneau, 1 Carlos R. Felix, 1 Carlos A. O. Ricart, 1Francilene Lopes da Silva, 1 Andrei Stecca Steindorff, 1 Cirano J. Ulhoa, 2 , * and Eliane F. Noronha 1. Analysis of Phaseolus vulgaris Response to Its Association with Trichoderma harzianum (ALL-42) in the Presence or Absence of the Phytopathogenic Fungi Rhizoctonia solani and Fusarium solani. PLoS One. 2014; 9(5): e98234.	doi: 10.1371/journal.pone.0098234
		Bacillus subtilis CA32 and Trichoderma harzianum RU01	+	*Phaseolus vulgaris L.,*	Saman Abeysinghe. Biological control of Fusarium solani f. sp. phaseoli the causal agent of root rot of bean using Bacillus subtilis CA32 and Trichoderma harzianum RU01. RUHUNA JOURNAL OF SCIENCE Vol. 2, September 2007, pp. 82-88	http://www.ruh.ac.lk/rjs/rjs.html
		Rhizobium tropici and Bacillus subtilis	+	*Phaseolus vulgaris L.,*	C.Estevez de JensenaJ.A.PercichaP.H.Grahamb. Integrated management strategies of bean root rot with Bacillus subtilis and Rhizobiumin Minnesota. Field Crops Research Volume 74, Issues 2–3, 15 March 2002, Pages 107-115	https://doi.org/10.1016/S0378-4290(01)00200-3
	Fusarium solani f.sp. phaseoli	*Bacillus spp; Pseudomonas spp, Rhodococcus spp*		bean seeds 'BRS Valente	Bianca Obes Corrêa , Jaqueline Tavares Schafer, Andrea Bittencourt Moura, 2014. Spectrum of biocontrol bacteria to control leaf, root and vascular diseases of dry bean. Biological Control 72 (2014) 71–75	http://dx.doi.org/10.1016/j.biocontrol.2014.02.013
		Pseudomonas fluorescens, pseudomonas chlororaphis			diseases of dry bean	https://www.sciencedirect.com/science/article/pii/S1049964409000334
	Colletotrichum lindemuthianum	*Trichoderma viride, Trichoderma harzianum and Pseudomonas fluorescence*	+	*Phaseolus vulgaris L.,*	Mohammed Amin*, Jifara Teshele, Amare Tesf	DOI:10.12691/plant-2-1-5

Annex 4

Species	List of Pathogen	Natural compounds	*in vitro*	*in vivo (specify plant species)*	Bibliografy	DOI or link
Tomato	*Clavibacter michiganensis*	Extracts and pure metabolites of Origanum onites L	+	+	Kotana R, Cakir A, Ozer H , Kordali S , Cakmakci R, Dadasoglud F, Dikbas N , Aydinf T, Kazaz C, 2014.Antibacterial effects of Origanum onites against phytopathogenic bacteria: Possible use of the extracts from protection of disease caused by some phytopathogenic bacteria. Scientia Horticulturae 172 (2014) 210–220	http://dx.doi.org/10.1016/j.scienta.2014.03.016
	Pseudomonas syringae pv. tomato	Acqueos plant extracts	+	+	Karabuyuk and Aysan, 20186. Aqueous plant extracts as seed treatments on tomato bacterial speck disease.Acta Hortic. 1207, 193-196	DOI: 10.17660/ActaHortic.2018.1207.25
	Xanthomonas spp.	Plant extracts	+	+	Mbega, E.R., Mortensen, C.N., Mabagala, R.B. et al., 2012. The effect of plant extracts as seed treatments to control bacterial leaf spot of tomato in Tanzania. J Gen Plant Pathol (2012) 78: 277.	https://doi.org/10.1007/s10327-012-0380-z
	ToMV	Palnt extract		+	**Vinayarani, S.A. Deepak, S.R. Niranjana, H.S. Prakash, G.P. Singh, A.K. Sinha and B.C. Prasad, 2011. Antiviral Activity of Plant Extracts and other Inducers against Tobamoviruses Infection in Bell Pepper and Tomato Plants. International Journal of Plant Pathology, 2: 35-42.10.3923/ijpp.2011.35.42**	https://scialert.net/abstract/?doi=ijpp.2011.35.42
	Fusarium oxysporum f.sp. radicis lycopersici	Chitosan	+	+	Benhamou, N., Lafontaine, P.J., Nicole, M. 1994. Induction fo systemic resistance of Fusarium crown and root rot in tomato plants by seed treatment with chitosan. Phytopathology 84:1432-1444	https://www.apsnet.org/publications/phytopathology/backissues/Documents/1994Articles/Phyto84n12_1432.pdf

Broccoli	*Xanthomonas campestris pv. campestris*	Acetone extracts of Cymbopogon citratus	+	rape (Brassica napus L.),	G. Mandiriza, Q. Kritzinger, T.A.S. Aveling. The evaluation of plant extracts, biocontrol agents and hot water as seed treatments to control black rot of rape in South Africa Crop Protection 114 (2018) 129–136	DOI: 10.1016/j.cropro.2018.08.025
		Zataria multiflora essential oil (thymol and carvacrol)	+	*Brassica oleracea var. capitata (Cabbage Glory of Enkhuizen)*	Leila Aminia, Mohammad Reza Soudia,⁎, Azra Saboorab, Hamid Mobasheric,d. Effect of essential oil from Zataria multiflora on local strains of Xanthomonas campestris: An efficient antimicrobial agent for decontamination of seeds of Brassica oleracea var. capitata. Scientia Horticulturae 236 (2018) 256–264 Van Der Wolf, J.M., Birnbaum, Y., Van Der Zouwen, P.S., and Groot, S.P.C. (2008). Disinfection of vegetable seed by treatment with essential oils, organic acids and plant extracts. Seed Sci. Technol. 36 (1), 76–88	https://doi.org/10.1016/j.scienta.2018.03.046 http://dx.doi.org/10.15258/sst.2008.36.1.08.
	Alternaria spp.	thyme oil	+	+	Amein T et al., 2011. Evaluation of non-chemical seed treatment methods for control of Alternaria brassicicola on cabbage seeds.	DOI: 10.1007/BF03356406
		garlic bulb extract		Indian mustard [Brassica juncea (L.)]	Meena, P. D. et al. Comparative study on the effect of chemicals on Alternaria blight in Indian mustard -A multi-location study in India. J. Environ. Biol. 32, 375–379 (2011).	https://search.proquest.com/docview/876868474?accountid=15599

	Phoma lingam(Leptosphaeria maculans)	Phytoalexinsa from Brassica napus ssp. Rapifera (Rutabaga)	+	+	M. Soledade C. Pedras,* Sabine Montaut, and Mojmir Suchy Phytoalexins from the Crucifer Rutabaga: Structures, Syntheses, Biosyntheses, and Antifungal Activity J. Org. Chem. 2004, 69, 4471-4476	https://pubs.acs.org/doi/pdf/10.1021/jo049648a
Bean	*Pseudomonas savastanoi pv. phaseolicola*	garlic extract	+	bean seedlings	O. Hassan Eman and Z.A. El-Meneisy Afaf, 2014. Biocontrol of Halo Blight of Bean Caused by Pseudomonas phaseolicola . International Journal of Virology, 10: 235-242.	https://scialert.net/abstract/?doi=ijv.2014.235.242
	Fusarium solani f.sp. phaseoli	PLANT POWDER AND ESSENTIAL OIL FROM ARTEMISIA MONOSPERMA	+	+	Hend A. Hamedo 2009. CONTROL OF ROOT ROT DISEASE USING PLANT POWDER AND ESSENTIAL OIL FROM ARTEMISIA MONOSPERMA. Egypt. J. Exp. Biol. (Bot.), 5: 169 – 173 (2009).	https://www.ejmanager.com/mnstemps/15/15-1430505174.pdf?t=1556798679
	Colletotrichum lindemuthianum	Acetone, ethyl acetate and water extracts of Syzygium cordatum Hochst.ex Krauss, Chlorophytum comosum cv. Variegatum, Agapanthus caulescens Spreng., Ipomoea batatas (L.) Lam, Allium sativum L. and Carica papaya L.	Agar infusion tecnique	+	JIG Masangwa. The effect of plant extracts on anthracnose of Phaseolus vulgaris L. and Vigna unguiculata (L.) Walp. July 2012 PhD Thesys. University of Pretoria	https://repository.up.ac.za/bitstream/handle/.../dissertation.pdf?.
		Agapanthus, Allium, Carica and Syzygium	"+	Bean seeds	Masangwa, J. I. G., T. A. S. Aveling, and Quenton Kritzinger. "Screening of plant extracts for antifungal activities against Colletotrichum species of common bean (Phaseolus vulgaris L.) and cowpea (Vigna unguiculata (L.) Walp)." The Journal of Agricultural Science 151.4 (2013): 482-491.	https://doi.org/10.1017/S0021859612000524

		Extracts from M. argyrophylla, M. fallax, O. vulgare, S. arianeae and S. pohlii	+		Joyce Mendes Andrade Pinto, Elaine Aparecida de SouzaElaine Aparecida de Souza, Denilson Ferreira Oliveira. Use of Plant extract in the control of common bean anthracnose. August 2010Crop Protection 29(8):838-842.	DOI: 10.1016/j.cropro.2010.03.006

Annex 5

Species	List of Pathogen	Physical Agents	*in vitro*	*in vivo (specify plant species)*	Bibliografy	DOI or link
Tomato	*Clavibacter michiganensis*	Hot temperature	+	+	Diego, M., Wilma, W. 2012. Evaluación de métodos para desinfectar semillas de tomate contra cancro bacteriano (Clavibacter michiganensis subsp. michiganensis). Agrociencia Uruguay 16:134-141.	http://www.scielo.edu.uy/scielo.php?script=sci_arttext&pid=S2301-15482012000100016
		Hot temperature	+	+	Fatmi, M. (1991). Seed Treatments for Eradicating Clavibacter michiganensis subsp. michiganensis from Naturally Infected Tomato Seeds. Plant Disease. 75. 383.	DOI: 10.1094/PD-75-0383
		Fermentation of fruit pulp	+	+	Dhanvantari, B.N. 1989. Effect of seed extraction methods and seed treatments on control of tomato bacterial canker. Canadian Journal of Plant Pathology 11:400-408	https://www.tandfonline.com/doi/abs/10.1080/07060668909501087?journalCode=tcjp20
	Pseudomonas syringae pv. tomato	Hot temperaure	+	+	Grondeau, C., Samson, R., Sands, D.C. 1994. A review of thermotherapy to free plant materials from pathogens, especially seeds from bacteria. Critical Reviews in Plant Sciences 13:57-75.	https://www.tandfonline.com/doi/abs/10.1080/07352689409701908
	Xanthomonas spp.	Hot temperaure	+	+	Grondeau, C., Samson, R., Sands, D.C. 1994. A review of thermotherapy to free plant materials from pathogens, especially seeds from bacteria. Critical Reviews in Plant Sciences 13:57-75.	https://www.tandfonline.com/doi/abs/10.1080/07352689409701908

	ToMV	Hot temperaure	+	+	Silva, P.R., Freitas, R.A., Nascimento, W.M. 2011. Detection of Tomato mosaic virus in tomato seeds and treatment with thermotherapy. Acta Horticulturae 917:303-308.	https://www.actahort.org/books/917/917_43.htm
	Fusarium oxysporum f.sp. radicis lycopersici					
Broccoli	*Xanthomonas campestris pv. campestris*	Hot temperature	+	+	Nega, E., Ulrich, R., Werner, S., Jahn, M., 2003. Hot water treatment of vegetable seed -an alternative seed treatment method to control seed-borne pathogens in organic farming. J. Plant Dis. Prot. 110, 220–234.	https://www.jstor.org/stable/43215507
	Alternaria spp.	Warm water: 50°C for 30 minutes, 51°C for 25 minutes	+	+	Koch, Eckhard & Groot, Steven. (2015). Health management for seeds and other organic propagation material.	DOI: 10.1094/9780890544785.015
		Warm water: 50°C for 30 minutes, 51°C for 25 minutes	+	+	Jahn, M., Koch, E., Blum, H., Nega, E., & Wilbois, K. P. (2007). Leitfaden Saatgutgesundheit im Ökologischen Landbau-Gemüsekulturen.	http://orgprints.org/11675/1/Leitfaden_Gem%C3%BCsekult__100326.pdf
	Phoma lingam(Leptosphaeria	Warm water: 50°C for 30 minutes, 51°C for 25 minutes	+	+	Koch, Eckhard & Groot, Steven. (2015). Health management for seeds and other organic propagation material.	DOI: 10.1094/9780890544785.015
		Warm water: 50°C for 30 minutes, 51°C for 25 minutes	+	+	Jahn, M., Koch, E., Blum, H., Nega, E., & Wilbois, K. P. (2007). Leitfaden Saatgutgesundheit im Ökologischen Landbau-Gemüsekulturen.	http://orgprints.org/11675/1/Leitfaden_Gem%C3%BCsekult__100326.pdf

	maculans)	Hot water treatment	+	+	Cynthia M. Ocamb and Briana J. Claassen, Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331 A CLINIC CLOSE-UP Management of Black Leg in Brassica Vegetable Crops Oregon State University Extension Service September 2016.	https://pnwhandbooks.org/sites/pnwhandbooks/files/plant/document/broccoli-brassica-oleracea-black-leg-phoma-stem-canker/cliniccloseupblacklegmanagementinvegetables2016finaldraft.pdf
Bean	*Pseudomonas savastanoi pv. phaseolicola*	Steam air			Ralph, W. "Steam-air treatment of bean seed infected with Pseudomonas phaseolicola." Seed Science and Technology 5.3 (1977): 559-565.	https://www.cabdirect.org/cabdirect/abstract/19771340357
	Fusarium solani f.sp. phaseoli					
	Colletotrichum lindemuthianum	Warm water: 50°C for 30 minutes, 51°C for 25 minutes	+	+	Jahn, M., Koch, E., Blum, H., Nega, E., & Wilbois, K. P. (2007). Leitfaden Saatgutgesundheit im Ökologischen Landbau-Gemüsekulturen.	http://orgprints.org/11675/1/Leitfaden_Gem%C3%BCsekult__100326.pdf
		Warm water: 50°C for 30 minutes, 51°C for 25 minutes	+	+	Koch, Eckhard & Groot, Steven. (2015). Health management for seeds and other organic propagation material.	DOI: 10.1094/9780890544785.015
		Hot water, hot air	+	+	Thomas, G. J., and K. G. Adcock. "Exposure to dry heat reduces anthracnose infection of lupin seed." Australasian plant pathology 33.4 (2004): 537-540.	https://link.springer.com/article/10.1071/AP04057
					Haesen, Esther. Efficacy of non-synthetic seed treatments against anthracnose (Colletotrichum lupini) in white lupin. Diss. Research Institut of Organic Agriculture (FiBL), CH-Frick ETH Zurich, CH-Zurich, 2018.	https://orgprints.org/34403/1/MThesis_EfficacyofNonSyntheticSeedTreatmentsAgainstLupinAnthracnose_EHaesen.pdf

Annex 6

Species	List of Pathogen	Registered active substances for seed coating*	Registered active substances (Italy)
Tomato	*Clavibacter michiganensis*		Serenade max (Bayer) - Amilo-x (Biogard)
	Pseudomonas syringae pv. tomato		
	Xanthomonas campestris pv. vesicatoria		Tusal (Certis) - Trianum P (Koppert) - Amylo-X (Biogard)
	ToMV		
	Fusarium oxysporum f.sp. radicis lycopersici		Tusal (Certis) - Trianum P (Koppert) - Amylo - X (Biogard)
Broccoli	*Xanthomonas campestris pv. campestris*		Serenade max (Bayer) - Amilo-x (Biogard) - Cerall (Serbios)
	Alternaria spp.		Serenade max (Bayer) - Amilo-x (Biogard) - Cerall (Serbios)
	Phoma lingam(Leptosphaeria maculans)		Serenade max (Bayer) - Amilo-x (Biogard) - Cerall (Serbios)
Snap Bean	*Pseudomonas savastanoi pv. phaseolicola*		
	Fusarium solani f.sp. phaseoli		Tusal (Certis) - Trianum P (Koppert) - Amylo-X (Biogard) - Mycostop
	Colletotrichum lindemuthianum		Tusal (Certis) - Trianum P (Koppert) - Amylo-X (Biogard)

***Cerall and Mycostop are registered on seed coating in cereals and general seeds**

Company	Product	Active substance
Bayer	Serenade max	Bacillus amyloliquefaciens ,(former subtilis) ceppo QST 713,
BioGard	Amilo-X	Bacillus amyloliquefaciens, sottospecie palntarum , ceppo D747.
Certi	Tusal	Trichoderma asperellum (T25) g 0,5 (1 x 108 UFC/g); Trichoderma atroviride (T11) g 0,5 (1 x 108 UFC/g)
Koppert	Trianum	Trichoderma harzianum T-22
	Mycostop	Streptomyces griseoviridis ceppo K61
Serbios	Cerral	Pseudomonas chlororaphis strain MA 342
Landor	Agri-Sem*	Horsetail manure, clay
Full Service	Fulltack*	Adhesive for hydroseeding
BASF Schweiz	Hi Stick Soy*	Bradyrizobium japonicum
Hauenstein	Nitragin Gold*	Clay, Sinorhizobium meliloti
3folium	Promos*	Botanical extract not subject to the fertilizer ordinance
3folium	RhizoFix-10*	Bradyrizobium japonicum
3folium	RhizoFix-20*	Rhizobium fabae
3folium	RhizoFix-30*	Rhizobium pisi
3folium	RhizoFix-40*	Rhizobium leguminosarum
3folium	RhizoFix-50*	Ensifer meliloti
Biocontrol	RootWin S*	Bradyrizobium japonicum, rhizobia for Soja
Biocontrol	T-Gro Easy-Flow*	Trichoderma harzianum

*Substances allowed for seed treatment in Switzerland. Reference: FiBL inputs list for Switzerlan. https://shop.fibl.org/chfr/mwdownloads/download/link/id/76/

Annex 7

Fungal-bacterial target	Reference	Pathogen inoculation	Seed treatment	Notes	
Fusarium oxysporum f.sp. radicis lycopersici	Domenech, J., Reddy, M.S., Kloepper, J.W.,2006 Combined Application of the Biological Product LS213 with Bacillus, Pseudomonas or Chryseobacterium for Growth Promotion and Biological Control of Soil-Borne Diseases in Pepper and Tomato. Biocontrol 51, 245. https://doi.org/10.1007/s10526-005-2940-z	Fusarium oxysporum f. sp. radicis-lycopersici isolate AU-TF1 and Rhizoctonia solani (AG-2) isolate AU-TR1 were grown on PDA for 5–6 days at 28 C° and five to six plates of each were mixed with sterile distilled water in a Waring blender for 2 min.Pathogen inoculum (FORL 10^{6-7} conidia /ml +Rs 10^{6-7} conidi/ml), was spread on the seeds.	PGPR (Bacillus licheniformis CECT 5106; Pseudomonas fluorescens CECT 5398; Chryseobacterium balustinum CECT 5399) and the biological product LS213 (Gustafson Inc., Dallas, Texas, B. subtillis strain GBO3 and B. amyloliquefaciens strain IN937a) were applied as a seed drench method immediately after seeding (1 ml/seed of 10^{8--9} cfu/ml).		
	Datnoff L E, .Nemec S., PerneznyK.1995 Biological Control of Fusarium Crown and Root Rot of Tomato in Florida Using Trichoderma harzianum and Glomus intraradices - Biological Control 5 (3): 427-431https://www.sciencedirect.com/science/article/pii/S1049964485710511	plants translated into commercial fields with with a previous history of FCRR	amended soil mixed	Plants were first cultivated in soil where the biocontrol agent was inoculated and then transplanted in a field fumigated with ethidium bromide where the pathogen was already present	1
	Thomas F. C. Chin-A-Woeng, Guido V. Bloemberg, Arjan J. van der Bij, Koen M. G. M. van der Drift, Jan Schripsema, Bernadette Kroon, Rudy J. Scheffer, Christoph Keel, Peter A. H. M. Bakker, Hans-Volker Tichy, Frans J. de Bruijn, Jane E. Thomas-Oates, and Ben J. J. Lugtenberg, 1998 . Biocontrol by Phenazine-1-carboxamide-Producing Pseudomonas chlororaphis PCL1391 of Tomato Root Rot Caused by Fusarium oxysporum f. sp. radicis-lycopersici Molecular Plant-Microbe Interactions 11:11, 1069-1077	One third of a 10-day-old PDA petri dish culture of F. oxysporum f. sp. radicis-lycopersici was homogenized and inoculated in 200 ml of Czapek-Dox medium in a 1-liter Erlenmeyer flask. After growth for 3 days at 28°C under shaking the fungal material was placed on top of sterile glass wool and the filtrate was adjusted to a concentration of 5×10^5 spores/ml. For inoculation, spores were mixed thoroughly with potting soil (3.0×10^6 spores per kg)	Tomato (Lycopersicon esculentum Mill.) seeds (cv. Carmello) were coated with bacteria (P. chlororaphis strain PCL1391) by dipping the seeds in a mixture of 1% (wt/vol) methylcellulose (Sigma) and 1×10^9 CFU/ml bacteria in phosphate-buffered saline (PBS) buffer.		
Alternaria spp.	Manhas, R. K. & Kaur, T. Biocontrol Potential of Streptomyces hydrogenans Strain DH16 toward Alternaria brassicicola to Control Damping Off and Black Leaf Spot of Raphanus sativus. Front. Plant Sci. 7, 1–13 (2016). - 10.3389/fpls.2016.01869	Seeds surface sterilized, were first artificially infected with the pathogen prior to antagonist treatment; immersed for 4h in fungal spore suspension in presence of 1% carboxymethyl cellulose	soaked in different concentrations (5,10,and20%v/v) of culture supernatant of antagonist/(ii)soaked in cell suspension of antagonist prepared in 1% CMC (107–108/ml).	Seeds were first immersed in a suspension with the pathogen and then immersed at different concentrations in a suspension with the biocontrol agent.	2
	Sharma, R., Sindhu, S. & Sindhu, S. S. Suppression of Alternaria blight disease and plant growth promotion of mustard (Brassica juncea L.) by antagonistic rhizosphere bacteria. Appl. Soil Ecol. 129, 145–150 (2018). - 10.1016/j.apsoil.2018.05.013	Fungal growth suspension (100 ml) was mixed in the 10 kg soil: sand mixture in earthen pots in coinoculation treatments.	Growth suspension of rhizobacterial isolates grown for 48 h on LB medium slopes was made in 5 ml of sterilized water. Seeds of mustard var. RH749 were inoculated with 5 ml bacterial growth suspension for 1 h.	Seeds were immersed in the suspension containing the biocontrol agents and then sown on a substrate inoculated with a suspension of the pathogen	3
	Hassan, N. et al. Biocontrol Potential of an Endophytic Streptomyces sp. Strain MBCN152-1 against Alternaria brassicicola on Cabbage Plug Seedlings. Microbes Environ. 32, 133–141 (2017). - 10.1264/jsme2.ME17014	A challenge inoculation was performed by spraying a conidial suspension of A. brassicicola (105 conidia mL–1) onto cabbage seedlings 7 days after sowing until run off occurred.	Sterilized seeds were sown in 128-cell plug trays containing an autoclaved commercial soil mix, One d after sowing, a mycelial suspension (ca. 106–107 CFU mL–1) of each strain was dropped onto the seeds (1 mL per seed) and they were grown for a further 6 d.	Sterilized seeds were sown on an autoclaved substrate. One day after sowing, the biocontrol agents were inoculated on the seeds, then the pathogen was inoculated on the seedlings by sprayin 6 days after sowing.	4
	Meena, P. D. et al. Comparative study on the effect of chemicals on Alternaria blight in Indian mustard -A multi-location study in India. J. Environ. Biol. 32, 375–379 (2011). - https://search.proquest.com/docview/876868474?accountid=15599	The experimental sites represented hot spots for *Alternaria* blight disease in different dominant Indian mustard growing areas mainly as a field crop under non-limiting soil moisture conditions in semi-arid and sub humid agro-climatic zones of India.	Twelve treatments including a control plot with only water spray were considered;Uniform spray solution of required concentration for chemicals at all the locations ;	the plants were grown in different fields subject to Alternaria infections, and the treatments were carried out by sprayng	5

	Sharma, S., Singh, J., Munshi, G. D. & Munshi, S. K. Effects of biocontrol agents on lipid and protein composition of indian mustard seeds from plants infected with Alternaria species. Arch. Phytopathol. Plant Prot. 43, 589–596 (2010). - 10.1080/03235400801972350	seeds from plants infected with Alternaria species	Finally the mass inoculum was used for the preparation of dry formulation, were individually formulated as described earlier (Sharma et al. 2008). The seeds were treated with each of the three formulations separately and sown along with the untreated control in experimental area; Seed treatments were followed by sprays with biocontrol agents at 30 and 60 days after sowing as described earlier (Sharma et al. 2008).		6
	Shikha Thakur and N.S.K. Harsh, 2014. Phylloplane fungi as biocontrol agent against Alternaria leaf spot disease of (Akarkara) Spilanthes oleracea. Biosci. Disc., 5(2):139-144. - http://jbsd.in/Vol%205%20No.%202%20July%202014/Shikaha139-144.pdf	only in vitro	only in vitro		7
Phoma lingam(Leptosphaeria maculans)	Hammoudi, O., Salman, M., Abuamsha, R. & Ehlers, R.-U. Effectiveness of Bacterial and Fungal Isolates to Control Phoma lingam on Oilseed Rape Brassica napus. Am. J. Plant Sci. 03, 773–779 (2012). - 10.4236/ajps.2012.36093	1)Cotyledons were punctured with a needle and 10 µl of pycnidiospore suspension ; 2) OSR plantlets at growth stage of BBCH 14/15 were inoculated at the stem base either with V8 agar disks (7 mm diameter) grown with P. lingam or with 40 µl pycnidiospors; 3) Experiments were conducted in naturally infested fields with P. lingam.	Seeds were treated with the antagonists by soaking 1 g of seeds in 1-ml bacterial suspension for 5 h at 20°C.	The seeds were first treated by immersion with the biocontrol agents, then their activity was tested at three different times: 1) inoculating the cotyledons; 2) inoculating the seedlings with the mycelium; 3) in a naturally infected field	8
	Hammoudi, O., Salman, M., Abuamsha, R. & Ehlers, R.-U. Effectiveness of Bacterial and Fungal Isolates to Control Phoma lingam on Oilseed Rape Brassica napus. Am. J. Plant Sci. 03, 773–779 (2012). - 10.4236/ajps.2012.36093	"	"	"	"
	Abuamsha, R., Salman, M. & Ehlers, R. U. Effect of seed priming with Serratia plymuthica and Pseudomonas chlororaphis to control Leptosphaeria maculans in different oilseed rape cultivars. Eur. J. Plant Pathol. 130, 287–295 (2011). - 10.1007/s10658-011-9753-y	Ten days after sowing, the cotyledons were wounded in the centre of each leaf lobe with a sterile needle and 10 µl droplets of the conidial suspension were deposited onto each wound.	Seeds were bio-primed by soaking them in bacterial suspensions ; One g of seeds of the different OSR cultivars were treated with 1 ml bacterial suspension and incubated for 5 h at 20oC. When seeds were treated with both antagonists, 0.5 ml of each of the bacterial suspensions was mixed prior to the seed bio-priming. During incubation, seeds were agitated at 150 rpm on a rotary shaker. Seeds were then air dried over night at 20oC.	The seeds are first treated with biocontrol agents and the cotyledons are inoculated with the pathogen	9
	Dawidziuk, A., Popiel, D., Kaczmarek, J., Strakowska, J. & Jedryczka, M. Optimal Trichoderma strains for control of stem canker of brassicas: molecular basis of biocontrol properties and azole resistance. BioControl 61, 755–768 (2016). - 10.1007/s10526-016-9743-2	Inoculations were made on 12-day old plants, each half-cotyledon was punctured with a needle, Spore suspensions of plant pathogens were deposited directly onto each plant wound.	When the plants reached BBCH stage 16 they were sprayed with spore suspensions of the studied Trichoderma species		10

	Ramarathnam, R., Fernando, W. G. D. & de Kievit, T. The role of antibiosis and induced systemic resistance, mediated by strains of Pseudomonas chlororaphis, Bacillus cereus and B. amyloliquefaciens, in controlling blackleg disease of canola. BioControl 56, 225–235 (2011). - 10.1007/s10526-010-9324-8	All the assays were carried out at the seedling stage, Cotyledons of B. napus cv Westar were used for the assays. Both the bacteria and the pathogen were inoculated in the same wound spot	All the assays were carried out at the seedling stage, Cotyledons of B. napus cv Westar were used for the assays. Both the bacteria and the pathogen were inoculated in the same wound spot. Effect of time of inoculation of the bacteria: 1. Bacteria inoculated 24 h prior to inoculation of the pathogen. 2. Bacteria inoculated 48 h prior to inoculation of the pathogen. 3. Bacteria and pathogen inoculated at the same time (co-inoculation). 4. Pathogen inoculated 24 h prior to inoculation of bacteria. 5. Pathogen inoculated 48 h prior to inoculation of bacteria.		11
Fusarium solani f.sp. phaseoli	Filion M, St-Arnaud M, Jabaji-Hare SH. Quantification of Fusarium solani f. sp. phaseoli in Mycorrhizal Bean Plants and Surrounding Mycorrhizosphere Soil Using Real-Time Polymerase Chain Reaction and Direct Isolations on Selective Media. Phytopathology. 2003 Feb;93(2):229-35. - doi: 10.1094/PHYTO.2003.93.2.229.	After 28 days of growth, the seedling compartment of each experimental unit with the Fusarium treatment was inoculated with 5 ml of a F. solani f. sp. phaseoli conidial suspension	The seedling compartment of each experimental unit with the mycorrhizal treatment was inoculated at planting with 2.5 × 103 spores of G. intraradices delivered in a 1-ml volume. The inoculum was mixed with the soil	First the seeds were germinated, and the seedlings were transplanted onto a substrate in which the biocontrol agents were inoculated. After 28 days the inoculation was carried out by pouring 5ml of suspension of the pathogen	12
	Jackeline L. Pereira, 1 Rayner M. L. Queiroz, 1 Sébastien O. Charneau, 1 Carlos R. Felix, 1 Carlos A. O. Ricart, 1Francilene Lopes da Silva, 1 Andrei Stecca Steindorff, 1 Cirano J. Ulhoa, 2 , * and Eliane F. Noronha 1. Analysis of Phaseolus vulgaris Response to Its Association with Trichoderma harzianum (ALL-42) in the Presence or Absence of the Phytopathogenic Fungi Rhizoctonia solani and Fusarium solani. PLoS One. 2014; 9(5): e98234. - doi: 10.1371/journal.pone.0098234	The soils samples were previously infected with the phytopathogenic fungi. the colonized sorghum was triturated, sifted (20 mesh) and used for soil infection.	Rinsed seeds were immersed in a T. harzianum spore suspension containing 2.4x 10^8 spores per mL and sown in 500 mL cups containing sterile soil		13
	Saman Abeysinghe. Biological control of Fusarium solani f. sp. phaseoli the causal agent of root rot of bean using Bacillus subtilis CA32 and Trichoderma harzianum RU01. RUHUNA JOURNAL OF SCIENCE Vol. 2, September 2007, pp. 82-88 - http://www.ruh.ac.lk/rjs/rjs.html	Ten days after planting when the primary leaves were fully expanded, Five milliliters of spore suspension was applied by pipette just below the collar region around the hypocotyls of each plant.	The concentration of cells in the suspension was spectrophotometricaly adjusted to 108 CFU/mL and used for seed bacterization		14
	de Jensen C.E. ,Percich J.A., Graham P.H.,2002. Integrated management strategies of bean root rot with Bacillus subtilis and Rhizobiumin Minnesota. Field Crops Research Volume 74, Issues 2–3: 107-115 - https://doi.org/10.1016/S0378-4290(01)00200-3	the soils samples were previously infected with the phytopathogenic fungi. the colonized sorghum was triturated, sifted (20 mesh) C19:C21nd used for soil infection.	Biocontrol agent were applied alone ar in combiantion of fungicide as seed treatment or to the seed prior to sowing	soil was pasteurized, seeds surface sterilized	15
	Bianca Obes Corrêa , Jaqueline Tavares Schafer, Andrea Bittencourt Moura, 2014. Spectrum of biocontrol bacteria to control leaf, root and vascular diseases of dry bean. Biological Control 72 (2014) 71–75 - http://dx.doi.org/10.1016/j.biocontrol.2014.02.013	Bean seedlings, 10-days after emergence, were exposed to each isolate, separately, by pouring 5 mL of the in conidial suspension in each of two holes made in the soil around the plants	The bean seeds 'BRS Valente' were microbiolized by immersing and agitating for five hours at 10 °C in the cell suspension (20 seeds/20 mL).	non-sterile soil in pots	16
Colletotrichum lindemuthianum	diseases of dry bean - https://www.sciencedirect.com/science/article/pii/S1049964409000334	Inoculations with C. lindemuthianum were made by root dipping, during transplantation, in a conidial suspension of 10^6 conidia per ml, at the first leaf stage (10-day-old plants).	Bacteria were applied by covering pre-germinated bean seeds with a 1:1 mixture of 1% (w/v) methyl cellulose (Sigma, St. Louis, MO, USA) and 2 109 cfu per ml, in PBS. Application of bacteria was repeated 5 days later, at the cotyledon stage, by means of irrigation onto the soil with a bacterial suspension of 2 109 cfu per ml, in PBS	Plants were first grown in nurseries, from pre-germinated seeds, Bacteria were applied at this stage of growthas described below. Subsequently, 10-day-old plants were transplanted in pots, in a soil mixture same as above. The pathogenicfungus was applied during transplantation	17
	Mohammed Amin*, Jifara Teshele, Amare Tesfay. Evaluation of Bioagents Seed Treatment Against Colletotrichum Lindemuthianum, in Haricot Bean Anthracnose under Field Condition. Research in Plant Sciences, 2014, Vol. 2, No. 1, 22-26 - 10.12691/plant-2-1-5	Naturally infected seeds of the variety Mexican 142 were treated with each bioagent separately and dried overnight before sowing.	Talc based formulations (28 x 10-6 cfu/g product) of T. viride and T. harzianum [11] were used as seed treatments at 40 g/Kg of seeds soaked in 1 L of water for 24 hrs. Similarly, the talc based formulation of P. fluorescence by the method of Kloepper and Schroth, [12] was used as a seed treatment @ 10 g/Kg of seeds soaked in 1 L of water for 24 hrs		18
NATURAL COMPOUNDS					
Fusarium oxysporum f.sp. radicis lycopersici	Benhamou, N., Lafontaine, P.J., Nicole, M. 1994. Induction fo systemic resistance of Fusarium crown and root rot in tomato plants by seed treatment with chitosan. Phytopathology 84:1432-1444 - https://www.apsnet.org/publications/phytopathology/backissues/Documents/1994Articles/Phyto84n12_1432.pdf	tomato seedlings at the three-leaf stage were inoculated by introducing disks of actively growing FORL mycelium close to the root system.	Seeds of tomato surface sterilized, then immersed into each of the chitosan solution; gentle stirring for 15 min	treatments with chitosan	19

Alternaria spp.	Amein T et al., 2011. Evaluation of non-chemical seed treatment methods for control of Alternaria brassicicola on cabbage seeds. - 10.1007/BF03356406	Naturally infected seed lots of a white and a red head cabbage (Brassica oleracea) were used.	Thyme oil was used as an emulsion prepared by sonication in 40°C warm water. The seeds were placed in the different solutions/emulsions (usually in 100 ml beakers) for 4 hours with continuous stirring. The seeds were immersed for 15 min in the microbial cultures or spore suspensions, respectively, and thereafter used immediately or allowed to dry overnight and sown the following day.	In this study, both microbial consortia and thyme oil were evaluated	20
	Meena, P. D. et al. Comparative study on the effect of chemicals on Alternaria blight in Indian mustard -A multi-location study in India. J. Environ. Biol. 32, 375–379 (2011). - https://search.proquest.com/docview/876868474?accountid=15599	The experimental sites represented hot spots for *Alternaria* blight disease in different dominant Indian mustard growing areas mainly as a field crop under non-limiting soil moisture conditions in semi-arid and sub humid agro-climatic zones of India.	Twelve treatments including a control plot with only water spray were considered;Uniform spray solution of required concentration for chemicals at all the locations ;	the plants were grown in different fields subject to Alternaria infections, and the treatments were carried out by sprayng	5
Clavibacter michiganensis	Kasselaki, A.M., Goumas, D., Tamm, L., Fuchs, J., Cooper, J., Leifert, C. 2011. Effect of alternative strategies for the disinfection of tomato seed infected with bacterial canker (Clavibacter michiganensis subsp. michiganensis). NJAS - Wageningen Journal of Life Siences 58:145-147. - https://www.sciencedirect.com/science/article/pii/S157352141100039X	Seeds (50 g) of the tomato cultivar Packmore (Geoponiko Spiti, Athens, Greece) were packed in a cheesecloth bag and placed in a 1-l flask (Millipore, Schwalbach, Germany) containing 400 ml of the bacterial suspension. A vacuum was created by applying negative pressure (−40 kPa) for 5 min, after which the seeds were left to dry completely on sterile blotting paper, at room temperature, in a laminar flow cabinet.	Application of treatments by soaking; following treatments were applied to Cmm infected tomato seeds; Treatments were applied by immersion of the seed into prepared solutions for 10 min except for the compost extracts, where the seeds were soaked overnight.		21
	Umesha S., 2006. Occurrence of bacterial canker in tomato fields of Karnataka and effect of biological seed treatment on disease incidence. Crop Protection 25: 375-381 - https://doi.org/10.1016/j.cropro.2005.06.005	(Effect of Clavibacter michiganensis ssp. michiganensis on seed germination and seedling vigor) Seeds of the three tomato cultivars (collected from diseased plants) .	Seeds of the three tomato cultivars (collected from diseased plants) were treated with a suspension of P. fluorescens by shaking seeds in a pure culture (1 108CFUml1) of the antagonist for 12 h. Other seeds were treated with the above formulation of P. fluorescens in the form of a slurry treatment at the rate of 8 and 10 g/ kg of seeds.	Biocontrol agents were tested on naturally infected seeds while artificially inoculated seeds were used to study the germination of infected seeds	22
	Boudyach, E.H., Fatmi, M., Akhayat, O., Benziri, E., Ait Ben Aoumar, A. (2001). Selection of antagonistic bacteria of Clavibacter michiganensis subsp. michiganensis and evaluation of their efficiency against bacterial canker of tomato. Biocontrol Science and Technology (11), 141-149. - doi.org/10.1080/09583150020029817	After 3 weeks, the seedlings were removed and a suspension of C. m. subsp. michiganensis (~ 108 cfu ml - 1) was sprayed on the roots to give a ® nal concentration of 104 cfu/root system. The seedlings were then transplanted to individual pots.	Seeds were treated with antibiotic resistant bacteria in phosphate buffer (PBS; 0.05 m PO3 -4 , pH 7.4) containing 0.5% xanthan gum as an adhesive (Suslow & Schroth, 1982)		23
Pseudomonas syringae pv. Tomato	Bashan Y, Luz E., 2002.Protection of Tomato Seedlings against Infection by Pseudomonas syringae pv. Tomato by Using the Plant Growth-Promoting Bacterium Azospirillum brasilense. Appl Environ Microbiol. 68(6): 2637–2643. - 10.1128/AEM.68.6.2637–2643.2002	Leaves were inoculated at the three- to five-true-leaf stage with a handheld pneumatic sprayer from a height of 25 to 35 cm above the plant. Plants were sprayed until runoff occurred.	To increase the A. brasilense Cd population on the leaves, diluted malic acid was sprayed onto the leaves prior to inoculation. (Bashan, 2002). Inoculation with the bacterial suspension was done by spraying it, until runoff using an atomizer, onto the plant leaves and the root system, which was extracted carefully from the sand and rinsed to remove adhering particles (Bashan ,1998) .		24
	Ji P, Campbell H.L., Kloepper J, Wilson M, Jones J, Suslow T, 2006. Integrated biological control of bacterial speck and spot of tomato under field conditions using foliar biological control agents and plant growth-promoting rhizobacteria. Biological Control 36(3):358-367 - 10.1016/j.biocontrol.2005.09.003	The pathogen, Pst strain PT12, was spray inoculated onto upper and lower leaf surfaces 2 weeks after transplanting.	Tomato seeds (cv. Rutgers, Michael–Leonard, Grant Park, IL) were soaked in the bacterial suspensions for 30 min and then planted in seed trays with 3x3x4-cm cells containing Promix (Premier Peat, Riviere-du-Loup, Quebec) and incubated in the greenhouse. Soil drenches were applied at the time of seedling transplanting by pouring 100ml of bacterial suspensions (107CFU/ml) into the Promix in each pot.		25
Xanthomonas spp. (tomato)	Silva HSA, da Silva Romeiro R , Macagnan D , de Almeida Halfeld-Vieira B , Baracat Pereira MC, Mounteerd A, 2004. Rhizobacterial induction of systemic resistance in tomato plants: non-specific protection and increase in enzyme activities.Biological Control 29, 288–295 - 10.1016/S1049-9644(03)00163-4	Thirty days after planting, the plants were inoculated by spraying with the fungal and bacterial pathogen conidial/cell suspensions.	Twenty-four-hour cultures of B101R and B212R and 48-h cultures of A068R were used to microbiolize tomato seeds. Enough tap water was added to the tubes containing these isolates to cover two thirds of the culture medium. The tubes were then shaken vigorously to obtain homogeneous cell suspensions that were adjusted to OD540 ¼ 0:5, corresponding to approximately 1012 cfumL 1. Volumes of the suspensions sufficient to cover the seeds were transferred to 20mL plastic cups. The seeds were then immersed in these suspensions for24 h in the laboratory after which they were ready for planting.		26
Xanthomonas campestris pv. Campestris	Massomo, S.M.S., Mortensen, C.N., Mabagala, R.B., Newman, M.-A., Hockenhull, J., 2004. Biological control of black rot (Xanthomonas campestris pv. campestris) of cabbage in Tanzania with Bacillus strains. J. Phytopathol. 152, 98–105. - doi.org/10.1111/j.1439-0434.2003.00808.x	In the greenhouse seedling assay, 10-day-old seedlings were inoculated by dispensing a 30 ll drop of Xcc suspension at the sinus area of each cotyledon. In field experiments, inoculation with Xcc was done 3 weeks after transplanting by spraying in the evening (from 18:00 h) with freshly prepared inoculum until runoff, using a knapsack sprayer.	Seed inoculation was carried out by immersion of seeds in individual antagonist suspensions for 6 h followed by air-drying overnight in a flow cabinet. Inoculated seeds were sown immediately after treatment. For cotyledon inoculation, 10-day-old seedlings were sprayed with antagonist suspension, placed in plastic dew chambers for 12 h, returned to the greenhouse and grown for a further 11 days before planting in the field.	Evaluated the biocontrol efficacy of strains of Bacillus from Tanzania against the black rot pathogen, Xanthomonas campestris pv. campestris, in cabbage and the influence of the method of application under field conditions. significantl reduced, especially when antagonists were applied through the roots as compared to application through the seeds or foliage	27
	Ednar G. Wulff1,, Cames M. Mguni2, Carmen N. Mortensen3, Chandroo L. Keswani2 and John Hockenhull1, Biological control of black rot (Xanthomonas campestris pv. campestris) of brassicas with an antagonistic strain of Bacillus subtilis in Zimbabwe European Journal of Plant Pathology 108: 317–325, 2002 - https://link.springer.com/content/pdf/10.1023%2FA%3A1015671031906.pdf	One week after inoculation with the antagonist, the pathogen was applied to plots to be treated with Xcc by spraying 2ml of inoculum suspension (1 × 108 CFU/ml) per plant	1-monthold cabbage seedlings were lifted from their pots and the roots were carefully washed with tap water to eliminate most of the soil. The root tips were cut (0.5 cm) with a pair of scissors and the plant roots were immersed for 2 h in the inoculum suspension of the antagonist (5 × 108 CFU/ml).	Seeds were certified as free fromXcc. However, they were surface disinfected to avoid the presence of any pathogenic microorganisms on the seed surface. Sterility control was performed after seed disinfection by plating 100 seeds per disinfected seed lot on tryptic soy agar (TSA, Difco Laboratories, Detroit, MI, USA) and incubating at 25 ◦C	28

	Ghazalibiglar, H., 2014. Discovery of Paenibacillus Isolate for Control of Black Rot in Brassicas. PhD thesis. Lincoln University, Christchurch, New Zealand - https://researcharchive.lincoln.ac.nz/handle/10182/6322	Seeds of cabbage cv. Kameron were immersed in Xcc suspensions of 1 × 109 CFU/ml (3 ml suspension per 1 g seed = 3 × 109 CFU/g seed) or in sterile 0.1% BP (non-inoculated control) in a conical flask. These seed suspensions were gently mixed under vacuum (c. 50 mm Hg) for 5 min. Seeds were collected by filtration through sterile Mira Cloth, and air-dried in open Petri dishes in a laminar flow cabinet overnight in the dark.	The next day, 0.6 ml of Paenibacillus suspensions (5 × 109 CFU/ml) or sterile 0.1% BP (non-inoculated control) was added to 1 g of these seeds (3 × 109 CFU/g seed). Inoculated seeds were incubated in closed Petri dishes (non-sealed) in the laminar flow cabinet overnight in the dark.	A grow-out test was performed to determine the incidence of Paenibacillus on individual inoculated cabbage seeds.	29
	Hoda Ghazalibiglar, John G. Hampton, Eline van Zijll de Jong & Andrew Holyoake .Evaluation of Paenibacillus spp. isolates for the biological control of black rot in Brassica oleracea var. capitata (cabbage). Biocontrol Science and Technology · December 2015 - dx.doi.org/10.1080/09583157.2015.1129052	Cabbage seeds of cultivar Kameron (South Pacific Seeds (NZ) Ltd) were immersed in Xcc5 suspension at the concentration of 1 × 109 CFU ml-1 (3 ml suspension per 1 g seed) or in sterile 0.1% (w/v) BP in a conical flask and mixed gently under vacuum (c. 50 mm Hg) for 5 min.	Paenibacillus suspensions (P1, P6, P9, P10, P16, P20 and P24 isolates which had demonstrated different bioactivity in dual culture assay) at the concentration of 5 × 109 CFU ml-1 or sterile 0.1% BP (w/v) were then added to these seeds (0.6 ml/g seed) and mixed well using a sterile spatula. Inoculated seeds were incubated in closed Petri dishes (non-sealed) overnight in the dark.	Grow-out test was performed for both pot experiments to determine the incidence of Xcc or Paenibacillus on individual cabbage seeds.	30
	Shruti Mishra•Naveen K. Arora. Evaluation of rhizospheric Pseudomonas and Bacillus as biocontrol tool for Xanthomonas campestris pv campestris February 2012World Journal of Microbiology and Biotechnology (Formerly MIRCEN Journal of Applied Microbiology and Biotechnology) 28(2):693-702 - 10.1007/s11274-011-0865-5	Disinfected seeds were incubated with Xccrif+ for 30 min and left for air-drying in a laminar flow cabinet overnight (16 h).	Two methods of antagonist application were used : The dilution was adjusted to give final concentration of 109 c.f.u. ml-1 for seed and soil inoculation; Next day, 0.3 g pre-incubated seeds were soaked for 6 h under agitation (150 rev min-1) in 10 ml of the inoculum suspension made from the respective antagonists followed by air-drying overnight in a laminar flow cabinet. Inoculated seeds were sown after treatment. Antagonist suspension was mixed in soil to achieve soil drenching. For foliar treatment (conducted after 3 weeks) the bacterial suspensions (107 c.f.u. ml-1) were spray inoculated onto the abaxial and adaxial surface until run-off	For seed treatment, B. campestris seeds were surface disinfected by immersing in 70% ethanol for 1 min, followed by 1% sodium hypochlorite for 2 min and subsequently rinsed three times with sterile distilled water.	31
	Y. A. KAVATHIYA, R. L. KALASARIA, J. D. TALAVIA AND M. A. VADDORIA. MANAGEMENT OF BLACK ROT CAUSED BY Xanthomonas campestris (PAMMEL) DOWSON IN CABBAGE. PESTOLOGY VOL. XLI NO. 10 OCTOBER 2017 - 10.13140/RG.2.2.31993.36967	A field trial under the All India Co-ordinate Research Project on Vegetable Crops were conducted at the Research farm. First spraying was done when initial symptoms of the disease were observed during fourth week of January	Twelve treatments comprising of hot water seed treatment, seed treatment with streptomycin sulphate (Streptocycline) and Pseudomonas fluorescens, combination of seed treatment and spraying with streptomycin sulphate, copper oxychloride, and Pseudomonas fluorescens, and combination of seed treatments, root dipping and foliar spray, both with Pseudomonas fluorescens were evaluated disease. First spraying was done when initial symptoms of the disease were observed during fourth week of January		32
	Sharanaiah Umesha and Raheem K. Roohie. Role of Pseudomonas fluorescens and INA against Black Rot of Cabbage. J Phytopathol 165 (2017) 265–275 2017 Blackwell Verlag GmbH. - 10.1111/jph.12558	1)Effect of X. campestris pv. campestris on seed germination and seedling vigour of cabbage under greenhouse conditions: Seeds of both resistant and highly susceptible cultivars were treated with X. campestris pv. campestris pure culture at the rate of 1 9 108 cfu/ml (ISTA, 2005). A total of 1000 seeds were shaken for 12 h in 10 ml of bacterial suspension. 2)Effect of seed treatment with P. fluorescens on black rot disease incidence under greenhouse conditions: Four-week-old seedlings were inoculated by spraying 50 ml of the bacterial suspension showing the bacterial concentration to 1 * 10^8 cfu/ml	The seeds were then pretreated with P. fluorescens by placing the seeds in a 100-ml solution containing 20 ll Tween 20 and the 1 ml of P. fluorescens inoculum on a rotary shaker at 37°C for 5 h, and then the seeds were air-dried and used for further experiments		33
Pseudomonas savastanoi pv. Phaseolicola	O. Hassan Eman and Z.A. El-Meneisy Afaf, 2014. Biocontrol of Halo Blight of Bean Caused by Pseudomonas phaseolicola . International Journal of Virology, 10: 235-242. - https://scialert.net/abstract/?doi=ijv.2014.235.242	Seedlings, 7-10 days old, with fully expanded primary leaves were used for inoculation. The bacterial suspension was sprayed ontothe abaxial surface of the leaves using aomizer until completely wet .	Phage: Bean seedlings were sprayed with phage isolates either individual or mixed before inoculation with P. syringae phaseolicola. Bioagent tretment (P.fluorescence e P.putidae): The seedlings were treated with bioagents (20 mL per seedling) one week before and after inoculation.		34
Clavibacter michiganensis	Kotana R, Cakir A, Ozer H , Kordali S , Cakmakci R, Dadasoglud F, Dikbas N , Aydinf T, Kazaz C, 2014.Antibacterial effects of Origanum onites against phytopathogenic bacteria: Possible use of the extracts from protection of disease caused by some phytopathogenic bacteria. Scientia Horticulturae 172 (2014) 210–220. - http://dx.doi.org/10.1016/j.scienta.2014.03.016	Seeds Pathogen bacteria were grown in 50 ml flasks containing 20 mlof TSB medium on a rotary shaker at 27◦C for 24 h. Absorbance of the bacterial suspensions was measured spectrophotometrically at 600 nm and appropriately diluted to 1 × 10^8cfu/ml in sd. H2O.Approximately, 0.2 g of sucrose (10 mg/ml) was added to each Erlenmeyer flasks, and 90 g of the surface-sterilized seeds were soaked separately in this suspension. The seeds were incubated inthe flasks by shaking at 80 rpm for two days at 28◦C to coat the seeds with the pathogens.	1) Determination of the germination percentage and numberof infected seedlings of tomato seeds treated with the extracts on petri plate assays: The seeds surface disinfected and coated with the pathogens separately(C. michiganensis ssp. michiganensis, X. axonopodies pv. vesicatoriaand X. axonopodies pv. vitians) soaked in the suspensions, and then incubated by shaking at 80 rpm for 3 h at 28◦C until the seeds were uniformly coated with the suspensions 2) Determination of the effect of the extracts on seed germination, disease severity and growth promotion on pot assays The extracts (5, 10 and 20 mg/ml) were prepared by dissolvingin 10% DMSO: distilled-water in 10 ml flasks. Lettuce and tomato seeds were coated with pathogens (C. michiganensis ssp. michiga-nensis, X. axonopodies pv. vesicatoria and X. axonopodies pv. vitians),and treated with different concentrations of the extracts (5, 10, 15and 20 mg/ml) and streptomycin (0.5 mg/ml)		35

Pseudomonas syringae pv. Tomato	Karabuyuk F. and Aysan Y., 2018. Aqueous plant extracts as seed treatments on tomato bacterial speck disease.Acta Hortic. 1207, 193-196 - 10.17660/ActaHortic.2018.1207.25	The suspension was prepared from purified Pst in distilled water and adjusted to 108 cfu mL-1 with the aid of a spectrophotometer. Tomato seeds were added to the suspension and shaken for 30 min at 150 rpm at room temperature on a shaker	Artificially inoculated tomato seeds were soaked in aqueous plants extracts for an additional 30 min on a rotary shaker at 150 rpm	Immersed tomato seeds were subsequently air-dried at room temperature (20±2°C) for 1 day. Treated seeds were sown in plastic trays containing sterilized soil as five replicates consisting of 30 seeds per tray.	36
Xanthomonas spp.	Mbega, E.R., Mortensen, C.N., Mabagala, R.B. et al., 2012. The effect of plant extracts as seed treatments to control bacterial leaf spot of tomato in Tanzania. J Gen Plant Pathol (2012) 78: 277. - doi.org/10.1007/s10327-012-0380-z	One thousand seeds of tomato were vacuum-infiltrated for 30 min with 10 mL of the bacterial suspension, and seeds were air-dried in the laminar air flow chamber at 4 °C until used.	Twenty tomato seeds pre-inoculated with X. perforans were treated with 1 mL of the 10 % plant extract in an Eppendorf tube and placed on an agitation table at 100 rpm overnight at 25 °C.	Seed samples that were free of infection by Xanthomonas spp. were used in the experiments. One thousand seeds per cultivar were surface-disinfested in 70 %ethanol for 1 min, then in 1 %sodium hypochlorite for 3 min and rinsed three times in sterile distilled water. The seeds were then transferred to Petri dishes containing sterile filter papers and allowed to air-dry overnight in a laminar flow chamber and stored at 4 °C until used.	37
Xanthomonas campestris pv. Campestris	G. Mandiriza, Q. Kritzinger, T.A.S. Aveling. The evaluation of plant extracts, biocontrol agents and hot water as seed treatments to control black rot of rape in South Africa Crop Protection 114 (2018) 129–136 - 10.1016/j.cropro.2018.08.025	Seeds of rape (cultivar English Giant), obtained from a seed company in South Africa, were artificially inoculated by soaking in bacterial suspension of Xcc, adjusted to 10^8 cfu/ml, for one hour with occasional hand shaking. After inoculation, the bacterial suspension was drained and seeds were left to dry for 48 h in a laminar flow cabinet.	1) Seed treatment with plant extracts: Evaluation of the plant extracts against black rot disease in the greenhouse was performed using acetone extracts of A. caulescens (15 mg/ml) and C. citratus (10 mg/ml), which showed the best activity in vitro as seed treatments. Artificially Xcc inoculated rape seeds were soaked in the respective extracts for 3 h at 25 °C in the dark with occasional hand shaking. 2) Seed treatments with commercial biological control agents The liquid formulations of Paenibacillus sp. and Bacillus sp. were applied at recommended rates of 40 ml/kg seed and at 1.6 ml/kg seed, respectively. The rate used for the powder formulation of Bacillus subtilis was 200 g/12.5 kg seed and a few drops of the supplied sticker were added to allow even mixing. The BCAs were applied as slurries for 2 h and seeds were then left to dry overnight in Petri dishes inside a laminar flow cabinet (in vitro tests) or sown immediately (greenhouse tests).		38
	Leila Aminia, Mohammad Reza Soudia,⁎, Azra Saboorab, Hamid Mobasheric,d. Effect of essential oil from Zataria multiflora on local strains of Xanthomonas campestris: An efficient antimicrobial agent for decontamination of seeds of Brassica oleracea var. capitata. Scientia Horticulturae 236 (2018) 256–264 - doi.org/10.1016/j.scienta.2018.03.046	The surface sterilized seeds (0.7 g, ∼ 230#) were then soaked in the bacterial suspension. The seeds were incubated in the shaking flasks at 28 °C and 150 rpm for 2 h and then collected and air dried on sterile Whatman filter paper sheets.	One group of the contaminated seeds was immersed in the emulsion of ZMEO(Z. multiflora essential oil) (463.5 µg/mL) in DMSO(dimethyl sulfoxide) (8 mg/mL) and incubated for 1, 2, 3 and 4 h at 28 °C at 150 rpm. The seeds of the second group were immersed in DMSO (8 mg/mL) in the absence of ZMEO to identify the effect of DMSO on the decontamination of the seeds.	The surface of cabbage seeds was disinfected to destroy any saprophytic and/or pathogenic microorganisms. In order to fulfill this task, the seeds were initially washed in running tap water for 1 h. They were then dipped in 70% (v/v) ethanol for 2 min, exposed to 1% (v/v) sodium hypochlorite for 15 min, washed three times with sterile distilled water, and finally dried at room temperature for 30 min	39
Pseudomonas savastanoi pv. Phaseolicola	O. Hassan Eman and Z.A. El-Meneisy Afaf, 2014. Biocontrol of Halo Blight of Bean Caused by Pseudomonas phaseolicola . International Journal of Virology, 10: 235-242. - https://scialert.net/abstract/?doi=ijv.2014.235.242	Seedlings, 7-10 days old, with fully expanded primary leaves were used for inoculation. The bacterial suspension was sprayed ontothe abaxial surface of the leaves using aomizer until completely wet .	Extract of garlic cloves was sprayed at 20%, wich inhibited P. syringae phaseolicola in vitro. Spraying was applied two days before inoculation by pathogenic bacterium		34

Annex 8

MILESTONE 9 – Trialling Plan

Task 4.2: Evaluation of alternative seed treatments to the use of chemical treatments to control sanitary quality of seed lots.

Organic farming prohibits the use of conventional chemicals to control pests and diseases, so alternative natural compounds as well as mechanical treatments will be evaluated on seed to reduce pathogen inoculum on seed and plant protection against seed-borne pathogens as well as for seed vigour enhancement.
The review of the detection tools for the main seed-borne pathogens of tomato, broccoli and beans and for the available or common seed treatments that are applicable in organic seed production (D4.1) was very useful as a starting step for the planning of systems to investigate in this task and for pointing our attention to the scarcity of diverse and sure methods to control seed pathogens under the organic conditions as well as the potential there is in procedures and methodologies using Biocontrol agents (BCAs) for plant and soil colonization and the use of natural compounds (NCs). The optimal treatments to control main seed borne pathogens affecting tomato, brassicas and snap bean will be described in deliverable **D4.2**.and the activities here described will represent the basis for its redaction.
The trialling plan foresees a series of treatments defined by the corresponding existing protocols coupled with new methods that will be tested. The results will be made accessible to the stakeholders by the dissemination activities planned in WP6.
Three to five pathogens per crop were selected according to deliverable D4.1. The binomial host-pathogen will be first screened in model/representative varieties for which biological seed are available and then possibly in the following steps in the breeding lines provided by WP2 and WP3. For the same pathogen, when available, different detection methods will be evaluated (Tab. 1).

Table 1 Seed borne pathogens and BRESOV partners involved in T4.2

species	**seedborne pathogen***	**Detection tool development****	**Treatment development**
Tomato	*Clavibacter michiganensis subsp. michiganensis*	ITAKA/UniCT (v)	ITAKA
	Pseudomonas syringae pv. tomato	ITAKA/UniCT (v)	ITAKA
	Xanthomonas spp pathogenic to tomato	UNICT (a)	UNICT
	ToMV	VEG (a)	ITAKA
	Fusarium oxysporum f.sp. radicis lycopersici	ITAKA (a)	ITAKA
Broccoli	*Xanthomonas campestris pv. campestris*	VEG (d), UNICT (v)	VEG
	Alternaria spp	VEG (d), UniCT (a)	UNICT
	Phoma lingam (Leptosphaeria maculans)	ITAKA (a)	ITAKA
Snap Bean	*Colletotrichum lindemuthianum*	FiBL (a)	FiBL
	Pseudomonas savastanoi pv. phaseolicola	FiBL (a)	FiBL
	Fusarium solani f.sp phaseoli	ITAKA (a)	ITAKA
*same as those of D4.1		**validation (v) development (d) application (a)	

Trialling plan was deeply discussed by the partners involved (P1-UNICT, P6-FiBL, P8-VEG, P18-ITAKA) with criticism and based on the different experiences and expertise. The main problem resides in the availability of infected seed lots to be used in the trials. At the same time, infection in naturally infected seed lots is rarely homogenous which make a standard detection and quantification of the infection rate more complex. Therefore, for these two reasons, and on advice from seed producers, it was decided to perform these experiences on artificially infected seeds, and then test the resulting most promising seed treatments methods on naturally infected seed lots, whenever available. Requests of naturally infected seed lots of these host-pathogen binomial have been already sent to a couple of seed producers.

According to the Description of Action (DoA):

UNICT will define the trialling plan for all 3 crops and agenda for the whole duration of the project, and will provide the harmonized trialling protocol and recommendations to the BRESOV partners involved in this task.
UNICT will supervise the trials and will use all the collected data and statistical results to determine the treatments (microorganisms/natural compound alone or in combination, or mechanical treatment alone, or in combinations) that ensure a high sanitary quality of organic seeds in tomato, brassicas and snap bean.

- UNICT will also organize trials on its own site, and especially evaluates the sanitary quality of the seed samples.

- ITAKA will provide BCAs (fungus and/or bacteria consortium), and different NCs for seed treatments. It will check mechanical treatments allowed in organic farming.
- ITAKA will assist UNICT in establishing the harmonized trialling protocols to evaluate the compounds and treatments among partners and will organize trials on its own site by applying the harmonized protocol.
- FiBL will conduct the experiments on its own site or in labs of industrial producers/distributers of organic seeds and it will be investigating other systems and other methods, although the general protocol of detection method validation application, seed treatments, detection and quantification would be common to all.
- FiBL will especially evaluate alternative and novel methods of disinfection.
- VEG will organize trials on their own sites by following UNICT & ITAKA's harmonized protocol.

The protocol proposed will be subjected to the appropriate adaptation by each research group.
According to the task description, ITAKA products to be tested are:

Microbial:
1. KONCIA XP191EV (*Bacillus subtilis and megaterium, Pseudomonas lurida, Glomus spp*)
2. KONCIA KMS1943 (*Bacillus subtilis,megaterium and amyloliquefaciens, Pseudomonas fluorescens and putida, Streptomyces griseus and lydicus, Trichoderma arzianum, asperellum and atroviride, Glomus spp*)
3. KONCIA KSK1967 (*Streptomyces griseus, Pseudomonas fluorescens and chlororaphis, Glomus spp)*
4. KONCIA KFC1980 *(Bacillus subtilis and megaterium, Azotobacter vinelandii, Glomus spp)*

Natural compound:
5. CH193EV (CHITOSAN based)
6. CR192 EV (mustard oil-glucosinolates and propolis)

Controls:
7. Control thesis – no treatment (pathogen only)
8. Control on healthy seed lot of the tested methods to assess the effect on seed germination and seedling health (PGPR effect control).

Crop/variety
1) One commercial cultivar for each crop will be utilized for the first cycle of trials (second year of the BRESOV project); best product/s for each crop could be selected for the other trials.
2) Two commercial cultivars for each crop will be utilized for the second cycle of trials (Third year)
3) Three Elite breeding lines provided by the CG will be utilized for the third cycle of trials (fourth year)
Each partner will use the commercial cultivars available on the seed market of each country (NOT TREATED). The elite breeding lines will be indicated by the WP3 leader.

Pathogen inoculation in seeds: UNICT and ITAKA have a protocol for *Pseudomonas, Xanthomonas, Clavibacter*. Each partner will propose their usual validated inoculation method or validate a new one.
Bacterial suspensions in sterile distilled water obtained re-suspending bacterial cells scraped from NDA grown 24 h at 27°C and adjusted to approximately 1x108 cfu mL-1 (OD600=0.1).
Seeds inoculated by immersion in the suspension of each bacterial strains for 30 min under vacuum, after which the seeds were left to dry completely on sterile blotting paper, in a laminar flow cabinet. Moreover, P1-UNICT and P-18-Itaka will also evaluated the mean number of bacteria adhering to the seed was determined for each host-pathogen combination following the respective ISTA protocol – Everyone can use here the detection method validated.

Seed coating
KONCIA Products (seed microbiolization)/CHITOSAN)
After pathogen inoculation, the dried seeds will be immersed in a dilution 1:10 of the microbial BCAs *consortium*; 1:100 for CH193EVfor about 10-30' and then the seeds were left to dry completely on sterile blotting paper. After check germination and mean germination time, the seedlings could be transplanted in containers with big holes in order to evaluate specific symptoms for each of the studied diseases.

Disease evaluation: those validated in the respective laboratory or according to literature.
Other evaluations: PGPR activity were evaluated.

PCR-based pathogen detection and quantification tests on seed lots for the targeted diseases: For bacterial or virus pathogen, PCR-based detection method (real time PCR or conventional PCR) according to official protocols (if available) or protocols derived from scientific literature will be used. Serological method could be selected for virus detection. Detection of fungal pathogen will be performed using official protocols or, when available, molecular method (PCR or real-time PCR).

Detection method tuning:

According to the pathogens selected for the trials, each group could work with its models crop/pathogen and produce sensitivity results.

Spiked seed lots will be prepared by adding infected seeds (artificially contaminated either with bacterial cells or fungal spores-see above) to healthy seed batches (e.g. 1:1000, 2:1000, 5:1000, 10:1000 and 0:1000). Although official protocol use 10.000 seeds, we propose 1000 seeds sub-lots

Each laboratory/task partner will propose the detection protocols with the appropriate justification.

Each T4.2 partner could try to grow in confined conditions or under natural inoculum short cycle for seed infection. Each partner will acquire the commercial cultivars and the strains needed, informing all the partners about the methodology. Whenever naturally-infected seeds are available, they will be used for testing of the resulting best methods for validation under normal conditions.

Time sheet

	Nov19-Jan20	Feb20-Apr20	May20-Jul20	Aug20-Oct20	Nov20-Jan21	Feb21-Apr21	May21-Jul21	Aug21-Oct21	Nov21-Jan22	Feb22-Apr22
detection method tryed	Broccoli snap bean tomato	Broccoli snap bean tomato								
biocontrol compound delivery (ITAKA)										
grow-out tests		no. 1 broccoli, snap bean, tomato commercial cv								
model plants		no. 1 broccoli commercial cv	no. 1 snap bean commercial cv	no. 1 tomato commercial cv	no. 2 broccoli commercial	no. 2 snap bean commercial	no. 2 tomato commercial			
elite breeding lines								no. 3 broccoli	no. 3 snap bean	no. 3 tomato