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SEED HEALTH TREATMENTS IN ORGANIC SEED PRODUCTION

SUMMARY

The basic principles for the development of organic agriculture has been prescribed by the International Federation of Organic Agriculture Movements - IFOAM and the European Union (Commission Regulation No 209/91), on whose standards EU regulations are founded. The field of organic production at the international level and issues of seeds and planting material are regulated by the IFOAM Basic Standards 2002, which stipulates that seed and planting material used in organic agriculture has to be produced in line with the regulations applicable to organic crops. Unlike the conventional seed production, in organic seed production, there is a higher risk of contamination with pathogens, i.e. seed-borne diseases. The aim of this study was to point out the existing methods of seed treatments in the organic production system in order to obtain healthy seeds. Seed-borne pathogens, including bacteria, fungi, viruses and viroids, are responsible for disease recurrence in subsequent cycles of seed multiplication and spread of diseases in new geographic regions. According to various authors, there are several classifications of treatments including physical treatments, application of natural compounds, such as plant extracts and oils, use of inorganic natural products and biological control (use antagonistic microorganisms). In order to overcome various pathogens different biocontrol strategies should be developed. Microorganisms can be used in diverse crop protection practices, i.e. several seed treatments can facilitate high levels of both disease control and production yield.

Keywords: seed borne diseases, microorganisms, plant extract, plant oils

INTRODUCTION

Organic farming is characterised by the production of organic, high-quality food, while preserving plant health and complete biodiversity, soil fertility, the environment and the entire ecosystem (Popović *et al*., 2016).

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The share of the land used for organic farming has been steadily increasing worldwide for many years, as well as, the organic products market (organic-world.net, 2022; Golijan *et al*., 2017; Golijan and Dimitrijević, 2018). Organic products are more beneficial and environmentally safe, similarly or more alimental and contain lower amounts of pesticide residues or do not contain any residues compared to conventionally produced food (Golijan *et al.,* 2021; Golijan & Sečanski, 2021a). The aim of organic farming can be achieved by the application of various practices including polycrop rotation, diverse combination of crops and farm animals, pulses, organic manure and biological pest control (Vaško and Kovačević, 2020; Golijan *et al*., 2021). Organic farming is frequently recommended as an option to farmers who cannot be lucrative in conventional farming. To achieve the optimal cost-benefit ratio, farmers take into consideration various combinations of production factors and their use. They very often face the dilemma whether to use a conventional or organic farming system. Organic farmers have not only moral commitment but also corporate social responsibility (Vaško and Kovačević, 2020).

The International Federation of Organic Agriculture Movements - IFOAM and the European Union (Regulation 209/91) prescribed the basic principles for the development of organic agriculture. Seed production is one of the most important segments of plant production. The field of organic production at the international level and issues of seeds and planting material are regulated by the IFOAM Basic Standards 2002. The 1991 EC Council Regulation (EEC) No 2092/91 is one of the first regulations at the level of the European Economic Community regulating the field of seeds and planting material in organic production. The field of seed production in organic plant production is regulated by the EU Directives 834/2007 and 889/2008. The Regulation (EC) No. 834/2007 imposes the need for each Member State to create a computerised database that is set up to contain varieties of which organic-produced seed is available in its territory (Golijan, 2020; Golijan and Sečanski, 2021b). The offer of organic varieties and organic seeds is at odds with the expansion of organic agriculture, which slows down the development of organic production (Ugrenović *et al*., 2010). The production and treatments of seeds, bulbs and tubers according to the principles of organic production represent an additional challenge for development of high-quality plant material for further propagation. Early harvest is one of the possible measures to improve seed health and there are various forms of seed treatments. High-quality seeds, i.e. seed production in harmony with principles of organic production, is of exceptional importance for organic farming (Kolašinac *et al*., 2017; Golijan *et al*., 2018). There are significant technical challenges in organic production of healthy seeds with germination of a high percentage. The aim of current regulations in many countries is to produce all seeds and planting material, used for organic farming, according to prescribed organic methods. A similar consideration applies to materials used for treatments, film coating and pelleting of seeds (Döring *et al*., 2012). The European Commission Regulation (EC) No. 1452/2003 states that only organic seeds can be used in organic agriculture. Deviations from this rule are allowed if organic seeds are not available on the market, and conventional seeds are not chemically treated. In case that a user wants to use organic seed of a certain variety and if seed is not listed in a database of all commercially obtainable organic seeds, the user is allowed to use conventional seeds (Spadaro *et al*., 2017). Furthermore, methods that can be applied in seed treatments used against pathogens have become scarce (Howard, 2009), i.e. as the application of chemicals is limited, risk of contamination with weed seeds and seed-borne pathogens are much more expressed in the organic seed production (Roschewitz *et al*., 2005).

The use of chemical fungicides is common in conventional seed production. This treatment reduces losses in seeds and seedlings caused by seed- and soil-borne diseases. The use of the majority of seed protectants in organic production is not allowed. Nevertheless, some seed treatments, such as priming, pelleting and the use of hot water could be an option for organic growers to improve seed traits (Gatch: https://eorganic.org/node/749). Organic production of seed is more subjected to risk of contamination with weed seeds and seed-borne pathogens than conventional seed production. Moreover, seed-borne pathogens can be accumulated and can become severe problem after several cycles of seed multiplication. According to Emily Gatch, Washington State University (https://eorganic.org/node/749):

„The purpose of any seed treatment is to improve seed performance in one or more of the following ways: 1) eradicate seed-borne pathogens or protect from soil-borne pathogens, 2) optimize ease of handling and accuracy of planting (reduce gaps in stand or the need for thinning of seedlings, particularly when mechanical planters are used), and 3) improve germination rates.“

SEED HEALTH TREATMENTS

These treatments are used to improve health of seeds and seedlings by killing seed-borne pathogens or by protecting germinating seeds from infestation of soil-borne pathogens. Many authors refer to different categories of seed treatments in organic production. For instance, Spadaro *et al*. (2017) classified treatments as follows: physical treatments, use of natural compounds, use of inorganic natural products, antagonistic microorganisms (biological control), and induced resistance. According to Kumari *et al*. (2013), the organic seed is produced under the organic system in which seeds are typically treated with materials from organic sources (Table 1).

Table 1. Seed treatments with materials from organic sources

Botanicals	Biofertilizers	Cow's product	Biocontrol agent	Other
Neem leaf extract	*Rhizobium*	Panchagavya	*Pseudomonas* spp.	Coconut milk
Mint leaf extract	*Azatobactor*	Cow milk	*Trichoderma* spp.	Tender coconut
Sarani leaf extract	*Azospirillum*	Curd		Vermicompost
Prosopis leaf extract	*Phosphobacteria*	Cow urine		Vermiwash
Arappu leaf extract		Cow dung		

Source: Kumari *et al.* (2013)

Since it is usually impossible to produce healthy, disease-free seed and since the application of conventional treatments with chemical is not allowed, many studies on alternative seed treatments have been done and are still in progress. According to Kumari *et al.* (2013), different treatments that have been tested can be grouped into the following categories:

1. **Thermal treatment**: Hot water seed treatments are quite efficient when applied in some crops, but they should be carefully applied to prevent seed destruction. The limiting factor is that seed have to be dried quickly after the treatment and it is difficult to achieve it in the process of industrial production. To elude this problem, the aerated steam method has been suggested. Due to the fact that the seed is not immersed in water but is exposed to hot humid air, drying is not t a problem anymore. The selection of the temperature and its control is essential.

2. **Use of antagonists**: A number of antagonists have been tested, and some results are as follows;

- *Trichoderma* spp. are used against the collar rot disease of groundnut caused by *Aspergillus niger*
- *Pseudomonas chlororaphis, Bacillus subtilis, Fusarium oxyporum, Streptomyces* spp. are used against *Alternaria* spp. on *Brassica* seeds.
- *Bacillus subtilis* is used against *Tilletia caries* on wheat.
- *Trichoderma viride* is used against *Fusarium* spp. and *Bipolaris sorokiniana* on wheat and barley.
- Several antagonists are used against *Rhizoctnia solani*.

3. **Natural compounds**: Essential oils, occasionally with chelator and natural detergent have been tested. Thyme and oregano oils are successful against *Botritis aclada*, *Alternaria dauci*, *Clavibacter michiganensis* pv. *michiganensis* and *Xanthomonas campestris* pv. *campestris*. The mustard powder Tellecur expresses good results against different pathogens, especially T*elletia caries* in wheat. On the other hand, Chitosan gives excellent results against *Fusarium* spp. and *Bipolaris sorokiniana* on wheat and barley. A complex product, Biokal (57% of medicinal herb extracts, 38% bio-humus extracts, 5% volatile oil and metal and trace elements) has proven to give some good results against *Ascochyta pisi* on pea seeds.

4. **Other products**: Tests of organic acids (acetic, ascorbic, citric, lactic and propionic) and antiseptic products such as potassium permanganate and copper sulphate are in progress.

PHYSICAL TREATMENTS

Thermotherapy is one of the oldest methods of heat treatments of seeds, but due to many practical limitations, it has never been widely used in conventional agriculture for control of seed-borne fungi. On the other hand, the progress in organic agriculture aroused interest in methods of physical seed treatments, such as thermotherapy, including the use of hot water, hot humid air, and microwave radiation (Szopińska and Dorna, 2021). According to Spadaro *et* *al*. (2017) physical treatments encompass mechanical treatments (sorting progress of organic farming and brushing), heat treatments (warm water, aerated steam or hot air), ultrasonic treatments and radiations (with microwaves resulting in higher temperatures), UV-C light and redox treatments as with cold plasma and electrons.

Hot water treatment. The hot water treatment destroys the majority of bacterial organisms that cause diseases on or within seeds, Miller and Lewis Ivey (2021). This treatment is recommended for seeds of eggplant, pepper, tomato, carrot, spinach, lettuce, celery, cabbage, turnip, radish, and other crucifers. Hot water can damage seeds of cucurbits, such as watermelons, pumpkins, squash, gourds, etc., and therefore this treatment should not be used for these seeds. Since the use of this treatment reduces seed vigour over time, seeds treated with hot water should be kept no longer than a season. The period between destruction of the pathogen and the seed injury is usually short and therefore a precise control of the intensity and the length of the treatment is needed. A successful thermotherapy without seed damage is difficult, particularly for large seeds, such as for legumes. Seed lots, even those of the same variety, can greatly differ in sensitivity for the hot water treatment. The sensitivity can depend on the seed maturity, water content, or the period of seed storage (Forsberg, 2004).

Miller and Ivey (2021) state that the seed treatment is performed according to the following steps:

„*Step 1: Wrap seeds loosely in a woven cotton bag (such as cheesecloth) or nylon bag.*

Step 2: Pre-warm seed for 10 minutes in 100°F (37°C) water.

Step 3: Place pre-warmed seed in a water bath that will constantly hold the water at the recommended temperature (see table that follows). Length of treatment and temperature of water must be exactly as prescribed.

Step 4: After treatment, place bags in cold tap water for 5 minutes to stop heating action.

Step 5: Spread seed in a single, uniform layer on screen to dry. Do not dry seed in area where fungicides, pesticides, or other chemicals are located.

Step 6: Dust seed with Thiram 75WP (1 tsp/1 lb seed) once the seed is completely dry.“

Table 2 presents a list of crop seeds and the temperatures and times recommended for the hot water treatment.

Groot *et al.* (2006) observed effects of seed maturity on the susceptibility to hot water, aerated steam and electron treatments. Two seed lots each of *Brassica oleracea* L. and *Daucus carota* L. commercially produced were chosen as they contained relatively great amounts of insufficiently mature seeds. Less mature *B. oleracea* seeds were more susceptible to hot water and aerated steam treatments, while *D. carota* seeds were more susceptible to the hot water treatment. On the other hand, seed maturity did not affect susceptibility to the applied electron seed treatments. Although seed lots were not selected for infections caused by pathogens carried on the seeds, it was observed that less mature seeds were more frequently infected. Thus, seeds should be harvested at full maturity and less mature seeds should be removed during seed processing. Categorisation of seeds by their level of chlorophyll fluorescence provides an effective method of sorting seed lots of *B. oleracea* and *D. carota*.

Table 2. List of crop seeds and the temperatures and times recommended for the hot water treatment.

Seed	Water temperature		Minutes
	°F	°C	
Brussels sprouts, eggplant, spinach, cabbage, tomato	122	50	25
Broccoli, cauliflower, carrot, collard, kale, kohlrabi, rutabaga, turnip	122	50	20
Mustard, cress, radish	122	50	15
Pepper	125	51	30
Lettuce, celery, celeriac	118	47	30

Source: Miller and Ivey (2021)

According to Nega *et al.* (2003) the hot water treatment at 50ºC for 20 to 30 min, or at 53ºC for 10 to 30 min controlled *Alternaria dauci*, A. *radicina*, *A. alternata*, and *A. brassicicola* on seeds of carrot, cabbage, celery, parsley, and lamb's lettuce. Pryor *et al.* (1994) applied the treatment of water or 1.0% NaOCl heated to 50ºC for 20 min on carrot seeds. This teatement led to eradication of *A. radicina* with a minimum reduction in germination. Du Toit and Hernandez-Perez (2005) performed spinach seed treatments in 1.2% NaOCl for 10 to 60 min, or hot water (40, 45, 50, 55, and 60°C) for 10 to 40 min, in order to evaluate eradication of *Cladosporium variabile*, *Stemphylium botryosum*, and *Verticillium dahliae* from seeds. A significant reduction in germination was recorded in the hot water treatment at 50°C for ≥30 min or 55 or 60°C for ≥10 min. Eradication of *C. variabile* was observed in seeds treated in 40°C water for 10 min. *V. dahliae* was eradicated from seeds treated at 55°C for ≥30 min or 60 °C for ≥10 min. Furthermore, eradication of *S. botryosum* was possible from seeds in a lightly infected seed lot (5% incidence) by hot water treatment at 55 or 60 °C for ≥10 min, but eradication was not possible from two heavily infected lots (>65% incidence), even at 60°C for 40 min. According to Hermansen *et al.* (2000) the treatment of carrot seeds with 54ºC water for 20 min eradicated *A. dauci*, but germination, emergence, or yield were not adversely affected.

Other physical treatments. The possibility of microwaves to raise temperatures in seeds has also beenstudied. The routine application of hot humid air (ThermoSeed technology) to control seed-borne pathogens in cereals has been common in Sweden and Norway for many years (Forsberg *et al.*, 2002; Forsberg *et al.*, 2005). There are quite a few advantages of microwave technology, including safety, high efficiency, and environmental protection. Microwave radiation, causing microbial inhibition, is based on the internal heating of the seeds that results from molecular movements in the pulsing electromagnetic field. As a result, the denaturation of proteins, enzymes, and nucleic acids occurs. Heating affects proteins and damages them directly because the bonds that hold them together are destroyed. This also implies the risk of loosing enzymatic activities, which are essential for carrying out metabolic processes (Schmidt *et al*., 2018; Wang *et al.,* 2019). According to Knox *et al*. (2013), fungal pathogens in wheat could not be significantly eliminated by microwave treatments without seed being damaged. The higher seed moisture content increases efficacy of microwave radiation against seed-borne fungi, Mangwende *et al*. (2020). As water molecules are polar, they rotate when exposed to microwaves. This rotation of water molecules produces heat. There are no effects on dried samples because of the lack of polar molecules, while those in the presence of water can reach lethal temperatures (Gartshore *et al*., 2021). Szopińska and Dorna (2021) recorded the highest seed germination (81%, 85% and 77%) in carrot cultivar Amsterdam when the microwave wet treatment at power output levels of 500 W, 650 W and 750 W was applied for 75 s 45 s 60 s, respectively. On the other hand, corresponding values of 46% and 43% were recorded in carrot seeds of cultivar Berlikumer when treated for 60 s at 500 and 650 W, respectively. Seeds of both samples soaked in water and treated with microwaves for over 30 s, regardless of the power output, were significantly less infested with *Alternaria* spp. Tylkowska *et al.* (2010) reported that the microwave treatment of dry seeds (9.5% m.c.) of common bean in a microwave oven with a power output of 650 W and frequency of 2450 MHz for 15-120 s did not affect *A. alternata* and *Fusarium* spp., but reduced the presence of *Penicillium* spp. Microwave radiation less affects dark, multi-celled, and thick-walled spores, as well as dark mycelium (e.g., *Alternaria* spp. or *Bipolaris* sp.) than hyaline and one-celled spores (e.g., *Aspergillus* spp. or *Penicillium* spp.). Schmidt *et al*. (2018) performed the study on *A. parasiticus* and established that the severity of DNA damage increased with higher temperatures.

Electroporation is one of the non-thermal effects that might be caused by microwave irradiation. Microwaves at sub-lethal temperatures stimulate the pore formation in a cellular membrane as a result of their interaction with polar molecules. These pores allow the content of cells, including DNA, to leak outside (Gartshore *et al*., 2021).

Another way to reduce the inoculum load of mainly fungal pathogens on seed is to apply ultrasound. Frequencies ranging from 20 to100 kHz are typically used to generate a powerful cavitation that can destroy and detach microorganisms from surfaces. According to Sagong *et al.* (2011) the combination of the ultrasound treatment with organic acids effectively increased the pathogen reduction in comparison with individual treatments without significantly affecting quality. These authors demonstrated the potential of this novel method in increasing microbial safety on organic fresh lettuce.

A low energy electron treatment of seeds was developed to control cereal seed-borne pathogens (Burth *et al.*, 1991). The electron penetration depth is limited to the seed surface and external parts of the seed coat (0.025-0.5 mm), and therefore pathogens in the endosperm and embryo remain unaffected. Waskow *et al.* (2021) observed and compared seed decontamination by the cold atmospheric-pressure plasma and the low-energy electron beam regarding their effects on quality of seeds and seedlings. Results showed that both technologies provided large potential for inactivation of microorganisms on seeds. Cold plasma yielded a higher efficiency with 5 log units than a maximum of 3 log units after the electron beam treatment. Regardless of the applied technique, the short plasma treatment (< 120 s), or all applied doses of the electron beam treatment (8–60 kGy)), seed germination was accelerated, defined by the percentage of hypocotyl and leaf emergence at 3 days. Nonetheless, even the lowest dose of the electron beam treatment (8 kGy) caused root abnormalities in seedlings, implying a detrimental effect on the seed tissue. The cold plasma treatment eroded the seed coat and increased seed wettability compared to electron beam treated seeds. A good effect was also achieved against *Xanthomonas hortorum* pv. *carotae* on carrot seeds (Jahn and Puls, 1998).

Selcuk *et al.* (2008) employed the low-pressure cold plasma system using air gases to inactive *Aspergillus* spp. and *Penicillium* spp. on seed surfaces. The fungal attachment to seeds was reduced below 1% of the initial load depending on the initial contamination level by applying the plasma treatment, while preserving quality of seed germination. A significant decrease of 3-log for both species was achieved in the course of 15 min of the SF6 plasma treatment time. The non-thermal plasma treatment of rice seeds for 76 s resulted in a 90% level of control against *Gibberella fujikuroi*, a fungal plant pathogen that causes bakanae disease (Jo *et al*., 2014).

Seed-borne pathogens can be eliminated by seed treatments with UV-C, UV light with a low wavelength (100-280 nm). UV-C has powerful germicidal properties and can cause the photochemical damage of the DNA of viruses and microorganisms. According to results gained with water-based solutions and with surfaces, pulsed UV light is more effective than continuous UV light. Practically three orders of magnitude of increased inactivation have been accomplished with the photosensitised UV process on surfaces (McDonald *et al*., 2000).

According to Brown *et al.* (2001), the optimum UV-C dose of 3.6 kJ m was effective in reducing black rot and the population density of *Xanthomonas campestris* pv. *campestris* in infected cabbage leaves. Plants grown from seeds treated with UV-C at 3.6 kJ m had the most desirable colour, greatest weight, largest head diameter and delayed maturity. The impact of the storage period at room temperature on the disease occurrence of black rot of cabbage grown from seeds treated with a low hormetic UV-C dose of 3.6 kJ m was 90% of black rot in plants from UV-C treated seeds stored for 2 days, 40% stored for a day, 60% stored for 5 days and 60% stored for 8 months, 8 weeks after transplanting cabbage plants.

Chlorine Treatment. The chlorine treatment successfully removes bacterial pathogens from the seed surface. Unlike the hot water treatment, it does not eliminate pathogens in the seed. This treatment is recommended for both large- and small-seeded vegetables if no other treatments were applied to the seeds and if the possibility of pathogens being carried inside the seeds is not a concern (Miller and Ivey, 2021).

According to Miller and Ivey (2021), the procedure of seed chlorine treatment should be performed in the following steps:
„*Step 1: Agitate seed in a solution of 25 oz Clorox plus 100 oz water with one teaspoon surfactant for 1 minute. Use 1 gallon of disinfectant solution per pound of seed (conversions provided below) and prepare a fresh solution for each batch.*
Step 2: Rinse seed thoroughly in cold running tap water for 5 minutes.
Step 3: Spread seed in a single, uniform layer on screen to dry. Do not dry seed in area where fungicides, pesticides, or other chemicals are located.
Step 4: Dust seed with Thiram 75WP (1 tsp/1 lb seed) once the seed is completely dry."

It is very important to test seed germination after a hot water and chlorine treatments. According to Miller and Ivey (2021) germination is tested in the following way:
"*1. Mix seeds in each seed lot and count out 100 seeds per seed lot.*
2. Treat 50 of the seeds exactly as described in the fact sheet.
3. After treated seeds have dried, plant the two groups of seeds separately in flats containing planting mix according to standard practice. Label each group as "treated" or "untreated."
4. Allow the seeds to germinate and grow until the first true leaf appears (to allow for differences in germination rates to be observed).
5. Count seedlings in each group separately.
6. Determine the % germination in each group:

$$\frac{\#\ \text{seedlings emerged}}{\#\ \text{seeds planted}} \times 100$$

7. Compare % germination in each group: they should be within 5% of each other."

Conversions:
8 oz = 1 cup
16 oz = 1 pint
32 oz = 1 quart
128 oz = 1 gallon

Du Toit and Derie (2003), observing the occurrence of *Stemphylium botryosum* in a spinach seed lot, determined that it reduced from 54.8 to 23.3% when the seed was soaked in 1.2% NaOCl for 10 min. This reduction was less than 20% for the seed soaked in chlorine for 20, 30, or 40 min. On the contrary, the reduction of the occurrence of *Corynebacterium. variabile* ranged from 49.0 to 0.3% after chlorine treatment for 10 min, and was not detected in seeds treated with chlorine for over 10 minutes. Moreover, Du Toit and Hernandez-Perez (2005) treated spinach seeds with 1.2% NaOCl for 10 to 60 min, and found out that *C. variabile* and *Verticillium dahliae* were largely eliminated by the chlorine treatment lasting 10 or more minutes. Although the chlorine treatment reduced the occurrence of *S. botryosum*, this fungus was not eliminated after 60 min in chlorine. Even after the 60-min chlorine treatment seed germination was not negatively affected.

USE OF NATURAL COMPOUNDS – PLANT EXTRACTS AND OILS

A number of plant oils including oils produced from garlic, savory, clove, oregano, thyme, lemongrass, and cinnamon express some potential in suppressing damping-off (Golijan and Sečanski, 2022). Thyme oil is used in Europe as a seed treatment. The majority of the studies carried out on seed disinfection with natural compounds have been aimed on cereal seed-borne pathogens. It has been determined that pure soya bean or mineral oils had reduced storage moulds not only of soya bean but also of maize. In order to establish feasibility of seed treatment protocols based on essential oils it is necessary to continue with research on the disease suppressive potential of these oils (https://eorganic.org/node/749).

Antifungal activities of essential oils against *Fusarium* spp. have been reported in many studies previously performed for different laboratory media and plant materials (Kumar *et al*., 2016; Matusinsky *et al*., 2015; Ferreira *et al*., 2018). According to Schmitt *et al.* (2009), thyme oil (1%) was effective against *Phoma valerianellae* on seeds of lamb's lettuce. Different essential oils, organic acids and plant extracts were tested by Van Der Wolf *et al.* (2008) with the intention to use them to disinfect vegetable seeds. Thirty-minute treatments with certain essential oils eliminated 99% of the bacteria on cabbage seeds. Furthermore, it reduced fungi in blotter tests. High concentrations of organic acids (>2.5%) reduced bacteria on seeds. However, a concentration higher than 1% of certain products such as propionic acid, cinnamon oil and Biosept, negatively affected seed germination, whereas thyme oil had the best efficiency. Perczak *et al.* (2019) reported that essential oils have great potential for the inhibition of the growth of *Fusarium* fungi on maize seeds.

Shukla *et al.* (2002) stated that the toxicity of the ajowain (*Trachyspermum ammi*) oil was fungicidal at 0.1%, which inhibited heavy doses of inocula (25 fungal discs, each of 5-mm diameter) and killed the test pathogen in no more than 2-3 s. This oil also exhibited a wide antifungal activity against *Aspergillus flavus*, *A. parasiticus, Curvularia lunata*, *Cladosporium cladosporioides*, *Alternaria alternate*, *Colletotrichum capsici*, *Colletotrichum falcatum*, *Helminthosporium maydis*, *Helminthosporium oryzae*, *Penicillium implicatum*, *P. italicum* and *P. minio-Iuteum*, and was more active than some commercial synthetic pesticides (benlate, captan, mancozeb, and thiram). Mohamed *et al.* (2020), determined antifungal activities of *Origanum majorana* essential oils against four seed-borne fungi in rice: *Fusarium verticilliodies, F. graminearum, Bipolaris oryzae*, and *Curvularia lunata*. Chen *et al*. (2014) demonstrated the effectiveness of a citronella (*Cymbopogon nardus*) essential oil on *A. alternate* in in vitro and in vivo assays. Moumni *et al.* (2021) tested in vivo seven essential oils for disinfection of squash seeds (*Cucurbita maxima*) that had been naturally contaminated with *Stagonosporopsis cucurbitacearum, Alternaria alternata, Fusarium fujikuro, Fusarium solani, Paramyrothecium roridum, Albifimbria verrucaria, Curvularia spicifera*, and *Rhizopus stolonifer*. The seeds were treated with essential oils produced from *Cymbopogon citratus, Lavandula dentata, Lavandula hybrida*, *Melaleuca alternifolia*, *Laurus nobilis*, and *Origanum majorana* (#1 and #2). The occurrence of *S. cucurbitacearum* was decreased, ranging from 67.0% in *L. nobilis* to 84.4% in *O. majorana* #2. Seed germination was not affected by treatments at 0.5 mg/mL essential oils, even though radicles were shorter than control ones, except for *C. citrates* and *O. majorana* #1 essential oils. The occurrence of *S. cucurbitacearum* was reduced by approximately 40% in plantlets developed from seeds treated with *C. citratus* essential oil. Xu *et al.* (2014) demonstrated that 0.5 mg/mL of their *L. nobilis* essential oil protected cherry tomatoes from infection with *A. alternata*. Moreover, Xu *et al.* (2017) showed an exceptional antifungal activity of essential oil made from bay tree in tests against *A. alternata*. Eke *et al.* (2020) reported that essential oil made from lemon grass protected young plants of French bean from diseases caused by *F. solani*, under conditions both of the laboratory and the greenhouse.

One of difficulties of using plant extracts are high amounts of water that are needed and a drying step that is necessary afterwards. In order to obtain a stable emulsion of essential oils in water, a sonication procedure was developed. The activity may be possibly enhanced if chelating divalent cations are added. They stabilise the anionic lipopolysaccharide layer in the outer membrane of Gram-negative bacteria (Skandamis *et al*., 2001).

BIOLOGICAL SEED TREATMENTS (USE OF ANTAGONISTS)

Other approaches to seed treatments encompass in-field applications to plants for the reduction of disease-causing fungi and bacteria that can develop on the seed. Biological seed treatments control seed pests by parasitising the pest organisms, which defeat them in the competition for food on the root system, or which produce toxic compounds that inhibit pathogen growth. Various organisms such as filamentous fungi, bacteria and yeast have been used as biocontrol agents (BCAs) against seed-borne pathogens. In order to be more successful against plant pathogens, a BCA have to be able to colonise strongly the plant rhizosphere, to prevent the attack of pathogens and to compete with other microorganisms in the plant rhizosphere. Disease control can be effective if BCAs survive and develop in the spermosphere before sowing and in the rhizosphere after seed germination (Spadaro *et al*., 2017).

Comprehending the mechanism of action is vital for developing optimal commercial formulations and application procedures with the intention to maximise the efficacy of BCAs (Spadaro and Gullino, 2005). Microbial antagonists use diverse mechanisms to control plant pathogens, such as food competition, hyperparasitism, production of lytic enzymes, secretion of antibiotics, and interference with quorum sensing. Moreover, microorganisms can also elicit localised and systemic host defences (Mukerji and Chincholkar, 2007).

In the study with commercially formulated micro-organisms, Tinivella *et al.* (2009) established that two out of seven products tested were effective. When specific strains of microorganisms are combined, numerous traits that antagonise the pathogen can also be combined and this may result in more efficient protection. De Boer *et al.* (2003), controlled *Fusarium* wilt of radish by combining *P. putida* strain WCS358, which competed for iron through the production of its pseudobactin siderophore, with *P. putida* strain RE8, which induced systemic resistance against *F. oxysporum* f.sp. *raphani*. Studies of Bennett *et al.* (2009) and Wharton *et al.* (2012), showed that *Trichoderma* spp. effectively controlled soil- and seed-borne pathogens such *as Pythium*, *Phytophthora*, *Rhizoctonia* and *Fusarium* spp. in different crops. Raupach and Kloepper (1998) applied a mixture of three different plant growth-promoting rhizobacteria (PGRP), as a seed treatment. PGRPs intensively promoted the pant growth and reduced numerous cucumber diseases. Many strains of bacteria or fungi used in biocontrol produce antibiotics that inhibit the growth of other fungi. The introduction of the gene encoding the housekeeping sigma factor into a strain of *P. fluorescens* increased the production of pyoluteorin and 2,4-diacetylphluoroglucinol (DAPG) (Schnider *et al*., 1995).

Pseudomonas and *Bacillus* species as PGPRs have attracted much attention for their role in mitigating and reduction of plant diseases. When applied as seed treatments, PGPR resulted in significant reduction of *Phytophthora* blight disease of squash (Zhang *et al*., 2010). The development of mixtures containing strains communicating with each other to maximise antibiotic production and disease control could be another approach in the improvement of control of soil-borne diseases (Becker *et al*., 1997; Davelos *et al*., 2004). According to De Sousa *et al.* (2021), inoculation with *Trichoderma* increased the length of the radicle and hypocotyl and showed no fungi in the seeds. Some treatments increased the height and the plant root dry mass in seedlings.

Pseudomonas chlororaphis has a suppressive ability against the pathogens as it produces antifungal metabolites with broad activities. This bacterium efficiently controls many seed-borne diseases present on seeds or near seed coats but it does not control soil-borne diseases and pathogens located deeper in seeds. Although active on the seed in the soil, this bacterium does not provide sufficient effect later (Kilany *et al.*, 2015; Shah *et al.*, 2017, BioAgri, 2019).

According to Emily Gatch, Washington State University (https://eorganic.org/node/749), Kodiak (*Bacillus subtilis*), Mycostop (*Streptomyces grieseoviridis*), SoilGard (*Gliocladium virens*), T-22 Planter Box (*Trichoderma harzianum*), Actinovate (*Streptomyces lydicus*) are products used for biological treatments of seeds that can be purchased on the world market.

CONCLUSIONS

Organic seed production in comparison to conventional seed production is more prone to risk of contamination with weed seeds and seed-borne pathogens that can accumulate and become a problem after several cycles of seed multiplication. It is currently very difficult to achieve the desired seed quality standards for a large number of crops. Weed, disease and pest control is a particularly sensitive segment as many difficulties occur due to the almost complete absence of chemical measures. Various simple and complex methods regarding seed treatments have been developed and tested for the past several decades. There are numerous effective and sustainable seed treatments used to control seed-borne diseases that are successfully applied world-wide: 1) physical seed treatments, including mechanical treatments, thermal treatments, radiations, and redox treatments, 2) the use of natural compounds, in which organic compounds comprise plant extracts, essential oils, as well as purified microorganism compounds, and 3) biological control, based on the use of antagonistic microorganisms. Since the use of pesticides is not allowed in organic agricultural production, seed production in compliance with the prescribed principles for such a production mostly faces difficulties within the filed of plant protection and therefore further studies are necessary in order to coin measures for successful control of pathogens, especially seed-borne diseases.

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