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Producing the Biocontrol Fungi Trichoderma and Beauveria

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Introduction

In nature, dozens of species of harmful fungi can quickly kill a plant, including *Fusarium* spp., the causal agents of Fusarium wilt, and *Phakospora pachyrhizi*, the causal agent of soybean rust (Figure 1). Fungi are unable to produce nutrients on their own, so they must find another source; sometimes that source is old bread, orange peels, a rotting tree trunk, or a plant's translocation tissues. These pathogenic fungi thrive in conditions of poor air circulation, slow water drainage, over-irrigation or too much rainfall. Such poor conditions can often be prevented by spacing plants properly, following an irrigation schedule, and removing fungus-prone debris, such as old plant material and weeds. No matter what we do, though, there is a good chance that pathogenic fungi will infect our plants at one time or another.

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Figure 1. Fusarium wilt on a tomato plant. Fusarium oxysporum is a fungal pathogen that can affect hundreds of plant species. (Photo: Mercure, 1998)

Unfortunately, in our modern world, chemical fertilizers and pesticides have become the norm in agricultural production, causing severe and serious environmental pollution. Use of these agricultural methods can lead farmers to become dependent on more and more inputs, as environmental imbalance ensues.

Fortunately, Fungi Kingdom is not exclusively populated by pathogenic intruders, dwelling unpoliced in the murky corners of the invisible world. Two particular beneficials of the fungal world, *Trichoderma* spp. and *Beauveria bassiana*, have been widely studied for their beneficial properties in agricultural production. The potential of these fungi species is especially exciting because of their ability to improve agricultural productivity while decreasing the development of fungicide-resistant pathogens (Studholme, 2012).

Beauveria bassiana

Beauveria bassiana is a common soilborne fungus occurring worldwide. It is an insect-pathogenic fungus. When spores of *B. bassiana* contact the shell of an insect, they penetrate the exoskeleton and begin producing a toxin called Beauvericin, which weakens the host's immune system until the insect dies (Caldwell, 2013). Eventually, the fungus will fill the entire body cavity. Under high humidity conditions, the fungus will grow through softer body parts, creating a characteristic "white bloom" appearance as the fungus covers the body (Figure 2). The whole process is slow, happening over three to seven days, so it takes time to suppress the insect population and a single application would not be sufficient. Also, note that *Beauveria* will only suppress and not eradicate an insect population, killing between 50 and 75 percent of the population on average. Spraying during times of higher humidity and at earlier insect life stages will increase effectiveness (Caldwell, 2013).

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Figure 2. The 'white bloom' characteristic of Beauveria bassiana under more humid conditions. (Photo: Surendra, 2013)

Beauveria is recommended mainly for the control of chewing pests, white fly and beetles. Since contact with the pest is required, it can be applied at any time without great risk to bee populations, as the hive is elsewhere. Even so, avoid spraying at times when bees are most active. In addition to controlling agricultural pests, certain strains of *Beauveria* are effective against bed bugs and termites. Before you purchase a commercial strain, do some research or read the label to find out what that strain is effective against. While most research has been done on its insecticidal activity, some studies have shown *Beauveria* to act against soil fungi such as *Rhizoctonia*, a pathogenic fungi that causes damping off, root rot and many other pathogenic conditions in a wide variety of plants (Ownley, 2004).

Trichoderma sp.

Trichoderma sp. is a genus of fungi also commonly occurring worldwide, with different species native to different areas; hence the 'sp.' designation above. The species are frequently found isolated from

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Figure 3. Trichoderma fungus occurring naturally on wood bark. (Photo: Samuels, 2014)

agricultural soils or are seen as green spots on tree bark and deteriorating wood (Figure 3). Several strains of *Trichoderma* have been developed as biocontrol agents. As a decomposer, *Trichoderma* fungi can be used to help speed up compost piles. *Trichoderma* sp. are most effective as a seed treatment, to prevent death from other fungi in emerging and young plants; in this role, *Trichoderma* can offer improvement over chemical seed treatments. Both encourage germination, yet *Trichoderma* subsists in the soil and the plant, promoting long-term benefits that a chemical treatment cannot (Harman, 1997). As a soluble spray, *Trichoderma* is also beneficial when applied to flowers, to prevent fungal growth that decreases fruit set.

Until recently, the benefits of the *Trichoderma* fungus was thought to be from its ability to produce enzymes that target and degrade chitin, the structural component in the cell walls of fungi and insects. However, recent research has shown that the diverse benefits of *Trichoderma* are caused by different mechanisms (Contreras-Cornejo, 2009). It has been shown to enhance general plant biomass production and lateral root growth, through interactions that promote auxin production in the host plant. Auxins are a class of plant hormones integral in plant growth and development. *Trichoderma* is able to form a symbiotic relationship with some plants, even living inside the plant's vascular system and between cells (Hermosa, 2011), helping the plant resist foliar fungal pathogens (such as rice blast on rice plants) (Studholme, 2012). The fungus can also help plants better tolerate abiotic stress compared to non-inoculated plants (Hermosa, 2011). However, its anti-fungal ability can make *Trichoderma* harmful in the vicinity of mushroom production.

Producing *Beauveria* and *Trichoderma*

Strands of both *Beauveria* and *Trichoderma* fungi have been studied in lab conditions. Strains with the most effective beneficial properties have been isolated and reproduced, and are commercially available in Southeast Asia. Enzymes produced by these fungi differ depending on the strain, making their impact potentially unpredictable (Contreras-Cornejo, 2009). For this reason, we do not recommend that you attempt to find and reproduce your own 'local' variety. We do recommend, however, that you work to create soil conditions that will promote the growth of these beneficial fungi in your soil. You can do this by using minimal tillage or no-till techniques, by mulching, and by not spraying chemical fungicides.

Fortunately, producing your own *Beauveria* or *Trichoderma* spray at home is possible and quite easy. First, purchase a commercial strain, usually available as a dry powder. Be sure to keep this as your starter source for growing more batches of the fungus. Fungi can evolve easily over many generations; if you continually inoculate your substrate with spores from your previous batch, after only a few generations you may have something quite different (and much less effective) than the original. It is worth the cost to use the commercial strain each time you create a new batch.

Supplies

Be careful to avoid getting any fungus, no matter how beneficial, into

your lungs or eyes. We recommend wearing goggles, a dust mask and gloves when inoculating the growing media. *Beauveria*, in particular, can affect humans, but only in extraordinary circumstances. All four reported cases have occurred when the host's immune system is already severely compromised.

Other supplies that you will need include: clean rice or sorghum, water, a rice cooker and spoon, large clear plastic bags, rubber bands, a needle, and your *Trichoderma* or *Beauveria* powder (Figure 4).

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Figure 4. Supplies for producing a fungal spray. The container pictured contains the fungus Beauveria. (Photo: Brock Mashburn)

Procedure

1. Mix three parts rice to two parts water (3:2) in the rice cooker. Sorghum can be used in place of the rice, if it is cheaper or more readily available. Make enough to fill your rice cooker, and turn the rice cooker on.
2. When the rice has finished cooking, place two to three large spoonfuls (serving spoons) of cooked rice into a new plastic bag. Pack rice down, then flatten the bag and fold the bag over itself to prevent air (containing foreign spores) from getting in while the rice cools. Let the rice cool until it is comfortable to hold against skin.
3. Open the bag and sprinkle ½ teaspoon of *Trichoderma* or *Beauveria* powder on the rice (Figure 5). Close the bag and seal tightly with a rubber band at the top of the bag (Figure 6). Don't try to force all the air out of the bag. Mix the rice around to spread the spores among the rice, then pack the rice down again.
4. With a needle, poke 10-15 holes in the upper part of the bag where there is no rice. The holes will allow for air exchange. Alternatively, a few changes can be made to the procedure to reduce the likelihood of contamination. Instead of sealing the bag with a rubber band and poking holes in the bag with a needle, you can thread the bag through a 3 cm section of thin PVC pipe and use cotton balls or shreds of cloth to fill the hole. The cotton balls or shreds of cloth will allow

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Figure 5. Not many spores are needed to populate the bag of rice.

for air exchange while preventing contamination, which is more likely with the holes in the rubber-banded bags (Figure 6).
5. Store the bags in a clean (preferably disinfected) indoor location at room temperature. Store in a an area with natural or artificial lighting.
6. After two days, mix the rice again inside the bag and pack it down.
7. After seven days, the fungi should have taken over the whole bag. It is now usable, but can live in the bag for three to four weeks longer. Healthy *Trichoderma* should have a sweet coconut odor and is most often dark green (Figure 8) but can be white or light yellow. *Beauveria* is scentless and should be white (Figure 6).


(/resources/255e3849-a2ed-4681-bed3-4343cce8eb95)


(/resources/3c5774e7-e4fd-4e21-acfd-0366d04eeecb)Figure 6. Two methods of storing. Above: The bags are closed with rubber bands, and then holes are poked around the top. Below: The bags are threaded through a pvc section, then banded down. Cloth or cotton is used to plug the holes.

Your bags are contaminated by other fungi if you see different colors, especially black, and if the smell is putrid. Don't use any of the contaminated mixture, even if part of it looks pure. Discard the whole bag.

If problems persist, try completing the entire process under a biohood or a similar biosanitary machine. Directions for building a homemade biohood are available from ECHO on ECHOcommunity.org (http://ECHOcommunity.org) (Figure 7).

For a different method of inoculating corn with *Trichoderma*, see "PhilRice," under "Other Helpful Sources" below.


(/resources/2b2f0cd6-9b9a-43fa-badc-8a9832de44d5)Figure 7. Niemeet Chomopoothong works under ECHO's homemade biohood. (All Above Photos: Brock Mashburn)

Using *Beauveria* and *Trichoderma*

When your *Trichoderma* or *Beauveria* bags are finished, dilute 1 kg of inoculated rice into 200 liters of water* to make a sprayable solution. Make sure to rinse the bag and rice clean to get all the possible spores. Separate the rice from the liquid. Once the *Trichoderma* or *Beauveria* is mixed with water it must be used or discarded. Mixed solution will not remain effective long term.

Spray every three or four days while pests persist, especially on the underside of leaves. Sprays are especially effective when insects are young, during high humidity and when the spray is highly concentrated with spores. You can continue to spray once per week when the fungus or insect pest is under control. Do not mix with other sprays and avoid spraying other products for four days before or after spraying *Trichoderma* or *Beauveria* (Caldwell, 2013).

The *Trichoderma*-inoculated rice can also be added straight to compost piles, potting soils or planting stations (three days before planting).


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Figure 8. Mass production of Trichoderma in Thailand. (Photo: IPM Thailand, 2013)

Conclusion

"Natural Farming" methods encourage the use of nature's processes to replace potentially harmful chemical or inorganic pesticides, fungicides and fertilizer. Many of these methods are not yet widely recognized in Western agriculture or in the academic realm. However, the effects of the fungi *Trichoderma* and *Beauveria* as beneficial biocontrol agents are widely researched and are commercially available worldwide. Both fungi are easy to reproduce at a low cost, and their potential makes them deserving of the attention they are receiving in the academic world and among farmers in Asia.

**This is the rate used by Maejo University, Chiang Mai, TH: Boonsong Thansrithong, Agriculture Program Manager, ECHO Asia*

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