USE OF PLANT EXTRACT-BASED BIOPESTICIDES AS A SAFE ALTERNATIVE TO MEBR.

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The toxicity and negative environmental effects of most agricultural soil fumigants has created interest in using antimicrobial volatile plant secondary metabolites to control plant pathogens. Previously, mixtures of essential oils (EO) from plants belonging to *Labiatae* and *Umbellifera* families i.e. thyme and related herbs (named as BioFume^{TM}) were found to have broad-spectrum activity against fungal, bacterial and nematodal phytopathogens. BioFume formulations contain components generally regarded as safe (GRASS) status by FDA and since the components are highly volatile and/or degraded by natural soil microbes they do not pose any health, residue or environmental problems at the concentrations, which they are used. Over the years of experiments we have observed that soil treatment with BioFume protected plants from fungal, bacterial and nematodal pathogens under field conditions, but BioFume was more effective in unpasteurized soil than in pasteurized soil. We have found a significant shift in soil microbial content, where an increase in a number of plant beneficial organisms, including Actinomycetes, and overall dehydrogenase activity was observed in unsterilized soil upon treatment with BioFume. Part of the plant-protective effect of BioFume may therefore be due to plant-beneficial soil organisms that are enriched by application of EO. To test this hypothesis, we isolated several bacteria that belong to *Pseudomonas* spp. from soil treated with 1000 ppm EO. *Pseudomonas fluorescens* TR97, an isolate found to degrade components of thyme oil and grow well in the presence of 1000 ppm EO, was chosen for further study.

The effect of TR97 on pepper and tomato plants exposed to a number of pathogens naturally present in field soil was determined. In growth chamber trials, agricultural soil containing various pathogens including *Phytophthora capsici* and root knot nematodes was treated with 100-400 ppm EO, emulsified in water and mixture of EO and TR97. Field trials were also conducted with similar treatments. Plant root growth, root gall formations, discoloring on roots and the number of healthy plants were monitored.

Inoculation with TR97 alone was found to protect growth chamber plants from *P. capsici* (when zoospore suspension was added additionally), and natural pathogens in soil obtained from field locations known to be heavily infested with *P. capsici* and root knot nematodes despite the fact that TR97 has minimal antifungal activity. A new formulation of BioFume containing 1.5-2% thyme oil and 10^8 colonies/ml of

strain TR97 as emulsified in 1000 ppm thyme oil per liter was added into drip irrigation water in a rate of 10 gallon/acre at the time of transplanting and with a half of this rates afterwards at bi-weekly intervals in fields where tomato, pepper and cucumber plants were transplanted. We have observed an increase in root growth, reduction in browning and gal formation caused by root-knot nematode, resulting in early flowering and fruit set and an increase in overall healthy plants upon BioFume applications.

Since many Plant Growth-Promoting Rhizobacteria (PGPR) species, including fluorescent Pseudomonads, found to be inducing systemic resistance (ISR) in plants we have studied the mod of action of BioFume as formulated with TR 9 under green house conditions. Although, higher concentrations of new formulations of BioFume had direct activity against foliar pathogens, BioFume treatment via soil protected cabbage and tomato plants against foliar bacterial and fungal pathogens at lover concentrations, indicating systemic nature of activity. Both in mixture or TR 97 alone application induced formation of small microscopic hypersensitive reaction (HR) -type lesions, commonly associated with ISR, increased overall peroxidase activity and lignin formation, which may further indicate involvement of host defensive responses. Induced resistance, results in similar reactions which are commonly found in conventional breeding lines that contain multiple genes for resistance (multigenic or horizontal resistance), considered to be a safe method for protecting plants against variety of pathogens and pests. New formulations of BioFume acts as a multiple layered protection agent by initially reducing the pathogenic organisms thus enabling and supporting colonization and growth of the fluorescent Pseudomonads (i.e. TR 97), and by acting as an activator of defense responses, especially in the root of plants. Moreover, BioFume provide better root growth, thus enable increased uptake of nutrients, increases overall growth of plants and reduce time of flowering and fruit set.

These results indicate that (1) EO soil treatments can inhibit pathogens indirectly, by enriching levels of soil bacteria with plant-beneficial effects, and (2) mixtures of the EO and EO-tolerant plant-beneficial bacteria may have useful agricultural applications, especially as a replacement for methyl bromide. This unique approach to use antimicrobial volatile plant extracts together with conventional biological control agents, which are adapted to use components of plant extracts as carbon source, may provide a niche in development of novel biopesticides. For example combination of EO and biological components in BioFume formulations have advantages by reducing the cost of fermentation of biological control agents since heat is not needed for sterilization (only certain adapted bacteria can grow in 1000 ppm EO); oil component increases the life of biological entity by covering the surface of emulsified bacterial suspension, therefore cutting the contact with air and prevent from contamination with other organisms. Overall this combination reduces the cost of production, hence reduces the cost of BioFume below to the levels of MeBr, providing an environmentally friendly, safe alternative.