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Control of Bacterial Wilt Disease Caused by Ralstonia solanacearum in Pepper using Arbuscular Mychorrhizal Fungi (Mykovam)

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Abstract
Soil-borne antagonists Arbuscular Mycorrhizal Fungi (AMF) Mykovam were evaluated for their ability to suppress the growth of Ralstonia solanacearum. The experiment utilized two hundred (200) hot pepper seedlings as test plants that were grown in the seedling trays. The recommended rate of AMF Mykovam were introduced to half of the seedling population (100 seedlings) while leaving the other half untreated. Introduction of AMF was borne by incorporating them in the soil seven (7) days prior to the inoculation of pure culture of Ralstonia solanacearum. Fastest rate of infection caused by R. solanacearum was observed on untreated seedlings three (3) days after inoculation while the highest infection were on the second (31%) and third (28%) week. T-test results showed highly significant difference between the seedlings with and without AMF treatments. Rate of infection from seedlings treated with AMF ended on the early days of second week possibly due to the presence of AMF colonizing the roots of hot pepper seedlings which compete for the nutrients and produces substance that caused to antagonize the disease -causing pathogens. Hence, applying inoculum of AMF at early seedling stage of hot pepper to infect the roots and protect them for the entry of pathogenic microorganism R. solanacearum is recommended.
Keywords: Mycorrhizal Fungi, *Ralstonia solanacearum*

Introduction

Bacterial wilt occurs widely in tropical, subtropical and some temperate regions of the world (Kelman, 1998) and is caused by a soil-borne, vascular pathogen, *Ralstonia solanacearum* (formerly *Pseudomonas solanacearum*) (Yabuuchi*et al*., 1995). An alarming disease wide-reaching, bacterial wilt set boundaries in the production of solanaceous crops such as tomato, pepper, eggplant, tobacco and potato as well as other important crops like peanut, banana, ginger and geranium. Approximately around 450 crop species have been reported as hosts of this pathogen (Grimaultet al., Ranamukhaarachchi, 1994; Swanson et al., 2005).

In Vietnam, bacterial wilt causes significant damage on many important crops under disease-favorable weather conditions (Doan and Nguyen, 2005). Control is difficult due to high variability of the pathogen, limited possibility for chemical control, high capacity of the pathogen to survive in diverse environments and its extremely wide host range. The use of resistant varieties has been used to reduce disease. However, crop resistance is often overcome by the genetic diversity of the pathogen as well as genotype and environment interactions (Wang, 1998).

Disease mechanism is being struggled with some cultural management practices including crop alternation, growing other plants like ornamentals in between the main plant as intercrop, the use of organic fertilizers that may encourage beneficial microorganism that can be used to antagonize entry of pathogen and the use of resistant cultivars. However, multiple crop based control of bacterial wilt is often hampered by the pathogen's wide host range. Biological mechanism technique is still in its research phase according Overbeek et. al., 2002, and other researchers with few studies reported for bacterial wilt like Shekhawat, 1993; Lwin and Ranamukhaarachchi, 2006; and Messiha, 2007.

Biological control not only increases crop yield and suppresses disease but also avoids environmental pollution. It is important to develop methods for evaluating antagonistic microorganisms and incorporating them into successful disease management. Some research on microbial antagonists, such as *Candida ethanolica* has shown promise for bacterial wilt control according to Lwin and Ranamukhaarachchi, 2006. Toyota and Kimura (2000) described the oppressive effect of some unfriendly bacteria on *R. solanacearum*. Furthermore, Ciampi-Panno et al. (1989) showed that antagonistic pathogens were effective in conquering *R.solanacearum* under field conditions.

Several microorganisms' competitors have been evaluated with variable success as cited by Shekhawat, 1993. Lwin and Ranamukhaarachchi (2006) described a satisfactory suppression of the bacterial wilt pathogen by the application of a commercially obtainable mixture of effective microorganisms (EM). Advance studies have recognized many different microorganisms that have the potential of suppressing bacterial wilt causing microorganisms, although they have not yet been evaluated for effectiveness according to Hoang et al., 2004.

Arbuscular Mycorhhizal Fungi (AMF) is one of those microorganisms with potentiality in suppressing

bacterial wilt causing microorganism. AMF is a group of fungi based bio-fertilizer developed by UPLB-BIOTECH. This mycorrhizal inoculant is composed of several strains of fungi spores, which can be used to infect the roots of the plants and other communicable propagules of endomycorrhizal fungi. When use and functional, it is projected to replace 60-85 percent of the plants' chemical fertilizer requirement. Results of laboratory observations have also exhibited that Mykovam is capable bio-fertilizer input to protect high value crops including vegetables, ornamentals, fruit crops, and forest trees.

AMF when inoculated to seedlings the fungi may possibly infect the roots and benefit the plants in search of water and supply of nutrients predominantly phosphorus, which is needed by the plants for plant growth. The mycorrhizal fungus that colonizes the plants roots may prevent the entry of infectious pathogens and increase plant tolerance to drought and heavy metals (Zarate, 2012).

This study was conducted to evaluate the potential effect of mycorrhizal fungi (Mykovam) as bio-control agent on the growth of *R. solanacearum* pathogen on hot pepper seedlings.

Materials and Methods

Preparation and conditioning of test seedling plant s with Mykovam

Two (2) 100 holes seedling trays were filled with sterilized soil mixed with organic substrate composed of carbonized rice hull and coconut coir dust in a ratio of 1:1:1. The imbibed hot pepper seeds were placed in each hole and were allowed to germinate. After germination, protective cover was installed by using a fine mosquito net to protect the seedlings from insect pest and diseases. When seedlings have true leaves, approximately 12 days old, the culture media was sterilized using the recommended rate of fungicide to ensure that organisms present in the growing media were inactive or at minimal level. Five (5) days after treatment of fungicide, the recommended rate of AMF (Mykovam) was introduced to the first hundred seedlings, the second hundred seedlings from another seedling tray was allowed to grow without AM fungi. Seedlings inoculated with AMF were allowed to grow for seven (7) days before the inoculum of *Ralstonia solanacearum* were introduced.

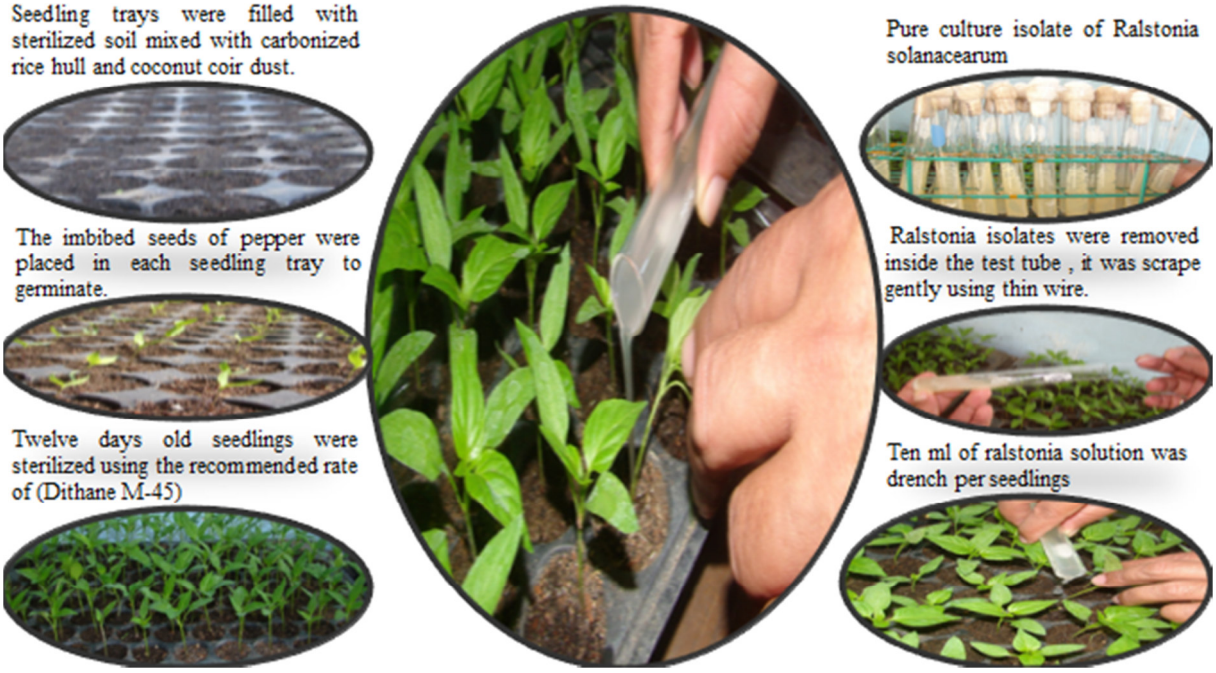
Preparation of Ralstonia solanaciarum isolate

Five (5) slightly infected pepper plants with *Ralstonia solanacearum* was collected in the field. To ensure that the infection of the plant was caused by *Ralstonia*, an expert from the UPLB Crop Protection Cluster Laboratory was consulted for proper identification of the diseased sample including collection and reproduction of isolate to produce pure isolates. The preparation of virulent *Ralstonia* pathogen was done by disinfecting diseased portion of the sample plant using 1% Sodium Hypochlorite solution for 15 minutes. Different portions of diseased plants were detached and put in the culture media after disinfecting. Five (5) isolates were monitored to identify the strain of *Ralstonia* intended for reproduction. After two days, pure isolates of target pathogen was identified and reproduced.

Introduction of Ralstonia solanaciarum to the test plants

Thirty (30) test tubes containing the isolates of *Ralstonia solanacearum* were divided into two groups. Fifteen (15) test tubes, each with more or less equal amount of *Ralstonia* isolates based on their hyphae, were removed by putting few volume of water inside the test tube then scraped gently using thin wire. The collected isolates from fifteen (15) test tubes were placed in a container with one thousand ml of water. Ten (10) ml of the solution with *Ralstonia* isolates was equally distributed by drenching to one hundred (100) seedlings of hot pepper treated with AMF. The same procedure was done to the remaining one hundred seedlings un-inoculated with mykovam (Figure I).

Figure I. Introduction of *Ralstonia solanaciarum*



Data Gathering

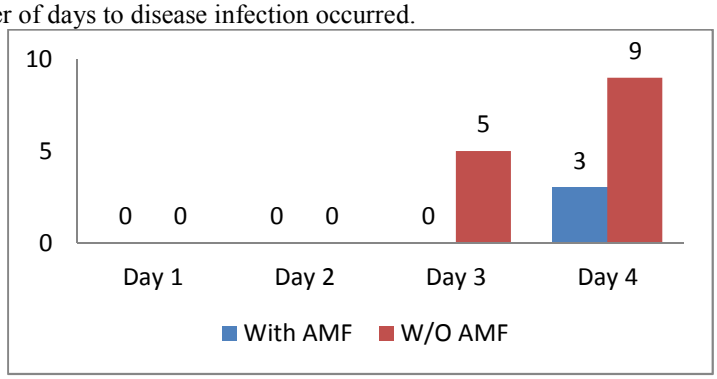
To compare the severity of infection by the *Ralstonia* pathogen to the seedlings inoculated with AMF and without AMF, the number of days before the appearance of first symptom of infection was recorded. Infected seedlings were counted and recorded daily to compare the rate of infection and severity of disease. Final count of diseased seedlings was done during termination of the study, seventeen (17) days after treatment. Data gathered from treated with AMF and untreated seedlings were compared and the degree of severity was analyzed.

Treatment	With Ralstonia solanacearum
T1 With AMF (Mycovam)	10 ml solution each seedling
T2 Without AMF	10 ml solution each seedling

Results and Discussion

Degree of infection by disease causing microorganisms *Ralstonia solanacearum* was observed on seedlings inoculated with Arbuscular Mycorhhizal Fungi (AMF) as well as in seedlings untreated with AMF. In just three (3) days after inoculation of pathogen, a total of five (5) seedlings from untreated plants with AMF exhibited signs of disease infection and turn out to be wilted. However, on the fourth (4) day, there are three (3) seedlings treated with AMF presented signs of infection. A short interval in the application of mycorrhizal fungi to seedling and the introduction of *R. solanacearum* is suspected to be the cause of the manifestation of the diseases in seedling treated with AMF. Because the extent to take possession of the seedling roots by the mycorrhizal fungi was not fully understand, it is assumed that the roots were not fully colonized by AMF before the *Ralstonia* was presented. However, even though some of the seedlings treated with AMF fungi were diseased by the pathogen, it was found out that the degree of infection based on the number of infected seedlings by *Ralstonia* was not severe and limited unlike to those seedlings untreated with AMF. Highly significant difference was experiential in terms of percentage of infection between seedlings inoculated with AMF and seedling with no AMF on the roots, using T-test with 2.22 differences (t-Stat 5.00000) compared to t-Critical value (2.77644).

Figure 2. Initial number of days to disease infection occurred.

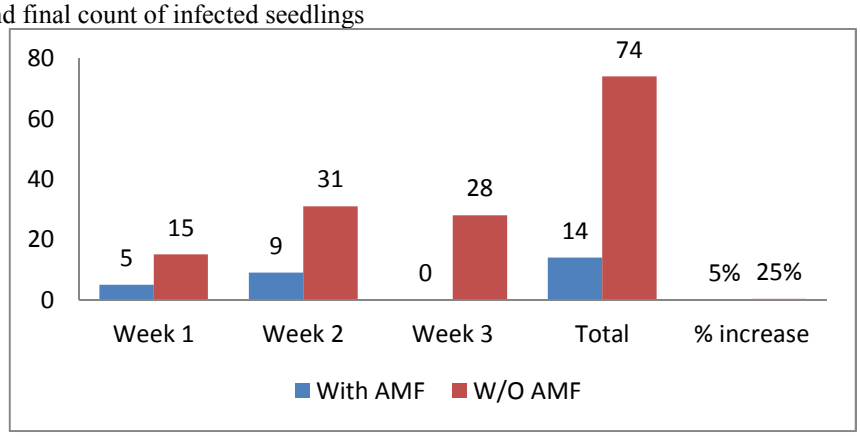


The higher weekly rate of infection was recorded on seedlings not treated with AMF from the first to the last week of observation. Weekly total of infected seedlings of 15, 31 and 28 were recorded, correspondingly. The disease manifestation was severe on the second and third week with 31% and 28% diseased seedlings. However, no infection was observed from seedling treated with AMF on the third week.

Zhu, et al 2004, describe the inhibition of *R. solanacearum* as a result of increased phenols induced locally or systemically by an Arbuscular mycorrhizal (AM) fungi. In pot cultures, *R. solanacearum* populations in the rhizosphere, on root surfaces and in the xylem were decreased by 26.7, 79.3 and 81.7%, respectively, following inoculation of tomato plants (*Lycopersicon esculentum* Mill.) with *Glomus versiforme* Berch. Colonization of the plants by both *R. solanacearum* and *G. versiforme* increased the contents of soluble phenols and cell-wall bound phenols in root tissue, but with different patterns. Whereas *R. solanacearum* preferably promoted the cell-wall bound phenol content, *G. versiforme* preferably enhanced the soluble phenol content. Split root experiments revealed that *R. Solanacearum* was inhibited by *G. versiforme*, and that *G. versiforme* also increased the phenol content systemically, but to a lesser extent than locally. Ciampi-Panno *et al.* (1989) showed that antagonistic pathogens were effective in suppressing *R. solanacearum* under field conditions.

T-test result was highly significant in terms of disease severity with difference of 2.371 from seedlings without AMF fungi compared to treated seedlings with AMF. Mohumad Tahat, Monther (2009) confirmed that the local species of AMF were more able to support and enhance plant growth compared to the introduced species. *G.mosseae* was able to control totally the bacterial wilt causal agent *R. solanacearum* under glasshouse conditions. The total infected hot pepper seedling was fourteen (14) from plants inoculated with mycorrhizal fungi while seventy four from seedling untreated with Mycorhzal fungi (Figure 3).

Figure 3.Weekly and final count of infected seedlings



Conclusion

Based on the results of the study, applications of AMF inoculant at early seedling stage has the potential to provide boundary or prevents the development of *R. solanacearum* disease causing organisms on hot pepper seedlings.

Recommendations

Based on the result of the study, applying inoculum of AMF fungi at early seedling stage of hot pepper communicate an infection to the roots and protect them for the entry of disease causing microorganisms like *Ralstonia* is recommended.

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