BIOLOGICAL CONTROL OF *PYTHIUM APHANIDERMATUM* CAUSING DAMPING OFF DISEASE IN ETHIOPIAN KALES

P. Juma[1], L. Murungi[2] and T. Losenge[3]
[1,2,3]Horticulture Department, Jomo Kenyatta University of Agriculture and Technology, Nairobi-Kenya
E-mail: patricjumah@gmail.com

Abstract
Damping-off disease caused by *Pythium aphanidermatum* has the potential to cause severe loss in greenhouse and field grown vegetables. *P. aphanidermatum* is common in soils from all climates, and capable of surviving for long periods without a host. In Kenya, this disease is mainly controlled through application of synthetic fungicide. The negative effects of this chemical has necessitated the search for an environmental and health friendly management method. Therefore study was undertaken to determine the efficacy of *Bacillus subtilis* BS-01 and *Trichoderma asperellum* TRC-900 against damping off in Ethiopian kale. *B. subtilis, T. asperellum* and combination of the two microorganisms were applied as seed coat. The coated Ethiopian kale seeds were planted on *pythium aphanidermatum* inoculated media. Pre and post emergence damping off incidence were observed. In *B. subtilis, T. asperellum* and combination of the biological control agents disease incidence of 16.9% - 29.8% compared to control which had 60.6%. The incidence of Post- emergence damping off reduced to range of 11 -25.4%, and control had an incidence of 64.8%. Damping off disease incidence in the *T. asperellum* and *B. subtilis* combinations was significantly reduced compared to the *B. subtilis* and *T. asperellum* isolates alone. This study revealed that using mixtures of *B. subtilis* and *T. asperellum* can provide a potential control for damping off disease in Ethiopian kales. Therefore utilization of *Bacillus subtilis* BS-01 and *Trichoderma asperellum* T-800 alone or in a combination as seed coat can offer a solution to control damping off disease.

Key words: Damping off, biological control agents, *Brassica carinata*, *pythium aphanidermatum*

1.0 Introduction

Seedlings damping-off caused by *Pythium aphanidermatum* is an important diseases that affect the vegetable crop causing serious economic losses in seedlings(Seebold and Ward, 2012). This disease cannot be prevented by rotational cropping since the causative agent *(Pythium aphanidermatum)* has a wide host range. Currently control measures of these disease is majorly through application of fungicides, this is responsible for chemical accumulation and low quality of the harvestable produce(Gravel et al., 2006). Chemical control is inappropriate and nondiscriminatory putting human and animal health at risk, as well as contaminating the environment (David, 2001). Therefore biological control can offer an alternative approach in control of this disease in Ethiopian mustards. *Bacillus spp* and *Trichoderma spp* are generalist biological control agents that have been successful in control various fungal disease in many vegetable crop.
Trichoderma spp is a fungi belonging to the Deuteromycetes class and has been utilized as biological control agents against a variety of plant pathogenic fungi because of their antagonistic properties towards plant pathogens (Junaid et al., 2013;Ithaca and Harman, 1997).

Bacillus spp. is Gram-positive bacteria having numerous strains that have presented biological control activity on a wide range of plant diseases (Cook and Baker, 1983). These *Bacillus* spp. strains are attractive candidates as biological control agents against plant pathogens due to their ability to form endospores that are tolerant to heat and desiccation, giving them lengthy shelf lives compared to other biological control agents (Sathiyaseelan et al., 2009).
Several studies have indicate that combinations of biological control agents can increase disease suppression (Singh and Nautiyal, 2012)) However, information pertaining to combined inoculations of *Trichoderma* and *Bacillus* species especially on damping off disease control appears to be very sparse. Therefore this study aims at evaluating the ability of *B. subtilis* and *T. asperellum* isolates alone and combinations to control damping off disease.

2.0 Material and Methods

2.1 Biocontrol Isolates and Seeds

Isolates of *Bacillus subtilis* and *Trichoderma asperellum* isolates was obtained from Real IPM Company in Thika, Kenya (Latitude: 1°01'59" S and Longitude: 37°04'09" E), while seeds for Ethiopian kale was sourced from the World Vegetables Centre (AVRDC) in Tanzania.

2.2 Isolation and Preparation of Pathogen

Tomato seedling showing symptoms of damping off was obtained from the JKUAT vegetable nursery. The seedlings were washed in running tap water and surface sterilized by dipping in 50% sodium hypochlorite for 2 minutes, and then rinsed twice in distilled water and blotted dry using sterile filter paper. Portions of the seedling near the affected area was placed in petri-dishes containing potato dextrose agar PDA amended with 300 µg/ml of sodium ampicillin on a plate and incubated at 25℃ for 2 days. The emerging hyphal tips was picked using sterile needles and sub-cultured in water agar and incubated for 5 days. *Pythium* spp identification and confirmation was done using the keys and description of (Dick, 1990). Pythium inoculum was prepared by introducing into freshly prepared PDA media. When the plates were full all the mycelia was scrubbed off using a painting brush and the fruiting bodies allowed to form. The fruiting bodies were harvested in distilled water and inoculated in planting media.

2.3 Seed Coating

The concentration of *B subtilis, T asperellum* and combination of the bio-control was determined using a haemocytometer and adjusted to 10^7 spores/ ml. African nightshade seeds was surface sterilized by soaking in 0.5% hypochlorite sodium (NaClO) for 2 min, washed and rinsed in sterile distilled water. The seeds were inoculated by immersion in spore suspension for 10 minutes while the control seeds were dipped in distilled water. The seeds were air dried and planted one day after coating at a rate of one seed per cell in 66-cell trays.

2.4 Experimental Design

A factorial layout consisting of three treatments (*Bacillus subtilis*, *Trichoderma asperellum* and a combination) and one pathogen *Pythium* spp was used. The treatments were laid out in a completely randomize design in three replicates. The experiments were repeated three times.

2.5 Data Analysis

A general linear model (GLM) was used to run an ANOVA on all data collected. If the ANOVA was significant, ($P \leq 0.05$) the means were separated using the Tukey's test using SAS

3.0 Results

B. subtilis and *T. asperellum* significantly reduced the incidence of pre-emergence damping off in Ethiopian kale compared to control however, combination of the two biological control agent's showed a significantly lower incidence of pre-emergence damping off compared to the control and the single application of the two biological control agents.

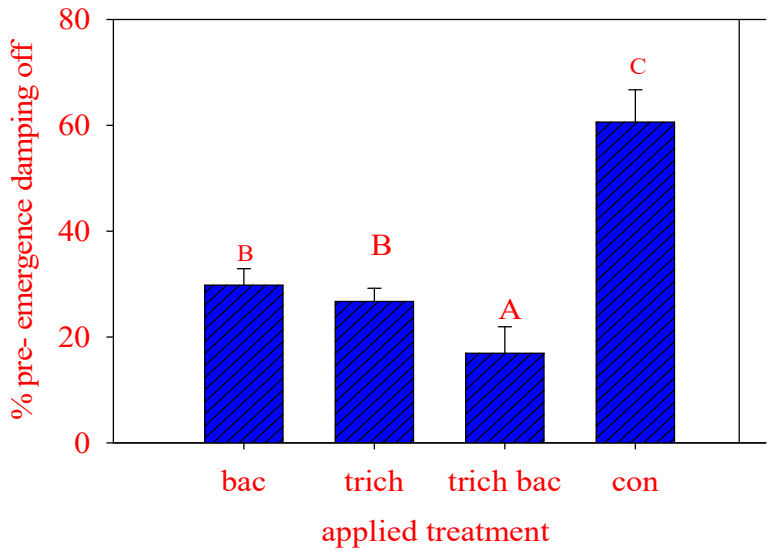

Fig 1: arcsine square root transformation of pre-emergence damping off Ethiopian kales seeds coated with B. subtilis, T. asperellum combination of the two biological control agent's and uncoated seeds control.

Post emergence damping off was significantly reduced by application of the biological control as alone application and as combined application. Application of *B. subtilis,* significantly reduced post emergence compared to *T. asperellum* and control (figure 2). However, combination of the two biological control agents had lower significant reduction in the incidence of damping off disease.

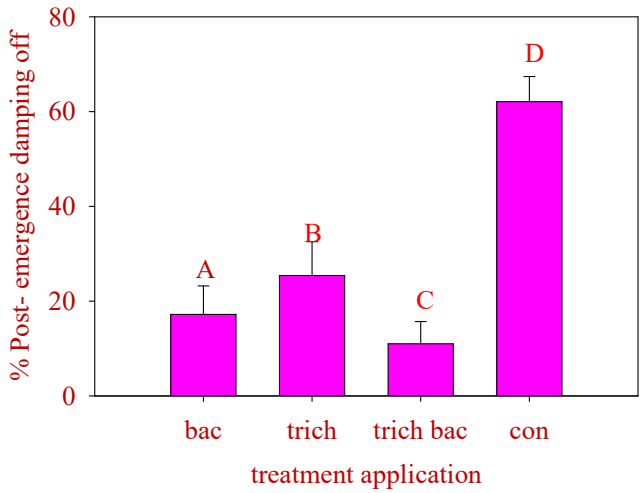

Fig 2: post- emergence damping off disease incidence in Ethiopian kale treated with B. subtilis, T. asperellum combination of the two biological control agent's and uncoated seeds arcsine square root transformed

4.0 Discussion

The main objective of this study was to investigate the ability of *B. subtilis, T. asperellum* combination of the two biological control agents to control damping off disease in Ethiopia kale. Biological control agent's trials in the greenhouse demonstrated that single inoculations of *B. subtilis, T. asperellum* and combination of the two biological control agent's reduced *P. aphanidermatum* pre and post-emergence damping-off disease. Various *Trichoderma* and *Bacillus* spp. have been reported as being able to successfully control several plant pathogens (Yobo *et.al*, 2011). The results obtained for the biological control agents trials support the finding of (Burns and Benson, 2000;Maketon et al.,2008) both of which demonstrated that *T. asperellum* and *B. subtilis* formulations were able to reduce damping-off caused by *P. aphanidermatum.* Itachi et al., (2000) showed that *Trichoderma spp* were shown to be hyperparasitic against *P. aphanidermatum in vitro* and also exhibited chitinase activity and siderophore production. These mechanisms are all thought to contribute to biological control ability of *Trichoderma spp* (Naziha, 2012). However inhibitory activity against *P. aphanidermatum* by *Bacillus spp* can be attributed to production of antibiotic such as iturin A and surfactin (Maketon et al., 2008)

A broad trend shows that combined application of *B. subtilis* and *T. asperellum* increased suppression of damping-off disease compared to single inoculations of the biological control agents. The difference in the mechanisms of action of B*. subtilis* and *T. asperellum* may explain why combinations of the two biological control agents resulted to increased disease control than some single (Yobo et al., 2011). Multiple biological control agents can enhance the level and consistency of control by providing several mechanisms of action (Maketon et al., 2008).

5.0 Conclusion

In conclusion, the results showed that protection of Ethiopian kale plants by *B. subtilis, T. asperellum* and combination of the two biological control agents against damping-off reduced the incidence of this disease. Combination treatment increased the survival of the seedling compared to single application. Therefore this biological control can be used in combination to provide protection to seedling against damping off disease.

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