MANAGEMENT OF DAMPING OFF DISEASE AND ENHANCEMENT OF AFRICAN NIGHTSHADE (*SOLANUM SCABRUM*) GROWTH THROUGH THE USE OF *BACILLUS SUBTILIS* AND *TRICHODERMA ASPERELLUM*

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ABSTRACT

Damping off, caused by Pythium aphanidermatum, is one of the diseases of economic importance in African Nightshade (Solanum scabrum) production in Kenya and can result to up to 100% seedling loss. The efficacy of antagonistic bacterial and fungal isolates of Bacillus subtilis and Trichoderma asperellum respectively were evaluated against P. aphanidermatum in African Nightshade in greenhouse studies. Seeds of three broad leaved varieties viz. 'Olevolosi', 'Abuku1' and 'Abuku2' were coated with 10^7 CFU/gram of B. subtilis and T. asperellum either singly or in combination. The treated seeds were planted in coco peat media inoculated with 10^7 CFU/ml Pythium aphanidermatum. The pre- and post-emergence damping off was suppressed in the seeds treated with isolates of B. subtilis and T. asperellum either singly or in combination in the three varieties. Furthermore, among the growth parameters, unit leaf rate and Leaf weight fraction were significantly promoted among the three African Nightshade varieties. Similarly, seeds of Abuku1 and Olevolosi varieties coated with isolates of the biological control agents produced enhanced the yield 1.5 times relative to the uncoated. All three African nightshade varieties seeds coated with B. subtilis and T. asperellum had expressed sufficient root colonization. Application of with B. subtilis and T. asperellum may reduce the incidence of damping off disease and consequently promote growth and yield of African nightshade.

Keywords: Solanum scabrum, Bacillus subtilis, Pythium aphanidermatum, Trichoderma asperellum, seed coating

INTRODUCTION

African Nightshade has become one of the most popular African leafy vegetables (ALVs) widely cultivated in East Africa particularly in Kenya (Grubben *et al*, 2014). It plays a major role as a source of calcium, iron, vitamin A and C (Ondieki *et al,* 2011) to the rural poor as well as gaining importance as a food nutritional security crop. Despite its importance, production of African Nightshades is threatened by pest and diseases. Damping off disease caused by *Pythium spp.* results to significant losses at the seedling stage (Mbugua *et al,* 2008). Infected seeds rot before germination or vesicular tissue rot at the soil line after germination (Sallam *et al,* 2008). Although synthetic chemical fungicides are commonly use for the management of the disease, (Subash *et al*, 2013; Jiskani *et al,* 2007) an increased awareness of fungicide related hazards has necessitated the search for alternative methods of controlling the disease.

Biological disease control methods as integral component of pest management in pursuit of eco-friendly solutions are being used as alternatives to chemical pesticides over the past 20

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years (Singh and Nautiyal, 2012). Isolates of non-pathogenic beneficial soil borne fungi and bacteria have been characterized and used as antagonistic biological control agents against persistent and troublesome soil borne pathogens *(Çakmakçi et al,* 2007). Isolates of fungal and bacterial antagonists such as *Pseudomonas sp, Bacillus spp, Burkholderia cepacia and Trichoderma spp* are being commercialized as bio-pesticides for control diseases caused by pathogens including members of genus *Pythium, Fusarium oxysporum, Phytophthora infestans* and *Verticillium spp* in vegetables such as tomato and cucumber (Kipngeno *et al*, 2015; Loliama *et al,* 2013; Mansoori *et al*, 2013).

Some of the biological control agents are also known to promote growth in various crops such as tomatoes, cucumber and beans (Azarmi *et al*, 2011; Abd-El-Khair *et al*, 2002). However, the information on the ability of *Bacillus subtilis* and *Trichoderma asperellum* to control damping off disease and the effect on the growth and yield of African nightshade is limited.

This study hypothesized that *Bacillus subtilis* and *Trichoderma asperellum* have the potential to control damping off disease and promote growth and yield of African nightshade. We tested this hypothesis by coating seeds of three African nightshade varieties namely Olevolosi, Abuku1 and Abuku2 with the two biological control agent agents and examined their efficacy against damping-off disease at pre- and post-emergence stages. The impact of the biological control agents on growth and yield of the three African nightshade varieties was assessed.

MATERIALS AND METHODS

This study was carried out in the greenhouse at Jomo Kenyatta University of Agriculture and Technology (JKUAT) (Altitude: 1537 MASL; Latitude: -1°5'47.04" Longitude: 37°0'46.08") between 2014 and 2016. Temperature of 28±3 °C and relative humidity of 72±6% was recorded in the greenhouse using a data logger (MIC 98583, Linz, Austria). Formulations of *Trichoderma asperellum* TRC 900 and *Bacillus subtilis* BS 01 were obtained from Real IPM Company (K) Ltd, and stored in a refrigerator at 5 °C. The biological control agent formulations were composed of clay (90%), glue (2%) and BCA conidia (8%). African nightshade seeds, *Solanum scabrum* var. Olevolosi were obtained from the World Vegetable Center in Arusha, Tanzania, while *Solanum scabrum* var. Abuku1 and Abuku2 were acquired from JKUAT.

Preparation of *Pythium aphanidermatum* inoculum

An isolate of *P. aphanidermatum* stored in sterilized soil slants was obtained from Kenya Agricultural and Livestock Research Organization (KALRO) and cultured on potato dextrose agar media then incubated at 25°C for 3 days. After *P. aphanidermatum* culture had fully covered the plates, mycelia were scrubbed off to initiate sporulation. A week later, the plates were filled with double distilled water, and the conidia removed using an artist's brush into a 500 ml beaker containing distilled water. The conidia in the beaker were subsequently poured into a 500 ml conical flask and sealed using Parafilm®. Immediately, after extraction the content of conical flasks was shaken in a reciprocal shaker at 80 rpm (Taiyo Reciprocal Shaker SR-1, Tokyo Japan) for five consecutive days. The concentration of conidia in the conical flask was determined using a Neubauer hemocytometer (Model A116, Tokyo, Japan).

Effect of biological control agents against *Pythium aphanidermatum*

Seeds (3 g) of each variety were coated with a powdered formulation of *B. subtilis* and *T. asperellum* either singly or in combination in the ratio 1:2 of biological control agent to that

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of African nightshade seeds (g/g). Seed coating was done by wetting the seeds for one minute to moisten the seed surface followed by mixing the seeds with the biological control agent. The coated seeds were then spread on filter papers (Whatman No.1) and dried at 25 °C for 24 hours. The seeds were sown immediately after drying in trays (66 plugs/ tray) filled with *Pythium* infected coco peat media. The experiment had four treatments comprising of 66 seeds per treatment that were replicated four times. The incidence of damping off disease was assessed daily for 42 days after sowing (DAS).

Effect of *B. subtilis* and *T. asperellum* on growth and leaf yield of African nightshade

Two grams of African nightshade seeds were coated with *B. subtilis* and *T. asperellum* either individually or in combination as described previously (see section on efficacy experiment). Seeds treated with the biological control agents were air dried at 25°C before sowing at a rate of five seeds per 2 L pot. The pots were filled with 3 Kg of soil in a mixture of soil and sand in the ratio of 3:2. After two weeks, the seedlings were thinned to a single plant per pot. *B. subtilis, T. asperellum,* a combination of the two biological control agent and uncoated treatments had five plants each and were replicated three times. Destructive harvesting was carried at the 6th week which is the stage at which the first harvesting of African nightshade and again at the 16th week after sowing just before flowering. The leaf area of harvested African nightshade leaves was determined using a leaf area meter (LI-COR Li-3000, Lincoln, NE). The fresh weight of leaves was determined immediately after harvesting using a weighing balance. To determine plant growth rate parameters, plant parts were partitioned into respective below and above-ground parts and oven dried at 70°C for 72 hours. The dry weight of respective plant parts was determined using a weighing balance.

Colonization of African nightshade roots by *T. asperellum* and *B. subtilis*

Seeds treated with the biological control agent were sown in trays as described previously (see section on efficacy experiment). Seedlings from the three African Nightshade varieties were sampled six weeks after sowing. African nightshade roots were excised, ground using a mortar and pestle and mixed with 10 ml of distilled water. 50 µl of suspension of 1:1000 dilutions each treatment was plated on a plate of potato dextrose agar (PDA) and nutrient agar (NA) for *T. asperellum* and *B. subtilis* respectively. Inoculated Petri-dishes were incubated at 25 °C for 48 hours after which the colony forming units on the media were assessed at 24 hours’ time interval for 3 days.

DATA ANALYSIS

The percentage of pre and post-emergence damping off, colony forming unit counts and fresh weight data of harvested leaves were subjected to the analysis of variance (ANOVA) using the generalized linear models (GLM) procedure of SAS (version 9.2; SAS Institute, Inc., Cary, NC). Plant growth parameters including relative growth rate (RGR), unit leaf rate (ULR), leaf area ratio (LAR), Leaf weight fraction (LWF) and specific leaf area (SLA) were estimated using the software developed by Hunt et al. (2002). All tests were performed at 5 % level of significances.

RESULTS

Effect of *Bacillus subtilis* and *Trichoderma asperellum* on *Pythium aphanidermatum*

The incidence of pre-emergence damping off disease was significantly reduced ($P \leq 0.001$) at least two times lower in seeds that were coated with the biological control agents compared to the uncoated seeds (control) in the three African nightshade varieties (Fig 1). The pre-emergence damping off disease incidence on Abuku 1 and Olevolosi was significantly

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lowered (P≤ 0.041) two times in seeds coated with *B. subtilis* and a combination of both biological controls compared to those treated with *T. asperellum* (Figure 1a,c). Single and combined application of *B. subtilis* and *T. asperellum* successfully suppressed the incidence of pre-emergence damping off. The post-emergence damping-off disease incidence was significantly reduced (P ≤ 0.024) by at least 3 folds in seeds coated with the biological control agents compared to uncoated seeds (Fig 1). Coating Abuku2 and Olevolosi Seeds with *T. asperellum* reduced post-emergence damping off 3 times lower compared to Abuku 1 *T. asperellum* coated seeds (Fig 1).

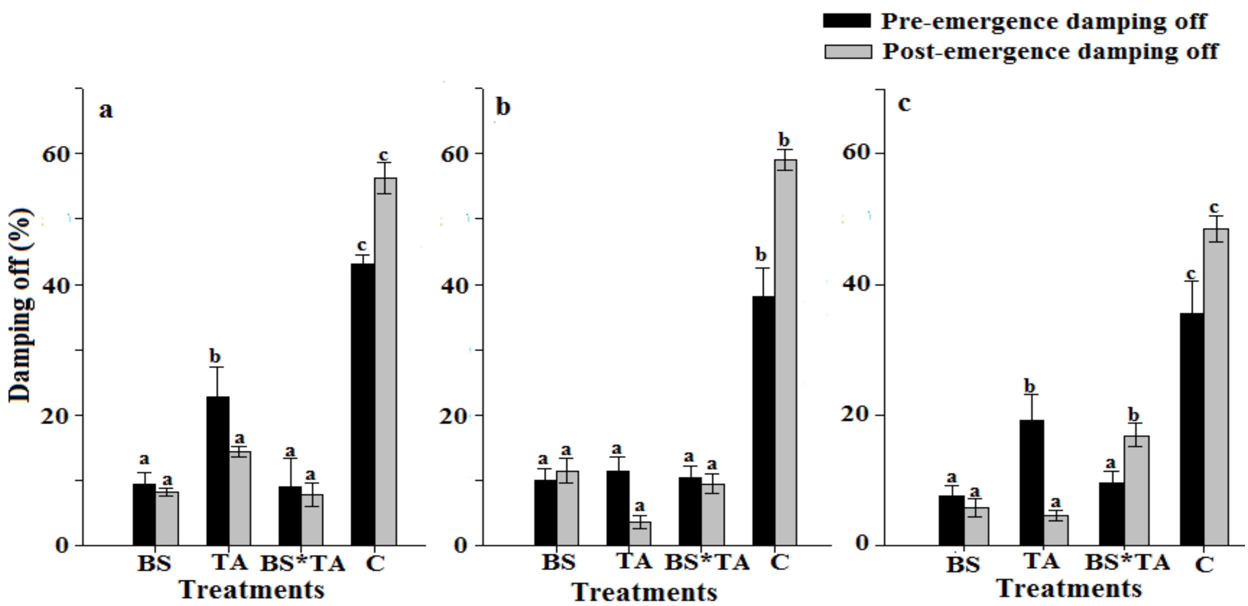

Figure 1: Damping-off disease incidence in three African nightshade varieties; Abuku 1 (a), Abuku 2 (b) and Olevolosi (c) treated with BS*TA= *Bacillus subtilis* and *Trichoderma asperellum*; BS= *Bacillus subtilis;* TA: *Trichoderma asperellum* C= Control. Vertical bars followed by the same letter are not significantly different (P <0.05).

Effect of *B. subtilis* and *T. asperellum* on growth and leaf yield of African nightshade

The RGR is an overall index of plant growth which describes the increase in dry weight per unit total weight, SLA a measures the leaf area of a plant by total dry weight and LAR described as the ratio of leaf area and total plant weight were not significantly different among the treatments. The ULR (the ratio of increase in dry weight per unit leaf area) was significantly different among the treatments with the ULR ranging between 0.01-0.06 cm^2 g^{-1} $week^{-1}$. While, the LWF, which measures the ratio of leaf area of a plant to the total dry weight of the plant, was significantly different among the treatment ranging between 0.13-0.48 (Table 1).

Application of the biological control agents in Abuku 1 and olevolosi varieties resulted to a significant increase in yields. Treatment of Abuku 1 with the biological control agents resulted to significantly higher yield up to 1.5 times compared to the control (P ≤ 0.001, Fig. 2 a). In Abuku 2 variety the increase of yield was not significantly different (P ≤ 0.365) among the treatments (Fig. 2 b). Seed coating with the biological control agents resulted to significant increase of yields (P ≤ 0.027) of up to 1.2 times higher in olevolosi variety compared to uncoated treatment (Fig. 2 c).

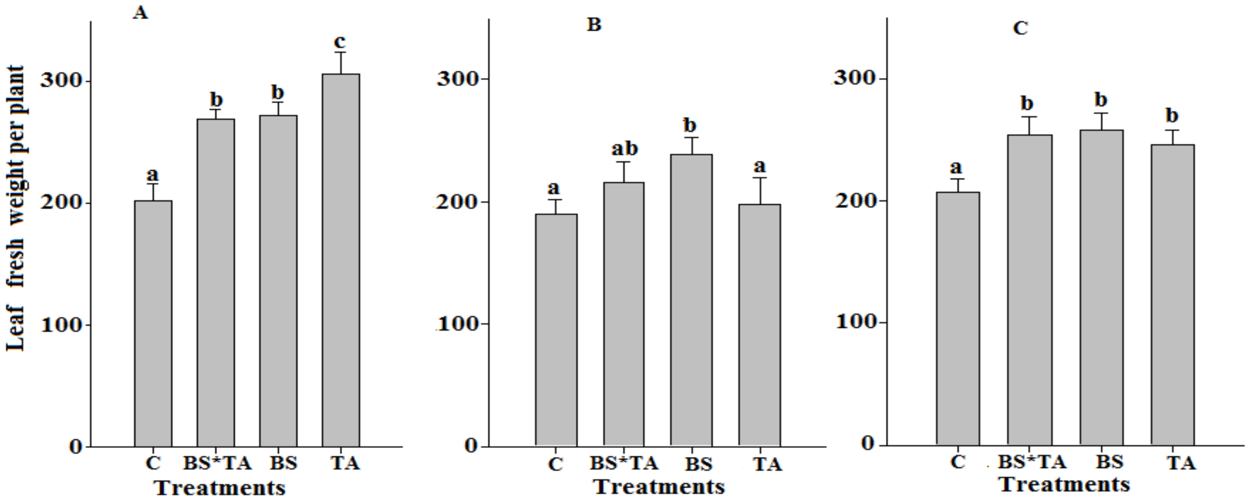

Figure 2: African nightshade varieties yield from plants inoculated with either biological control agents. BS ≤ *Bacillus subtilis,* TA ≤ *Trichoderma asperellum.* Abuku 1 (a), Abuku 2 (b) and Olevolosi (c). Vertical bars followed by the same letter are not significantly different (P <0.05).

African nightshade roots colonization

Colonization of African nightshade roots by either *B. subtilis* or *T. asperellum* in either single or combined application were significantly different from the control (P ≤ 0.001). The root colonization was significantly lower in *T. asperellum* compared to other treatments in variety Abuku 1 (P ≤ 0.042, Fig. 3 a). Root colonization in the variety Abuku 2 was significantly high in the seeds treated with *T. asperellum* in single application compared to other treatments (P ≤ 0.036, Fig. 3 b). In the variety Olevolosi, *T. asperellum and B. subtilis* in combination treatment had a significantly lower efficiency in root colonization than other biological control agent treatments (P≤ 0.035, Fig. 3 c).

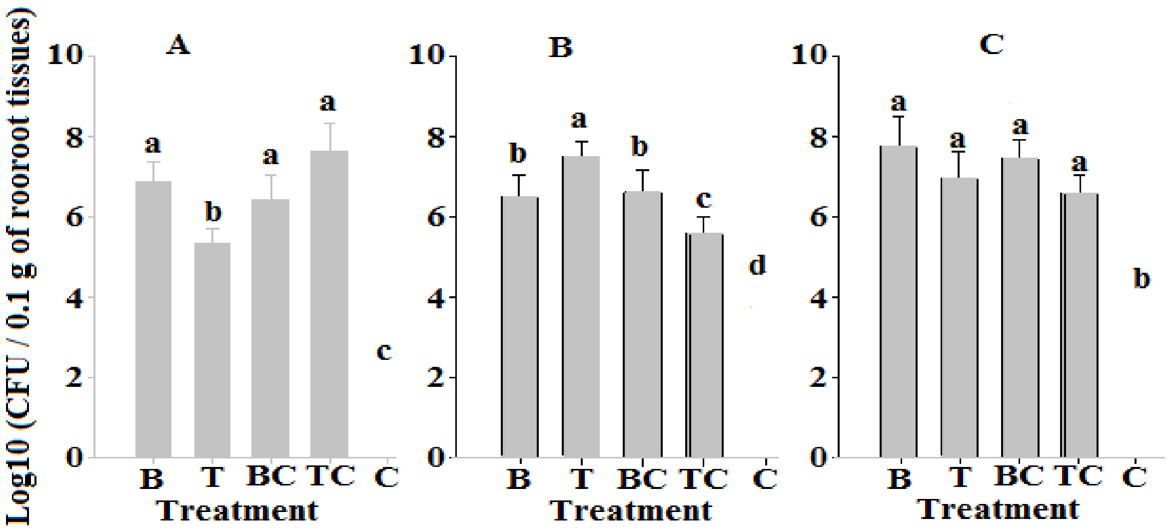

Figure 3: Root colonization of three African nightshade varieties *viz*. Abuku 1 (a), Abuku 2 (b) and Olevolosi (c) by *Bacillus subtilis* and *Trichoderma asperellum* in single or combined application at sixth week after inoculation. B≤ *Bacillus subtilis;* T≤ *Trichoderma asperellum;* BC≤ *Bacillus subtilis* in combined application TC≤ *Trichoderma asperellum* in combined application C ≤ Control. Vertical bars followed by the same letter are not significantly different (P<0.05).

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Table 1. Relative growth rate, unit leaf rate, leaf area ratio, Leaf weight fraction and specific leaf area of African nightshade varieties inoculated with either *Bacillus subtilis* or *Trichoderma asperellum* in a single and combined application. Bac≤ *Bacillus subtilis;* Trich≤ *Trichoderma asperellum;* Con: control.

Treatment	Relative growth rate (g week $^{-1}$)	Unit leaf rate (cm2 g^{-1} $week^{-1}$)	Leaf area ratio (cm^2 g^1)	Leaf weight ratio	Specific leaf area (cm^2 g^1)
BacAbuku 1	0.32 ± 0.04 (0.08)	0.02 ± 0.01 (0.02)	28.74 ± 12.69 (26.00)	0.48± 0.02 (0.03)	101.86 ± 73.71 (151.03)
BacAbuku 2	0.29 ± 0.06 (0.14)	0.04 ± 0.01 (0.03)	8.65 ± 4.84 (9.91)	0.28 ± 0.12 (0.24)	35.59 ± 23.96 (49.09)
BacOlevolosi	0.31 ± 0.07 (0.14)	0.06 ± 0.05 (0.11)	17.93 ± 12.71 (46.53)	0.45 ± 0.24 (0.49)	68.95 ± 53.19 (149.97)
TrichAbuku 1	0.33 ± 0.06 (0.13)	0.05 ± 0.04 (0.08)	19.75 ± 4.92 (71.56)	0.27 ± 0.19 (0.58)	93.6 1± 61.31 (124.63)
TrichAbuku2	0.34 ± 0.06 (0.11)	0.06 ± 0.02 (0.04)	31.04 ± 3.33 (3.79)	0.13 ± 0.13 (0.33)	202.14 ± 81.71 (67.43)
TrichOlevolosi	0.28 ± 0.06 (0.13)	0.02 ± 0.02 (0.04)	18.45 ± 9.73 (19.93)	0.19 ± 0.12 (0.25)	131.74 ± 68.66 (140.69)
TrichBac Abuku 1	0.29 ± 0.06 (0.13)	0.05 ± 0.02 (0.03)	18.73 ± 4.92 (71.56)	0.47 ± 0.02 (0.04)	66.89 ± 44.50 (214.33)
TrichBac Abuku 2	0.22 ± 0.05 (012)	0.03 ± 0.01 (0.03)	19.04 ± 12.96 (26.36)	0.36 ± 0.06 (0.03)	66.58 ± 57.74 (118.31)
TrichBacOlevol osi	0.27 ± 0.07 (0.15)	0.03 ± 0.01 (0.03)	17.21 ± 11.11 (22.77)	0.29 ± 0.12 (0.27)	92.86 ± 4.52 (11.72)
Con Abuku1	0.16 ± 0.05 (0.10)	0.01 ± 0.01 (0.02)	11.16 ± 5.24 (10.73)	0.21 ± 0.15 (0.31)	45.67 ± 31.37 (64.14)
Con Abuku2	0.23 ± 0.06 (0.13)	0.03 ± 0.01 (0.04)	9.73 ± 4.97 (10.20)	0.15 ± 0.15 (0.31)	66.97 ± 50.84 (104.18)
Con Olevolosi	0.18 ± 0.09 (0.19)	0.01 ± 0.05 (0.10)	9.39 ± 7.32 (5.01)	0.14 ± 0.10 (0.20)	61.69 ± 42.93 (87.97)

DISCUSSION

The two biological control agents, *B. subtilis*, *T. asperellum* and their combined application resulted to reduction of damping off disease incidence. This results concur with Sawant,(2014) and Kipngeno *et al,* (2015), who reported that *Bacillus spp* and *Trichoderma spp* was effective in control of various diseases including botrytis blight, damping off, powdery mildews and downy mildew in tomatoes. However, the combination application did not work to improve the efficacy as reported by Yobo *et al,* (2011) and Christy *et al,* (2012). It might be lack of organic matter in the planting media (coco peat) making the root exudates as the only source of nutrition which is not enough to support a high population density *B. subtilis* and *T. asperellum* hence lower efficacy recorded. The *B. subtilis* and *T. asperellum* can provide a potential environmental friendly control for damping off disease in African nightshade.

Seed coating with *B. subtilis* and *T. asperellum* in the single and combined application resulted to significant unit leaf rate, Leaf weight fraction in African nightshade. Previous studies acknowledge the ability of various biological control agents such as *Trichoderma, Glomus* and *Agrobacterium* to enhance dry matter accumulation in plants (Tsvetkov *et al,* 2015). However, the increase of dry matter in relation to the plant leaves has not been done. In this study the unit leaf rate which ratio of increase in dry weight per unit leaf area and the leaf weight fraction which is described as quotient of total dry leaf weight per total dry weight were significantly different among the treatments. Therefore, the application of the two biological control agents can result to high accumulation of dry matter to the leaf which is important for leafy vegetables such as African nightshade.

The ability of the two biological control agents to promote African nightshade growth through various mode action such as increased mineral uptake and production of plant growth promoters can explain the observed increase in yield in plants treated with the biological control agents. Seed coating with *Bacillus spp* or *Trichoderma spp* is suggested to enhance nutrient uptake particularly phosphates and zinc as well as production of plant growth promoters (Habibzadeh *et al,* 2012). The result from this study concur with the results of Almaghrabi *et al*, (2013) and Lo and Lin, (2002) which indicated that *Trichoderma spp* and *Bacillus cereus* promoted tomato plant growth and yield. Hence, *B. subtilis* or *T. asperellum* in single and combined application can enhance African nightshade growth and yield.

The root colonization is an important prerequisite for the effectiveness of biological control agent against pathogens and plant growth promotion (Ramarao *et al* 2011). High root colonization by *B. subtilis* and T. *asperellum* in single application was recorded among the treatments. This concur with studies conducted by Filonow and Dole, (1999) which indicated that application of single biological control agents resulted to sufficient root colonization. However, in the combination of the *B. subtilis* and T. *asperellum* the root colonization was significantly lower which contradict the previous studies by Singh *et al,* (2012) that indicated the combination of the two biological control agents was not significantly different from individual application. This can be explained by the fact that African nightshade seeds have limited carrying capacity for microorganisms on the surface. Hence, inoculated antagonists competed for the attachment sites on seed resulting to low initial inoculum population density. Therefore, the reduction of damping off incidence and improved growth and yield of African nightshade in *B. subtilis* or *T. asperellum* treatments can be attributed to the root colonization ability of the biological control agents.

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CONCLUSION

Seed coating with the *B. subtilis* and T. *asperellum* in singled and combined application reduced pre and post-emergence damping off disease caused by *P.aphanidermatum* in African nightshade although the combination did not work well *regarding* root colonization. Consequently, the application can efficiently increase growth and yield of African nightshade. Application of the *B. subtilis* and *T. asperellum* as African nightshade seed coat is efficient to provide enough inoculum for root colonization up to six weeks after sowing.

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REFERENCE

[1] Abd-El-Khair, H., Khalifa, R.K., & Haggag, H. E. (2002). Effect of Trichoderma species on damping off diseases incidence, some plant enzymes activity and nutritional status of bean plants. *Journal of American Science, 7*, 156–167.

[2] Almaghrabi, O.A., Massoud, S.I., & Abdelmoneim, T. S. (2013). Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. *Saudi Journal of Biological Sciences, 20*, 57–61.

[3] Azarmi, R., Hajieghrari, B., & Giglou, A. (2011). Effect of Trichoderma isolates on tomato seedling growth response and nutrient uptake. *African Journal of Biotechnology, 10*, 5846–5855.

[4] Çakmakçi R, & Dönmez, M. F. (2007). The effect of plant growth promoting rhizobacteria on barley seedling growth, nutrient uptake, some soil properties, and bacterial counts. *Turkish Journal of Agriculture and Forestry, 31*, 189–199.

[5] Christy, J. E., Tharmila, S., & Niranjan, K. (2012). Antagonistic activity of Trichoderma spp. and Bacillus spp. against Pythium aphanidermatum isolated from tomato damping off. *Scholars Research Library Archives of Applied Science Research, 4*, 1623–1627.

[6] Filonow, A.B., & Dole, J.M. (1999). Biological control of pythium damping-off and root rot of greenhouse-grown geraniums and poinsettias. *Procedings of Oklahoma Academy of Science, 79*, 29–32.

[7] Grubben, G., Klaver, W., & Womdim, R. (2014). Vegetables to combat the hidden hunger in Africa. *Chronica Horticulturae, 54*, 24–32.

[8] Habibzadeh, F. S., Ali, P., Hemmatollah, S., & Seyyed, A. M. (2012). A comparison between foliar application and seed inoculation of biofertilizers on canola (Brassica napus L.) grown under waterlogged conditions. *Australian Journal of Crop Science, 6*, 1435–1440.

[9] Jiskani, M.M., Pathan, M.A., Wagan, K.H., & Abro, H. (2007). Studies on the control of tomato damping-off disease caused by Rhizoctonia Solani Kuhn. *Pakistan Journal*

of Botechnology, 39, 2749–2754.

[10] Kipngeno, P., Losenge, T., Maina, N., Kahangi, E., & Juma, P. (2015). Efficacy of Bacillus subtilis and Trichoderma asperellum against Pythium aphanidermatum in tomatoes. *Biological Control, 90*, 92–95.

[11] Lo, C., & Lin, C. (2002). Screening strains of trichoderma spp for plant growth enhancement in Taiwan. *Plant Pathology, 22*, 215–220.

[12] Loliama, B., Morinagab, T., & Chaiyanana, S. (2013). Biological control agent of Pythium aphanidermatum by the cellulolytic actinomycetes Streptomyces rubrolavendulae S4. *Science Asia, 39*, 584–590.

[13] Mansoori, M., Heydari, A., Hassanzadeh, N., Rezaee, S., & Naraghi, L. (2013). Evaluation of pseudomonas and bacillus bacterial antagonists for biological control of cotton verticillium wilt disease. *Journal of Plant Protection Research, 53*, 11–14.

[14] Mbugua, G., Muriuki, A., Anyango, J., Ndungu, B., Gathambiri, C., & Manyeki, L. (2008). Enhancing production of African Nightshade (Solanum scabrum), Spider Plant (Cleome gynandra) and Amaranthus (Amaranthus cruentus) amongst small-scale farmers in Maragua District, Central Kenya. *KARI 11TH Biennial Scientific Conference*, 1–13.

[15] Ondieki, M. J., Aguyoh, J. N., & Opiyo, A.M. (2011). Fortified compost manure improves yield and growth of African nightshades. *International Journal of Science and Nature, 2*, 231–237.

[16] Ramarao, N., Lereclus, D., Sorokin, A, & Oh-Mi, H. (2011). Effect of Trichoderma isolates on tomato seedling growth response and nutrient uptake. *African Journal of Biotechnology, 10*, 5850–5855.

[17] Sallam, N.M.A., Abo-Elyousr, K.A.M., & Hassan, M.A.E. (2008). Evaluation of Trichoderma species as biological control agent agents for damping-off and wilt diseases of phaseolus vulgaris L. and efficacy of suggested formula. *Journal of Phytopathol, 36*, 81–93.

[18] Sawant, I. (2014). Trichoderma foliar pathogen interactions. *Open Mycology Journal, 8*, 58–70.

[19] Singh, P., & Nautiyal, C. S. (2012). A novel method to prepare concentrated conidial biomass formulation of Trichoderma harzianum for seed application. *Journal of Applied Microbiology, 113*, 1442–1450.

[20] Singh, V., Mawar, R., & Lodha, S. (2012). Combined effects of biological control agent agents and soil amendments on soil microbial populations, plant growth and incidence of charcoal rot of cowpea and wilt of cumin. *Phytopathologia Mediterranea, 51*, 307–316.

[21] Subash, N., Meenakshisundaram, M., Unnamalai, N., & Sasikumar, C. (2013). Effect of Trichoderma harizanum to control damping off disease and growth promotion in chilli (Capsicum annuum). *International Journal of Pharma and Bio Sciences, 4*, 1076–1082.

[22] Yobo, K. S., Laing, M. D., & Hunter, C. H. (2011). Effects of single and combined inoculations of selected Trichoderma and Bacillus isolates on growth of dry bean and biological control of Rhizoctonia solani damping-off. *African Journal of Biotechnology, 10*, 8746–8756.

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