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Development of a Rapid Bio-Test to Study the Activity Potential of Biofertilizers

Entwicklung eines schnellen Bio-Tests zur Untersuchung des Wirkungspotentials von mikrobiellen Pflanzenstärkungsmitteln

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Schlagwörter: Entwicklung Ökolandbau, Bodenfruchtbarkeit, Bodenbearbeitung, Pflanzenernährung, Pflanzenstärkungsmittel, *Trichoderma*

Abstract:
Plant-growth-promoting soil microorganisms are increasingly distributed on the world market. Nutrient mobilization, stimulation of root growth, enhanced resistance to environmental stress factors are discussed as possible mechanisms. These assumptions are based only on scarce scientific evidence due to limited reproducibility of pot and field experiments, limited information concerning the conditions for successful application, limited standardization of inoculum preparation and quality. Thus, the development of rapid screening tests is to demonstrate the principle effectiveness of biofertilizers prior to set-up of labourous pot or field experiments is urgently required.
*In this study, a rapid bio-test with cucumber (*Cucumis sativa *L.) as an indicator plant was developed to evaluate the effectiveness of five commercial biofertilizers based on* Trichoderma spp. *and* Bacillus spp. *(Biohealth-G, Biohealth-WSG, Biomex, Vitalin T50 and SP11) using germination rate, root and shoot biomass, maximum root length, and leaf area as test parameters. The experiment was repeated twice with 6 replicates in hydroponic culture under controlled conditions (pH 5.5, 22° C; Light: 230 mmol cm^{-2} sec^{-1}). Biofertilizers were applied at the rate of 3 g per 2.5 l mineral nutrient solution. Germination rate was increased by 20 - 25% in all biofertilizer treatments compared to the control. After 2 weeks culture period, root dry weight and leaf area of Biohealth-G, Vitalin T50, SP-11 and Biomex-treated cucumber seedlings were significantly increased. Biohealth-G and Vitalin T50 showed significantly higher main root length and Biohealth-G higher shoot dry weight than the remaining treatments, while Biohealth-WSG did not cause differences compared to untreated control plants. The pathogen-antagonistic potential of Trichoderma strains can be easily tested by co-inoculation with the pathogenic fungus Gaeumannomyces graminis on malt extract peptone agar plates. The results suggest that the activity potential of different Trichoderma-based biofertilizers could be easily screened by using the described bio-test with cucumber seedlings.*

Introduction and Objectives:
Bio-effectors based on plant-growth-promoting soil microorganisms are increasingly distributed on the world market. Mobilization of sparingly available plant mineral nutrients, stimulation of root growth, enhanced resistance to environmental stress factors and direct or indirect suppression of plant pathogens and induced resistance are discussed as possible mechanisms for the effectiveness of these products. However, these assumptions are based only on scarce scientific evidence which is further abused by a lack of standards for production and quality control. Therefore, rapid

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screening tests to evaluate the potential effectiveness of a given product, prior to more detailed and labouous investigations are urgently needed.
In this study test plants with bioindicator potential for toxins and plant growth regulators (*Lepidium* and Cucumber) were investigated for responses to biofertilizer treatments under controlled conditions.

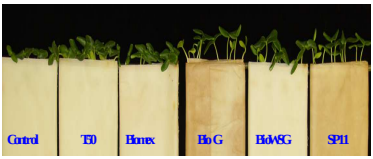

Fig. 1: Seed germination in filter paper.


Fig. 2: Seedling establishment test.

Methods:
(1) Germination test:
Seed surface sterilization: 3 min in 30 % H_2O_2, 2 min in 70% ethanol, Two times washing with sterile distilled H_2O. Seeds of *Cucumis sativa* cv. Vorgbirgstrauben and *Lepidium* were sown in rolls with 4 layers of moist filter paper (MN 710, Machery and Nagel, Dueren, Germany). The filter rolls were soaked with 2.5 mM $CaSO_4$ containing 1% (w/v) of different commercial bio-fertilizers. Different biofertilizers, such as T-50 and Sp-11 were collected from Vitalin Pflanzengesundheit GmbH, Ramstadt, Germany; BioHealth-WSG and BioHealth-G from Humintech GmbH, Düsseldorf, Germany and Biomex from Omex Agriculture Inc., Manitoba, Canada. Rolls with each 15 seeds were placed in upright position into a closed plastic box and incubated in the dark for 4 d at 22°C with 6 replicates per treatment (Fig. 1).
(2) Seedling establishment test:
Seedlings, germinated for 4 days in filter rolls (see (1) were subsequently grown for 7 days under controlled conditions (22°C; 230 mmol light cm^2 sec^{-1}) in 2.5 L aerated nutrient solution (mM: 2 $Ca(NO_3)_2$; 0.7 K_2SO_4, 0.5 $MgSO_4$, 0.25 KH_2PO_4, 0.1 KCl; µM: 10 H_3BO_4, 0.5 $MnSO_4$, 0.5 $ZnSO_4$, 0.2 $CuSO_4$, 0.01 $(NH_4)_6Mo_7O_{24}$, 20 Fe-EDTA; pH 5.5) supplied with biofertilizers 1.5 - 3g pot^{-1}. Each pot contained 10 seedlings in 6 replications (Fig. 2).

Results and Discussion:
Most consistent responses were obtained with cucumber (cv. Vorgebirgstrauben).

Germination test: biofertilizers increased germination rate by 20-25 % at 4 days after sowing (DAS) (Fig. 4A). Seedling emergence and plant development was accelerated by approximately 2 days after inoculation with the biofertilizers.
Similar responses have been reported by other authors (HARMAN et al. 2004, OZBAY et al. 2004, ARORA et al. 1992) and enhanced seed germination induced by seed inoculation with *Trichoderma* spp. has been related to the production of growth factors such as auxin, cytokinin or gibberelic acid (GA3) and even ethylene.

Seedling establishment test in hydroponics: Biofertilizers increased dry matter of roots (50-90%) and shoots (30-80%) and particularly leaf area (70-100%) during early seedling growth (7d culture period in nutrient solution, Fig. 4B and C). A differential effect on root morphology was observed in hydroponic culture. Maximum root length

increased by 30% in T50 and BioHealth-G (Fig. 4D), while T50 stimulated particularly lateral root formation (Fig. 3). The increase in shoot growth and leaf area in *Trichoderma* treated seedlings suggests a common beneficial role of *Trichoderma harzianum* in improving plant growth (Yedidia et al., 2001). The mechanisms involved in increasing growth responses induced by *Trichoderma* sp might be the production of growth-stimulating compounds (GRAVEL et al. 2006, HARMAN et al. 2004, YEDIDIA et al. 2001, ALTOMARE et al. 1999, CHANG et al. 1986).

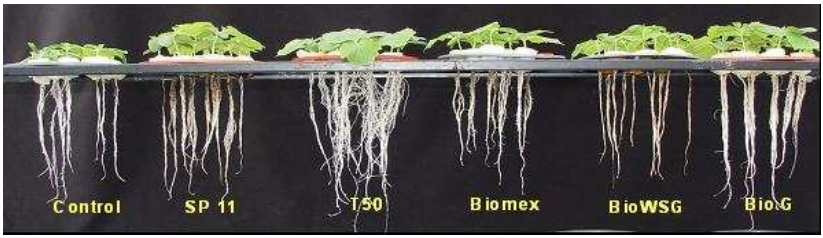

Fig. 3: Shoot and root development of cucumber in hydroponics with and without *Trichoderma* treatments

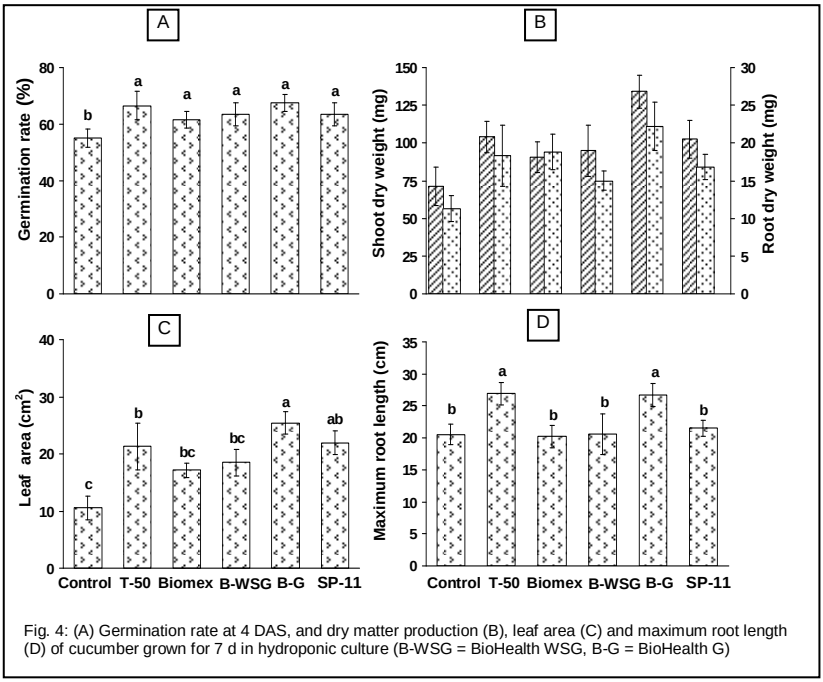

Fig. 4: (A) Germination rate at 4 DAS, and dry matter production (B), leaf area (C) and maximum root length (D) of cucumber grown for 7 d in hydroponic culture (B-WSG = BioHealth WSG, B-G = BioHealth G)

Conclusions:

Cucumber (cv. Vorgebirgstrauben) is a suitable test plant to demonstrate effects of variouscommercial *Trichoderma*-based biofertilizers on germination, plant development, and root growth within culture periods of 4 – 12 days in simple culture systems (filter paper germination test and hydroponic culture).

Best results were obtained by using seeds with sub-optimal germination rates (approx. 50%), For high quality seeds, this is easily achieved by artificial seed aging treatments: e.g. 1-2 days incubation enclosed in plastic bags at 40-45℃ in a water bath (data not shown).

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