Effect of a *Bacillus subtilis* Isolate on Southern Blight (*Sclerotium rolfsii*) and Lipid Composition of Peanut Seeds

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**INTRODUCTION**

Southern blight caused by *Sclerotium rolfsii* Sacc. is a severe seed- and soilborne disease of peanuts in Egypt (45) and other countries (34,37), especially during the warm season. The pathogen attacks the roots, collar, stems, gynophores and pods of peanut plants (34). Alteration in lipid metabolism during infection by fungal pathogens can provide a sensitive monitor of the physiology of host-pathogen interactions (15,28,29,44), but such comparisons have not been extended to biocontrol of these fungal pathogens. Biocontrol of *S. rolfsii* was achieved by using preparations of *Bacillus subtilis* (4,33). The current recommendation suggests an integrated approach that combines the induction of systemic resistance with production of antifungal compounds by *B. subtilis* throughout biocontrol of fungal pathogens, especially *via* lipid metabolism (14) of the host plant.

The present study was carried out to obtain information regarding the enhanced effect of *B. subtilis* on seed quality as well as the lipid profile of peanuts throughout biocontrol of *S. rolfsii*.

**MATERIALS AND METHODS**

*Microorganisms, host plant and experimental soil* A strain of *S. rolfsii* was isolated from a peanut plant (cv. ‘Giza 6’) grown in Kassasien, Ismailia, Egypt, showing the symptoms of blight disease. The pathogenicity of the isolate against peanut was investigated according to Zayed *et al.* (45). The biocontrol agent *B. subtilis* was previously isolated from the rhizosphere of tomato seedlings (cv. ‘Peto 86’) grown in Sharkia, Egypt. The *in vitro* antagonistic potential of *B. subtilis* was considered in a previous study (3). The peanut seeds (*Arachis hypogaea*) used were cv. ‘Shulamit’, Hazera Co., Israel, provided by the Blue Seed Co., Nasr City, Egypt. The soil used in this study was a loamy sand with the following properties (%): moisture content, 22.57; total soluble salts, 0.27; organic carbon, 0.74; total carbonates, 4.20; total nitrogen, 0.034; and pH 7.5.
Preparation of inocula

*Sclerotium rolfsii* Based on a preliminary experiment (data not shown), the soil was artificially inoculated with sclerotia of *S. rolfsii* [collected from 10-day-old potato dextrose agar media (PDA, Difco Laboratories, Detroit, MI, USA)] with a final sclerotia concentration of 0.1 g kg\(^{-1}\) soil (36). Non-inoculated soil was used as a control.

*Bacillus subtilis* Talc was chosen as a carrier for *B. subtilis* (5). Its pH was adjusted to 7.0 using calcium carbonate. Carboxy methyl cellulose (10 g kg\(^{-1}\) carrier) was used as adhesive. *B. subtilis* was grown in King’s broth medium (22) for 72 h on a rotatory shaker (200 rpm) at 28°C to obtain \(9 \times 10^8\) cfu ml\(^{-1}\). The bacterial suspension was mixed with the sterile carrier (400 ml kg\(^{-1}\)) and air-dried (43). Peanut seeds were coated with thin film of 1.0\% (w/v) carboxy methyl cellulose and mixed with the respective formulation at 4 g kg\(^{-1}\) seed.

Greenhouse application Seeds of peanut for each treatment were sown in the experimental soil (plastic boxes, 10X10X5 cm) and maintained in a greenhouse at the Kassasien Agriculture Research Station at 24–30°C. Disease incidence was recorded as the percent of stand plants showing no symptoms suggestive of southern blight disease at 75 days after sowing. The experiments were repeated three times.

Seed analysis At harvest time, the pods were removed, shaken to remove the attached soil, counted per plant and the fresh weight expressed as g per pod. A fresh peanut seed sample of each treatment was used for lipid extraction. Lipid contents were extracted using chloroform : methanol (2:1, v/v), with 0.05% (w/v) of butylated hydroxytoluene (BHT, 2,6-di-tert-butyl-p-cresol) added to all solvents to prevent lipid peroxidation (9). Total lipid contents were expressed as mg per g fresh weight. Acid number, peroxide value, iodine value, refraction index and unsaponified value were determined according to the methods of Kates (19). Fatty acid methyl esters were prepared by methanolation in \(\text{H}_2\text{SO}_4\)-MeOH (19). Esters were analyzed by gas liquid chromatography (GLC; Perkin-Elmer Model 910, Perkin Elmer, Shelton, CT, USA) equipped with a flame ionization detector (18). A dual-open recorder and a computing integrator (Perkin-Elmer Model M1) were attached to GLC for recording. The separation and quantitation of peak fatty acid methyl esters were identified by comparing their retention times with those of an authentic methyl ester standard (Sigma Co., St. Louis, MO, USA).

Statistical analysis The experiment was repeated three times and the treatment means were compared using Least Significant Difference (LSD) analysis (10).

**RESULTS AND DISCUSSION**

The *B. subtilis* preparation was highly effective in controlling the disease, achieving up to 92\% reduction in disease incidence (data not shown). The efficiency of formulated *B. subtilis* for control of *S. rolfsii* under greenhouse conditions has been demonstrated by other investigators in various crops such as cotton (32), grapevines (21), peanut (3), soybean (25), sugarbeet (37), tomato (4), and wheat (24). In the present study, the incidence of southern blight of peanut caused a significant decrease in the number of pods per plant, fresh weight of pod and number of pegs per pod (Table 1) by 19.4\%, 51.1\% and 65.7\%, respectively, compared with the healthy control. The inhibitory effect of *S. rolfsii* on yield and growth parameters of peanut pods agrees with the results of Zayed *et al.* (45). Such