التاثير المضاد للأنواع البكتيرية *Bradyrhizobium spp* , *Pseudomonas spp* , and *Bacillus subtilis* ضد الفطر الممرض *Rhizoctonia solani* على نبات الفول السوداني

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الملخص:

الكائنات الحية الدقيقة مثل الفطريات والبكتيريا والفيروسات والديدان الخيطية هي جزء لا يتجزأ من النظم البيئية الزراعية. بعضها ضار بمسببات الأمراض النباتية، في حين أن البعض الآخر محايد أو مفيد في تأثيره على نمو النبات. إن الخسائر الزراعية الكلية للمحاصيل الاقتصادية والتي تصل إلى حوالي 50-75٪ ناتجة عن الفطريات الممرضة التي تنتقل عن طريق التربة من نوع*.Rhizoctonia spp* ، *Fusarium spp.*، *Verticillium spp.*، *Sclerotinia spp.*، Pythium *spp.* و *Phytophthora spp.* تعود الخسائر إلى تعفن البذور وتعفن الجذور وأمراض الذبول في حقول المحاصيل المختلفة والصوبات الزراعية

تهدف الدراسة الحالية إلى تقييم التأثير المضاد للبكتيريا المدروسة مثل *Pseudomonas fluorescens* و *Pseudomonas chlororaphis* و *Bacillus subtilis* وتم اختبارها ضد ثلاث عزلات مختبرة من*.R. solani* أجريت اختبارات المكافحة الحيوية باستخدام عوامل بيولوجية فطرية وبكتيرية. كانت العوامل الحيوية الفطرية المختبرة ثلاث عزلات من .Trichoderma viride جمع المزرعة، تم الحصول مسبقًا على ثلاث عزلات لـ *Rhizoctonia spp* من نباتات الفول السوداني ذات الجذور المريضة. يشمل المضاد البكتيري عزلة واحدة من *.B subtilis* وعزلة واحدة من.Pseudomonas fluorescens . وأوضحت النتائج أن Bacillus subtilis كان لها أعلى تأثير قمعي (100٪) مع العزلات المختبرة من *Rhizoctonia solani* مقارنة بالعزلات *Pseudomonas fluorescens* و *P. chlororaphis*.

كان لبكتيريا *Pseudomonas fluorescens* أقل تأثير جرثومي في تثبيط نمو الفطريات المختبرة(16.7 ٪R. solani 5 (5.6 ،٪R. solani 3 (0.0 ،٪R. solani 12 .(بذور الفول السوداني المنبتة بالمعاملة بعزلات Bradyrhizobium أي 208، 209، 210 ومخلوطة برادير هيزوبيوم، برادير هيزوبيوم + ترايكوديرما هارزيانوم، ترايكوديرما وحدها، برادير هيزوبيوم + سيودوموناس فلورية أظهرت النتائج التالية: عزلة برادير هيزوبيوم (208) أعطت أفضل البذرة (208) 4.1667 سم). إنبات بذور الفول السوداني المعتدل مع؛ *Trichoderma harzianum* و *Bradyrhizobium* و*Bradyrhizobium + P.* فلورية أعطت (2.73، 2.40، 2.33 سم من طول الجذور). وسجلت أقل نسبة إنبات لبذور الفول السوداني في المعاملة (المقارنة) (1.43). تأثير مثبط معنوي على النمو الشعاعي لعزلات R. solani الثلاثة المختبرة بسبب عزلات *Trichoderma viride*. علاوة على ذلك، أظهرت عزلة *T. viride* 1 أعلى تأثير مثبط مع عزلات *R. solani* المختبرة (100٪) مع عزلات *R. solani* 5 و12 و*R. solani* 3 (88.9 ٪).

الكلمات المفتاحية : *P. fluorescens - P. chlororaphis - B. subtilis* - antagonism - *R. solani*

Antibacterial effect of *Bradyrhizobium spp., Pseudomonas spp., and Bacillus subtilis* against pathogenic fungus *Rhizoctonia solani* on peanut plant

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Abstract

Background: Microorganisms such as fungi, bacteria, viruses, and nematodes are integral parts of agro-ecosystems. Some of them are harmful to plant pathogens, whereas, others are neutral or beneficial in their effects on plant growth. The total agricultural losses of economic crops which amount to about 50–75% are caused by soil-borne pathogenic fungi of *Rhizoctonia spp., Fusarium spp., Verticillium spp., Sclerotinia spp., Pythium spp*. And *Phytophthora spp.* The losses are due to seed rot, root rot, and wilt diseases in different crop fields and greenhouses

Aimes: the present study aimed to evaluate the antagonistic effect of the studied bacteria i.e., *Pseudomonas fluorescens*, *Pseudomonas chlororaphis,* and *Bacillus subtilis* were tested against three tested isolates of *R. solani*
Methods: The biocontrol tests were carried out using fungal and bacterial bioagents. The tested fungal bioagents were three isolates *of Trichoderma viride*. culture collection, three isolates for *Rhizoctonia* spp were Previously obtained from peanut plants with diseased roots. The bacterial antagonistic include one isolate of *Bacillus subtilis* and one isolate of *Pseudomonas fluorescens*.
Results: Showed that *Bacillus subtilis* had the highest suppression effect (100%) with the three tested isolates of *Rhizoctonia solani* compared to *Pseudomonas fluorescens* and *P. chlororaphis*.

Pseudomonas fluorescens had the lowest bacterial in its suppression effect on the tested fungal growth *R. solani* 5 (16.7%), *R. solani* 3 (5.6%), and *R. solani* 12 (0.0%). Peanut seeds germinated with treated by *Bradyrhizobium* isolates i.e., 208, 209, 210and *Bradyrhizobium* mixed, *Bradyrhizobium* + *Trichoderma harzianum*, *Trichoderma* alone, *Bradyrhizobium* + *pseudomonas fluorescence* results showed that followings: The isolate *Bradyrhizobium* (208) gave the best germination percentage of peanut seeds (4.1667 cm length). The moderate germination of peanut seeds with the; *Trichoderma harzianum*, mixed *Bradyrhizobium*, and *Bradyrhizobium* + *P. fluorescences* had given (2.73, 2.40, and 2.33 cm of roots length). The lowest germination of peanut seeds was obtained in the (control) treatment (1.43). A significant inhibitory effect on radial growth of the three tested *R. solani* isolates due to *Trichoderma viride* isolates. Moreover, *Trichoderma viride* isolate 1 showed the highest inhibitory effect with three tested *R. solani*, (100%) with *R. solani* isolates 5 and 12, and *R. solani* isolates 3 (88.9%).
Key words: *P. fluorescens- P. chlororaphis - B. subtilis – antagonism - R. solani*

Introduction

Biocontrol of plant pathogens involves using biological processes to reduce the inoculum density of pathogens and maintain their soil population below the disease threshold level. The global trend appears to be shifting towards reduced use of fungicides on produce and hence, there is a strong public and

scientific desire to seek safer and eco-friendly alternatives for reducing the decay loss in the harvested commodities. Among different biological approaches, use of the microbial antagonists like yeasts, fungi, and bacteria is quite promising and gaining popularity (1,2,3).

The organic amendment of cornmeal improved colonization for a long time. It was an effective biocontrol agent of *T. harzianum* to suppress the growth and pathogenicity of *R. solani* inciting root and hypocotyl diseases of beans and increasing vegetative and dry weights of the bean shoot system (4).

Caviedes *et al.*, 2021 concluded that Streptomyces M2A2 disease was selected and demonstrated in vitro and in vivo biocontrol efficacy against *R. solani* causal agent of rice sheath blight. In addition to inhibiting the growth of *R. solani*, Streptomyces spp. M2A2 delayed the onset of symptoms and affected the progress of the pathogen in susceptible plants of the cultivar Fedearroz 68 without differences with the difenoconazole treatment and producing better results than *Trichoderma spp*. M2H1 isolate. The results highlight the possibilities for using Streptomyces spp. in *R. solani* management (5).

Microorganisms such as fungi, bacteria, viruses, and nematodes are integral parts of agro-ecosystems. Some of them are harmful to plant pathogens, whereas, others are neutral or beneficial in their effects on plant growth. Control of disease-causing organisms is essential in every crop production system (6).

Numerous soil microorganisms are reported to be antagonistic to plant pathogens few are available as commercial products (7). Some species of fungi can secrete substances or metabolites that have very specialized activity, being lethal to a particular group of life forms **(8).** However, Soil-borne plant pathogens affecting agricultural plants can be controlled by the use of species of *Trichoderma*, *Bacillus subtilis,* and *Pseudomonas fluorescence* (9,10).

The anamorphic fungal genus *Trichoderma* Pers. (*Hypocreales, Ascomycota*) contains cosmopolitan soil-borne species which also are frequently found on decaying wood, of which some are economically important producers of industrial enzymes and antibiotics, or are applied as biocontrol agents of plant pathogens (11).

Review of previous studies

1- *Trichoderma*

Antagonists belonging to the genus *Trichoderma* are among the most commonly isolated soil fungi. Due to their ability to protect plants and contain pathogen populations under different soil conditions, these fungi have been widely studied and commercially marketed as biopesticides, biofertilizers, and soil amendments. *Trichoderma* spp. also produces numerous biologically active compounds, including cell wall degrading enzymes, and secondary metabolites (12).

Seed treatment with *Trichoderma viride* eliminated seed-borne infection of pigeon pea by *A.alternata* (Fr.) Keissler, *Rhizoctonia bataticola* (Taub.)Butler. *Rhizoctonia solani* Khunand *Curvularia lunata* (Wakker) Boed with a significant increase in seed germination, vigor I, index, and fresh weight of seedling over untreated control (13).

Trichoderma is biotrophic mycoparasite, the primary antagonistic response between *Trichoderma* and the phytopathogen involves growth towards the susceptible hyphae, probably by positive chemotropism. Once *Trichoderma* detects its host, its hyphae develop a profuse branching by which the antagonist gets contwitht on it. Once the mycoparasite reaches the host its hyphae often roll or grow up along the pathogen mycelium (14).

Isolates of *T.harzianum* can produce lytic enzymes and (15), antifungal antibiotics (16,17), they can also be competitors of fungal pathogens (18), and promote plant growth (19). However, Dunlop *et al.,* (1989) reported that the production of metabolites from different *Trichoderma* strains depends on ecological factors, and so the strains show varying effects on pathogens (20). *In vitro* studies show that *T. harzianum* was the most effective bioagent in reducing the growth of *Phytophthora infestans* (21), *R. Batticaloa* (22), *R. solani* and *Pythium ultimum* (23), and *Fusarium oxysporum* f. sp. *lycopersici* (24)

Trichoderma, *Aspergillus*, vesicular-arbuscular mycorrhizas (VAM), and *Penicillium* were found effective to control Fusarium wilts. Among these biocontrol agents, *Trichoderma* produced the highest control of the pathogen and the highest host growth and yield (25). Abdel–Kader and Elmougy (2002) study the activity of *T. harzianum* (T1&T3) and *T. viride* (T2) against the

growth of faba bean root rot pathogens *in vitro* as well as for controlling disease incidence in greenhouse and field conditions than Rizolex-T treatment. They found that application of *Trichoderma* spp. as soil drenchresultedd in a more efficient reduction in root rot incidence than treatment with *Trichoderma* and fungicide as seed coating at both pre and post-emergence stages (26).

2- *Bacillus subtilis:*

Bacillus subtilis are stthe able in soil as spores. This is advantageous for using this bacterium as a biocontrol agent mainly because of the spore's stability and ease of handling (27). Asaka & Shoda, 1996found that treatment with the culture broth, cell suspension, or centrifuged culture broth will be effective as a biological control against several phytopathogens(28).

Bacillus subtilis produce a wide variety of antibacterial and antifungal antibiotics (29,30). The antifungal secondary includes chitinases and other cell wall-degrading enzymes, volatiles, and compounds that elicit plant resistance mechanisms. Lin *et al.*, 2011suggested that the *B. subtilis* isolate BS-99 is a potential spoof for an effective commercial biofungicide for the management of wax apple disease in Taiwan (31).

Biocontrol of damping-off diseases has been successfully applied using *B. subtilis* (32). *B. subtilis* used to be a bioagent against *Alternaria* spp., *A. flavus, A. niger, Cercospora nicotiana, Colletotrichum gloeosporioides, F. oxysporum, Helminthosporium* spp., *R. solani, Penicillium chrysogenum, Phytophthora parasitica, Pythium aphanidermatum* and *Verticillium dahliae* (33,34,35).

Heidi, 2006 studied two bacterial strains of *B. subtilis* on the mycelial growth of five isolates of *Pythium ultimum in vitro*. She also reported that the usage of *B. subtilis* as seed treatment reduced the percentage of damping-off incidence of sugar beet under greenhouse conditions (36). Hussein *et al.,* 2007 (37) mentioned *B. subtilis* as a biocontrol agent against Stemphylium blight caused by *Stemphylivesicationium* in onion plantKelaniani *et al.,* 2011evaluated *B. subtilis* against cowpea fungal pathogens in the laboratory. The antibiosis exhibited by *B. subtilis* against *F. verticilloides, F. eqexquisite*and *R. solani* was highly significant. However, there were little or no inhibition effects on *F. solani*, and *F. oxysporum* (38).

Karimi *et al.*, 2012 evaluated the efficacy of *Pseudomonas* spp. and *B. subtilis* strains against *Fusarium oxysporum* f.sp. *ciceris in vitro* and *in vivo*. Some bacterial isolates showed high inhibition activity on the pathogen. The ability of bacterial isolates was variety d in the production of cyanide hydrogen, siderophore, protease, and indole acetic acid (IAA). The growth parameters were significantly increased by B28, P12, and P112 isolates in seed treatment and soil-inoculation compared to the untreated control. Results indicated that Plant Growth Promoting Rhizobacteria (PGPR) improve growth parameters in this plant and can help in the biocontrol of pathogen (39).

Music Music & Quimio, 2006 reported that the Formulated *B. subtilis* BR23 used as seed treatment had no detrimental effects on corn seed germination and seedling vigor. Seed treatment with the same formulation suppressed *R. solmicroplates*roplots and increased grain yield by 27% compared to that of the control captan. Seed treatment with 14.4%. *B. subtilis* BR23 h a potential for commercialization as a seed treatment for the control of banded leaf and sheath blight disease (*R. solani*) in corn (40).

3-*P. Pseudomonas*

Fluorescent *Pseudomonas* has great importance in nature. *Pseudomonas* sp. is ubiquitous in agricultural soils, well adapted to growing in the rhizosphere. *Pseudomonas* possesses many traits that make them well suited as biocontrol angrowthrompromotingnts (41,42). The several strains of *P. fluorescens* degrade xenobiotics and can survive in harsher environmental conditions. Notably, *P. entomophila* could secrete many degradative enzymes (proteases and lipases), putative toxins, and secondary metabolites (43).

In recent years, the genus *Pseudomonas* has drawn attention worldwide because o the opposition of secondary metabolites such as siderophore (44), volatile compounds (45), hydrogen cyanide (HCN) (46), enzymes such as Chitinase (47,) and β-1, 3-glucanase (48), phytohormones and antibiotics such as 2, 4-diacetylphloroglucinol (49). Fluorescent *Pseudomonas* isolates PGC1 and PGC2 were checked for the antifungal potential against *R.solani* and *Phytophthora capsici*. Both the iswe havehaveduced range an of antifungal compounds. They also indicated the role of chitinase and β-1, 3-

glucanase in the inhibition of *R. solani*; however, antifungal metabolites of a non-enzymatic nature were responsible for the thee inhibition. *capsici* (50).

Van Peer *et al.,* 1991reported that signals provided by *Pseudomonas* strain WCS417r at the root system, induce the stem sensitization of defense responses against *Fusarium oxysporum* f. sp. *dianthus*, such as synthesis and accumulation of phytoalexins (51).

Aishah *et al.,* 2011 reported that *Pseudomonas* sp. is the most extensively studied plant growth-promoting rhizobacteria (PGPR) and is known to protect the plant from many deleterious soils and foliar plant pathogenic microorganisms. The fresh culture and formulations (Talc and Sodium alginate) of *P. fluorescens* isolate 2 (Pf2), were found to be effective in reducing the tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* under greenhouse conditions when applied as a seed treatment. They also found that fresh cultures of Pf2 isolate increase seedling emergence, reduce Fusarium wilt disease incidence, and increased plant growth (plant height, fresh weight, and dry weight) under greenhouse conditions when compared to the control and the formulations (52).

Register *et al.,* 2012 found that bacterial colonization in infested soil increased plant growth and dry weight more than the control inoculateplants'ts growth-promoting bacteria (PGPR) causing a shift in the rhizosphere microbial population. Seed inoculation with antagonistic bacteria is an efficient method to control certain soil-borne plant pathogens (53).

Velusamy *et al.,* 2011 reported that a new strain of *Pseudomonas* sp. A3 possesses strong chitinolytic activity, which exhibited an antagonism toward *F. oxysporum*. Moreover, the crude chitinase isolated from strain A3 can be directly applied for suppressing the growth of viable fungal hyphae (54).

Aishah *et al.,* 2011 reviewed *P. fluorescent,* and the chemical fungicides aminobutyric acid, as the most effective biocontrol solution for Fusarium wilt in bananas caused by *Fusarium oxysporum* f. sp. *cubense*. Application of *P. fluorescens* to chickpea seeds significantly reduced Fusarium wilt incidence(52) and increased grain yields over the control by more than 100% (55). *Pseudomonas fluorescens* isolates effectively controlled rice sheath blight (*R. solani*) when it was applied to seed coating or soil drenching or foliar spray. A combined application of bacteria suspension isolates was with

being for seed coating and foliar spray was the most effective method for control of rice disease in the field (56).

Howell & Stipanovic, 1979 mentioned that treating cottonseed with *P. fluorescens* or pyrrolnitrin at the time of planting in *R. solani* infested soil increased seedling survival from 30 to 79% and from 13 to 70%, respectively. Pyrrolnitrin persisted for up to 30 days in moist nonsterile soil with no measurable loofioactivityty (57).

Aly *et al.,* 2002 reported that *P. fluorescens* CW1 was the most effective bacterial isolate in reducing *P. infestans* mycelial growth followed by CW2 isolate. Culture filtrate and bacterial suspension significantly inhibited the release of zoospores and cysts germination compared with the control **(21)**. Also, they found that different isolates of *P. fluorescence* produced salicylic acid with different concentrations in their culture media. Salicylic acid production was responsible for inducing resistance against different plant pathogens. The antagonist *P. fluorescens* inhibits the growth of *Penicillium* sp. and *Botrytis cinerea* causing green and gray molds of apple fruits by 100& 97.1%, respectively (58).

Parke & Rand, 1992 found that two strains of bacteria *Pseudomonas cepacia* AMMD and *P. fluorescens* PRA25 were effective in the biological control of root rot (*Aphanomyces euteiches* f. sp. *pisi*) and Pythium damping-off when applied to pea seeds singly or combined with captan (59).

Okigbo & Emeka, 2010 suggested that biological control was in operation and that *T. harzianum, P. syringae* and *P. chlororaphis* against *B. theobromae* and *F. solani* acted by either producing antifungal substances or colonizing the microsites faster than naturally occurring surface pathogens. They found that *T. harzianum* was the most effective in controlling *B. theobromae* and *F. solani* (60).

Saikia *et al.,* 2005 Suggested that isolates of *P. fluorescens* (Pf4-92 and PfRsC5) and *P. aeruginosa* (PaRsG18 and PaRsG27) can control the Fusarium wilt of chickpea as well as promote the growth and colonized the roots of chickpea. They also found induced systemic resistance (ISR) caused by *Pseudomonas* spp. against Fusarium wilt of chickpea is related to iron availability (61).

Rhizoctonia solani, the most important species within the genus *Rhizoctonia*, is a soilborne plant pathogen with considerable diversity in cultural morphology, host range, and aggressiveness. Despite its history as a destructive pathogen of economically important crops worldwide, the understanding of its taxonomic relationship with other Rhizoctonialike fungi, incompatibility systems, and population biology is rather limited. Among the host of diseases, it has been associated with, seedling diseases inflicted on soybean are of significant importance, especially in the soybean growing regions. Due to the dearth of resistant soybean genotypes, as well as the paucity of information on the mechanisms of host-pathogen interactions and other molecular aspects of pathogenicity (62). Natural polymers are produced and extracted by biological agents such as microorganisms, fauna, and flora. The result of Gagne-Bourque *et al.* 2015 indicated that *Bacillus subtilis* B26weres is encapsulated in alginate and pea protein as wall materials. The provision of healthy and nutritious food and agricultural products to continue living has always been one of the challenges of human societies. The adoption of technologies to increase the quantity and quality of agricultural products can be an excellent strategy to address these challenges (63).

Pathogenicity tests revealed that all *Rhizoctonia solani* isolates were pathogenic on chickpea and the disease severity values of 23 isolates varied between 42.8% and 100%. Based on the virulence, the isolates were grouped into two categories: 5 of them exhibited moderately virulence, and 18 of them exhibit d highly virulence reaction on chickpea. The high virulent isolate level (>50% disease severity) was determined as 78.2% of all 23 isolates. This is the first report of *R. solani* AG-4 as a pathogen of chickpea in Turkey (64). Furthermore, Ganeshamoorthi & Dubey 2015 characterized 50 isolates in terms of cultural variability obtained from chickpea, and among four isolates of AG-4 of *R. solani* two isolates showed light brown, while two isolates had dark brown colony color (65).

R. solani is a necrotrophic plant pathogen with a wide host range. *R. solani* is a species complex consisting of thirteen anastomosis groups (AGs) defined by compatibility of hyphal fusion reaction and subgroups based on cultural morphology. The relationship between such classifications and host specificity remains elusive. the pathogenicity of seventeen *R. solani* isolates

(AG-1 to 7) in Japan towards Arabidopsis thaliana using leaf and soil inoculations was studied. The tested AGs, except AG-3 and AG-6, induced symptoms in both methods with variations in pathogenicity. The virulence levels differed even within the same AG and subgroup. Some isolates showed tissue-specific infection behavior (66).

Materials and methods

The biocontrol tests were carried out using fungal and bacterial bioagents. The tested fungal bioagents were three isolates of *Trichoderma viride* obtained from plant Plant Pathology Department, culture collection, three isolates for *Rhizoctonia* spp were Previously obtained from peanut plants diseased roots. The bacterial antagonistic include one isolate of *Bacillus subtilis*, one isolate of *pseudomonas fluorescens*

Bacterial - Fungal Interaction.

Glycerol agar (GA) and nutrient agar (NA) media were used to grow cultures of *Bacillus subtilis, Pseudomonas fluorescens, and pseudomonas chlororaphis* respectively. Potato dextrose agar and nutrient agar (PDA+ NA) medium were used to test the *in vitro* antagonistic interaction between virulent isolates of *R. solani* and the biocontrol agents *B. subtilis, P. fluorescens,* and *P. chlororaphis* according to the dual culture method.

The antagonistic medium was poured into sterilized Petri dishes 9 cm in diameter. Two straight parallel lines were drawn each at 1.5 cm from the dish edge. On the medium tracing those two lines, a full loop of the tested bacterial suspension was streaked. The Petri plates were incubated at 28ºC for 48 h before the fungus inoculum was introduced in a central position between the lines. Three plates were prepared for each treatment. The inoculated Petri dishes were incubated at 30oC for 5 days. The antagonistic effect was determined by measuring the longest and shortest free growth zone between the antagonistic bacteria and the tested fungal isolate.

Effect of *Bradyrhizobium* on Germination of Peanut Seeds cv. *in Vitro*.

The bacterium *Brady rhizobium* was grown on flasks containing yeast extract mannitol (YEM) broth medium with 10 ml Congo red per liter. *Bradyrhizobium* was inoculated on (YEM) broth medium then left incubated for 48 h, 50 seeds of peanut cv. Giza 6 were submersion in *Brady rhizobium*

broth medium for 10 minutes, then plated on potato dextrose agar medium and incubated at room temperature 22± 2^{o}C.

After the germination of peanut seeds, the root length was measured in cm.

3. 3. Fungal – Fungal Interaction.

In the dual culture test, mycelial discs of 6 mm for each of the causal pathogens and one of the bioagents obtained from actively growing colonies were placed on the two halves of the solidified PDA medium. Plates were incubated at 30 ^{o}C. Three plates were prepared for each treatment besides the control treatment. The radial growth of tested pathogens in treated and control plates was recorded after 5 days of incubation and the percent inhibition of mycelial growth of the pathogens was calculated by using the following equation **(67):**

RI=100*(R2-R1)/R2.

RI= percent inhibition of mycelial growth.

R1= radial growth of the pathogen in dual culture with the antagonist (cm).

R2= radial growth of the pathogen in control plates (cm).

Results and discussion

Bacterial - Fungal Interaction.

To study the antagonistic effect of the studied bacteria i.e., *Pseudomonas fluorescens*, *Pseudomonas chlororaphis*, and *Bacillus subtilis* were tested against three tested isolates of *Rhizoctonia solani* after five days of incubation. The inhibition zone between the tested bacterium and the pathogenic fungus was measured by the dual culture method.

Data in table (1) Showed that *Bacillus subtilis* had a higher suppression effect (100%) with the three tested isolates of *Rhizoctonia solani* compared to *Pseudomonas fluorescens* and *Pseudomonas chlororaphis*. *Pseudomonas fluorescens* had the lowest bacterial in its suppression effect on the tested fungal growth *Rhizoctonia solani* 5 (16.7%), *Rhizoctonia solani* 3 (5.6%), and *Rhizoctonia solani* 12 (0.0%).

Table 1. Effect of *Bacillus subtilis, pseudomonas chlororaphis,* and *pseudomonas fluorescence* on linear growth of the three tested isolates of *Rhizoctonia solani* growing on PDA +NA medium.

Tested Bioagents	liner growth (cm) of *Rhizoctonia solani* isolates		
	Rhizoctonia solani 3	*Rhizoctonia solani* 5	*Rhizoctonia solani* 12
B.subtilis	0.00 d	0.00 d	0.00 d
P. chlororaphis	4.6 c	5.5 c	7.86 ab
P.fluorescens	8.5 ab	7.5 b	9.0 a

*Means of three replicates

Means followed by the seam letters, in each row are not significantly different according to the LSD test at (P=0.05).

Effect of *Bradyrhizobium* on Germination of Peanut Seeds cv. Giza 6 *in Vitro*.

Data in Table 2. indicated that peanut seeds germinated treated by *Bradyrhizobium* isolates i.e., 208, 209, 210 and *Bradyrhizobium* mixed, *Bradyrhizobium + Trichoderma harzianum*, *Trichoderma* alone, *Bradyrhizobium + pseudomonas fluorescence* results showed the followings:

1. The isolate *Bradyrhizobium* (208) gave the best germination percentage of peanut seeds (4.1667 cm length), followed by isolate 209, 210, and *Bradyrhizobium + Trichoderma* (3.600, 3.733and 3.6667) respectively.
2. The moderate germination of peanut seeds with the; *Trichoderma harzianum*, mixed *Bradyrhizobium*, and *Bradyrhizobium + pseudomonas fluorescence* had given (2.73, 2.40, and 2.33 cm of roots length).
3. The lowest germination of peanut seeds was obtained in the (control) treatment (1.43).

These results were in harmony with those of (66, 58) who reported that the influence of soil-borne fungi *R. solani*, *S.rolfsii*, *F. solani*, and *F. oxysporum* and *M. javanica* singly or in combinations on peanut plants indicated that the mixture of all fungi gave the highest incidence of pre-, post-emergence damping off. Furthermore, all the tested fungi alone or in

combination reduced the growth and yield of peanuts. Generally, infection with *M. javanica* plus each of the tested fungi decreased growthsponse and yield of peanuts more than when plants were infected by either nematode or fungus alone.

Table 2 Root length (cm) of peanut seeds cv. Giza 6 was treated with *Bradyrhizobium* isolates *in vitro*.

Treatments							
**Control	208	209	210	Br Mix	br +tri	tri	br +pf
1.4333 d *	4.1667 a	3.6000 ab	3.7333 ab	2.4000 cd	3.6667 ab	2.7333 bc	2.3333 cd

*Means of three replicates **Untreated plants

Means followed by the seam letter(s), in each Colum, are not significantly different according to the LSD test at (p=0.05).

Biological control involves the use of beneficial organisms that reduce the negative effects of plant pathogens and promote a positive re by the plant. Disease suppression, as mediated by control agents, is the consequence of the interactions between the plant, pathogens, and microbial community **(68).** Biological control is thus being considered as an alternative or a supplementary way of reducing the use of chemicals in agriculture **(69).** It could be cleared that, the application of *Trichoderma viride*, *Pseudomonas fluorescens*, *Pseudomonas chlororaphis* and *Bacillus subtilis* as biocontrol agents against *R. solani* isolate 3, 5 and 12. showed significant inhibition of the tested pathogens. These findings or data are supported by the results of **(29, 30, 41).**

Fungal – Fungal Interaction.

In a dual culture test using a PDA medium and three *Trichoderma viride* isolates to study their antagonistic affection against three *Rhizoctonia solani* isolates. Data Table 3 indicated that there is a significant inhibitory effect on radial growth of the three tested *Rhizoctonia solani* isolates due to *Trichoderma viride* isolates. Moreover, *Trichoderma viride* isolate showed the highest inhibitory effect with three tested *Rhizoctonia solani*, (100%) with *R. solani* isolates 5 and 1 and *R. solani* isolates 3 (88.9%).

These results are in agreement with those of **Seema and Devaki 2012 (70)** who studied the efficacy of four fungal and one bacterial bioagent viz, *Trichoderma viride, Trichoderma harzianum, Aspergillus niger, Penicillium spp., and Bacillus subtilis* were evaluated in vitro condition against, *R solani*. In the dual culture assay, the percentage inhibition of growth by *T. viride, T. harzianum, A. niger, B. subtilis, and Penicillium spp. on R. solani* were 70%, 67%, 57%, 50%, and 44% respectively. All the antagonists suppressed the formation of sclerotia. The volatile metabolite studies revealed that *T. viride* and *T. harzianum* showed 50% and 40% inhibition in mycelial growth respectively. Microscopic observations of the dual cultures revealed the inhibitory effect was caused by the hyphal interaction between the biocontrol agent and the pathogen causing the lysis of pathogen hyphae.

Table 3. Effect of three *Trichoderma viride* isolates on the linear growth of the three tested isolates of *Rhizoctonia solani*.

Trichoderma isolate	**liner growth cm. of the tested isolates**		
	R. solani 3	*R. solani* **5**	*R. solani* **12**
1	1.00 cd *	0.00 d	0.00 d
2	2.83 ab	2.40 ABC	3.83 a
3	2.07 bc	3.43 ab	3.67 ab

*Means of three replicates.

Means followed by the seam letters, in each row are not significantly different according to the LSD test at (P=0.05).

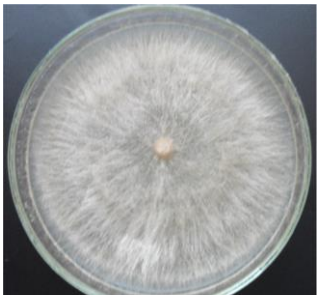
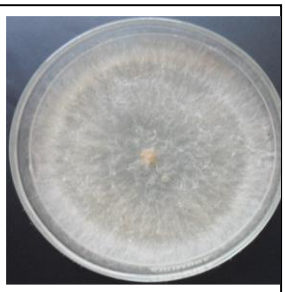
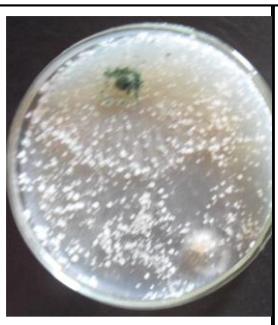
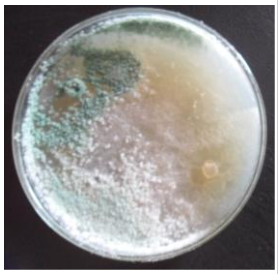
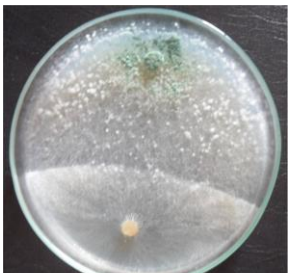
	R.solani 3	*R.solani* 5	*R.solani* 12
Contr.			
T. isol. 1			
T. isol. 2			
T. isol. 3			

Fig. 1. Effect of *Trichoderma viride* isolates on radial growth of three tested isolates of *Rhizoctonia solani.*

References

1) Eckert, J. W. and Ogawa, J. M. 1988. The chemical control of postharvest diseases: deciduous fruits, berries, vegetables, and root tuber crops. Annual Review of Phytopathology 26: 433-469

2) Droby, S.; Chalutz, E. and Wilson, C. L. 1991. Antagonistic microorganisms as biocontrol agents of postharvest diseases of fruit and vegetables. Postharvest News Information 2: 169-173.

3) Droby, S. 2006. Improving the quality and safety of fresh fruit and vegetables after harvest by the use of biocontrol agents and natural materials. Acta Horticulturae 709: 45-51.

4) Mayo-Prieto S., Rodríguez-González A., Lorenzana A., Gutiérrez S., Casquero P.A. 2020. Influence of substrates in the development of bean and in pathogenicity of *Rhizoctonia solani* JG Kühn. Agronomy 10: 707. org/10.3390/agronomy10050707.

5) Caviedes, E. Z., Silva, A. P., Mogollón, A.M. O. 2021. Biocontrol of rice sheath blight with microorganisms obtained in rice cultivated soils. Brigantia, 80, e0921.

6) Hyeongwon, S.; Duku, L.; GyeongSoo, L.; SeungHun, Y.; Shim, H. K.; Lee, D. K.; Lee, S. S. and Yu, S. H. 1996. Fungi are associated with the seed and seedling disease of peanuts. RDA-Journal-of-Agricultural-Science,-Crop-Protection. 38: 1, 507-515.

7) Meyer, S. L. F., and Roberts, D. P. 2002. Combinations of biocontrol agents for the management of plant-parasitic nematodes and soil-borne plant pathogenic fungi. Journal of Nematology 34: 1-8.

8) Turhan, G. and Gossmann, F. 1994. Antagonistic activity of five *Myrothecium* species against fungi and bacteria *in vitro*. J. Phytopathology 140: 97-113.

9) David, M. W. 2007. *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. Phytopathology 97: 250-256.

10) Anitha, B. S. 2011. Biocontrol of fusarium wilt by *Bacillus pumilus*, *Pseudomonas alcaligenes*, and *Rhizobium* sp. on lentil. Turk Journal Biology 34: 1-7.

11) Boureghda, H.; Z. Bouznad and C. Decock. 2008. Cultural and molecular characterizations of some isolates of *Trichoderma* spp. Arab J. Pl. Prot. 26: 75-80.

12) Vinale, F.; Sivasithamparam, K.; Ghisalberti, E. L.; Marra, R.; Woo, S. L. and Lorito, M. 2008. Trichoderma-plant-pathogen interactions. Soil Biology and Biochemistry 40: 1-10.

13) Pradeepkumar, A. and Kumudkumar, 2000. Biocontrol of seed-borne fungal pathogens of pigeon pea. *annals of plant protection*, **8**: 30-32.

14) Dwivedi, B. P., and Shukla, D. N. 2002. Biocontrol of Fusarium wilt of guava (*Psidium guajava* L.) using *Trichoderma* and *Gliocladium* species. Karnataka Journal of Agricultural Sciences 15: 399-400.

15) Haran, S.; Schickler, H. and Chet, I. 1996. Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. Microbiology 142: 2321-2331.

16) Brewer, D.; Mason, F. G., and Taylor, A. 1987. The Production of alamethicins by *Trichoderma* spp. Can. J. Microbiol. 33: 619-625.

17) Almassi, F.; Ghisalberti, E. L. and Narbey, M. J. 1991. New antibiotics from strains of *T. harzianum*. J Nat Product 54: 396-402.

18) Whipps, J. W. 1987. Effect of media on growth and interactions between a range of soil-borne glasshouse pathogens and antagonistic fungi. New Phytol. 107: 127-142.

19) Inbar, J.; Abramsky, M. and Cohen, D. 1994. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. European Journal of Plant Pathology 100:337-346.

20) Dunlop, R. W.; Simon, A. and Sivasithamparam, K. 1989. An antibiotic from *Trichoderma koningii*active against soilborne plant pathogens. Journal of Natural Products 52: 67-74.

21) Aly, A. Z.; Buchenauer, H.; Abou-Zaid, M. I.; Shalaby, M. S. and Atia, M. M. 2002. Induced resistance against tomato late blight disease using bioagents. Egypt. J. Phytopathol. 30: 25-43.

22) Kumar, S.; Sharma, S.; Pathak, D. V. and Beniwal, J. 2011.Integrated management of Jatropha root rot caused by *Rhizoctonia bataticola*. Journal of Tropical Forest Science 23: 35-41.

23) Marchetti, R.; Nipoti, P.; Ercole, N. D. and Guerzoni, M. E. 1992. Competition at an atmospheric level as biocontrol mechanism in *Trichoderma* spp. Petria 2: 137-147.

24) Lubna, S. N. 2005. Chitosan and three *Trichoderma* spp. to control Fusarium crown and root rot of tomato in Jedda, Kingdom Saudi Arabia. Egypt. J. Phytopathol. 33: 45-58.

25) Singh, P. K., and Kumar, V. 2011. Biological control of Fusarium wilt of *Chrysanthemum* with *Trichoderma* and botanicals. Journal of Agricultural Technology 7: 1603-1613.

26) Abdel-Kader MM, El-Mougy NS 2002. Integrated mycoherbicides and certain herbicide against Orobanche crenata infestation in a fava bean field. Egyptian Journal of Phytopathology 30, 27–39.

27) Kugler, M.; Loeffler, W.; Rapp, C.; Kern, A. and Jung, G.1990. Rhizocticin A, an antifungal phosphono-oligopeptide of *Bacillus subtilis* ATCC 6633: biological properties. Arch. Microbiol., 153: 276-281.

28) Asaka, O. and Shoda, M. 1996. Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB14. Applied and Environmental Microbiology 62: 4081-4085.

29) Islam, M. R.; Jeong, Y. T.; Lee, Y. S., and Song, C. H. 2012. Isolation and identification of antifungal compounds from *Bacillus subtilis* C9 inhibiting the growth of plant pathogenic fungi. Mycobiology 40: 59-66.

30) Rebib, H.; Abdeljabbar, H.; Rousset, M.; Abdellatif, B.; Ferid, L. and Najla, S. 2012. Biological control of Fusarium foot rot of wheat using fengycin-producing *Bacillus subtilis* isolated from salty soil. African Journal of Biotechnology 11: 8464-8475.

31) Lin, H. F.; Chen, T. H. and Liu, S. D. 2011. The antifungal mechanism of *Bacillus subtilis* against *Pestalotiopsis eugeniae* and its development for commercial applications against wax apple infection. African Journal of Microbiology Research 5: 1723-1728.

32) Schmidt, C. S.; Agostini, F.; Leifert, C.; Killham, K. and Mulins, C. E. 2004. Influence of soil temperature and matric potential on sugar beet seedling colonization and suppression of Pythium damping-off by the antagonistic bacteria *Pseudomonas fluorescens* and *Bacillus subtilis*. Phytopathology 94: 351-363.

33) Zongzheng, Y. X. L.; Zhong, L.; Jinzhao, P.; Jin, Q., and Wenyan, Y. 2009. Effect of *Bacillus subtilis* SY1 on antifungal activity and plant growth. Int. J. Agric. and Biol. Eng. 2: 55-61.

34) Elkahoui, S.; Djébali, N.; Tabbene, O.; Hadjbrahim, A.; Mnasri, B.; Mhamdi, R.; Shaaban, M. and Limam, F. 2012. Evaluation of antifungal activity from Bacillus strains against *Rhizoctonia solani*. African Journal of Biotechnology 11: 4196-4201.

35) Seema, M. and Devaki, N. S. 2012. *In vitro* evaluation of biological control agents against *Rhizoctonia solani*. Journal of Agricultural Technology 8: 233-240.

36) Heidi, I. G. Abo-Elnaga. 2006. *Bacillussubtilis* is a biocontrol agent for controlling sugar beet damping-off disease. Egypt J. Phytopathol. 34: 1-59.

37) Hussein, M. A. M.; Hassan, M. H. A.; Allam, A. D. A. and Abo-Elyousr, K. A. M. 2007. Management of Stemphylium blight of onion by using biological agents and resistance inducers. Egypt. J. Phytopathol. 35: 49-60.

38) Killani, A. S.; Abaidoo, R. C.; Akintokun, A. K. and Abiala, M. A. 2011. Antagonistic effect of indigenous *Bacillus subtilis* on root-/soil-borne fungal pathogens of cowpea. Researcher 3:11-18.

39) Karimi, K., Amini, J.; Harighi, B. and Bahramnejad, B. 2012. Evaluation of biocontrol potential of *Pseudomonas* and *Bacillus* spp. against fusarium wilt of chickpea. Australian Journal of Crop Science 6: 695-703.

40) Music, A. and Quimio, A. J. 2006. Biological control of banded leaf and sheath blight disease (*Rhizoctonia solani* Kuhn) in corn with formulated *Bacillus subtilis* BR23. Indonesian Journal of Agriculture Science 7: 1-7.

41) Weller, D. M. 2007. *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. Phytopathology 97: 250-256.

42) Anitha, A. and Dass, M. A. 2011. Activation of rice plant growth against *Rhizoctonia solani* using *Pseudomonas fluorescens, Trichoderma,* and salicylic acid. Research in Biotechnology 2: 7-12.

43) Vallet-Gely, I.; Novikov, A.; Augusto, L.; Liehl, P.; Bolbach, G.; Péchy-Tarr, M.; Cosson, P.; Keel, C.; Caroff, M. and Lemaitre, B. 2010. Association of hemolytic activity of *Pseudomonas entomophila*, a versatile soil bacterium, with cyclic lipopeptide production. Applied and Environmental Microbiology 76: 910-921.

44) Páez, M., Martínez-Nieto, P. and Bernal-Castillo, J. 2005. Siderophore producing Pseudomonas as pathogenic Rhizoctonia solani and Botrytis cinerea antagonists. Universitas Scientiarum 10: 65-74.

45) Nourozian, J.; Etebarian, H. R. and Khodakaramian, G. 2006. Biological control of *Fusarium graminearum* on wheat by antagonistic bacteria Songklanakarin J. Sci. Technol. 28: 29-38.

46) Voisard, C.; Keel, C.; Haas, D. and Défago, G. 1989. Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. The EMBO Journal 8: 351-358.

47) Gupta, C. D.; Dubey, R. C.; Kang, S. C. and Maheshwari, D. K. 2001. Antibiotics mediated the necrotrophic effect of *Pseudomonas* GRC2 against two fungal plant pathogens. Current sci. 81: 91-94.

48) Fridlender, M.; Inbar, J. and Chet, I. 1993. Biological control of soil-borne plant pathogens by a β-1, 3-glucanase-producing *Pseudomonas cepacia*. Soil Biol. Biochem. 25: 1211-1221.

49) Gupta, C. P.; Kumar, B.; Dubey, R. C., and Maheshwari, D. K. 2006.The chitinase-mediated destructive antagonistic potential of *Pseudomonas aeruginosa* GRC1 against *Sclerotinia sclerotiorum* causing stem rot of peanut. Biocontrol 51: 821-835.

50) Naveen, K.; Arora, Khare, E.; Oh, J.H.; Kang, S. C., and Dinesh K. Maheshwari. 2008. Diverse mechanisms adopted by fluorescent *Pseudomonas* PGC2 during the inhibition of *Rhizoctonia solani* and *Phytophthora capsici*. World J. Microbiol. Biotechnol. 24: 581-585.

51) Van Peer, R.; Niemann, G. J. and Schippers, B. 1991. Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. Phytopathology 81: 728-734.

52) Aishah, M. M.; Laith K. T. AL-Ani.; Lyazzat B. and Baharuddin S. 2011. Biological control of *Fusarium oxysporum* f. sp. *cubense* by *Pseudomonas fluorescens* and BABA *in vitro.* World Applied Sciences Journal 15: 189-191.

53) Registeri, R.; Taghavi, S. M. and Banihashemi, Z. 2012. Effect of root colonizing bacteria on plant growth and Fusarium wilt in *Cucumis melo*. J. Agr. Sci. Tech. 14: 1121-1131.

54) Velusamy, P.; Ko, H.S. and Kim, K.Y. 2011. Determination of the antifungal activity of *Pseudomonas* sp. A3 against *Fusarium oxysporum* by high-performance liquid chromatography (HPLC). Agriculture, Food and Analytical Bacteriology 1: 15-23.

55) Vidhyasekaran, P. and Muthamilan, M. 1995. Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. Plant Disease 79: 782-786.

56) Kazempour, M. N. 2004. Biological control of *Rhizoctonia solani*, the causal agent of rice sheath blight by antagonistic bacteria in greenhouse and field conditions. Plant Pathol. J. 3: 88-96.

57) Howell, C. R., and Stipanovic, R. D. 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology 69: 480-482.

58) El-Moggy, N. S.; Abd El-Kareem, F. and Abd Alla, M. A. 2002. Postharvest diseases control the preventive effect of chitosan and bioagents against green and gray molds of apple fruits. Egypt. J. Phytopathol. 30: 99-112.

59) Parke, J. L. and Rand, R. E. 1992. Biological control of Pythium damping-off and Aphanomyces root rot of peas by application of *Pseudomonas cepacia* or *P. fluorescens* to seed. Plant Disease 75: 987-992.

60) Okigbo, R. N. and Emeka, A. N. 2010. Biological control of rot-inducing fungi of water yam (*Dioscorea alata*) with *Trichoderma harzianum*, *Pseudomonas syringae*, and *Pseudomonas chlororaphis*. Journal of Stored Products and Postharvest Research 1: 18-23.

61) Sakhuja, P. K. and C. L. Sethi. 1985. Frequency of occurrence of various plant-parasitic nematodes and root-rot fungi on groundnut in Punjab. Indian Journal of Nematology 15:191–194.

62) Ajayi-Oyetunde O. O. and C. A. Bradley (2018) Rhizoctonia solani: taxonomy, population biology, and management of Rhizoctonia seedling disease of soybean. Plant Pathology (2018) 67, 3–17.

63) Gagne-Bourque F, Xu M, Dumont MJ, Jabaji S. 2015. Pea protein alginate encapsulated Bacillus subtilis B26, a plant biostimulant, provides controlled release and increased storage survival. J. Fertil. Pestic. 6: 157

64) Basbagci, G.; Unal, F.; Uysal, A.; Dolar, F. S. 2019. Identification and pathogenicity of *Rhizoctonia solani* AG-4 causing root rot on chickpea in Turkey. Spanish Journal of Agricultural Research, Volume 17, Issue 2, e1007.

65) Ganeshamoorthi P, Dubey SC, 2016. Morphological and pathogenic variability of *R. solani* isolates associated with wet root rot of chickpea in India. Legume Res 383): 389- 395.

66) Abdelghany, M.M.A.; Kurikawa, M.; Watanabe, M.; Matsui, H.; Yamamoto, M.; Ichinose, Y.; Toyoda, K.; Kouzai, Y.; Noutoshi, Y 2022. Surveillance of Pathogenicity of Rhizoctonia solani Japanese Isolates with Varied Anastomosis Groups and Subgroups on Arabidopsis thaliana. Life 2022, 12, 76.

67) Grondona, I.; Hermosa, R.; Tejada, M.; Gomis, M.; Mateos, P. F.; Bridge, P. D.; Monte, E. and Garica. Acha, I. 1997. Physiological and Biological characterization of *Trichoderma harzianum*, a Biological control agent against soil-borne fungal plant pathogen Applied and Environmental Microbiology. 63 (8): 3189-3198.

68) Sivan, A. and Chet, I. 1986. Possible mechanisms for control of *Fusarium* spp. by *Trichoderma harzianum.* British crop protect. 2: 865-872.

69) Compant, S.; Duffy, B.; Nowak, J.; Clément, C. and Barka, E. A. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles,

mechanisms of action, and prospects. Applied and Environmental Microbiology 71: 4951-4959.

70) Seema1. M. and N.S. Devaki.2012 In vitro evaluation of biological control agents against *Rhizoctonia solani*. Journal of Agricultural Technology 2012 Vol. 8(1): 233-240.