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Effect of Carbon Disulfide (CS_2) Emission by *Acacia tortilis* subsp. *raddiana* on Microbial Population in the Rhizoshpere

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ABSTRACT

Roots of *Acacia tortilis* subsp. *raddiana* seedlings were proved to emit carbon disulfide (CS_2); a low molecular weight, volatile, highly flammable, sulfur-containing molecule with bacteriostatic, fungicidal, nematicidal and insecticidal properties that has inhibition influence on microbial communities in the rhizosphere of *A. tortilis* seedlings. Total count of bacterial cells, colony forming unites (CFU) of actinomycetes and population of nitrifying bacteria around roots of *A. tortilis* seedlings grown on different soil types, were dramatically inhibited compared to the control. Inhibition by CS_2 was represented by reduction percentage of bacterial count which reached its maximum value (99.8%) by the end of the experiment (eight weeks after transplanting seedlings) on sandy-loam soil. However, with nitrifying bacteria highly significant inhibition was recorded at six weeks with 96.1%. Loamy-sand soil significantly recorded less reduction percentage of nitrifying bacteria that reached the peak by the end of six week with 80.0%. Inhibition of CFU of actinomycetes was significantly less in compare to other organisms in different tested soils. Sandy soil was less affected by CS_2 emission and showed temporary growth inhibition. Growth recovery of actinomycetes was observed after six weeks of transplanting. Growth of *A. tortilis* significantly varied with soil type and showed the best growth in loamy-sand soil. Sandy soil showed promising growth rate even it was significantly less than other soil types. The obtained results suggest that CS_2 production is regulated by plant growth which is in turn affected by soil chemical and physical characteristics. The study recommends that *Acacia tortilis* subsp. *raddian* plants may have an agronomic use in crop rotations or intercropping because root-produced CS_2 may act as a deterrent to soil pathogens.

Key words: Carbon disulfide, *Acacia tortilis* subsp. *raddiana*, rhizosphere, biocontrol.

INTRODUCTION

Carbon disulfide (CS_2) is a clear colourless, toxic, highly flammable, highly volatile, foul-smelling liquid (Peyton *et al.*, 1978; Newhook and Meek, 2002) that exists everywhere (atmospheric, aquatic and terrestrial habitats). It has a potent to inhibit microbes and has been long and widely used as fumigant for the stored seeds and grains as well as a soil fumigant against phyto-pathogens due to its bactericidal (Bremner and Bundy, 1974; Hartel and Haines, 1992; Meister, 1995), fungicidal (Banwart and Bremner, 1975; Filip and Roth, 1977; Lewis and Papavizas, 1971), nematicidal (Guba, 1932) and insecticidal (Punj and Girish, 1969) properties. Meanwhile, CS_2 is expected to have little or no tendency to bioaccumulation or biomagnifying in animals, owing to its relatively low log Kow value (2.14) and rapid metabolism in most animals (Beauchamp *et al.*, 1983). Therefore, it is sometimes used as a raw material to make some pesticides (Newhook and Meek, 2002). Although *Trichoderma viride* is relatively tolerant to CS_2, most fungi are eradicated by high concentration of CS_2 (Mazen *et al.*, 1988).

CS_2 has two major sources, natural and anthropogenic, from which it is released to global context. Industrial, manufacturing and processing facilities involved in viscose, rayon, and cellophane productions and others produce 700 kilotons of CS_2 annually (Newhook and Meek, 2002). CS_2 used in these processes is likely to end up in the atmosphere as a gaseous state. It is also released as by-product in oil and gas processing. However, it is released in larger quantities from a wide variety of natural sources such as volcanic eruption, weathering of sulphide minerals and forest and grass fires. It also released from soil, marshes and coastal regions which have a tendency to be the largest biogenic sources. Microorganisms are another natural source for CS_2 which is produced by during their metabolic reactions in costal and ocean waters.

A major natural source of CS_2 is the higher plants which emit it in considerable amount. The emission rate increases when their roots are injured or wetted after drought stress (Whitfield *et al.*, 1981; Haines *et al.*, 1987). Once CS_2 is released from roots, it moves quickly to air. It stays close to the ground because it is heavier than the surrounding air. It breaks down into simpler components within 12 days (Newhook and Meek, 2002). However, CS_2 moves through soil fairly quickly and does not bind tightly to its particles. The amount that does not evaporate can easily move down through the soil into ground water. Species belong to Fabaceae, subfamily Mimosoideae, including some *Acacia* species have been proved to be related to direct increase of CS_2 in the rhizosphere zone that affect the nearby micro biota (Hartel and Reeder, 1993; Feng and Hartel, 1996; and Piluk *et al.*, 1998).

There are twenty five species of *Acacia* growing in dry areas of the Middle East, ten of them grown in Egypt including *Acacia tortilis* subsp. *raddiana* (Forssk.) Hayne which is the most wide spread tree in Egyptian deserts due to its ability to stand drought conditions and resistant to high temperature (Halevy

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and Orshan, 1972; Zohary, 1973; Boulos, 1999). It grows in desert wadis and sandy plains, usually in water catchments areas, and is found in Red Sea coast, Eastern Desert, Gebel Elba, and Sinai (Boulos, 1999). It dominates mainly rocky wadi-bed habitats near the foothills and sometimes the slopes of metamorphic mountains (Moustafa *et al.*, 2000). It is capable to grow in areas with annual rainfall as low as 40mm. The tree favours alkaline soil but will colonize saline and gypsum soil, and form deep tap root in sandy soil. On shallower soil, it can develop hose-pipe root extended over twice the width of the crown. It survives sites where temperature regularly reaches 50^{o}C at mid-day and fall to near freezing at night (NFTA, 1991). It is a pioneer species easily generated from seeds.

Very considerable socioeconomic benefits can flow from cultivation of acacias that it may play a dual role contributing to industrial forestry plantations, for timber, pulpwood and tannin, and at the Bedouin's community level in the provision of fuel wood, land rehabilitation and as a component of agro-forestry systems (Moustafa *et al*., 2000). Moreover, *A. tortilis* is natural source for soil-nitrogen fertilization as it fixes huge quantity of atmospheric nitrogen as a result of symbiotic integration with soil bacterium genus *Rhizobium* or *Bradyrhizobium* (Ferro *et al.*, 2000). Therefore, *A. tortilis* is a promising multiple-use plantation species in arid ecosystems having poor soil conditions.

The present study addresses three questions: (1) Does *Acacia tortilis* subsp. *raddiana*, grown in Egyptian deserts, emits carbon disulfide in its rhizoshpere, (2) If yes, does emitted CS_2 affects soil microflora (total bacteria, nitrifying bacteria, and actinomycetes) in different soil types (sandy-loam, loamy-sand and sandy) common in Egypt, and (3) Is there any difference in reflection of this effect on growth of *Acacia tortilis* seedlings itself in different soil types?

MATERIALS AND METHODS

Carbon disulfide detection

There is no simple method for the detection of carbon disulfide in biological samples other than smell. However, we were able to detect CS_2 qualitatively by relying on the fact that it is metabolized easily into inorganic or organic (e.g. TTCA) sulfate. The compound formed can catalyses the decolonization of a solution of iodine by sodium azide following Newhook and Meek (2002). To carry out this experiment, seeds of *Acacia* were surface sterilized for two minutes in H_2O_2 with few drops of detergent after they have been chocked with heat using boiling water. Clorox was added, after washing with sterilized distilled water, for one minute with continuous steering. Sterilized washed seeds were transferred to semi-solid medium of 1/4-complete Hoagland's solution with pH 6.7 and kept at room temperature with photoperiod 16/8 day/night till the observation of radical emergence. At begging and after seed germination, CS_2 was detected regularly, every 24h, up to first two compound leaves appeared.

Seed collection and plant propagation

Bulked open-pollinated seeds of *Acacia tortilis* subsp. *raddiana* were collected randomly from populations growing naturally in Wadi Mander and Wadi Lithi located in Southern Sinai. Seeds were treated with 95% sulfuric acid for 20-25 minutes and rinsed thoroughly with water several times to scarify the impervious seed coat to facilitate germination. Treated seeds were germinated in flats containing a sterilize mixture of sand - beat moss - vermiculite (1:1:1), kept under laboratory conditions and watered at regular bases. Germinated seeds were allowed to grow until giving out, at least two compound leaves before transplanting to run the experiment. Growing seedlings were irrigated with 1/4-complete Hoagland's solution once a week.

Experimental Design

To determine the capability of *Acacia tortilis* subsp. *raddiana* as a CS_2-producing plant, three different soil types were used. For each soil type, four sets (with five pots each) represent harvesting time were used. In each pot, one healthy acacia seedling (three weeks old) was transplanted. Five plant-free pots from each soil type were run in parallel and served as negative control. Pots containing the acacia seedlings were transferred to greenhouse under 32±2/28±2^{o}C day/night temperature for 16/8 hours. After two weeks of transplanting, soil rhizosphere and acacia seedlings microbial population in coincident with plant growth parameters were measured. The measurements were repeated three times with two weeks interval period (i.e. at four, six, and eight weeks).

Microbial inhibition by CS_2 emission

To determine the potential effect of CS_2 emission on microbial population in acacia rhizosphere, soil samples (collected at regular intervals; two, four, six and eight weeks) were collected from shacked soil rhizosphere in each pot and were investigated for microbial population using serial dilution technique. Three to five replica of fifteen combined rhizosphere soil, for each collected time per each soil types, were carried out. In parallel, soil samples from plant-free pots kept under the same conditions (control) were collected at 5-8 cm depth and were investigated for microbial population at the same time intervals.

Total count of bacterial cells and actinomycetes forming units

Total bacterial and actinomycetes forming units counts were estimated by pouring plates technique (Black, 1996). Nutrient and starch-casein agar medium

were used respectively. Counting by serial dilution technique was carried out in which ten grams of collected soil was agitated in fifty ml of sterile saline solution and then serial dilutions up to 10^{-7} were made. Medium inoculation with 0.1 ml of different saline dilution was carried out. Three replicas from each dilution per each soil type at each sampling time were done. Inoculated plates were incubated at 30±2°C for 48 hours and seven days for bacteria and actinomycetes respectively. An appropriate dilution was used for counting Cell Forming Unit (CFU) per gm soil.

Estimation of total count of nitrifying bacteria

Nitrification occur in soils by nitrifying bacteria is highly affected by interfering S-containing molecule (Kyveryga *et al.*, 2004). As nitrifying bacteria could not be examined by usual microbial techniques, the Most Probable Number (MPN) method was used (DiGeronimo *et al.*, 1978). The MPN technique has been the most widely used method to enumerate nitrifying bacteria although it has distinct drawback having a high inherent statistical error. Soil extracts were made by blending ten grams of soil, collected as described before, with 95 ml of sterile buffer for 30-60 seconds. Serial dilutions of these soil extracts were made till 10^8 for sandy-loam, 10^6 for loamy-sand, and 10^5 for sandy soil. From the highest five dilutions, one ml of the diluted extract was transferred to tubes of media which were then incubated at 25°C – 28°C in dark. The counting was done after three week.

Plant growth and survivorship

Growth of *Acacia tortilis* subsp. *raddiana* seedlings, expressed as plant height and number of compound leaves, were measured four times at two weeks interval to estimate the difference in growth of *A. tortilis* seedlings planted on different soil types in response to CS_2 emission. Mean values of fifteen *A. tortilis* seedlings were calculated. Meanwhile, survivorship of *A. tortilis* seedlings was estimated as the percentage of survived individuals after the time period (eight weeks) of the experiment (Townsend *et al.*, 2003). Correlation between reduction percentage in microbial counts, soil type and plant growth parameters were investigated.

Soil sampling and analysis

Soil used in planting *A. tortilis* subsp. *raddiana* were collected from different localities, within Ismailia province, with different history and practices of cultivation representing the most common types of soil in Egyptian agro-ecosystems; sandy-loam, loamy-sand, and sandy soil. Quantitative physical (soil texture) and chemical (pH, EC, and water soluble cations) analyses were carried out to characterize the collected soil. Typically, particle size analyses, as well as other standard soil analyses, are made on the fine fraction of the soil that is on soil material of less than 2 mm equivalent spherical diameter (Ball, 1976; Hausenbuiller, 1985). Therefore, soil samples were air-dried and sieved through 2mm sieve to exclude large particles that are relatively less reactive (Robertson *et al.*, 1999).

Particle size distribution was determined by dry sieving for the coarse sand and by pipette method for fine sand, silt, and clay (Richards, 1954). Soil aggregation was treated by 5% Sodium Hexametaphosphate as a dispersing agent. Soil texture were classified based on the percentages of clay, silt and sand using USDA limits of the basic soil textural classes (Gee and Bauder, 1986).

Soil pH was measured electrometrically, using pH meter in soil suspension of ratio 1:2.5 soil to water. The soil-water mixture was first shacked for two hours, and then pH was measured. Electrical conductivity (EC) was measured in soil water extract 1:1 using electrical conductivity meter.

Soil organic matter was measured using loss-on-ignition method. This method gives quantitative oxidation of organic matter (Nelson and Sommers, 1996). About ten gm of air-dried soil in a tarred porcelain crucible was first dried at 105°C for 24 hours, cooled in a desiccators, and weighed then ignited at 600°C for three hours using muffle furnace. The crucibles were cooled to room temperature, and loss on ignition was calculated as a percent of the oven-dried sample weight.

Water soluble cations were measured in soil water extract (1:1) using Flame Photometer for Na and K (Helmke and Sparks, 1996) and titration for Ca and Mg (Suarez, 1996).

Data analysis and statistical evaluation

Statistical analyses were done following Zar (1984) using MINITAB 15 computer software (MINITAB, 2007). Significance of differences between two groups of variables was assessed by T-test. Significance of variation between multiple groups of variables were done using one way Analysis of Variance (ANOVA), while Tukey's pairwise comparisons were was used to assign the inter-groups significance of variation.

RESULTS

Soil physical and chemical properties

The soil fraction analysis showed that the collected soil samples belong to three main soil texture classes representing the major soil types in Egyptian deserts and reclaimed soils. These types are sand, loamy-sand, and sandy-loam (Table 1). The sand soil was collected from desert non-cultivated site. This type of soil is characterized by low water-holding capacity, well aerated and rapid drainage, low organic matter, and poor supply of plant nutrients. The loamy-sand soil was collected from reclaimed site with a 20-years history of

cultivation. Loamy-sand soil is characterized by low to medium water-holding capacity, moderate to high aeration and medium drainage, medium organic matter, and moderate supply of plant nutrients. The sandy-loam soil was collected from a site with long history of cultivation practices more than 50 years. This type of soil is characterized by medium to high water-holding capacity, moderate aeration and slow drainage, medium to high in organic matter, and moderate to good supply of plant nutrients.

The collected three different soil types are alkaline in soil reaction with slightly alkaline in loamy-sand and moderate to high alkaline in sandy-loam and sand soil. Results of electric conductivity indicate that all of the three soil samples are non saline with a range of EC varies from 0.11 dS m^{-1} in sand soil to 1.14 dS m^{-1} in sandy-loam soil. As Sodium content is highly and directly correlated with soil electric conductivity, sandy-loam soil has the highest content of Na content (4.07 meq l^{-1}) followed by loamy-sand (3.94 meq l^{-1}) and sand soil (1.12 meq l^{-1}) (Table 1).

Soil organic matter varied from 0.38% in sand soil to 1.79% in sandy-loam soil. Loamy-sand soil has a moderate percentage of soil organic matter (0.81%). Water soluble cations measured in these soils were highly correlated with the soil organic matter percentages. While sand soil has the lowest content of K, Ca, and Mg (0.16, 1.17, and 0.24 meq l^{-1}, respectively), loamy-sand soil showed the highest values of these parameters (0.78, 4.17, and 1.28 meq l^{-1}, respectively). An intermediate concentration of K, Ca, and Mg was recognized in loamy-sand soil (0.5, 2.69, and 0.84 meq l^{-1}, respectively) (Table 1).

Potentiality of *A. trotilis* as CS_2-emitor

CS_2 emission from germinated seeds of acacia was detected only after radical emergence and during the formation of main and lateral roots. No CS_2 emission was detected at any time before radical emergence. The yellow-brown color of iodine solution was decolorized within few seconds. Faster decolonization was observed after complete seedlings have been well developed. Also, the results indicated that CS_2 emission increased with increasing the emergences and formation of new lateral roots during seedlings growth.

Effect of CS_2 emission on bacterial population

The effectiveness of CS_2 emitted by *Acacia tortilis* roots in rhizosphere, grown on different soil types as inhibitor for bacterial colonization was determined for eight weeks at regular intervals (Table 2). There were marked differences in the reduction percentage of bacterial population in response to CS_2 emission. The highest reduction percentage was recorded (99.8%) for sandy-loam (Fig. 1A) soil followed by 89.2% for loamy-sand soil. Although bacterial population in sand soil (Fig. 1B) was the lowest significantly affected by CS_2 emission, the reduction percentage is still significant (79.5%).

In contrast, bacterial growth showed significant increase in control pots (not planted soil) in compare to initial bacterial counts (21 and $17X10^4$ g^{-1} for sandy-loam and loamy-sand soil, respectively). The highest counts were reached after transplanting by six weeks for sandy-loam soil ($20X10^8$ g^{-1}) and four weeks for loamy-sand soil ($20X10^7$ g^{-1}). Sand soil showed significant less count at zero time of transplanting ($1X10^4$ g^{-1}), but the bacterial counts in control samples showed significant continuous increase with time course of the experiment except for four and six weeks where no significant difference was recorded.

The variation in total bacterial count in pots with acacia seedlings (Table 2) in compare to count in control pots (without seedlings) was highly significantly

Table (1): Physical and chemical characterization of three soil types used.

Soil texture	Soil fraction %			pH	EC (dS m^{-1})	Organic matter %	Water soluble cations (meq l^{-1})			
	Clay	Silt	Sand				Na^+	K^+	Ca^{2+}	Mg^{2+}
Sandy-loam	2.4	28.6	74.8	8.01	1.14	1.75	4.07	0.78	4.17	1.28
Loamy-sand	1.4	19.7	79.5	7.72	0.81	1.39	3.94	0.5	2.69	0.84
Sand	1.4	0.7	97.9	8.42	0.11	0.38	1.12	0.16	1.17	0.24

Table (2): Effect of CS_2 emission from *Acacia* roots, grown in different soil types on bacterial population estimated as Colony Forming Unites (CFU) per gram soil. Zero-time reading was 21, 17 and 1 ($X10^4$) CFU per gram soil of sandy-loam, loamy-sand and sand, respectively.

Counting time after transplanting (weeks)	Colony Forming Units ±SE (10^4 g^{-1})								
	Sandy-loam			Loamy-sand			Sand		
	1	2	Red. %	1	2	Red. %	1	2	Red. %
Two	220±5.8	41±6.1	81.4	55±2.9	17±1.2	69.1	1.6±0.06	1.5±0.06	6.3
Four	1500±11.5	246±12.4	83.6	20000±1154	900±57.7	95.5	26±0.6	19±2.3	26.9
Six	200,000±3511	850±5.8	99.6	3900±404.1	400±50.2	89.7	26±0.6	15±1.5	42.3
Eight	150000±14550	350±46.1	99.8	1900±57.7	205±25.9	89.2	39±0.6	8±0.6	79.5

1 = Soil without *Acacia* seedlings, 2 = Soil with transplanted *Acacia* seedlings, Red.% = Reduction %.

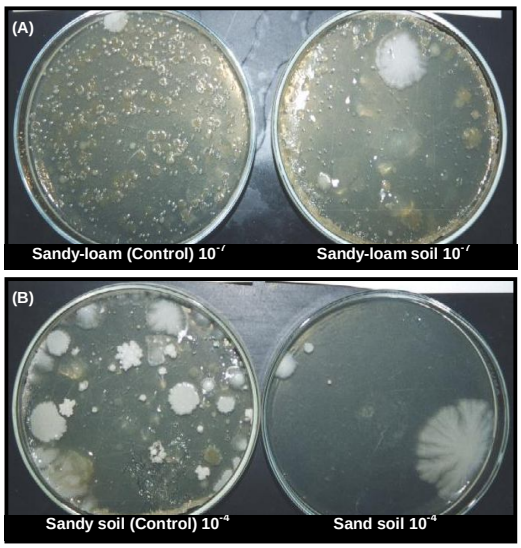

Figure (1): Effect of CS_2 emission from *Acacia tortilis* subsp. *raddiana* roots on bacterial population of (A) sandy-loam and (B) sand soil.

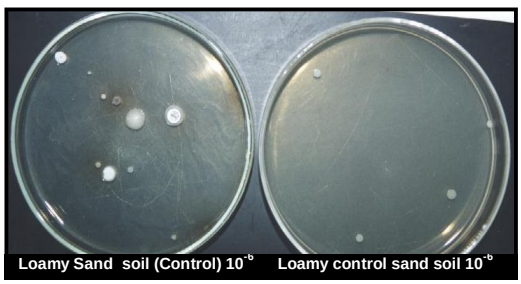

Figure (2): Effect of CS_2 emission from *Acacia tortilis* subsp. *raddiana* roots on actinomycetes population of loamy-sand soil.

different ($P \leq 0.000$). The variation was also significant between different soil types used and amount of CS_2 emitted, where loamy-sand soil was the highest at fourth weeks (9.0 X 10^6 g^{-1} with 95.5% reduction) followed by sandy-loam after sixth weeks (8.50 X 10^6 g^{-1} with 99.6% reduction) of transplanting.

Effect of CS_2 emission on actinomycetes

Although not as much as in case of bacterial population, actinomycetes population was reduced in different soil types planted with *Acacia*. Generally, the reduction was higher in sandy-loam than loamy-sand (Fig. 2) than sand soil. The maximum reduction percentage was recorded in loamy-sand soil after six (75.0%) and eight (70.0%) weeks of transplantation. Percentage of reduction varied between readings within each soil type. While the peak of reduction was reached after eight weeks of transplantations in sandy-loam soil, it was reached after six weeks in loamy-sand soil and after four weeks in sand soil (Table 3). After reaching the peak, reduction percentage decreased but still significant in compare to control.

Influence of CS_2 emission on nitrifying bacteria

Emission of CS_2 from roots of *A. trotilis* seedlings reduced the population of NH_4-oxidizing bacteria in all tested soil types (Table 4). Although the highest reduction in bacterial population was recorded in sandy-loam soil at six week of transplantation ($158.3X10^4g^{-1}$) in compare to control and $4033.0X10^4g^{-1}$, significant and continuous growth inhibition ($136.7X10^4g^{-1}$) was observed two weeks later but with less reduction

Table (3): Effect of CS_2 emission from *Acacia* roots, grown in different soil types, on actinomycetes population estimated as Colony Forming Unites (CFU) per gram soil. Zero-time reading was 8, 14 and 0.7 (X104) CFU per gram soil of sandy-loam, loamy-sand and sand, respectively.

Counting time after transplanting (weeks)	Colony Forming Units ±SE (10^4 g^{-1})								
	Sandy-loam			Loamy-sand			Sand		
	1	2	Red. %	1	2	Red. %	1	2	Red. %
Two	20±1.2	18±3.5	10.0	3.9±0.06	2.1±0.5	46.2	1.3±0.07	1.2±0.06	7.7
Four	30±5.8	20±1.15	33.3	75±1.9	40±0.6	46.7	20±2.9	15±0.6	25.0
Six	20±1.8	10±0.6	50.0	72±4.3	18±0.6	75.0	16.2±1.2	13±1.8	19.8
Eight	48±1.2	18±1.2	62.5	50±10.0	15±5.0	70.0	8.2±1.8	7.0±1.8	14.6

1 = Soil without *Acacia* seedlings, 2 = Soil with transplanted *Acacia* seedlings, Red. % = Reduction %.

Table (4): Effect of CS_2 emission from *Acacia* roots, grown in different soil types, on nitrifying bacterial population estimated as Colony Forming Unites (CFU) per gram soil.

Counting time after transplanting (weeks)	Colony Forming Units ±SE (10^4 g^{-1})								
	Sandy-loam			Loamy-sand			Sand		
	1	2	Red. %	1	2	Red. %	1	2	Red. %
Two	3000.0±57.7	3200.0±57.73	-6.7	0.04±0.0	0.07±0.0	-75.0	0.03±0.0	0.02±0.0	33.3
Four	3566.7±233.3	160.0±5.7	95.5	0.83±0.03	0.15±0.01	82.0	0.16±0.03	0.09±0.0	43.8
Six	4033.0±88.1	158.3±7.26	96.1	0.80±0.06	0.67±0.03	16.3	0.22±0.06	0.18±0.02	18.2
Eight	733.3±120.2	136.7±18.6	81.4	1.1±0.26	0.45±0.03	59.0	0.13±0.04	0.08±0.0	38.5

1 = Soil without *Acacia* seedlings, 2 = Soil with transplanted *Acacia* seedlings, Red.% = Reduction %.

percentage (81.4%) in compare to control (733.3X $10^4 g^{-1}$). It was different in loamy-sand soil where there was a significant increase ($p \leq 0.05$) in nitrifying bacteria population in control set with a significant increase in growth inhibition at harvesting time (0.07, 0.15, 0.32 and $0.45X10^4 g^{-1}$, respectively). Less reduction percentage was recorded by the end of the experiment (Table 4). Sand soil was significantly the lowest in NH_4-oxidizing bacteria population and in the reduction percentage. In sandy-loam and loamy-sand soil, the reduction percentage in nitrifying bacteria population reached its highest value (96.1 and 80.0%, respectively) after six weeks of transplanting. For sand soil, the highest reduction percentage (43.8%) was recorded after four weeks followed by six weeks (40.9%) of transplanting with non significant difference. Two weeks later, bacterial growth subsequently recovered but the size of the population remained significantly lower than in control (soil without acacia seedlings).

Plant growth and survival rate

Acacia tortilis seedlings showed very highly significant variation ($P \leq 0.000$) in growth parameters on different soil types (Table 5, Fig. 3). The highest growth was recorded for seedlings growing on loamy-sand soil at the four measurement times (two, four, six, and eight weeks). *A. tortilis* showed a relatively good growth in sandy soil even it is significantly lower than in other two soil types. Meanwhile, highly significant variation in terms of change in heights ($P \leq 0.000$) and number of compound leaves ($P \leq 0.004$) was recorded in all soil types. The highest mean of change in height (9.45 ± 3.51 cm $plant^{-1}$) and number of compound leaves (2.20 ± 2.11) were recorded in loamy-sand soil by the eighth weeks of transplantation (Table 5). Tukey's test didn't recognize a significant difference between change in number of compound leaves of plants in sandy-loam and loamy-sand soil. This high growth of *A. tortilis* plants in loamy-sand soil was accompanied by higher survival percentage (94.3 %) than plants in sandy-loam soil (89 %). No difference was recorded in survival percentage among plants in sandy and loamy soils.

Figure (3): Growth of *Acacia tortilis* subsp. *raddiana* on different soil types: **(A)** Sand, **(B)** Loamy-sand, and **(C)** Sandy-loam**.**

DISCUSSION

Acacia tortilis subsp. *raddiana*, a multipurpose tree belongs to Fabaceae (subfamily Mimosoideae) has been proved through present study to emit CS_2 at juvenile stages (seedling). This subspecies will be added to the list of other CS_2-emitor acacias (Piluk *et al.*, 1998). In their elegant study, they reported 29 species (73%) belong to six genera out of 40 species belong to nine genera within subfamily Mimosoideae as CS_2-emitors. Acacias represent 93.1% (27 species) of these CS_2-emitors plants. The mechanism of CS_2 production occur through hydrolysis of L-djenkolic acid by a cysteine lyase as described by Piluk *et al.*, (1998), who proved that all CS_2-producing plants possess a djenkolic acid and a cysteine lyase. Therefore, *Acacia tortilis* subspecies *radiana* grown in Egyptian deserts may possess cysteine lyase enzyme which capable of CS_2 production in the presence of djenkolic acid as other Mimosoideae CS_2-producers possess.

CS_2 has powerful inhibition effect on microbial population when emitted from roots of *A. tortilis* seedlings in soil types common in Egyptian agro-ecosystems. Our results are in agreement with many studies carried before (e.g., Feng *et al.*, 1998; Hartel and Haines, 1992) proving that CS_2-producing plants inhibit microbial growth in their rhizosphere. However, the appearing general trend of increment in bacterial population size in both planted (despite emitting of CS_2) and control sets maybe explained by assuming that emission of CS_2 is more prevailing during new roots formation which maybe concurrent with harvesting time from soil rhizosphere. Alternatively, soil properties might play a role in such increment as discussed in the studies done by Hartel and Reeder (1993). Presence of other microorganisms that have the ability to degrade emitted CS_2 can also explain the general increment of microbial community during the time course of the study. *Jordan et al.* (1995) isolated four eubacteria strains able to grow on CS_2 as a sole energy substrate for growth and producing carbonyl sulfide and hydrogen disulfide which can further be oxidized to form SO_4^{2-}. The former is considered as source for enhancement of some microbial communities.

The present study showed that growth of nitrifying bacteria is inhibited by emitted CS_2. In a five-days study of the effect of carbon disulfide on nitrification in soils using sealed containers, Bermner and Bundy (1974) reported nearly complete inhibition at nominal concentration as low as 0.5mg/g. The inhibition is more in sandy-loam soil than in other soil types. These data are in agreement with results obtained by Kyveryga *et al.*, (2004). The increase in inhibition could be attributed to increase in CS_2 emission. Staubes *et al.* (1987) found that the increment in soil moisture, temperature, organic content, and light result in a direct increase in the rate of CS_2 production.

Table (5): Growth of *Acacia tortilis* seedlings, grown in different soil types, at different time intervals of transplanting (2, 4, 6 and 8 weeks).

Measuring Intervals (Weeks after Transplanting)	Plant height (cm plant^{-1})±SE			Number of leaves plant^{-1}±SE		
	Sandy-loam	Loamy-sand	Sand	Sandy-loam	Loamy-sand	Sand
Two	3.66±0.18	5.74±0.34	3.62±0.19	4.00±0.16	5.00±0.15	3.00±0.20
Four	4.81±0.16	7.36±0.24	4.70±0.23	5.00±0.16	6.00±0.16	4.00±0.22
Six	5.31±0.19	8.61±0.32	5.57±0.21	6.00±0.12	7.00±0.19	5.00±0.21
Eight	7.08±0.21	12.80±0.52	6.28±0.29	7.00±0.17	9.00±0.24	6.00±0.31

The present study also indicated that soil type is the key factor which regulates CS_2 emission by growing *Acacia* seedlings. The study detected a highly significant variation in total count of microbial flora where sandy-loam soil supported the highest CFU counts g-1. In contrast, sandy soil was significantly the poorest in counts. The soil is an extremely complex physical-chemical-biological system of interactions. Soil organic matter influences many soil properties, including the capacity of soil to supply N, P, and S and trace metals to plants; infiltration and retention of water, degree of aggregation and overall structure that affect air and water relationships; cation exchange capacity; and soil colour, which in turn affects temperature relationships (Nelson and Sommers, 1996). Like clay, humus colloids hold nutrient cations (potassium, calcium, magnesium, etc.) in easily exchangeable form. Organic acids associated with humus also accelerate the release of nutrient elements from mineral structures. In addition, nitrogen, phosphorus, sulphur, and micronutrients are stored as constituents of soil organic matter until released by mineralization. Therefore, *Acacia tortilis* seedlings showed very highly significant variation in growth parameters on different soil types (Table 5) and the highest growth was recorded for seedlings growing on loamy-sand soil.

Finally, the present study recommends that *Acacia tortilis* subsp. *raddian* plants may have an agronomic use in crop rotations or intercropping because root-produced CS_2 may act as a deterrent to soil pathogens.

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تأثير إنبعاث ثانى كبريتيد الكربون من نبات السيال *Acacia tortilis* subsp. *raddiana* على المجتمع الميكروبى فى منطقة محيط الجذر

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الملخص العربـــى

تتميز أشجار السيال التى تنتمى لعائلة البقوليات (تحت العائلة الطلحية Mimosoideae) بكونها متعددة الإستخدامات. فبجانب إستخدامها كغذاء ومأوى للحيوانات فى الصحارى، يستخدمها السكان المحليون من البدو كوقود وكدعامات خشبية. ولقد أشارت بعض الدراسات السابقة على بعض الأشجار التابعة لنفس تحت العائلة إلى إنبعاث ثانى كبريتيد الكربون ذو الرائحة المميزة من جذورها. وترجع أهمية ثانى كبريتيد الكربون فى قدرته على تثبيط نشاط الكائنات الحية الدقيقة فى التربة المحيطة. لذا هدفت هذه الدراسة إلى الاجابة عن ثلاثة تساؤلات هى: (1) هل أشجار السيال النامية طبيعياً فى الصحارى المصرية تنتج ثانى كبريتيد الكربون؟ (2) هل ثانى كبريتيد الكربون - إذا كان ينبعث فعلاً من جذور أشجار السيال – قادراً على تثبيط نشاط الكائنات الحية الدقيقة فى منطقة محيط الجذر فى الظروف الفعلية للتربة السائدة فى الأراضى المصرية؟ وأخيراً (3) هل لهذا الإنبعاث تأثير على نمو أشجار السيال وما هو دور خصائص التربة فى ذلك؟

لتفسير ذلك تم تقدير المحتوى البكتيرى والأكتينوميسيتات والبكتريا المؤكسدة للنشادر فى ثلاثة أنواع من التربة (طفلية – رملية و رملية – طفلية ورملية) تم زراعتها ببادرات السيال ومقارنة ذلك بتربة فى ظروف مماثلة خالية من بادرات السيال. وتم تقدير هذا المحتوى أربع مرات على فترات زمنية متساوية (كل أسبوعين بداية من أسبوعين بعد الشتل). بالإضافة إلى معدل نمو البادرات فى نفس الشتلات فى نفس مواعيد أخذ عينات التربة للتقدير الميكروبى.

ولقد أظهرت النتائج أن نبات السيال تنتج بالفعل ثانى كبريتيد الكربون فى تربة محيط الجذر فى المراحل العمرية التى تم دراستها للبادرات. وأن ثانى كبريتيد الكربون المنتج له تأثير مثبط واضح على المحتوى البكتيرى والأكتينوميسيتات والبكتريا المؤكسدة للنشادر بنسب معنوية متفواته بين أنواع الترب بالغاً أشده بعد ثمانية أسابيع من الشتل بنسبة (99.8%) بالنسبة للبكتريا وبعد ستة أسابيع من الشتل بنسبة (96.1%) بالنسبة للبكتريا المؤكسدة للنشادر وبنسبة (75%) بالنسبة للأكتينوميسيتات وذلك فى التربة الطفلية – الرملية. ولذا فلقد تميزت البادرات النامية فى التربة الطفلية – الرملية بأعلى معدلات نمو.

وأخيراً أوصت الدراسة بإمكانية زراعة أشجار السيال على حافة الحقول أو كمحصول بينى فى الأراضى ذات المحتوى العالى من الميكروبات الممرضة كأحد الوسائل الفعالة للمقاومة الحيوية.