



ANATOMICAL STUDIES OF *JATROPHA CURCAS* LINN. AND *J. GOSSYPYFOLIA* LINN. AND ASSOCIATED MICROFLORA UNDER DIFFERENT WATERING CONDITIONS

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ABSTRACT

Two species of the genus Jatropha namely Jatropha curcas and J. gossypifolia were propagated with seeds and watered at predetermined watering intervals of daily, weekly and monthly. After six weeks of watering the seedlings were harvested, uprooted, anatomically studied and soil samples were analysed in order to isolate the bacteria and fungi present in each sample of daily, weekly and monthly watered soils. The two species possess the same types of stomatal complexes namely paracytic and brachyparacytic but with different stomatal density, index and size. Bacteria (Bacillus subtilis, Clostridium spp. and Micrococcus luteus) and fungi (Penicillium italicum, Aspergillus spp., Mucor mucedo and Penicillium chrysogenum) were implicated to have certain influence on leaf formation, while Bacillus subtilis, Clostridium spp., Proteus mirabilis, Corynebacterium spp, Penicillium chrysogenum, P. italicum and Humicola grisea were found to influence the rates of transpiration along with stomatal features such as density, index and size.

Keywords: *Jatropha curcas, J. gossypifolia, Bacteria, Fungi, growth, Anatomy*

INTRODUCTION

Soil is known to harbour many macro- and micro-flora and fauna. Among the organisms found in the soil are fungi and bacteria. These organisms associate with plants growing in the soil, and most of the time the fungus and plant formed what is known as mycorrhiza; the association is beneficiary to both partners. Pathogenic microorganisms affecting plant health are a major and chronic threat to food production and ecosystem stability worldwide. As agricultural production intensified over past few decades, it has become more and more dependent on agrochemicals as a relatively reliable method of crop protection (Compant *et al.*, 2005). However, increasing use of chemical inputs causes several negative effects, i.e. development of pathogens resistance to the applied agents and their non target environmental impacts (De Weger *et al.*, 1995; Gerhardson, 2002). Furthermore, the growing cost of pesticides, particularly in less-affluent regions of the world, and consumer demand for pesticide-free food has led to a search for substitutes for these products. There are also a number of fastidious diseases for which chemical solutions are few, ineffective, or nonexistent (Gerhardson, 2002). Biological control is thus being considered as an alternative or supplemental way of reducing the use of chemicals in agriculture (De Weger *et al.*, 1995; Gerhardson, 2002; Postma *et al.*, 2003; Welbaum *et al.*, 2004).

Jatropha species are known for their medicinal and ornamental purposes (Morton, 1968). For instance, the leaves of *J. gossypifolia* are used for intermittent fevers, carbuncles eczema, itches, and sores on the tongues of babies, swollen mammae, stomachache,

and venereal diseases (Balee, 1994). The leaf decoction is used for bathing wounds (Morton, 1968). The bark contains the alkaloid jatrophone and a lignin (jatroiden) is found in its stem. Seeds are emetic, purgative, and used for body pain. Leaves and Bark of the plant is antibiotic, insecticidal and used for treating toothache and as blood purifier (Balee, 1994; Oduola *et al.*, 2005). The stem latex of *J. gossypifolia* is routinely used in southern Nigeria by herbalists, rural dwellers and some people in urban centers to stop bleeding from nose, gum and skin (Oduola *et al.*, 2005).

J. curcas has numerous uses. The young leaves may be safely eaten, steamed stewed; pounded leaves are applied near horses' eyes to repel flies in India. *J. curcas* leaves help in dressing wounds. Nuts are sometimes roasted and eaten, although they are purgative. Nuts can be burnt like candlenuts when strung on grass. Also, nuts and seeds are used as contraceptive in south Sudan. Seeds can be used as bio-diesel for any diesel engine without modification. The oil obtained from the seeds has been used for illumination, soap and candle making, cooking, in engines, varnishes, pest control and medicine for treatment of skin diseases, as purgative (Balee, 1994). This study investigated the association between microflora isolated in the soil samples in relation to the growth and development of *J. curcas* and *J. gossypifolia*.

MATERIALS AND METHODS

Soil sampling and propagation of seeds

Soil samples were collected according to the method of the United State Department of Agriculture (1972).



Soil samples with a well drained texture and moderate humus was collected from a fallow land at the University of Ilorin, Main Campus. Eighty (80) grams of the sterilized soil sample was deposited in a sterile plastic bucket and then planted with seeds of *J. curcas*. A duplicate of the set up was made. The same set up was carried out for *J. gossypifolia*. 20cc of sterile distilled water at varying intervals of watering was added to the plastic buckets which represented duplicate buckets for daily watered, weekly watered and monthly watered for *J. curcas* and *J. gossypifolia* respectively. The seeds were planted according to the method described by Alofe and Somide (1982).

Determination of mean leaf area

Mean leaf area was measured using the formula: $L \times B \times 0.75$ (Abayomi and Adedoyin, 2004; Moll and Kamprath, 1977).

Where: L = Length of leaf
 B = Breadth of leaf

Stomatal anatomy

Slides on epidermal peels were prepared from the leaves of *J. curcas* and *J. gossypifolia*. Leaf segments of an area of 1cm square from each species were cut and immersed in concentrated solution of nitric acid for maceration (Alvin and Boulter, 1974) for certain time intervals depending on the species. The adaxial and abaxial surfaces of the macerated leaves were separated with dissecting needles and forceps, and rinsed with clean water. Using the field of view at X40 objective as quadrat, the number of subsidiary cells per stoma was noted to determine the frequency of stomatal complex type present in each species. Terminology used followed those of Dilcher (1974) as well as Metcalfe and Chalk (1988).

Stomatal density was determined as the number of stomata per square millimetre.

Stomatal index was determined as number of stomata divided by number of stomata plus number of epidermal cells multiplied by 100.

Mean stomatal size of a species was determined by measuring length and breadth of a sample of 35 stomata using eye-piece micrometer.

Determination of transpiration rate

A cobalt chloride paper method was used to determine the transpiration rate (Obiremi and Oladele, 2001; Dutta, 2003; Oyeleke *et al.*, 2004). Strips of filter paper of 2cm x 6cm dimension was cut and immersed in 20% cobalt chloride solution. The strips were thoroughly dried in an oven. The dried strips were placed in a sealed, airtight polythene bags and weighed (W1) using mettler balance. They were transferred quickly to the seedlings and placed on the adaxial and abaxial leaf surfaces, and covered with glass slides. The time (in seconds) taken for the strips to turn pink was noted. Once turned pink, the bags were quickly untied and sealed again, and transferred to the laboratory to weighed (W2). Weight of water transpired was determined as W2 minus W1.

Media used and preparations

All the materials used were adequately sterilized before and after use. The media used for the bacterial and fungal analyses of the soil samples were Nutrient agar (Oxoid) and Potato Dextrose agar (Oxoid) respectively. The media were prepared according to the manufacturer's instructions.

Isolation of fungal and bacterial isolates

Soil dilution plating method by spread plating was employed according to the method described by Oyeiola (1992). One gram each of the soil samples planted with *J. curcas* and *J. gossypifolia* for the daily, weekly and monthly watered respectively as serially distributed to 10^{-3} dilution. Zero point one millilitres (0.1ml) was inoculated into nutrient agar and potato dextrose agar plates and was incubated at 37°C for 24 hours and at 25°C for 72 hours for bacteria and fungi respectively. Duplicate plates for each plant were made.

Identification of fungal and bacterial isolates

Colonies seen were repeatedly sub-cultured until pure cultures of isolates were obtained. Fungal identification was carried out using the illustration described by Domsch and Gams (1972). Bacterial isolates were identified using colonial cellular, microscopic and biochemical characteristics with the aid of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbon, 1974).

RESULTS

Early germination of 7 days was observed in *J. gossypifolia* watered weekly while least germination of 9 days occurred in *J. curcas* watered daily (Table 1). Large number of 10 leaves was formed in *J. gossypifolia* watered weekly while least number of 5 leaves was formed in daily watered *Jatropha curcas* and monthly watered *Jatropha curcas* and *J. gossypifolia* (Table 2). Largest leaf area of 6391mm² was observed in *J. gossypifolia* of weekly watering frequency while smallest leaf area of 27.70mm² was observed in *J. curcas* watered monthly (Table 3). High transpiration rate of 10.46×10^{-5} mol./m²/sec⁻¹ occurred in daily watered *J. gossypifolia* and low transpiration rate of 1.09×10^{-9} mol./m²/sec⁻¹ occurred in daily watered *J. curcas* (Table 3).

The two species had the same types of stomatal complex, namely paracytic and brachyparacytic on both leaf surfaces (Figures 1 and 2) with 100% occurrence. High stomatal density of 15mm⁻² occurred on abaxial surface while low stomatal density of 2mm⁻² occurred on adaxial surface of *Jatropha curcas* watered daily and weekly respectively (Table 4). Stomata occupied large area, 17% on abaxial surface of *Jatropha gossypifolia* and lower area, 1.67% on adaxial surface of *Jatropha curcas* watered weekly. Large stomata of 100.75µm occurred on adaxial surface of *Jatropha curcas* while stomata of 23µm occurred on adaxial surface of *Jatropha gossypifolia* of daily and monthly watering frequencies respectively (Table 4).

**Table 1: Germination and leaf emergence in two species of *Jatropha* raised with different watering intervals**

Watering intervals	Species	Time taken for leaf emergence/germination (in days)
Daily	<i>J. curcas</i>	9
	<i>J. gossypifolia</i>	8
Weekly	<i>J. curcas</i>	8
	<i>J. gossypifolia</i>	7
Monthly	<i>J. curcas</i>	8
	<i>J. gossypifolia</i>	8

Table 2: Rate of leaf formation in two species of *Jatropha* raised with different watering intervals

Watering intervals	Species	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Daily	<i>J. curcas</i>	2	1	-	1	1	-
	<i>J. gossypifolia</i>	2	1	1	1	1	1
Weekly	<i>J. curcas</i>	2	1	-	1	1	1
	<i>J. gossypifolia</i>	3	1	1	1	2	2
Monthly	<i>J. curcas</i>	2	-	1	-	-	2
	<i>J. gossypifolia</i>	2	-	1	-	1	1

Table 3: Leaf area and transpiration rate in two species of *Jatropha* raised with different watering intervals

Watering intervals	Species	Mean leaf area (mm ²)	Transpiration rate (mol./m ² /sec ⁻¹)	
			Abaxial	Adaxial
Daily	<i>J. curcas</i>	31.80a	1.09x10 ⁻⁹ a	4.85x10 ⁻¹⁰ c
	<i>J. gossypifolia</i>	3704.00b	10.46x10 ⁻⁵ b	8.65x10 ⁻⁶ c
Weekly	<i>J. curcas</i>	26.70a	8.83x10 ⁻⁹ c	3.91x10 ⁻⁹ b
	<i>J. gossypifolia</i>	6391.00b	7.00x10 ⁻⁶ c	3.00x10 ⁻⁶ b
Monthly	<i>J. curcas</i>	27.70a	8.60x10 ⁻⁹ c	3.78x10 ⁻⁹ b
	<i>J. gossypifolia</i>	687.00b	1.95x10 ⁻⁵ a	1.41x10 ⁻⁵ a

Means along the columns are significantly different at $p < 0.05$

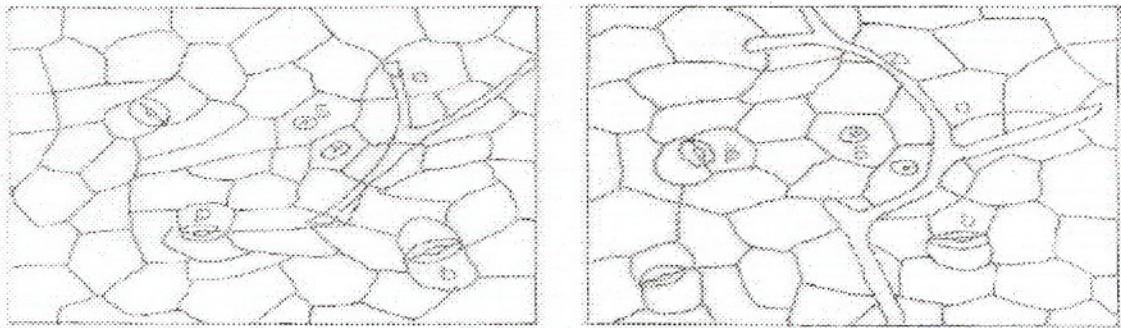
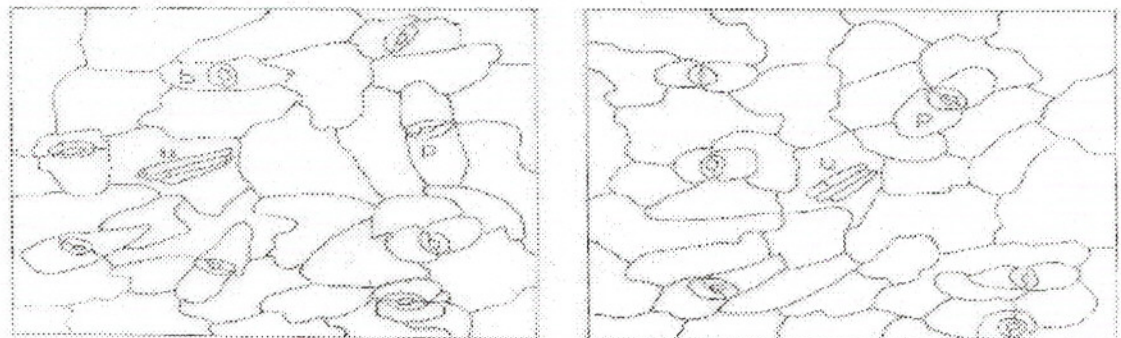
**Figure 1: Surface view of leaf epidermis, abaxial (left) and adaxial (right) of *J. curcas* showing paracytic (p) and brachyparacytic (b) stomata, and long-branched unicellular (a) and scale (s) trichomes x600****Figure 2: Surface view of leaf epidermis abaxial (left) and adaxial (right) of *J. gossypifolia* showing paracytic (p) and brachyparacytic (b) stomata, and unicellular trichomes x600**


Table 4: Stomatal anatomy in two species of *Jatropha* raised with different watering intervals

Watering intervals	Species	Leaf surface	Stomatal Complex Types	Frequency (%)	Stomatal Density (mm ⁻²)	Stomatal Index (%)	Stomatal Size (µm)
Daily	<i>J. curcas</i>	Abaxial	Paracytic and brachyparacytic	100.00	15.00a	9.31a	53.73a
		Adaxial	Paracytic and brachyparacytic	100.00	5.00b	5.18b	100.75b
	<i>J. gossypifolia</i>	Abaxial	Paracytic and brachyparacytic	100.00	9.67b	8.82a	54.28a
		Adaxial	Paracytic and brachyparacytic	100.00	4.46b	3.43b	48.00a
Weekly	<i>J. curcas</i>	Abaxial	Paracytic and brachyparacytic	100.00	11.33a	10.18a	57.45a
		Adaxial	Paracytic and brachyparacytic	100.00	2.00b	1.67b	50.83a
	<i>J. gossypifolia</i>	Abaxial	Paracytic and brachyparacytic	100.00	13.01a	17.00a	66.00b
		Adaxial	Paracytic and brachyparacytic	100.00	10.20a	8.00a	50.04a
Monthly	<i>J. curcas</i>	Abaxial	Paracytic and brachyparacytic	100.00	11.91a	4.89b	75.76b
		Adaxial	Paracytic and brachyparacytic	100.00	3.60b	2.78b	52.25a
	<i>J. gossypifolia</i>	Abaxial	Paracytic and brachyparacytic	100.00	10.02a	4.11b	49.45a
		Adaxial	Paracytic and brachyparacytic	100.00	10.01a	2.01b	23.00c

Means along the columns are significantly different at $p < 0.05$

The results also revealed a wide variety of pathogenic fungi which includes *Penicillium italicum*, *Humicola grisea*, *Penicillium chrysogenum*, *Cladosporium* spp, *Mucor racemosus*, *Mucor mucedo*, *Saccharomyces cerevisiae*, *Geotrichum candidum*, *Aspergillus flavus*, *Candida albicans*, *Botrytis* spp, *Rhizopus stolonifer*, *Rhizopus nigricans*, *Aspergillus niger* and *Aspergillus terreus* for all soil samples watered at varying intervals

(Table 5). Also common to all soil samples analysed were the presence of some pathogenic bacteria which includes *Bacillus subtilis*, *Clostridium* spp, *Proteus mirabilis*, *Micrococcus luteus*, *Corynebacterium* spp, *Klebsiella* spp, *Staphylococcus aureus*, *Lactobacillus* spp, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Bacillus licheniformis* for *J. curcas* and *J. gossypifolia* planted respectively (Table 6).

Table 5: Distribution of fungal isolates in the soil samples planted with *Jatropha* species at different watering intervals

Species	Daily	Weekly	Monthly
<i>J. curcas</i>	<i>Penicillium italicum</i>	<i>Penicillium italicum</i>	<i>Penicillium italicum</i>
	<i>Humicola grisea</i>	<i>Penicillium chrysogenum</i>	<i>Penicillium chrysogenum</i>
	<i>Penicillium chrysogenum</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>
	<i>Cladosporium</i> spp	<i>Humicola grisea</i>	<i>Rhizopus stolonifer</i>
<i>J. gossypifolia</i>		<i>Botrytis</i> spp	<i>Rhizopus nigricans</i>
	<i>Rhizopus stolonifer</i>	<i>Penicillium italicum</i>	<i>Aspergillus terreus</i>
	<i>Penicillium italicum</i>	<i>Penicillium chrysogenum</i>	<i>Rhizopus stolonifer</i>
	<i>Mucor mucedo</i>	<i>Aspergillus flavus</i>	<i>Penicillium italicum</i>
	<i>Geotrichum candidum</i>	<i>Mucor racemosus</i>	<i>Aspergillus flavus</i>
	<i>Penicillium chrysogenum</i>		<i>Mucor mucedo</i>
	<i>Humicola grisea</i>		<i>Saccharomyces cerevisiae</i>
	<i>Aspergillus flavus</i>		
	<i>Candida albicans</i>		
	<i>Aspergillus terreus</i>		

**Table 6: Distribution of bacterial isolates in the soil samples planted with *Jatropha* species at different watering intervals**

Species	Daily	Weekly	Monthly
<i>J. curcas</i>	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>	<i>Clostridium</i> spp
	<i>Clostridium</i> spp	<i>Clostridium</i> spp	<i>Bacillus cereus</i>
	<i>Proteus mirabilis</i>	<i>Bacillus subtilis</i>	<i>Bacillus subtilis</i>
	<i>Micrococcus luteus</i>	<i>Proteus mirabilis</i>	<i>Corynebacterium</i> spp
	<i>Corynebacterium</i> spp	<i>Micrococcus luteus</i>	<i>Pseudomonas aeruginosa</i>
<i>J. gossypifolia</i>		<i>Proteus vulgaris</i>	
	<i>Klebsiella</i> spp	<i>Proteus mirabilis</i>	<i>Micrococcus luteus</i>
	<i>Clostridium</i> spp	<i>Micrococcus luteus</i>	<i>Corynebacterium</i> spp
	<i>Staphylococcus aureus</i>	<i>Clostridium</i> spp	<i>Lactobacillus</i> spp
	<i>Micrococcus luteus</i>	<i>Klebsiella</i> spp	<i>Bacillus cereus</i>
	<i>Lactobacillus</i> spp	<i>Bacillus subtilis</i>	<i>Proteus mirabilis</i>
	<i>Bacillus subtilis</i>	<i>Proteus vulgaris</i>	<i>Bacillus licheniformis</i>
	<i>Proteus mirabilis</i>		<i>Bacillus subtilis</i>
	<i>Pseudomonas aeruginosa</i>		

DISCUSSION

Early germination within 7 days was recorded in the seeds of *J. gossypifolia* watered weekly, least germination was observed in 9 days of *J. curcas* watered daily (Table 1). It shown that in all watering intervals *J. gossypifolia* produced more leaves than the *J. curcas* with higher number of leaves (10 leaves) in weekly watering interval and lower number of 5 leaves in monthly watering interval (Table 2). Different bacteria and fungi were found in association with each of these two species. *J. gossypifolia* which produced higher number of leaves was found to possess in its soil sample some fungal isolates such as *P. italicum*, *Mucor* spp and *Aspergillus* spp and some bacterial isolates like *Micrococcus luteus* and *Bacillus subtilis*, while in *J. curcas* with fewer number of leaves also have *P. italicum* and *P. chrysogenum* fungal isolates, and *Clostridium* spp as bacterial isolate present in its soil sample (Tables 5 and 6). Probably, the presence of certain fungal and bacterial isolates in the soil samples may be interpreted to mean that these microorganisms aid in decomposing organic materials in the soil thereby increasing the soil fertility which will in turn increase plant growth as in *J. gossypifolia* where more leaves are produced. Similarly, some isolates of fungi and bacteria are associated with parameters such as stomatal density, index and size, rate of transpiration of the two species of *Jatropha* (Tables 3 and 4). Such bacterial isolate like *B. subtilis* and fungal isolate *P. chrysogenum*, were found in association with *J. curcas* whose transpiration was low ($1.09 \times 10^{-9} \text{ mol./m}^2/\text{sec}^{-1}$). *B. subtilis*, *Corynebacterium* spp. and *P. mirabilis*, and fungal isolate like *P. italicum* were found in *J. gossypifolia* with low transpiration rate of $1.95 \times 10^{-5} \text{ mol./m}^2/\text{sec}^{-1}$. Bacterial and fungal isolates were also found in species with high rate of transpiration, *B. subtilis*, *P. mirabilis*, *Clostridium* spp. and *H. grisea* are found in *J. curcas* with high rate of transpiration ($8.83 \times 10^{-9} \text{ mol./m}^2/\text{sec}^{-1}$ and $8.60 \times 10^{-9} \text{ mol./m}^2/\text{sec}^{-1}$) and in *J. gossypifolia* with high transpiration rate

($8.65 \times 10^{-6} \text{ mol./m}^2/\text{sec}^{-1}$ and $7.00 \times 10^{-6} \text{ mol./m}^2/\text{sec}^{-1}$).

Since transpiration rate is mostly determined by the rate at which stomatal pores open and close, it may therefore be deduced that the microorganisms found in association with rate of transpiration may aid to accelerate opening or reduce closing of the stomatal pores as the case may be. *J. curcas* and *J. gossypifolia* possess stomata on both leaf surfaces with more on abaxial or lower surface than on the adaxial or upper surface (i.e. they are amphihypostomatic), and both possess the same types of stomatal complexes namely paracytic and brachyparacytic (Table 4). Meanwhile, Carr and Carr (1990) reported that large number of subsidiary cells per stoma may be responsible for a more precise and rapid regulation of stomatal opening, and therefore encourage rapid water loss or transpiration. Earlier studies by Obiremi and Oladele (2001) and Oyeleke *et al.* (2004) confirmed this pattern in some *Citrus* species and afforestation tree species respectively, where species with higher number of subsidiary cells per stoma transpired faster than those with lower number. But the two *Jatropha* species possess the same types of stomata where two subsidiary cells surround the guard cells, the rate of opening and closing of stomatal pores are likely to occur at same rate. However, variations in their transpiration rate may be due to the presence of certain bacterial and fungal isolates in conjunction with stomatal features like density, index and size. The principle behind this mechanism is not yet understood.

The performance of the two *Jatropha* species varied depending on the frequency of watering; daily, weekly or monthly (Tables 1 – 4), with these watering intervals are certain bacteria and fungi (Tables 5 and 6) associated with them. This may translate to mean that even though water play a major role in influencing growth and development of plant, the presence or absence of these microorganisms (bacteria and fungi) also exert some effects on this role.



The roles of microorganisms have been dealt with by many workers. These roles range from beneficial to harmful effects on plants and plant products. The occurrence of *Bacillus subtilis* is in agreement with the work of Mukherjee (1987). *Bacillus subtilis* has been implicated in food spoilage (Ellem, 1990). Some *Proteus* species are normal faecal flora (Ellem, 1990). Some activities of the microbes help in improving soil fertility. Denitrification is also carried out by some members of the genus *Pseudomonas* (Payne *et al.*, 1980). *Staphylococcus* species has been recognised as a virulent and important pathogen implicated in food poisoning. Its capacity to produce human disease has not diminished with the introduction of antibiotics. It also causes scalded skin syndrome which affect children (Prescott *et al.*, 2005). *Bacillus cereus* has also been implicated in diarrhoea, food poisoning and food borne intoxication. It is a common cause of gastroenteritis (Jensen *et al.*, 1997). *Micrococcus luteus* are saprophytic organisms and are often isolated from soil (Ellen, 1990).

The occurrence of Ascomycetes predominate other classes of fungi. The species of Ascomycetes grow on all kinds of foodstuff including butter, bread, fruits and vegetables. They also grow in leather, fabrics and books. This probably explains the reason for their abundance in the soil samples which could be limited to their decomposing abilities. Ascomycetes are heterotrophs and obtain nutrients from dead or living organisms (Camoll and Wickhow, 1992). The occurrence of *Aspergillus* and *Penicillium* is also in agreement with the work of Abdul-Hafez *et al.* (1982). *Candida albicans*, *Saccharomyces cerevisiae* is among the emerging causative agents of opportunistic mycoses in individuals who are immuno-compromised (Sutton *et al.*, 1998). However, some microorganisms have been associated with growth of plants. Some of them are beneficial while others are harmful. Increasing use of chemical inputs causes several negative effects, i.e. development of pathogen resistance to the applied agents and their non target environment impacts (De Weger *et al.*, 1995; Gerhardson, 2002). Furthermore, the growing cost of pesticides, particularly in less-affluent regions of the world, a consumer demand for pesticide-free food has led to a search for substitutes for these products.

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There are also a number of fastidious diseases for which chemical solutions are few, ineffective, or nonexistent (Gerhardson, 2002). Biological control is thus being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture (De Weger *et al.*, 1995; Gerhardson, 2002; Postma *et al.*, 2003; Welbaum *et al.*, 2004). This statement could be relevant in this present study where certain microorganisms were found in association with positive growth and development of plant parts in two *Jatropha* species studied. Plant-growth promoting rhizobacteria (PGPR) encourage plant growth by promoting growth regulators, facilitating nutrient uptake, accelerating mineralization, reducing plant stress, stimulating nodulation, providing nitrogen fixation, promoting mycorrhizal fungi, suppressing plant diseases, and functioning as nematocides (Malik, 2006). As plant roots grow in the soil, bacteria and fungi also grow living off the nutrients leaking from the plant roots. Some of these organisms may be disease causing bacteria, while other organisms may be beneficial bacteria to the plant helping it grow. These beneficial bacteria are called plant growth-promoting rhizobacteria (PGPR) and have been studied for several years. They promote plant growth by providing plant growth regulators, inhibiting disease bacteria, facilitating nutrient uptake, supplying nitrogen, and promoting nodulation in legumes (Malik, 2006). The strongest plant growth promoting (PGR) strain was *Penicillium* sp KC6-W2, which stimulates significant increase in shoot growth and dry mass in seven of the nine experiments conducted (Walkelin *et al.*, 2007). The supply of photosynthates and decaying plant materials to the root-associated microbiota is a key issue for rhizosphere formation and functioning. The release of organic material is known to occur mainly as root exudates, acting as either signals or growth substrates (Barea *et al.*, 2007).

In conclusion, influence of watering frequencies is a critical factor in plant growth and development. Thus, the amount of water in the soil and availability of growth promoting-microorganisms with appropriate stomatal features do influence rate of transpiration and indeed the whole plant growth and development.



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