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EFFICACY OF AM FUNGI IN IMPROVING PETROLEUM CONTENT OF *CALOTROPIS PROCERA* UNDER STRESSED SOIL CONDITIONS

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Overexploitation of petroleum has resulted in global energy crisis. Now-a-days, petroleum is not relied upon as a stable and economic raw material to satisfy tremendous demand for energy. Worldwide efforts are being made to explore the possibility of using Petroleum derived from bio-crude, extracted from the petro crops as an alternate source of energy. The present study has been undertaken to confirm the efficacy of *Glomus claroideum* in improving the biomass and bio-crude content in *Calotropis procera* in a variety of wasteland soils *viz.*, alkali soil, brick-kiln soil, calcareous soil, saline soil and silica mining soil. All the wasteland soils being poor in available plant nutrients, very low microbial population and high pH caused adverse effect on the growth and bio-crude content however; *G. claroideum* improved the biomass and bio-crude content in *C. procera* in all the wasteland soils. The maximum improvement was recorded in calcareous soil and minimum in alkaline soil.

Key words- Biomass, Bio-crude content, *Calotropis procera*, Petro-crop, Wasteland soils

Petroleum currently accounts for a larger share of world energy consumption than any other energy source and is expected to remain in that position throughout the forecast period. World crude oil and liquid fuel consumption grew to 86.7 million barrel/ annum in 2010. EIA expects that world liquid fuel consumption will grow by 1.4 million barrel per day to 1.6 million barrel/day in 2012 (US Energy Information Administration, 2011). Petroleum resources of world may not last forever. There is need to develop alternative and renewable sources of petroleum. Biomass resources are potentially the world's largest renewable energy source. Biomass conversion to fuel and chemicals has once again become an important alternative to replace oil and coal. Petrocrops (wild and waste plants) are renewable source of petroleum hydrocarbons (Charoenpakdee *et al.* 2010). Photosynthetically produced hydrocarbons and phytochemicals from plants have been suggested as substitute for conventional petroleum resources (Calvin 1977, Nielson *et al.* 1977). Latex of petro plants is a rich source of biocrude, a complex mixture of lipids, triglycerides, waxes, terpenoids, phytosterols, polyphenols and other modified isoprenoid compounds that can be catalytically upgraded for use as liquid fuels (Erdman and Erdman, 1981). These are all alternative of petrochemicals and source of long chain aliphatic compounds or liquid fuel.

The selected petrocrop *Calotropis procera* (R.Br.) is a leticiferous shrub growing easily and well adapted to the environmental conditions. The latex of *Calotropis procera* reported to contain Trypsin, Calotropin ($C_{29}H_{32}O_6$), Uscharin ($C_{31}H_{41}O_8$) and Calotoxin ($C_{29}H_{40}O_{10}$). These photosynthetically produced hydrocarbons have been suggested as a substitute for conventional petroleum resources (Erdman and Erdman 1981). Concerted efforts are being made to develop strategies to improve the quality of petro crops through scientific inputs, treatments and practices (Jeffery and Robinson 1979, Kephart 1981, Palsson *et al.* 1981, Campbell 1983, Naqvi *et al.* 1987). Due to economic consideration it is advocated that the petro crops should be raised in the wastelands and problematic soils. Through the use of such areas for bio-crude production, competition with conventional crops and consequent economic disadvantages can be avoided (Bassham 1977, Lepinsky and Kresovich 1979, Slesser and Lewis 1979). Raising of vegetation on wastelands and problematic soils, however, requires a well-planned programme involving use of convenient and economical scientific inputs and strategies which may overcome the adversaries associated with such lands and soils. AMF technology has proved its potential in overcoming various constraints of wastelands and problematic soils (McGee *et al.* 2003, Turnau and Haselwandter 2002, Lalitha and Santhaguru 2010). It has been successfully used in reclamation of various types of degraded soils and in improving the quality of petro crops or their establishment in wastelands and problematic soils

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(Ahiabor and Hirat 1994, Sharma and Roy 1991).

In our earlier study we screened a number of AM fungi for improving the performance of *Calotropis procera* (Chandra *et al.*, 2007) and *Glomus claroideum* was found to be the most efficient. The present study has been undertaken to confirm the efficacy of *Glomus claroideum* in improving the overall performance of *Calotropis procera* in a variety of wasteland and problematic soils.

MATERIALS AND METHODS

Performance of selected AMF in wasteland and problematic soils

Soils of variety of wastelands *viz.*, alkali soil, brick-kiln soil, calcareous soil, saline soil and silica mining soil were collected aseptically in sterilized gunny bags. Seedlings of *Calotropis procera* were raised in sterilized mixture of sand and soil (1:1 ratio) in plastic trays from seeds surface sterilized with 3% sodium hypochlorite solution. The trays were provided with sterilized water and Hoagland solution periodically to ensure proper growth of the seedlings. They were kept under green house conditions.

Later on, seedlings of equal size were transplanted (@ 3 seedlings/pot) in earthen pots (30 X 30 cm) filled with unsterilized soils (@ of 5kg/pot) of wastelands. Before transplanting the seedlings in the pots, upper layer of the soil up to a depth of 4cm was removed and a mycorrhizal inoculum consisting of infected root pieces and spores (250 spores) collected from the pot culture was spread as a layer over the surface. The soil removed earlier was replaced in the pots. The seedlings were transplanted in the pots in such a way that their root system and inoculum layer came in contact with each other and the development of a symbiotic association was fully ensured. A control series without inoculum was also maintained. The pots were watered appropriately and maintained under greenhouse conditions.

Six plants from each treatment as well as control series were uprooted every two months after transplanting, upto eight months. Samples of root with adhering soil were collected and processed for determining the mycorrhizal intensity in roots by the method of Phillips and Hayman (1970) and population of AMF spores in rhizosphere by wet sieving and decanting method given by Gerdemann and Nicolson (1963). Length, fresh and dry weight of roots and shoots, and bio-crude content were also recorded.

For mycorrhizal intensity in roots and spore population in the rhizosphere, average of four samplings was considered to be the mycorrhizal status of plants during a period of six months. The difference in length, fresh and dry weight of root/shoot and bio-crude content of the plants of first and fourth samplings was taken to be their growth performance and bio-crude productivity during the same period.

The efficacy of AMF inoculant for *Calotropis* in the wasteland soil was judged on the basis of improvement it caused in the performance of the petro crop especially in terms of productivity of biomass and bio -crude content over control.

Estimation of bio-crude content

The bio-crude content of plant was estimated by the method outlined by Adams and Mc Chesney (1983). Plant material was collected two and eight months after transplant and dried at 70°C for 48 hours. Five g fine powder of dried plant material was extracted with cyclohexane in Soxhlet's apparatus for 20 hours. The extract was evaporated (100°C, 24 hours) to remove the solvent. The weight of extract was determined and the cyclohexane fraction was calculated and recorded in terms of yield/g dry plant material. The residue of the plant material was dried (4 hours, 100°C) and re-extracted with methanol in Soxhlet's apparatus for 20 hours. The extract was evaporated (100°C, 24 hours). The weight of extract was determined and the methanol fraction (polyphenol and oil) was calculated and recorded in terms of yield/g dry plant material.

Analysis of physico-chemical and microbiological characteristics of different wasteland soils

Samples of soils from different waste lands were collected aseptically and brought to the laboratory in sterilized polythene bags.

Table 1. Analysis of wasteland soils.

Soils	Soil Texture (Groups)	pH	Organic Matter (%)	Nitrogen (%)	Phosphorus (ppm)	Potassium (%)	Fungi ($1X10^4$)	Bacteria ($1X10^6$)	Actinomycetes ($1X10^6$)
Field soil	Loam	6.4	0.620	0.064	16.50	0.012	94.01	14.24	10.54
Alkali soil	Silt loam	9.0	0.275	0.025	8.03	0.010	21.80	7.47	3.11
Brick Kiln Soil	Sandy loam	7.7	0.120	0.012	15.03	0.015	14.88	4.11	2.67
Calcareous Soil	Coarse sandy loam	8.0	0.775	0.078	13.16	0.014	28.98	9.85	5.79
Silica soil	Sand	8.2	0.482	0.050	6.02	0.011	24.39	4.33	3.25
Saline soil	Silt loam	8.6	0.534	0.050	6.02	0.010	40.49	7.47	6.54

The soil texture was determined by International pipette method of mechanical analysis outlined by Daji (1980), pH by digital pH meter, organic matter in terms of organic carbon by the method outlined by Piper (1944), nitrogen content in soil by Kjeldahl method (Jackson 1973), phosphorus content by the method outlined by Mishra (1968), potassium by flame photometer (Jackson 1973).

The population of fungi, bacteria and actinomycetes in soil was determined by soil dilution plate method (Timonin 1940). For estimating the population of fungi, soil suspension of 1:1000 was plated on the dishes containing potato dextrose agar medium, for bacteria and actinomycetes, soil suspension of 1:10,000 was plated on Petri dishes containing soil extract agar medium.

Data collected on the physicochemical and microbiological characteristics of field soil and soils of wastelands are given Table 1.

All the wasteland soils were characterized by high pH (7.7 to 9.0), poor physical properties (silt, sandy, coarse sandy, sand silt etc.), low organic matter, low availability of nutrients like nitrogen, phosphorus and potassium and low microbial population in comparison to field soil.

Statistical analysis

The experiments were performed in pot condition and for each set 4 replicates were taken. All the data were statistically analyzed by the method of Panse and Sukhatme (1985) and the minimum difference required for significance (CD) at 5% level was calculated and recorded.

RESULTS AND DISCUSSION

In the present investigation, the potentiality of *Glomus claroideum* inoculant in improving the biomass and bio-crude content of *Calotropis procera* in the soils of 5 different wasteland soils *viz.* alkali soils, brick-kiln soils, calcareous soils, saline soils and silica mining soils was evaluated under pot condition.

Mycorrhizal Status

The mycorrhizal status in roots of *Calotropis procera* in terms of % root bits infected and spore population in rhizospheric (number/20g air dried soil) soils of different wastelands is presented in Table-2. In general, the AMF inoculants improved the mycorrhization in roots of *C. procera* in all the wasteland soils. However, the improvement varied with the type of wasteland soil. The data revealed that in AMF inoculated series average mycorrhizal intensity in the roots of *C. procera* ranged from 33 to 61% while in non-inoculated series it ranged from 14 to 32%. Likewise AMF spore population in inoculated series ranged from 68 to 100 spores/20 g air dry soils and in non-inoculated series it ranged from 26 to 53 spores/20 g air dry soil. Maximum improvement in mycorrhizal infection and spore population was recorded in calcareous soil while minimum in alkaline soil. Lowest mycorrhizal status in alkaline soils may be attributed to high soil pH, low nutrients and low microbial

Table 2: Effect of *G. claroideum* on the mycorrhizal intensity in the roots, spore population in the rhizospheric soil, length, fresh weight and dry weight of roots and shoots in *Calotropis procera* in different wasteland soils under pot condition.

Soil*/sampling	Mycorrhizal intensity (% root bit infection)	AM spore population (number/20g air dry soil)	Root			Shoot		
			Length (cm)	Fresh weight (g/plant)	Dry weight (g/ plant)	Length (cm)	Fresh weight (g/plant)	Dry weight (g/plant)
AKL (-AMF)	14.75	37.75	17.0	13.33	4.33	27.0	24.57	11.30
BRK (-AMF)	25.75	45.25	26.1	18.29	5.85	38.4	29.39	13.81
CAL (-AMF)	32.75	53.00	27.4	19.78	5.85	40.1	30.19	14.34
SMS (-AMF)	19.50	26.25	20.0	13.83	4.46	29.5	26.60	12.31
SS(-AMF)	20.75	39.75	23.1	18.29	4.90	32.5	28.41	13.82
AKL (+AMF)	32.25	72.50	23.8	17.36	5.88	34.3	28.54	13.70
BRK (+AMF)	50.00	84.00	30.1	23.37	7.80	46.7	37.25	17.84
CAL (+AMF)	61.00	100.2	35.0	30.59	10.53	48.5	39.79	19.12
SMS (+AMF)	33.50	68.50	23.5	18.81	6.32	35.7	30.79	14.78
SS (+AMF)	46.25	78.25	30.4	23.37	7.47	39.8	34.66	16.60

Minimum difference required for significance (CD.) at 5% level
Mycorrhizal intensity: 0.172 AM spore population: 0.680
Root Length: 0.0485 Root Fresh weight: 0.0437 Root Dry weight: 0.0442
Shoot Length: 2.817 Shoot Fresh weight: 0.259 Shoot Dry weight: 0.955 Soils*
AKL- Alkali soil, BRK-Brick-kiln soil, CAL- Calcareous soil, SMS- Silica mining soil,
SS-Saline soil

Table 3: Effect of *G. claroideum* on the biocrude yield of *C. procera* (mg/5g biomass) in different wasteland soils under pot condition.

SOILS*/ SAMPLINGS*	I After two months of transplant		II After eight months of transplant	
	C-HEXANE EXTRACTED	MeOH EXTRACTED	C-HEXANE EXTRACTED	MeOH EXTRACTED
AKL (-AMF)	130	786	137	796
BRK (-AMF)	128	784	136	799
CAL (-AMF)	129	785	138	801
SMS (-AMF)	129	786	134	795
SS(-AMF)	129	785	136	798
AKL (+AMF)	130	788	155	825
BRK (+AMF)	130	787	158	837
CAL (+AMF)	128	786	161	840
SMS (+AMF)	130	787	152	826
SS (+AMF)	130	786	156	830

Minimum difference required for significance (CD.) at 5% level:
C-Hexane Extracted: 0.842 MeOH Extracted: 0.046
Soils*
AKL- Alkali soil, BRK-Brick-kiln soil, CAL- Calcareous soil, SMS- Silica mining soil,
SS-Saline land

ROOT GROWTH **SHOOT GROWTH**

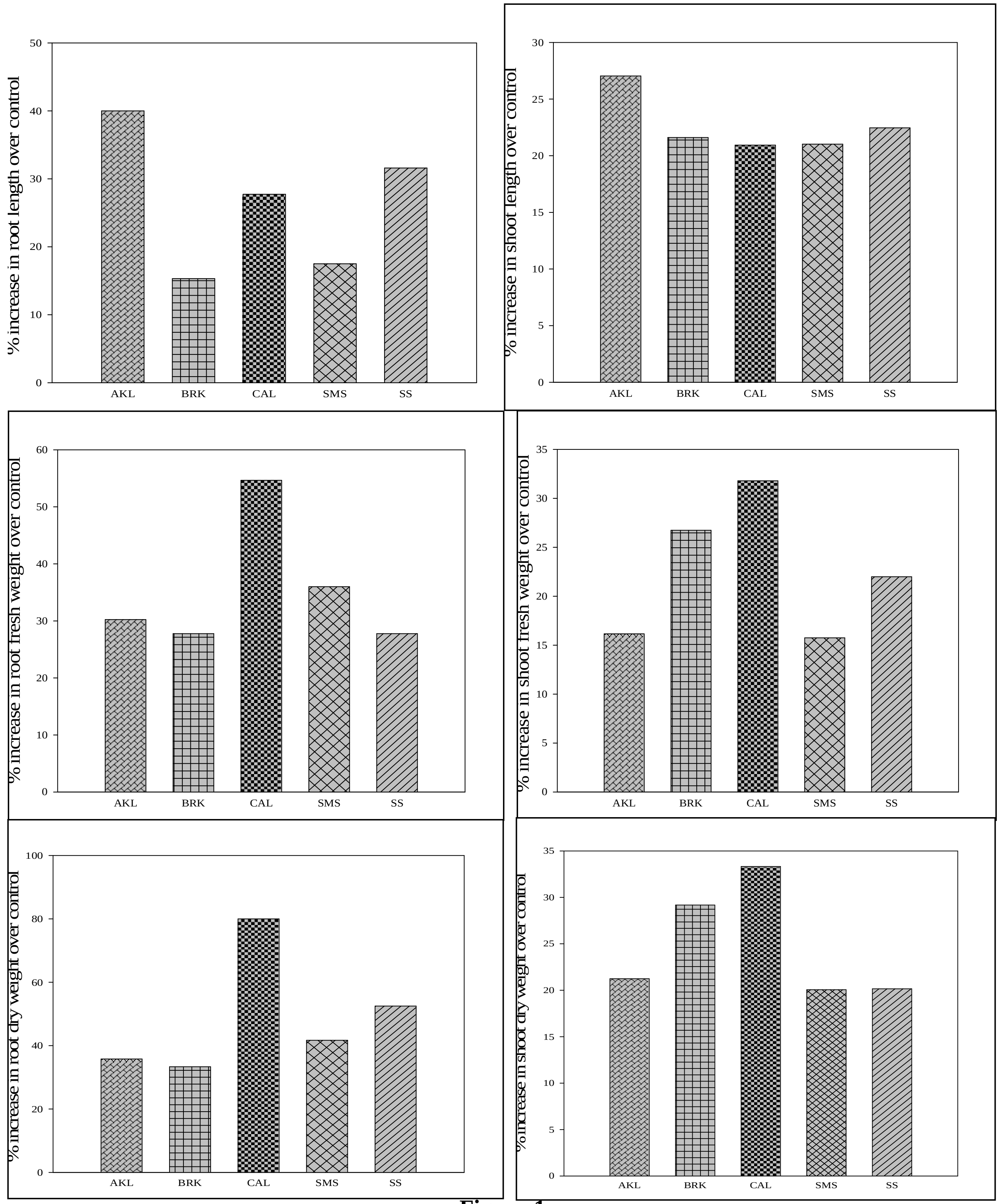

Figure-1

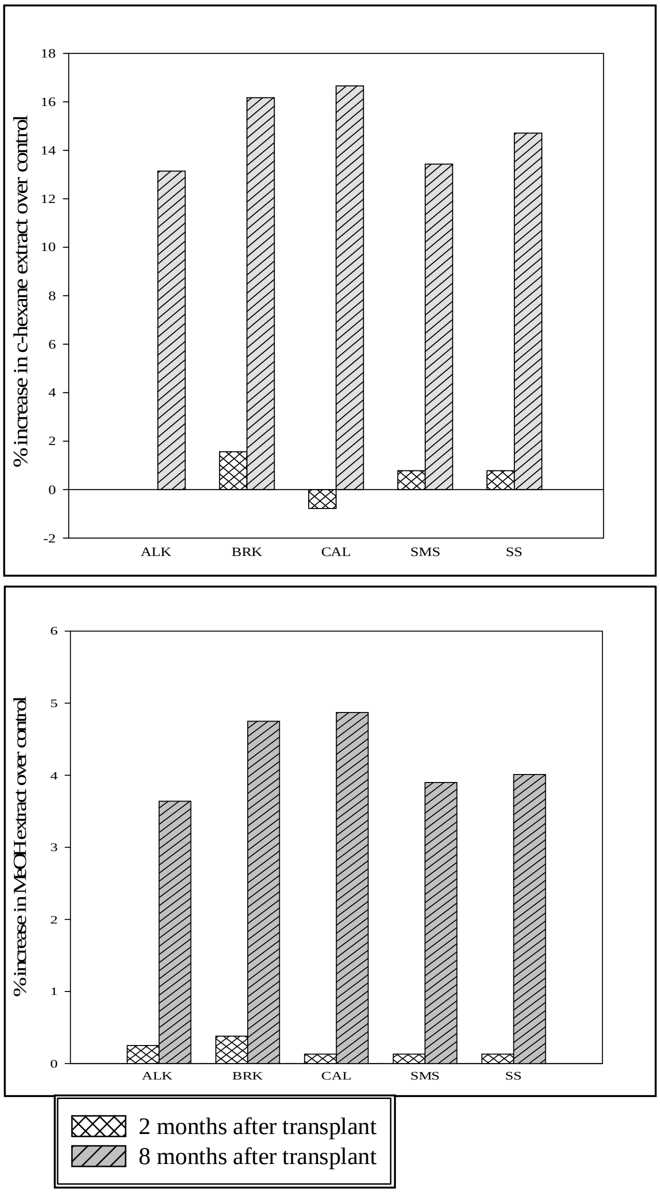

Figure-2

population (Aliasgharzadeh *et al*. 2001, Wang *et al*. 2004).

Growth of roots and shoots

Growth of roots and shoots of *C. procera* in terms of their improvement in length (cm), fresh weight (g/plant) and dry weight (g/plant) in different wasteland soils with *G. claroideum* are presented in Table 2 and Figure 1. In general AMF inoculant caused an improvement in growth of roots and shoots of *C. procera* in all types of wasteland soils. However, the extent of improvement varied with the types of wasteland soils. The data revealed that in AMF inoculated series root length ranged from 23.8 cm to 35.0 cm, fresh weight from 17.36 g/plant to 30.59 g/plant and dry weight 5.88 g/plant to 10.53 g/plant, while in non -inoculated series root length ranged from 17.0 cm to 27.4 cm, fresh weight 13.33 g/plant to 19.78 g/plant and dry weight 4.33 g/plant to 5.85 g/plant. Likewise shoot length in inoculated series ranged from 34.3 cm to 48.5 cm, shoot fresh weight from 28.54 g/plant to 39.79 g/plant and dry weight from 13.70 g/plant to 19.12 g/plant and in non-inoculated series, shoot length ranged from 27.0 cm to 40.1 cm, fresh weight 24.57 g/plant to 30.19 g/plant and dry weight from 11.30 to 14.34 g/plant. The highest improvement in fresh weight and dry weight of the roots and shoots was recorded in calcareous soil while lowest improvement was recorded in alkaline soil. Per cent increase in root/shoot biomass in AMF inoculated plants over control (non-inoculated plants) is presented in Figure-1. It shows highest improvement in calcareous soil. In many earlier reports, efficacy of AMF inoculants in improving the performance of their respective hosts has been shown to vary with the soils of wastelands (Allen and Cunningham 1983, Bloss 1985, Pfeiffer and Bloss, 1988, Logan *et al.* 1989).

Bio-crude Yield

The biocrude yield of the *C. procera* in soils of different wastelands is presented in Table-3. The data show that the % increase in bio-crude content in AMF inoculated plants over control (non-inoculated plants) was negligible in the beginning (after two months of transplant), however, it increased up to 16% in C-Hexane extract and more than 5% in MeOH extract over control after eight months of transplant (Figure 2). Improvement in the bio-crude yield (Cyclo-hexane extracted and Methanol extracted) of the *C. procera* was observed in all the wasteland soils but the highest improvement was recorded in calcareous soil.

Data shows that the AMF inoculant improved not only the mycorrhizal status of the *C. procera* in soils of different wastelands but also their biomass and biocrude yield. However, with AMF inoculant i.e., *G. claroideum*, *Calotropis* showed its best performance in the calcareous soil. According to Joshi and Singh

(1995) and Charoenpakdee *et al.* (2010) the improvement in growth parameters may not be because of inoculated AMF alone but also its interaction with indigenous AMF fungi, physic-chemical characteristics of the soils and other microbes present. The soils of different wastelands included in the present study differed in their physico-chemical and microbial characteristics. Thus, the difference in magnitude of improvement in the performance of *Calotropis* due to its AMF inoculant in the soils of different wastelands may be safely attributed to the differences in their physico-chemical and microbiological characteristics.

High pH (9) of the alkali soils along with excessive concentration of soluble salts and exchangeable sodium, unavailability of nutrients like nitrogen, phosphorous, potassium etc. caused adverse effect on the growth of the plants. Without AMF inoculant *Calotropis* suffered in the alkali soil due to above constraints. However, with *G. claroideum* it showed improvement not only in its mycorrhizal status, but also in its biomass and bio-crude content. AMF inoculants have been shown to help the plants to overcome their constraints of alkali soil by increasing their tolerance to high pH (Poss *et al.* 1985, Medeiros *et al.* 1994) and making the soil more porous thereby increasing the water absorption (Thomas *et al.* 1986, Logan *et al.* 1989).

Soil in brick-kiln area is characterized by lack of available nutrients and lower population of microbes. Some of the nutrients required by plants are in unavailable form. In the present study, AMF inoculant caused an improvement in the mycorrhizal status, biomass and bio-crude content of the petro crop in soil of brick-kiln area indicating that it could overcome some of the constraints of this soil.

Calcareous soil is characterized by high pH value (8.0) and alkalinity of soil, creating a poor nutrient status of some of the nutrients like iron, manganese, boron, copper, zinc, phosphorus, and potassium etc. Findings of present study are supported by many earlier reports where AMF fungi have been shown to help the plants in overcoming the constraints of calcareous soil (Medeiros *et al.* 1994).

Saline soil differs from alkali soil in having pH less than 8.5. High salt concentration and pH are the major constraints for normal growth of plants in this soil. Soil structure is ill developed therefore, drainage and aeration are poor and oxygen supply is considerably reduced. Many reports are available where AMF fungi have shown their potentiality to help the plants to overcome the constraints of saline soil (Porras-Soriano *et al.* 2009, Poss *et al.* 1985, Bolan *et al.* 1984) and ensure better uptake of nutrients (Hirrel and Gerdemann 1980, Allen and Cunningham 1983, Pfeiffer and Bloss 1988, Rosendahl and Rosendahl 1991, Dixon *et al.* 1993, Phosri *et al.* 2010).

Soils of silica mining sites contain silica, sand and other coarser particles. Their microbial population is very low in comparison to field soil and their nutrient status in terms of macronutrients like nitrogen, phosphorus and potassium is poor. Soil reaction is moderately alkaline (pH 8.2). High pH, poor physical property and less availability of nutrients are the major constraints of silica mining soil. The water holding capacity is low due to high porosity. Better performance of *Calotropis* in soils of silica mining site may be attributed to an improved water flow through AMF hyphae or a secondary response due to improved nutrition or physiological alteration of the host (Powell and Bagyaraj 1984, Tisdall 1994). In the present study, variation in the extent of improvement in the performance of *Calotropis* due to selected AMF inoculant in different wasteland soils is expected. This is in conformity with the fact that host genotype and soil characteristics govern the activity of AMF fungi.

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