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SELECTION OF ARBUSCULAR MYCORRHIZAL INOCULANTS FOR IMPROVING BIOMASS AND HYDROCARBON CONTENT OF THREE POTENTIAL PETROCROPS

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Now a day, petroleum is not relied upon as a stable and economic raw material to satisfy tremendous demand for energy. There is a need to develop alternative and renewable sources of petroleum. Biomass conversion to fuel and chemicals is once again becoming an important alternative to replace petroleum and coal. Petrocrops (wild and waste plants) are renewable sources of petroleum hydrocarbons. *Calotropis procera* (Ait.) R. Br., *Euphorbia tirucalli* L., *Pedilanthus tithymaloides* (L.) Poir.,var. *cuculatus* are some of the potential candidates for petrofarming. The latex obtained from these plants may be hydrocracked to obtain hydrocarbons. AM technology plays a vital role in mineral cycling, energy flow and plant establishment. This technology has proved to be helpful not only in establishment but also the growth and productivity of plants.

The present study was undertaken to select efficient arbuscular mycorrhizal fungi to improve the overall performance of these petrocrops especially in terms of biomass as well as their biocrude content. The magnitude of mycorrhizal status varied with AM fungi and the petrocrops. All the AM inoculants showed an improvement in the length, fresh and dry weight of roots and shoot as well as in biocrude content. The findings suggest that *Glomus claroideum, G. mosseae* and *G. aggregatum* proved highest potential for improving the overall performance of *Calotropis procera, Euphorbia tirucalli* and *Pedilanthus tithymaloides* var. *cuculatus* respectively.

Key words: AM fungi, Petrocrops, Hydrocarbons

Worldwide efforts are being made to explore the possibility of using Petroleum derived from biocrude, extracted from the petrocrops as an alternate source of energy. Petrocrops include those plants, which may be used as substitute of petroleum. The latex of petrocrops yields variety of products that are competitive with the synthetic petrochemicals, collectively called as "hydrocarbons". Along with hydrocarbons, latex of petroplants 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 petrocrops *viz. Calotropis procera* (Ait.) R. Br., *Euphorbia tirucalli* L., *Pedilanthus tithymaloides* (L.) Poir., var. *cuculatus* are leticiferous shrubs growing easily and well adapted to the environmental conditions. The latex of *Pedilanthus tithymaloides* reported to contain polyhydrocarbons, oils and polyphenols (Mclaughlin and Hoffmann, 1982; Srivastava, 1989) while Trypsin, Calotropin ($C_{29}H_{32}O_6$), Uscharin ($C_{31}H_{41}O_8$) and Calotoxin ($C_{29}H_{40}O_{10}$) are present in the latex of *Calotropis procera* (Murti and Sheshadari, 1945). The latex of *Euphorbia tirucalli* contains low molecular weight polyhydrocarbons (Nielson *et al.*, 1979), Traxasferol ($C_{30}H_{50}CH_3OH$) Isoeuphrol and Tirucallol ($C_{30}H_{50}O$)(Haines and Warren, 1950). These photosynthetically produced hydrocarbons have been suggested as a substitute for conventional petroleum resources (Erdman and Erdman, 1981).

The economics of hydrocarbon production from plant material depends on the rate of biomass production and cost of conversion (Weisz and Marshall, 1979). In view of this, it has been emphasized that attempts must be made to improve the biomass of petrocrops through scientific inputs, treatments or practices. AM technology has proved its importance in increasing the growth, biomass and productivity of plants. The present study has been undertaken to select efficient AM mycorrhizal fungi

to improve the overall performance of these plants especially in terms of biomass as well as their biocrude content.

MATERIALS AND METHODS

Selection of AM inoculants for selected petrocrops: Three petrocrops (*Calotropis procera* (Ait.) R. Br., *Euphorbia tirucalli* L. and *Pedilanthus tithymaloides* (L.) Poir., var. *cuculatus*) were included in this study . They were selected on the basis of the properties of the respective constituents, which are used in the petroleum industry. In order to select suitable AM inoculants for them, the six species of *Glomus*, *viz. G. aggregatum, G. claroideum, G. fasciculatum, G. macrocarpum, G. mosseae, G. multicaule* which were recovered from the rhizosphere of petrocrops under natural conditions, were evaluate for their potentiality to improve the productivity of above mentioned petroplants in terms of increase in the biomass and biocrude content.

Seedlings of all the three petrocrops were raised in sterilized mixture of sand and soil (1:1 ratio) in plastic trays. The seedlings of *Calotropis procera* were raised from seeds surface sterilized with sodium hypochlorite solution, while those of other two petrocrops from cuttings treated with a solution of Gibberellic acid. The seedlings were watered with sterilized water and Hoagland solution periodically to ensure proper growth of the seedlings. They were kept in greenhouse under aseptic conditions. Later on, seedlings of equal size were transplanted in sterilized/unsterilized field soil (loamy, pH 6.4, organic mater 0.06%, 4 AM spores/g air dried soil) in earthen pots (30x30 cm) at the rate of 3 seedlings/ pot. Twenty-four pots (with a control series without inoculum) were maintained for each treatment.

Plants from treatment as well as control series were uprooted after every two months 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 AM spores in rhizosphere by the wet sieving and decanting method given by the Gerdemann and Nicolson (1963) using the sieves of different sizes (500 µm, 210 µm, 150 µm, 90 µm and 60 µm). Length, fresh and dry weight of roots and shoots, and biocrude content were also recorded.

Estimation of biocrude content : The biocrude content of plants was estimated by the method outlined by Adams and McChesney (1983). 5 g fine powder of dried plant material (70^0C for 48 hours) was extracted with cyclohexane in Soxhlet's apparatus for 20 hours. The extract was evaporated (100^0C, 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 plant material was dried (4 hours, 100^0C) and re-extracted with methanol in Soxhlet's apparatus for 20 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.

Statistical analysis : The experiments were performed in pot condition and for each set 7 replicates were taken. Experiment was designed as Randomized block method. All the data were analyzed statistically by the method of analysis of variance (Panse and Sukhatme, 1954) and standard error was calculated.

RESULTS

For the establishment of AM infection in the roots of the host a lag period is required. According to Black and Tinker (1979) soon after the seedling emergence the rate of infection and its level is low. The assessment of the real benefit from the symbiont to the host can be made only after the lag period when the infection has established well. To overcome this constraint, the first two months of the experimental period were avoided in the present investigation while considering the benefits from the AM inoculant to the petrocrop.

Mycorrhizal Status : The mycorrhizal status in

Table 1: Effect of different AM inoculants (*Glomus spp.*) on the length, fresh and dry weight of roots and shoots of three petrocrops in sterilized and unsterilized soil under pot conditions.

	AM inoculants/ Samplings*	STERILIZED SOIL						UNSTERILIZED SOIL					
		Length		*Fresh weight*		*Dry weight*		*Length*		*Fresh weight*		*Dry weight*	
		Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
C.procera													
	Control	24.1	26.0	13.08	24.14	3.95	11.10	27.8	33.9	16.22	30.14	5.34	14.17
	G.aggregatum	26.0	35.1	15.56	29.43	4.82	13.83	29.0	37.0	18.42	32.40	5.71	15.23
	G.claroideum	**31.0**	**39.4**	**17.92**	**35.00**	**5.73**	**16.55**	**31.2**	**43.6**	**21.00**	**37.32**	**6.83**	**18.10**
	G.fasciculatum	26.1	36.1	16.34	31.00	5.06	14.57	28.6	41.0	19.00	34.66	5.89	16.01
	G.macrocarpum	25.4	33.0	15.52	27.62	4.81	12.98	27.9	34.2	13.41	30.00	5.49	14.70
	G.mosseae	27.2	35.1	16.05	31.00	4.97	14.57	28.9	38.2	17.00	33.41	5.37	15.70
	G.multicaule	24.9	33.0	15.46	28.46	4.79	13.38	29.0	36.4	19.43	32.10	6.02	15.01
E.tirucalli	Control	30.4	40.0	10.41	56.00	3.58	13.30	30.0	41.6	11.53	59.92	3.83	14.06
	G.aggregatum	32.9	43.6	13.07	66.00	3.49	16.29	33.6	41.9	13.00	65.15	4.29	16.00
	G.claroideum	32.0	44.2	12.99	62.52	3.41	15.61	32.2	40.0	12.12	62.12	4.10	15.54
	G.fasciculatum	32.6	44.5	13.17	64.00	3.61	15.95	33.6	40.8	12.44	63.02	4.23	15.76
	G.macrocarpum	32.1	42.2	12.28	60.10	4.05	14.81	32.1	39.6	11.30	59.00	3.61	14.76
	G.mosseae	**35.0**	**51.6**	**13.73**	**70.42**	**4.94**	**18.46**	**35.9**	**51.2**	**13.40**	**67.21**	**4.70**	**19.92**
	G.multicaule	33.8	42.2	13.07	60.31	4.44	15.11	33.9	40.5	12.13	60.15	4.00	15.04
P.tithymaloides var. cuculatus	Control	28.0	39.2	14.00	21.00	4.20	8.19	32.4	49.3	16.42	27.00	5.09	11.48
	G.aggregatum	**38.0**	**57.1**	**17.58**	**30.00**	**6.38**	**12.60**	**34.6**	**58.9**	**22.40**	**33.50**	**7.39**	**14.41**
	G.claroideum	32.1	44.2	16.34	26.42	5.00	10.83	36.0	53.2	18.02	28.41	5.59	11.65
	G.fasciculatum	32.1	46.1	16.24	27.00	5.13	11.07	38.0	54.6	18.50	29.01	5.73	11.90
	G.macrocarpum	30.1	45.7	15.22	26.50	4.94	10.87	35.2	51.7	17.25	27.22	5.34	11.56
	G.mosseae	36.0	46.2	17.01	27.50	5.47	12.09	39.0	57.0	20.46	32.92	6.38	13.50
	G.multicaule	31.1	45.6	16.27	28.00	5.26	11.48	37.1	54.0	18.22	29.00	5.56	11.90

Samplings*: Average of eight months

Standard error in Sterilized soil

Calotropis procera	*Euphorbia tirucalli*	*Pedilanthus tithymaloides* var. *cuculatus*
Root Length: 0.329	Root Length: 0.663	Root Length: 0.374
Shoot Length: 0.829	Shoot Length: 0.985	Shoot Length: 0.830
Root Fresh wt.: 0.010	Root Fresh wt.: 0.119	Root Fresh wt.: 0.138
Shoot Fresh wt: 0.142	Shoot Fresh wt: 0.336	Shoot Fresh wt: 0.241
Root Dry wt.: 0.053	Root Dry wt.: 0.079	Root Dry wt.: 0.096
Shoot Dry wt. :0. 072	Shoot Dry wt.: 0.041	Shoot Dry wt.: 0.097

Standard error in Unsterilized soil

Calotropis procera	*Euphorbia tirucalli*	*Pedilanthus tithymaloides* var. *cuculatus*
Root Length: 0.364	Root Length: 0.467	Root Length: 0.338
Shoot Length: 1.026	Shoot Length: 1.631	Shoot Length: 0.801
Root Fresh wt.: 0.064	Root Fresh wt.: 0.097	Root Fresh wt.: 0.076
Shoot Fresh wt: 0.061	Shoot Fresh wt: 0.529	Shoot Fresh wt: 0.431
Root Dry wt.: 0.007	Root Dry wt.: 0.082	Root Dry wt.: 0.059
Shoot Dry wt.: 0.059	Shoot Dry wt.: 0.013	Shoot Dry wt.: 0.008

roots of different petrocrops in terms of % root bits infected and spore population in rhizosphere (number/20g air dried soil) in unsterilized and sterilized soils induced by different AM inoculants are presented in Table 1. All the AM inoculants improved the mycorrhization but the magnitude of improvement varied with the petrocrops, sampling periods and AM inoculants. Results show that in unsterilized soil, with the application of *G. macrocarpum*, *G. mosseae* and *G. multicaule*

Table 2: Effect of different AM inoculants (*Glomus spp.*) on the mycorrhizal intensity in the roots and spore population in the rhizosphere of three petrocrops in sterilized and unsterilized soil under pot conditions

	AM inoculants/ Samplings*	STERILIZED SOIL		UNSTERILIZED SOIL	
		Mycorrhizal intensity (% root bits infected)	AM spore population (Number/20 g air dry soil)	Mycorrhizal intensity (%root bits infected)	AM spore population (Number/20 g air dry soil)
C. procera	Control	.3	9	44.5	88
	G.aggregatum	46.8	83	49.0	117
	G.claroideum	**56.8**	**96**	**57.8**	**144**
	G.fasciculatum	42.8	80	47.8	113
	G.macrocarpum	37.8	70	41.0	102
	G.mosseae	39.5	83	41.5	106
	G.multicaule	37.0	73	41.0	95
E.tirucalli	Control	1.8	8	33.7	105
	G.aggregatum	42.0	102	36.5	133
	G.claroideum	34.0	99	31.5	123
	G.fasciculatum	40.3	104	35.8	139
	G.macrocarpum	32.0	91	29.8	116
	G.mosseae	**52.5**	**121**	**48.5**	**158**
	G.multicaule	33.9	92	29.3	131
P.tithymaloides var. cuculatus	Control	3.0	9	34.0	96
	G.aggregatum	**52.5**	**113**	**54.8**	**162**
	G.claroideum	33.3	92	36.0	126
	G.fasciculatum	36.0	99	42.75	136
	G.macrocarpum	29.8	89	32.3	113
	G.mosseae	38.5	101	42.8	135
	G.multicaule	31.5	92	28.3	114

Samplings*: Average of eight months

Standard error in Sterilized soil

Calotropis procera
AM infection: 0.833
AM spore population: 1.106

Euphorbia tirucalli
AM infection: 0.885
AM spore population: 1.114

Pedilanthus tithymaloides var. cuculatus
AM infection: 1.123
AM spore population: 0.972

Standard error in Unsterilized soil

Calotropis procera
AM infection: 1.048
AM spore population: 0.790

Euphorbia tirucalli
AM infection: 0.9194
AM spore population: 0.856

Pedilanthus tithymaloides var. cuculatus
AM infection: 1.234
AM spore population: 0.953

mycorrhizal intensity in *C. procera* and *E. tirucalli* was less while *G. claroideum, G. mosseae* and *G. aggregatum* exhibited highest potential to develop mycorrhization in the roots and to produce spores in the rhizosphere of *C. procera*, *E. tirucalli*, *P. tithymaloides* var. *cuculatus* respectively.

Growth of roots and shoots : The effect of different AM inoculants on the growth of roots and shoots of selected petrocrops in terms of their improvement in length (cm), fresh weight (g/plant) and dry weight (g/plant) in sterilized and unsterilized soil with selected AM inoculants are presented in Table 2. All the AM inoculants caused an improvement in growth of roots and shoots of all the petrocrops in sterilized/unsterilized soil. However, the magnitude of improvement varied with the petrocrops and inoculants. On one side, *G. claroideum, G. mosseae* and *G. aggregatum* exhibited highest potential to improve the growth of *C. procera*, *E. tirucalli*, *P.*

Table 3: Effect of different AM inoculants (*Glomus* spp.) on the biocrude yield of *Calotropis procera* in mg/5g biomass in unsterilized soil under pot condition.

Am Inoculant/ Sampling*	UNSTERILIZED SOIL			
	I		IV	
	C-Hexane Extracted	MeOH Extracted	C-Hexane Extracted	MeOH Extracted
Control	130	807	138	839
G. aggregatum	132	808	140	842
G. claroideum	**137**	**809**	**151**	**848**
G.fasciculatum	132	807	141	837
G.macrocarpum	129	800	134	827
G. mosseae	130	807	141	842
G. multicaule	127	793	134	821

Minimum difference required for significance (C.D.) at 5% level :
C-Hexane Extracted: 0.609
MeOH Extracted: 0.627

SAMPLING*:
I - Two months after transplant
IV- Eight months after transplant

Table 4: Effect of different AM inoculants (*Glomus* spp.) on the biocrude yield of *Euphorbia tirucalli* in mg/5g biomass in unsterilized soil under pot condition.

Am Inoculant/ Sampling*	UNSTERILIZED SOIL			
	I		IV	
	C-Hexane Extracted	MeOH Extracted	C-Hexane Extracted	MeOH Extracted
Control	225	950	250	986
G. aggregatum	230	950	257	988
G. claroideum	211	955	244	992
G.fasciculatum	231	957	255	995
G.macrocarpum	232	958	254	990
G. mosseae	**239**	**969**	**268**	**999**
G. multicaule	234	956	259	992

Minimum difference required for significance (C.D.) at 5% level:
C-Hexane Extracted: 0.455
MeOH Extracted: 0.468

SAMPLING*:
I - Two months after transplant
IV- Eight months after transplant

tithymaloides var. *cuculatus* respectively while on other side *G. macrocarpum* less effect on all the growth parameters especially in *C. procera* and *E. tirucalli*.

Biocrude Yield : All the inoculant showed an improvement in the biocrude yield (Cyclo-hexane extracted and Methanol extracted) of the selected petrocrops in unsterilized soil (Table 3-5). However, the extent of improvement in the biocrude yield varies with the petrocrops and inoculants. The results presented in the Tables show that out of six AM inoculants only two *viz., G. multicaule* and *G. macrocarpum* show slight decreasing trend in the C-Hexane as well as MeOH extracted biocrude yield in *C. procera* and in MeOH extracted biocrude yield of the *P. tithymaloides* var. *cuculatus*. *G. claroideum, G. mosseae* and *G. aggregatum* showed highest potential to improve the biocrude yield of *C. procera, E. tirucalli, P. tithymaloides* var. *cuculatus* respectively.

DISCUSSION

Experiment in sterilized soil do not provide the real assessment of the potential of AM inoculants in improving the performance of the host because under field conditions they have to interact with the other AM fungi and variety of the other microbes present in the soil. In view of this all the studies with sterilized soil are of no relevance as far as the field application is concerned. In the present study, the AM inoculants for the petrocrops were selected by examining their effect on the performance of petrocrop in sterilized as well as unsterilized soil. Many recent studies have shown that the AM inoculants introduced in unsterilized soil compete with the indigenous AM fungi and other microbes and cause a favourable effect on the growth and performance of the host plant (Khan,1972; Bagyaraj and Manjunath, 1980; Mclean *et al.*,1994). According to Abbott and Robson (1984), the experiment designed to evaluate the potential of AM inoculants in improving the performance of plants and to select efficient inoculants for them should be conducted in sterilized as well as unsterilized soils so as to get a clear picture of interactions between indigenous and introduced AM fungi. In the present study, the AM inoculants for the petrocrops were selected by examining their effect on the performance of petrocrops in sterilized as well as unsterilized soils. However, more emphasis was given on the results of the experiments in

Table 5: Effect of different AM inoculants (*Glomus* spp.) on the biocrude yield of *Pedilanthus tithymaloides* var. *cuculatus* in mg/5g biomass in unsterilized soil under pot condition.

Am Inoculant/ Sampling*	UNSTERILIZED SOIL			
	I		IV	
	C-Hexane Extracted	MeOH Extracted	C-Hexane Extracted	MeOH Extracted
Control	430	715	452	738
G. aggregatum	**439**	**721**	**469**	**748**
G. claroideum	432	716	456	738
G.fasciculatum	433	717	460	738
G.macrocarpum	437	718	458	735
G. mosseae	433	717	461	741
G. multicaule	432	716	456	735

Minimum difference required for significance (C.D.) at 5% level:
C-Hexane Extracted: 0.576
MeOH Extracted: 0.540

SAMPLING*:
I - Two months after transplant
IV- Eight months after transplant

unsterilized soil.

In AM symbiotic association, the extent of benefits from the symbiont to the host does not depend only on its potential to colonize the root but also on its efficiency to absorb the nutrients as well as water from soil and transfer it to the host. According to Daniel and Menge (1981) the level of root colonization by AM fungi is not always related to the extent of benefit offered by them to their host. Therefore, in the present study the potentiality of AM inoculants was adjusted on the basis of their efficacy in improving the performance of the petrocrops in terms of growth of roots and shoots, as well as biocrude content. However, their potentiality to colonize the roots and produce spores was also given due consideration.

All the six species of *Glomus* forming mycorrhizal association with petrocrops in nature could induce mycorrhization in selected petrocrops in sterilized soil under pot conditions showing a clear compatibility between them and petrocrops. Their introduction improved the mycorrhizal status of the petrocrops in unsterilized soil indicating that they could adjust with the indigenous AM fungi and other microbes in the soil and could associate with petrocrops symbiotically. A careful analysis of the result show that all three petrocrops responded to all the six AM inoculants favorably in both sterilized as well as unsterilized soils. However, *G. claroideum, G. mosseae* and *G. aggregatum* may be recommended as possible AM inoculants for *C. procera, E. tirucalli, P.tithymaloides* var. *cuculatus* respectively.

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