Bioengineering of Crops for Biofuels and Bioenergy

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Abstract

Biomass contributes a significant share of global primary energy consumption and its importance is likely to increase in future world energy scenarios. Current biomass use, although not sustainable in some cases, replaces fossil fuel consumption and results in avoided CO_2 emissions, representing about 2.7 to 8.8 % of 1998 anthropogenic CO_2 emissions. The global biomass energy potential is large, estimated at about 104 EJ/a. Hence, biomass has the potential to avoid significant fossil fuel consumption, potentially between 17 and 36 % of the current level and CO_2 emissions potentially between 12 and 44 % of the 1998 level. Modern biomass energy use can contribute to controlling CO_2 emissions to the atmosphere while fostering local and regional development. There is significant scope then to integrate biomass energy with agriculture, forestry and climate change policies.
Further the advantages from utilization of biomass include: liquid fuels produced from biomass contain no sulfur, thus avoiding SO_2 emissions and also reducing emission of $N0_x$. The production of compost as a soil conditioner avoids deterioration of soil. Improved agronomic practices of well managed biomass plantations will also provide a basis for environmental improvement by helping to stabilize certain soils, avoiding desertification which is already occurring rapidly in tropical countries. The creation of new employment opportunities within the community and particularly in rural areas will be one of the major social benefits. The specific research work carried out in the areas of biomass production and utilization in less fertile areas will provide satisfactory answers to the double challenge of energy crisis and forced deforestation in the country and semi-arid and arid regions of Rajasthan. The possibility of conversion of biomass into liquid fuels and electricity will make it possible to supply part of the increasing demand for primary energy and thus reduce crude petroleum imports which entail heavy expenditure on foreign exchange. The families Euphorbiaceae (*Euphorbia antisyphilitica, E.tithymaloides, E. caducifolia E. royleana E. neerifolia* etc. and Ascelpiadaceae (*Calotropis gigantea* and *C. procera*) which have been worked out in previous years (Kumar, 2000) will form the basis for further studies.

Introduction

Worldwide energy consumption is projected to grow by 59 % over the next two decades, according to International Energy Outlook 2001 (IEO 2001), released by theUS Energy

Information Administration (EIA). One half of the projected growth is expected to occur in the developing nations of Asia (including China, India and South Korea) and in Central and South America, where strong economic growth is likely to spur demand for energy over the forecast period. Renewable energy use is expected to increase by 53 % between 1999 and 2020, but its current 9 % share of total energy consumption is projected to drop to 8 % by 2020. Oil 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 oil use is projected to increase from 75 million barrels per day in 1999 to 120 million barrels per day in 2020.

Biomass resources are potentially the worlds largest renewable energy source – at an annual terrestrial biomass yield of 220 billion oven dry tonnes. Biomass conversion to fuel and chemicals is once again becoming an important alternative to replace oil and coal. Biodiesel from the rape seed oil methylester (RME) produced by farmer cooperatives is about 2000 t RME per year. A large facility of 15000 t RME per year is located at the oil mill at Bruck/Leitha in Austria. RME is excellent substitute for diesel. Already, European countries, mainly France, Italy, Germany and Austria are leading in biodiesel production, nearing 500,000 tons in 1997 out of which 250,000 was produced in France.(Statt, 1998) The production capacity of biodiesel in Germany was fully utilized in1997, the sold quantity amounting to roughly 100,000 t (Groenen,1998). The technologies for producing bio-oil are evolving rapidly with improving process performance , larger yield and better quality products. The challenge is to develop a process technology which can cope with the significant variation in the composition of the raw material. Another line of action is *Camelina sativa* . This plant was a traditional oilseed in Europe. It is considered a “low input high yield” plant which could enhance the environmental aspect of biodiesel. However, it has a higher Iodine number (160).

Carbon dioxide emission is projected to grow from 5.8 billion tonnes carbon equivalent in 1990 to 7.8 billion tonnes in 2010 and 9.8 billion tonnes by 2020. The Kyoto conference agreement last year is not far reaching but indicates the role clean energy sources will play in the future. Biomass is renewable, non pollutant and available world wide as agricultural residues, short rotation forests and crops . Thermochemical conversion using low temperature processes are among the suitable technologies to promote a sustainable and environmentally friendly development. Biomass can play a dual role in greenhouse gas mitigation related to the objectives of the United Nations Framework Convention on Climate Change (UNFCC) i.e. as an energy source to substitute for fossil fuels and as a carbon store.

The sustainable development of large areas of the world is today one of the greatest challenges . How will it be possible to provide the means for improving the socio-economic conditions of the increasing population in developing countries, a large part of which lives in villages and rural areas of Asia, Africa and South America. Biomass currently supplies about a third of the developing countries energy varying from about 90 % in countries like Uganda, Rwanda and Tanzania to 45 % in India, 30 % in China and Brazil and 10-15 % in Mexico and South Africa. Tropical deforestation is currently a significant environmental and development issue. The annual tropical deforestation rate for the decade 1981-1990 was about 15.4 million ha (Mha). According to some estimates the forest cover is 64.01 Mha accounting for 19.5 % of India’s geographic area. At present there is hardly 0.4 % forest cover below the 25 cm rainfall zone and 1.3 % above 30 cm. Since the annual photosynthetic production of biomass is about eight times the worlds total energy use and this energy can be produced and used in an environmentally suitable manner and mitigating net CO_2 emission, there can be little doubt that the potential source of stored energy must be carefully considered for future energy needs. The fact that nearly 90 percent of the worlds population will reside in developing countries by about 2050 probably implies that biomass energy will be with us forever unless there are drastic changes in the world energy trading pattern.

Biomass should be used instead of fossil energy carriers in order to reduce i) CO_2 emissions ii) the anticipated resource scarcity of fossil fuels and iii) need to import fuels from abroad.

Current commercial and non-commercial biomass use for energy is estimated at between 20 and 60 EJ/a representing about 6 to 17 % of the world primary energy. Most of the biomass is used in developing countries where it is likely to account for roughly one third of primary energy. As a comparison, the share of primary energy provided by biomass in industrialized countries is small and is estimated at about 3 % or less.

Global land availability estimates for energy crop production vary widely between 350 and 950 million hectares (Alexandratos , 1995). An energy potential of about 37.4 EJ/a is estimate based on country specific biomass yield and an average land availability The worldwide technical biomass energy potential is then estimated at about 104 EJ/a corresponding to approximately one third of the global 320 EJ/a primary energy consumption of oil, gas and coal (BP-Amoco 1999).

The bio-oil consortium of the UK received huge grants (1.16 million pounds) to enable the commercial production and testing of an integrated bio-oil and electricity generating plant. UK´s energy minister Peter Hain ascribed “ high priority to research and development of sustainable energy sources “. Commercial processing plants for the medium scale production of biodiesel from inter-esterification of triglycerides have been developed in France, Germany (CARMEN), Austria (ENERGIA Biodiesel Technology) USA (Ensyn Group Inc.) and in the EU (Eubia).
Liquid and gaseous transport fuels derived from a range of biomass sources are technically feasible. They include methanol, ethanol, dimethyl esters, pyrolytic oil, Fischer-Tropsch gasoline and distillate and biodiesel from (i) Jatropha , Pongamia pinnata, Salvadora persica, Madhuca longifolia and (ii) hydrocarbon from Euphorbia species.
Biomass energy is experiencing a surge in interest in many parts of the world due to a greater recognition of its current role and future potential contribution as modern fuel in the world energy supply, its availability, versatility and sustainable nature; a better understanding of its global and local environmental benefits, perceived potential role in climate stabilization, the existing and potential development and entrepreneurial opportunities. Technological advances and knowledge which have recently accumulated on many aspects of biomass energy, e.g. greater understanding of the possible conflict of food versus fuel etc. A recent World Bank report concluded that “Energy policies will need to be as concerned about the supply and use of biofuels as they are about modern fuels. (and) they must support ways to use bio-fuels more efficiently and in sustainable manner (World Bank, 1996)
Biomass resources are potentially the worlds largest and sustainable energy source a renewable resource comprising 220 billion oven dry tones (about 4500 EJ) of annual primary production. The annual bio-energy potential is about 2900 EJ though only 270 EJ could be considered available on a sustainable basis and at competitive prices.
Most major energy scenarios recognize bio-energy as an important component in the future worlds energy. Projections indicate the biomass energy use to the range of 85 EJ to 215 EJ in 2025 compared to the current global energy use of about 400 EJ of which 55 EJ are derived from biomass (Hall and Rosillo-Calle. 1998).

Despite the fact that biomass represents about one third of the energy consumption in developing countries, it is not taken very well into account in energy studies. A set of factors explain the slow growth on the biomass utilization . They include:

1. High costs of production
2. Limited potential for production
3. Lack of sufficient data on energy transformations coefficients.
4. Low energy efficiency
5. Health hazard in producing and using biomass.

In the large scale use of biomass for energy risks are insecurity in raw material supply and prices, doubts about adequate quality assurance and hesitance for a wider acceptance by the diesel engine manufacturers, missing marketing strategies for targeting biodiesel differential advantages into specific market niches and last not least missing legal frame conditions similar to the clean air act in the USA.

Energy Plantation Demonstration Project Center

Development of agrotechnolgies

The work on the development of suitable agro-technology for hydrocarbon yielding plants was initiated at the University of Rajasthan, Jaipur in 1980 with seeds of *Euphorbia lathyris* provided by Professor Melvin Calvin (Kumar, 1984). DST (Later on DNES) granted a research project to the principal investigator at the University of Rajasthan in 1982 to work on hydrocarbon yielding plants which was later raised to practical demonstration on 5 ha in 1985 after successful completion of first phase. This area was totally barren with only one tree as seen in the Figure 1 and 2.
After the successful demonstration of the second phase a project called Energy Plantation Demonstration Project for 50 has was granted in which a three tier system was followed as per the details of the work given below. A brief summary of the work done and important achievements is given below.

A 50 ha Energy Plantation Demonstration Project Center (EPDPC) in the semi- arid region of Rajasthan was used to conduct the investigations.
A large number of hydrocarbon yielding plants are able to grow under semi arid and arid conditions and they also produce valuable hydrocarbons (up to 30 % of dry matter) which could be converted into petroleum like substances and used as fossil fuel substitute. During the last 18 years investigations have been carried out on the optimization of yield and production of hydrocarbons by such plants at the 50 ha EPDPC of the University of Rajasthan, Jaipur. Their yield could be increased several fold making their commercial cultivation feasible.

Hydrocarbons from plants

Some of the laticiferous plants identified by Bhatia et al. (1983) were investigated in detail at Jaipur (for review see Kumar et al., 1995; Kumar 2000 and 2001) .

Certain potential plants were selected and attempts were made to develop proper agro-technology for their large scale cultivation. Initially work was initiated at 5 ha and subsequently extended to the 50 ha EPDPC.

Methodology employed

Certain potential plants were selected and attempts were made to develop agro-technology for their large scale cultivation (Kumar, 1984; 1994; Kumar et al, 1995; Kumar 1996; Kumar,1998; Roy, 1998 – for review see Kumar, 1995 and Kumar, 2000). A 50 ha bio-energy plantation demonstration project center has been established on the campus of the University of Rajasthan to conduct the experiments on large scale cultivation of selected plants with the objective of developing optimal conditions for their growth and productivity, besides conserving the biodiversity.

The work done included

i) Hydrocarbon yielding plants, ii) high molecular weight hydrocarbon yielding plants, (iii) non edible oil yielding plants

(I) Hydrocarbon yielding plants included :

1.*Euphorbia lathyris* Linn., 2. *Euphorbia tirucalli*. Linn., 3. *Euphorbia antisyphilitica*, Zucc.,4. *Euphorbia caducifolia* Haines.,5.*Euphorbia neriifolia* Linn, 6.*Pedilanthus tithymalides* Linn, 7.*Calotropis procera* (Ait.)R.Br.,8.*Calotropis gigantea(*Linn) R.Br.

II) High Molecular Weight Hydrocarbon Yielding Plants :

Parthenium argentatum Linn.

III) Non edible oil yielding plants

1. *Jatropha curcas*. 2. *Simmondsia chinenesis*

Considerable work has ben carried out on these plants (Kumar, 1987;1994; 1995; 1996; Kumar and Roy, 1996; Roy and Kumar, 1998; 1990). Investigations on several plant species have been carried out at our center including *Euphorbia lathyris* (Garg and Kumar, 1987a ; 1987b; 1989a; 1989b; 1990; Kumar and Garg, 1995) *Euphorbia tirucalli* (Kumar and Kumar, 1985, 1986;; Kumar and Kumar 1986) *Euphorbia antisyphilitica* (Johari et al., 1990b,1991; Johari 1992; 1993a; 1995) *Pedilanthus tithymaloides* (Rani et al. 1991;Rani and Kumar, 1994a); *Calotropis procera* (Rani et al, 1990) ; *Euphorbia neeriiifolia* and E. caducifolia (Kumar 1990, 1994) Jatropha curcas (Roy ,1990, 1991, 1992b, 1994, 1996; Roy and Kumar,1990) and *Simmondsia chinensis* (Roy, 1992a). The following aspects have been studied in detail:

(A) Propagation

In general these plants are easily propagated through cuttings. The optimum period for raising cuttings is June -July and March -April. Cuttings from the apical and middle portions of *E.antisyphilitica* exhibited 100 percent survival rate while none of the cuttings from the basal portions survived. Besides, cuttings treated with growth regulator IAA showed longest root length in a certain time period. Spacing among the planted cuttings is also a crucial factor for survival of cuttings. It was noted that initially up to a period of two months the survival percentage was maximum in closest planting density . However for better results in later stages they must be transferred to beds having a minimum distance of approximately 45 cm. At this optimum density productivity of *E. antisyphilitica* was the best. (Johari, 1992). Regarding environmental variations, the March to October period was best suitable for *E.antisyphilitica* because a linear increase in growth was recorded in the period (Kumar, 1990). During these months, maximum sprouting was observed in *Pedilanthus tithymaloides, E.antisyphiliitca* and *E.tirucalli*. Cuttings measuring around 15 cm in length and 1 cm in diameter gave optimal growth. Seeds of *Jatropha curcas* and *E.lathyris* also showed maximum germination during these months. Overall growth and productivity was lowest in the winter months from November to February. Higher accumulation of hexane extractables corresponded with higher temperatures of the summer season (Johari and Kumar, 1992).

(B) Edaphic factors

Among different soil types sand was best for the growth of *E. lathyris* (Garg and Kumar,1990) and *P. tithymaloides* (Rani et al., 1991) while red loam soil was best for *E. antisyphilitica*. However, for *E.lathyris* latex contents were maximum on sand gravel. Red soil was rich in nitrate, sodium, potassium and phosphorus pentaoxide (Johari and Kumar, 1992). *E. antisyphilitica* plants were relatively tall in sandy soil and less branched as compared to red soil. Plants grown in red soil branched more instead of increasing much in height. When different combinations of these soil types were made biomass of *E.antisyphilitica* was maximum in red loam+sand+gravel (Johari et al., 1990a). While the red loam+sand combination in equal amounts was best for *P.tithymaloides* (Rani et al., 1990). A mixture of gravel+sand favored maximum increase in plant height fresh weight and dry weight in *E. lathyris* (Garg and Kumar, 1990; Kumar and Garg, 1995). Environmental factors influenced the growth and yield of *Calotropis procera* (Rani et al.,1990)

(C) Growth Curve

Growth of these plants was promoted by relatively higher temperatures. Maximum growth was observed during June-July to October-November and also from February March to May June. Increase in hexane extractables (HE) was recorded up to 6-7 months; thereafter HE did not increase significantly in *E.lathyris. E.antisyphilitica* and *P.tithymaoildes*. Higher levels of HE were recorded in leaves as compared to the stem in *E. lathyris* and in fruits of *Calotropis procera*. The active phase of growth exhibited greater amounts of HE.

(D) Fertilizer application

Application of NPK singly or in various combinations improved growth of all the selected plants. In general NP combination gave better growth which was only slightly improved by the addition of K for *E.tirucalli*.(Kumar and Kumar, 1986). When best doses of NPK were applied in different combinations like NP, NK, KP and NPK the last combination gave best results in the form of biomass, latex yield, sugars and chlorophyll in *E.lathyris* (Garg and Kumar, 1990) and *P.tithymaloides* (Rani and Kumar, 1994a). In *E. antisyphilitica,* however, NP combination gave best results, followed by NPK for biomass production. Chlorophyll, sugars and latex yield was best in combination (Johari and Kumar, 1993a).

Addition of farm yard manure (FYM) alone and in combination with urea improved the growth and productivity of *E.antisyphilitica, E.lathyris* (Kumar and Garg, 1995), FYM + Urea application improved the productivity in comparison with FYM application alone. In *E.lathyris* addition of FYM increased the plant height fresh weight and dry weight to varying degrees. Hexane and methanol extractables also increased (Garg and Kumar, 1896; 1987a)

Micronutrients, B, Zn, Cu, Mn, Fe, and Mo were applied to *E.antisyphilitica, E. lathyris* and *P. tithymaoildes* in different concentrations. Their soil application resulted in general promotion in fresh and dry biomass, latex and chlorophyll yield. Foliar spray was given to E.lathyris. In this plant best results were obtained by Mg application followed by Cu, B, Fe, Mo, Zn and Mn (Garg and Kumar,1987a).

Salinity stress studies were also made on *Euphorbia tirucalli* (Kumar and Kumar, 1986). Salinity was applied in the form of irrigation water. Lower concentrations of salinity improved plant growth of *E. antisyphilitica* (Johari et al.,1990a) but higher concentrations inhibited further increase in growth. Sugars, however, did not increase in any saline irrigation. A slightly higher level of salinity impaired chlorophyll synthesis also. At higher level of salinity leaves of *E.antisyphilitica* became yellow and fell down but the stem did not show any visible adverse effects. *E. lathyris* could also tolerate lower salinity levels but its tolerance was lower than *E.antisyphilitica*. In *E.lathyris* salinity adversely affected root growth (Garg and Kumar, 1990). *P.tithymaloides* also exhibited increases in biomass and yield at lower salinity levels and higher concentrations adversely affected the plant. Its underground part could tolerate slightly higher salinity concentration (Rani et al., 1991). Saline irrigation was also given with different percentage of FYM added in the soil. Both *E.antisyphilitica* and *P.tithymaloides* exhibited tolerance of higher salinity levels with increasing percentage of FYM in the soil, biomass sugars, biocrude and chlorophyll all increased in proportion with increasing FYM levels in the soil and along with saline irrigation. It was found in *Euphorbia lathyris* that up to a certain level FYM causes increase in overall growth and yield along with different concentrations of saline irrigation. Beyond a certain level increased FYM did not improve growth and productivity. *P. tithymaloides* required still higher percentage of FYM in the soil for best yield and biomass.

Lower salinity levels increased the sugar contents in sand. Higher saline concentrations adversely affected the chlorophyll contents but with increase in manure supply the chlorophyll accumulation was promoted in *P.tithymaloides.* The effect of water stress was also studied. Five different percentages of field capacity (FC) were determined and plants were irrigated. Above ground plant biomass improved significantly with increasing percentages of FC, maximum being 100 percent FC irrigation. In *E. antisyphilitica* as well as in *P. tithymaoildes* plant height also increased linearly with increasing soil water status. However, under ground length was found to increase up to a certain level only. Irrigation beyond an optimum level tended to reduce biocrude, sugar and chlorophyll in *E. antisyphilitica.* In *P. tithymaloides* lowest FC gave maximum yield of HE and chlorophyll. Sugar, however, increased with increasing levels of field capacity irrigation. Percent dry matter yield also decreased with increasing the quantity of irrigation water to the soil in *E.antisyphilitica* and *P. titymaloides* (Rani and Kumar, 1994a)

(E) Application of growth regulators

Exogenous application of growth regulators has been reported for several horticultural and ornamental plants and sugarcane. In *Euphorbia antisyphilitica* in the present experiment maximum plant height was observed with GA_3, followed by CCC, NAA, 2,4,5-T and IAA treatment. Spray of growth regulators resulted in enhanced fresh and dry weight production (Johari et al., 1994b). However bio-crude synthesis occurred more with the auxins NAA and IAA in *E. antisyphilitica*. Out of all the growth regulators employed on *P. tithymaoildes,* IAA supported maximum plant growth in terms of fresh weight and dry weight of above ground and under ground plant parts. 2,4,5-T showed minimum plant growth, and certain nodular structures were observed on the stem of the plants. Biocrude yield was best in IAA followed by 2,4,5-T, GA_3, CCC, NAA and control. Application of growth regulators on *P. tithymaloides* resulted in a slight decrease in chlorophyll, whereas on *E.lathyris* they induced favorable results regarding chlorophyll (Garg and Kumar, 1987a).

In *E.lathyris* IBA caused maximum fresh weight productivity followed by IAA, GA_3 and NAA. NAA sprayed plants exhibited more production of HE. A favorable influence of growth regulators was also observed in sugar yield maximum with NAA followed by IBA, GA_3 and IAA (Garg and Kumar 1987b).

The cultivation of these plants suffers from plant pathogenic diseases affecting at the root level. Investigations on pathogenicity and control aspects of Charcoal rot of *E.lathyris.* (Garg and Kumar, 1987c); *E.antisyphilitica* (Johari and Kumar, 1993b) were also carried out.

(F) Micropropagation

Plant tissue culture has been successfully employed to achieve rapid clonal propagation of *E.lathyris* (Kumar and Joshi,1982); *Pedilanthus tithymaloides*(Rani and Kumar,1994 b) and *E.antisyphilitica* (Johari and Kumar, 1994). Likewise propagation of jojoba has also been carried out (Roy 1972a). *Jatropha curcas* L. is potential diesel fuel yielding plant and details about this are given in Roy and Kumar, 1988 and Roy, 1999.

Development of wasteland

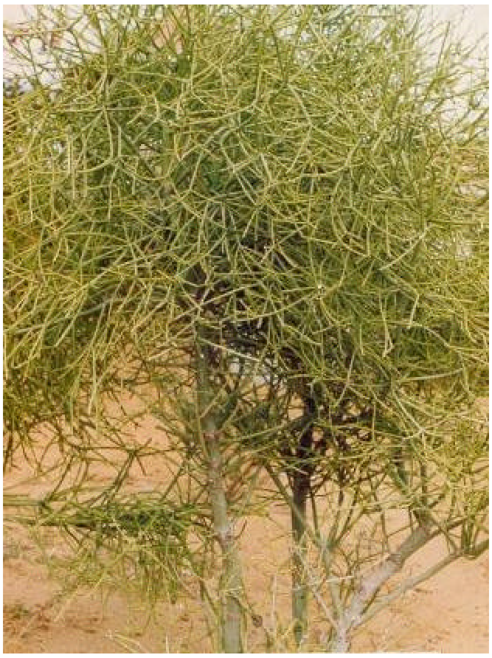
A protocol was set up for developing the wasteland following the three tier system in which small shrubs, shrubs and trees were used at a close spacing and this yielded a dry matter production of over 40 dry tonnes in a three year rotation .
The *Euphorbia antisyphilitica* in the lower tier, *Jatropha curcas* in the middle tier and *Acacia totilis* in the upper tier were used to colonize the EPDPC. The picture below represents the area as seen originally in Figure 1 and 2 which has been developed at EPDPC as greenland from the wasteland


(1)

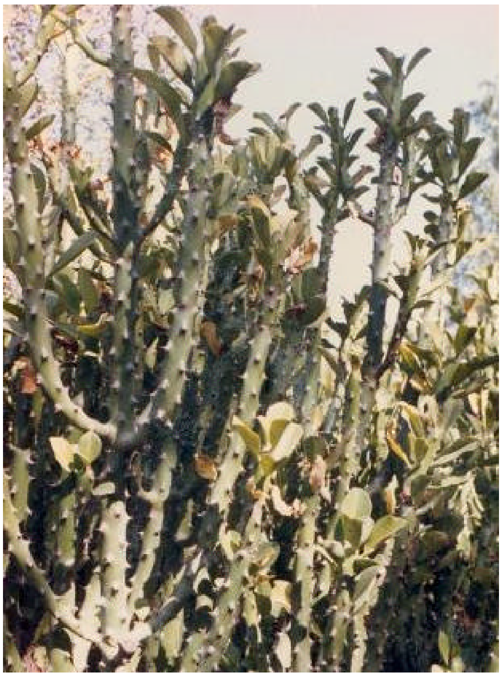

(2)


(3)


(4)


(5)


(6)



(7)

(8)

(9)

(10)

(11)

(12)

Figure 1: Energy plantation Demonstration Projekt Center (EPDPC) University of Rajasthan Jaipur 1984. Barren Land with only one tree (Holoptelia integrifolia).

Figure 2: Another View of wasteland at EPDPC. Pitting was done at 1 Meter x 1 Meter For Plantation.

Figure 3: Euphorbia Antisyphilitica Nursery stage, with close spacings.

Figure 4: Calotropis Procera a hydrocarbon plant used to colonise

Figure 5: *Euphorbia tirucalli*

Figure 6: *Euphorbia caducifolia*

Figure 7: *Euphorbia neeriifolia*

Figure 8: *Jatropha curcas* and *Calotropis procera* in background

Figure 9: *Euphorbia antisyphilitica* and *Calotropis procera*

Figure 10: *Euphorbia antisyphilitica* and *Jatropha curcas* in background

Figure 11: Three tier system with *E.antisyphilitica* in foreground and *Acacia tortilis* in background

Figure 12: A well developed EPDPC following the three tier system.

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