African Journal of Biotechnology Vol. 7 (5), pp. 613-616, 4 March, 2008
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB07.880
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Effect of phytoprotein treatment on *Jatropha curcas* for wasteland reclamation

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Accepted 31 January, 2008

***Jatropha curcas* (Euphorbiaceae) is a perennial oil yielding plant. *Jatropha* is also host to a large number of pathogens such as *Cercospora* sp. The systemic resistance and overall performance was enhanced by seed soak treatment with leaf extract of *Clerodendrum aculeatum*. On treatment, the treated plants not only showed systemic resistance but also showed marked enhancement in the plant vegetative growth, rooting, flowering, fruiting and seed formation. Mechanisms underlying this may due the synthesis of a new virus inhibitory phytoprotein produced in cells perceiving signals from systemic resistance inducers or ribosome-inactivating functions of these antiviral proteins. These treated Jatropha *curcas* can prove to be a miracle plant by turning wasteland into a money making land.**

Key words: *Jatropha curcas,* biodiesel, phytoprotein , *Clerodendrum aculeatum.*

INTRODUCTION

For a country reeling under the burden of a large oil import bill and spiraling oil prices biodiesel is a promising indigenous and renewable source of energy. Production of bio-fuel from plant materials is a major step toward harnessing renewable energy resources. *Jatropha curcas* (Euphorbiaceae*)* is a drought resistant, perennial plant yielding 5 - 12 tones per hectares oil seeds and produces 2 - 4 tones of biodiesel. It may also transform the poorest people and the most marginalized land into the source for this energy (Kochar et al., 2005). It is a multipurpose tree with a long history of cultivation in the tropical and subtropical regions of the world. It is a native of he central America and occurs mainly at low altitudes in areas with annual temperature of well above 30°C.The seeds are toxic due to the presence of curative ingredients but after the treatment the seeds or the seed cakes can be used as animal feed. Jatropha is grown as a boundary fence to protect field from the grazing animals and as a hedge to prevent erosion. The problem of great concern regarding the plant is the rate of vegetative growth and seed yield. The plant has profuse vegetative growth but the number of seeds produced per plant is very low. Besides the plants produces seeds after approximately 2 - 3 years depending on the environmental conditions. Seeds have limited viability and the plant is also prone to pathogens like *Cercospora*, *Rhizopus oryzae,* and insects.

In spite of all these properties research on cultivation and propagation of *J. curcas* is limited. Thus it was considered useful to undertake a systematic study and to develop a management strategy so that losses can be minimized. Phytoproteins derived from a few non hosts healthy plants have recently shown to have potential use as biocontrol agents (Baranwal and Verma, 2000). In Ayurvedic system of medicine, certain plants and their extracts have been used to control viral diseases of human beings and a number of plan products have been identified through photochemistry (Sukh Dev, 2006). These agents appear to act by sensitizing the plant for activation of resistance mechanism. The leaves of *Clerodendrum aculeatum (*Verbenaceae) and roots of *Boerhaavia diffusa* (Nyctaginaceae) have been shown to contain potent endogenous virus inhibitory proteins called the BD-SRI and CA-SRI (Awasthi and Verma, 2006). These phytoproteins confer strong systemic resistance in

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Table 1. Effect of phytoprotein treatment in *J. curcas*.

Plant	Control	Treated
1. Height (cm)	183.488±1.05	366.97±1.84
2. Leaf: stem	0.79 ± 0.11	1.14 ± 0.15
3. Stem		
a. Number of branches of stems	12 ± 2.04	29 ± 2.55
b. Diameter of the stem (cm)	23.5±0.05	34.1± 0.65
4. Root		
a. Length (cm)	15 ± 0.10	60± 0.54
b. Branching	5 ± 0.11	15±0.12
5. Flowers		
a. male	39 ± 1.26	20 ± 1.35
b. female	10 ± 0.86	25±0.55
6. Fruits		
a. size (in cm)	3.1 ± 0.0.1	3.4 ± 0.0.1
b. number per branch	10 ± 0.008	6 ± 0.01
7. Seeds		
a. Size (in cm)	1.2± 0.23	1.5±0.46
b. Oil content (%)	30 ± 0.11	32 ± 0.25

Each value represents the mean ±SD, n = 10.

several plants against a large number of plant viruses (Srivastava et al., 2004) within 4 - 6 h and at the same time enhance vegetative growth. These phytoproteins have a molecular mass of 30 and 34 KDa respectively. The phytoproteins are highly stable and could be purified. This can be a better approach towards introducing resistance against virus in crops and simultaneously allow these antiviral compounds to act as bioenhancers and increase their plant growth simultaneously (Verma et al., 1996). The present investigations were carried out to study the effect of seed soak treatment of *J. curcas* with the antiviral resistance inducing phytoprotein extracted from leaves of *Clerodendron aculeatum* (Verbenaceae).

MATERIALS AND METHODS

During the present study the seeds of *J. curcas* were soaked and given an overnight pretreatment in 12% *C. aculeatum* plant extract. One set of seeds was also soaked in plain distilled water and kept as control. The overall growth, vegetative, rooting, flowering, fruiting and seed formation were recorded at frequent intervals and compared with control plants. The oil content of the dried, crushed seeds of treated and the control plants were estimated using petroleum ether as solvent by Electronic SOCS PLUS Automatic Solvent Extraction System.

RESULTS AND DISCUSSION

In the present study there was better faster germination rate in the treated seeds as compared to the control. There was a rapid increase in the vegetative growth rate in the treated plants (Table 1). After 3 months there was vigorous rate of vegetative growth with increased branching and overall better growth of the plants in comparison to the control, untreated plants (Figure 1A, B). Cutting of these plants can be a viable source of vegetative propagation. From the treated plants more than 20 - 25 such propagules for cutting can be easily obtained for macropropagation. The height, stem diameter and number of leaves were also more in comparison to the control plants. The number of the leaves and leaf: stem ratio was also evaluated and found to be more in comparison to the control plants (Figure 1C) .These treated plants can be thus useful as planting material for the vegetative propagation.

The roots also showed profound differences in growth. The treated *J. curcas* plants were deep rooted and penetrating in comparison to the control plants. When the root and rootlet length of the tap root was compare, marked differences were observed. The treated plants showed very long, thin (0.5 – 5 cm) roots that were extendable upto 60 cm in comparison to the control plants having short (15 cm) and thick (2 – 3 cm) roots after 3 months of growth. The roots gave rise to profuse rootlets. The rootlets in the treated plants were many in number with further branching. In the treated plants the branches was longer as well as more in number (Figure 1D). The treated plants with their deep penetrating roots can be used for soil reclamation as well as phytoremediation of various metal ions and minerals. This observation led to the conclusion that the root characteristics of the treated and the control plants had marked differences in their morphological and functional abilities.

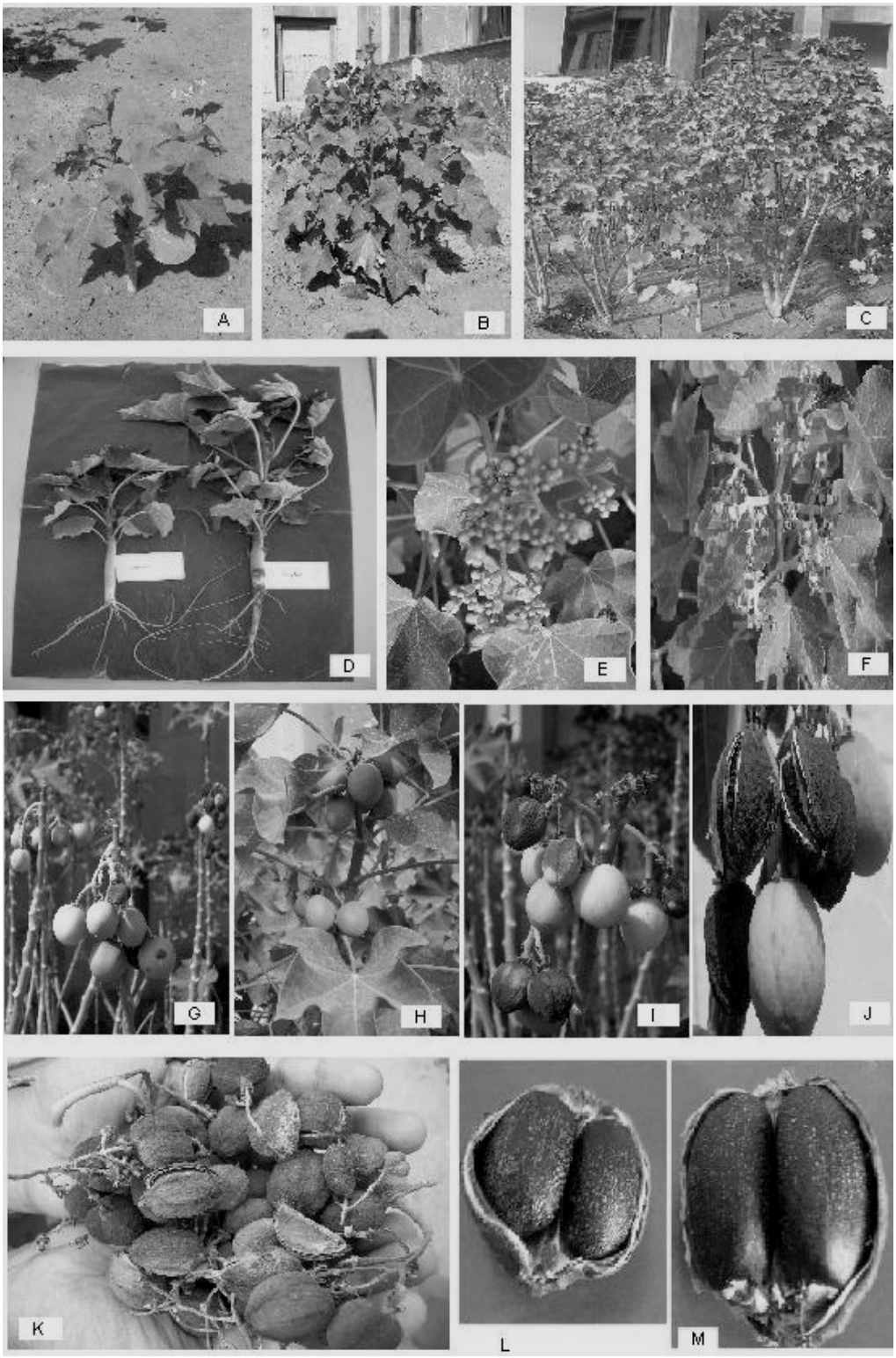

Figure 1. A. Vegetative growth of *Jatropha curcas* control plants after 3 months of growth. **B.** Vegetative growth of treated plants after 3 months of growth. **C.** Enhanced branching and height of treated in comparison to control plants. **D**. Deep rooting with enhanced branching in roots and rootlets of treated (left) in comparison to control (right) plants. **E.** More flowers in control plants. **F.** More female flowers in treated plants. **G.** Fruits are more in number and small in size in control plants. **H.** Fruits are less in number and bigger in size in treated plants. **I**. Fruits of control plants in various stages. **J.** Fruits of treated plants in various stages. **K.** The fruits and seeds after first yield. **L.** Seeds of control plants. **M.** Seeds of treated plants.

Early flowering was observed in the plants. Flowering was observed after 9 months in the treated plants. There were more number of flowers in the control plants (Figure 1E) as compared to the phytoprotein treated plants. In the 1st flowering phase there was maximum number of flowers in the control plants but all the flowers withered and resulted in no fruit set in both control and treated plants. In the 2nd flowering phase, 10 months after sowing, there was enhanced flowering in both control and treated plants. The number of female flowers was slightly increased in the treated plants (Figure 1F) and the fruit characteristics were also recorded on the 11th month. The numbers of the fruits in a single branch were more in number in the control plants as compared to the treated plants (Figure 1G, H). But when the size of the fruits was correlated it was found that there was increased fruit size and numbers of seeds were also more in treated plants. The numbers of fruits in the treated plants were bigger in size (Figure 1I, J and K). The seeds were also bigger in size in the treated plants (Figure 1 L and M). The oil content of the seeds of treated and the control plants were evaluated and a 2% increase in the treated plants was estimated.Thus it was concluded that the soak treatment of the leaves of *C. aculeatum* resulted in miraculous increase not only in vegetative stages (branching of the shoots, deep penetrating roots) as well as in the reproductive stages (early flowering, increase in female flowers, increase in fruit and seed size and oil content). These results clearly indicate that the plants on seed soak treatment are not only resistant to many pathogens but also show improved growth characteristics. Several reports are available on the effectiveness of the abiotic agents as elicitors of resistance in susceptible plant host. These agents appear to act by sensitisizing the plants for activation of resistance mechanism (Gupta et al., 2004; Srivastava et al., 2004). Such plant substances are known as systemic resistance inducers (SRI). The induction of the resistance is expressed as reduction in lesion number in host reacting hypersensitivity to viral infection. Till now these SRI were sprayed after every week (Verma and Verma, 1993; Awasthi and Kumar 2003; Gupta et al., 2004). There are also reports of *in vitro* induction of these phytoprotein and enhancement of growth in certain selected plants (Verma et al., 1979; Verma et al., 1984; Verma et al., 1996). Mechanisms underlying this can be due the synthesis of a new proteinaceous virus inhibitory agent produced in cells perceiving signals from systemic resistance inducers. Apart from resistance induction, ribosome-inactivating function of these antiviral proteins have also been shown.

This is the 1st report of seed soak treatment of *J. curcas* with 12% leaves extract of *C. aculeatum.* The development of disease resistant variety itself can provide the best option but breeding disease resistance varieties is tedious and time consuming. The enhanced growth by the activity of these bioenhancers was reflected by substantial increase in height, leaf area, fresh shoot weight, fresh root weight, root length, increased number of flowers, fruits, seeds, oil content and better resistance towards the development of systemic disease symptoms of viral infections. The overall crop performance was satisfactory and these treated plants were least affected by biotic and abiotic factors like rainfall, frost and infes-tation by insects and diseases. These treated *J. curcas* can prove to be a miracle plant by turning wasteland into a money making land.

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