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Effect of raw, defatted and detoxified Jatropha seed cake on germination of green gram (Vigna radiata)

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Abstract: ***Large amount of Jatropha seed cake is generated every year as a by-product of Jatropha biodiesel industry. Jatropha seed cake significantly affected seed germination and root growth of Vigna radiata because of the presence of phorbol esters which are known to cause various physiological and morphological changes in the plant. Germination was not affected by the addition of 1 and 5% defatted seed cake while a decrease in germination rate was observed in soil containing 10% defatted seed cake. Addition of higher percentage of defatted Jatropha seed cake showed an inhibitory effect on the growth parameters of the green gram. Higher percentage of detoxified Jatropha seed cake did not affect the root length and very less reduction in the number of secondary roots observed. The vigor index was affected at 10% levels of all the treatments with detoxified seed cake having least effect. There was improvement in the number of leaves and leaf area compared to other two seed cakes. Raw Jatropha seed cake affected the chlorophyll a content while no significant changes were observed in chlorophyll b content at lower levels. Addition of detoxified seed cake concentrations at 1% and 5% level matched with the control in chlorophyll b content. It was observed that at 5% level of defatted seed cake, chlorophyll a content drastically reduced compared to raw and detoxified seed cake.***

I. INTRODUCTION

Jatropha is a large shrub or small tree usually 3-5 m in height with a smooth grey bark which is well adapted to soils with low nutrient content. The study of Jatropha curcas seeds showed that it contains; 6.62% moisture; 18.2%; protein; 38.0% fat; 17.30% carbohydrates; 15.50% fibre and 4.5% ash (Gubitz et al., 1999). According to Arab and Salem (2010), Jatropha seeds are also rich in micro nutrients (mg/kg) such as Mn (28.37), Fe (0.38) and Zn (47.13) and some macro-elements like K (34.21), Ca (103.13), Na (8.44), Mg (109.89) and P (185.17). Jatropha plant has also been used for fencing and preventing wind and soil erosion (Openshaw, Keith. 2000) thereby increasing the fertility of the agricultural fields. Many researchers have successfully used Jatropha husk to produce gas, however, high ash content in Jatropha shell limits its use to be converted as gas (Cooke, 2009). Therefore, shell can be made use by converting it into briquettes and for burning stoves for cooking purposes. The shell after combustion helps in soil enrichment as it contains a good amount of sodium and potassium. The shell contains 34% cellulose 10% hemicellulose and 12% lignin. A good chemical composition of shell and husk may reduce the need of adding chemical fertilizers in the field, when these are left in field and ploughed. Therefore, Jatropha seed cake can also be used as a substitute for chemical fertilizers because of good content of Nitrogen, phosphorus and potassium (NPK) which is much higher than the NPK values of chicken or cow manure (Achten et al., 2008). Also, the biological decomposition of Jatropha seed cake helps in improving soil fertility which enhances crop production.

The oil content of the Jatropha seeds ranges between 25-30 % by weight of the seeds which can be used for bio-diesel production and the remaining percentage is of seed cake (Staubmann et al., 1997; Singh et al., 2008). In bio-diesel industry, 3 tons of seed cake renders one ton of biodiesel depending upon the seed quality (Mahajani, 2009). Jatropha seeds also contain certain antinutritional compounds like curcin, phytates, lectins, saponins and toxic compounds like phorbol esters which restrict the use of Jatropha for food/feed applications. The toxicity of Jatropha seed, Jatropha oil and Jatropha seed cake is predominantly because of this toxin i.e. phorbol esters. Phorbol esters are widely distributed in different parts of the Jatropha plant but they are mainly concentrated in the seed kernel (Ahmed and Salimon, 2009)

During oil extraction from Jatropha seeds, a major part (70-75%) of phorbol esters being lipophillic in nature goes with the oil while 25–30% remains bounded with seed meal matrix. The content of phorbol esters varies in the toxic and non-toxic varieties of Jatropha, while the highly studied species, *Jatropha curcas*, contains about 1–3 mg/g of phorbol esters in Jatropha meal and 3–

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6 mg/g in Jatropha oil (Gogoi et al., 2014). Anti- nutritional factors such as phytates, tannins, saponins are present in 9.4, 0.04 and 2.60 % respectively in Jatropha kernel meal (Makkar and Becker, 2009a). So the handling and disposal of Jatropha seed cake should be guarded or it should be detoxified before disposing for environmental and health concern.

In this paper we are presenting the effect of addition of raw Jatropha seed cake, defatted seed cake and detoxified seed cake on germination and vigor of green gram (Vigna radiata).

II. MATERIALS AND METHODS

A. *Materials*

Green gram seeds used for the study were purchased from the local market. Seeds of uniform size and weight were selected for study. Soil was collected from Delhi Technological University ground and was sieved before use. Whatman No.1 filter papers were used in the present study. *Jatropha* seed cake (raw and defatted) was received from centre for Advance Studies and Research in Automotive Engineering, DTU. A part of this was detoxified by subjecting it to microbial remediation of phorbol esters for 12h as described by Shilpi *et al.*, (2017).

B. *Sterilization*

Soil was autoclaved at 121°C for 40 min at 1.05 Kg/cm^2 (115 lb psi) every alternate day for a week ensuring proper sterilization. Distilled water and glass wares were sterilized by autoclaving at 121°C/20 min at 115 lb psi. Petri dishes, plastic cups, forceps and needles were cleaned with 90% ethanol before use. Green gram seeds were surface sterilized using teepol and were washed thoroughly under tap water. The seeds were then treated with an antioxidant i.e. Citric acid solution (1%) in a flask, placed on rotary shaker (100 rpm for 10min) followed by washing with distilled water. The seeds were re-sterilized with freshly prepared $HgCl_2$ solution (0.1%) under constant shaking (100 rpm/ RT) for 5 min and washed with sterile distilled water. Distilled water, cups, tissue paper, lamp, etc. except seeds, were exposed to UV light in a laminar air flow cabinet for 30 min prior to use. Laminar air flow surface was cleaned with 70% ethanol before starting with the seeds germination experiments.

C. *Germination Test in Soil*

Different concentrations of raw, defatted and detoxified *Jatropha* seed cake were mixed thoroughly with sterilized soil according to Table 1 and filled in plastics cups (8.0 cm diameter). Controls were maintained separately. The sides and bottom of plastic cups were pricked facilitating the air and extra water removal. 10 seeds per cup equidistant and at 1 cm depth were sown with help of flame- sterilized forceps. After sowing, soil was moistened by adding 10 ml of sterile distilled water with the help of pipette. 10 replicates of each concentration were made and kept in air conditioned room (28±2°C) under natural light. During the incubation period, sterile distilled water (2 ml) was added daily in the evening in each cup to maintain the water content. The experiments were terminated after five days and the growth parameters of seedling such as morphogenesis, rhizogenesis and leaves surface area etc. were measured and calculated.

Table 1: Different combinations of soil and *Jatropha* seed cake

	1% (Ratio of seed cake to soil)	5% (Ratio of seed cake to soil)	10% (Ratio of seed cake to soil)
Raw Jatropha seed cake	1:99	5:95	10:90
Defatted seed cake	1:99	5:95	10:90
Detoxified seed cake	1:99	5:95	10:90
Control	100:0	100:0	100:0

D. *Analytical*

After five days, seedlings were counted, and their root and shoot lengths were measured. Seedling vigor index was calculated according to Bidlan *et al.* (2004) as

$$. = \frac{(\quad h + \quad h \quad h) \quad \%}{10}$$

1) *Growth of seedling:* Observations were recorded after 5 days of sowing of seeds. The following observations were made: a) number of seedlings b) Percentage seed germination c) Average shoot length (cm) d) Average root length (cm) e) Average

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number of leaf per shoot f) Average number of roots per shoot. The *in vivo* raised shoots were excised immediately and maintained at 4°C until further analysis for photosynthetic pigments.

2) *Photosynthetic pigments:* Chlorophyll was estimated by using method described by Holden (1960) with slight modifications. Fresh leaf samples of 0.5g were weighed and homogenized using mortar-pestle with 80% acetone until all the color was extracted from the tissue. The extract was then centrifuged at 1200 rpm for 10min and supernatant was collected. The residue was re-extracted with 10ml of 80% acetone and centrifuged and the supernatants were pooled together. The test tubes were covered with black sheet so as to protect chlorophyll from photolytic degradation. The Bio-spectrophotometer (Eppendorf) was set at wavelengths 663 nm, 645 nm, and 480 nm for chlorophyll *a*, chlorophyll *b*, and carotenoid respectively. The optical density was measured and the content of chlorophyll *a*, chlorophyll *b*, and carotenoid (in $mg.g^{-1}$) was calculated using standard formula. For total chlorophyll, the values of chlorophyll a and chlorophyll b were added.

$$h \quad h \quad (\quad ^{-1}) = \frac{12.3 \quad _{663} - 0.86 \quad _{645}}{1000}$$

$$h \quad h \quad (\quad ^{-1}) = \frac{19.3 \quad _{645} - 3.6 \quad _{663}}{1000}$$

$$h \quad h \quad (\quad ^{-1}) = h \quad h \quad + \quad h \quad h$$

$$(\quad ^{-1}) = \frac{4}{h \quad h}$$

where, OD = Optical Density
V = Final volume of chlorophyll extract in 80% acetone
W = Dry weight of plant material
a = Path length (usually 1 cm)

III. RESULTS AND DISCUSSION

A. *Effect of addition of raw seed cake on seeds of green gram*

The effect of seed cake at different levels (1, 5, and 10%) on the germination and growth of green gram was observed. A maximum of 96.6% seeds germinated with an average of 20.95 cm length of seedling on addition of 1% raw seed cake to the soil. However, in control (without seed cake) 100% seeds germinated with an average length of 29.98 cm. Interestingly, higher germination rate was observed in 5% as compared to 1% of seed cake while at 10% a much lower percentage of seed germination and survival was observed. Inhibition in germination of tobacco plant on the application of Jatropha kernel cake was also observed by Xing et al. (2013). In another study by Larissa et al. (2014), Jatropha curcas oil significantly affected the germination of lettuce with increasing concentrations of the Jatropha oil indicating the phytotoxicity of the oil. Mitodepressive effects on the cell cycle were observed on analyzing meristematic cells along with chromosomal and nuclear alterations indicating the cytogenotoxicity of the oil (Andrade et al., 2010). As the percentage of raw Jatropha seed cake was increased, a significant decrease in root and shoot length was observed (Figure 6).

No secondary roots and leaves were observed in samples containing 10% raw Jatropha seed cake. According to Xing et al. (2013), there was no significant improvement in the tobacco seedling growth on the application of the kernel cake to the soil, but the root growth decreased by 48% indicating the phytotoxicity of the kernel cake on root growth. Phorbol esters are mainly responsible for the toxicity of Jatropha plant. After extraction of oil from Jatropha meal, most of the phorbol esters (70-75%) being lipophilic show affinity towards oil and 25-30% is left in the seed cake. Phorbol ester toxicity is known to cause various physiological and morphological changes in the plant. Reduction in mitotic index results in decreased root growth which can be seen in the percentage of dividing cells (Andrade et al. 2010). Phorbol esters are responsible for the activation of many iso-forms of protein kinase - C (PKC) which is a cell protein that regulates many cellular processes in the cell cycle and results in interaction between DNA and toxic compounds which leads to reduction in mitotic index (Zhang et al., 1995; King et al., 2009). Overall an inhibitory growth pattern was observed with increase in %age of raw Jatropha seed cake as shown in Figure 1.

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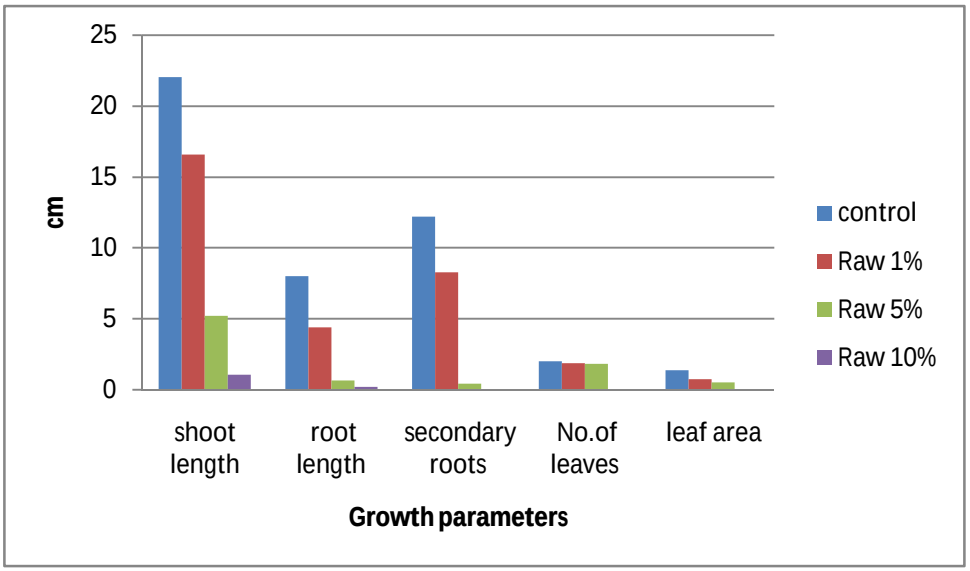


Figure 1: Effect of different concentrations of raw seed cake on growth parameters of green gram

B. Effect of addition of defatted seedcake on seeds of green gram

The effect of defatted seedcake at different levels (1, 5, 10%) on the germination and growth of green gram was observed. Germination was not affected by the addition of 1 and 5% defatted seed cake while a decrease in germination rate (93.3%) was observed in soil containing 10% defatted seed cake. Addition of higher percentage of defatted *Jatropha* seed cake showed an inhibitory effect on the growth parameters of the green gram. There was reduction in root, shoot length and secondary roots (Figure 2). Not much difference was observed in growth parameters in samples containing 1% and 5% defatted seed cake but in samples containing 10% seed cake difference was clearly visible. A better root & shoot length and leaf area was observed as compared to samples containing raw *Jatropha* seed cake as defatted seed cake contained less amount of oil and hence the phorbol esters. In a study done by Penjit *et al.* (2012), treatment of Chinese kale with the low rate chemical fertilizer and high rate of *Jatropha* deoiled seed cake resulted in best marketable yield which was comparable to that obtained with full rate of chemical fertilizer. Chemical fertilizer combined with any grade of *Jatropha* seed cake (low, medium, and high) gave the maximum tomato and tuber yield. The vigor index (Table 2) ranged from

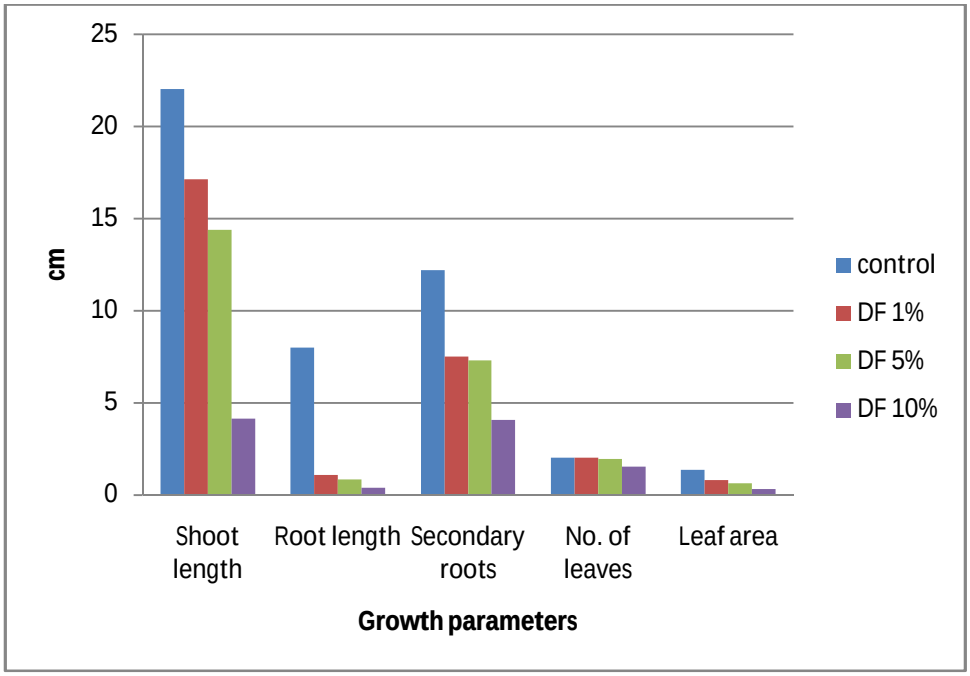


Figure 2: Effect of different concentrations of defatted seed cake on growth parameters of green gram

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C. Effect of addition of detoxified seedcake on seeds of green gram

Detoxified seedcake at three different levels (1, 5, 10%) was added and its effect was studied on the germination and growth of green gram. No effect on seed germination was observed in samples containing 1 and 5% detoxified seed cake while 10% had a decreased germination rate (93.3%). Addition of higher percentage of detoxified *Jatropha* seed cake affected the root length but interestingly there was very less reduction in the number of secondary roots and leaves (Figure 3). There was improvement in the number of leaves and leaf area compared to other two seed cakes. In a study by Xing *et al.* (2013) application of the kernel cake fermented by *S. fimicarius* YUCM 310038 significantly improved the leaf length and tobacco seedling growth. This implies that the *Jatropha* seed cake can be used as organic fertilizer after it has been detoxified by various micro-organisms which have the potential to degrade phorbol esters. Chaturvedi *et al.* (2009) observed increase in growth and yield of tuberose (a commercial flower) when *Jatropha* press cake was used as an organic fertilizer. Wheat yield also improved significantly when inorganic fertilizer was substituted with *Jatropha* press cake (Ghosh *et al.*, 2012).

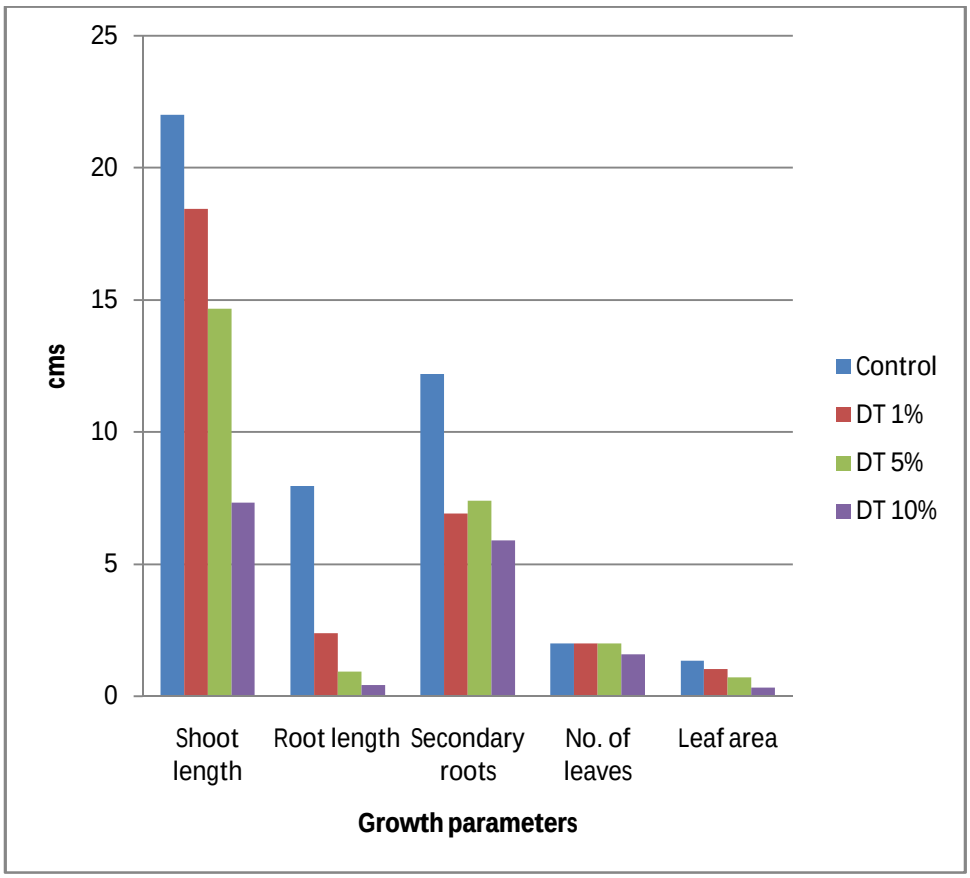


Figure 3: Effect of different concentrations of detoxified seed cake on growth parameters of green gram

The relative growth and germination patterns for different treatments of green gram are depicted in Figures 4-6. The apparent growth indicates the toxic effects on the germination with increasing concentrations in all the treatments. The highest concentration studied i.e. 10% show very little growth even at the end of 5 days of plantation.

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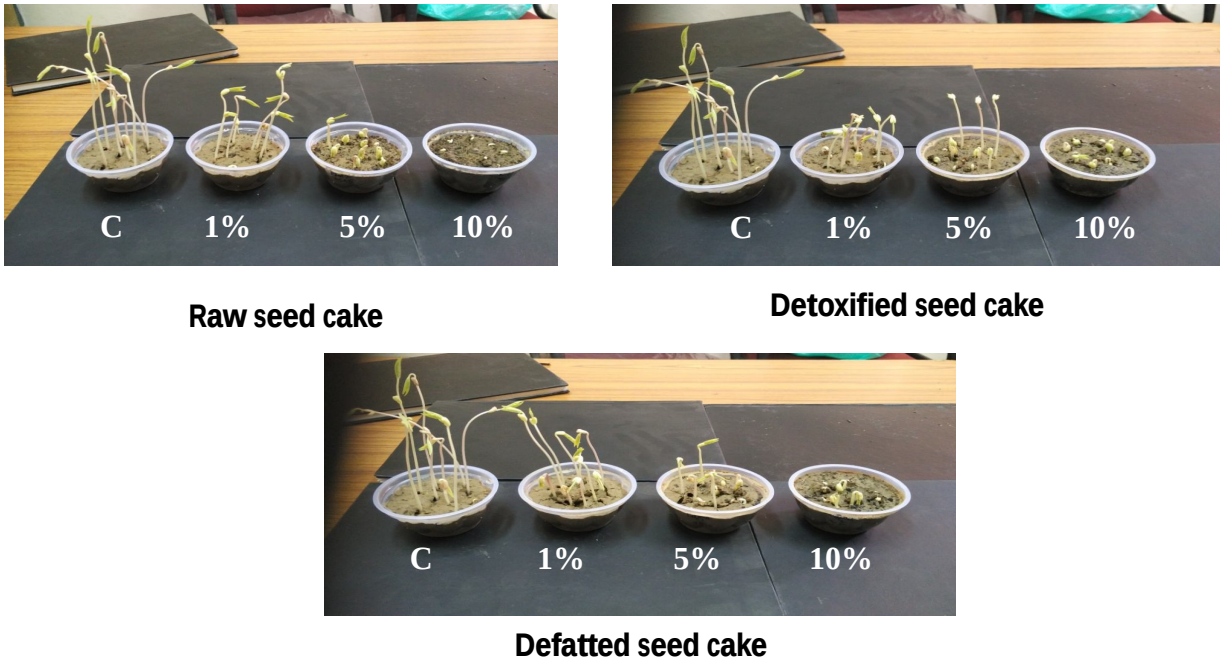


Figure 4: Seed germination observed after three days at different concentrations of raw, detoxified and defatted Jatropha seed cake.

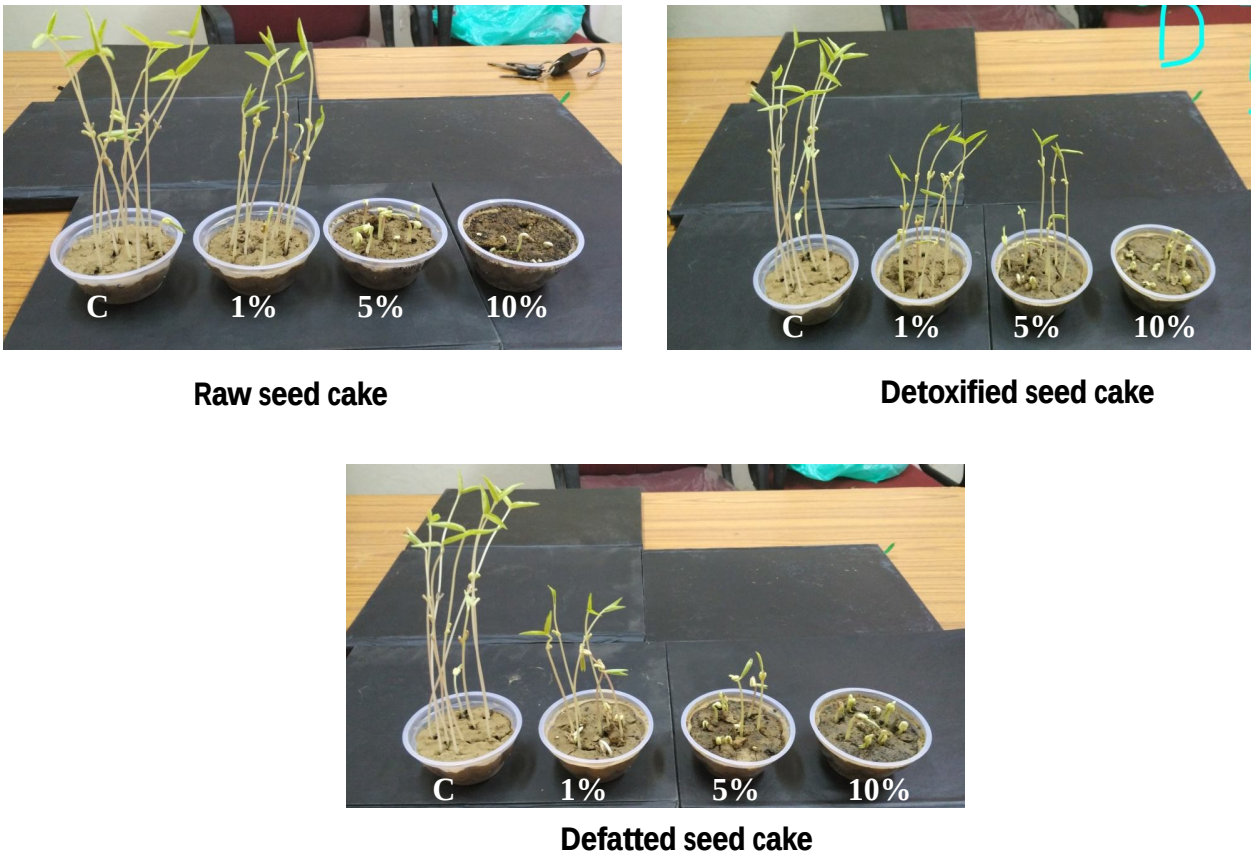


Figure 5: Seed germination observed after five days at different concentrations of raw, detoxified and defatted *Jatropha* seed cake. C: Control.

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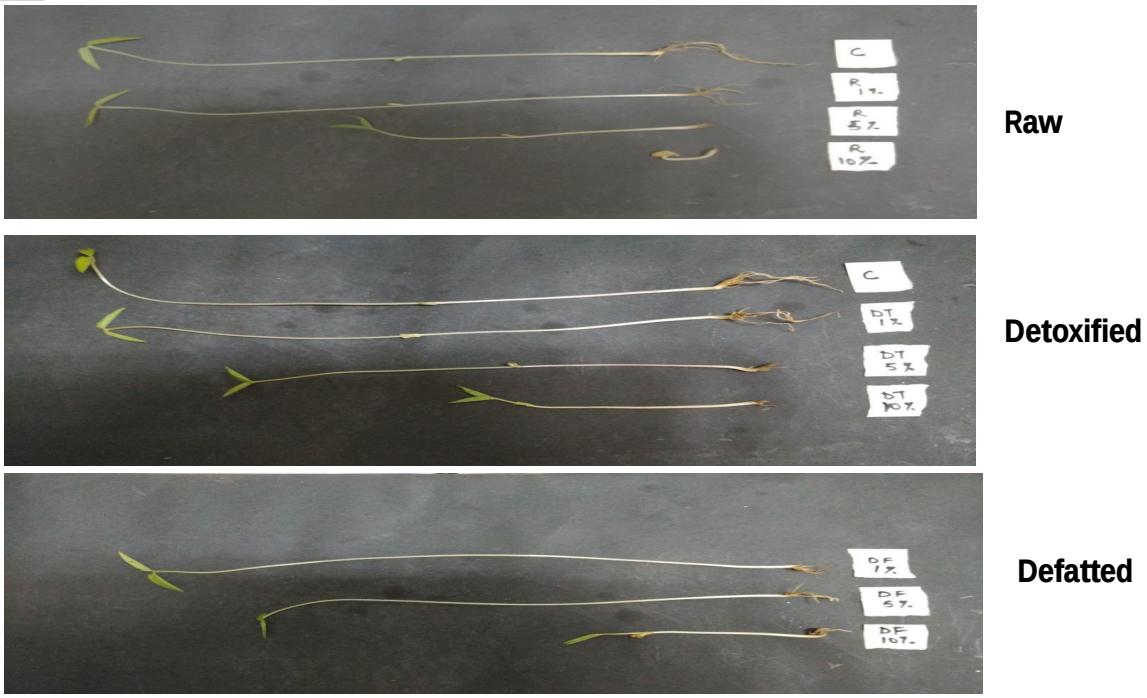


Figure 6: Comparative lengths of seedlings under treatment with different concentrations of raw, detoxified and defatted seed cake

D. Effect of different treatments on vigor index (V.I) of green gram

The effect of different seed cakes (raw, defatted and detoxified) on vigor index of green gram is shown in Table 2. The highest value of vigor index was obtained from detoxified seed cake (1%) and raw seed cake (1%) which recorded 208.2 and 202.3 respectively. Vigor index was significantly affected by the different concentrations of the raw *Jatropha* seed cake in soil. Soil with 1% raw seed cake had the higher *V.I.* than 10%. Not much difference was observed in vigor indexes at 1 and 5% levels of defatted and detoxified seed cake, however difference increased when the level was raised to 10%. This reveals the negative impact of higher concentrations of the seed cake on the overall health of the seedlings. The 10% level of detoxified seed cake, though had a lower impact on the plant health compared to the other two treatments. This indicates that the detoxified seed cakes can be used as fertilizer with further treatments after strategic analyses. We can say that the detoxification reduces the inhibitory effects of *Jatropha* seed cake.

Table 2 Effect of different treatments on vigor index of green gram

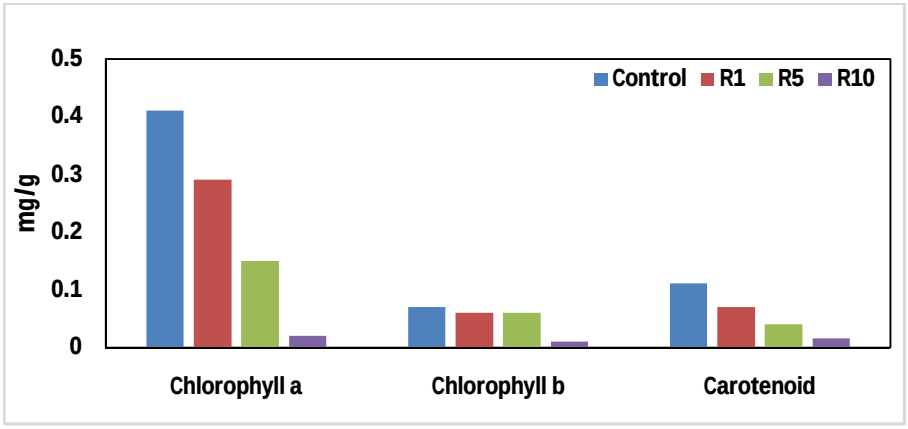
	Raw	Defatted	Detoxified
1%	202.3	181.9	208.2
5%	57.9	152.4	156.1
10%	6.2	41.9	72.4
Control	299.8	299.8	299.8

E. Effect of different concentrations of seed cake on photosynthetic pigments in green gram

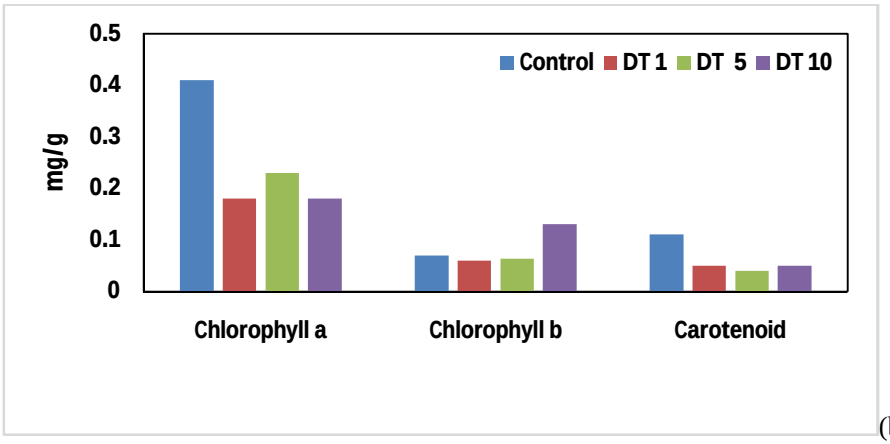
In the present investigation, 6 days old seedling was evaluated for the analysis of various photosynthetic pigments, chlorophyll a, chlorophyll b and carotenoid. Chlorophyll is the most valuable primary metabolite which captures sunlight and is responsible for photosynthesis. The effects of *Jatropha* seed cake on chlorophyll a, chlorophyll b is shown in Figure 7. It was observed that increase in concentration of raw *Jatropha* seed cake affected the chlorophyll a content (Figure 7a) which implies that raw *Jatropha* seed cake inhibits the production of chlorophyll in green gram. There were no significant changes observed in chlorophyll b content at lower levels, it drastically decreased at higher concentration of 10% raw *Jatropha* seed cake. Addition of detoxified seed cake concentrations at 1% and 5% level matched with the control in chlorophyll b content (Figure 7b). At all levels of detoxified seed cake addition, carotenoid and chlorophyll a content decreased by more than 50%. It was also observed that chlorophyll b content at 10% detoxified seed cake was comparatively higher (almost double) than the control. In the presence of defatted seed cake at all

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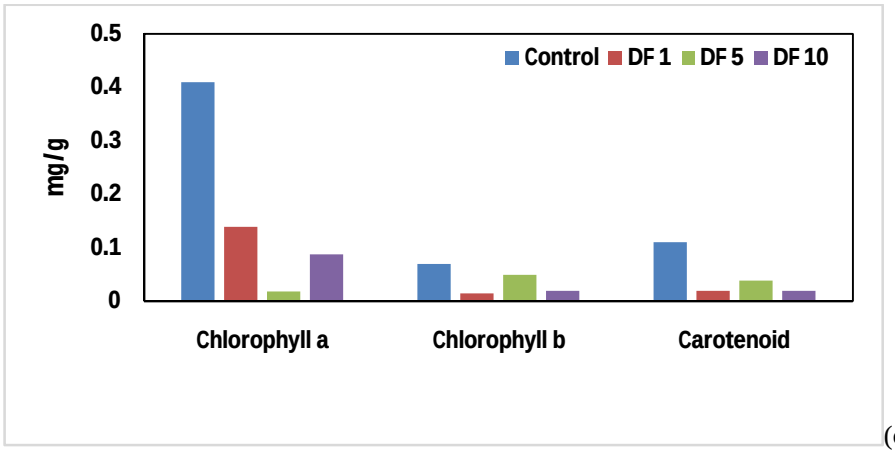
concentrations, there was significant reduction in chlorophyll b and carotenoid content while at 1%, chlorophyll a and at 5% chlorophyll b were observed to be slightly higher and at 10% level, chlorophyll a was comparatively more. However, chlorophyll and carotenoid contents were better in all the controls (Figure 7c).



(a)



(b)



(c)

Figure 7: Effect of different treatments on pigments of green gram

The presence of *Jatropha* seed cake shows significant changes at all concentrations under study. Exceptional enhancement in chlorophyll b content at 10% level of detoxified seed cake was observed indication metabolic interference under normal conditions in the absence of factors that are being supplied by detoxified seed cake for its bio synthesis. It was also observed that at 5% level of defatted seed cake, chlorophyll a content drastically reduced compared to raw and detoxified seed cake while not much difference was observed at 1% level in case of detoxified and defatted seed cakes, but it was almost two-third as compared to 1% raw seed cake level.

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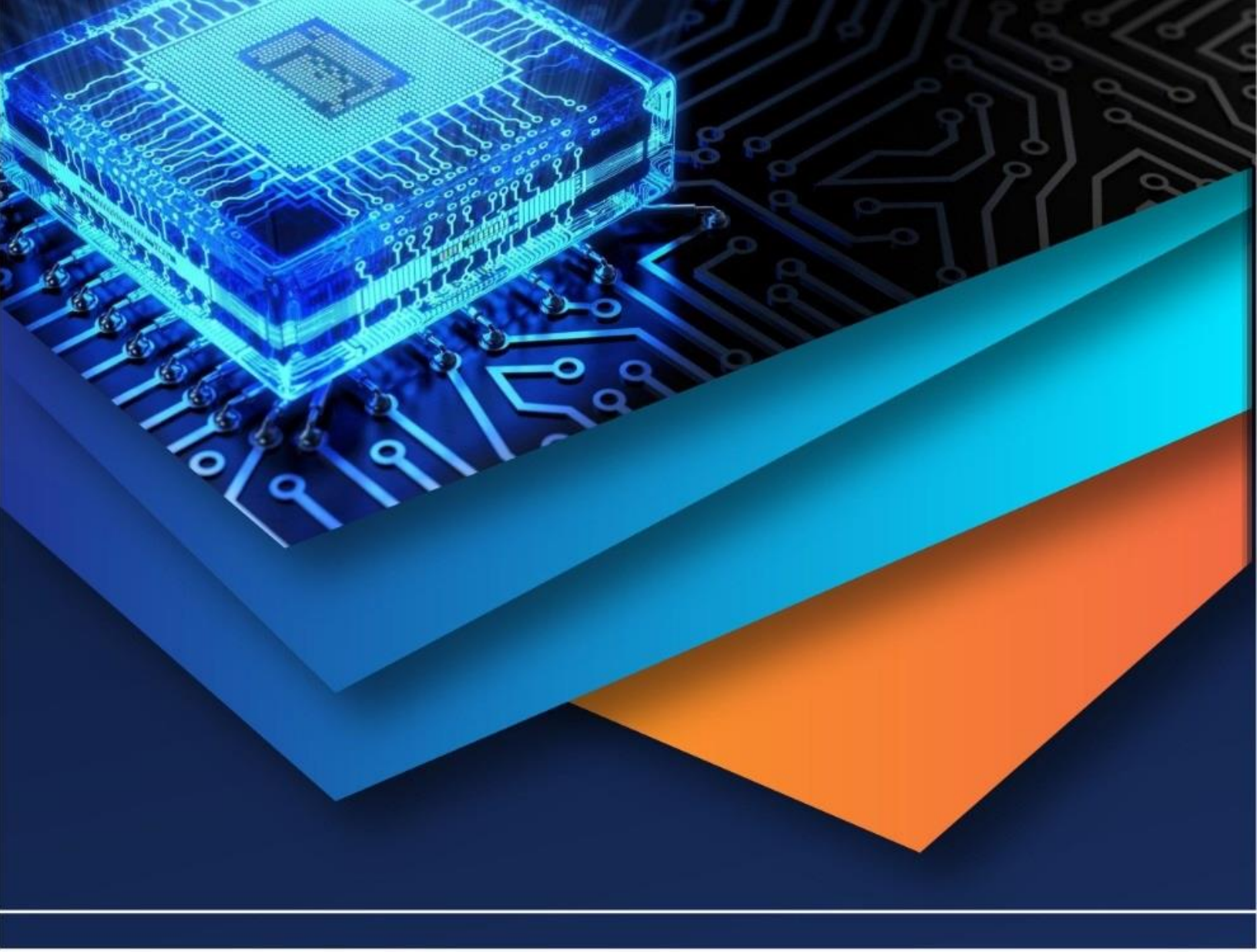
IV. CONCLUSION

Jatropha seed cake in all forms was found to have inhibitory effects on green gram seed germination and overall seedling health. The plant pigments were also affected and found to have reduced in raw and defatted seed cake treatments. The detoxified seed cake was found to have positive and eliminatory effect on the toxicity leading to improvement in both vigor index and pigmentation. Though, this is a prospective step towards the use of *Jatropha* seed cake as fertilizer after detoxification, the avenues are open for various applications.

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