Discussion
Triacontanol is known as the plant growth regulator which was extracted from the rice bran wax. It is lipid in nature hence it is found associated with the lipid molecules. In the present investigation it was isolated from the rice bran wax by saponification reaction by treating with the alkali, NaOH saponification reaction is used for the detection of fats. The saponified material was then treated with acetone to form crude extract of triacontanol. Wang et al. (2007) indicated that the extract obtained by dry saponification has the highest contents of octacosanol and triacontanol among extracts by all used extraction methods including dry saponification, saponification in alcohol, saponification in water (neutralized and non-neutralized), and transesterification. In the present work also the fatty alcohols were extracted along with the crude triacontanol. The extract was then filtered under vacuum. It was then tested by determining its melting point. Melting point of the compound is a unique property by which the compound can be identified and separated from mixture the melting point of compound is the temperature at which they show equilibrium with its liquored stage (Fevre, 1997). Out of the crude extract of triacontanol 23.3 % triacontanol was estimated to be pure. This was possible because of the use of Gas Chromatography. The triacontanol crude extract was subjected for gas chromatographic analysis. The application of GC lies in the detection of several compounds. It is also used for the detection of hormones therefore this technique was used in the current work of investigation. Choi et al. (2006) supports the findings. They identified the steroids hormones in pomegranate by following same technique. The preparation of pure n-Hexacosanol from the wax of cock’s foot has already been described (Pollard et al., 1931). Luzbetak et al. (1979) also supports the methodology. The crude extract of triacontanol was used further. The triacontanol was mixed with
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water to form colloid as it is hydrophobic in nature, therefore when mixes with water forms colloid. The colloidal mixture was then diluted and sprayed on the foliage of *Vigna radiata* plants which is supported by the work of Laughlin *et al.* (1983).

The triacontanol treated plants were kept in light and in shade conditions. In light the growth was increased and in shade it was found reduced. Only the plants treated with 5 ppm concentration of triacontanol could survive. Ries and Wert (1977) showed that the plants treated with triacontanol could survive in the dark with sufficient gain of dry weight. The result obtained in the current work therefore shows resemblances for the 5 ppm concentration only.

The growth parameters were studied in terms of height of the plant, number of leaves, number of pods per plant, number of seeds per pod and yield of the plant. Verma *et al.* (2009) also used similar growth parameters to analyze the effect of plant growth regulators on the plants.

The changes in the biochemical contents were measured by estimating the total proteins, total moisture, total fats, total carbohydrates and total calories. The changes in the metabolites due to the plant growth regulators are commonly analyzed by several workers Taspiner *et al.* (2009) worked on the changes in the isoenzymes in bean at chilling temperature induced due to plant growth regulators. As triacontanol is known to enhance the rate of photosynthesis, the end products of photosynthesis, carbohydrates were determined. In the present work the estimation of carbohydrates, fats and proteins was done. Kumaravelu *et al.* (2000) supports the findings. Naeem *et al.* (2009) also studied the action of triacontanol on the nitrogen fixation. Therefore in present work the estimation of nitrogen was also carried out. There are several evidences to know the action of
triacontanol during the process of photosynthesis. More et al. (1991) showed the stimulation of NADH oxidase of the soybean hypocotyls plasma membrane due to triacontanol.

Triacontanol increased the cell division rate in plant cell cultures of haploid, *Nicotiana tabacum* (Hangarter et al., 1978). In the present investigation the percentage of protein was found decreased with the increasing concentration of triacontanol. Kumaravelu et al. (2000) observed that the seedlings of green gram [*Vigna radiata* (L.) Wilczek] cultivar KM-2 were sprayed with different concentrations of triacontanol (TRIA) (0, 0.5, 1.0, and 2.0 mg dm\(^{-3}\)) at 15 and 25 days after sowing. Foliar spray of 0.5 mg dm\(^{-3}\) TRIA significantly promoted the plant height, fresh mass, and contents of chlorophylls, saccharide, starch, soluble proteins, amino acid and phenols. It is therefore attributed to the low concentration of triacontanol. High concentration of triacontanol was found inhibitory to the plant growth. The study by Moorthy and Kathiresan (1993) on mangroves supports the findings. The increase in the carbohydrates up to certain extent due to triacontanol was explained by Naeem et al. (2009), and it was later found decreased with the increasing concentration of triacontanol. The content of carbohydrates was studies in order to assess productivity. A light source of different spectrum showed enhancement in the growth of the plants by direct or indirect Methods such as the interaction of mycorrhizae with the pin plant were found to be enhanced (Niemi et al., 2005).

*In vivo* studies in the cell division of green gram showed that the rate of cell division was enhanced with triacontanol concentration at 8 ppm. Many workers showed that the triacontanol was found responsible for increasing growth and yield of the plants which is the direct effect of cell division. The germination of seed was found to be enhanced in combination
with other plant growth regulators; however, triacontanol alone could not enhance the germination in darkness but could show positive effect with the light treatment. Lewak and Skowronska (2006) observed the controversial nature of the triacontanol. The treatment of triacontanol to the green gram results into the growth and yield \textit{in vivo}.

\textit{In vitro} assessment showed that the cell division and differentiation was found faster. It was evident from the degree of the change in callus leading to the elongated sickle shaped curved cells. Differentiation in plants refers to the process by which distinct cell types arise from precursor cells and become different from each other. The change in shapes of cells showed the differentiation. Glover (2011) and Fukuda (2011) support the findings that showed plant vascular cells originate from procambial cells, which are vascular stem cells. Recent studies shows that signal from plant hormone increased the rate of differentiation (Zheng \textit{et al.}, 2002). The overall work on the triacontanol effect on the cell division, elongation and differentiation is less, therefore it needs further research.