CHAPTER 9
SUMMARY
The present investigation deals with the Changes in Morphometric and Biochemical characteristics of *Stevia rebaudiana* Bertoni variety SRB 128 under the effect of distillery spent wash. The different growth aspects such as seed germination, shoot length, root length and biomass of seedling, growth analysis, biomass and productivity of different plant parts, energy content, total sugars and chlorophylls, oil, protein, total nitrogen, phosphorus and potassium content have been studied under test concentrations of spent wash. Physico-chemical characteristics of spent wash and residual soil have also been analyzed.

There is vast growth of distilleries in western Uttar Pradesh. These distilleries use molasses to produce alcohol and in the process, produce wastewater called spent wash. The spent wash is an acidic effluent loaded with inorganic and organic salts. Being plant origin, the spent wash contains considerable amount of N, P, K, Ca, Mg and S. Disposable of this wastewater is often done by discharge it into water bodies. The farmers, for irrigation purpose have used water of these bodies as fertilizer.

*Stevia rebaudiana* Bertoni is a small, shrubby, herbaceous and perennial plant. It is a medicinal, economical, non-caloric, natural sweetener contains steviosides, which are 250-300 times sweeter than table sugar. It is used for a number of medicinal purposes such as for diabetes, cardiotonic, hypotensive, antiviral, antibacterial, antifungal, depression, fatigue, candidians and for reducing the cravings for sweet and fatty food products. Although *Stevia* is a medicinal and economical plant and Indian professionals are interested in cultivation of the plant, not much work has been done on different aspects of *Stevia rebaudiana* in India. Therefore it is of interest to study the
Morphometric and Biochemical aspects of the plant under the effect of different concentrations of spent wash.

**Physico-chemical characteristics of distillery spent wash** have been analyzed for colour, odour, pH, and temperature, biological oxygen demand, chemical oxygen demand, dissolved oxygen and nitrogen, phosphorus and potassium content. Different concentrations of spent wash from first release point of M/S Sir Shadilal Distillery and Chemical Works Mansurpur, Muzaffarnagar distillery were prepared by diluting appropriate amount of distillery waste with water. These test concentration of spent wash selected are 5.0%, 10.0%, 15.8%, 25.1%, 36.6% and 39.8%.

The spent wash analysis revealed the spent wash to be in highly effluent ridden status. High temperature, acidic pH, excessive quantity of salts had been observed in the high concentration of spent wash. A good amount of nitrogen, phosphorus and potassium has been observed in different concentration of spent wash. Dissolved oxygen has not been detected in any concentration of spent wash. Biological oxygen demand and chemical oxygen demand has been very high in all concentration of spent wash. Both BOD and COD depicted an increasing trend with the increase in the concentration of spent wash analyzed. The spent wash analysis revealed the high concentration of spent wash to be in highly effluent state in comparison to the tolerable quantities.

**Morphometric characteristics** have been observed in the present investigation such as seed germination, seedling growth, biomass and leaf area and growth parameters (LAR, RGR, NAR and NPP) of *Stevia rebaudiana* Bertoni variety SRB 128 plants treated with different concentration of spent wash
Seed germination in *Stevia rebaudiana* Bertoni variety SRB 128 have been studied at 3, 5, 7 and 9 days of germination. At low concentration of distillery spent wash i.e. 5.0% and 10.0%, seed germination percentage increases from 3 to 9 days of germination but at high concentration of spent wash inhibited/delayed the seed germination percentage has been observed.

Propagules of *Stevia rebaudiana* Bertoni variety SRB 128 procured from Forest Research Institute, Dehradun, Uttranchal were allowed to propagate and grow under different treatments in the laboratory as well as research plots at the study site. The experimental plots were irrigated with control and/or different dilutions of spent wash at various phenological stages of *Stevia rebaudiana* Bertoni.

Seedling growth has been observed at 15, 30 and 45 days of growth for fresh weight, dry weight, length of hypocotyls and radical and leaf area. Seedling growth has been promoted by low concentration of spent wash in comparison to high concentration of spent wash. Initially fresh and dry weight of seedling in *Stevia rebaudiana* has been promoted even by high concentration of spent wash that is up to 25.1% spent wash, but as seedling progressed, high concentrations inhibited it. Length of hypocotyls and radical, both have been promoted by low concentration of spent wash that is in the presence of 5.0% and 10.0% spent wash as compared to other treatments. Leaf area of seedling showed the same growth pattern as other parameters of seedling.

Slight activation of seed germination and seedling growth under the treatment of 5.0% and 10.0% spent wash indicated that the low concentration of ions might favour better plant growth while they are directly toxic in higher concentration.
For plant growth observations, samples of plants from *Stevia* have been uprooted randomly from 60 to 135 days of growth at fortnight intervals. Such samples have been observed for fresh weight as well as dry weight of different plant parts, measuring the leaf area and to calculate the various growth parameters such as Leaf Area Ratio (LAR), Net Assimilation Rate (NAR), Relative Growth Rate (RGR) and Net Primary Productivity (NPP).

Fresh as well as dry weight of different plant parts and plants of *Stevia rebaudiana* continued to increase from 60 to 135 days of growth irrespective of the spent wash treatment. Pattern of increase in total biomass and biomass of plant parts depicted 60 to 90 days as the most active period of vegetative phase and 105 to 135 days to be period of accumulation. Diversion of food from vegetative parts towards the reproductive parts of *Stevia rebaudiana* Bertoni during the period of reproductive phase is supported by- (i) increase in biomass of inflorescence with in 105 to 135 days and (ii) no decrease in biomass of leaves and stem.

Initial increase in leaf area has been followed by a decrease in the maturity phase of the plant. More and more steep decrease in leaf area value with increase in concentration of spent wash suggested nitrogen and phosphorus deficiency in the plant due to mineral absorption inhibition.

Reduced dry matter accumulation suggested decreased photosynthetic efficiency of the seedlings due to high salt concentration in the spent wash. Reduced pH, decreased aeration due to high biological oxygen demand and chemical oxygen demand, increased amount of osmotically active substances, fluctuation in the amount of salts with increase in the concentration of spent wash caused malnutrition to the plants.
Growth parameters have been analyzed with the help of sample plant from *Stevia rebaudiana* Bertoni SRB 128 uprooted randomly from 60 to 135 days of growth. Relative growth rate, Net assimilation rate and Net primary productivity depicted either no change or slight increase under the treatment of lower concentration of spent wash. However, no significant relationship could be established between these growth parameters and different concentration of spent wash.

**Biochemical characteristics** have been observed such as total nitrogen, phosphorus and potassium values, calorific values, protein, sugar, chlorophyll and oil content of *Stevia rebaudiana* Bertoni variety SRB 128 plants treated with various concentration of spent wash.

The total nitrogen, phosphorus and potassium content of *Stevia rebaudiana* plants continued to increase with increasing age. Percentage of potassium is manyfold than phosphorus and nitrogen in plant tissue. Fluctuation in the mineral contents of plants under various irrigation regimes pointed a reciprocal relationship between the concentration of spent wash and nutrient contents in different fractional plant parts.

Continuous decrease in leaf area, root length and shoot length under the treatments of higher concentration of spent wash possibly due to nitrogen deficiency. Phosphorus deficiency is considered to enhance the effect of nitrogen deficiency on plant growth. Progressively weaker plants and lesser potassium content under the treatments of higher concentration of spent wash indicated the rate of potassium in restricting shoot growth and hampering root development.

Continued mineral absorption, greater seedling growth and improvement in biomass under the treatments of 5.0% and 10.0% spent wash suggested the
role of lower concentration of the spent wash as fertilizers. Chemical analysis of spent wash, growth analysis of plants and initially increased values of leaf area after irrigation with high concentrations of spent wash revealed stunting as the major effects of salinity indicating negative effects of higher concentration of spent wash irrigation on growth and biochemical characteristics of *Stevia rebaudiana*.

Energy contents in all plant parts increased with age mainly due to altered rate of oil, protein and sugar in dry matter. This view point is supported by decrease in the energy content of roots (from 60 days of growth onwards), an organ of practically no accumulation of fat, protein etc. Fluctuation in the energy contents of different plant parts during various phenological stages suggested that the leaves act as the site of synthesis of nutrients during the period of vegetative growth and the components of inflorescence act as nutrient sinks during the period of reproductive growth.

Lower calorific values in various plant parts irrigated with higher concentrations of spent wash depicted reduced synthetic activities under such treatments. This view point is supported by the facts – (i) no appreciable changes in calorific values of various plant parts under the treatments which did not reduced dry weight or biomass (ii) increase in the extent of decrease in the calorific values with increase in concentrations of spent wash used for irrigation (iii) most appreciable decrease in the energy contents of root, stem and leaves in the plants irrigated with 36.6% and 39.8% spent wash.

Protein content increases with increase in the age of *Stevia rebaudiana* plant. Protein content per plant has been slightly higher in 5.0% and 10.0% spent wash while it has been slightly lower in the presence of 15.8% spent wash as compared with the control. Total protein content per plant continued to
decrease with the increase in the concentration of spent wash at all growth intervals studied.

Total sugars of leaf increase up to 90 days of growth and thereafter decrease up to 135 days of growth. Sugar content of leaf has been higher in low concentration of spent wash (5.0% and 10.0%) while it has been lower in high concentration of spent wash (15.8% to 39.8%) in comparison to the control. Lower protein and total sugars in plants of *Stevia rebaudiana* irrigated with high concentration of spent wash depicted reduced plant metabolism under such treatments.

Total chlorophylls of leaf have been estimated and were found to increase with the increase in the age of the plant form 60 to 135 days of growth. Total chlorophylls of leaf have been higher in those plants, which were irrigated with 5.0% and 10.0% spent wash as compared to other concentrations of spent wash. With the increase in the concentration of spent wash there has been decrease in the total chlorophyll content of leaf.

Oil content of leaf has been found to be very low in quantity. The oil content of leaf continued to increase form 60 to 135 days of growth. Oil content has been slightly higher in the presence of low concentration of spent wash (5.0% and 10.0%) whereas it has been slightly lower in 15.8% spent wash over control. The remaining concentration of spent wash (25.1% to 39.8%) depicted an inversely proportionate relationship with the oil content of leaf.

**Mineral dynamics** of the plants irrigated with different concentrations of spent wash depicted more efficient mineral transfer by the plants irrigated with 5.0% and 10.0% spent wash while it became lesser efficient after irrigating the plants with higher concentrations. The differences between mineral transfer rate of vegetative and reproductive plant parts have been
wider in the plants irrigated with lower concentration of spent wash and have been narrower in those irrigated with higher concentration of spent wash.

**Different physico-chemical characteristics of the residual soil** treated with different concentration of spent wash have also been analyzed. Different parameters observed were pH, water holding capacity, wilting coefficient, volume weight analysis and total nitrogen, phosphorus and potassium.

Soil pH decreased when irrigated with 25.1% to 39.8% spent wash. Volume weight analysis and water holding capacity remained unaffected under all the treatments of spent wash. More percentage of nitrogen, phosphorus and potassium in soil irrigated with 15.8% to 39.8% spent wash depicted reduced mineral absorption by the plants under these treatments. Nil dissolved oxygen, high biological oxygen demand and chemical oxygen demand in the higher concentration of spent wash created anaerobic conditions in the soil adversely affecting seed germination, seedling growth and biomass.

It may be concluded form the present study that 5.0% and 10.0% concentration of spent wash may be used as a fertilizer. Up to 10 to 20 times dilution, the distillery spent wash may be used to improve morphometric and biochemical characteristics of *Stevia rebaudiana* Bertoni variety SRB 128 such as crop growth, total sugars and chlorophylls, oil, and protein content. Thus spent wash concentration of 5.0% and 10.0% has been found to be optimum with sufficiently rich nutrients while the inhibitory factors are either absent or inactive. Different growth parameters of *Stevia rebaudiana* studied showed an inverse effect when the plants were irrigated with higher concentrations of spent wash. In other words in higher concentrations of spent wash inhibitory factors were present which adversely affect the growth of the plant. Physicochemical characteristics of soil are also affected adversely due to irrigation with higher concentration of spent wash. Besides
conventional, biological and chemical treatments by the distilleries, the spent wash should be properly diluted with water before discharge on land and use for irrigation purposes as fertilizer. The utilization of distillery spent wash in agriculture would save cost on fertilizer, better crop productivity and facilitate reduction in pollution load on aquatic system.