CHAPTER 5

CONCLUSIONS

Secondary compound production from root cultures of *Stemona curtisii*

The time profiles of the production of oxyprotostemonine, stemocurtisine and stemocurtisinol in root cultures have been determined. It was found that 16 weeks was the optimum period for root growth (411±4.7 mg dw) in semi-solid culture media, which was also the peak of oxyprotostemonine accumulation (2,713.6±18.1 μg/g dw, 5 folds higher than of intact root) in the root extract.

The affects on alkaloid production by the addition of elicitors (salicylic acid, methyl jasmonate, yeast extract and chitosan) and precursors (sucrose, sodium acetate and tyrosine) and culture conditions (culture temperatures, media pH and illumination) to *S. curtisii* root cultures on semi-solid MS medium for 16 weeks were examined because 16 weeks was the optimum period for root growth and oxyprotostemonine (the most potent insecticidal compound) production. The results are summarized below:

-The maximum root growth (729±61 mg dw) was induced by treatment with 4% sucrose (Figure 5.1).

-The highest total oxyprotostemonine (7,192±138.2 μg/g dw, 13 folds higher than intact root) (Figure 5.2) and stemocurtisine (39±0.4 μg/g dw, 39 folds higher than control) contents (Figure 5.3) were observed in cultures treated with 500 mg/L salicylic acid.

-The highest content of stemocurtisinol was 1,333±15 μg/g dw (37 folds higher than in control) from roots treated with 20 mg/L tyrosine (Figure 5.4).

These results indicate that the addition of SA and tyrosine to *S. curtisii* root cultures can enhance the production of oxyprotostemonine and stemocurtisinol higher

than that of intact root. These methods could be potentially developed for the future large scale production of *Stemona* alkaloids. However, it is possible to increase the production of stemocurtisinol and stemocurtisine if *in vitro* root cultures will be added with SA or tyrosine for 8 and 12 weeks, respectively because they were the optimum period for these alkaloids production.

Time profile study of 1′,2′-didehydrostemofoline production in root cultures of *Stemona* sp.

A time profile study of root growth and 1′,2′-didehydrostemofoline accumulation from root cultures of *Stemona* sp. showed that the highest 1′,2′-didehydrostemofoline production (31.04±1.62 mg/g dw) was observed at the end of stationary phase (week 16), which also corresponded to the maximum root dry weight (255 mg dw). However, it was found that natural root could produce this alkaloid at 47.46 mg/g dw (53% higher than that of cultured root at 16 weeks). Therefore, the effects of precursors, elicitors and culture condition will not be investigated for this species.

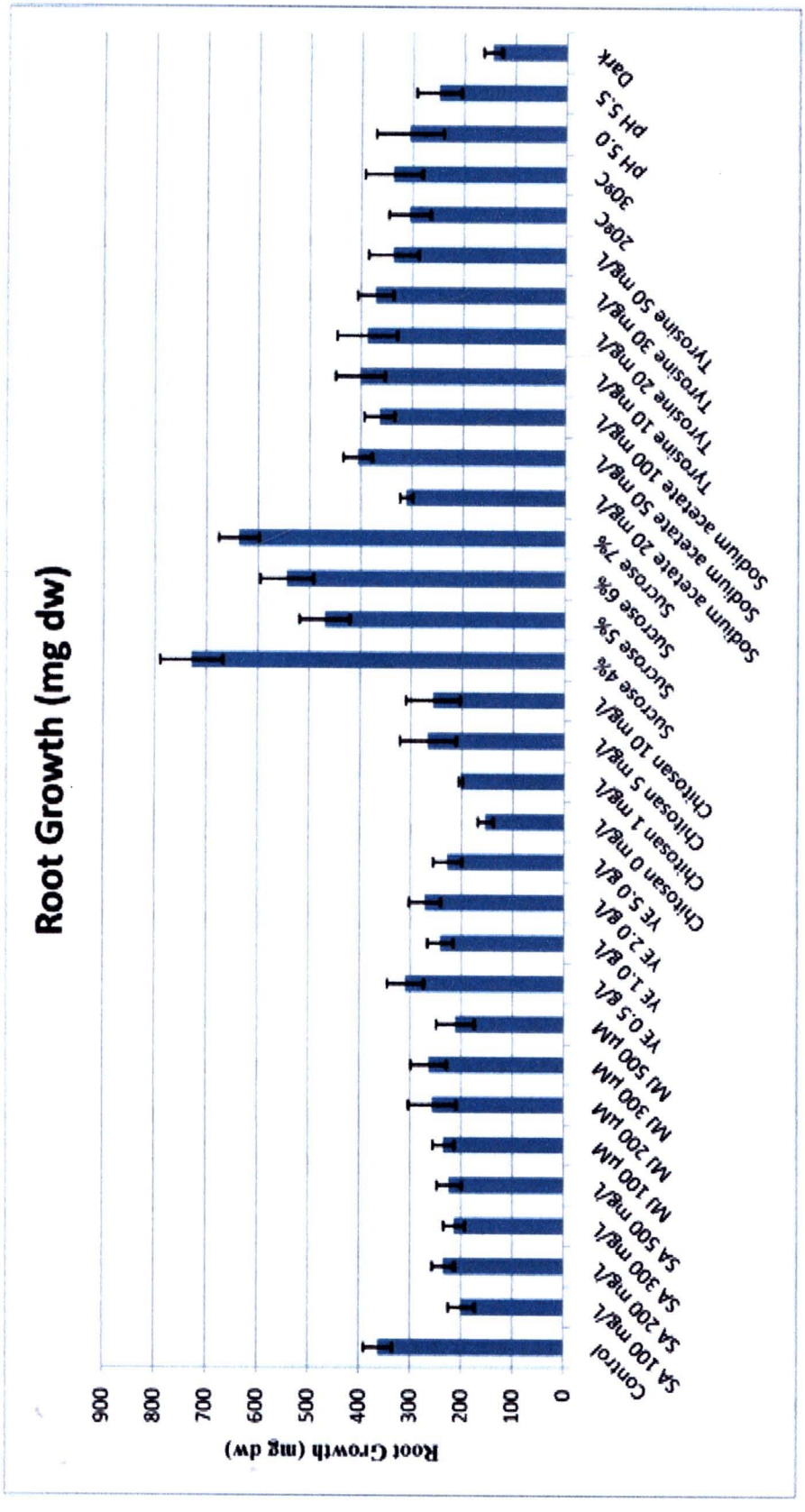

Figure 5.1 Effects of elicitors, precursors and culture condition on root growth.

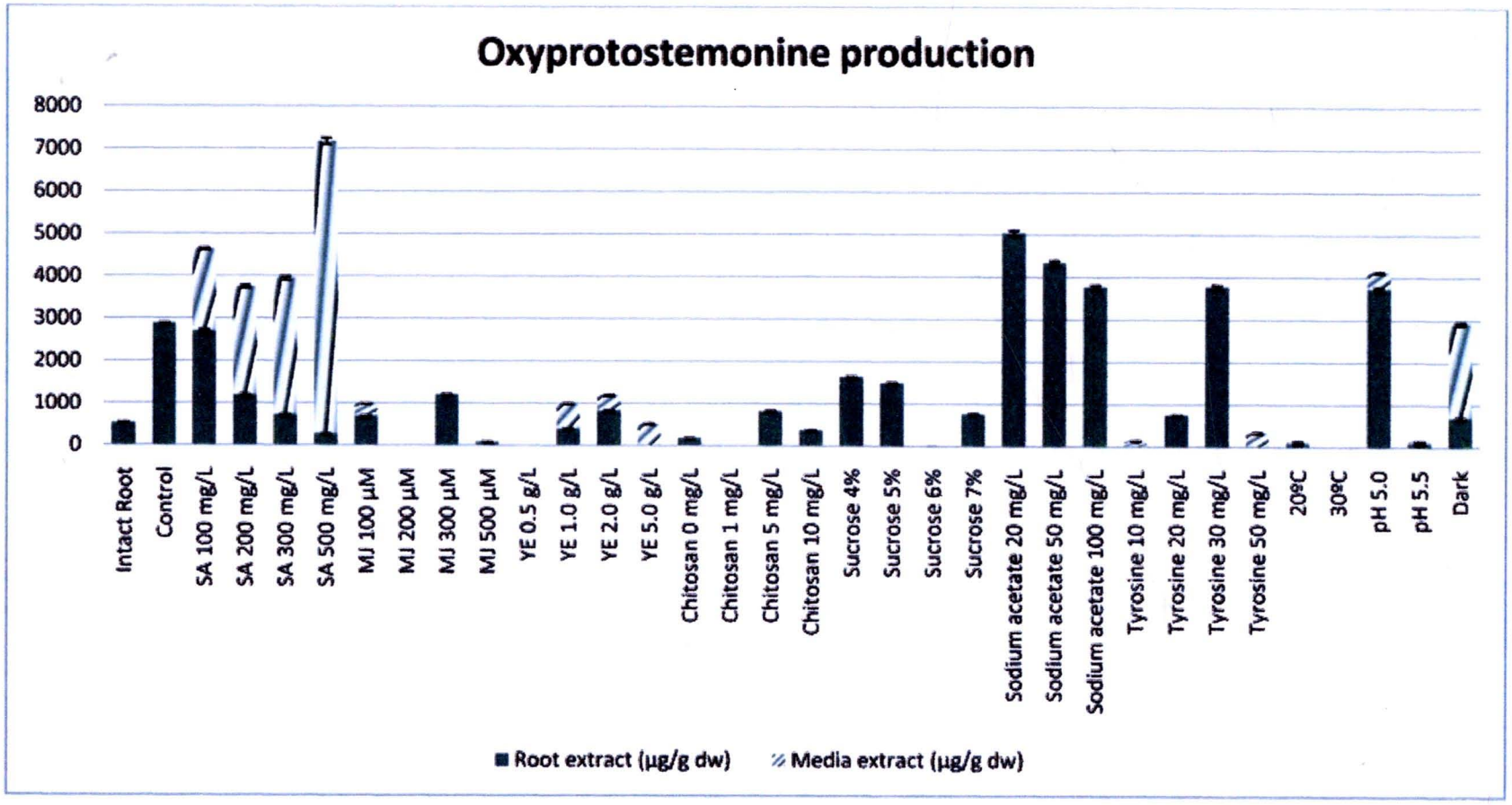

Figure 5.2 Effects of elicitors, precursors and culture condition on oxyprotostemonine production.

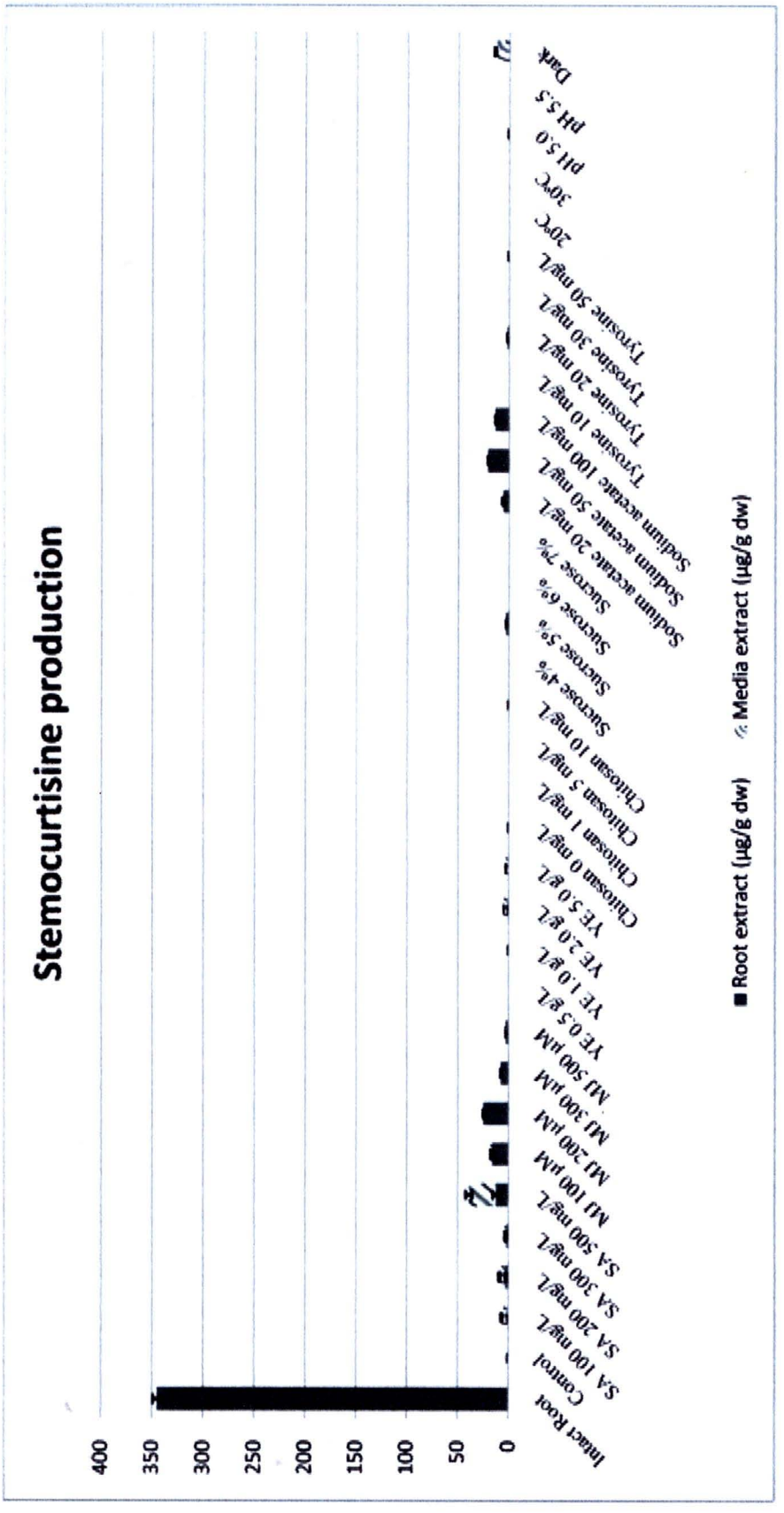

Figure 5.3 Effects of elicitors, precursors and culture condition on stemocurtisine production.

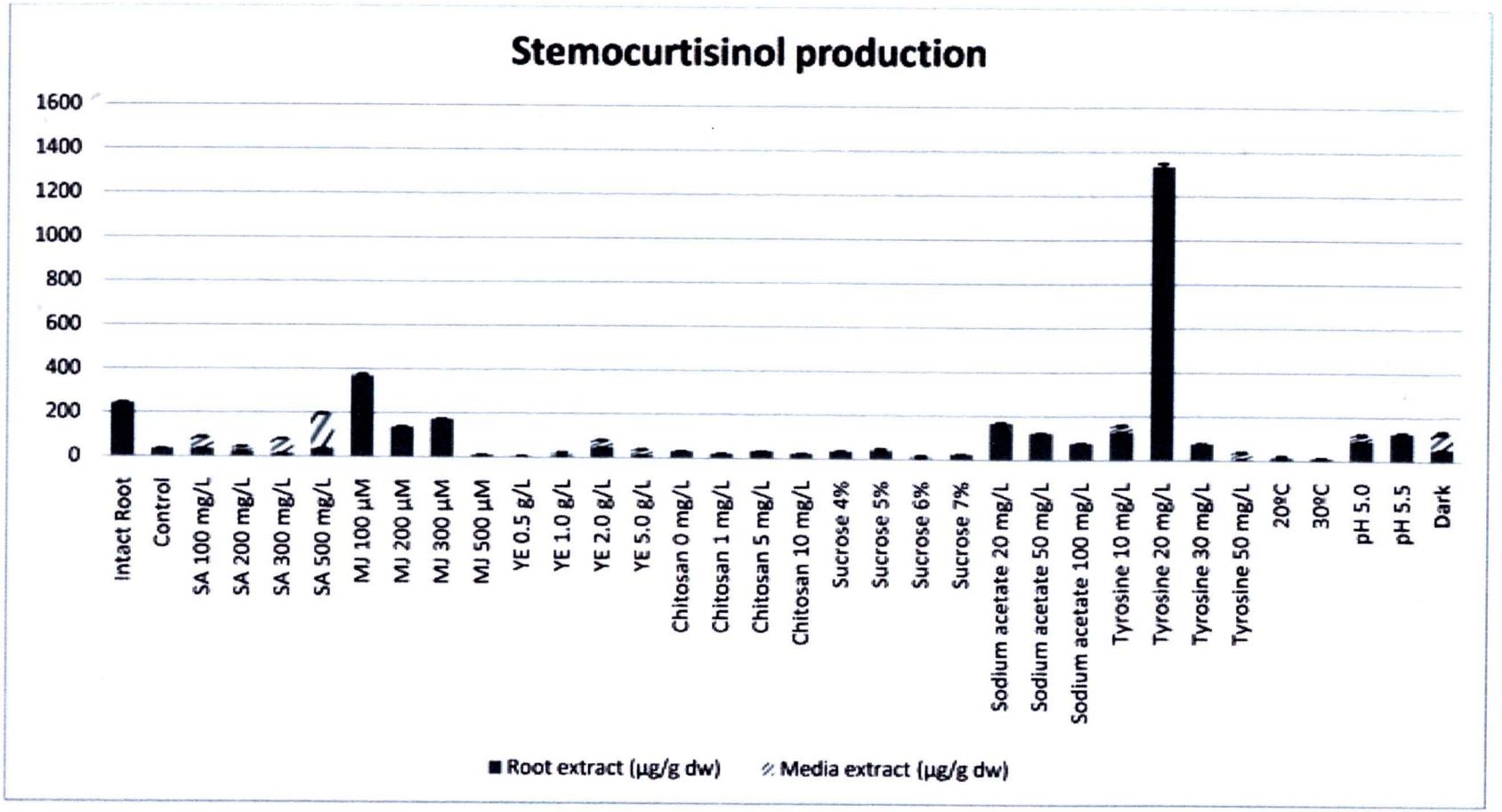

Figure 5.4 Effects of elicitors, precursors and culture condition on stemocurtisinol production.