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Studies on growth and biochemical contents of blackgram varieties (*Vigna mungo* (L.) Hepper) under sago effluent stress

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Abstract

Industrial waste water is being used for irrigation in dry areas and agricultural lands. These effluents not only contain nutrients that enhance the growth of crop plants but also have other toxic materials. An experiment was carried out to study the effect of sago effluent on germination and growth behaviour of blackgram varieties such as VBN – 2, VBN – 1, T – 9, Co – 5 and TMV – 1. The analysis of the effluent showed high concentration of BOD, COD, solids, dissolved solids and chloride. The result obtained after germination showed that the lower concentrations (2.5 and 5%) of effluent increased the germination. At higher concentrations (above 50%) of the effluent, no germination was observed. The highest seedling length was observed in the variety, VBN – 2 (33.50cm) at 2.5% effluent concentration and lowest seedling length was observed in TMV – 1 (15.35cm) variety at 50% effluent concentration. The variety, VBN – 2 is tolerant to the sago effluent in all concentrations studied, than the other varieties on the basis of germination and seedling growth performance. The biochemical contents were also increased in the lower concentration of effluent in all the varieties studied. Hence, it is recommended that the farmers in and around the sago factory can cultivate the blackgram variety, VBN – 2 in the effluent concentration up to 5% per cent.

Keywords: blackgram varieties, biochemical contents, germination, growth, sago effluent, tolerant variety

INTRODUCTION

Industrialization is an important tool for the development of any nation. Consequently, the industrial activity has expanded so much all over the world. Today, it has become a matter of major concern in the deterioration of the environment. With the rapid growth of industries (sago, paper, tannery, textile, sugar, dye, etc.) in most of the countries, pollution of natural water by industrial waste water has increased tremendously (Baskaran *et al*., 2009). Among them, sago industry is an agro based seasonal industry. In India, sago is mainly manufactured from starch obtained from tubers of tapioca. Tapioca is a native of tropical south America and forms an important source of food material for Latin Americans. It is believed to have been introduced into India by Portuguese in 17th century. It forms an important food for a larger percentage of population in the west coast of south India (Denial and Thulasidas, 1993). It is estimated that in India, tapioca is grown over an area of 3.5 million hectares with a production of over 60 lakh tonnes of tubers annually. Kerala ranks first in the cultivation and production of tapioca (FAO, 1990). Tamil Nadu stands first in respect of processing of tapioca into starch and sago throughout the country and meeting about 80% of the country's demand (Sengottaian and Ramanathan, 1998). The total area under tapioca cultivation in Tamil Nadu is about 85,000 hectares.

There are around 1000 sago and starch industries in small scale sector scattered throughout the Tamil Nadu state, of which about 800 units are located in and around the districts of Salem, Dharmapuri and Namakkal which make a substantial amount of economic return. However, it causes serious environmental problem also. It has been reported that 50,000 litres of water is used for crushing 10,000 kg of tapioca (Savitha *et al*., 2009). The effluent has obnoxious odour and irritating colour. The effluents are mainly discharged into the river and sometimes directly into wastelands and agricultural lands. As this polluted water is being used for irrigation to cultivate the crops, it is necessary to conduct experiments to find out the impact of this effluent on agricultural crops and give recommendations for irrigation. Hence, the present study was carried out on the physico-chemical characteristics of sago effluent and its effect on seed germination, growth performance and biochemical contents of five varieties of blackgram (*Vigna mungo* (L.) Hepper).

MATERIALS AND METHODS

The effluent sample was collected at the point of discharge from a sago factory of Sellappam patti, Namakkal district, Tamil Nadu, South India . The

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physico-chemical characteristics of the effluent were analysed by the method of American Public Health Association (1992). The five varieties of blackgram seeds such as VBN – 1, VBN – 2, T – 9, Co – 5 and TMV – 1 were procured from Tamil Nadu Agricultural University, Coimbatore, South India. Healthy seeds of uniform size were divided into batches of 25 each. The seeds were surface sterilized with 0.1% mercuric chloride for 1 – 2 minutes, rinsed repeatedly with sterile distilled water and soaked in petriplates containing equal volume of different concentrations (2.5, 5, 10, 25, 50, 75 and 100%) of sago effluent. Each Petri plate was irrigated with uniform amount of different concentrations of the effluent. In addition, sets of Petri plates were irrigated with water treated as control. Each treatment including control was replicated for three times. The Petri plates were kept and maintained under diffused light at room temperature. The germination study was made upto 7th day from the date of sowing and germination percentage was calculated. Five seedlings from each replicate were selected for recording the morphological parameters such as seedling length, fresh and dry weight of the seedling and biochemical contents like protein (Lowery *et al.*, 1951), chlorophyll (Arnon, 1994) and free amino acids (Moore and Stein, 1948) were studied on the 7th day.

RESULTS AND DISCUSSION

In Tamil Nadu, tapioca figures predominantly as an industrial crop for the production of sago, vermicelli and starch. The Namakkal, Salem and Dharmapuri districts offers good raw materials, cheap labour and conducive climatic conditions throughout the year. This

Table 1. Physico-chemcial properties of sago effluent.

S. No.	Characteristics	Values
1	pH	4.75
2	Colour	Pale white
3.	Temperature	30°C
4	Total solids	6230
5	Total dissolved solids	6030
6	Suspended solids	200
7	Biological oxygen demand	5822
8	Chemical oxygen demand	6800
9	Calcium	380.80
10	Magnesium	270.00
11	Sodium	0.062
12	Potassium	0.017
13	Fluoride	0.120
14	Sulphate	166,86
15	Chloride	1050.00
16	Ammonical nitrogen	17.78
17	Per cent sodium	00.0010

All parameters are expressed in mg/l except pH, colour and temperature.

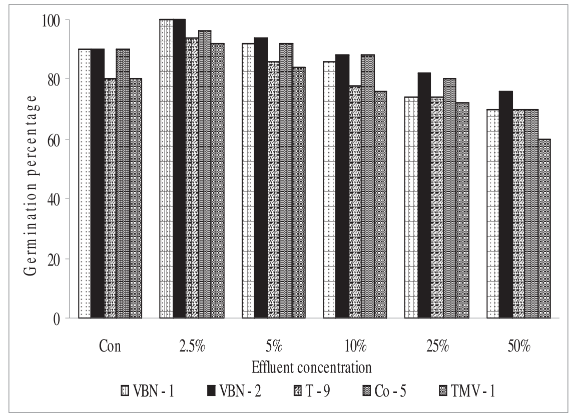

Figure 1. Effect of sago factory effluent on seed germination percentage of five varieties of blackgram. *No germination was observed in 75 and 100 % effluent concentrations. Values are means of five replicates.

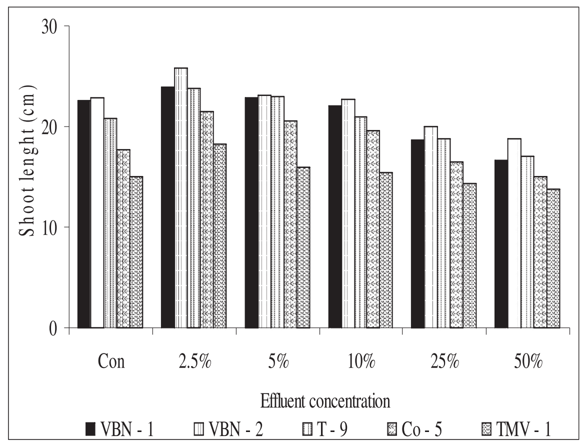

Figure 2. Effect of sago factory effluent on shoot length (cm) of five varieties of blackgram.

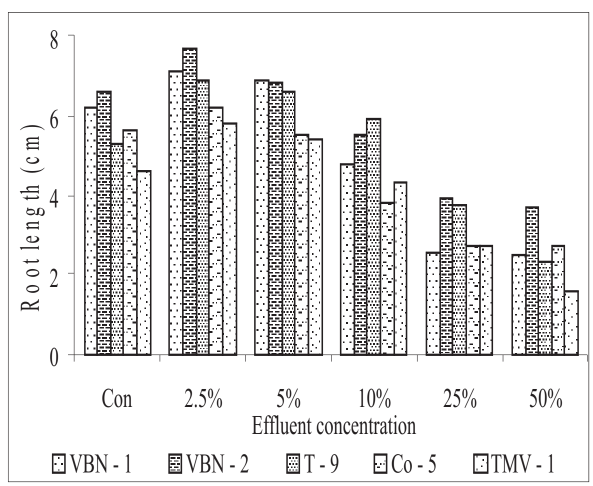

Figure 3. Effect of sago factory effluent on root length (cm) of five varieties of blackgram.

Table 2. Effect of sago factory effluent on seedling length (cm) and fresh weight (gm/fw) and dry weight (gm/dw) of five varieties of blackgram.

Effluent concentration (%)	Blackgram varieties				
	VBN – 1	VBN – 2	T – 9	Co – 5	TMV – 1
Control	SL – 28.80 FW – 1.70 DW – 0.30	SL – 29.50 FW – 1.90 DW – 0.32	SL – 26.10 FW – 1.76 DW- 0.30	SL – 23.24 FW – 1.74 DW – 0.28	SL – 19.62 FW – 1.41 DW – 0.28
2.5	SL – 31.00 (+7.64) FW – 1.94 (+14.11) DW – 0.33 (+9.09)	SL – 33.50 (+13.54) FW – 2.13 (+12.10) DW – 0.35 (+6.66)	SL – 30.76 (+17.62) FW – 1.88 (+6.81) DW – 0.32 (+6.66)	SL – 27.70 (+19.19) FW – 1.73 (+12.64) DW – 0.33 (+17.85)	SL – 24.14 (+23.03) FW – 1.52 (+19.14) DW – 0.30 (+7.14)
5	SL – 29.70 (+3..12) FW – 1.74 (+7.64) DW – 0.31 (+3.33)	SL – 29.90 (+1.35) FW – 1.84 (+1.52) DW – 0.33 (+3.12)	SL – 29.60 (+13.40) FW – 1.80 (+2.22) DW – 0.31 (+3.33)	SL – 26.10 (+12.30) FW – 1.73 (-0.57) DW – 0.32 (+14.28)	SL – 21.30 (+8.56) FW – 1.52 (+7.80) DW – 0.28 (0.00)
10	SL – 26.80 (-6.94) FW – 1.74 (+1.33) DW – 0.29 (-3.33)	SL – 28.20 (-4.40) FW – 1.84 (-3.15) DW – 0.31(-3.22)	SL – 26.20 (+3.06) FW – 1.77 (+0.56) DW – 0.28 (-6.66)	SL – 23.40 (+0.68) FW – 1.07 (-2.29) DW – 0.27 (-3.57)	SL – 19.77 (+0.76) FW – 1.38 (-2.12) DW – 0.26 (-7.14)
25	SL – 20.92 (-27.36) FW – 1.33 (-21.76) DW – 0.27 (-10.00)	SL – 23.93 (-18.88) FW – 1.50 (-21.06) DW – 0.28 (-12.50)	SL – 22.56 (+13.56) FW – 1.36 (-22.72) DW – 0.26 (-10.71)	SL – 19.33 (-20.22) FW – 1.45 (16.66) DW – 0.25 (-10.71)	SL – 17.02 (-13.25) FW – 1.29 (-8.10) DW – 0.23 (-17.85)
50	SL – 19.62 (-28.22) FW – 1.20 (-29.14) DW – 0.24 (-20.10)	SL – 22.56 (-23.52) FW – 1.40 (-26.31) DW – 0.25 (-25.00)	SL – 19.90 (-23.75) FW – 1.26 (-28.40) DW – 0.22 (-21.48)	SL – 17.77 (-25.77) FW – 1.30 (-25.28) DW – 0.32 (-21.48)	SL – 15.35 (-27.76) FW – 1.06 (-24.12) DW – 0.20 (-28.57)

No germination was observed in 75 and 100 % effluent concentrations. Values are means of five replicates. Percentage over control values are expressed within parentheses.
SL – Seedling length; FW – Fresh weight; DW – Dry Weight.

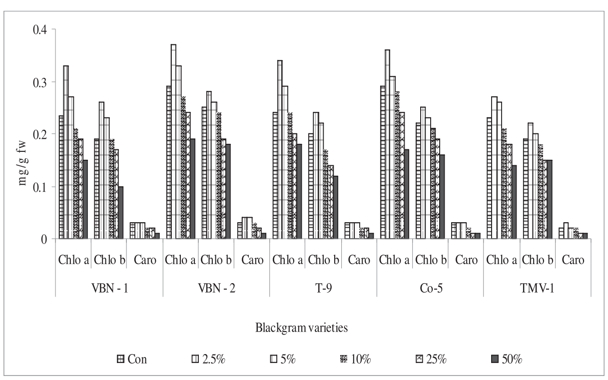

Figure 4. Effect of sago factory effluent on pigment content of five varieties of blackgram.
Chlo a – chlorophyll a; Chlo b – chlorophyll b;
Caro – carotenoid

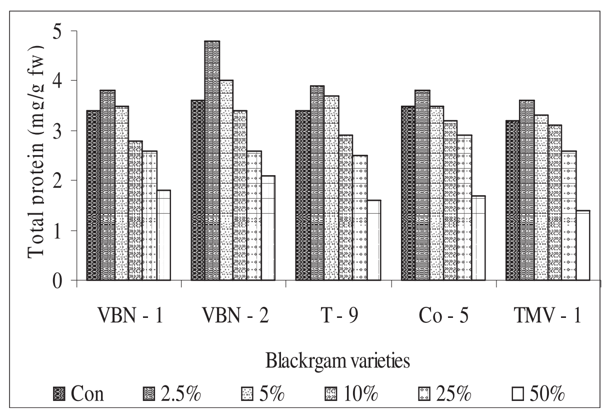

Figure 5. Effect of sago factory effluent on total protein of five varieties of blackgram.

helps manufactures to produce more tapioca based products and therefore this area is known as a '**Land of Sago**' even at the industrial level. The productivity of tapioca is about 20-30 tonnes/hactare in this area, which is the highest in the world. The national average is 19 tonnes/hactare while the world's average production stand at 10 tonnes/hectare only. In sago factories, during the process of sago a small amount of sulphuric acid (H_2SO_4 is added for whitening of the powder). It was reported that 100 kg of starch was produced from 400 kg of tapioca. The powder is directly used or heated to produce sago. During this process a considerable amount of waste water is discharged as effluent.

The physico-chemical properties of the effluent are given in Table 1. The pH of the effluent is acidic (4.75) in nature. It may be due to the enrichment of chemicals in the

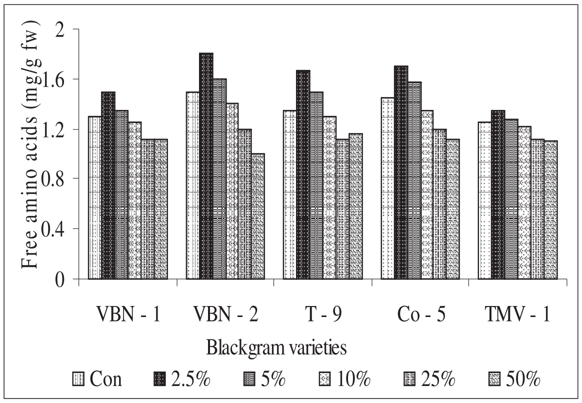

Figure 6. Effect of sago factory effluent on free amino acids of five varieties of blackgram.

effluent. The effluent discharged in the surrounding areas and nearby water bodies which results in the toxification of drinking water. This also affects the growth and reproduction of aquatic organisms (Gorman and Garden, 1963). The effluent contain higher amount of total solids (6230mg/l) and dissolved solids (6030mg/l) which result in higher amounts of BOD (5822mg/l) and COD (6800mg/l). The high levels of BOD in the effluent might cause respiratory problems to the aquatic life, which might results in changes in the species composition. In addition to the above, the waste water may also act as a source of disease causing vectors and create public health problems in and around the sago units (Prabhakara Rao and Prasada Rao, 2002). A considerable amount of calcium (380.80mg/l), magnesium (270mg/l), sulphate (166mg/l) and chloride (1050mg/l) are also present in the effluent. This may be due to the colour, chemicals and quality of the effluent. This inturn depends on the type of sago factories and the amount of H_2SO_4 and chloride used by them (Sivashanugam, 2003).

The effects of the different concentrations of effluent on germination percentage of black gram are shown in Fig. 1. In all the varieties, the germination have been observed up to 50% effluent concentration and no germination was recorded in the 75% and 100% effluent concentrations. This reduction in the germination at higher concentration of effluent might be due to the presence of excess amount of solids in the effluent, this seems to be responsible for inhibition of germination, because of osmotic relationship between seed and water, thus reducing the amount of obsorbed water and retarding seed germination (Adrranio, 1973; Rita *et al.*, 2008). In addition, the reduction in germination at higher concentrations may be due to the excess amount of minerals and nutrients present in the effluent (Baskaran *et al.*, 2009; Sivananada, 2010). Among the studied varieties, generally in all concentrations of effluent treatment the variety VBN – 2 (2.5% effluent concentration) showed better performance in terms of per cent germination (100%). On the other hand, the variety, TMV – 1 exhibited relatively poor germination percentage (92%). The increase in germination percentage over the control at 2.5 and 5% effluent concentrations in all the varieties indicates the stimulation by the effluent treatment at lower concentration (Sivaraman and Thamizhinian, 2010). Jeratha and Sahai (1982) also reported that the lower concentrations of effluent provided optimum conditions for germination. In addition, the increase in germination percentage might be due to the reduction in the level of toxic metabolites by dilution and better utilization of nutrients in the effluent (Kannan, 2001; Baskaran *et al.*, 2009).

The shoot, root and seedling lengths of five varieties of black gram at different concentration of the effluent are given in Table 2 and shown in (Figs. 2-3). The seedling length was increased at lower concentrations (2.5 and 5%) of effluent but decreased at higher concentrations. Similar effect was noticed for various effluents by Balashouri and Prameeladevi (1995); Sundaramoorthy and Kunjithapatham (2000). The germinated seeds get low amount of oxygen in dissolved form due to the presence of high concentration of solids in the higher effluent concentrations. This reduces their energy supply through anaerobic respiration that results in restricting the growth and development of seedling (Saxena *et al*., 1986). The highest seedling length was found in the variety VBN – 2 (33.50cm) in 2.5% effluent concentration, while the lowest seedling length (15.33cm) was observed in the variety TMV – 1 at 50% effluent concentration. Sharon and Garg (1983) reported that, excess accumulation of chloride iron (Cl^-) under salt stress reduce turgor pressure inside the cell of the treated seedlings which in turn causes reduction in growth of seedling. The inhibition of seedling growth by sago effluent might be due to the excess accumulation of chloride ions (Cl^-).

The dry matter accumulation/production reflects the actual physiological status of the plant. It is rather an indicator parameter of growth performance of plant (Kumawat *et al*., 2001). The fresh and dry weights of the five varieties of black gram at different concentrations of the effluent are given in Table 2. The variety, VBN – 2 pronounced more fresh and dry weight in comparison to other varieties studied in all the concentrations, whereas the variety, TMV – 1 recorded low fresh and dry weights. The reduction of fresh and dry weights of plant materials might be due to the poor growth under effluent treatment (Balashouri and Prameela devi, 1994). The highest fresh and dry weight of seedlings was observed in the variety VBN – 2 at 2.5% effluent concentration and lowest weight of seedlings was recorded in the variety TMV – 1 at 50% effluent concentration. Rajannan and Oblisamy (1979) and Somasekar *et al.* (1990) suggested that the interaction between the various constituents of the effluent and native microbes might be responsible for the inhibition of seedling growth. It may be due to the reduction of fresh and dry weights of seedlings. Based on the morphological parameters observed, in the present study the variety, VBN – 2 was found to be tolerant than the other varieties studied. Similarly, among the various concentrations of the effluent, up to 5% concentration favoured the seedling development when compared to the other concentrations studied.

Chlorophyll estimation is one of the important biochemical parameters which is used as the index of production capacity. The production of pigments namely Chlorophyll a, b, total chlorophyll and carotenoid were higher in lower concentrations of the effluent treatment (Fig. 4). They decrease in the seedling growth under higher concentrations of sago effluent. Similar kind of results was observed by Ayyasamy *et al.* (2008) and the lower concentration of effluent seems to play a role in increasing the pigment concentration (Strongonor *et al.*, 1979). The increase of chlorophyll content at lower concentration might be due to the favourable effect of elements present in the effluent on the pigment system (Cottenie, 1973).

The seedling grown under the treatment of lower concentration of effluent registered higher protein content (Table 5). It might be due to adsorption of most of the necessary elements by plants (Rita *et al*., 2010). In addition, higher nitrogen uptake occurred in the seedling grown under lower concentrations of the effluent (Tunaka *et al*., 1964; Srivastava *et al*., 1988). The protein content decreased with the increasing effluent concentrations. It has been reported that high concentrations of various cations and anions are present in the effluent might reduce protein synthesis and nutrient levels in the plants, as physiological activity was decreased (Swaminathan *et al*., 1992; Muthusamy and Jayabalan, 2001). The aminoacid content increased in the heat ments up to 5 per cent effluent concentration and then decreased at higher concentrations (Fig. 6). Such a decreasing activity might be due to the inhibitory effect of the effluent on protease activity, the major enzyme during the early stage of germination (Joshi and Iyangar,1982 and Balashuri and Prameeladevi, 1994).

CONCLUSION

It is concluded from the results obtain in the present study that the lower concentrations of sago effluent enhanced the germination and seedling growth of black gram in all the five varieties studied. Among them, the variety VBN – 2 was found to be more tolerant than the other varieties studied. On the other hand, the variety, TMV – 1 was found to be highly susceptible against sago effluent. Similar trend was observed with regard to the biochemical contents as well in all the varieties of blackgram. Hence, it is concluded that the farmers in and around the sago factories of Salem, Namakkal and Dharamapuri districts may cultivate the black gram variety, VBN – 2 in the effluent concentration of up to 5 per cent.

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