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ORIGINAL ARTICLE

Allelopathic Stress Produced by Bitter Gourd (*Momordica charantia* L.)

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The present study deals with in vitro effects of allelochemicals present in leaf and fruit leachate of *Momordica charantia in vitro* on plant growth and metabolism of *Lycopersicon esculentum*. *Momordica* was selected as a donor plant and tomato as recipient. Seeds of tomato were shown in pots and after germination different concentrations viz. 25, 50, 75 and 100% of leaf and fruit leachates were applied as treatment. Twenty days old seedlings were harvested for biophysical and biochemical analyses. The root and shoot length, fresh and dry weight of the seedlings decreased in dose dependent manner. The reduction in pigment and protein contents and nitrate reductase activity was concentration dependent. Membrane leakage increased as the concentration of leachates increased. Activities of antioxidant enzymes viz. superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) activities significantly enhanced under allelopathic stress. Inhibition of various metabolic activities under allelopathic stress resulted in decreased plant growth and development. The fruit leachate of *Momordica* was more inhibitory than leaf leachate.

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Key words: allelopathy, antioxidants, electrolyte leakage, leachates, Momordica

Abbreviations: CAT, Catalase; DW, Dry weight; EDTA, Ethylene diamine tetra acetic acid; EL, Electrolyte leakage; FW, Fresh weight; NBT, Nitro blue tetrazolium; NEDD, N-1-naphthyl-ethylene diamine dihydrochloride; NR, Nitrate reductase; POX, Peroxidase; ROS, Reactive oxygen species; SOD, Superoxide dismutase; SL, Shoot length; RL, Root length

Plants produce various bioactive secondary metabolites which have favourable or unfavourable effects on growth and development of neighbouring plants or microorganisms (Rice, 1984; Singh *et al*., 2009). This phenomenon is known as allelopathy and the bioactive secondary metabolites involved are called allelochemicals (Narwal *et al*., 1997; Singh *et al*., 2010). Allelopathy

becomes more apparent in crop rotation and in mix or intercropping systems. Allelochemicals are released in the environment as root exudates, plant residues decomposition, leaf leachates and microbial metabolic activity and caused allelopathic stress (Crutchfield *et al*., 1985). In natural ecosystems and agro-ecosystems, allelochemicals influence the growth and development of recipient plants (Inderjit and Duck, 2003). Allelochemicals have adverse effects on the target plants and they cause a biotic stress called allelopathic stress. Allelopathins are accumulated in the soil and affect the growth and metabolism of receipient plants (Crut-Ortega *et al*., 2002). Allelochemicals may also adversely affect the basic characteristic of the soil which in turn affects the growth of the plants (Batish *et al*., 2002, Singh *et al*., 2010). The crop productivity, vegetation pattern and growth were adversely influenced under the allelopathic stress condition (Weir *et al*., 2004; Singh *et al*., 2009). Allelochemicals cause alternation in various cellular processes in plants viz. stomatal closure (Barkosky *et al*., 2000), water balance in plants (Barkosky and Einhellig 2003), membrane permeability (Galindo *et al*., 1999) and respiration (Abrahim *et al*., 2000). Reactive oxygen species are generated under stress condition and they cause oxidative damage (Bias *et al*., 2003; Cruz-Ortega *et al*., 2002). Plants have a detoxifying defense mechanism to tolerate stress and avoid oxidative damage (Doblinski *et al*., 2003; Yu and Matsui 1997). Antioxidant defence system includes enzymes viz. superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX). SOD, CAT and POX detoxify highly reactive oxygen species (Rubio *et al*., 2002, Únyayar *et al*., 2005). *Momordica* is one of the important medicinal vegetable crops worldwide. It abundantly contains several phenolic compounds viz. gallic, chlorogenic, ferulic acids etc with allelopathic activities, which are beneficial for human health (Singh *et al*., 2011).

The aim of the present study was to investigate the allelopathic potential of leaf and fruit leachate of bitter gourd on tomato seedlings. This type of study will help to understand how one vegetable crop which is beneficial for human health can adversely affect other vegetable crop.

MATERIALS AND METHODS

Preparation of leachate

Plants of *Momordica charantia* were sown and grown to fruiting stage in the Roxburgh Botanical Garden, Department of Botany, University of Allahabad, Allahabad (24° 47′ and 50° 47′ N latitude; 81° 91′ and 82° 21′ E longitude; 78 m above the sea level). Leaves and fruits were taken for leachate preparation. Leaves and fruits were cut into small pieces and soaked separately in distilled water in the ratio 1:4 (w/v). After 3 days the collected leachate was filtered and centrifuged at 1500g. The supernatant was collected and stored at low temperature to avoid biodegradation. The leachate was used undiluted (100%) and diluted with distilled water to 25, 50 and 75% concentrations.

Growth and stress treatment

Seeds of tomato (*Lycopersicon esculentum* L. var. Pusa ruby) were obtained from the certified seed agency of Allahabad, U.P, India. Healthy seeds of tomato were surface sterilized in 0.001 M $HgCl_2$ and washed with double distilled water thoroughly. The 3 seeds were sown in each pot. The experimental pots were divided into two sets. One set was treated with fruit leachate and other set with leaf leachate. After seed germination the treatment of leaf and fruit leachates of different concentrations i.e., 25, 50, 75 and 100% was

applied. The treatment was given in alternate days. Pot treated with distilled water were taken as control. The experiment was conducted in a culture room at a temperature 28 ± 2°C, photoperiod 18/6h, humidity 61±5% and photon flux density 240 µmol m^{-2} s^{-1}. 21 days old seedlings were harvested. The first fully expanded leaves were sampled for bioanalyses.

Measurement of pigment and protein content

Chlorophyll of experimental plant was extracted with 80% acetone. The amount of photosynthetic pigments was determined as per the method of Lichtenthaler (1987). Ten mg fresh leaf was homogenized in 10 mL of 80% acetone and centrifuged. Supernatant was taken and optical density was measured at 663nm, 645nm and 470nm. Protein content was determined following the method of Lowry *et al*. (1951). The amount of protein was calculated with reference to standard curve obtained from bovine serum albumin.

Nitrate reductase

Nitrate reductase (EC 1.6.6.1) activity was assayed by modified procedure of Jaworski (1971) based on incubation of fresh tissue (0.25g) in 4.5 mL medium containing 100 mM sodium phosphate buffer (pH 7.5), 3% (w/v) KNO_3 and 5% propanol. About 0.4 mL aliquot was treated with 0.3 mL 3% sulphanilamide in 3 N HCL and 0.3 mL 0.02% N-(1-Naphthyl) ethylene diamine dihydrochloride (NEDD). The absorbance was measured at 540 nm. NR activity was calculated with a standard curve prepared from $NaNO_2$ and expressed as µ mol NO_2 g^{-1} FW h^{-1}

Electrolyte leakage

Membrane integrity was measured in terms of electrolyte leakage. Fresh leaves (0.1g) were placed in a vial containing 10 mL of double distilled water kept in dark for 24h at room temperature. Electrical conductivity (EC_1) of the bathing solution was measured at the end of incubation period. The tissue with bathing solution was then heated in water bath at 95°C for 20 min the electrical conductivity (EC_2) was again measured after cooling. EL was calculated as percentage of EC_1/EC_2.

Extraction and assay of antioxidant enzymes

Enzyme extract was prepared by homogenizing 500 mg leaves in 10 mL of 0.1 M sodium phosphate buffer (pH 7.0). The homogenate was filtered and centrifuged at 15000 g at 4° C for 30 min. The supernant was collected and used for measurement of activities of SOD (EC 1.15.11), CAT (EC 1.11.1.6) and POX (EC 1.11.1.7).

SOD activity was estmated by the nitroblue tetrazolium (NBT) photochemical assay method following Beyer and Fridovich (1987). The reaction mixture (4mL) contained 63 µM NBT, 13 mM methionine, 0.1 mM ethylene diamintetra acetic acid (EDTA), 13 µM riboflavin, 0.5 M sodium carbonate and 0.5 mL clear supernatant. Test tubes were placed under fluorescent lamps for 30 min and identical unilluminated assay mixture was used as blank. The absorbance was recorded at 560 nm. One unit of enzyme was defined as the amount of enzyme which caused 50% inhibition of NBT reduction.

Catalase activity was assayed as per the method of Cakmak and Marschner (1992). The reaction mixture (2mL) contained 25 mM sodium phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.2 mL enzyme extract. The activity was determined by measuring the rate of disappearance of H_2O_2 for 1min at 240 nm and calculated using extinction coefficient of 39.4 mM^{-1} cm^{-1} and expressed as enzyme unit g^{-1} fresh weight. One unit of CAT was defined as the

amount of enzyme required to oxidize 1 µM H_2O_2 $min^{-1.}$

Peroxidase (EC 1.11.1.7) activity was assayed following the method by Mc Cune and Galston (1959). Reaction mixture contained 2.0 mL enzyme extract, 2 mL potassium phosphate buffer, 1.0 mL 0.1 N pyrogallol and 0.2 mL 0.02% H_2O_2 and determined spectrophotometrically at 430 nm. One unit of enzyme activity was defined as the amount which produced an increase of 0.1 OD per minute.

Statistical analysis

Standard errors of means were calculated in triplicates. In addition, analysis of variance was carried out for all the data generated from this experiment, employing one way ANOVA test using GPIS software 3.0 (GRAPHPAD California USA).

RESULTS

The results showed the allelopathic potential of *Momordica* leaf and fruit leachates on tomato seedlings. Both leaf and fruit leachates caused significant alternations in the growth and metabolism of the seedlings. Seedling height and biomass significantly ($p \leq 0.001$) decreased under allelopathic stress as compared with control. RL, SL and FW and DW significantly decreased in dose dependent manner under both leachates. Maximum reduction was recorded in 100% concentration of leachates as compared with control. Reduction in growth was more prominent under fruit leachate than leaf leachate. Seedlings under control showed maximum growth (Table 1).

Allelochemical stress gradually decreased photosynthetic pigment content in the seedlings. Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents were significantly ($p \leq 0.001$) decreased in dose dependent manner in the seedlings under the influence of both leachates. The minimum amount of pigment content was recorded in 100% concentration of both leaf and fruit leachates. Fruit leachate caused maximum decrease in pigment content as compared with leaf leachate. The seedlings of control group exhibited maximum amount of photosynthetic pigment (Table 2).

Protein gradually reduced in seedlings under all treatments. Both leachates were found to be inhibitory. The inhibition was concentration dependent. Maximum reduction in protein content was recorded in highest concentration of leachates. The seedlings in control exihibited maximum amount of protein content. NR activity was adversely affected under allelopathic stress. Decrease in NR activity was proportional to concentration. The leaf and fruit leachates significantly ($p \leq 0.001$) inhibited the NR activity with drastic decrease in seedlings treated with highest concentration. Maximum NR activity was recorded in the leaves of the seedlings under control. Allelopathic stress caused membrane damage and electrolyte leakage. Higher concentration of leaf and fruit leachates of *Momordica* increased the electrolyte leakage (EL) which was concentration dependent with maximum in 100% concentration (Tale 3).

Antioxidative defense system consists of an important components viz. SOD, CAT and POX to avoid oxidative damage caused by stress. The activities of antioxidative enzymes were significantly ($p \leq 0.001$) enhanced under both leaf and fruit leachates. The increase in SOD, CAT and POX activities were concentration dependent. Maximum activities of SOD, CAT and POX were recorded in the seedlings treated with 100% concentration of the leachates (Table 4). The fruit leachate was more phytotoxic than leaf leachate.

Table 1 Allelopathic effects of leaf and fruit leachates of *Momordica* on shoot length, root length, fresh weight and dry weight of tomato seedlings.

Treatment	Root length (cm)	Shoot length (cm)	FW (g/plant)	DW (g/plant)
C	20.5+0.86	27+0.28	9.14+0.23	1.05+0.038
L25	15.2+0.17[a]	25.5+0.86	7.12+0.29[a]	0.86+0.004[a]
L50	10.9+0.23[a]	23.8+0.11[a]	6.70+0.30[a]	0.82+0.016[a]
L75	10.4+0.08[a]	23.7+0.72[a]	6.14+0.14[a]	0.70+0.002[a]
L100	9.2+0.43[a]	20.5+0.86[a]	4.25+0.33[a]	0.37+0.002[a]
F25	6.5+0.46[a]	23.2+0.14[a]	6.09+0.09[a]	0.82+0.001[a]
F50	5.2+0.11[a]	22.2+0.72[a]	5.31+0.30[a]	0.52+0.017[a]
F75	4.8+0.14[a]	19.5+0.86[a]	4.29+0.31[a]	0.48+0.028[a]
F100	4.4+0.05[a]	15.7+0.14[a]	3.66+0.05[a]	0.39+0.011[a]

Data are mean of three replicates ± SEM. [a] $p<0.001$ versus C. C, control, L25, L50, L75, L100 , F25, F50, F75 and F100 are 25%,50%, 75% and 100% concentrations of leaf and fruit leachates of *Momordica*, respectively.

Table 2 Allelopathic effects of leaf and fruit leachates of *Momordica* on the pigment contents of tomato seedlings

Treatment	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Carotenoid (mg/g FW)
C	0.89+0.036	0.76+0.030	1.66+0.005	0.72+0.004
L25	0.89+0.013	0.71+0.001[c]	1.61+0.014	0.69+0.006
L50	0.77+0.025[a]	0.68+0.006[a]	1.46+0.019[a]	0.64+0.010[a]
L75	0.66+0.002[a]	0.64+0.009[a]	1.31+0.011[a]	0.58+0.001[a]
L100	0.65+0.033[a]	0.60+0.009[a]	1.25+0.023[a]	0.55+0.014[a]
F25	0.65+0.031[a]	0.58+0.019[a]	1.23+0.012[a]	0.52+0.024[a]
F50	0.64+0.005[a]	0.54+0.004[a]	1.18+0.001[a]	0.46+0.002[a]
F75	0.57+0.006[a]	0.53+0.015[a]	1.10+0.009[a]	0.45+0.008[a]
F100	0.52+0.014[a]	0.43+0.015[a]	0.95+0.030[a]	0.40+0.002[a]

Data are mean of three replicates ± SEM. [a] $p<0.001$, [c] $p<0.05$ versus C. C, control, L25, L50, L75, L100 , F25, F50, F75 and F100 are 25%,50%, 75% and 100% concentrations of leaf and fruit leachates of *Momordica*, respectively.

Table 3 Allelopathic effects of leaf and fruit leachates of *Momordica* on protein and sugar content and nitrate reductase activity of tomato seedlings

Treatment	Protein (mg/g FW)	NR (µmol NO_2 g^{-1} FW h^{-1})	EC(%)
C	15.45+0.43	38.75+0.72	58.71+0.11
L25	14.1+0.49[c]	33.25+0.43[a]	58.76+0.31
L50	13.27+0.04[a]	26+0.14[a]	61.19+0.68[a]
L75	11.75+0.02[a]	18.37+0.36[a]	66.21+0.38[a]
L100	9.25+0.02[a]	12.87+0.50[a]	70.64+0.21[a]
F25	14.1+0.34c	21.25+0.28[a]	68.56+0.41[a]
F50	11.95+0.34[a]	18.62+0.50[a]	68.92+0.06[a]
F75	11.52+0.73[a]	11.12+0.07[a]	71.37+0.[21a]
F100	3.32+0.18[a]	9.5+0.28[a]	74.23+0.82[a]

Data are mean of three replicates ± SEM. [a] $p<0.001$, [c]$p<0.05$ versus C. C, control, L25, L50, L75, L100 , F25, F50, F75 and F100 are 25%,50%, 75% and 100% concentrations of leaf and fruit leachates of *Momordica*, respectively.

Table 4 Allelopathic effects of leaf and fruit leachates of *Momordica* on enzymes activity of tomato seedlings

Treatment	SOD (EU g^{-1} FW)	CAT (EU g^{-1} FW)	POX (EU g^{-1} FW)
C	18.45+0.42	7.45+0.115	15.02+0.03
L25	22.36+0.91[a]	9.19+0.002[b]	20.55+0.26[a]
L50	27.99+0.48[a]	11.19+0.885[a]	28.35+0.82[a]
L75	31.25+0.58[a]	12.25+0.923[a]	33.39+0.40[a]
L100	38.55+0.51[a]	12.92+0.346[a]	39.53+0.76[a]
F25	21.34+0.19[a]	7.49+0.057	26.18+0.18[a]
F50	29.3+0.27[a]	8.35+0.019	27.63+0.03[a]
F75	33.54+0.24[a]	8.77+0.028	30.17+0.37[a]
F100	38.57+0.76[a]	9.42+0.057[b]	39.57+0.71[a]

Data are mean of three replicates ± SEM. [a] $p<0.001$; [b]$P<0.01$ versus C. C, control, L25, L50, L75, L100 , F25, F50, F75 and F100 are 25%,50%, 75% and 100% concentrations of leaf and fruit leachates of *Momordica*, respectively.

DISCUSSION

The results clearly showed that the allelopathic potential of *M. charantia* on growth of tomato seedlings. Reduction in plant growth under allelopathic stress was previously studied (Batish *et al*., 2006; Singh *et al*., 2009, Singh *et al*., 2008). In

case of *Trianthema portulacastrum* and *Sicyos deppei* aqueous leachate inhibited the plant growth (Randhawa *et al*., 2002; Romero-Romero *et al*., 2005). Seedlings growth significantly decreased by increase leachate concentrations. The leaf and fruit leachates of *M. charantia* adversely affected the growth of the test plant. Tomato seedlings were sensitive to both leachates. In the experiment, we observed reduction in the photosynthetic pigments under allelopathins present in the leachate of donor plant. Similar results were also reported in radish and sorghum (Venkateshwarlu *et al*., 2001; Bagavathy and Xavier 2007). Previous studies have indicated, that allelochemicals reduce the accumulation of chlorophyll content (Hejl *et al*., 1993; Singh *et al*., 2010). Jia *et al*., (2008) found that chlorophyll synthesis could be stopped by allelopathy. The reduced chlorophyll content by allelochemical was also reported (Kanchan and Jayachandra 1980; Singh *et al*., 2010). Oxidative damage, caused by ROS, may cause arrested biosynthesis of pigment or degradation by impaired metabolic processes (Singh *et al*., 2010).

The protein degradation or inhibition was earlier reported under allelochemical stress (Thaper and Singh, 2006). NR activity decreased in stressed seedlings. NR activity was found to be dependent on energy, e^- donor and carbon skeleton which were provided by the process of photosynthesis (Kaiser *et al*., 1993, Singh *et al*., 2010). NR activity is induced by substrate regulated absorption of nitrate is also responsible for the decrease of NR activity. Inhibited synthesis of enzymes may be another possible reason for reduction in NRA (Chen and Sung, 1983). Decreased NR activity was also reported in sorghum (Bagavathy and Xavier, 2007) and *V. radiata* (Tripathi *et al*., 2000).

The membrane damage is the common mark of allelopathic stress (Singh *et al*., 2006). The allelopathins present in the both leachates increased the membrane damage of test plant. Allelochemicals caused disturbance in membrane permeability and diversion of oxygen towards oxidative damage (Nürnberger *et al*., 1994; Singh *et al*., 2010). Plants overcome from the oxidative damage by activating the enzymes of antioxidative defense system (Singh *et al*., 2010).

Under stress condition a number of antioxidative enzymes increased tremendously to avoid the oxidative damage caused by ROS (Foyer and Noctor, 2003; Singh *et al*., 2009). These antioxidative enzymes act as stress markers. SOD is considered to be the first line of defense (Gomez *et al*., 2004). Allelochemicals enhanced the activities of SOD, CAT and POX (Doblinski *et al*., 2003; Curtze-Ortega, 2002). Present results show that the enzyme activities increased with increasing concentration of leachates. Decrease in FW, DW, SL and RL of tomato seedlings clearly indicated that the allelopathic nature of *Momordica* leaf and fruit leachates. This decrease was the manifestation of impaired metabolic activities due to allelochemicals present in the leaf and fruit leachate of donor plant. POX, SOD and CAT activities increased with the free radicals production. Leachate was inhibitorier in its higher concentration (Singh *et al*., 2008). The reduction in growth and alternation in metabolic processes due to the allelopathic potential of *Momordica* is accompanied by reduction in the various physiological parameters of treated plants.

CONCLUSIONS

The present study showed that the allelochemicals present in leachates of *Momordica* leaf and fruit caused deleterious effect on growth

and metabolism of tomato. Results clearly indicated that fruit leachate has more allelopathic potential than leaf leachate.

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