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Allelopathic Efficacy of *Zingiber officinale* Rosc Aqueous Leaf, Stem and Rhizome Extract on Biochemical Parameters of *Zea mays* L.

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In the present study an attempt was been made to assess the allelopathic effect of ginger on biochemical parameters of maize. The aqueous leaf, stem and rhizome extract of ginger showed inhibitory effects on protein, carbohydrate, chlorophyll and phenol content. The chl. a, b, total chlorophyll and carotenoid content were found to be decreased as the concentration of the extract increased. The total carbohydrate, total phenol and total protein in root –shoot axis decreased as the concentration of the extract increased on the other hand in endosperm all these parameters were found to be increased as the concentration of the extract increased. The results of present investigation showed that negative effect of ginger aqueous leaf, stem and rhizome extract may be due to the presence of allelochemicals *viz*. phenols, terpinoids and steroids.

Key Words: Ginger, Biochemical, Protein, Phenol.

Agriculture production is the main aim for rapid increasing population. Allelopathy is a mechanism in which chemical compounds produced by plants may increase or decrease the associated plant growth (Jabeen and Ahmed, 2009). Allelochemicals are different classes of chemicals such as phenolic compounds, flavanoids, terpenoids, alkaloids, amino acids and some time mixture of different compounds having greater allelopathic effect. Allelochemicals are commonly involved in plant-plant, plant-insect or plant-herbivore chemical communication (Weir *et al.,* 2004).

Allelopathy is an interference mechanism. It may also play an eminent role in intraspecific and interspecific competition and may determine the interspecific association. There is no common mode of action or physiological target site for all allelochemicals, they effect on cell division, pollen germination, nutrient uptake, photosynthesis and specific enzyme function. The current world wide demand for cheaper, more eco friendly weed management technologies has motivated a number of studies on the allelopathic interaction between crops and weeds (Dudai *et al.,*1999; Om *et al.,* 2002; Albuquerque *et al.,* 2010). The new synthetic chemicals with new target sites are decreasing. Natural compounds pose a great field for discovery of new environment friendly herbicides, so called “Bioherbicides” which based on compounds produced by living organisms. Among these bioherbicides allelochemical are significantly more in number. Plant allelochemicals can be successfully used in integrated weed management (Soltys *et al*., 2013).

Ginger (*Zingiber officinale Rosc.*) belongs to the family zingiberaceae, is one of the most important horticultural crop in south east Asia, grown as an annual crop for its spicy underground rhizome (Singh *et al.*, 1999). Maize (*Zea mays L.*) belongs to the family Poaceae. It is second most important cereal crop, cultivated worldwide. The genus Zea consists of four species, out of which species *Zea mays. L* is economically important. The major maize growing states are Bihar, Rajasthan, Uttar Pradesh, Andhra Pradesh Madhya Pradesh, Punjab, Himachal Pradesh, West Bengal, Karnataka, Jammu and Kashmir. In India about 28% of maize produced is used for food purpose, maize seeds used for many purposes, about 11% as livestock feed, 48% as poultry feed, 12% in wet milling industry (oil, starch production) & 1% as seed (Anonymous., 2010).

Materials and Methods

Procurement of certified seed samples and plant materials

The certified three varieties of maize- NAC-6002, NAC-6004 and NAH-6004 were obtained from University of agricultural sciences, Naganahalliy. Ginger plants were procured from fields of Channapura village. To select best variety among these three, NAC-6002, NAC-6004 and NAH-6004 varieties were subjected to preliminary germination studies. All the seeds were treated and seedlings were maintained up to 8 days, among the three varieties NAC 6004 showed maximum percentage of germination, it showed maximum resistance to fungal infection and suitable for laboratory experimental condition. So NAC 6004 maize variety was selected for further studies.

Preparation of Ginger aqueous extracts:

Plants were washed properly with water to remove the impurities then plants were separated into leaves, stem and rhizome, dried for 30 days. The dried leaves and stems were chopped into 1cm long pieces and rhizomes were chopped into 0.5cm thick slices. The components were then oven dried at 60^0 C for 2days. 100g of dried rhizomes, leaves and stems were soaked in 1lt deionised water at 25^0 C for 24hrs in a shaker to get a concentration of 100g dry tissue/litre. The extracts were filtered through filter paper to remove the debris and centrifuged at 3000rpm for 4hour. The supernatant was filtered again. Fresh stock extract were kept in a refrigerator at 3^0C until used.

Biochemical assay:

Stock extracts (rhizome, stem, leaf) were diluted with sterile distilled water to get different concentrations of 20%, 40%, 60%, 80% and 100%. Biochemical studies like total chlorophyll (Arnon,1949), carotenoids (Kirk and Allen 1965), total protein (Lowry.s *et al,.* 1951), total carbohydrate (Hedge and Hofreiter 1962) and total phenol (Malick and Singh 1980) were analysed.

Results and Discussion

Effect of ginger stem, leaf and rhizome extract on pigment content of maize seedlings is represented in table- 1. The mean value of pigment content in leaves differed significantly when treated with different concentration of aqueous extracts. The maximum amount of chlorophyll ‘a’ was recorded in control (1.02 mg/g F. Wt.). The seedlings showed a decrease in chlorophyll ‘a’ from 0.66 to 0.09 mg/g F.Wt. 20% and 100% concentration in ginger stem extract respectively. In leaf extract amount of chlorophyll ‘a’ was decreased from 0.58 mg/g F.Wt. to 0.13 mg/g F.Wt. 20% to 100% concentration respectively. In rhizome extract chlorophyll ‘a’ decreased from 0.57 mg/g F.Wt. 0.14 mg/g F.Wt.to 20% to 100% concentration respectively.

Chlorophyll ‘b’ showed same pattern as of chlorophyll ‘a’. Maximum amount of chlorophyll ‘b’ was recorded in control (1.44

mg/g F.Wt.). In ginger stem aqueous extract the maximum and minimum amount of chlorophyll 'b' (0.98mg/g F.Wt. and 0.33 mg/g F.Wt.) was recorded in 20% and 100% concentration respectively. In leaf extract of ginger maximum and minimum amount of chlorophyll 'b' (1.07mg/g F.Wt. and 0.42mg/g F.Wt.) was recorded in 20% and 100% concentration respectively. There was a significant decrease in the total chlorophyll of leaves of maize seedlings with increase in the concentration of extracts. The maximum value with respect to total chlorophyll was recorded in control (0.96 mg/g F.Wt.). In stem extract of ginger total chlorophyll decreased from 0.61 to 0.25 mg/g F.Wt. at 20% to 100% concentration respectively. In leaf extract of ginger the maximum and minimum total chlorophyll in leaves (0.72mg/g F.Wt. and 0.30mg/g F.Wt.) was recorded in 20% and 100% concentration respectively.

Table 1: Leaf pigments of maize seedlings treated with different concentrations of stem, leaf and rhizome extract of Ginger.

Concentration	Chlorophyll a (mg/g F.Wt..)	Chlorophyll b (mg/g F.Wt.)	Total Chlorophyll (mg/g F.Wt.)	Carotenoid (mg/g F.Wt.)
	Different concentrations of Ginger stem extract			
Control	1.00±0.8815^{a}	1.44±0.0015^{a}	0.95±0.0015^{a}	0.69±0.0155^{a}
20%	0.66±0.0014^{b}	0.98±0.0045^{b}	0.61±0.0035^{b}	0.45±0.0082^{b}
40%	0.57±0.0028^{c}	0.93±0.0036^{b}	0.60±0.0033^{b}	0.33±0.0265^{c}
60%	0.50±0.0049^{d}	0.62±0.0046^{c}	0.42±0.0033^{c}	0.32±0.0179^{c}
80%	0.18±0.0088^{e}	0.57±0.0039^{d}	0.36±0.0055^{d}	0.05±0.0097^{d}
100%	0.09 ±0.0026^{f}	0.33± 0.0076^{e}	0.25±0.0071^{e}	0.02±0.0373^{e}
Different concentrations of Ginger leaf extract				
Control	0.99±0.0300^{a}	1.37±0.0031^{a}	0.95±0.0017^{a}	0.72±0.0068^{a}
20%	0.58±0.0088^{b}	1.07±0.0026^{b}	0.72±0.0020^{b}	0.43±0.0061^{b}
40%	0.54±0.0070^{c}	1.00±0.0013^{b}	0.66±0.0014^{c}	0.30±0.0138^{b}
60%	0.49± 0.0006^{d}	0.86±0.0034^{c}	0.57±0.0028^{d}	0.27±0.0124^{c}
80%	0.37±0.0015^{e}	0.60±0.0014^{d}	0.39±0.0006^{e}	0.21±0.0196^{d}
100%	0.13±0.0043^{f}	0.42±0.0017^{e}	0.30±0.0021^{f}	0.04±0.0311^{e}
Different concentrations of Ginger rhizome extract				
Control	1.02±0.0376^{a}	1.42±0.0031^{a}	0.96±0.0017^{a}	0.71±0.0057^{a}
20%	0.57±0.0021^{b}	0.92±0.0076^{b}	0.59±0.0068^{b}	0.43±0.0381^{b}
40%	0.47±0.0003^{c}	0.84±0.0060^{c}	0.56±0.0017^{b}	0.26±0.0138^{c}
60%	0.31±0.0014^{d}	0.53±0.0116^{d}	0.35±0.0096^{c}	0.17±0.0277^{d}
80%	0.27±0.0003^{e}	0.47±0.0042^{e}	0.31±0.0036^{d}	0.15±0.0078^{e}
100%	0.14±0.0149^{f}	0.28±0.0090^{f}	0.19±0.0124^{e}	0.07±0.1506^{f}

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level.

In rhizome extract there was no significant difference between 20% and 40% concentrations. The maximum and minimum total chlorophyll in rhizome extract was recorded in 20% and 100% concentration (0.96mg/g F.Wt. and 0.19mg/g F.Wt.) respectively. The carotenoid content was found to be decreased with increase in the concentration of the aqueous extracts of ginger stem, leaves and rhizome. The maximum carotenoid content observed in leaves of maize seedlings in control (0.71 mg/g F.Wt.). In stem extract of ginger the maximum and minimum amount of carotenoid was recorded in 20% and 100% concentration (0.45mg/g F.Wt. and 0.02 mg/g F.Wt) respectively. There was no significant difference between 40% and 60% concentration with respect to amount of carotenoid recorded. In leaf extract the maximum and minimum carotenoid was recorded in 20% and 100% concentration (0.43mg/g F.Wt. 0.04 mg/g F.Wt.) respectively. There was no significant difference between 20% and 40% concentrations of leaf extract. In rhizome extract the maximum and minimum carotenoid was recorded in 20% and 100% concentration (0.43mg/g F.Wt. and 0.07 mg/g F.Wt.) respectively.

Table 2: Total carbohydrate content of Maize seedlings treated with different concentrations of stem, leaf and rhizome extract of Ginger.

Concentration	Root-shoot axis(mg/g F.Wt.)	Endosperm (mg/g F.Wt.)
Different concentrations of Ginger stem extract		
Control	$51.33{\pm}0.881^{a}$	$25.33{\pm}0.812^{f}$
20%	$48.00{\pm}0.577^{b}$	$45.00{\pm}0.241^{e}$
40%	$46.00{\pm}0.512^{c}$	$75.00{\pm}0.561^{d}$
60%	$44.00{\pm}0.574^{d}$	$87.00{\pm}0.412^{c}$
80%	$37.00{\pm}0.576^{e}$	$164.00{\pm}0.577^{b}$
100%	$31.33{\pm}0.881^{f}$	$261.33{\pm}0.881^{a}$
Different concentrations of Ginger leaf extract		
Control	$51.33{\pm}0.881^{a}$	$15.00{\pm}0.245^{f}$
20%	$46.00{\pm}0.577^{b}$	$28.00{\pm}0.142^{e}$
40%	$42.00{\pm}0.452^{c}$	$31.00{\pm}0.325^{d}$
60%	$35.33{\pm}0.881^{d}$	$75.00{\pm}0.554^{c}$
80%	$31.00{\pm}0.572^{e}$	$104.00{\pm}0.65^{b}$
100%	$25.00{\pm}0.241^{f}$	$125.00{\pm}0.814^{a}$
Different concentrations of Ginger rhizome extract		
Control	$51.33{\pm}0.881^{a}$	$11.33{\pm}0.881^{f}$
20%	$45.00{\pm}0.254^{b}$	$35.00{\pm}0.652^{e}$
40%	$34.00{\pm}0.614^{c}$	$74.00{\pm}0.821^{d}$
60%	$32.00{\pm}1.124^{d}$	$81.33{\pm}0.881^{c}$
80%	$25.00{\pm}0.577^{e}$	$161.00{\pm}0.577^{b}$
100%	$19.00{\pm}0.814^{f}$	$187.66{\pm}0.881^{a}$

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level.

Table 3: Total phenol content of Maize seedlings treated with different concentrations of stem, leaf and rhizome extract of ginger.

Concentration	Root-shoot axis (mg/g F.Wt..)	Endosperm (mg/g F.Wt..)
Different concentrations of Ginger stem extract		
Control	$3.60{\pm}0.115^{a}$	$2.63{\pm}0.088^{f}$
20%	$2.50{\pm}0.057^{b}$	$2.83{\pm}0.012^{e}$
40%	$1.63{\pm}0.120^{c}$	$2.93{\pm}0.058^{d}$
60%	$1.43{\pm}0.121^{d}$	$3.63{\pm}0.096^{c}$
80%	$1.36{\pm}0.088^{e}$	$3.76{\pm}0.081^{b}$
100%	$1.03{\pm}0.082^{f}$	$3.96{\pm}0.088^{a}$
Different concentrations of Ginger leaf extract		
Control	$3.20{\pm}0.057^{a}$	$2.56{\pm}0.088^{f}$
20%	$2.76{\pm}0.145^{b}$	$2.86{\pm}0.033^{e}$
40%	$2.33{\pm}0.088^{c}$	$2.93{\pm}0.088^{c}$
60%	$1.63{\pm}0.012^{d}$	$2.96{\pm}0.083^{c}$
80%	$1.31{\pm}0.044^{e}$	$4.66{\pm}0.086^{b}$
100%	$0.33{\pm}0.881^{f}$	$5.50{\pm}0.057^{a}$
Different concentrations of Ginger rhizome extract		
Control	$3.28{\pm}0.044^{a}$	$3.43{\pm}0.066^{f}$
20%	$2.30{\pm}0.057^{b}$	$3.71{\pm}0.060^{e}$
40%	$2.06{\pm}0.145^{c}$	$4.00{\pm}0.154^{d}$
60%	$1.96{\pm}0.120^{d}$	$5.23{\pm}0.881^{b}$
80%	$0.96{\pm}0.088^{e}$	$5.30{\pm}0.577^{b}$
100%	$0.46{\pm}0.120^{f}$	$5.76{\pm}0.457^{a}$

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level.

The results are similar to studies of Al-saadawi *et al.,* 1986. They mentioned that Allelochemicals reduces the chlorophyll content and ion uptake. Peng *et al*, 2004 reported that allelochemicals affects the photosynthetic activity in plant by destroying chlorophyll. Allelochemicals alters the physiological functions adversely in target plants (Gniazdowska and Bogatek, 2005). Leaf chlorophyll content is a major parameter to understand the response of plant to environment in which it inhabits. Hussai *et al.*, 2011 reported that allelochemicals significantly inhibit chlorophyll synthesis in target plant and suppress the photosynthesis. Above finding is supported by Oyerinde *et al.*, 2009 who reported the decrease in chlorophyll-a, chlorophyll-b and total chlorophyll accumulation in young plants of maize after being treated with fresh shoot aqueous extract of *Tithonia diversifolia* which possess allelopathic characteristics.

Table 4: Total protein content of *Zea mays L.* seedlings treated with different concentrations of stem, leaf and rhizome extract of ginger.

Concentration	Root-shoot axis (mg/g F.Wt.)	Endosperm (mg/g F.Wt.)
Different concentrations of Ginger stem extract		
Control	24.52±0.120[a]	5.65±0.006[f]
20%	17.15±0.008[b]	6.54±0.012[e]
40%	14.30±0.008[c]	9.30±0.017[d]
60%	10.15±0.014[d]	15.59±0.008[c]
80%	5.43±0.014[e]	19.14±0.008[b]
100%	3.42±0.006[f]	21.39±0.005[a]
Different concentrations of Ginger leaf extract		
Control	27.14±0.011[a]	10.21±0.168[f]
20%	23.45±0.012[b]	15.35±0.014[e]
40%	20.03±0.008[c]	19.30±0.008[d]
60%	17.14±0.008[d]	21.14±0.010[c]
80%	13.25±0.008[e]	22.30±0.008[b]
100%	9.49±0.017[f]	24.19±0.025[a]
Different concentrations of Ginger rhizome extract		
Control	26.31±0.008[a]	13.24±0.014[f]
20%	23.04±0.028[b]	16.08±0.014[e]
40%	21.13±0.012[c]	18.14±0.008[d]
60%	18.45±0.058[d]	22.01±0.006[c]
80%	16.35±0.010[e]	26.54±0.011[b]
100%	12.06±0.002[f]	31.93±0.008[a]

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level.

Total carbohydrate content estimation:

Total carbohydrate content present in the root-shoot axis and endosperm of maize seedlings treated with different concentrations of ginger stem, leaf and rhizome extract were presented in the table 2. The difference in total carbohydrate was significant among maize seeds treated at 20% to 100% concentration of ginger aqueous extracts, when compared to control (51.33 mg/g F.Wt.). In stem extract of ginger the total carbohydrate content was decreased from 48.00 mg/g F.Wt. at 20% concentration to 31.33 mg/g F.Wt. at 100% concentration. In leaf aqueous extract total carbohydrate content decreased from 46.00 mg/g F.Wt. to 25.00mg/g F.Wt. in 20% and 100% concentrations respectively. In rhizome extract of ginger, total carbohydrate content decreased from 45.00mg/g F.Wt. to 13.00 mg/g F.Wt. in 20% to 100% concentration respectively.

A significant increase was observed in total carbohydrate content of the endosperm of maize with increase in the concentration of aqueous stem, leaf and rhizome extracts of ginger compared to control. In stem extract an increase of total carbohydrate content was observed from 45.00mg/g F.Wt. to 261.33 mg/g F.Wt. in 20% to 100% concentration respectively. In leaf extract the total carbohydrate content was increased from 28.00mg/g F.Wt. in 20% concentration to 125.00 mg/g F.Wt. in 100% concentration. Rhizome extract also showed similar results. Highest total carbohydrate content was recorded in 100% concentration (187.66mg/g F.Wt.) and lowest total carbohydrate content was recorded in 20% concentration (35.00mg/g F.Wt.). When compared to control, stem extract treatment at 100% concentration recorded highest carbohydrate content in endosperm and lowest carbohydrate content in endosperm was recorded in 20% concentration of leaf extract (28.00mg/g F.Wt.). The results correlated with report of Abdulghader *et al.*, 2008. They reported that leaf extracts of *Heliotropium foertherianum* (heliotrope) increases concentration of soluble sugar in radish. Similar increase in soluble sugars of maize in response to leaf extracts of *Acacia* and *Eucalyptus* has been reported by Sahar *et al.*, 2005.

Total phenol content

The results on mean values of phenol content in maize seedling root-shoot axis and endosperm under different concentrations of ginger stem, leaf and rhizome extract are tabulated in table-3. Root-shoot axis of control seedlings showed maximum phenol content (3.60mg/g F.Wt.). The phenol content was decreased in root-shoot axis of maize with increase in the concentration of ginger extracts. In stem aqueous extract of ginger the phenol content decreased from 2.50mg/g

F.Wt. in 20% concentration to 1.03mg/g F.Wt. in 100% concentration. In leaf extract also maximum phenol content in root-shoot axis was recorded in 20% concentration (2.76mg/g F.Wt.) and minimum phenol content was recorded in 100% concentration (0.33mg/g F.Wt.). In rhizome extract maize seedlings at 20% concentration showed maximum phenol content in root-shoot axis (2.30mg/g F.Wt.). In 100% concentration of rhizome extract the minimum phenol content in root-shoot axis was recorded (0.46mg/g F.Wt.).

In endosperm of maize seedling, significant increase in total phenol content with increase in the concentration of ginger extracts was observed. In stem extract the lowest phenol content was recorded in 20% concentration (2.83mg/g F.Wt.) and highest phenol content was recorded in 100% concentration (3.96mg/g F.Wt.). In leaf extract of ginger the total phenol content increased from 2.86mg/g F.Wt. in 20% concentration to 5.50mg/g F.Wt. in 100% concentration. In rhizome extract the lowest total phenol content was recorded in 20% concentration (3.71mg/g F.Wt.) and highest total phenol content was recorded in 100% concentration (5.76mg/g F.Wt.). Chum *et al.*, (2012) noted that by the effect of 2-benzoxazolinone phenolic content in some vegetable crops was increased over control.

Total protein content

The mean value of protein content of maize seedlings grown in different concentrations of ginger stem, leaf and rhizome extract are tabulated in table-4. In stem extract of ginger the protein content decreased from 17.15mg/g F.Wt. to in 3.42mg/g F.Wt. in 20% and 100% concentration respectively. In leaf extract total protein content in root-shoot axis was more in 20% concentration (23.14mg/g F.Wt.) and it is decreased with increased concentration. In rhizome extract total protein content decreased from 23.04mg/g F.Wt. to 12.06mg/g F.Wt. (20% to 100% concentration respectively). The total protein content significantly increased in endosperm with increase in concentration of ginger extracts. The endosperm of seedlings grown in stem extract of ginger showed highest protein content in 100% concentration (21.39mg/g F.Wt.) and lowest protein content was recorded in 20% concentration (6.54mg/g F.Wt.). In leaf extract the protein content increased from15.35mg/g F.Wt. to 24.19mg/g F.Wt. in 20% to 100% concentration respectively. In rhizome extract, the minimum and maximum amount of protein content was observed (16.08mg/g F.Wt. to 31.93 mg/g F.Wt.) in 20% to 100% concentration respectively. The opposite results were obtained by El-Rokiek and El-Nagdi, (2011) who worked on the effect of *Eucalyptus citriodora* on sunflower. They reported that treating sunflower seeds with extract of *E. citriodora* showed significant increase in total protein content of seeds when compare to control.

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