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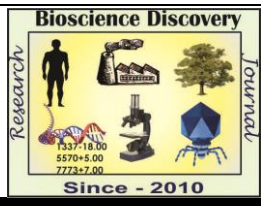

Effect of *Chromolena odorata* leaf extract and leaf leachate on nutrient uptake potential of *Crotalaria verrucosa* L. and *Crotalaria retusa* L.

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Abstract

Allelopathy is biological phenomenon in which biochemical or phytochemicals produced by one organism affects on growth, development and reproduction of other organisms. Such toxicity developing organism is also called as invasive species. *Chromolena odorata* (L.) R. M. King & H. Rob is one of the invasive species from family Asteraceae were selected for study their allelopathic effect on nutritional uptake potential (N, P, K, Ca, Mg, S, Zn, Fe, Mn and …) of *Crotalaria verrucosa* L. and *Crotalaria retusa* L. from this study it is clear that leaf leachate and leaf extract of this invasive weed highly influence micro and macro nutrient contents of studied plant species and directly or indirectly this species inhibit growth and development of other plants. Therefore this plant *Chromolena odorata* can be used to prepare weedicide or herbicide.

INTRODUCTION

Allelopathy refers to the beneficial or harmful effects of one plant on another plant that may be weed species. Allelochemicals are phytochemicals produced by plants that may be released by plant by process like leaching, root extraction, volatilization, residue decomposition and by many other experiment or processes (Patil, 2011).

All living organisms require a continuous supply of large number of substances from outside to complete their life cycle. This supply is called as nutrition. The essential nutrients required by higher plants are exclusively of inorganic nature. A plant for normal optimal growth requires sixteen different elements. Green plants have comparatively simple nutrient requirements and that these are classified as macronutrients (N, P, K, Ca, Mg, S, and Na) and micronutrients (Fe, Mn, Cu, Zn, Mo, B and Cl). Macronutrients are found and needed in plants in relatively higher amounts than micronutrients. But both are essential for almost all metabolic processes.

Allelopathy is biological phenomenon in which biochemicals or phytochemicals produced by one organism affects on germination, growth, development and reproduction of other organisms (Molisch, 1937; Madane and Patil, 2017). Such toxicity developing organism is also called as invasive species. *Chromolena odorata* (L.) R. M. King & H. Rob is one of the invasive species from family Asteraceae were selected and studied in current research for their allelopathic effect on nutritional uptake potential of *Crotalaria verrucosa L. and Crotalaria retusa L.*. Results have been depicted in table 1 and Fig. 1-10.

MATERIALS AND METHODS

Various inorganic constituents like Na^{+}, K^{+}, Mg^{2+}, Fe^{3+}, Mn^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+} were estimated from the *Crotalaria verrucosa*L. and *Crotalaria retusa* L. under allelopathic treatment of *Chromolena odorata* leaf extract and leaf leachates treatments. Oven dried plant material was powdered and 0.5 g of sample was acid digested following the standard method of Toth *et al.* (1948).

Plant material samples were taken in a 150 ml clean borosil beaker and to that 10 ml concentrated HNO_3 were added. It was covered with watch glass and kept for an hour till the primary reactions subsided. It was then heated on hot plate till all the material was completely dissolved. It was allowed to cool to room temperature and then 10 ml of Perchloric acid (60%) were added to it and mixed thoroughly. It was then heated strongly on the hot plate until the solution became colourless and reduced to about 2-3 ml. While heating, the solution was not allowed to dry. After cooling, it was transferred quantitatively to 100 ml capacity volumetric flask, diluted to 100 ml with distilled water and kept overnight. Next day it was filtered through Whatman No. 44 filter paper. The filtrate was stored properly and used for inorganic constituents analysis which was estimated by using flame photometer and Atomic absorption spectrophotometer (Perkin-Elmer, 3030 A) as prescribed by AOAC (1995) and Sangle (2015).

RESULTS AND DISCUSSION

Mineral uptake potential in any plant is always influenced by many edaphic factors such as soil moisture, soil pH and soil microflora. Leaf litter also shows effect on composition of nutrient availability in soil. But sometime it may adversely affects due to allelochemicals released by these litter (Pawar and Chavan, 2007). Many plant species release allelochemicals that (positive or negative) influence on germination, growth and development of other plants (Kadioglue *et al*., 2005 & Madane and Patil, 2017). Here an attempt have been made to study effect of allelopathic compounds from *Chromolenaodorata* on mineral uptake potential in *Crotalaria verrucosa* L. *and Crotalaria retusa*L. plants. The influence of leaf leachates and leaf extract of *Chromolena odorata* was investigated on *C. verrucosa* L. *and C. retusa*. Results have been depicted in table 1 and Fig. 1-10.

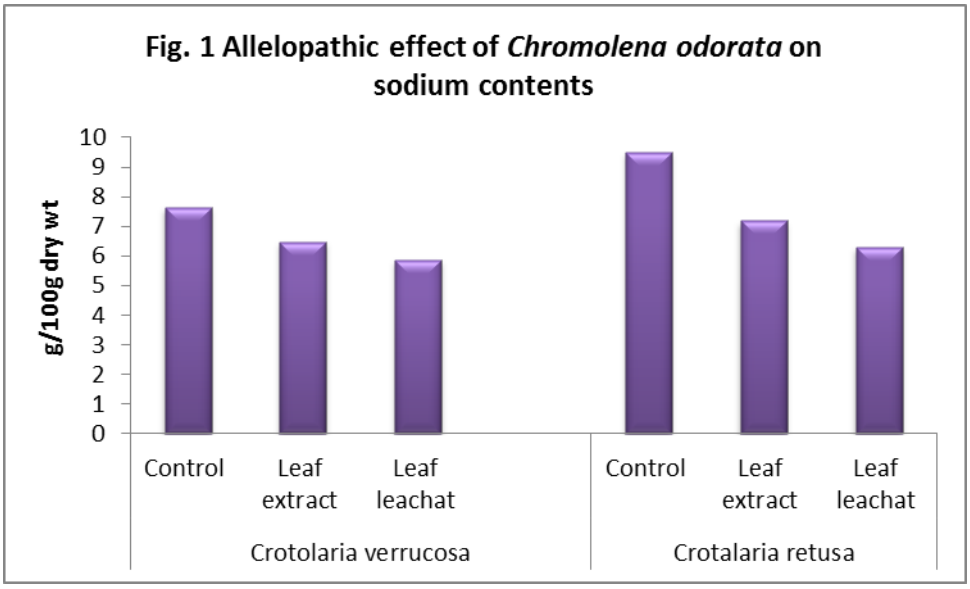
Table 1. Study the effect of leaf leachate and aqueous leaf extract of *Chromolaenaodorata*on mineral uptake potential of *Crotolariaverrucosa and Crotalaria retusa*

Plants under treatment →	*Crotolariaverrucosa*				*Crotalaria retusa*		
Inorganic constituents ↓	Control	Leaf extract	Leaf leachat		Control	Leaf extract	Leaf leachat
Sodium %	7.65	6.47	5.88		9.5	7.2	6.3
Potassium %	6.9	5.4	4.5		7.12	6.37	4.45
Nirogen %	3.24	2.16	1.97		3.68	2.84	2.41
Phosphrous %	0.28	0.21	0.19		0.53	0.32	0.19
Calcium %	8.5	6.2	5.9		9.89	9.05	7.36
Magnesium %	0.27	0.23	0.18		0.94	0.89	0.81
Sulphur %	0.38	0.27	0.22		0.28	0.19	0.13
Ferrous PPM	547.53	490.68	430.63		879.32	764.26	662.84
Mangenese PPM	116.99	102.65	95.62		62.1	59.32	54.68
Copper PPM	46.43	38.28	20.76		183.16	142.42	135.07
Zinc PPM	6.73	6.16	5.08		24.74	15.22	14.47
SD	162.918	146.108	128.648		261.653	227.140	206.635
SEM	49.122	44.053	38.789		78.891	68.485	62.303
P value		0.907	0.803			0.882	0.849

Sodium contents:-

It is evident from result shown in Fig. 1 that due to allelochemicals there is decline in sodium contents were observed in both species *Crotolaria verrucosa* and *C. retusa*. *In Crotolaria verrucosa* treatment of leaf extract responsible for declining sodium content up to 15.4% and due to leaf leachate 23.1% as compare to control (7.65gm/100gm dry wt.).In *Crotolaria retusa* of leaf extract responsible for declining sodium content up to 24.21% and leaf leachate responsible to decline 33.68% as compare to control (9.5gm/100gm dry wt.). Sodium is considered as accessory element for glycophytes except for few saline angiosperms. Sodium inspires growth at scarcity of potassium supply. It stimulates growth through enhanced cell expansion. It is also required in maintaining membrane integrity (Brownell, 1979).

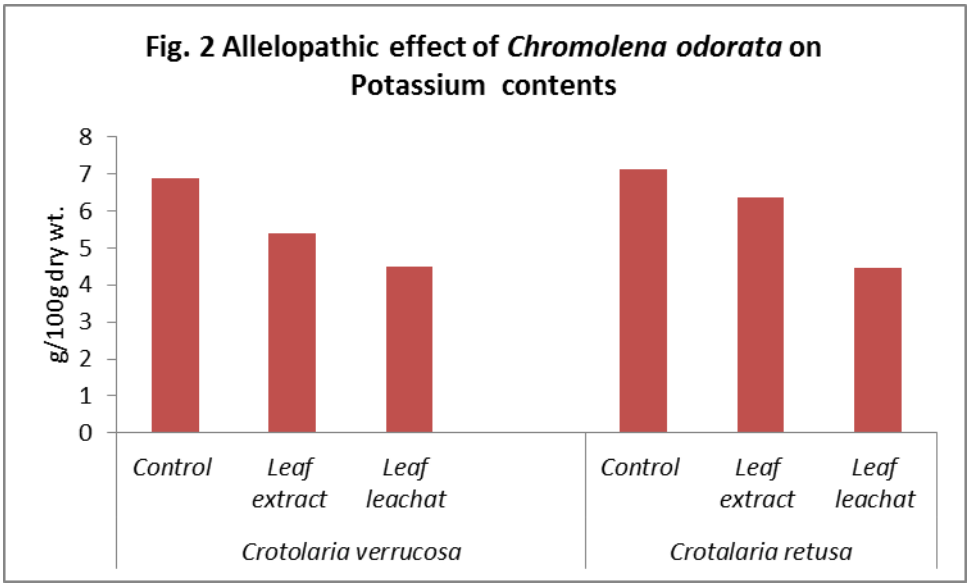
Under sodium deficiency the plants exhibit chlorosis and necrosis, or even fail to form flower. Many C_3 species also benefit from exposure to low levels of sodium ions. According to Chirputkar (1969) adequate level of sodium for glycophytes is 0.6 to 1.4% dry wt. The range of sodium in glycophytes as given by Gauch (1972) is 0.1 to 1.4% dry wt. In crop plants like sugarcane, 0.11% sodium content was recorded by Nimbalkar (1973) and in finger millet it was 0.09% (Chavan, 1980).



Potassium contents

It is evident from result shown in Fig. 2 that due to leaf extract and leaf leachate treatment of *Chromolena odorata*, there is decline in potassium contents were observed in both species *Crotolaria verrucosa* and *C. retusa*. *In C. verrucosa* treatment of leaf extract responsible for declining potassium content up to 21.7% and due to leaf leachate 34.8% decline observed as compare to control (6.9 g/100g dry wt.). In *Crotolaria retusa* of leaf extract responsible for declining potassium content up to 10.53% and due to leaf leachate 37.50% as compare to control (7.12g/100g dry wt.). Plants require 1% potassium for their optimal growth (Epstein, 1972). Potassium is indispensable for plant growth. Potassium is involved in wide range of metabolic activities such as carbohydrate metabolism, glycolysis, phosphorylation and adenine biosynthesis in plants. It also plays a significant role in plant growth and developmental processes such as photosynthesis (Peoples and Koch, 1979), translocation of proteins and carbohydrates (Marschner, 1997), stability of ribosomes, protein synthesis, nitrogen turnover, activation of enzymes, stomatal movement, nyctinastic and seismonastic movements, cell extension, etc. (Suelter 1970, Humble and Raschke 1971, Shankar *et al*., 2013)

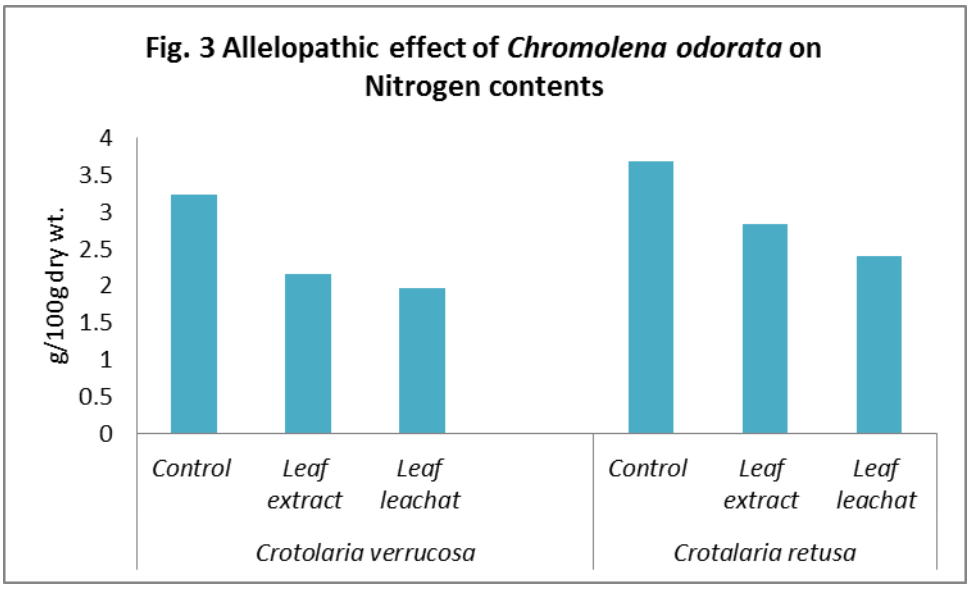
As potassium is highly mobile element (Mengel and Krikby, 1982), in plants its deficiency is usually observed in older leaves, which become chlorotic and develop necrotic patches. Potassium deficiency is usually noted in mature leaves which become chlorotic and develop dark necrotic lesions. Potassium deficiency causes reduction in nitrate reductase activity, disturbance of protein metabolism and accumulation of amino acids and soluble organic nitrogenous compounds.



Nitrogen contents

It is depicted from result shown in Fig. 3 that due to leaf extract and leaf leachate treatment of *Chromolena odorata*, there is decline in nitrogen contents were observed in both *C. verrucosa* and *C. retusa*.*In Crotolaria verrucosa* treatment of leaf extract responsible for declining nitrogen content up to 33.3% and that due to leaf leachate 39.2% as compare to control(3.24 g/100g dry wt.).Similarly in *C. retusa* of leaf extract responsible for declining nitrogen content up to 22.83% and that due to leaf leachate 34.51% as compare to control (3.68g/100g dry wt.) one. Nitrogen is the most essential macronutrient required in life cycle of plants. Nitrogen is a fundamental constituent of amino acids, proteins and nucleic acids, biochemistry of enzymes, pigments, secondary metabolites and polyamines (Maathuis, 2009). It involve in the biosynthesis of several vitamins such as biotin, thiamine, niacin and riboflavin.

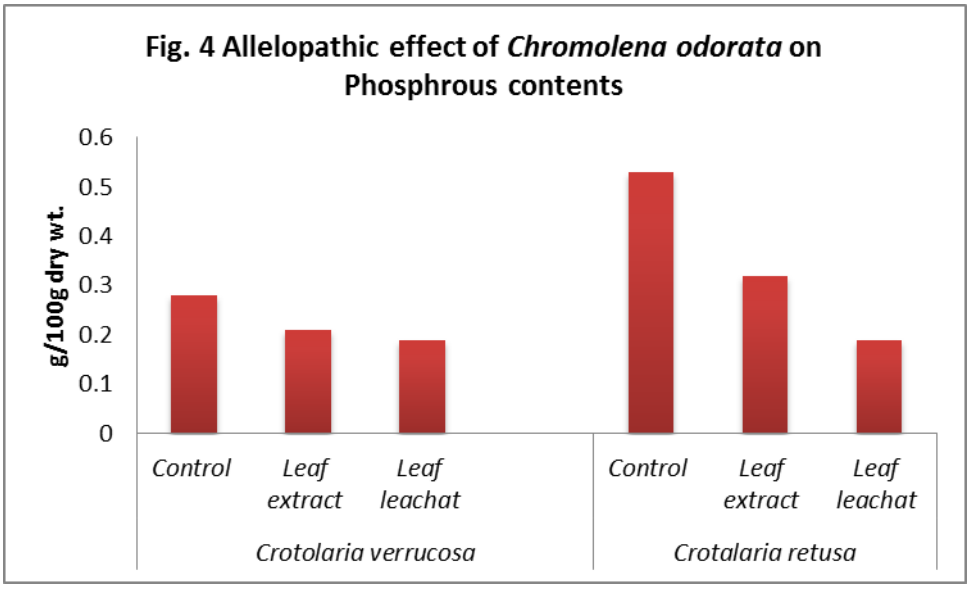
The critical amount of nitrogen 1to 2% requires by plants and maximum amount ranges in between 4 to 6% (Parsa and Bagheri, 2008). Many researchers reported and supported to Marschner's (1986) suggestion as the requirement of nitrogen for optimal growth of plants ranges in between 2 to 5% of the dry weight (Pawar 2004, Zhao *et al.* 2008, Shen *et al.* 2008 Rasmussen 2008, Tambe 2009, Desai, 2010 and Sonar, 2014). N deficiency seriously affects the growth, development and yield in the crop plants.



Phosphrous contents

It is depicted from result shown in Fig. 4 that due to leaf extract and leaf leachate treatment of *Chromolena odorata*, there is decline in phosphorus contents were observed in both *C. verrucosa* and *C. retusa*. *In Crotolaria verrucosa* treatment of leaf extract responsible for declining phosphorus content up to 25.0% and that due to leaf leachate 23.1 % as compare to control (0.28 g/100g dry wt.).Similarly in *C. retusa* of leaf extract responsible for declining phosphorus content up to 39.62% and that due to leaf leachate 64.15% decline as compare to control (0.53g/100g dry wt.) one.

Phosphorus is one of the most essential components of large number of metabolites it has vital functioning in various life processes. It is absorbed in the form of dihyrogen phosphate ion from the soil solution. It act as backbone in DNA and RNA molecules, act as structural component of ATP molecule. In plant body phosphorous in its inorganic state plays a very important role in regulation of various pathways like photosynthesis and carbohydrate metabolism (Marschner, 1986) and optimal growth of plants the requirement of P is in the range of 0.3 to 0.5% of plant's dry weight.

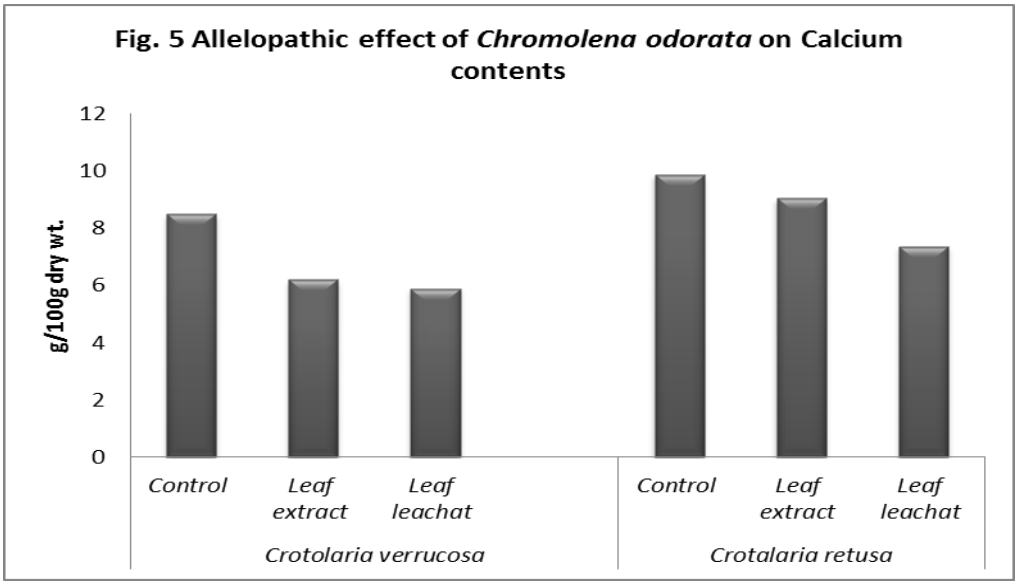


Calcium content

It is clear from result shown in Fig. 5 that due to leaf extract and leaf leachate treatment of *Chromolena odorata*, there is decline in calcium contents were observed in both *C. verrucosa* and *C. retusa*. *In Crotolaria verrucosa* treatment of leaf extract responsible for declining calcium content up to 27.1% and that due to leaf leachate 30.6 % as compare to control(8.5 g/100g dry wt.). Similarly in *C. retusa* of leaf extract responsible for declining calcium content up to 8.49% and that due to leaf leachate 25.58% decline as compare to control (9.89g/100g dry wt.). Calcium has many roles in plants and is required in differing amount depending on the process in which it is involved, from minute amount in regulating some aspects of cytosolic metabolism to macro amount in cell wall structure. The importance of calcium in functioning of membrane and maintenance of cell integrity as well as synthesis of pectin in middle lamellae of cell wall is very well documented.

Calcium has long been known to be essential for structural and functional integrity of plant membrane (Epstein1972 and Miller et al., 1992),. According to Clark (1984), the activities of many enzymes have been either stimulated or inhibited by calcium. Calcium is also a non- toxic mineral nutrient, even in high concentration and is very effective in detoxifying high concentrations of other mineral elements in plants. The major role carried out by calcium in plants is to bind with proteins, nucleic acids and lipids to affect cell adhesion, membrane chromatin organization and enzyme conformation (Clarkson and Hanson, 1980).

Under calcium deficiency, activities of glutamine synthase and nitrate reductase are decreased, while that of GDH is increased, root tips appear slimy, death of terminal buds takes place, young leaves develop rugged edges and die back of leaves takes place.

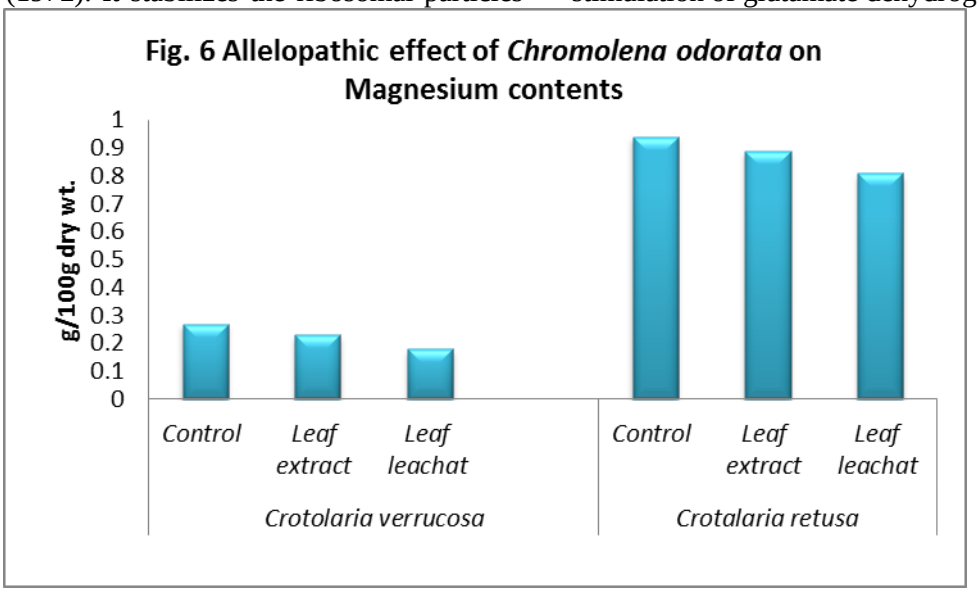


Magnesium content

From result shown in Fig. 6 it is evident that due to allelopathic treatment of *Chromolena odorata*, there is decline in magnesium uptake were observed in both *C. verrucosa* and *C. retusa. In Crotolaria verrucosa* treatment of leaf extract responsible for declining magnesium uptake up to 14.8% and that due to leaf leachate 33.3% as compare to control (0.27 g/100g dry wt.). Similarly in *C. retusa* of leaf extract responsible for declining magnesium uptake up to 5.32% and that due to leaf leachate 13.83% decline as compare to (0.94g/100g dry wt.) control one. In the plants, 2% Mg on dry weight basis has been regarded as critical value by Epstein (1972). It stabilizes the ribosomal particles in the configuration necessary for protein synthesis (Mengel and Kirkby, 1982).

Magnesium is a small, mobile and strongly electropositive divalent cation in the plants, found both in bound as well as free form (Gilbert, 1957). Most well known role of Mg is its contribution to the center of the chlorophyll molecule. It is a part of ring structure of chlorophyll molecule, the photosynthetic pigment in chloroplast. It is a cofactor of several enzymatic reactions involved in organic acid synthesis.

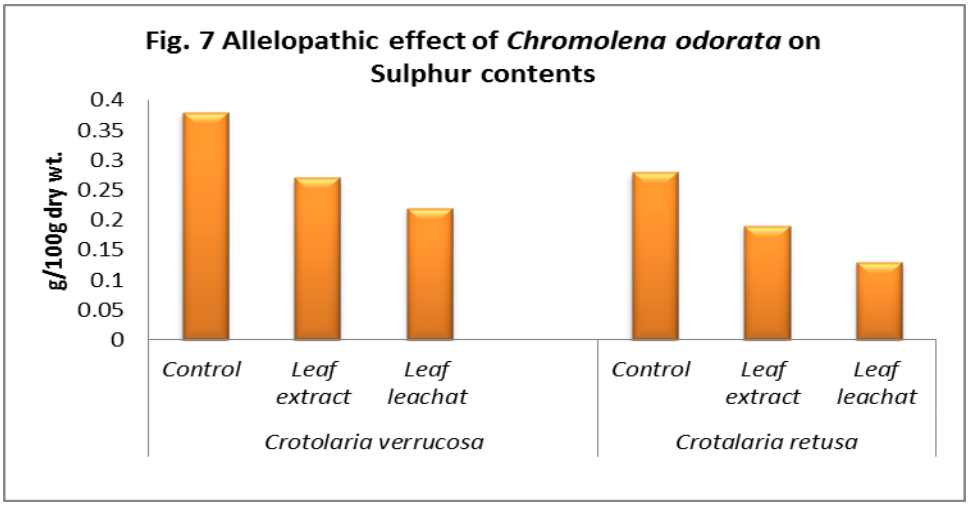
Mg deficiency leads to mottled and chlorotic leaves, interveinaly following or necrosis of older leaves, reduction in activities of nitrate reductase and stimulation of glutamate dehydrogenase.



Sulphur contents

According to result as shown in Fig. 7 it can be said that allelopathic treatment of *Chromolena odorata* responsible for decline in sulphur uptake. Which were observed in both *C. verrucosa* and *C. retusa. In Crotolaria verrucosa* treatment of leaf extract responsible for declining sulphur uptake up to 28.9% and that due to leaf leachate 42.1% as compare to control(0.38 g/100g dry wt.). Similarly in *C. retusa*of leaf extract responsible for declining Sulphur uptake up to 32.14% and that due to leaf leachate 53.57% decline as compare to control (0.28g/100g dry wt.). Sulphur act as secondary essential plant nutrient is usually required by crops in amounts comparable to phosphorus. The optimum value of sulphur in plant species is in the range 0.08 to 1.56 % (Munson, 1998). Mengel and Kirkby (1978) reported that total sulphur requirement of different crops depends on the plant biomass production and varies among the crop species and crops with a high production of organic material have a high demand for sulphur. Sulphur being a part of amino acids as cysteine and methionine. It is involved in the formation of chlorophyll (Tandon 1991, higher levels of structural organization in proteins. Rouhier*et al.* (2006) reported that sulphur present in tripeptide glutathione which helpful in oxidative stress tolerance. The lower sulphur content of proteins influences nutritional quality considerably.

Deficiency of sulphur results in the inhibition of protein synthesis. Griffiths *et al.* (1995) reported that decline in the enzyme activity can cause a reduction in S level in senescent leaves.

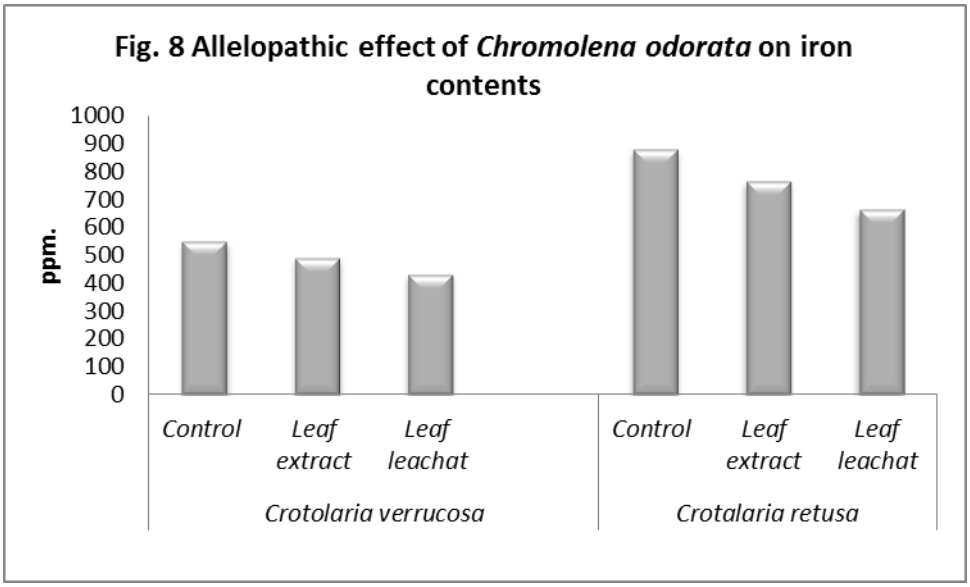


Iron contents

In current research as result shown in Fig. 8 indicates that due to allelopathic treatment of *Chromolena odorata*, there is decline in iron accumulation were observed in both *C. verrucosa* and *C. retusa. In Crotolaria verrucosa* treatment of leaf extract responsible for declining iron accumulation up to 89.6% and that due to leaf leachate 78.6% as compare to control(547.53 ppm). Similarly in *C. retusa*of leaf extract responsible for declining iron accumulation up to 13.09% and that due to leaf leachate 24.62% decline as compare to control (879.32 ppm) one.

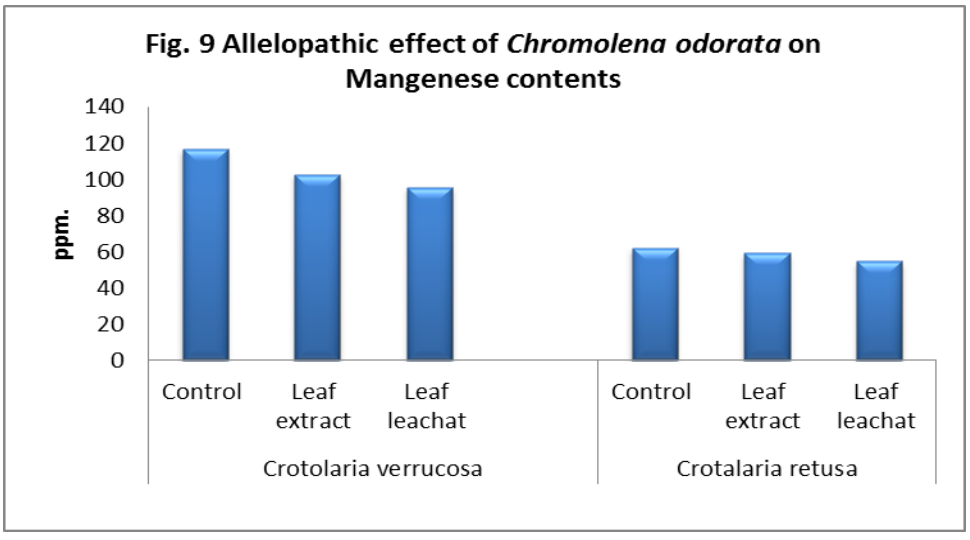
Iron is an immobile element in living cells. It is absorbed by plant roots as Fe^{2+} or as Fe chelate. Fe chelates are soluble and therefore available to roots. The adequate value of iron for optimal growth of plants is 100 ppm (0.01%) (Stout, 1961 and Epstein, 1972). The cytochromes and ferredoxins are the examples of hemeprotein and iron sulphur protein respectively, which act as electron transmitters in a number of basic metabolic processes in chloroplasts and mitochondria. According to Machold and Stephan (1969), iron has role in the synthesis of common precursors of chlorophyll. It is involved in oxidation, reduction reactions, ferredoxin formation and chlorophyll synthesis (Spillar and Teny, 1980). Fe is stored in stroma of chloroplast as phytoferritin, which can store about 5000 atoms of Fe^{3+} (Marschner, 1986).

Iron deficiency causes reduction of nitrate reductase activity and chlorosis (Perur*et al.*, 1961). However, increase in leaf Fe content may cause severe cellular damage.



Manganese contents

It can be depicated from result shown in Fig. 9 that due to allelopathic treatment of *Chromolena odorata*, there is decline in manganese content were observed in both *C. verrucosa* and *C. retusa*. *In Crotolaria verrucosa* treatment of leaf extract responsible for declining manganese content up to 12.3% and that due to leaf leachate 18.3% as compare to control(116.99ppm). Similarly in *C. retusa* of leaf extract responsible for declining manganese content up to 4.48% and that due to leaf leachate 11.95% decline as compare to control (62.1 ppm) one. Manganese plays an important role in the chloroplast membrane system as well as in photolysis of water and O_2 evolution during photosynthesis. Manganese is associated with photosynthesis, respiration, oxidation of carbohydrates and IAA and activation of enzymes of nitrogen metabolism. Enzymes of Kreb's cycle require manganese as a cofactor. According to Marschner (1986), it is directly involved as a component of the biotin enzyme in the biosynthesis of fatty acids.. Manganese is involved in shikimic acid pathway and enhances the resistance of plants to various diseases. Superoxide dismutase incorporating manganese in mitochondria plays an important role in scavenging of free radical (Jimenez *et al.*, 1998). Dietary recommendations established by the Food and Nutrition Board of the Institute of Medicine (IOM) (2004) suggested that 1.8 to 2.3 mg/day intake of Mn is required daily.

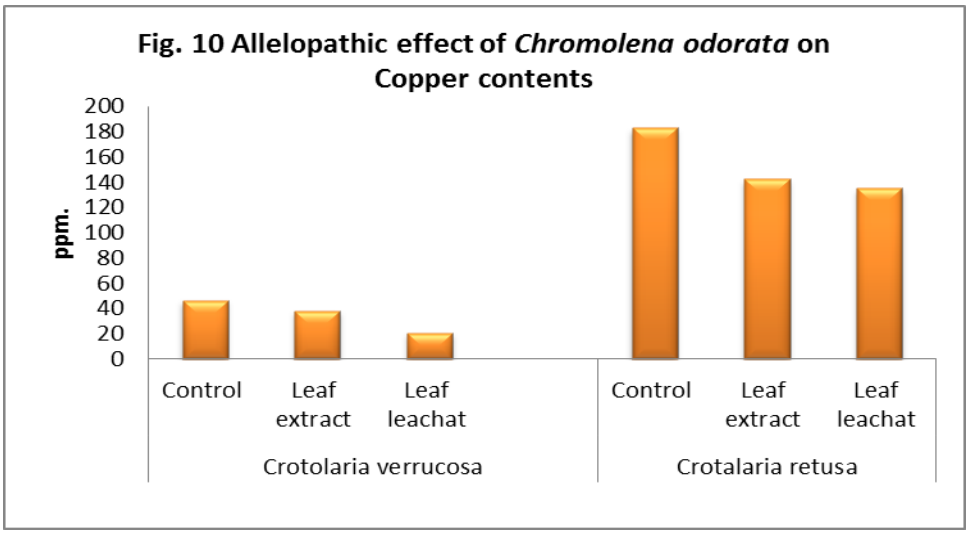


Copper contents

From result shown in Fig. 10 it is evident that due to allelopathic treatment of *Chromolena odorata*, there is decline in copper content were observed in both *C. verrucosa* and *C. retusa. In Crotolaria verrucosa* treatment of leaf extract responsible for declining copper uptake up to 17.6% and that due to leaf leachate 55.3% as compare to control(46.43ppm). Similarly in *C. retusa*of leaf extract responsible for declining copper uptake up to 22.24% and that due to leaf leachate 26.26% decline as compare to control (183.16 ppm).

Copper as a cupric ion is an essential trace element for algae and higher plants (Sommer, 1945; Walker, 1953). Copper provides metabolic control over auxin synthesis (Skoog,1940) and is also involved in protein and carbohydrate metabolism. It plays a vital role in reproductive growth as well as another trace element whose requirement is known in photosynthesis. It also plays an important role in nitrogen metabolism (Hallsworth *et al.,* 1960), being involved in the reduction of nitrate.The critical deficiency level of copper in vegetative parts is generally in the range of 3 to 5 µg g^{-1} (0.0003-0.0005%) dry wt. depending on the plant species, plant organ, developmental stage and nitrogen supply, this range can be larger (Robson and Reuter, 1981).

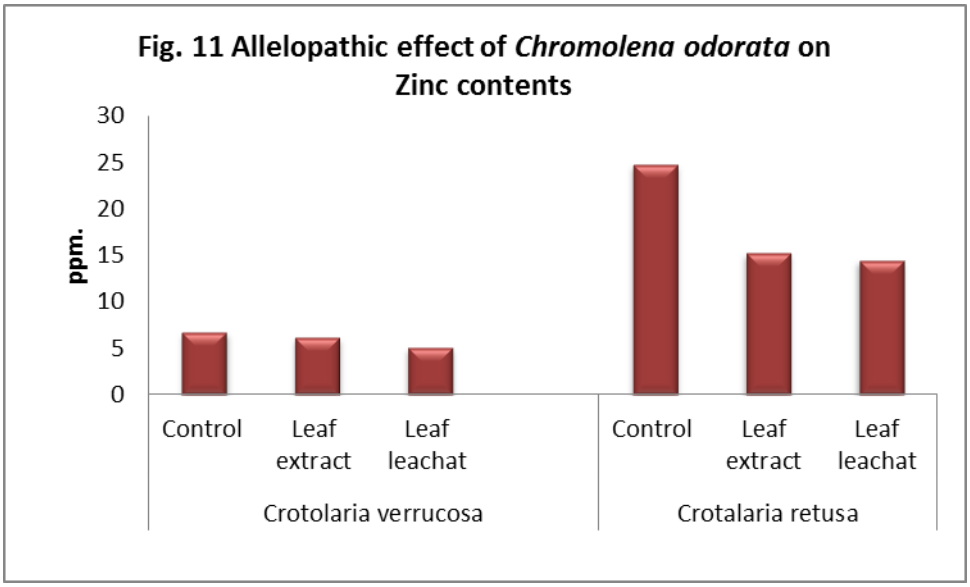
Deficiency of copper induces the activity of ferric reductase enzyme, involved in Fe uptake (Kochian, 2000). According to Mizumo *et al*. (1982), the copper deficient leaves exhibit low soluble carbohydrates than normal leaves during vegetative stage.



Zinc contents

Zinc is essential for carbohydrate metabolism and regulation of consumption of sugars, nitrogen metabolism, protein synthesis, auxin synthesis, particularly IAA synthesis, as well as for sexual fertilization and development of reproductive parts. Many enzymes require zinc for their activity. Zinc acts either as a metal component or as a functional, structural or regulatory cofactor of a large number of enzymes. It is required for chlorophyll biosynthesis. It participates in synthesis of indole acetic acid from its precursor, tryphtophan (Skoog, 1940 and Tsui, 1948). From result shown in Fig. 11 it is clear that due to leaf extract and leachate both treatments of *Chromolena odorata* responsible to decline in zinc content in both *C. verrucosa* and *C. retusa.In Crotolaria verrucosa* treatment of leaf extract responsible for declining zinc content up to 8.5% and that due to leaf leachate 24.5% as compare to control(6.73 ppm). Similarly in *C. retusa*of leaf extract responsible for declining zinc uptake up to 34.48% and that due to leaf leachate 41.51% decline as compare to control (24.74 ppm)). Zn plays a role in membrane stability by regulating the level of oxidizing O_2 species (Pinton *et al.*, 1994).The critical deficiency levels of Zn are below 15–20 mg kg^{-1} dry weight of leaves and critical toxicity levels of zinc in leaves of crop plants are more than 400–500 mg kg^{-1} dry weight basis (Marschner, 1986).

Zinc deficiency is characterized by a reduction in internodal growth resulting into rosette habit of a plant. Zn deficiency in wheat plant decreases NR activity and NO_3 content (Harper and Paulsen, 1969).



It can be concluded from result that *Chromolena odorata* responsible to decline in nutrient uptake potential due to having allelochemicals in it. Leaf leachate of *Chromolena odorata* highly responsible to decline inorganic composition of other species. i. e. it inhibit growth and development of other plants by affecting nutrient uptake potential. Therefore this plant *Chromolena odorata* can be used to prepare weedicide or herbicide.

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