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Allelopathic effect of ursolic acid on growth and physiology of green gram cultivar KM-2

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SUMMARY

Allelopathic effect of ursolic acid, a secondary metabolite obtained from the leaves of *Canthium dicoccum* on the germination, growth and some physiological aspects of green gram (*Vigna radiata)* cultivar KM-2 was investigated in this study. The negative influence on the green gram was maximum in ursolic acid treated plants (2.0mm) than control was evident from the bioassay and pot culture studies. The inhibition of seed germination and the decrease of seedling growth increased with increasing concentration of ursolic acid was very well understood from the morphometric measurement on root, shoot length, leaf area and biomass. Correspondingly the total number of lateral roots and the number of root nodules were very much reduced proportionately with increasing concentrations of ursolic acid treatment. There was considerable reduction in the level of photosynthetic pigments, total sugars, starch as well as soluble protein in the treated plants. The increased level of Malondialdehyde (MDA) suggested the negative influence of test solutions on membrane integrity and the increased activity of the oxidative enzymes such as Polyphenol oxidase (PPO) and Peroxidase (PO) through light on the production of enzymes to over come the effect of stress produced by the allelo chemical.

Key words : Allelochemical, Growth, Green gram, Photosynthetic pigment, Antioxidants.

Allelopathy, the term to refers the Inhibitary or stimulatory interaction between all types of plants including micro-organisms (Molisch, 1937). It refers to both autotoxic and hetrotoxic effect. Harbone (1989) studied that variety of chemicals may be released in the surrounding during the process of leaching of fallen leaves, residues of the previous crops. They contain water soluble inorganic and organic compounds (Rice, 1971).

Target species are affected by the toxins of allelo chemicals in many ways (Einhellig, 2002) and the toxic chemicals may inhibit shoot /root growth (Jayakumar *et al.*, 1998). They may inhibit nutrient uptake or they may attack a naturally occurring symbiotic relationship there by the destroying the plant's usable source of a nutrient (Elemore, 1980)

They also reduce the photosynthetic pigments, thereby reducing the amount of sugar, starch and soluble protein in the treated plants (Jayakumar *et al.*, 1998). The structural and fundamental integrity of the membrane is very much disturbed and it is indicated by the increase in the content of malondialdehyde (MDA) level.

Ursolic acid is a secondary metabolite of a sacred grove tree, *Canthium dicoccum*, and the green gram cultivar KM-2 *(Vigna radiata)* is a legume crop grown nearby field of the sacred grove. Hence, the allelopathic effect of the allolochemical, ursolic acid on the growth and physiology of the legume was studied in this project.

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MATERIALS AND METHODS

Certified seeds of *Vigna radiata* were used in this experiment. The molar solution was prepared by dissolving one mole of ursolic acid, first dissolved in chloroform (1ml), and then in methanol (1ml) later in distilled water and then its volume was made upto 1000ml. From this molar solutions, different milli molar concentrations (0.5mM, 1.0mM and 2.0mM) were prepared by diluting it with distilled water.

Bio assay was carried out in sterilized Petridish lined with whatmann. No.1 filter paper at 28 $\pm$2°c. The seeds were initially surface sterilized with 0.1% mercuric chloride solution for 60 sec. and then washed thoroughly with tap water and were used for both Petriplates and pot culture studies. Sterilized seeds were soaked in tap water for 24 hrs and were treated with different concentrations of ursolic acid (T_1 - 0.5 mM ; T_2 - 1.0 mM ; T_3 - 2.0 mM) water was used in Control (T_0 – 0.0 mM) in bioassay.

The seedlings grown under normal photoperiodic conditions, were irrigated with different concentrations of ursolic acid (250ml/pot) on alternate days and control plants were irrigated with tap water. The plants were removed from the pots on 15 / 30 DAS, washed

thoroughly and used for estimation. Germination percentage, radicle length, root/shoot ratio were measured periodically.

The contents of pigments, sugars and proteins and the activity of oxidative enzymes of the leaves were estimated following the procedures of Shoaf and Lium, 1976 (pigment estimation), Lowry *et al.*, 1951 (proteins) Mc Cready *et al.*, 1950 (starch), Dubois *et al.*, 1956 (sugars) and Kumar and Khan, 1982 (peroxidase and poly phenol peroxidase estimation).

Three replicates were maintained for all the experiments including treatments and control. The experiments were repeated to confirm the trends. All the data were analysed statistically and standard errors were mentioned where ever applicable (Zar, 1984).

RESULTS AND DISCUSSION

The allelopathic effect of ursolic acid, a secondary metabolite obtained from leaves of *Canthium dicoccum* on the germination growth and some physiological aspects of *Vigna radiata* is investigated in this study.

Germination of seeds is critical to the establishment of the plant, but this process suffers heavily from the ursolic acid. When all the seeds in the control recorded 100% germination by 24 hours, the inhibition was 70% for 2.0 mM ursolic acid at T_3, 50% for T_2 and 45% for T_1. The inhibitions at 0.5 mM and 1.0 mM levels were evident even after 72 hours indicating a concentration effect. (Table 1). However, the degree of inhibition was less when the ursolic acid was supplied through irrigation of soil. Perhaps the soil particles and variety of chemicals in the substratum would have either attenuated the inhibition capacity. Viewed together, data on Petri plate assay and pot culture confirmed the toxic potential of the allelochemical ursolic acid in tandem. The above results agree with the previous report of inhibition of leaf extract of *Rumex crispus* on the seed germination and seedlings growth of Sorghum and also the inhibitory effect of *Bambusa arundinacea* on *Arachis hypogea*.

Radicle elongation is an index of successful germination; plumule emerges only later. Hence, the radicle length was measured at 24 and 48 hrs after sowing of pre-soaked and unsoaked seeds treated with different concentrations of ursolic acid. In the unsoaked seed treatment, the inhibition was severe compared to pre-soaked seed treatment. The radicle length was inhibited severely and it decreased with increasing levels of ursolic acid. At any rate, 37.5% to 75 % decrease was in length points to a severe inhibition.

Morphometric measurements (length and dry biomass of root, shoot and whole plant) were made at 5,10,15 and 30 DAS to monitor the effect on seedling growth. Accordingly, not only the length of the root and shoot were lowered but also the accumulation of dry biomass. The reductions were observed at all stages and in all organs. The above data also confirmed the previous report of Sathiya (2007) and Rice (1971).

Study revealed a severe inhibition of lateral root initiation with a range of 25-50% inhibition. T_3 Plants were least efficient in developing nodule compared to control. Though T_1 and T_2 had more nodules than T_3, a strong inhibition of nodule development was recorded invariably. The number of root nodules per plant were also decreased with increasing concentration of ursolic acid. This is in

Table 1 : Germination and growth of *Vigna radiata* treated with ursolic acid

Sr. No.	Characteristics	Treatments				
		Age	Control (0 mM)	T_1 (0.5 mM)	T_2 (1.0 mM)	T_3 (2.0 mM)
1.	Seed germination (%) (Presoaked)	24h	20	14	12	9
2.	Radicle elongation (cm) (Presoaked)	48h	4.0	2.5	2.0	1.0
3.	Seed germination (%) (un soaked)	24h	20	11	10	6
4.	Radicle length (cm)	48h	3.5	1.5	1.0	0.4
5.	Shoot length (cm)	10 DAS	4.1	3.8	3.4	3.0
		30 DAS	8.0	7.5	7.1	6.7
6.	Root length (cm)	10 DAS	22	21	14	11
		30 DAS	40	39	32	25
7.	Plant height (cm)	10 DAS	26.1	24.8	17.4	14.0
		30 DAS	48.0	46.5	39.1	31.7
8.	No. of Lateral roots	30 DAS	20	15	9	6
9.	Nodulation	30 DAS	19	11	9	5
10.	Total leaf area (TF_1 cm^2)	30 DAS	18.5	15.2	11.7	8.5
11.	Dry biomass / plant / mg	30 DAS	480	473	348	308

Table 2 : Biochemical changes in *Vigna radiata* treated with ursolic acid

Sr. No	Characteristics	Age	Treatments			
			Control (0 mM)	T_1 (0.5 mM)	T_2 (1.0 mM)	T_3 (2.0 mM)
1.	Chl a+b mg /g^{-1} fw	15 DAS	1.786	1.741	1.216	1.028
2.	Carotenoids mg /g^{-1} fw	15 DAS	0.584	0.530	0.390	0.280
3.	Car. / Chl ratio	15 DAS	0.326	0.304	0.321	0.272
4.	Total sugar mg /g^{-1} fw	20 DAS	29.56	27.50	16.60	11.50
5.	Soluable protein mg /g^{-1} fw	20 DAS	52.10	50.70	35.60	29.50
6.	Soluable starch mg /g^{-1} fw	20 DAS	18.70	17.36	13.57	9.80

Table 3 : Estimation of MDA at 20 DAS of *Vigna radiata* treated with ursolic acid

Sr. No.	Characteristics	Age	Treatments			
			Control (0 mM)	T_1 (0.5 mM)	T_2 (1.0 mM)	T_3 (2.0 mM)
1.	MDA Malondialdehyde µ mol /g^{-1} fw	20 DAS	8.35	9.10	13.50	20.36

Table 4 : Changes in Enzyme activities of *Vigna radiata* treated with ursolic acid

Sr. No.	Characteristics	Age	Treatments			
			Control (0 mM)	T_1 (0.5 mM)	T_2 (1.0 mM)	T_3 (2.0 mM)
1.	Polyphenol oxidase (Δ A min^{-1} mg^{-1} protein)	20 DAS	3.15	4.112	6.10	8.56
2.	Peroxidase	30 DAS	2.13	3.70	4.71	6.81

conformity with the reports of Rice (1971) where the pioneer plant species inhibited the nodulation in legume.

With the suppression of growth, the cellular metabolites, total soluble proteins, sugars and starch were estimated towards obtaining a clue. With reference to Table 2, both the carbohydrates declined sharply in the treated leaves and the effect was proportional to concentrations. As these two metabolites are the products of photosynthesis, the development of the leaves, the photosynthetic pigments content of leaves were followed at 15 day old plants grown at growth chambers. Not only the leaves became progressively smaller in the treated samples but also there was a corresponding decline in the photosynthetic pigments too. While the cholorophyll and carotenoid development was depressed by the treatment, the ratio of carotenoid / chlorophyll was not affected significantly. This trend is also supported by Jayakumar *et al*. (1998) who observed the similar negative influence of aqueous abscissed leaves of *Eucalyptus globules* on *Arachis hypogea* and *Zea mays*

Plasma membrane is the primary target of all the stresses. The membrane is a bilipid layer integrated with proteins. To confirm the damages to the membrane, MDA levels were estimated. Data show the highest release in T_3 and lowest in the control (Table 3).

The MDA levels were more than control even at T_1 and T_2, (Table 3) suggesting that the structural integrity of the membrane has been eroded and the damage is concentration dependent. The similar enzyme activity in tomato was reported by Cruz Ortega *et al*. (2008). The enhanced production of Reactive Oxygen Species (ROS) would accelerate the ROS scavenging enzymes, especially polyphenol oxidase (PPO) and peroxidase (PO) and superoxidase dismutase. (SOD). Two of the three anti oxidative enzymes were assessed to gauge the resilience of *Vigna radiata* in resisting ursolic acid. Two enzymes were tested on this count, namely, polyphenol oxidase (PPO) and peroxidase (PO) which have recorded manifold increase in treated samples (Table 4). In fact, even the T_1 plants which have been suffering only mildly so far, have shown 40% more of PPO activity and two fold increase of peroxidase activity. The strength of the test solution (T_3), elicited a three fold rise of PPO and four fold rise of peroxidase. Such a stupendous acceleration of these antioxidative enzyme is significant in developing resistance to the ursolic acid.

The present study reveals that the ursolic acid, a secondary metabolite of *Canthium diccoccum* has an allelopathic effect of strong inhibition on germination, growth and physiology of the legume, *Vigna radiata* and

also confirmed the previous study of the strong negative influence of the crude leaf extract of *Canthium dicoccum* on *Vigna radiata* (Sathiya, 2007) The negative influence of the allelochemical can be exploited to control weeds and pathogenic microorganisms in crop production and management.

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