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BIO-EFFICACY OF CROTON *BONPLANDIANUM* BAILL.ON SOME WEED AND CROP PLANTS

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ABSTRACT

Allelo - chemicals were extracted from the leaves of *Croton bonplandianum*Baill.using various solvent systems. The seeds of the test plants i.e. *Triticum. aestivum*L.,*Spinaceaoleracea*L.,*Vicia sativa* L.and *Madicagohispida*Gaertn.were treated with different extract fractions i.e. Aqueous leachates (AL), Petroleum ether Fraction (PF) Methanolic Fraction (MF) Chloroform Fraction (MF) WaterFraction (WF)} thus obtained, various germination parameters viz, percent germination, seed vigour and mean seedling growth was observed. From the results of this experiment it is explicit that the seeds of *T. aestivum, S. oleracea, V. sativa M. hispida*. do not germinate in an environment that possesses allelo-chemics from *C. bonplandianum.* This result gains support from the observation. The toxic effect of the aqueous leachates and organic extract fractions on the seeds of the test plants assumes a lot of significance since seeds is produced by the plants to ensure the perpetuation of their types and for the spread of the species to newer areas.

KEY WORDS:*Croton bonplandianum***Bailland Alelochemicals**

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INTRODUCTION

The phenomenon of allelopathy has received increasing attention within the past two decades as a mechanism for explaining vegetation patterns in plant communities (Muller 1969,Rice 1979) and as an important aspect of weed crop interactions (Tukey 1969,Whittaker 1970,Bell and Koeppe 1972). These interactions amongst plants often lead to a superiority of one species to the detriment of another under natural conditions.The *Croton bonplandianum* is an obnoxious weed of family Euphorbiaceae, native of South America and was reported from India during late 1890 (Kaul, 1967). It now occurs widely along roadsides, railway abandoned field, in wide open ravines, paddy or sugarcane fields and on sandy or sandy clay soils. This species is seldom found in areas enclosed by shrubs and trees where free movement of air gets hindered. *C. bonplandianum* is strongly invasive species and forms its own colonies wherever it invades. Several factors contribute to its ability of quickly invading and establishing in different habitats. These include fast growth rate, high vegetative and reproductive potential, wide ecological amplitude, strong adaptability and allelopathic properties.

MATERIALS AND METHODS

Extraction of Leachable Allelo-Chemicals (Protocol: I)

Based on the methods devised by Kumari*et al*., (1985), healthy and freshly collected leaves of *C. bonplandianum*Baill.were cut roughly in to pieces after clearing their surface and their dry weight per unit fresh weight were determined by desicating the tissue in the oven.The weighed amount of fresh leaf pieces of the plant was soaked in requisite amount of pure water (resistively more 18.5 mega ohms cm. and conductivity less than 0.05 µ Simons cm at 25°C) for a period of 20 hrs. at room temperature. It was filtered completely through triple layer of muslin cloth and the requisite concentration was made with water. One half of this filtrate referred to as the aqueous leachates was used as such, while the other part was chilled and subjected to acid hydrolysis using pre-chilled, 3N HCl. The precipitates so formed were recovered through centrifugation (2000 rpm). These were washed 5-6 times with pure water. Every time the recovery was made through centrifugation. For experimental purpose, requisite amount of the precipitate was dissolved in a few drop of ethyl alcohol and the final volume was made with pure water. A drop of tween 20 was added to it, to serve as surfactant. This is referred to as aglycone or aglyconic or organic component of aqueous leachates. In this study however, aglyconic components have not been taken into consideration due to insignificant formation of the precipitates when 3N HCl was added.

Extraction of Organic Fraction (Protocol: II)

Freshly collected, surface cleaned and healthy leaves of the requisite plant were dried under shade and powdered. The powder was immersed in Petroleum ether (60°-80°C) for 20 hrs. The liquid was separated from the residue (marc), through mild centrifugation (500rpm for 2 min). From the liquid portion the solvent (Petroleum ether) was recovered on a hot water bath. Requisite amount of the residue so obtained, was weighed and a few drops of Xylene, a part from a drop of tween-20 (to act as surfactant) were added to it. Final volume was made with pure water. This was termed Petroleum ether fraction (PF). The marc (residue from Petroleum ether suspension) was suspended in methanol for 20 hrs and filtered, from one half of the filtrate, Methanol was recovered on a hot water bath. The residue, so obtained, was dissolved in a drop of methanol and the final volume was made with pure water. It has been called Methanol fraction (MF). From another half of methanol filtrate the solvent was removed and the residue was partitioned between chloroform and water (1:1 V/V). The two layers, so formed, were separated in a separating funnel. The chloroform was recovered over a

hot water bath. To the requisite amount of residue a few drops of methanol were added and the final volume was made with pure water. This has been termed as the chloroform fraction (CF). The water from the aqueous layer after separating chloroform fraction was dried under low pressure on a rotary flash evaporator. The solution made with water has been termed as the water fraction (WF).

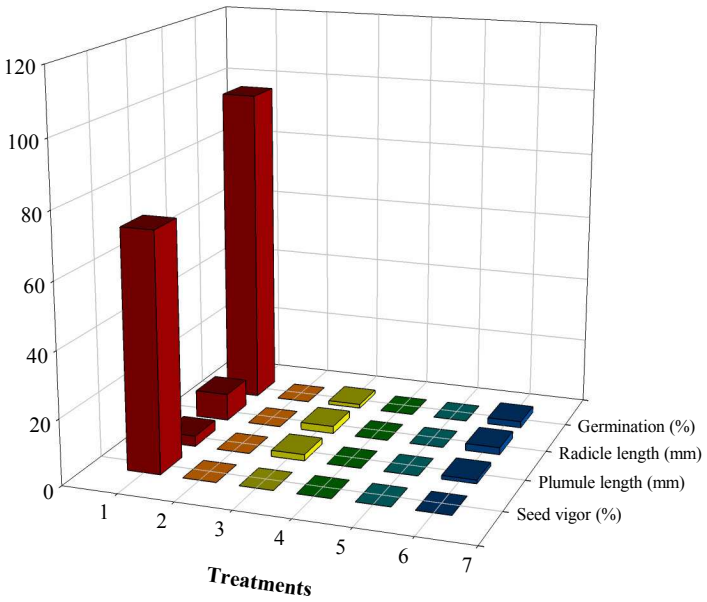
The mature healthy seeds of *T.aestivum, S. oleracea, V. sativa*and *M. hispida* were collected .Aqueous leachates (g fresh wt/ml pure water 0.1% w/v) petroleum ether fraction (PF) (0.1% w/v), methanolic fraction (MF) (0.1%w/v), chloroform fraction (CF) (0.1% w/v), and water fraction (WF) (0.33% w/v) derived from leaves of *C. bonplandianum* formed the treatment solutions. The comparable treatment with pure water served as control in any of the cases. The concentrations of various fractions under experimentation were decided on the basis of a pilot experiment on the % of germination where a wide range was used. The one that showed 50 ± 10 percent values served as LC 50(Lethal concentration) killing 50% of the population. For all the treatment solutions a minimum of three replicates were maintained. Data represent mean S.D.(standard deviation) of three sets. Germination Parameters Sample size of 300 uniforms, viable, healthy seeds of all species under test were taken. The seeds of each sample were soaked in respective concentrations of the treatment solution for 20 hrs. at room temperature. Treatment with pure water served as control. The seeds were arranged in Petri dishes with a thin wad of absorbent cotton lined with Whatman no.40 filter paper and the bed was moistened with respective treatment solution. The set up was maintained in a seed germinator and the test for percent germination and seed vigour was carried following ISTA rules (1976). Observations on the length of radicle and plumule were taken after 7 days of germination of the seeds

STATISTICAL ANALYSIS

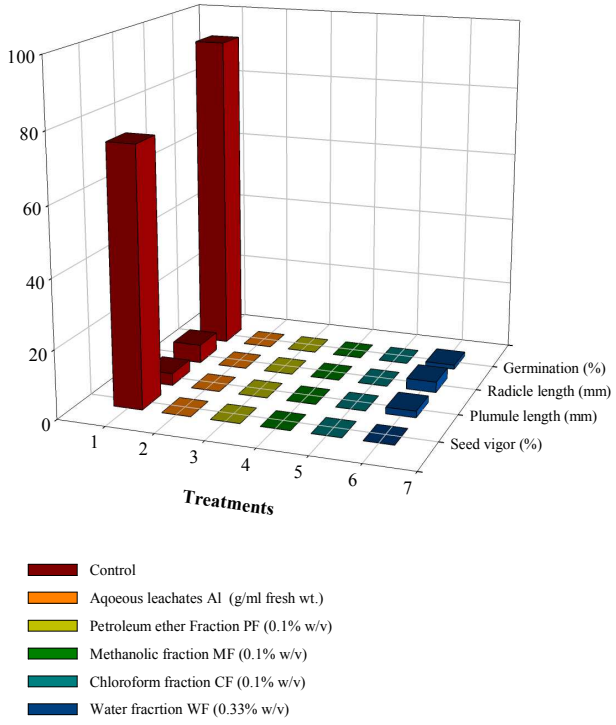
The data on the parameters under study was subjected to statistical analysis employing analysis of Variance (ANOVA) to asses the significance of difference of treatment over that of control.

RESULTS

In case of *T.aestivum* L. 97.42% seeds germinated with water as control, the treated seeds completely failed to germinate (Table.1). The entire fraction had an equal effect on germination percentage, radicle and plumule length. Seed vigour of *T. aestivum* was about 73% in control; it dropped to zero in the treated samples (Fig.1).92.82 % seed of *S.oleracea*germinated on third day with water as control. Except the water fraction, aqueous leachates, petroleum ether fraction, methanolic fraction and chloroform fraction completely inhibited germination. Water fraction (WF) showed a negligible about 2% germination (Fig.1). A very small protuberance visible only with a hand lens confirmed germination. Seed vigour of *S.oleracea*was 74.97% which was completely lost by the treatment solution (Table.1). 98.43% seeds of *V. sativa* with water as control germinated on the second day. Aqueous leachates, petroleum ether fraction and chloroform fraction inhibited germination completely (Fig.2). The water fraction and methanolic fraction allowed a very small percentage of seeds germination. Seed vigour of *V. sativa* was 85.13% which was completely lost by the treatment solution (Table.2).In *M.hispida* 96.66% seeds germinated were reported with water as control.Aqueous leachates, petroleum ether fraction and methanolic fraction all the other allelo-chemic extract fractions completely inhibited germination. Chloroform fraction and water fraction showed a about 3% and 4% germination respectively. Seed vigour of *M. hispida* showed same pattern (Table.2). All the results are highly significant statistically (Fig.2)

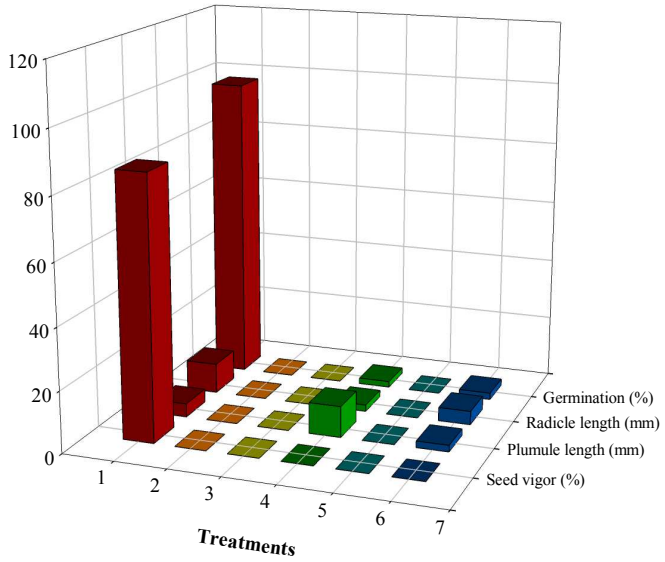


(A)



(B)

Figure 1
Effect of aqueous leachates and organic extract fractions of leaves of C. bonplandianumBaill. on germination parameters of seeds of (a) Triticumaestivum L. and (b) Spinaceaoleracea L.



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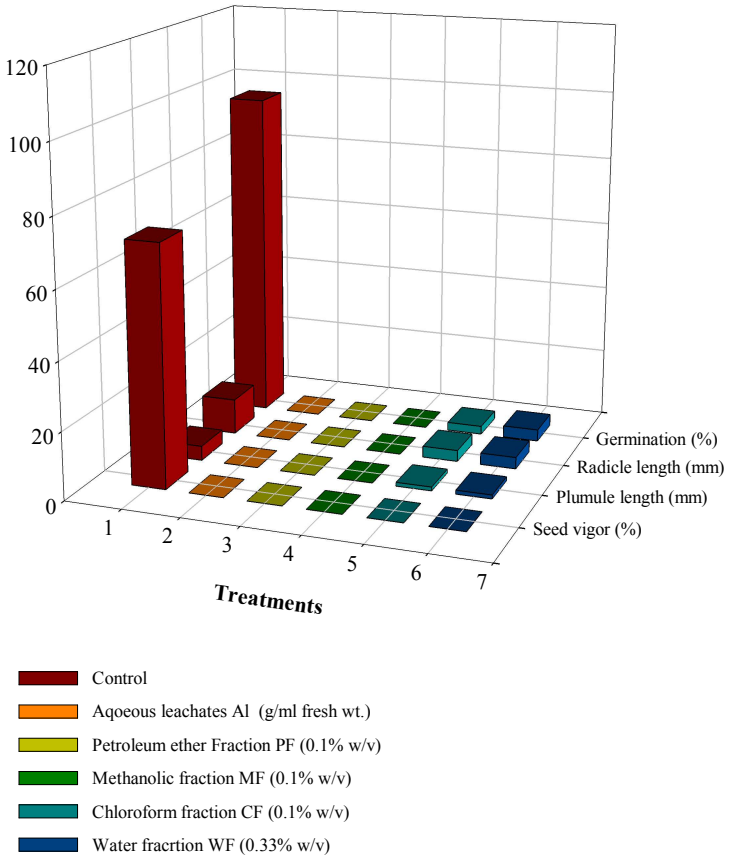

(B)

Figure 2

Effect of aqueous leachates and organic extract fractions of leaves of C. bonplandianumBaill. on germination parameters of seeds of (a) Vicia sativa L. and (b) MedicagohispidaGaertn.

Table 1

Effect of aqueous leachates and organic extract fractions of leaves of C. bonplandianumBaill.on germination parameters of seeds of Triticumaestivum L. and Spinaceaoleracea L.

Treatment	*T. aestivum*				*S. oleracea*			
	Germination %	**Radicle Length (mm)**	**Plumule length (mm)**	**Seed vigour %**	**Germination %**	**Radicle Length (mm)**	**Plumule length (mm)**	**Seed vigour %**
Control	97.42	8.34	3.19	72.37	92.82 ± 0.36	5.42 ± 0.63	3.52 ± 0.11	74.97 ± 0.21
Aqoeous leachates Al (g/ml fresh wt.)	0	0	0	0	0	0	0	0
Petroleum ether FractionPF (0.1% w/v)	0	0	0	0	0	0	0	0
Methanolic fraction MF (0.1% w/v)	0	0	0	0	0	0	0	0
Chloroform fraction CF (0.1% w/v)	0	0	0	0	0	0	0	0
Water fracrtion WF(0.33% w/v)	0	0	0	0	1.25 ± 0.22	3.12 ± 0.14	1.91± 0.06	0

± represents standard deviation

Table 2

Effect of aqueous leachates and organic extract fractions of leaves of C. bonplandianumBaill.on germination parameters of seeds of Vicia sativaL. and MedicagohispidaGaertn.

Treatment	*V. sativa*				*M. hispida*			
	Germination %	**Radicle Length (mm)**	**Plumule length (mm)**	**Seed vigour %**	**Germination %**	**Radicle Length (mm)**	**Plumule length (mm)**	**Seed vigour %**
Control	98.43 ± 0.15	9.73 ± 0.10	4.35 ± 0.44	85.13 ± 0.17	96.66	10.42	4.24	70.73
Aqoeous leachates Al (g/ml fresh wt.)	0	0	0	0	0	0	0	0
Petroleum ether Fraction PF (0.1% w/v)	0	0	0	0	0	0	0	0
Methanolic fraction MF (0.1% w/v)	1.92 ± 0.12	2.13 ± 0.11	10.1 ± 0.95	0 ± 0.0	0	0	0	0
Chloroform fraction CF (0.1% w/v)	0	0	0	0	2.42	3.29	1.12	0
Water fracrtion WF (0.33% w/v)	2.14 ± 32	4.42 ± 56	2.13 ± 0.22	0 ± 0.00	3.44	3.42	1.15	0

± represents standard deviation

DISCUSSION

Leaf leachates of *C.bonplandianum* totally inhibited the germination and growth of *Parthenium* in many crops (Thaper and Singh 2006; Datta and Roy, 1975, 1973). From the results of the previous experiments it is clear that *C. bonplandianum* are allelopathic in various degrees. The study of the response of allelopathic plant to the allelopathic agents of other plant, therefore, assumes significance. This aspect fetches more importance since all the four plants are crops/ weeds themselves and hence, some control measures need to be adopted for these crops/ weeds From the results of this experiment it is explicit that the seeds of *T. aestivum, S. oleracea, V. sativa M. hispida*. do not germinate in an environment that possesses allelo-chemics from *C. bonplandianum.* This result gain support from the observation.Allelo-chemics or the secondary plant products are leached into the environment in a variety of ways i.e., either by exudation or volatilization or leaching (Rice, 1984, Kayode, 2006). Allelo-chemics or secondary metabolites escape into the environment in their glycosidic forms. Glycosidic bonds, not only facilitate the movement of allelo-chemics in and outside the plant, but also lessen their toxic nature towards the donor plant itself (Goss, 1973). The presence of glycosides in the aqueous leachates of *C.bonplandianum* was confirmed by Fehling solution test. The glycosides, however, could be separated from the organic part either by enzymatically action or by acid hydrolysis.

The toxic effect of the aqueous leachates and organic extract fractions on the seeds of the test plants assumes a lot of significance since seeds is produced by the plants to ensure the perpetuation of their types and for the spread of the species to newer areas. A true seed is defined as a fertilized mature ovule that possesses an embryonic plant, stored material (some time absent) and a protective coat (Kozlowski and Gunn, 1972). For most of the annuals seeds are the only means of multiplication and their continued existence. Webb (1966) defined seed germination as resumption of growth of the embryonic plant contained by the seed in an active condition. Seeds germinate only when conditions of moisture, temperature, light and nutrition are favourable for their growth. If any one of these conditions is lacking the seeds fail to germinate. Different kinds of compounds are known to affect seed germination. Low concentration of hydrogen cyanide kills the growing embryos. Effect of a compound on seed germination may result from its influence on the moisture status of the immediate seed environment. Thus, high salt concentration in contact with the seed may prevent the seed from obtaining enough water to initiate germination. How exactly the allelo-chemicals affect seed germination still remains unclear whether they inhibit imbibitions of water or they act by killing the growing embryos after entering the seed coat is still not clear.

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