Journal of Computational Intelligence in Bioinformatics.
ISSN: 0973-385X Volume 4 Number 1 (2011) pp.87—92

http://www.ripublication.com/jcib.htm

Effect of Thunder (Weedicide) on the Growth and Nodulation of *Vigna mungo L:* Insilico and In Vitro Approach

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Abstract

Vigna mungo L. commonly known as the Urd bean is a important proteinaceous pulse crop in the food industry. Weedicides are sprayed commonly just to protect the crop from unwanted plants (weeds) but these chemicals are also having side effects and affect the growth and nodulation of plant . Thunder is one such type of the weedicide that affects metabolically important proteins and hence the growth and nodulation of the plant. Effect of the thunder on *Vinga mungo L.* has been observed and proved in the experimental plot. The phytotoxic effect of chemical on different growth parameters as plant hight, lateral spread, root length, shoot length, leaf area, number of lealets, nodes, branches, flowers, pods and nodules is observed. In general the reduction in all the growth parameters and nodulation is observed. In order to identify the specific proteins which are very important for growth and nodulation targeted by the thunder (ligand) we opted for the bioinformatics approach .This approach will help us for the support of findings of wet lab. We followed the docking protocol developed by Scripps Research Institute, Inc, using the AUTDOCK and VINA tutorial. After following this protocol we came to conclusion that annexin 1, catalase,UDP – glycose: flavanoid glcosyltransferase, flavonoid 3-0-galactosyl transferase, cysteine protease were the most essential of the proteins that were targeted by the thunder (ligand), hence affecting the plant growth and nodule development . The selected proteins were docked with Thunder (ligand) showing the interaction between proteins and chemical resulting in the disintegration of proteins. Results of docking concludes that protein - UDP – glycose:flavanoid glcosyltransferase binded best with the ligand. Therefore it is justified that UDP – glycose : flavanoid glcosyltransferase is the essential protein affected by the ligand resulting in functional disitigration of the protein. Inhibitory effect of Thunder on the growth and nodulation of *Vigna mungo L.* may be due to interaction of essential proteins with thunder.

Keywords: *Vigna mungo L.,* phytotoxicity, Thunder weedicide, AUTDOCK VINA, DOCKING.

Introduction

It is being increasingly understood that plants are saviors of environment and they help to maintain the balance and good health of ecosystem. Growth is the resultant of interaction between many factors both environmental and physiological. The role of environmental pollution to produce various types of deleterious effects on diverse living system has been well established. Any change in environment as chemical treatment disrupts the metabolic process and cellular functions affecting the overall growth of plant. The Thunder in combination with moisture status undergo acidification and reduce the soil pH, which in turn reduces the bacterial population responsible for root nodulation.

Vigna mungoL.

Commonly known as Urd, Urd bean and Black gram .An erect, bushy, pubescent, herbaceous legume reaching 30-100 cm in height with trifoliate leaves, small, light yellow flowers and short flat pods reaching 4-6 cm in length. Annual herb, growing 60-80 days before it can be harvested for green pods, and 75-130 days for mature beans. In India, it is grown both as a summer and winter crop scientific synonym: *Phaseolus mungo*. Urd bean originated in South East Asia. In India it is grown from sea level to 2000 m in elevation. It prefers low to medium humidity. It is best grown during dry weather with residual soil moisture.

Thunder (A Weedicide)

Weedicides are used to control weeds. Thunder is one of the most important weedicide. It is an important weed killer and pre-emergence weedicide. It is much toxic to microbial population and to plants. It is chemically composed of active ingredient- Butachlor 50% W/W and inert ingredient 50% W/W its chemical name is 2-chloro-2,6-diethyl-N-(Butoxy-methyl) and chemical formulae is $C_{17}H_{26}ClNO_2$.

Materials and Methods

This experiment was conducted to study the effect of thunder a weedicide on the growth and nodulation of *Vigna mungo L.* The seeds of *Vigna mungo L.* var. T9 (Urd bean) were procured from Government seed agency. The seeds of uniform size were selected, surface sterilized with mercuric chloride for two minutes. Two sets of experiments were conducted; one treated and other one is control. Treatment was done by spray method at the time of sowing. Four conc. of chemical were made as 1ppm, 5ppm, 50ppm and 100ppm. The seeds of *Vigna mungo L.* were sown in earthen pots filled with garden soil. The plants were grown with proper agronomic care. The growth parameters were taken are plant height, shoot length, root length, lateral spread, leaf area, number of nodes/plant, number of branches/plant and number of leaflets/plant.

Docking

Molecular docking is used for drug design where a ligand, which is a relatively small molecule, docks onto a receptor, which is usually a much bigger molecule. We have got a ligand molecule from the in vitro approach named: 2-chloro-2,6-diethyl-N-(Butoxy-methyl). We have designed it in insilico by tha software named: Chemsketch.

The AOTODOCK, using an empirical scoring function and a patented search engine to dock ligands into a protein's binding site, was applied to study molecular docking [12]. The crystal structure of essential proteins of *Vigna mungo L.* was not reported in RCSB Protein Data Bank therefore we modeled the structure by using MODELLER software. The RCSB Protein Data Bank structure was utilized in subsequent docking experiments without energy minimization. The ligands were docked into the corresponding protein's binding site by an empirical scoring function and a patented search engine in AUTODOCK and VINA tutorial. All ligands and water molecules were removed and the polar hydrogen atoms were added. In this paper, the automatic docking was done using AOTODOCK and VINA tutorial.

Results

The Phytotoxic effect of Thunder a weedicide on growth parameters like lateral spread,plant height,shootlength,rootlength,no.of branches,no.of nodes,no. of leaves and total leaf area have seen.In general,the reduction of all growth parameters is observed intreated plants with increasing concentrations.In treated plants pulse crop showed maximum inhibition of 61.77%in plant height,60.30% in rootlength, 64.75 % in shoot length and 62.81 % in leaf area with 100 ppm.of chemical.

Table 1: Thunder application showed inhibitory effect on root nodulation of *Vigna mungo L.*

Growth parameters	control	1ppm	5ppm	50 ppm	100 ppm
Lateral spread (cm^2)	457.19	343.93	339.62	277.45	209.35
Root length (cm)	47.19	24.23	23.14	20.60	13.73
Shoot length (cm)	162.31	89.15		65.81	51.21
Plant height (cm)	209.96	140.28	119.95	91.30	80.26
Total leaf area (cm^2)	1525.54	740.36	650.36	585.36	570.25
No.of node/plant	58.00	38.00	36.00	31.00	25.00
No of branches /plant	18.00	14.00	14.00	13.00	12.00
No. of leaf lets /plant	93.00	39.00	36.00	32.00	30.00
No. of flower/plant	12.00	10.00	9.00	8.00	8.00
No.of pod/plant	30.00	20.00	15.00	14.00	14.00
No.of nodules	100.00	82.00	76.00	72.00	70.00

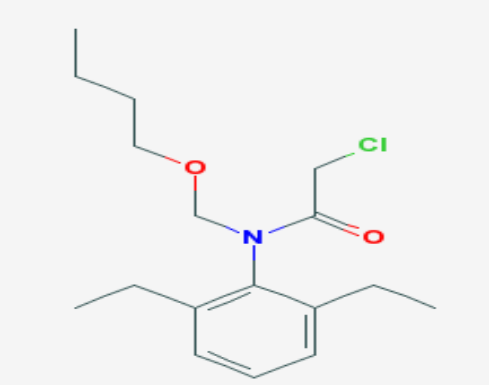

Figure 1: Chemical Structure of 2-chloro-2,6-diethyl-N-(Butoxy-methyl) ($C_{17}H_{26}ClNO_2$) A Thunder (weedicide) designed by Chemsketch.

Thunder showed a great reduction in growth parameters and nodule numbers as compared to control.These results are in support to the findings of Brock (1972),who while working with Trifluralin in loamy soil,observed decreased nodulation. Makawi et al (1971) have demonstrated that simazine reduces the nodulation in phaseolu vulgaris with increasing concentration

As UDPglucose:flavoniod glycotransferase had the best affinity score therefore it was selected from a list of 5 below listed proteins . therefore we can decipher form the results obtained that UDPglucose:flavoniod glycotransferase is essential protein in the metalobism of *Vigna mungo L.* and it is most affected by the thunder weedicide hence proving the results obtained in the wet lab this confirms that the action of thunder weed on the growth and development of *Vigna mungo L.* plant .

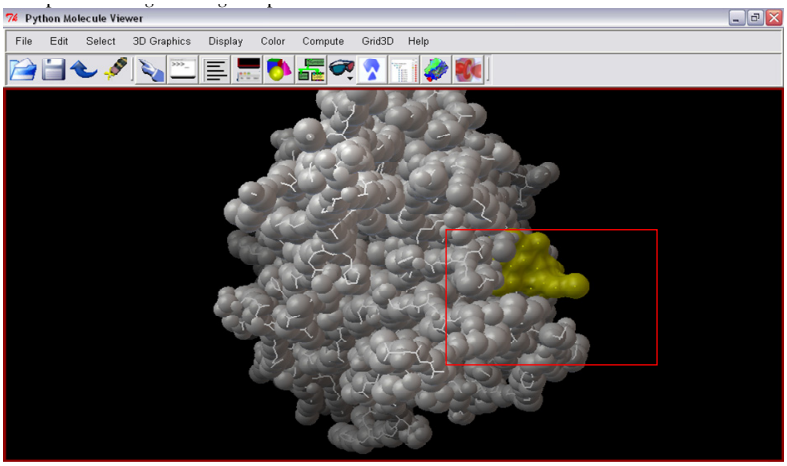

Figure 2: View of docking of ligand 2-chloro-2,6-diethyl-N-(Butoxy-methyl) (Thunder weediside) into the protein UDPglycose:flavoniod glycotransferase (an essential protein for metabolism in *Vigna mungo L.*) by -6.3 affinity in software PYTHON MOLECULER VIEWER.

Table 2: Binding affinity of ligand to five protein molecule which are essential to the metabolism of *Vigna mungo L.*

NCBI Accession number	Protein name	Affinity(kcal/mole)
ACZ57337	Annexin 1	-5.4
ADI58890	Catalase	-5.4
BAA36410	UDPglycose:flavoniod glycotransferase	-6.3
BAA36972	flavonoid3-0-galactosyl transferase	-5.0
BAA92495	Cysteine protease	-3.6

Conclusion

The Thunder (weedicide) in combination with moisture status undergoes acidification and reduces the soil pH, which in turn reduces the bacterial population responsible for root nodulation of plant *Vigna mungo L.* It is proved by in-vitro and in-silico approach in this practical. Therefore we can say that thunder (weedicide) is a chemical which affects the five essential protein of metabolism of the plant *Vigna mungo L.* (black gram).

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