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Research Article

BAVISTIN INDUCED ENHANCEMENT OF PAL AND PROLINE ACTIVITY OF *CAJANUS CAJAN* (L) MILLSP

[1]Bansod, P.G.

[1]Department of Botany, V.B.M.V. C.K. Naidu Road, Amravati 444602

ABSTRACT

Pesticides cause regular or sporadic damage to established vegetation within the vicinity of agricultural lands. For non-target plant species, there is sparse literature available about precise quantitative limits of tolerance. Therefore study was made to analyse the effect of pesticide Bavistin on some physiological aspects of *Cajanus cajan* (L) Millsp. For this The 24 hours presoaked seeds in different concentrations of bavastin solution (0.1 % and 0.2 %) in distilled water were used in experimentation, which were then transferred to germination trays. The 10 day old seedling of *Cajanus cajan* is used for the assay. Analysis was done on 11 day old seedling. Various parameters used for ascertaining pesticide stress to 11 day seedlings. The11 day seedlings were used to assay for ascertaining pesticide stress. The quantity of proline in the 11thday old seedling of *Cajanus cajan* (L) Millsp is shown to increase with increase in pesticide concentration.

Also the activity of enzyme PAL in 11th day old seedling of *Cajanus cajan* (L) Millsp treated with Bavistan increased continuously with increase in pesticide concentration.
Key Word- Bavistin, PAL, Proline, Abiotic stress, etc.

INTRODUCTION

In India, extensive use of the pesticides and insecticides in agriculture in recent years developed considerable interest in the study of their toxic effects on crop plants. Studies with many of them revealed inhibitory effects on percentage of seed germination and growth of crop plants.

Biochemically, seed germination requires the solublisation of stored polysaccharides. This is affected by *de nova* synthesis of amylase, which in turn is dependent on the embryonic growth and consequent release of gibberellic acid. *Cajnus cajan* (L.) is a commercial crop plant. In Vidarbha region of Maharashtra this crop is cultivated by farmer's at large scale. A large proportion of this crop grown today is cultivated in areas with less rainfall that obtain the water from irrigation (Lawal, 2012). The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others. Pesticide generally indicates any chemical, microbial agent or their mixture used as active ingredients of products for the control of crop pests and diseases, animals, ectoparasites and pests in public health. Modern agricultural practices have introduced numerous pesticides, bactericides, insecticides, fungicides, biocides, fertilizers and manures resulting in severe biological and chemical contamination of land.

Bavistin® DF is a fungicide for the control of fungus diseases in apples, beans, cereals, citrus, field tomatoes, lettuce, stone fruit, and the causal organism of facial eczema. It Contains 500 g/kg carbendazim in the form of a water dispersible granule. Chemical Group: Benzimidazole (MBC). BAVISTIN DF is a systemic fungicide with protectant and eradicant activity. It is recommended for the control of specific diseases in a range of crops. It is absorbed by the plant and transported to its apex by the sap stream in an upward and outward direction. It acts on fungal pathogens by inhibiting the development of germ tubes, the formation of appressoria and the growth of mycelia (Sammaiah et al., 2011).

It is a dry flow able formulation to be mixed with water for application as a spray. The addition of a spreader sticker is recommended for spraying hard-to-wet plants. It is compatible with most commonly used pesticides. Do not mix with strongly alkaline materials such as Bordeaux mixture and lime sulphur. It may be applied through all conventional ground and aerial spraying equipment. Dilution rates given are for high volume spraying and should be increased accordingly for concentrate application.

The cultivation of the pigeon pea goes back at least 3,500 years. The centre of origin is the eastern part of peninsular India, including the state of Odisha, where the closest wild relatives (*Mansi*) occur in tropical deciduous woodlands. Archaeological findings of pigeon pea include those from two Neolithic sites in Odisha, Gopalpur and Golbai Sassan dating between 3,400 and 3,000 years ago, and sites in South India, Sanganakallu and Tuljapur Garhi, also dating back to 3,400 years ago. From India it travelled to East Africa and West Africa. There, it was first encountered by Europeans, so it obtained the name Congo Pea. By means of the slave trade it came to the American continent, probably in the 17th century.

Cajanus cajan (L) Millsp. (Sanskrit: Adhaki, Hindi: Arhar, English: Pigeon pea, Bengali: Tur) (Family:Fabaceae), subfamily: *Faboideae* , tribe: *Phaseoleae* , subtribe: *Cajaninae* is the most important grain legume crop of rain-fed agriculture in semi-arid tropics. Except under cold conditions, laboratory studies have revealed a broad optimum temperature range (19-43ºC) for germination, with the most rapid seedling growth occurring between 29 and 36ºC. Germination is hypogeal, and the cotyledons remain underground. Under suitable field conditions the seedlings appear above the ground in about 5-6 days. It is a perennial, rarely annual, erect bush, 0.5 to 4 m, or creeper, or climber, strong or weak. Stem is ribbed, up to 15 cm diameter, show enormous secondary growth and become woody with age. (Ahmad et al., 2008)

A lot of work has been done on the role of pesticides in providing protection to plants against weeds in terms of crop yield. Only a little work has been established on the role of pesticides in affecting biochemical characteristics of the plant. Some of the pesticides are reported to be beneficial for plants growth if used at their lower concentration but becomes phytotoxic at their higher dose and to the change in the activity of some useful soil micro organisms. By repeated and extensive application of pesticides, it ultimately reaches the plant body and soil, which in turn may interact with plant growth and with soil organism and their metabolic activities. Pesticides have been shown to decreases the soluble Protein content in many plants, viz., Bavistin and

Monocrotophos in Trigonella and Xenobiotics in Sunflower. Janardhan (1988) and Noviel (1989) also reported Butachlor inhibit protein synthesis during shoot emergence. Studies on Mugbaen and *Vigna radiate* seedling germinated in different concentrations of Kitazin, showed that Protease activity was also affected by Kitazin (Paul and Mukherji and Murthy and Rao). Numerous researchers have reported on the adverse affects of pesticides on the germination and growth of the crop plants. Agriculture is the main destination for chemicals (Satake et. *al.,* 1997). Seeds are considered to be as a suitable host to maintain the pathogenic microorganisms even in the absence of the host. Treating such seeds with the fungi or bactericides will protect them from being attacked by fungi, nematodes or other pests Treating vegetable crops seeds with fungicides will protect them against. Oil-borne fungi which could cause diseases, especially root-rot (Buss *et. al*., 2001).

Phenylalanine ammonia-lyse (PAL) catalyses non oxidative deamination of L-phenylalanine to form trans-cinnamic acid and an equimolar amount of ammonium ion. In most system studies, accumulation of phenylpropanoid compounds under stress conditions is the result of increased PAL activity (Maldonado *et.al,* 2002).

PAL, as the bridge between primary metabolism and natural product biosynthesis, is a potential site for pathway regulation, and indeed PAL mRNA and enzyme levels are highly regulated spatially and temporally, associated with the tissue specific accumulation of phenylpropanoid products. The phenylpropanoid pathway leads to the biosynthesis of a wide range of plant natural products including flavonoids, hydroxycinnamic acids, coumarins, stilbenes, ligin and condensed tannins, which collectively have diverse biological functions as phytoalexins, signal molecules, structural components, flower pigments or UV protectants. PAL (L-phenylalanine ammonialyase plays a crucial role at the interface between plant primary and secondary metabiolism by catalyzing the deamination of L-phenylalanine to form trans-cinamic acid. The properties, regulation, expression and cellular distributions of PAL have been extensively studied and the crystal structures of PAL from parsley and the yeast *Rhodosporidiumtoruloides*, have been determined. (Kao et al, 2002).

Plants accumulate different types of organic and inorganic solutes in the cytosol to lower osmotic potential thereby maintaining cell turgor (Rhodes and Samaras, 1994). Under drought, the maintenance of leaf turgor may also be achieved by the way of osmotic adjustment in response to the accumulation of proline, sucrose, soluble carbohydrates, glycinebetaine and other solutes in cytoplasm improving water uptake from drying soil. The process of accumulation of such solutes under drought stress is known as osmotic adjustment which strongly depends on the rate of plant water stress. Wheat is marked by low level of these compatible solutes and the accumulation and mobilization of proline was observed to enhance tolerance to water stress (Nayyar and Walia, 2003). Of these solutes, proline is the most widely studied because of its considerable importance in the stress tolerance. Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce injury to cells.

Proline accumulates in many plant species in response to environmental stress. Although much is now known about proline metabolism, some aspects of its biological functions are still unclear. Here, we discuss the compartmentalization of proline biosynthesis, accumulation and degradation in the cytosol, chloroplast and mitochondria. We also describe the role of proline in cellular homeostasis, including redox balance and energy status. Proline can act as a signalling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress. Although the regulation and function of proline accumulation are not yet completely understood, the engineering of proline metabolism could lead to new opportunities to improve plant tolerance of environmental stresses (La´ szlo and Arnould, 2009).

Therefore the study is under taken to analyze the effect of various concentrations of Bavistan on various physiological and molecular parameters of *Cajanus cajan.* The present work revealed the effects of pesticides on germination of crop plant *Cajanus cajan.*

MATERIALS AND METHODS

Cajanus cajan seeds used for the present work was collected from krishikendra amravati. An assessment of in Vitro response of *Cajanus cajan* to pesticde stress was done on the basis of the pattern of response under in vitro condition. The 24 hours presoaked seeds in different concentrations of bavastin solution (0.1 % and 0.2 %) in distilled water were used in experimentation, which were then transferred to germination trays. The 10 day old seedling of *Cajanus cajan* is used for the assay.

Analysis was done on 11 day old seedling. Various parameters used for ascertaining pesticide stress to 11 day seedlings. The11 day seedlings were used to assay for ascertaining pesticide stress. The analysis is carried out by following assay.

Protein Estimation: - (Bradford Method)

The assay is based on the ability of proteins to bind commassie brilliant blue G 250 and form a complex whose extinction co efficient is much greater than that of the free dye. A series of protein samples in the test tubes of concentrations were prepared. Experimental samples in 100µl of PBS were prepared. 5ml of diluted dye binding solution was added to each tube. The sample in the test tubes was mixed well for at least 5 minutes but not more than 30 minutes to develop the colour. The red turns blue when it binds to protein. The absorbance was recorded at 595nm by using spectrophotometer. A standard curve was plotted using the standard protein absorbance Versus concentration and the protein in the experimental sample was calculated by using standard curve.

ESTIMATION OF PROLINE

Proline is a basic amino acid found in high percentage in basic protein. Free proline is said to play a role in plants under stress conditions. Though the molecular mechanism has not yet been established for the increased level of proline. Many workers have reported several- fold increase in the proline content under physiological and pathological stress conditions. Hence, the analysis of proline in plants has become routine in pathology and physiology divisions of agricultural sciences (Bates, 1973). 0.5g of fresh plant tissue was homogenated in 10ml of 3% aqueous sulphosalicyclic acid. The homogenate was filtered through whatman no. 2 filter paper. The filterate was used for assaying the proline activity. 2ml of filtrate was added in a test tube containing 2ml of glacial acetic acid and 2ml ninhydrin. It was heated in the boiling water bath for 1h. Reaction was terminated by placing the tube in ice bath. 4ml toluene was added to reactions mixture and stirred well for 20-30 second. The toluene was separated and warmed to room temperature. The red colour intensity was measured at 520nm. A standard curve by running a series of standard with pure proline in a similar way was prepared. The amount of proline was determined in the test sample from the standard curve.

PHENYLALANINE AMMONIA LYASE ASSAY

Phenylalanine ammonia lyase (PAL) is responsible for the conversion of L-phenylalanine to trans-cinnamic acid. Cinnamic acid serves as a precursor for the biosynthesis of Coumarins, Isoflavoniods and lignin. First 0.5 ml of Borate buffer was taken in test tube in that 0.2 ml enzyme solution and 1.3 ml water was added. The reaction was initiated by addition of 1ml of L-Phenylalanine solution. Test tubes were incubated for 60 minutes at 32°C. The reaction was stopped by addition of 0.5 ml of 1M Trichloro acetic acid. Then after absorbance was measured at 290 nm.

Result and Discussion

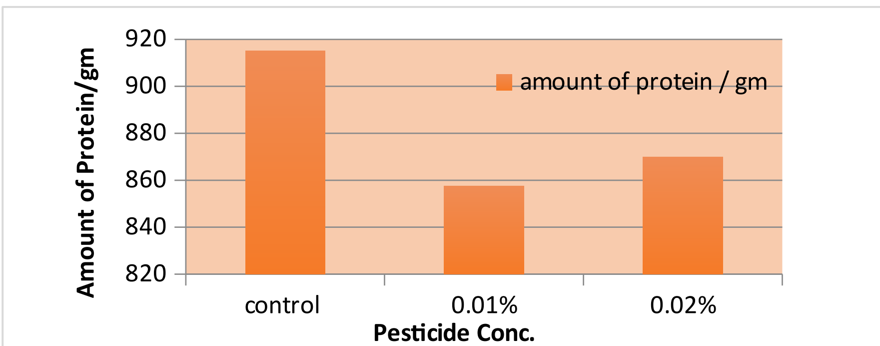

Diagram 1: Graphical sketch of amount of total protein per gm of tissue in 11 day old seedling of *Cajanus cajan* (L) Millsp

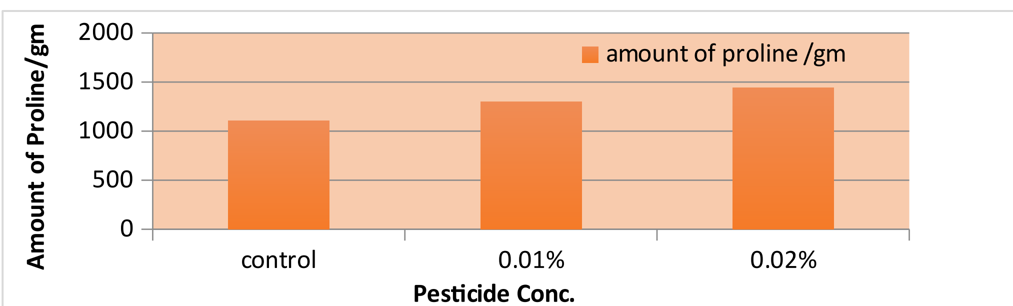

Diagram 2: - Graphical sketch of amount of total proline per gm of tissue in 11 day old seedling of *Cajanus cajan* (L) Millsp

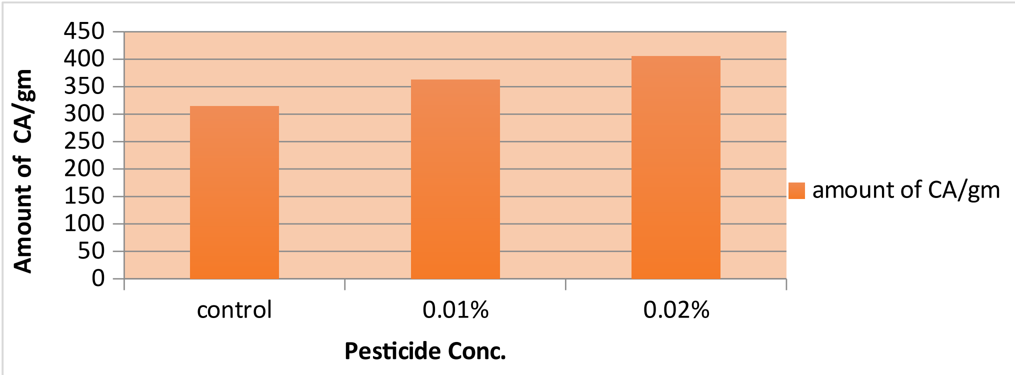

Diagram 3: Graphical sketch of transcinnamic acid amount of total cinnamic acid per gm tissue in 11th day old seedling of *Cajanus cajan* (L) Millsp

In the analysis the impact of pesticide stress on various physiological and molecular parameters on seedling of *Cajanus cajan* (L) Millsp have been studied. The outcome of the study reveals different observations and many conclusions can be drawn. Stress tolerance in plants has long been accepted as a multigenetic trait dependent on the coordinated expression of certain genes and silencing of others (Hare et el., 1996). The expression of these genes is influenced by multifarious environmental factors (Foolad, 2004). Changes in their expression can be detected by studying the protein pattern of expression.

The quantity of proline in the 11 thday old seedling of *Cajanus cajan* (L) Millsp is shown to increase with increase in pesticide concentration. For a long time, proline was considered as an inert compatible osmolyte that protects sub cellular structures and macromolecules under osmotic stress (Csonka and Cress., 1997 and Kavi Kishor et al., 2005). Proline has been shown to function as a molecular chaperone able to protect protein integrity and enhance the activities of different enzymes. Examples of such roles include the prevention of protein aggregation and stabilization of M4 lactate dehydrogenase during extreme temperatures (Rajendrakumar et al., 1994) protection of nitrate reductase during heavy metal and osmotic stress (Sharma and Dubey, 2005), and stabilization of ribonucleases and proteases upon arsenate exposure. Several studies have attributed an antioxidant feature to proline, suggesting ROS scavenging activity and proline acting as a single oxygen quencher (Smirnoff and Cumbes., 1989; Matysik, J. et al. 2002). Proline treatment can diminish ROS levels in fungi and yeast, thus preventing programmed cell death (Chen and Dickman., 2005), Proline pretreatment also alleviated Hg2+ toxicity in rice (Oryza sativa) through scavenging ROS, such as H_2O_2 (Wang, F. et al., 2009). Our study revealed the same increase in proline amount after pesticide stress imposed on the plant.

Proline is considered to act as an osmolyte, a ROS scavenger, and a molecular chaperone stabilizing the structure of proteins, thereby protecting cells from damage caused by stress (Verbruggen and Hermans, 2008) Proline accumulates in many plant species in response to different environmental stresses including drought, high salinity, and heavy metals. Proline levels are determined by the balance between biosynthesis and catabolism (Szabados and Savoure, 2010). Proline is produced in the cytosol or chloroplasts from glutamate, which is reduced to glutamate-semialdehyde (GSA) by D-1-pyrroline-5-carboxylate synthetase (P5CS). GSA can spontaneously convert to pyrroline-5-carboxylate (P5C), which is then further reduced by P5C reductase (P5CR) to proline. Proline is degraded in mitochondria by proline dehydrogenase (ProDH) and P5C dehydrogenase (P5CDH) to glutamate. Stress conditions stimulate proline synthesis while proline catabolism is enhanced during recovery from stress. Overexpression of P5CS in tobacco and petunia led to increased proline accumulation and enhanced salt and drought tolerance (Yamada et al., 2005). Over-expression of Arabidopsis d-OAT has been shown to enhance proline levels and to increase the stress tolerance of rice and tobacco (Roosens et al.,2002).

It has been analyzed that in a given study that the activity of enzyme PAL in 11^{th} day old seedling of *Cajanus cajan* (L) Millsp treated with Bavistan increased continuously with increase in pesticide concentration. Hura *et al*., (2007) indicated that determination of PAL enzyme activities should be explained during the stress period to obtain a better understanding of how resistant and sensitive varieties differ in their response. In another experiment, (Hura *et al*., 2008) also reported a correlation between PAL activity and phenolic compounds in leaves of hybrid maize in drought stress and considered the accumulation of phenolic compounds as the indication of activated defense reaction in the drought resistance of that genotype. Transformation of tobacco with bean PAL2 gene, modified by the inclusion of cauliflower mosaic virus 35s enhances sequences in its promoter, generates transgenic plants with severely reduced PAL activity and correspondingly lower levels of phenylpropaniod products (Elkind et al., 1990). The pal activity as earlier reported mainly enhances after the pathological infection with various microbes. From the present analysis it can be concluded that pal activity also increase due to pesticide stresses.

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