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Research Article

EVALUATION OF THE EFFECT OF BAVISTIN ON SOME PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF SPINACH

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ABSTRACT

Pesticide contamination in aquatic ecosystem is one of the reasons for global environmental concern. With the increased use of pesticides different physiological and biochemical problems are induced in crop plants. In order to know the stress response of Spinach plant against pesticide, spinach plant was treated with different concentrations of Bavistin and the impact was analyzed on the basis of physiological parameters like proteins, proline and PAL. A SDS- PAGE analysis was made to evaluate the protein profile of spinach plant after pesticide treatment. It was observed that proline and PAL activity increases considerably with pesticide concentrations.

Key words- Spinach, SDS- PAGE, proline, PAL, Bavistin etc.

INTRODUCTION

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 (Sammaiah et al., 2011)

Pesticides are the modern tools to the farmers to control pests, diseases and weeds and to increase crop yields. But they have to be applied on crops at proper times, uniformly and efficiently. Pesticides are used in agriculture mainly for the purpose of increasing plant productivity. 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. (Zamin et al., 2006)

Pesticide-induced oxidative stress as a possible mechanism of toxicity has been a focus of toxicological research for the last decade. Yet for certain pesticides, mechanisms leading to oxidative stress are only partly understood. Pesticide-induced oxidative stress is the final manifestation of a multi-step pathway, resulting in an imbalance between pro-oxidant and antioxidant defence mechanisms. Concomitantly, pesticide intoxication induces a derangement of certain antioxidant mechanisms in different tissues, including alterations in antioxidant enzymes and the glutathione redox system (Krishnan, 2008).

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).

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, Isoflavonoids and lignin. These compounds play an important role in pest and disease resistance

mechanism. Changes in PAL activity accompanying fungal, viral, and bacterial infection of plants have been reported.

Therefore the study is under taken to analyse the effect of various concentrations of Bavistan on various physiological and molecular parameters of *Spinacia oleracea* L. The present work revealed the effects of pesticides on germination of crop plant *Spinach oleracea*.

MATERIALS AND METHODS

Spinacia oleracea L. seeds used for the present work was collected from krishikendra Amravati. An assessment of in Vitro response of *Spinacia oleracea* L. to pesticide stress was made 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 *Spinacia oleracea* L. is used for the assay.

Analysis was done on 11 day old seedling. Various parameters used for ascertaining pesticide stress to 11th day seedlings. The 11 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.

Sodium dodecyl sulphate polyacrylamide slab gel electrophoresis (sds-page) for protein profiling assay (Laemellae

Electrophoresis is widely used to separate and characterized proteins by applying electric current. Electrophoresis procedure are rapid and relatively sensitive requiring only micro-weight of proteins.

Electrophoresis in the polyacrylamide gel is more convenient than in any other medium such as paper and starch gel. Electrophoresis of protein in the polyacrylamide gel is carried out in buffer gels (non-denaturing) as well as in sodium dodecyl sulphate (SDS) containing (denaturing) gel. Separation in buffer gel relies on both the charge and size of protein where as it depends only upon the size in the SDS gels. Analysis and comparison of protein in the large number of sample is easily made on polyacrylamide gel slabs.

ESTIMATION OF PROLINE

During selective extraction with aqueous sulphosalicylic acid, proline is precipitated as a complex. Other interfering materials are also presumably removed by adsorption to the protein sulphosalicylic acid complex.

The extracted proline is made to react with ninhydrin in acidic conditions (pH 1.0) to form the chromophore (red colour) and read at 520 nm.

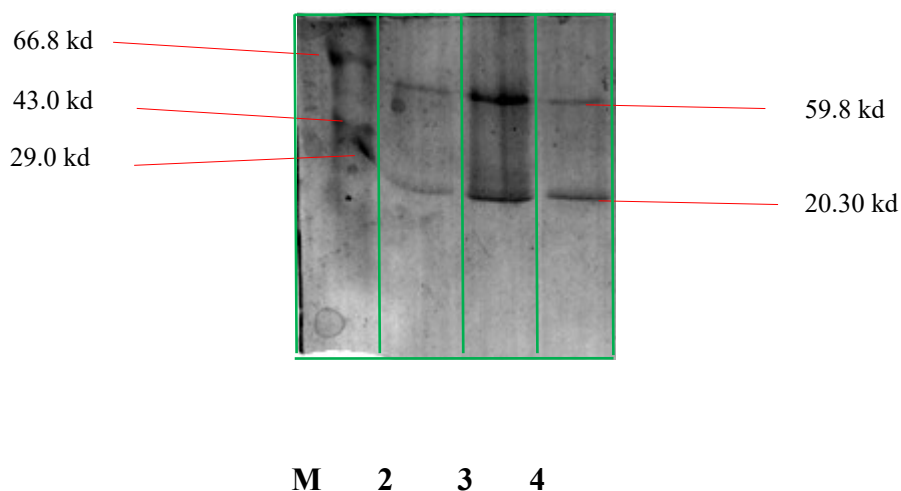
PHENYLALANINE AMMONIA LYASE ASSAY

Phenylalanine ammonia lyase activity is determined spectrophotometrically by following the formation of trans-cinnamic acid which exhibits an increase in absorbance at 290 nm (Crude enzyme)/ 270nm (Purified enzyme).

OBSERVATION AND RESULT

SDS-PAGE Electrophoresis for protein

Seedlings of *Spinacia oleracea L.* treated with various pesticide concentration treatments. The total protein were extracted from seedlings of control and treated with 0.01% and 0.02% Bavistan. This protein was analysis was performed on 11 day old seedlings. The bands of various molecular weights were observed. The protein bands of 0.01%, 0.02% and control were observed and compared with standard marker When SDS-PAGE electrophoresis was carried out, the gel after staining showed result as shown in photo plate.



Digramatic representation of SDS-PAGE of 11 day old seedling of *Spinacia oleracea* L.

From left to right:- Lanes M-Shows the bands of standard protein marker., Lane 2- Shows the bands of control., Lane 3-Shows the bands of 0.01%., Lane 4-Shows the bands of 0.02%

Effect of various concentrations of Bavistan on seedlings of *Spinacia oleracea* as analysed on SDS-PAGE. Lanes 2,3, and 4 from left to right represent proteins extracted from control, 0.01%, & 0.02% Bavistan treated seedlings of 11 day old and Lane M represents molecular marker. As visualized from SDS-PAGE intensity bands of molecular weight increases in temperature. The total protein content

Protein assay for 11 day old seedling of *Spinacia oleracea* L.

Diagram 1: Graphical sketch of amount of total protein per gm of tissue in 11 day old seedling of *Spinacia oleracea* L

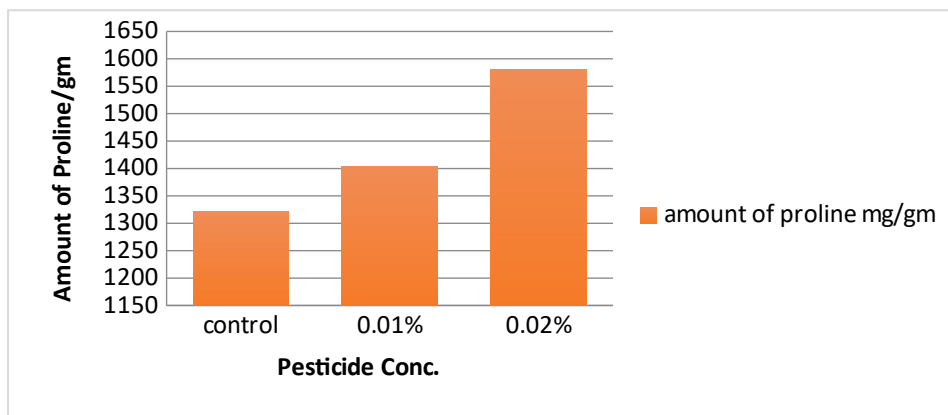
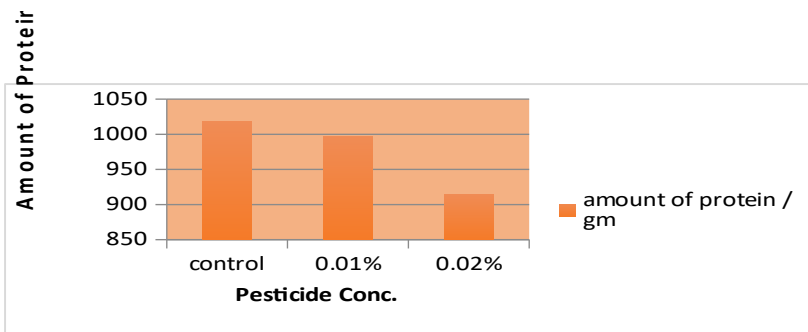


Diagram 2: - Graphical sketch of amount of total proline per gm of tissue in 11 day old seedling of *Spinacia oleracea L.*

PAL assay

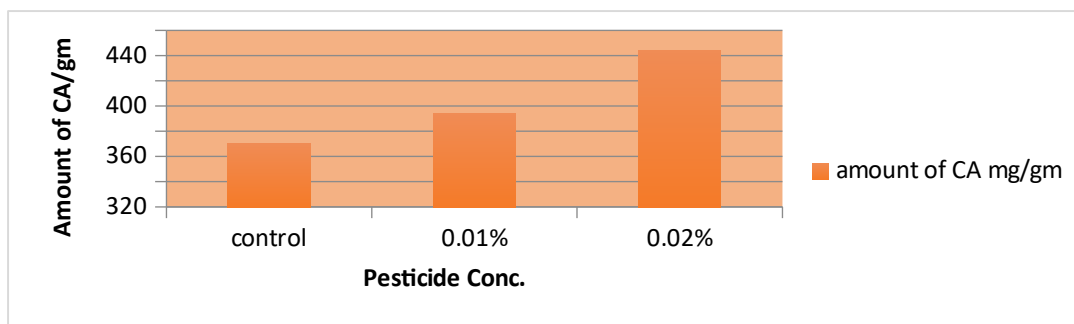


Diagram 3: Graphical sketch of transcinnamic acid amount of total cinnamic acid per gm tissue in 11th day old seedling of *Spinacia oleracea L.*

In the analysis the impact of pesticide stress on various physiological and molecular parameters on seedling of *Spinacia oleracea L* have been studied. The outcome of the study reveals following observations. The quantity of proline in the 11th day old seedling of *Spinacia oleracea L* 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; Hare & Cress 1997). 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) Proline treatment can diminish ROS levels in fungi and yeast, thus preventing programmed cell death (Chen and Dickman., 2005)

It has been analyzed that in a given study that the activity of enzyme PAL in 11th day old seedling of *Spinacia oleracea 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.

The pal activity as earlier reported mainly enhances after the pathological infection with various microbes. From the present analysis one can conclude that pal activity. 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. 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 fungi or bactericides will protect them from being attacked by fungi, nematodes or other pests (Buss *et. al.*, 2001)

Treatment with pesticide Endosulfan individually and in combination with Kitazin for different concentrations and durations were studied by D. Sammaiah *et al.*, (2011) on seed germination, seedling growth, number of lateral roots, fresh and dry weights of *Solanum melongena*

L. (Brinjal). A part from effect on early seedling growth the present investigation was also extended to study some enzymes such as Amylase, Protease and Protein content were studied in 48h germinating seeds. The effect of pesticides on various attributes studied was varied with level of concentrations and durations.

Pesticides are used in agriculture mainly for the purpose of increasing plant productivity. 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. In the present study, it was observed that pesticides individually and in combination might affect the enzyme activities.

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