



Morphological and Biochemical Changes in Mung Plant (*Vigna radiata* (L.) Wilczek): Respond to Synthetic Pesticide & Biopesticide

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Abstract – This experiment was designed with two treatments of synthetic pesticide & biopesticide. Laboratory experiment was carried out to investigate the effect of different concentration levels of dimethoate & neem extracts (10, 50, 100) mg^l on germination and radical length of mung plant. Seeds after 3 days of germination & 19 days old seedlings were exposed to same concentration of dimethoate & neem extracts through foliar spray. Analysis were done using F-test to find out the effect of synthetic pesticide & biopesticide on leaf area, shoot length, chlorophyll, protein, amino acid, carbohydrate & amino acid content of mung plant. 62.5 to 72% germination takes place in control & seed treated with biopesticide but 50% in seed treated with pesticide. Radical length showed maximum increase in biopesticide treated seed as compared to synthetic pesticide treated seed. Foliar application of biopesticide significantly improved the shoot length by 10.85 cm & leaf area by 5.46 cm² as compared to synthetic pesticide treated plant. Further increase in insecticide level had a negative impact upon biochemical characters studied. There is significant increase in protein & carbohydrate content of mung plant treated with biopesticide where as decrease in synthetic pesticide treated plant. The result also shows increase in chlorophyll & amino acid content in biopesticide treated plant but no significant difference found between the biopesticide & synthetic pesticide treated plant. The doses of biopesticide proved more efficient than doses of pesticide. Among these, Neem treated plant exhibit strong morphological and biochemical parameters to suppress pesticide treated plant.

Keywords – Neem Extract, Dimethoate, Chlorophyll, Carbohydrate, Protein, Amino Acid.

I. INTRODUCTION

Agriculture is the backbone of Indian economy. 70% of the population is engaged in farm sector directly or indirectly. Ever growing Indian population needs sufficient farm produce. So in recent years the importance of sustainable agriculture has risen to become one of the most important issues in agriculture. Increase in agricultural production is the key to all-over economic growth. In addition plant diseases continue to play a major limiting role in agriculture production. The control of plant disease using classical pesticides raises serious concern about food safety, environmental quality and pesticide resistance, which have dictated the need for alternative pest management techniques. The environment is currently under threat by the indiscriminate use of synthetic pesticides by the human activities and causing high risk to non-target organisms (Kumar et. al., 2010). In the search

for environmentally safe pesticides, much research has been done on the use of plants for the protection of crops in the field or in storage.

The pollution of the environment plays a crucial role in the occurrence of many diseases affecting plants, animals and man. One of the main factors causing pollution of the environment is the irrational use of organophosphorus insecticides (Al-Haj et al., 2005). In view of the environmental problems caused by the use of synthetic chemicals and the growing need for alternative methods of pest control that minimize this damage, there has been extensive research on pest control by substances from plants (Wan et al., 1996). One of the most promising natural compounds is azadirachtin, whose antiviral, antibacterial and antifungal properties have been known for several years (Isman et al., 1990; Harikrishnan et al., 2003; ICAR, 1993). It is generally considered less harmful to the environment than other more commonly used pesticides (Mordue [Luntz] and Blackwell, 1993). Therefore, neem-based insecticides are being investigated as alternatives to synthetic insecticides for the control of agricultural insect pests.

The environment of the world has changed drastically & dramatically. Environment pollution has increased tremendously during this period. Green belt is decreasing regularly. It is due to explosion of population, industrialization, mechanization & colonization. We have enough natural resources to full fill our requirement but our greed & unplanned way of their use is creating problems. Hence it is now necessary to take precautionary measure from the very beginning for saving this wonderful planet. Plant protection activity primarily follow the principle “**loose less feed more**” adopting prophylactic as well as curative measures of pest control causing little or no deleterious effect to man, non target organisms & the environment as a whole.

II. AIM & OBJECTIVE

II(a). Aim of the study: -

The aim of the present study is to use the plant extracts such as neem as bio pesticides which is the alternative means of pest control, which can minimize the use of synthetic pesticides like organophosphorous.

II(b). Objective:-

Monitor crops regularly to check the levels of pest populations & their damage. To know how much the pesticides are harmful to us particularly as pests of crops



and stored grain. Implement available non-chemical control practices. Maximize the benefits of naturally occurring biological controls by using bio-pesticides. To investigate the Morphological and Biochemical alteration in Mung plants responses to biopesticide & chemical pesticide. To compare the toxic effect of Biopesticide and Chemical pesticide.

III. MATERIALS & METHODS

III(A). Test species

III(a). Mung Plant:-

Mung plant was taken as test species. Pulses are the major sources of dietary protein in the vegetarian diet in our country. Besides being a rich source of protein, they maintain soil fertility through biological nitrogen fixation in soil and thus play a vital role in furthering sustainable agriculture. Mung bean performs best in fertile, well-drained sandy loam soil with a pH between 6.2 and 7.2 and will suffer in poorly-drained, heavy soils. An important feature of the mung bean crop is it has the potential of producing higher yield depending on the genotypes studied (Ullah et al., 2011).

III(B). Test chemical(s)

III(a). Biopesticide:-

The test chemical, Neem oil is used as biopesticide. It is a vegetable oil pressed from the fruits and seeds of the neem. This is the by-products of plant metabolism and is commonly referred to as volatile plant secondary metabolites. Neem seeds are very rich in fatty acids amounting to about 50 % of seed weight, like oleic acid, stearic acid, palmitic acid (Sindhu et al.,2004), & its oil contain some basic amino acids (Skellon et al.,1962). The commercial neem extract as the test biopesticide was purchased from the local market. It was prepared manually by taking out the neem oil extract from the neem kernel in the Gaisilet village of Bargarh District, Odisha.

III(b). Synthetic Pesticide:-

Dimethoate is used as synthetic pesticide. The chemical was purchased from Rallis Tata enterprises, CAS No. AK00612. Its chemical composition is dimethoate tech-35.500% w/w, Epichlorohydrin- 1.000% w/w (stabilizer), Emulsifier- 8.000% w/w(non-ionic-poly oxy ethylene ether), Aromax-10.500% w/w, xylene-30.000%, cyclohexanone-15.000% w/w.

III(C). Plant material & growth condition:-

The experiment was conducted in Sambalpur University, Dept of Env.sc in the period of September to December. Dimethoate & mung bean (*Vigna radiata* (L.)Wilczek) seeds were obtained from local market. Healthy and fresh seeds were used to avoid any pre storage infestation or egg laying of bruchids. The mung seed were soaked in water for 24 hours. After a day 8 numbers of seed was put in each petridish using cotton for analysis of germination. Pesticide & biopesticide of 10 mg^l⁻¹, 50 mg^l⁻¹ & 100 mg^l⁻¹ was applied except one petridish for control. Sowing was also done in field. The experimental area is divided into seven plots. Initially 30seeds of mung bean were sown in each plot. During the period of experiment, temperature was maintained at 22^oC

to 25^oC and irrigation was done based on plant requirements. All these foliar treatments were applied began just after the first leaflets were fully expanded except with control plants. All measurements were carried out after two days of application of pesticide. Plant height (cm), Leaf area (cm²) of plant was also calculated. Average leaf length and width was determined from 4 or 5 randomly selected leaves.

III(D). Morphological parameters:-

Morphological measurements were taken after application of pesticides. The experimental plots were maintained weed free. The following parameters were measured for at least 3-4 plants per plot. The heights of the plants were measured continuously during the period of experiment from the soil surface to apical tip. For germination 8 seeds per petri dish were calculated in all variables. Each radical length measured after 2 days of germination. Height of young shoots was measured on 19th days after germination. Photosynthetically active area of the plants was measured by recording the length of the leaves from the petiole to tips & width at its widest point. The product is multiplied by constant to find out the actual leaf area.

III(E). Biochemical analysis:-

Chlorophyll Pigment (chl-a, chl-b, total chl) were measured by the method by Arnon (1949). The amount of chlorophyll was expressed as mg/g fresh weight of leaf tissue, according to the formula of Witham et al (1971). Protein was estimated by Folin's Lowry Method. Total carbohydrate content was estimated by Anthrone method (Yemm & Wills) (1954). Amino acid was estimated by ninhydrin method (Moore & Stein) (1948).

IV. RESULT

IV(A). Result

Each experiment was repeated three times. The data are expressed as mean \pm SD (n=3). The data were analysed by F- test using MS Excel software.

IV(a). Morphological analysis :-

The effect of synthetic pesticide & biopesticide on the germination of seeds of mung plant after 48 hrs of the beginning process of germination was observed. The germination rate in 10 mg^l⁻¹, 50 mg^l⁻¹ & 100 mg^l⁻¹ of seed treated with synthetic pesticide was found to be 50 % where as in biopesticide treated seed it was found to be 62.5 % Fig [1]. It was noticed that the germination percentage of biopesticide treated mung seed was more than synthetic pesticide treated seed. No significant differences between the treatment were noticed. The high values of means of germination (72%) occurred on the distilled water at a control treatment, where as the low values of means occurred at high concentration of synthetic pesticide. A gradual decrease with increasing pesticide concentration was noticed.

Pesticides treatment in mung plant, shows the effects in the radical length as compared to biopesticide treated in mung plant. After 3 days the maximum radical length was observed in plant treated with biopesticide such as 2.74 cm in 10 mg^l⁻¹, 2.52 cm in 50 mg^l⁻¹, 1.54 cm in 100 mg^l⁻¹as



compared to 1.22 cm in control & synthetic pesticide treated plant such as 0.43 cm in 10 mg^l⁻¹, 0.4 cm in 50 mg^l⁻¹ & 0.3 cm in 100 mg^l⁻¹ Fig [2]. The difference in radical length in synthetic pesticide as well as biopesticide treated plant is significant subjected to F test. (FC=0.33>Ft=0.10).

Leaf area in mung plant treated with control & biopesticide showed maximum as compared to mung plant treated with synthetic pesticide. The leaf area in control plant was observed as 5.46 cm². The leaf area of the plant treated with biopesticide was observed as 5.37 cm² in (10, 50 & 100) mg^l⁻¹ as compared to plant treated with synthetic pesticide the observed value was 2.59 cm² in 10 mg^l⁻¹, 2.16 cm² in 50 mg^l⁻¹ & in 100 mg^l⁻¹ the plant totally die Fig [3]. The difference in leaf area in pesticide as well as biopesticide treated plant is significant subjected to F test. (FC= 2489.59>Ft=9.27).

From the result obtained in this present study showed that the growth of the plant with regards to seedling growth was effected due to the application of chemical pesticide in comparison to control & biopesticide treated mung plant. The height of the plant grown in control condition was recorded as 9.9 cm. The height of the plant treated with synthetic pesticide was observed as 5.2 cm in 10 mg^l⁻¹, 5 cm in 50 mg^l⁻¹ and at the concentration of 100 mg^l⁻¹ the plant totally die. In contrast the height of the plant treated with biopesticide was found as 10.85 cm in 10 mg^l⁻¹, 10.82 cm in 50 mg^l⁻¹, 10.7cm in 100 mg^l⁻¹ Fig [4]. The length of mung plant shows enhanced growth in biopesticide as compared to synthetic pesticide. Subjected to F test the height of the plant shows significant difference between synthetic pesticide treated plant & biopesticide treated plant. (FC = 80.84>Ft =9.27).

IV(b). Biochemical analysis:-

The chlorophyll-a, chlorophyll-b & total chlorophyll content in control set was found as 0.28g, 0.21g, 0.51g. In case of synthetic pesticide treated plant the chlorophyll-a contain 0.12g, chlorophyll-b contain 0.03g & total chlorophyll contain 0.15g in 10 mg^l⁻¹, 0.1g chlorophyll-a, 0.05g chlorophyll-b, 0.13 g total chlorophyll contain in 50 mg^l⁻¹ & the plant totally die when 100 mg^l⁻¹ of pesticide was applied whereas biopesticide treated plant contain chlorophyll-a 0.25g, chlorophyll-b 0.14g, chlorophyll-c 0.38g in 10 mg^l⁻¹, chlorophyll-a 0.20 g, chlorophyll-b 0.09 g, total chlorophyll 0.28g in 50 mg^l⁻¹, chlorophyll-a 0.15g, chlorophyll-b 0.06g & total chlorophyll 0.22g in 100mg^l⁻¹. Chlorophyll contents shows maximum in control & in mung plant treated with biopesticide but decreases in case of synthetic pesticide Fig [5]. The seedlings treated with dimethoate & neem extracts showed no significant difference in chlorophyll content in mung plant.

Protein content was showed inhibitory effect when treated with synthetic pesticide where as plant treated with biopesticide, protein content was more than control. Protein content in control was 1.43g, in biopesticide treated plant protein content was 1.83g in 10 mg^l⁻¹, 1.55g in 50 mg^l⁻¹, 1.50g in 100mg^l⁻¹, where as in synthetic pesticide treated plant protein content was found as 1.03g in 10mg^l⁻¹, 0.66g in 50 mg^l⁻¹ & total plant die when 100 mg^l⁻¹ of

synthetic pesticide was applied Fig [6]. Protein content in the plant is significant between the plants treated with pesticide & biopesticide when subjected to F- test. (Fc =12.00>Ft =9.27).

Amino acid showed decrease in amount in plant treated with synthetic pesticide where as there is increase in amount when treated with biopesticide. From present study it was found that plant treated with biopesticide, amino acid content was more than control & pesticide treated plant. In control plant amino acid content was 0.048g, in biopesticide treated plant amino acid content was 0.062g in 10 mg^l⁻¹, 0.068g in 50 mg^l⁻¹, 0.061g in 100 mg^l⁻¹ where as in synthetic pesticide treated plant amino acid content was 0.021g in 10 mg^l⁻¹, 0.019g in 50 mg^l⁻¹ & total plant die in 100 mg^l⁻¹ of pesticide Fig [7]. No significant difference found in plant treated with synthetic pesticide & plant treated with biopesticide.

There is decrease in the carbohydrate content in mung plant treated with synthetic pesticide as compared to biopesticide treated mung plant. Following results were found from the present study as 0.63g of carbohydrate content in control, carbohydrate content in synthetic pesticide treated plant showed 0.39g in 10 mg^l⁻¹, 0.1g in 50 mg^l⁻¹ & total mortality in 100 mg^l⁻¹ where as in biopesticide treated plant carbohydrate content was found as 0.69g in 10 mg^l⁻¹, 0.65g in 50 mg^l⁻¹, 0.60g in 100 mg^l⁻¹ Fig [8]. Significant difference found between synthetic pesticide & biopesticide, subjected to F- test. (Fc = 57.40>Ft =9.27).

V. DISCUSSION

Plant growth analysis is a necessary step in understanding the plant's performances and productivity. The data obtained were analysed statistically using F test. The present study showed total mortality in 100 mg^l⁻¹ after 2 days of treatment with dimethoate pesticide. The lower concentration of biopesticide significantly proved highly effective & seemed to be elevating the growth parameters. At higher concentration & plant treated with synthetic pesticide such as dimethoate the growth parameter reduced in all the growth phase under study.

The present study shows the effect of seed treatment on the germination rate & radical length at different doses of application of synthetic pesticide & biopesticide. Exposure to synthetic pesticide reduced normal germination & also significantly reduced the radical length. My results are in consistence with the result of Bashir et al. (2007). They observed the effect of seed treatment by mancozeb on various morphological parameters in *Lens culinaris* L. in different developmental stage and concluded that all the morphological parameters increased significantly only at lower dose (0.1%) whereas a linear decrease with increasing concentrations of mancozeb was observed. In the experiment of Stevens et al. (2008), continuous exposure to imidacloprid (4 days at 2000 mg AI L⁻¹) significantly reduced normal germination rate in rice plant.

Suppression was noted in plant height under the higher concentration of applied pesticide. This experiment of effect of plant height was also experimented previously

which shows effect in plant height due to the retarded cell growth and division in cell elongation in shoot length observed by Warabi et al., (2001), . Another reason could be explained on the basis of inhibition in the activity of 4-hydroxyl phenyl pyruvate dioxygenase (HPPD), an enzyme needed for the growth and development of meristematic tissue as suggested by Luscombe et al. (1995) following insecticide isoxaflutole treatment in maize and sugarcane. Similar observation has also been made on cucumber by Mishra et al. (2008) under insecticidal treatment.

In the present study there is reduction of the chlorophyll content in the plant treated with synthetic pesticide as compared to biopesticide. Pesticide application resulted no significant difference in chlorophyll content. But reduction was found from the previous study which may be due to the inhibition of their biosynthesis or breakdown of pigments or their precursors as suggested for cowpea seedling under stress by insecticide dimethoate (Mishra et al., 2008). Kaushik and Inderjit (2006) found that mung bean grown in soil treated with herbicides showed continuous decrease in Chlorophyll content with increasing doses and concluded that most of the biochemical symptoms associated with pesticide toxicity were Chlorophyll degradation and activation of oxidation process.

The higher concentration of protein & amino acid occurred in plant treated with biopesticide where the low concentration was observed in plant treated with dimethoate. No significant differences were observed. But from the previous experiments on effect of protein in mung plant was observed. The depression of plant growth was due to the effect of salinity on the protein bonds of green pigments . It was found that the adverse relationship between salinity and growth, that high salinity affected the protein bonds of green pigments and caused acute decrease on the chlorophyll content (Rivera and Heras , 1973) .

It was also observed from the study that the carbohydrate content in the plant treated with pesticide is less as compared to biopesticide. Significant difference was found between the plant treated with synthetic pesticide & biopesticide.

VI. CONCLUSION

The present study revealed that seed germination and seedling growth of Mung bean seed was suppressed by treatment with different concentration of dimethoate as compared to different concentration of Neem extracts. It is thus clear from the result that mung plant treated with biopesticide brought about significant improvement in germination rate, radical length, plant height, chlorophyll, protein, amino acid, carbohydrate content of leaves of mung plant where as reduction in pesticide treated mung plant. There is also significant increase in protein & amino acid content in mung plant treated with biopesticide as compared to control treated Mung plant which can also be proved to be a good fertilizer. The doses of biopesticide proved more efficient than doses of pesticide. Among

these, Neem treated plant exhibit strong morphological and biochemical parameters to suppress pesticide treated plant. This Neem extracts can be used as important source of natural herbicides to control weeds in crop fields.

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GRAPHS

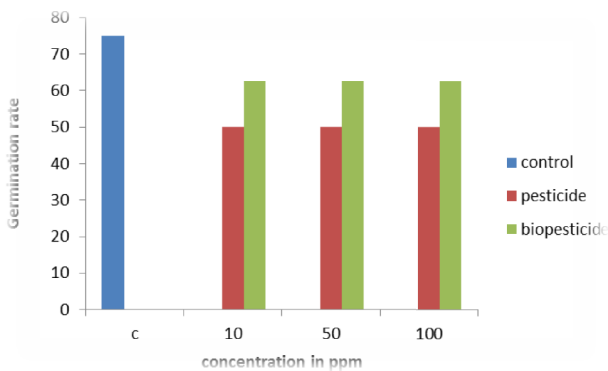


Fig.1. Germination percentage of mung plant treated with control, pesticide & biopesticide

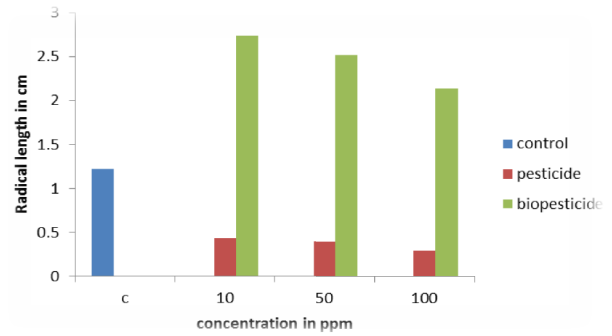


Fig 2: Radical length of mung plant treated with control, pesticide & biopesticide

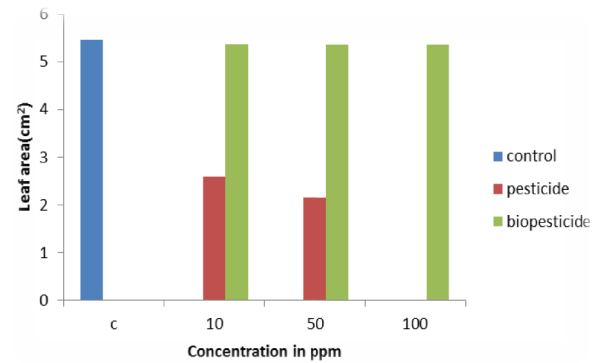


Fig 3: Leaf area of mung plant treated with control, pesticide & biopesticide

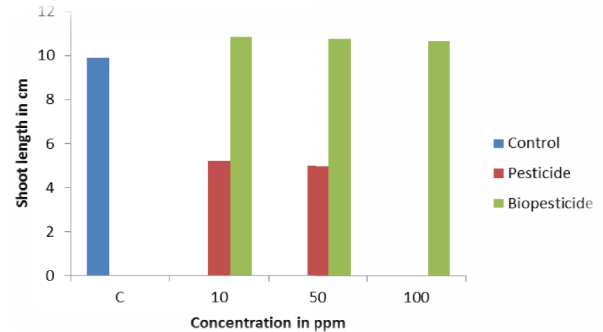


Fig 4: Shoot length of mung plant treated with control, pesticide & biopesticide

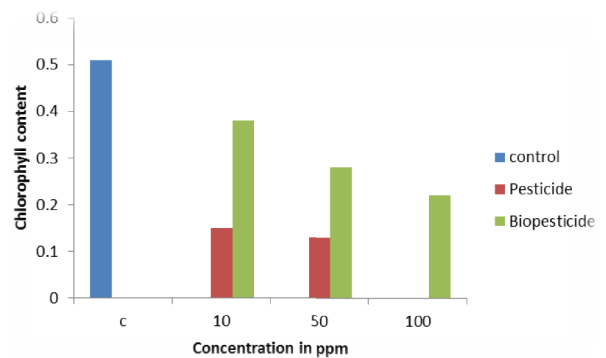


Fig 5: Chlorophyll content of mung plant treated with control, pesticide & biopesticide

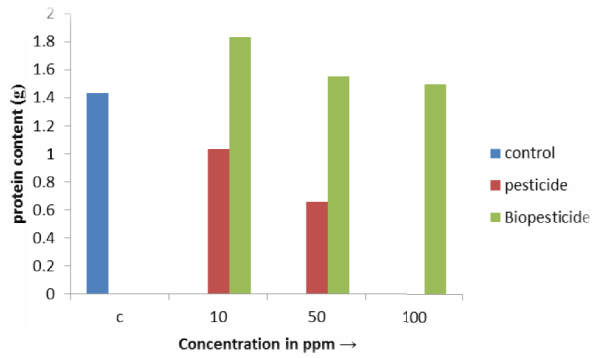


Fig 6: Protein content of mung plant treated with control, pesticide & biopesticide

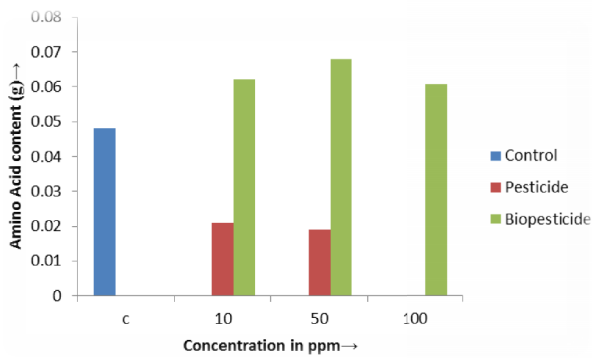


Fig 7: Amino acid content of mung plant treated with control, pesticide & biopesticide

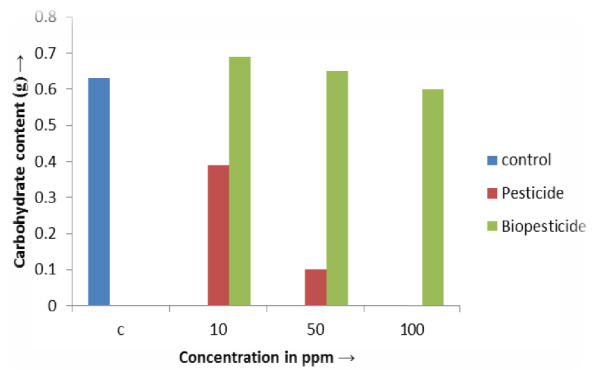


Fig 8: Carbohydrate content of mung plant treated with control, pesticide & biopesticide