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RESPONSES TO DIFFERENT DOSAGES OF MONOCROTOPHOS AND DELTAMETHRIN ON CHLOROPHYLL AND PROTEIN CONTENTS OF CHICKPEA

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ABSTRACT

Chickpea (*Cicer arietinum)* is a major legume food crop. Its seeds are rich in carbohydrates, protein, vitamin B, and other minerals. For better seed output and protein content, various factors viz; proper light conditions, and freedom from insect pests are necessary. Many insect pests play a damaging role in chickpea production and hence pest control is key to better production. This study was carried out at the experimental sites of Chhatrapati Shahu Ji Maharaj University, Kanpur, India to assess the responses to different dosages of monocrotophos pesticides on chlorophyll and protein contents in Chickpea. Different doses of monocrotophos viz., control, 0.5%, 1%, 1.5%, 2%, 2.5%, 5% 7.5%, and 10% were applied to seeds before sowing. Quantitative analysis of plants for chlorophyll content was done on a per-plant basis, The plant tissues were weighed and the analysis was done on an mgg^{-1} fresh weight basis. Chlorophyll content was estimated by extracting 80% acetone and determined spectrophotometrically and the quantification was done by the Arnon method (1949). The protein content was also estimated spectrophotometrically. The best results were obtained at 0.5% pesticide concentration. The total chlorophyll content was 1.662 mgg-1 for control and 1.671mgg-1 for 0.5% and decreased with the increasing concentration of monocrotophos pesticide. The protein content was highest, 28.41 mgg^{-1} at 0.5%, as compared to 27.45 mgg^{-1} at the control and decreased for increasing concentrations of monocrotophos. The same trend of results was recorded for different doses of deltamethrin.

Keywords: Catabolism, Chickpea, Chlorophyll, Monocrotophos, Deltamethrin, Protein.

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INTRODUCTION

Legumes are multipurpose crops and are consumed either directly as food or in various processed forms or as feed in many farming systems (Kumara et al., 2014). Legumes (dry beans and pulses) occupy a significant place in the human diet and are widely used both for subsistence farming and as value-added products being a good source of protein, fiber, starch, minerals, and vitamins (Siddiq and Uebersax, 2012). Among pulses, chickpea is chosen as food legumes (Siddique et al., 2000) in some regions because of their multiple uses. It is considered the sole food because of its high protein content, which accounts for almost 40% of its weight. It is a good source of energy, protein, minerals, vitamins, and fibers, and also contains potentially health-beneficial phytochemicals (Wood and Grusak, 2007). Moreover, the grain has potential health benefits, which include reducing cardiovascular, diabetic, and cancer risks (Merga and Haji, 2019).

Unfortunately, the production of chickpeas is constrained by diseases and insect pests. There are many reasons responsible for the poor production of this crop. In field conditions and storage conditions, insect pests and diseases play a very important role in crop production (Bentley and Clements,1989). About sixty insect species are known to feed on chickpea. Among the many arthropods gram pod borer is a major insect, accounting for twenty-one percent of crop yield losses and 50-60 percent of crop pod losses. (Kambrekar, 2012). Three important factors are required for the production of a legume seed crop whether it is pea or any of the legumes. Firstly, it is mandatory to provide the right conditions for the plants to grow. Secondly, the freedom from different pests should be there, and thirdly, stop insects at the blossoming time to ensure a comfortable seed set. They are all necessary and like a chain for better seed output and protein content.

Ahmed et al. (2013) worked on the Integrated Management of Insect Pests of Chickpea and the first step in developing an IPM approach should be to establish the economic threshold. Anandhi et al. (2011) carried out the Evaluation of bio-rational insecticides for the management of *Helicoverpa armigera.* Prasad and Kumar, (2002) explained the impact of intercropping and endosulfan on the incidence of gram pod borer infesting chickpea. Being a highly nutritious and demanding crop, it is necessary to protect the crop from insect pests. Keeping this in view, pre-sowing treatments with two different pesticides; organophosphate monocrotophos and pyrethroid deltamethrin were given and examined the values of protein, chlorophyll a, chlorophyll b, and total chlorophyll content.

MATERIALS AND METHODS

The present study was carried out at the experimental site of Chhatrapati Shahu Ji Maharaj University, Kanpur, Uttar Pradesh, India in 2016. The seeds were purchased from a certified dealer and were kept in standard conditions so that the seeds are not spoil. Healthy seeds of *Cicer arietinum* were selected and surface sterilized by using mercuric chloride ($HgCl_2$) and then washed with distilled water. The sterilized Petri dishes were taken and spread with wet filter paper and the seeds were transferred to these Petri dishes. 10 seeds were taken in each Petri dish. Different doses of monocrotophos, commonly known as Azodrin or Nuvacron and deltamethrin were prepared with distilled water, viz., control (no pesticide), 0.5%, 1%, 1.5%, 2%, 2.5%, 5%, 7.5%, and 10%. The Petri dishes with control seeds were sprayed with distilled water alone and other petri dishes having seeds were treated with increasing concentrations of pesticides i.e., 0.5%, 1%, 1.5%, 2%, 2.5%, 5%, 7.5%, and 10%. The seeds were germinated and the emergence of radicle and plumule was taken as confirmation of seed germination.

Quantitative analysis of plants for chlorophyll content was done on a per-plant basis, first of all, the plant tissues were weighed and the analysis was done on an mgg^{-1} fresh weight basis. Chlorophyll content was estimated by extracting 80% acetone and determined spectrophotometrically; quantification of chlorophyll content was done by the method of Arnon (1949). About 1gm of leaf sample (three replicates of each sample) was crushed into small pieces and homogenized in a precooled mortar and pestle using 80% acetone. A small amount of calcium carbonate is added to the mixture during grinding. The extract thus obtained was centrifuged at 5000 rpm for 5 to 10 minutes Finally, the supernatant was taken and mixed with 80% alcohol to make a 70 ml volume. The clear solution thus formed was transferred to a colorimeter tube and the optical density was measured at 645 nm 663 nm by spectrophotometer. The chlorophyll content was expressed as mg chlorophyll per gram fresh weight of the leaf mixture taken. By using Arnon’s (1949) formula the readings were calculated. Chlorophyll a, chlorophyll b and total chlorophyll concentrations were calculated using the following equation:

(a) Chlorophyll a= 12.7(A663) – 2.69(A645)

(b) Chlorophyll b= 22.9(A645) – 4.68(A663)

(c) Total Chlorophyll- 20.2(A645) + 8.02(A663)

The total soluble protein quantity of the plant was analyzed by the following method: First, the plants (both control and treated) were harvested on the 7^{th} day of growth using an ice medium with NaCl. The harvested plants were washed in distilled water and dried with the help of blotting paper. The shoots were weighed and finally crushed using mortar and pestle in buffer (50 ml Tris at 8.5 pH10 ml-EDTA, pH 8.2, 10 mM $MgCl_2$, 20 mM KCL, 5 mM mercaptoethanol. The slurry thus prepared is subjected to centrifugation at 10000 rpm for 20 minutes. After centrifugation, the total protein content of the seeds was analyzed by Bradford's (1976) method. The protein content was measured in mg/ g fresh weight per shoot basis.

The parameters' results were gathered, and statistics (Mean and Standard Deviation) were applied to the observed results. The chlorophyll content was expressed as mg chlorophyll per gram fresh weight of the leaf mixture taken. The Bradford Protein Assay method was used to estimate the concentration of proteins. The Coomassie dye was added to the sample under acidic conditions. The results were analyzed and can then measured using a spectrophotometer to determine the protein concentration in the given sample.

RESULTS AND DISCUSSION

It has been observed that when the seedlings of *Cicer arietinum* were treated with different concentrations of monocrotophos and deltamethrin, there was a general decrease in protein and chlorophyll content except for 0.5% where these parameters increased. The results are shown in figures 1 A, 1 B, and 2 A, 2 B. As per the results, the chlorophyll contents decreased with increasing concentrations of pesticides. On the other side, the effect was seen when the concentration of pesticides was less, i.e., at 0.5% pesticide concentration there was an increase in both parameters; chlorophyll and protein content. At high concentrations appx. 10% pesticide concentration there were no results at all.

Total chlorophyll contents also showed the same trend and the same happened with chlorophyll a and chlorophyll b, and protein content in seedlings samples at different concentrations of pesticides showed the same results in both treatments.

The effect of monocrotophos on *Cicer arietinum* seedlings is shown in figure 1A and 1B. The results showed that the Chlorophyll a (Fig. 1) content is 0.973 mgg^{-1} for control and 0.986, 0.871, 0.811, 0.745, 0.659, 0.538, and 0.319 mgg^{-1} respectively for increasing concentrations such as 0.5%, 1%, 1.5%, 2%, 2.5%, 5%, and 7.5%, of monocrotophos. The chlorophyll b contents (fig. 1A) were 0.541 mgg^{-1} for control and 0.550, 0.462, 0.375 mgg^{-1}, and 0.310, 0.219, 0.148 mgg^{-1}, and 0.101 mgg^{-1} respectively for increasing concentrations of pesticide. The total chlorophyll content (fig. 1A) was 1.662 mgg^{-1} for control and 1.671, 1.488, 1.347 mgg^{-1}, and 1.214, 1.078, 0.876 mgg^{-1}, and 0.513 mgg^{-1} fresh weight of plant tissue for different concentrations of pesticides. The protein content in the fresh plant (Fig. 2) was 27.45 mgg^{-1}as compared to 28.41, 24.41, 22.89, 19.68, 13.99,11.46 mgg^{-1}, and 10.54 mgg^{-1} respectively for increasing concentrations of monocrotophos, i.e., 0.5%, 1%, 1.5%, 2%, 2.5%, 5%, 7.5%. At 10% pesticide concentration no results were obtained.

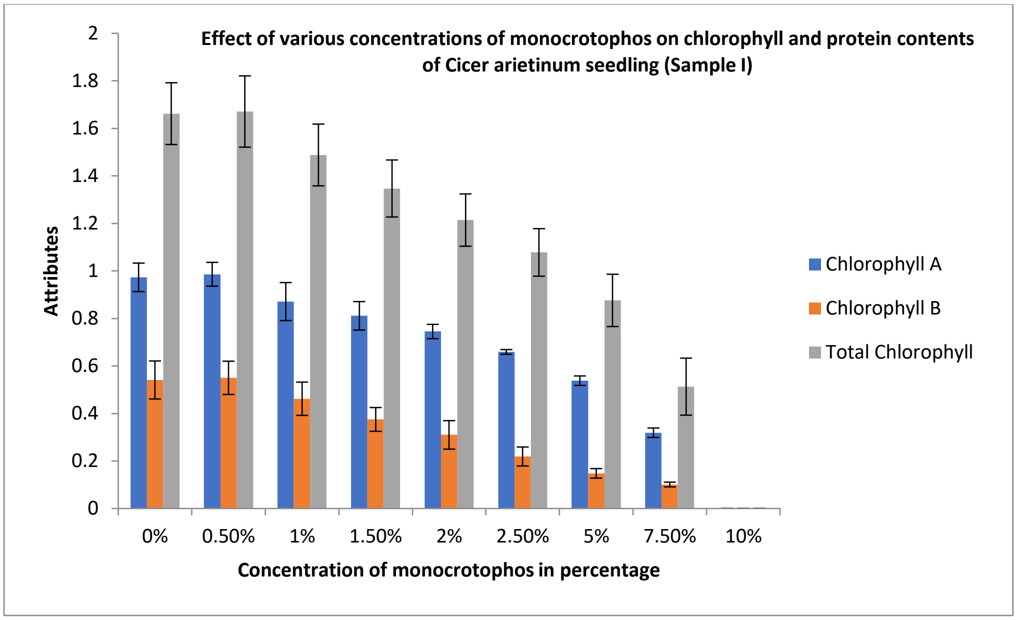

Figure 1: Effect of various concentrations of monocrotophos on chlorophyll a, chlorophyll b and total chlorophyll of chickpea seedlings

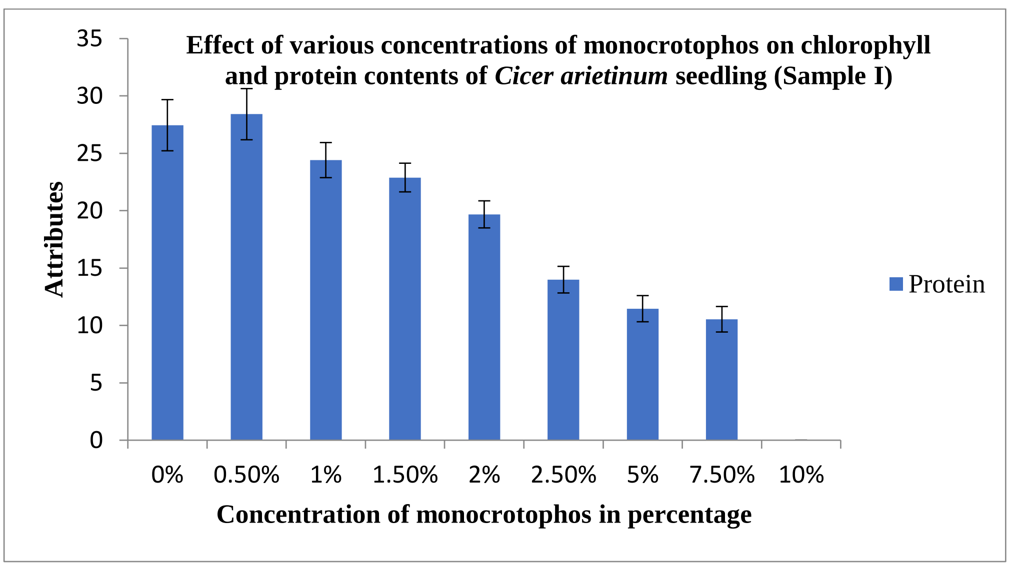

Figure 2: Effect of various concentrations of monocrotophos on protein contents of chickpea seedlings

Effect of deltamethrin on chickpea seedlings are shown in figure 2A and 2B. As per the observations, chlorophyll a (Fig. 3) was 0.931, 0.956, 0.891, 0.828, 0.748, 0.634, 0.565 mgg^{-1} and 0.319 mgg^{-1} fresh weight of leaf tissue in control and respective concentrations (0.5%, 1%, 1.5%, 2%, 2.5%, 5% and 7.5%) of pesticides. The chlorophyll b (Fig. 4) were 0.564, 0.589, 0.463, 0.387, 0.336, 0.211, 0.145 mgg^{-1} and 0.111 mgg^{-1} fresh weight of leaf tissue for control and increasing concentrations (0.5%, 1%, 1.5%, 2%, 2.5%, 5% and 7.5%) of pesticide respectively. The total chlorophyll contents were 1.91, 2.11,1.45, 1.32, 1.19, 1.05, 0.89 mgg^{-1} and 0.50 mgg^{-1} fresh weight.

Protein content for fresh weight of leaves tissue on increasing concentration (0.5%, 1%, 1.5%, 2%, 2.5%, 5% and 7.5%) of monocrotophos were 29.38, 30.30, 23.45, 22.17, 19.67, 13.45 mgg^{-1}, and 10.78, 9.58 mgg^{-1}, for different concentrations of pesticide i.e., 0.0%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 5.0%, 7.5% respectively. At 10.0% concentration results were not seen.

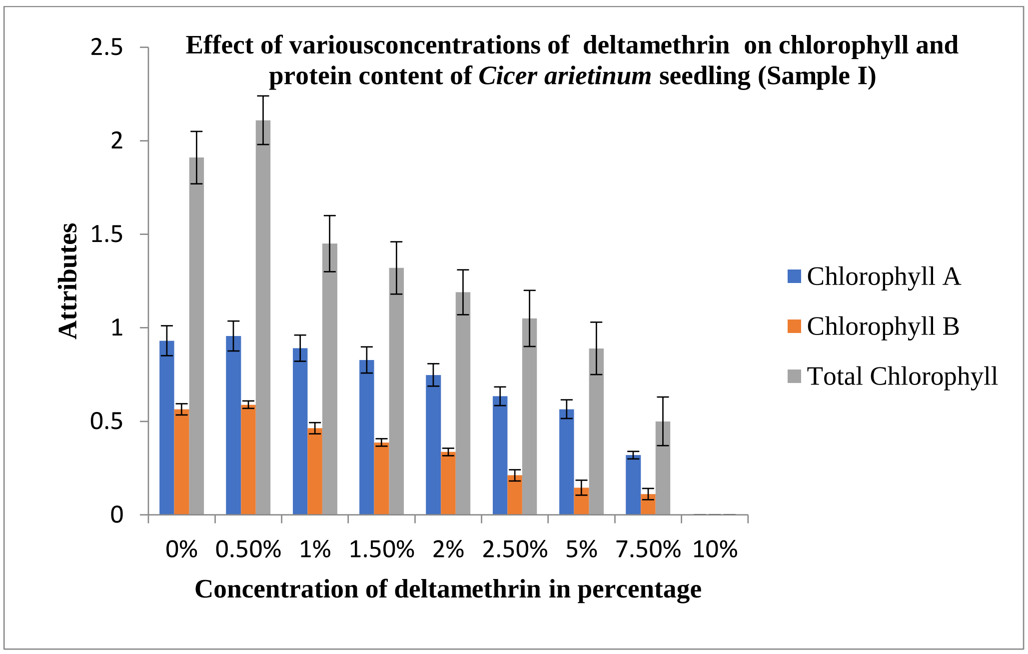

Figure 3. Effect of various concentrations of deltamethrin on chlorophyll a, chlorophyll b and total chlorophyll of chickpea seedlings

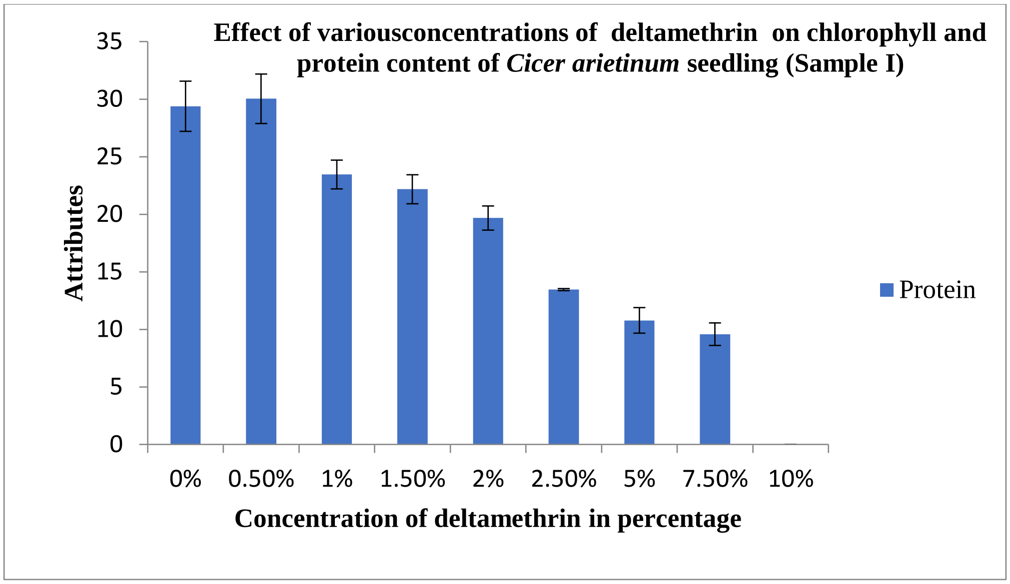

Figure 4. Effect of various concentrations of deltamethrin on protein contents of chickpea seedlings

The quantitative analysis of protein and chlorophyll contents gives an idea of the photosynthesis capacities and nitrogen contents of the plant. The chlorophyll content is used as an indicator of photosynthetic and other activities like catabolism. The reason for the decrease in chloroplast pigments and protein contents may be due to certain metabolic deformities. Some chemical compounds induce the uncoupling of photosynthetic electron flow to block ATP synthesis during photosynthesis (Mitra and Raghu, 1999)

Pesticides affect the germination and growth of the plant which leads to modification in biochemical, physiological, and different enzymatic and non-enzymatic processes which ultimately affect the yield and resulted in residues in plants, vegetables, and fruits. Generally, pesticides attack the target enzymes, which are normally inhibited; this may be due to overexpression or overstimulation of the target proteins. Talat Parween (2015) examined that the enzymes of non-target organisms and microbial enzymes can also be inhibited by a few pesticides However, when the effect of pesticides (chemical and biological) on chickpeas in Nandikandi village (Telangana) was studied, the pest population reduced with the increasing concentration of pesticides (Ramesh, 2017).

Duran et al. (2015) observed the consequence of deltamethrin on Maize, different dosages (0.01, 0.05, 0.1, and 0.5 ppm) of deltamethrin were given to seeds, and the maximum adverse effect on the radicle length was observed by the highest concentration of deltamethrin. Moreover, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were also found to decrease with the increasing concentration of deltamethrin. Anderson et al (2004) found that the herbicide residue inhibits the growth of some legume crops due to a direct effect on rhizobial growth and/or an indirect effect on plant growth. However, pesticides are not safe for the environment and health but they can be used as a weapon to protect against crop loss from insect pests and to gain more yield (Dixit et al., 2022).

CONCLUSION

It is concluded that in *Cicer arietinum* chlorophyll a, chlorophyll b, total chlorophyll, and total protein contents are negatively correlated with the increased concentration of any of the two pesticides (monocrotophos and deltamethrin). At 0.5% pesticide concentration, all the parameters i.e., chlorophyll a, chlorophyll b, total chlorophyll, and protein contents were reported higher. A dosage of 10.0% pesticide concentration is very harmful, even seeds do not germinate at this level of concentration. The present study suggests that for the maximum growth and production of chickpea, only a dosage of 0.5% pesticide can be given.

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