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Research Article

EFFECTS OF INSECTICIDE (IMIDACLOPRID AND PROFEX) AND BIOPESTICIDE (*PSEUDOMONAS* AND *TRICHODERMA*) ON GROWTH AND NUTRITIVE COMPOSITION OF MILLET (JOWAR) & SORGHUM (BAJRA)

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Abstract: Synthetic pesticides have an effect in the quantitative formation of their bio molecules which are used to control the pests of various crops. The present study was carried out to study the effect of Insecticide on the growth and nutritive composition of crop plants, formation of carbohydrate, total free amino acid, protein, total phenol and total chlorophyll contents of Millet (Bajra) and Sorghum (Jowar). A decrease in Protein, Lipid, Phenol content and Chlorophyll content was observed in the Sorghum and Millet Crop plants sprayed with insecticides Profex and Imidacloprid but no effect or increase in content was observed with bio insecticides used which were *Pseudomonas* and *Trichoderma* based.

Keywords: *Total Phenol, Chlorophyll Content, Profex, Imdiacloprid, Pseudomonas and Trichoderma*

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Introduction

A great impact on human health protection and preservation of foods fibre and other cash crops by controlling disease vectors is caused by pesticides use. In developing countries more than 55% of the land used for agricultural production for control of unwanted insects and plants, 26% of the total pesticides produced in the world is used. However, the rate of increase in the use of pesticides in developing countries, pesticides are necessary to protect crops and losses that may amount to about 45% of total food production worldwide. Most commonly used insecticides isprofenofos (0-4-bromo-2- chlorophenyl-O-ethyl-S propyl phosphorothioate) and Imidacloprid. These are non-systemic insecticide used against mites, leafhoppers, thrips, aphids, mealy bugs and cotton stainer. It is toxic to honeybees and birds [1].

Our knowledge of the adverse [2] effect of these pesticides on important plant enzyme and quality parameters of vegetables is quite meagre. The pesticides may affect some biochemical composition of plant like they alter the chemical composition and nutritive value of plant product. The common mechanism of its toxic action is inhibition of biological pathways such as photosynthesis and mitochondrial electron transport [3]. In agriculture, the pesticides are recurrently applied for three major objectives- (i) to produce a larger yield (ii) to produce crops of high quality and (iii) to reduce the input of labour and energy into crop production [4]. Millions of tons of pesticides are applied annually; however, less than 5% of these products are estimated to reach the target organism, with the remainder being deposited on the soil and non-target organisms, as well as moving into the atmosphere and water [5]. The surplus amount of pesticides, which does not reach the target organisms, is absorbed by the plants and hence, pesticide residues have been found in various fruits and vegetables; both raw and processed [6]. Pesticide exposure in consumers by food consumption is one of the most common routes. They can have numerous negative health effects on human consumers owing to their continual exposure in the form of contaminated food products [7]. The excessive use of pesticides is one of the major causes of reduction of the diversity of structural vegetation. Sensitive or stressed plants may be extra vulnerable to phytotoxicity.

Toxicity depends upon many factors such as use of pesticides, rate of application, spraying technique, climate conditions, and organization of flora, humidity and properties of soil such as moisture, temperature, pH, texture and microbial activity. It has been found that pesticide application negatively affects plant growth and development [8]. Pesticide application causes oxidative stress to plants as a result of the generation of reactive oxygen species (ROS) [9]. This oxidative stress results in degradation of chlorophyll pigments and proteins and it ultimately causes a reduction in the photosynthetic efficiency of plants. To cope up with oxidative stress, the antioxidative defence system of plants is activated, which involves enzymatic and non-enzymatic antioxidants.

The use of biopesticide has remained very low due to a number of socio-economic, technological and institutional constraints, in spite of the claimed efficacy of biopesticide [10]. The most common benefits of biopesticides are less toxicity, quick biodegradability and target to specific pest, maintain ecological balance, etc [11].

As a component of integrated pest management (IPM) programs, use of biopesticides can greatly decrease the use of chemical insecticides, while achieving almost the same level of crop yield. However, effective use of biopesticides demands the understanding of an excellent deal concerning managing pests especially by the end users [12].

The objectives of the present study were 1) to know the effect of Insecticides-Profex and Imidacloprid and Biopesticides -*Pseudomonas* and *Trichoderma* based on Sorghum and Millet crop plant growth throughout the growing season and 2) subsequent changes on nutritive composition of crop plants after the treatment of Insecticides and Biopesticides.

Materials and Methods

Insecticide acquisition and preparation: Insecticide -Profex and Imidacloprid and Biopesticide-*Pseudomonas* and *Trichoderma* were purchased from certified agrochemical shops in the Jaipur Market. The insecticides solutions were taken as it is applied in the farming by the farmers. Biopesticides were prepared by dissolving 10mg/l.

Soil preparation

Pots of the same size were filled with soil and labelled. For study, the control consisted of pot with no insecticides/pesticide and biopesticide applied (only water), another set of pot was labelled as (Insecticide), a third set was labelled (Biopesticide) treated Sorghum.

Seed sowing and insecticide application

The viable Sorghum (Bajra) and Millet (Jowar) seeds were purchased from certified agrochemical shops in the Jaipur Market. Seeds were treated with the Insecticides and Biopesticides for 24 hrs. After treatment the seeds were sowed in the pots and plant growth and nutritive composition was studied.

Plant growth and seed nutritive composition

Quantitative determination of Total Phenols

Leaf samples of treated crop plants (1.0g each) were collected randomly at vegetative and late fruiting stage and plunged into 2N HCl with the result that the tissues were killed immediately to restrict the enzymes peroxidase activity. Tissues were then crushed in pestle and mortar using 10 ml of 2N HCl. The crushed material was taken in a tube and boiled for half an hour in a water bath at 60˚C. Then it was filtered and the filtrate was left over anhydride CaCl2 at room temperature until dryness [13]. Quantitative determination was performed by the method described by Swain & Hillis [14]. Amount of total phenolic content was expressed as mg g^{-1} fresh weight using standard curve.

Extraction and estimation of total protein

Seeds (1.0 ± 0.002g) were randomly collected from the treated sites, then extracted in 10ml of 5%TCA (Tri-Chlorocetic acid), centrifuged at 4000rpm for 20minutes followed by the addition of 5ml of 0.5N NaOH in each respective residue, then incubated at 37ºC for 16h. This solution was then filtered through glass wool and OD was recorded at 260 and 280nm by using Shimadzu UV 1260 mini Spectrophotometer. Total protein in mg.ml-1was calculated using the formula adduced by Boyer, [15] as follows and expressed in g g^{-1} dry weight.

$$\text{Protein } (mg.ml^{-1}) = 1.55A280 - 0.76A260$$

Extraction and estimation of total lipid

Finely crushed seeds (1.0 ± 0.002g) of Sorghum treated with insecticide and biopesticide were transferred into a 125mL Erlenmeyer flask. Then 10ml of hexane isopropanol (3: 2) was added and warmed on a hot-plate for 15 minutes. Mixing was thoroughly carried out while heating. Extraction mixture was then filtered rapidly thorough Whatman No. 3 filter paper and poured an additional 10ml of warm hexane isopropanol. Removal of the solvent from the extract under vacuum on a rotary evaporator at 40ºC was performed to obtain a yellow oil or off-white solid. Total lipids were estimated on the basis of complex formation between lipids and ammonium ferrothiocyanate as an improved method [16]. The value of total lipid was expressed as g g-1 dry weight using standard curve.

Quantitative Determination of Total Carbohydrates

Fresh leaves of treated crop plant were taken into a boiling tube. Hydrolyse by keeping it in boiling water bath for 3 hours with 5mL of 2.5 N-HCl and cool to room temperature. Estimation of carbohydrates was done by Anthrone method [17]. A standard graph was plotted using concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph the amount of carbohydrate present in the sample tube was calculated.

Quantitative Determination of Total Chlorophyll content

Samples of leaves were taken before each application as control and after 24 and 96 hours of treatment then transferred immediately to the laboratory to determine chlorophyll (a) and chlorophyll (b). Leave samples of treated plants with insecticides and biopesticides were taken after 10 days from sowing. The photosynthetic pigments were extracted from fresh leaves using 85% acetone [18]. The optical densities were measured spectrophotometrically using spectronic 20D colorimeter at 662 and 644 to determine chlorophyll (a) and chlorophyll (b) respectively. The pigment concentrations were calculated using Wettstain's formula [19].

Chlorophyll (a) (mg /L) = (9.784× E662)-(0.99 × E644)

Chlorophyll (b) (mg /L) = (21.426 × E644)-(4.64× E662).

Where E is: reading of colorimeter.

Quantitative Determination of Total Oil content

The oil content of the mustard seed was determined by Folch *et al*, method [20]. One-gram mustard seed was taken in a mortar. The seeds were completely grinned with a pestle. Thirty ml of chloroform and fifteen ml of methanol (*i.e.* 2:1 ratio) solution was added to it. After through mixing the melt was filtered through Whatman no. 42 filter paper. The filtrate was taken in a beaker and allowed to stand for about six hours for air drying and then dried in an oven for about half an hour to determine total oil. Proper care was taken so that chloroform and methanol completely dry out. Oil content was calculated by the following formula:

*Percentage of Oil=(Weight of extract (gm))/(Sample weight (gm))*100*

Quantitative Determination of Proline Content

The proline content was determined using to the method of Bates *et al*., [21]. The extraction of proline from leaf samples, 100 mg FW was done using 2 ml of 40% methanol. Extract was mixed with a mixture of glacial acetic acid and orthophosphoric acid (6 M) (3: 2, v/v) and 25 mg ninhydrin. After 1 h incubation at 100°C, the tubes were cooled and 5 ml toluene was added. The absorbance of the upper phase was spectrophotometrically determined at 528 nm and total proline amount was calculated with the help of standard curve and expressed as µg proline g^{-1} FW.

Results and Discussion

The application of Insecticide and Biopesticide affects the nutritive composition of seeds. Significant decrease in protein was noted with an application of insecticide. Maximum decrease by 50% in protein of Sorghum (Jowar) and a decrease by 65% in Millet (Bajra) crop plant from the soil sprayed with insecticides (Imidacloprid and Profex) [Fig-1]. This may be due to the presence of amine group increase in nitrogen content because of the presence of amine group. It is also reported that amine group containing insecticides induce efficient synthesis of amino acids leading to build up of proteins [22].

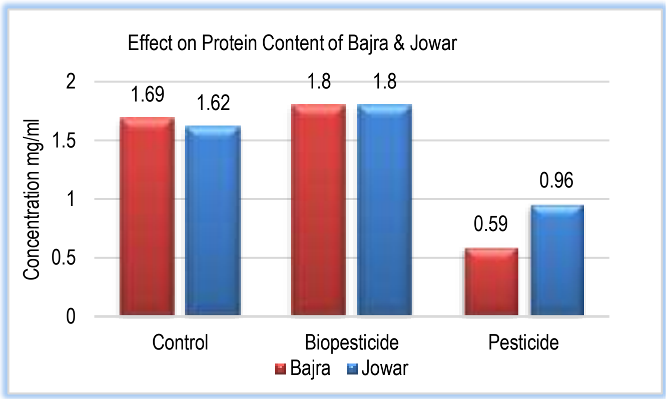

Fig-1 Effect of Insecticides and Biopesticides on Protein Content of Bajra & Jowar

Amar and Reinhold [23] had reported that plant growth is affected by an osmotic shock effect of systemic pesticides which causes release in structural protein and loss of transportability in the leave cells. Toxicant produced by the application of pesticides results in retardation of synthesis of protein and carbohydrate by inducing change in cytochrome oxidase activity, blocking alternative respiratory pathways and accumulation of succinate as reported in earlier studies [24].

Total phenols

Total phenols were tested at the vegetative phase of the growth in leaf of Sorghum (Jowar) which showed a decrease of 42% and 50% due to the application of insecticide (profex and imidacloprid) and 30% due to application of *Pseudomonas* biopesticide but an increase of 22% in Trichoderma biopesticide treated samples as compared with control.

The decrease in the total phenolic content can be attributed to the decomposition of phenols which are responsible for the non-enzymatic antioxidative properties [Fig-2]. Hesam [25] also reported a decrease in phenolic content in potatoes by application of insecticide from Iran [24]. The formation of toxin phenolic compound like flavones is due to the stress caused on plants by spraying pesticides such as fungicides and insecticides. These phytotoxins causes limitation of cell division, nodulation, respiration, photosynthesis, and disruption of cell membrane and also reduces total protein and carbohydrate contents of various plant species [25].

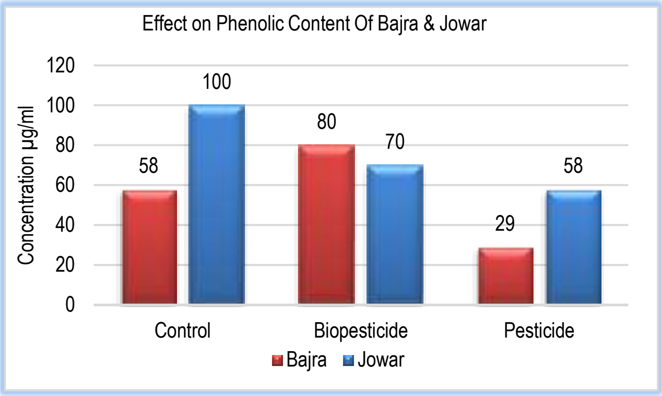

Fig-2 Effect of Insecticides and Biopesticides on Phenolic Content of Bajra & Jowar

Total Sugar

The sugar content was 20% higher in insecticide (imidacloprid) treated crop plant and 0.05% in biopesticide (*pseudomonas*) treated Sorghum (Jowar) crop plant [Fig-3]. The increase in the reducing sugar content in insecticide contaminated leaf might be due to some enzymatic changes which are responsible for the conversion of starch to some reducing sugars. It is well established that certain insecticides influence the chemical composition of the plants after they are applied. Insecticide residues also decreased the glucose content of potatoes [26]. The insecticide residue affects tomatoes and tomato products too, and it is reported that in the insecticide treated tomato samples the amount of glucose content was reduced by 4% as compared to the untreated tomato samples [27]. The soil treatment with biopesticide (Trichoderma) results in an increase of sugar content by 10 % and treatement with insecticides (prfofex) reduce the sugar content by 10 %.Similar results were reported by Abd ElMageed [28] who reported that there was a significant decrease of total soluble sugars in cyanophos treated cotton leaves but the result is in disagreement with Ismail *et al.* [29] who showed that total soluble sugars of treated tomatoes by profenofos increased during the test period.

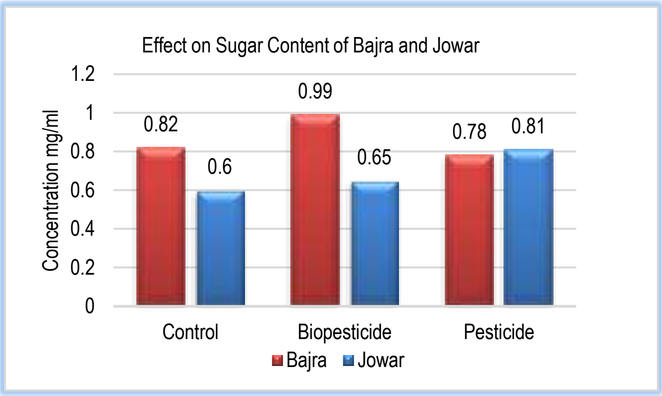

Fig-3 Effect of Insecticides and Biopesticides on Sugar Content of Bajra & Jowar

Total Lipid

Seeds of Millet (Bajra) and Sorghum (Bajra) treated with insecticide (Profex and Imidacloprid), lipid content was not affected as compared to the control but an increase of 10% and 5% was observed with biopesticide (*Trichoderma* and *Pseudomonas*) treatment [Fig-4]. Biochemically, herbicide overdose could lead to redox imbalance in the cell by the production of reactive oxygen species (ROS), leading to oxidative stress, DNA damage, lipid peroxidation in plasma membranes, etc, ultimately leading to cell death [27]

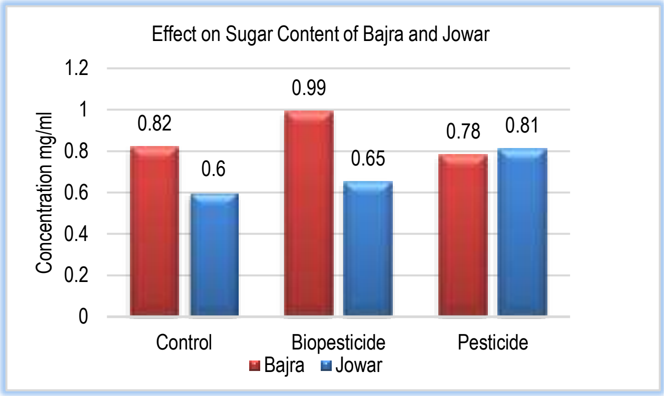

Fig-4 Effect of Insecticides and Biopesticides on Lipid Content of Bajra & Jowar

Cholorophyll

Our result showed that higher concentrations of both Insecticides (Profex& Imidacloprid) and Biopesticides (*Trichoderma* & *Pseudomonas*) significantly reduced chlorophyll a, chlorophyll b and total chlorophyll content ($P < 0.05$) [Fig-5]. It was interesting to note that the reducing potential of Insecticides was stronger than Biopesticide. The data were analyzed by applying t- test. The use of insecticides/pesticides has become a common practice in recent years in order to increase the productivity of various economically important plants, the present investigation has been undertaken to understand the deleterious effect of widely use insecticides/pesticides on the synthesis of chlorophylls in *Viciafaba* L. The genotoxic /cytotoxic effect have earlier been carried out in *Allium cepa* L. and *Viciafaba* L [30].

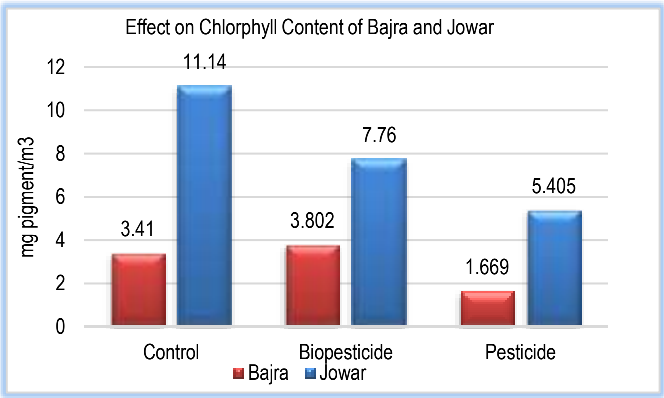

Fig-5 Effect of Insecticides and Biopesticides on Chlorophyll Content Of Bajra & Jowar

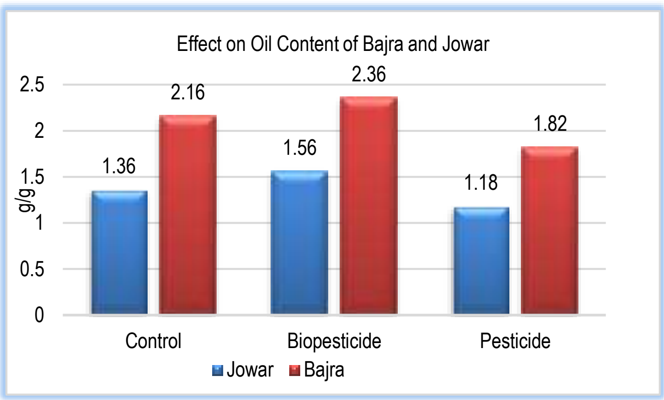

Fig-6 Effect of Insecticides and Biopesticides on Total Oil Content of Bajra & Jowar

Oil Content

Oil content in Sorghum and Millet seed showed statistically significant differences among the treatments. Maximum oil content was found in biopesticide treatment (*Pseudomonas* and *Trichoderma*) of crop plants. Inhibition in oil content was recorded with the Insecticides treatment (Profex and Imidacloprid) treated plants [Fig-6]. The oil content reduced due to insecticides application which confirms this study. Hossain [31] also observed that oil content reduced due to insecticides application which confirms this study.

Free Proline Content

In Sorghum and Millet crop plants, Insecticides (Profex and Imidacloprid) application resulted in reduction of free proline content [Fig-7]. This might be associated with the promontory effects of insecticides. Bhagat and De [32] reported the promotory effects of insecticides in tomato plants. Koleva *et al*., [33] found a reduction in essential aminoacids and amides in sunflower plants treated with carbendazim. The decrease of total free proline content may be associated with the inhibitory effect on various enzymes of proline synthesis pathway due to different pesticides. Different mechanisms have been suggested, how the higher concentration of insecticides retards the physiological and biochemical processes of the plants which could provide further insights into growth retardation as occurred in the present study. For instance, the presence of insecticide residues (solutes) in soil distresses the thermodynamic activity of water along with micro and macro nutrients in surrounding soil.

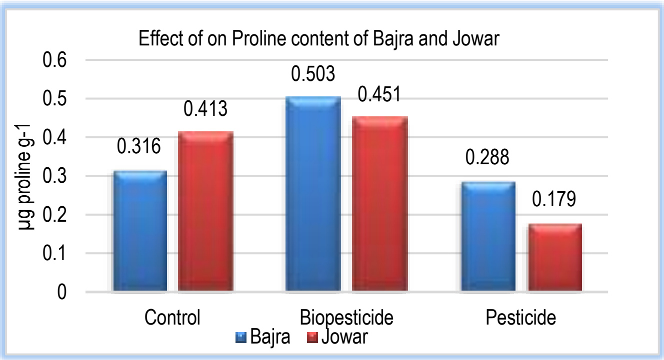

Fig-7 Effect of Insecticides and Biopesticides on Proline Content of Bajra & Jowar

In addition, investigations on total phenols provide insight regarding abiotic stress by insecticide with higher doses adversely affecting the leaves, shoot and fruits. In the past, several studies have attempted to explain the possible mechanisms of phenol induced stress in plants. Yang *et al*., [34] reported that phenolic stress on plant growth can occur in two ways (1) by the blockage of biosynthetic pathway of chlorophyll (an inhibition of supply orientation) or (2) by stimulating degradative pathway (*i.e.* a stimulation of consumption orientation) or both leading to a reduction of chlorophyll accumulation, which in turn causes reduction of photosynthesis and retardation in plant growth

Conclusion

From the above study it can be concluded that the injudicious application of insecticides on crops results in lower yields and persistence of high levels of insecticides residues in the crops at the time of harvest. While the application of biopesticides produces beneficial effects on the crop quality by enhancing the biochemical constituents including the total chlorophyll, carbohydrate and crude protein contents of crops which make the crop fit for consumption. More insight is needed to perform further studies related to phytotoxicity of insecticides so that the desired biochemical characteristics of the crop are retained and the residue levels are within the values. In this study, the application of biopesticides as green chemicals to control the agricultural pest to maintain the sustainability in the agricultural production is discussed.

Application of research: The use of Biopesticides enhances the beneficial effects on the quality of crop by enhancing the biochemical constituents of crops. So, the use of Biopesticides should be enhanced by the farmers instead of Pesticides.

Research Category: Agriculture, Environment

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Research project name or number: Research station study

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Jaipur Market

Cultivar / Variety / Breed name: Millet (Jowar) & Sorghum (Bajra)

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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