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Studies on biochemical resistance factors of maize plant against maize spotted stem borer, *Chilo partellus* (Swinhoe): A Review

Manish Kumar Yadav[1*], Deepayan Padhy[1], Lipsa Dash[1], Rajesh S Kalasare[2]
[1] Assistant Professor, Agricultural Entomology, M. S. Swaminathan School of Agriculture Science, Centurion University of Technology and Management, Paralakhemundi, Odisha, India
[2] Associate Professor, Agronomy and Agroforestry, M. S. Swaminathan School of Agriculture Science, Centurion University of Technology and Management, Paralakhemundi, Odisha, India

Abstract
Maize Spotted Stem Borer, *Chilo partellus* (Swinhoe) is a major pest of maize and sorghum and cause a very huge loss in these crops during *Kharif.* Here an effort has been made to evaluate the importance of different HPR traits related to biochemicals, present in maize plants and their significance in management of maize spotted stem borer, *Chilo partellus.* The term antibiosis is mainly dealing with biochemical components of maize plants which are Nitrogen, Phosphorus, Potassium, Protein and Chlorophyll. These components play vital roles in imparting resistance in maize plants and affect survivals of immature stages in many ways including the antibiosis. There many biochemical traits have been identified and discussed to be having greater impact against maize spotted stem borer in imparting resistance and the methods of estimation have been defined.

Keywords: resistance, HPR, maize, biochemicals, *Chilo,* chlorophyll

Introduction

India is an agriculture-based economy, having an average of 140 per cent cropping intensity which includes many groups of crops like cereals, pulses, oilseeds, fodders, vegetables and many others. Maize is the most important and second most widely grown cereal after rice. The origin place of maize or corn is Mexico and firstly domesticated about 10000 years ago by indigenous people of Mexico (Benz, 1999) [5]. It is also believed to have originated in Northern Guatemala.

Maize is a species of family Poaceae (tribe Maydeae), botanically known as *Zea mays* and firstly described by Carl Linnaeus (Gondim, *et. al.*, 2013) [8]. Maize is a diploid (2n=20) and cross pollinated (monoecious) crop. The leafy stalk of the plant produces pollen inflorescences and separate ovuliferous inflorescences called ears that yield kernels or seeds, which are fruits of maize. Maize is very rich in nutrients like proteins, minerals & vitamins and termed as queen of cereals due to having highest genetic potential for yield. The plant of maize grows straight up to 2.5 meter, unbranched and having leaves on every node. Maize is grown for many purposes including grains for food, for vegetable and for animals feed and fodders (Kakar *et. al.*, 2003) [10]. It is well-known for its role as food, feed and industrial crop in Indian subcontinent and other divisions of the new as well as old world. It is also being used for production of alcohol-based drinks, sweetener agents for food commodities, starch, oil and different proteins. Recently, it was recently has been came into light that sweet corn can also be used in the production of industrial-fuels. A Healthy plant of maize can constitute about 60 to 68 per cent of carbohydrate and 7 to 15 per cent of amino compounds. The edible embryo part which forms about 12 per cent share of the whole grain is the source of different nutrients like protein, fats and sugar. Yellow part of maize is known to be the richest sources of Vitamin-A. Maize has more riboflavin than wheat or rice and is rich in phosphorous and potassium. About 50 per cent of the total Indian produce is consumed as poultry feed and about 8 per cent is consumed by the starch industry. The sweet corn has 1.2 to 5.7 per cent of edible type of oils. Maize oil is more commonly being used as a cooking medium and for manufacturing of different hydrogenated oils. Maize serves as a source in the manufacture of different biochemicals like starch, syrup, dextrose, oil, gelatin, lactic acid etc.

Maize flour can be used as a thickening substance in the preparation of many edibles' items like soups, sauces and sweet custard powder. "Corn syrup is used as an agent in confectionary units. Corn sugar (dextrose) is used in pharmaceutical formulations as sweetening agent". "The gel of corn on account of its moisture retention character is used as a bonding agent for ice-cream cones, as a dry dusting agent for baking products according to Arnon, 1949 [4]". Maize in India is used as a source of poultry food (43%), human feed (23%), cattle feed (17%), starch industry (14%), brewery (2%) and seed (1%). It is extremely important for human and animal nutrition as staple food in a number of developed and developing countries. Maize consumption in India has grown up to 19 million tones (Anonymous, 2019).

Maize spotted stem borer, *Chilo partellus* is a major pest of maize and sorghum and mainly infest the crops during the *Kharif* season of the year. The pest belongs to Crambidae family of Lepidoptera order and characterized by having four metamorphic stages during its life cycle *i.e.*, *egg*, larva, pupa and adults. The pest mainly attacks the crop during *Kharif* as it requires hot and humid climate to develop into adults and in winters mostly, they hibernate inside the stems of their hosts in larval stages. The pest mostly attacks on the stem portion of the crops as the name reveals, neonate larvae use to make pinholes on the whorl leaves that later becomes windows while larvae use to transport themselves inside the stem. The larvae feeds on the inner content of stem and develop inside the stem. After approx. five moults, the larvae turn into the pupae inside the stem. Before take the pupation, larvae use to make an exit holes to make surety of a passage for their exit after turning into the adult (Weatherwax, 1955) [16].

Biochemicals like Nitrogen, Phosphorus, Potassium, Protein and Chlorophyll are largely important for imparting resistance in crop plants including maize and sorghum, there, the present review has been made to discuss the importance, significance, role of biochemicals in maize against maize spotted stem borer and methods to quantify these biochemicals.

Methodology of Assessment and Significance of Different Biochemical Traits of Maize in HPR

Biochemical plant characters likeNitrogen, Phosphorus, Potassium, Protein and Chlorophyllhave been studied at particular stage of crop, previously stated to be having maximum visible symptoms and have been presented below under their respective heads. The significance of all the biochemicals as well as impact as resistance factor against stem borer has been described below.

Major Plant Nutrients (N, Protein, P and K)

Sample Preparation

A sample must be a true representative of the crop under the field condition. For the estimation of biochemicals like nitrogen, phosphorus, potassium and protein samples should be prepared by selecting plants from each replication of each treatment randomly (use of random Table is suggested) and should be cut from the base at root before as the sample should represent the whole plant. These samples should be composited separately representing poor, vigorous and normal growths. These plants samples than brought to the laboratory, should be washed with running water in order to make them free from any other adhering substances and, chopped into very small pieces by mixing stem and leaves at the rate of 80:20. Later on, the chopped material was mixed thoroughly and dried in air for 1 day and later should be dried in the oven at 700C for a total period of 22-28 hours. These dried samples should again grinded upto finer dust. From these grinded samples, 5 gm of grinded material recommended for estimation of N, P and K and protein.

Nitrogen

Nitrogen is a macro-nutrient for plant and an essential dietary nutrient of insects for the fulfillment of the requirements of total essential amino acids. For quantification of nitrogen, Kjeldahl method should be used as its more authentic.

Materials Required

1. Plant Samples (5gm of each)
2. Electric Balance
3. Burette
4. Pipette
5. Conical Flask
6. Measuring Cylinder
7. Kel Plus
8. Digestion Unit with Digestion Tubes
9. K_2SO_4
10. Cupric Sulphate
11. Metallic Selenium Powder
12. NaOH (40%)
13. Boric Acid (4%)
14. Indicator

A. Methyl Red

B. Bromocresol Green

15. H_2SO_4 of 0.02 normality
16. Distilled Water
17. Concentrated H_2SO_4
18. K_2SO_4:$CuSO_4.5H_2O$: Metallic Selenium Powder at the rate of 50:10:1

Procedure

The total amount of samples should be 5gm and uniformly taken from each replication of each treatment for the estimation of nitrogen content. The plant samples should be placed in heating tubes (digestion tubes) and heated from 1000C to 4000C with 10 ml of concentrated sulphuric acid and Catalyst mixture for a period of total six hours with sulphuric acid of laboratory grade and prominent water supply, which decomposes the organic substance by oxidation. The samples should be heated upto the total amount decreased upto 5ml, the samples become light green or colourless at the end of the digestion process. In this step potassium sulphate should be added to increase the boiling point of the medium. Then the solution need be cooled and distilled with a small quantity of sodium hydroxide, which converts the ammonium salt into ammonia. After distillation, samples placed in Kel Plus unit in which Boric acid and Indicator are mixed. The samples collected from Kel Plus and the amount of nitrogen present in the sample should be determined by titration (AOAC, 1970) [3]

$$\textbf{Per cent Nitrogen} = \frac{(\textbf{SampleTV} - \textbf{BlankTV})\textbf{0.00007} \times \textbf{Volumeofdigation}}{\boldsymbol{b}\textbf{weightofsample} \times \textbf{Adiquot taken}} \textbf{X100}$$

Nitrogen is a well-known plant macro-nutrient and plants require it at all the stages of growth. Despite this, excess nitrogen leads to vigorous growth and resulted in more susceptibility to pest attack. Nitrogen shows a significant positive correlation with the infestation in many studies and similar findings have been observed for the pest relationship. In the case of Naveed *et. al*., 2015 [11], they have tested a total number of ten genotypes of maize for nutritional contents including nitrogen. They have concluded that increase level of nitrogen up to a certain level can result in increased infestation levels and they correlated it as positive between infestation and nitrogen level.It was observed in the studies that genotypes having greater amount of nitrogen, can be found susceptible to maize spotted stem borer, *Chilo partellus.* The correlation between the nitrogen and infestation was found to be positive and significant in all the studies conducted by different researchers. It was observed that greater nitrogen amount leads in excess vegetative growth and greater degree of insect feeding.

Protein

The protein content can be recorded at peak infestation stage in order to correlate protein level with the susceptibility levels. The protein was calculated by the formula followed by Winkleman *et al*., 1954

Protein per cent = Nitrogen per cent×6.25

Protein is basic element for developments in insects, higher amount of protein leads in susceptibility of plants towards the insect-pests. Genotypes having more amount of protein, identified as susceptible. It was also well studied by many researchers that protein and nitrogen were positively correlated with each other, genotypes having more proteinper cent showed more infestation *C. partellus*. Similar findings of the study have been reported by many workers on maize including Rao and Panwar, 2002 [12]; Naveed *et. al*., 2015 [11]; Kabre and Ghorpade, 1999 [9]; Ipshita *et. al*., 2019 and many others. Ipshita *et. al*., 2019, have conducted their work on maize with all the biochemicals including protein content. They have reported that protein is responsible for survival of immature stages inside the stem in large number. They have correlated protein with infestation and found positive relationship. Genotypes having more protein content, showed more immature stage after the tasseling stage at the time of splitting.

Phosphorus

The phosphorus is an essential plant nutrient and it occurs in many forms. Therefore, a reliable procedure for measuring the amount in plant is needed (Upadhyay and Sahu, 2012) [15].

Materials Required

1. Plant Sample
2. Spectrophotometer
3. Volumetric Flask (50ml)
4. Tri Acid Mixture (Sulphuric Acid (98%) + Nitric Acid (70%) + Perchloric Acid(70%), 1:3:10)
5. Digestion Chamber (Hot Plate)
6. Ammonium Molybdate
7. Distilled Water
8. Ammonium Metavandate
9. Burner

Procedure

A total amount of 5gm of grinded materials of all the made replications of each treatment should be taken for the estimation of phosphorus content. The procedure involve sampling, weighing, washing and grinding of the samples.

For the estimation of phosphorus, a tri acid mixture should be prepared with the help of Sulphuric Acid (98%) + Nitric Acid (70%) + Perchloric Acid (70%) in the ration of 1:3:10. After it, plant samples are placed in flasks with 10 ml of tri acid and placed on hot plate for a period of 12 hour for digestion. On the other hand, reagents should be prepared for solution preparation.

Solution A
for the preparation of solution-A, an amount of 25 ml of ammonium molybdate is taken and should be mixed in 400 ml of water.

Solution B
For preparation of solution-B, an amount of 1.25 gm of ammonium metavandate is taken and should be mixed in 300 ml of boiling water. After cooling, an amount of 250 ml of concentrated HNO3 of 70% should be added.After preparing both the solutions, they should mix together and total volume should be maintained 1000 ml by adding 50 ml of water (solution A=400ml+Solution B=300+250 ml HNO3 of 70%+50 ml of water). 10 ml of reagent is added in each sample and 50ml volume is prepared by adding distilled water. All the filtered samples are taken for spectrophotometer reading at 470nm.

Preparation of Standard Curve
Different volumes of 0, 1, 2, 3, 4 and 5 ml of 50 ppm P solution was transferred to volumetric flask of 50 ml capacity in order to get 0, 50, 100, 150, 200 and 250 μg P. 10 ml of vandomolybdate are added in all the solutions and mixed thoroughly. Later on, the absorbance should be read at 420nm with blue filter.
Phosphorus is a micro nutrient for plants and found in very minute quantity in different parts of plants. In case of maize genotypes, phosphorus has no any significant difference in quantity present in plants. Phosphorus per cent in different genotypes can be ranged from 0.25 to 0.45 per cent as per the earlier researches. A few researchers like Chen *et. al*., 2009; Showler and Patrick, 2014 conducted their works on biochemicals including Phosphorus and have reported no significant level of correlation between infestation and phosphorus content. However, it was recorded that phosphorus has a positive correlationwith infestation but not up to the significant level.

Potassium
Potassium is a major nutrient of plants, known to be associated with resistance and tolerance in various crops. The correlation between plant potassium content and infestation is found to be negative and significant.

Materials Required
1. Plant Sample
2. Flame Photo Meter
3. Plastic container of 20 ml
4. Tri Acid Mixture (Sulphuric Acid (98%) + Nitric Acid (70%) + Perchloric Acid(70%), 1:3:10)
5. Digestion Chamber (Hot Plate)
6. Distilled Water

Procedure
A total amount of 5gm of grinded materials of each made replication pf each treatment should be taken for the estimation of potassium content. The procedure involve sampling, weighing, washing and grinding of the samples same as nitrogen and phosphorus. For quantification of potassium, 5gm plant material is taken and should be placed in a 100 ml of flask with 10 ml digestion acid (tri acid) in the ratio of 3:10:1 (HNO3:HClO4:H2SO4).
Then the flasks are placed at low heat hot plate in digestion chamber and heated upto a higher temperature to reduce volume (3-5 ml) by evaporation and after cooling 50 ml of distilled water isused to add. The composition of digestion should be confirmed when the liquid became colourless and then the 10 ml from each sample is read in flame photometer for potassium. Potassium is a nutrient mostly related towards to resistance against pests and diseases. Genotypes having greater amount of potassium can be showing less infestation while the genotype comparatively less amount of potassium, shows more susceptibility towards the insect infestation as per the earlier researches.
Potassium content in the maize from genotype to genotype showed a greater variation and found to be in increased amount in genotypes partially resistant to pests and diseases. Researchers like Naveed *et. al*., 2015 [11]; Ali *et.al*., 2015 [1]; Dhillon *et. al*., 2015 [7] and many others have included potassium assessment in the host against maize pests and reported similar finding. They have concluded that potassium is directly related to resistance of crop. They have established a significant negative correlationwith infestation of maize spotted borer.

Chlorophyll
Chlorophyllis the component, responsible for green colour of plants.The chlorophyll content was estimated according to the method of Arnon (1949) [4].

Materials Required
1. Plant Samples
2. Spectrophotometer
3. Acetone
4. Mortar Pistil

5. Centrifuge Unit
6. Balance
7. Centrifuge Tubes

Procedure

About 1 gm of leaf samples should be taken, cut into small pieces and homogenized in a pre-cooled mortar and pestle using 80% of Acetone. A pinch of calcium carbonate is added while grinding. Later the extract should be centrifuged at 3000 rpm for 15 min and made up to 25 ml with 80% of Acetone. The clear solutions are transferred to a colorimeter tube and the optical density is measured at 645 nm and 663 nm, against an 80% acetone blank in Shimadzu 35 Double Beam spectrophotometer (UV 240). The levels of chlorophyll 'a' and chlorophyll 'b' are determined using the equation given below:

Chlorophyll-a [mg/mL] = 12.7 A663 - 2.69 A645
Amount of Chlorophyll-b [mg/ml] = 22.9 A645 - 4.68 A663

where:

A645 = absorbance at a wavelength of 645 nm
A663 = absorbance at a wavelength of 663 nm.
Total Chlorophyll (mg/mL) = Chlorophyll a + Chlorophyll b

The amount of chlorophyll *a* is found in higher amount in compare to chlorophyll *b* in maize as well as in other plants too. Earlier studies done by researchers like Rao and Panwar 2002 [12], Yadav *et. al*., 2019, indicated a positive correlation between plant ages and chlorophyll content while in case of infestation and chlorophyll association is also positive for all the types of chlorophyll. Dhillon and Chaudhary, 2015 [7] have conducted their works on maize with composite and hybrid group of genotypes and have concluded that chlorophyll *a*, chlorophyll *b* and total chlorophyll found significant positive correlation (r=0.431, 0.137 and 0.773) with the infestation. They have also confirmed that increasing plant age will results in decreased amount of total chlorophyll including chlorophyll *a* and chlorophyll *b*.

Conclusion

Nutritional of biochemicals are the components, responsible for proper growth and development of plants at all the stages of crop growth and they solely responsible for nutritional values of certain parts of plants. The nutritional contents directly affect the health and activity of insects by impacting on their energy levels as well as proper developments. There are many pests attract towards the crops having greater vigour and greater morphological visibility. The vigour of plants is largely depends upon certain biochemicals like nitrogen, protein and chlorophyll contents. Insects known to be causing heavy feeding on plants with greater amount of chlorophyll and greater vigour *i. e*., nitrogen and protein per cent. Nitrogen is solely source on insects to build non-essential amino acids, that's why they use to prefer vigorous plants. Plants nutrients like potassium and phosphorus are known to be related with stress and providing different degree of resistance in plants against many pests and diseases. In biochemicals, plant nitrogen, potassium and many other biochemicals reported to be directly related with various levels of infestation and known to be making plants suitable for infestation or providing resistance. Many earlier workers have reported that excess nitrogen is directly related to susceptibility of plants against various insects while potassium has been reported to be imparting tolerance and resistance in crops against various pests. studieshave reported in their study that plant chlorophyll contents have positive relationship with infestation various insects. Nutritional factors are largely responsible for imparting resistance into the maize plants against many insects including *Chilo partellus* (Swinhoe). The major plant nutrients like N, P, K and proteins are present in maize plant tissues have a significant role in insect-pest resistance and tolerance. Therefore, there are different methodology are used to assess the different levels of injury/damage caused by *Chilo partellus* in maize crop. So, there is need to explore the utilization of different level of resistance/tolerance through assessing the comprehensive relationship of injury/damage caused by *Chilo partellus* in promising genotypes of maize in view of major nutrition factors.

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